CHARACTERISATION, MONITORING AND TREATMENT OF SYSTEMIC AMYLOIDOSIS

Helen J Lachmann

Doctor of Medicine

University of London

National Amyloidosis Centre

Department of Medicine

Royal Free and University College Medical School

Rowland Hill Street

London NW3 2PF

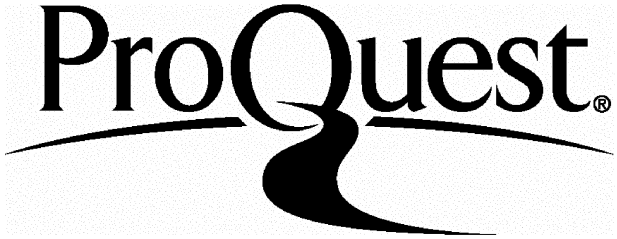
2003

ProQuest Number: 10014455

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.



ProQuest 10014455

Published by ProQuest LLC(2016). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code.
Microform Edition © ProQuest LLC.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ABSTRACT

Amyloidosis is a heterogeneous group of disorders caused by extracellular deposition of protein in a characteristic abnormal fibrillar form. The development of serum amyloid P component (SAP) scintigraphy has uniquely enabled non-invasive serial assessment of the amount and distribution of amyloid deposits in patients. The findings of SAP scintigraphy have refuted the traditional belief that amyloid deposition is both inexorably progressive and irreversible. In contrast, amyloid deposits exist in a state of dynamic equilibrium, and therefore may regress when underlying disorders can be treated. Unfortunately many treatments used in systemic amyloidosis carry considerable iatrogenic risks. The general hypothesis explored in this thesis is that the rationale for treatment and clinical outcome of patients with the various forms of amyloidosis can be improved by more precise characterisation and quantitative monitoring of the disease process that underlie amyloid deposition.

Hereditary systemic amyloidosis has hitherto been considered to be exceptionally rare, but the finding described in this thesis that it actually accounts for about 10% of patients with apparent sporadic acquired systemic amyloidosis demonstrates the critical importance of accurate diagnosis. This finding has already spared many patients undergoing inappropriate and dangerous chemotherapy, and, in some cases, has enabled liver transplantation to be performed with the objective of correcting the inherited metabolic abnormality. It has also facilitated the detailed clinical characterisation of the hereditary fibrinogen A α-chain amyloidosis phenotype, and has led to the introduction of

routine DNA analysis among NHS patients attending the National Amyloidosis Centre.

Precise quantitative monitoring of circulating amyloid fibril precursor proteins enabled the relationship between monoclonal immunoglobulin production and clinical outcome to be evaluated for the first time in AL amyloidosis, and in AA amyloidosis confirmed efficacy of surgical and pharmacological treatments in patients with Castleman's disease and familial Mediterranean fever (FMF) respectively. It also revealed for the first time the pattern and substantial intensity of sub-clinical inflammation in familial Mediterranean fever, explaining the high incidence of AA amyloidosis in this disorder.

ETHICAL APPROVAL

All individuals who participated in the clinical research studies described in this thesis gave full informed consent in a format approved by the Ethics Committee of the Royal Free Hospital. The dosage and administration of radioactive isotopes were approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health.

ACKNOWLEDGEMENTS

I am grateful to Professor Philip Hawkins for providing the opportunity for me to undertake this M.D. and for his intellectual stimulation and supervision. I owe thanks to everyone in the laboratory, particularly to Dr David Booth and Dr Alison Bybee who performed all of the gene sequencing work with help from Dorota Rowczenio, to Janet Gilbertson who provided much of the histology and all of the difficult immunohistochemistry; and to Ruth Gallimore who performed the serum protein assays and always knew were to find even the oldest samples. The Turkish patients were looked after by Professor Mehmet Tunça and he and his colleges from the Department of Rheumatology, Ismir University collected their blood samples. The clinical service relied on nursing support from Sheril Madhoo; echocardiography services from Jayshree Joshi; radiography from Dorothea Gopaul and much secretarial support and organisation by Jackie McLeod.

Most importantly I would like to thank the referring physicians and the patients themselves who have been unfailingly enthusiastic about research and tolerant of so much investigation and treatment.

CONTENTS

Page

Abstract ii
Ethical Approval iv
Acknowledgements v
Abbreviations xiii
List of Figures xvi
List of Tables xviii

Chapter One **INTRODUCTION**

..... 1
AMYLOID STRUCTURE 2
Protein fibrils 2
Non fibrillar components 4
Glycosaminoglycans 4
Serum amyloid P component 5

PATHOGENESIS OF AMYLOID 6

PATHOLOGICAL EFFECTS OF AMYLOID 10
Amyloid degradation 11

TYPES OF AMYLOID 11
Reactive systemic, AA, amyloidosis 11
Systemic amyloidosis associated with monoclonal immunocyte dyscrasias, AL amyloidosis 12
Systemic amyloidosis associated with monoclonal immunocyte dyscrasias, AH amyloidosis 14
Dialysis related amyloidosis (DRA) 14
Senile systemic amyloidosis 14
Hereditary systemic amyloidosis 15
Localised amyloidosis 15
Cerebral amyloidosis 16

DIAGNOSIS 16

GENERAL TREATMENT PRINCIPALS 18
Preservation and replacement of organ function 18

REACTIVE SYSTEMIC, AA, AMYLOIDOSIS 20
Underlying conditions 20
The inherited periodic fever syndromes 23
Familial Mediterranean fever 23
Muckle-Wells syndrome and familial cold urticaria 29
Hyper immunoglobulin D syndrome 31
Tumour necrosis factor receptor associated periodic syndrome 32
Clinical features 33
Treatment 35

SYSTEMIC AMYLOIDOSIS ASSOCIATED WITH MONOCLONAL IMMUNOCYTE DYSCRASIAS, AL AMYLOIDOSIS 36
Underlying conditions 36
Clinical features 37
Treatment 38

DIALYSIS RELATED AMYLOIDOSIS (DRA), B_2-MICROGLOBULIN AMYLOIDOSIS 39
Underlying conditions 39
Clinical features 40
Treatment 41

HEREDITARY AMYLOIDOSIS 42
Familial amyloid polyneuropathy (FAP) 42
Treatment 43
Familial amyloid polyneuropathy with predominant cranial neuropathy 46
Hereditary non-neuropathic systemic amyloidosis 46
Lysozyme 46
Apolipoprotein AI 47
Apolipoprotein AII 48
Fibrinogen A alpha chain 50

GENERIC THERAPIES FOR AMYLOIDOSIS 50
Prevention of fibril formation 51
Enhancement of amyloid regression 51
Immunotherapy 51
Targeting SAP 52

Chapter Two **METHODS**

PATIENTS 55

SAP SCINTIGRAPHY 55

ECHOCARDIOGRAPHY 57

CRITERIA FOR DIAGNOSIS OF AMYLOID RELATED MAJOR ORGAN INVOLVEMENT 58
Cardiac 58
Renal 58
Adrenal 58
Splenic 58
Hepatic 59
Peripheral nervous system 59
Autonomic nervous systemic 59

HISTOLOGY 59
Congo red staining 59
Immunohistochemistry 60

IMMUNOASSAYS 61
C-reactive protein (CRP) 61
Serum amyloid A protein (SAA) 61
Serum free immunoglobulin light chain assay 61
Interleukin 6 62

GENE SEQUENCING 62

STATISTICAL ANALYSIS 65

Chapter Three **FREQUENCY OF HEREDITARY SYSTEMIC AMYLOIDOSIS MASQUERADING AS IMMUNOGLOBULIN LIGHT CHAIN, AL AMYLOIDOSIS**

INTRODUCTION 66

PATIENTS 67

RESULTS 68
Gene sequencing 68
Histology 68
Detection of monoclonal immunoglobulin 71

DISCUSSION 74

Chapter Four **CHARACTERISATION OF THE PHENOTYPE OF HEREDITARY AMYLOIDOSIS ASSOCIATED WITH VARIANT FIBRINOGEN A ALPHA CHAIN**

INTRODUCTION 79

PATIENTS 80

RESULTS 81
Fibrinogen A alpha chain Glu526Val 81
Family history 81
Clinical Phenotype 87
Solid organ transplantation 91
Asymptomatic fibrinogen A alpha chain Val526Glu

heterozygotes detected by family screening 94
SAP scintigraphy 95
Biopsy appearances 95
Haplotype evidence 96
Fibrinogen A alpha chain Gly540Val 98

DISCUSSION 99

Chapter Five **MEASUREMENT OF CIRCULATING FREE IMMUNOGLOBULIN LIGHT CHAINS IN PATIENTS WITH AL AMYLOIDOSIS**

INTRODUCTION 105

METHODS 107
Patients 107
Chemotherapy 108
Clinical assessment 108
Serum free immunoglobulin light chain assay 109
Statistical analysis 109

RESULTS 110
Detection of excess FLC in patients with systemic AL amyloidosis prior to cytotoxic treatment 110
Detection of excess FLC in patients with localised AL amyloidosis 112
Detection of excess FLC in patients with uncomplicated MGUS 112
Definition of a normal range of serum FLC in renal failure 113
Serial assays of serum FLC after cytotoxic treatment in patients with systemic AL amyloidosis 114

DISCUSSION 120

Chapter Six **OUTCOME IN FIVE CASES OF UNICENTRIC CASTLEMAN'S DISEASE COMPLICATED BY SYSTEMIC AA AMYLOIDOSIS**

INTRODUCTION 125
CASE HISTORIES 127
Case 1 127
Case 2 131
Case 3 131
Case 4 133
Case 5 133

DISCUSSION 134

Chapter Seven **SERIAL MONITORING OF THE ACUTE PHASE RESPONSE IN HEALTHY INDIVIDUALS WITH WILD TYPE MEFV, HETEROZYGOUS CARRIERS OF THE FMF TRAIT AND PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER**

INTRODUCTION 138

METHODS 139
Patients 139
Gene sequencing 139
Statistical analysis 140

RESULTS 140
MEFV genotypes 140
Controls 140
FMF patients 140
First degree relatives 141
Serial measurements of the acute phase response proteins;
serum amyloid A protein (SAA) and C-reactive protein (CRP) 144
MEFV, wild type healthy controls 144
FMF patients 144

MEFV heterozygotes 147

DISCUSSION 148

Chapter Eight **CHARACTERISTICS AND OUTCOME OF PATIENTS WITH AA AMYLOIDOSIS COMPLICATING FMF**

INTRODUCTION 152

PATIENTS 153

RESULTS 154
Gene sequencing 154
Clinical features 154
Serial measurements of serum amyloid A protein (SAA) 157
Changes in ^{123}I-labelled SAP scintigraphy and renal function 159

DISCUSSION 158

GENERAL CONCLUSIONS 160

PUBLICATIONS ARISING FROM STUDIES IN THIS THESIS 162

REFERENCES 164

ABBREVIATIONS

Aβ	amyloid β protein
AA	amyloid A protein
AEF	amyloid enhancing factor
AL	monoclonal immunoglobulin light chain derived amyloid
ALP	alkaline phosphatase
apoAI	apolipoprotein AI
apoAII	apolipoprotein AII
AS	ankylosing spondylitis
B_2M	Beta-2-microglobulin
CAPD	continuous ambulatory peritoneal dialysis
CCF	congestive cardiac failure
CR	complete response
CRF	chronic renal failure
C-terminal	carboxyl terminal
CTx	cardiac transplant
CNS	central nervous system
CRP	C-reactive protein
CV	coefficient of variance
C-VAMP	cyclophosphamide, vincristine, Adriamycin, methyl prednisolone
Da	Dalton
DB	3,3' diaminobenzidine tetrachloride

DRA	dialysis related amyloid
ECG	electrocardiogram
ECM	extracellular matrix
EDTA	ethylenediaminetetra-acetic acid
ESRF	end stage renal failure
FAP	familial amyloid polyneuropathy
FLC	free immunoglobulin light chains
FCU	familial cold urticaria
FMF	familial Mediterranean fever
GAGs	glycosaminoglycans
HCl	hydrochloric acid
HIDS	hyper immunoglobulin D syndrome
HDL	high density lipoprotein
HDM	high dose melphalan
HRA	hereditary renal amyloid
IDM	intermediate dose melphalan
IgG	immunoglobulin G
IHD	ischaemic heart disease
IL-1	interleukin 1
IL-6	interleukin 6
IV	intravenous
JIA	juvenile idiopathic arthritis
KDa	kiloDalton
MBq	megaBecquerel
MEFV	Mediterranean fever gene

MHC	major histocompatibility complex
mSv	millisieverts
MWS	Muckle-Wells syndrome
N	number
N-terminal	amino terminal
NYHA	New York Heart Association
OLTx	orthotopic liver transplant
PAP	peroxidase-anti-peroxidase
PCR	polymerase chain reaction
PR	partial response
RA	rheumatoid arthritis
RTx	renal transplant
SAA	serum amyloid A protein
SAP	serum amyloid P component
SC	subcutaneous
SLE	systemic lupus erythematosis
TBS	Tris buffered saline
TNF	tumour necrosis factor
TRAPS	tumour necrosis factor associated periodic syndrome
TTR	transthyretin
ULN	upper limit of normal range
VAD	vincristine, Adriamycin and dexamethasone

LIST OF FIGURES

		Page
1.1	The structure of the CPHPC-SAP complex	54
3.1	Partial DNA sequence of the fibrinogen α-chain gene	69
3.2	Progression of amyloid deposits in a patient with fibrinogen A α-chain Glu526Val associated amyloidosis and an incidental monoclonal gammopathy	73
4.1	German kindred with familial renal amyloidosis due to variant fibrinogen A α-chain Glu526Val	84
4.2	Scottish kindred with familial renal amyloidosis due to variant fibrinogen A α-chain Glu526Val	83
4.3	New Zealand kindred with familial renal amyloidosis due to variant fibrinogen A α-chain Glu526Val	85
4.4	English kindred with familial renal amyloidosis due to variant fibrinogen A α-chain Glu526Val	86
4.5	Asymptomatic progressive accumulation of fibrinogen A α-chain amyloid in a renal transplant	92
4.6	Complete regression of fibrinogen A α-chain amyloidosis following hepatorenal transplantation	93
4.7	Characteristic renal histology in fibrinogen A α-chain Glu526Val amyloidosis	97
5.1	Serum concentration of free immunoglobulin light chains prior to treatment in 345 patients with AL amyloidosis	111

5.2 Concentration of the amyloidogenic class of free light chain after 6 months of chemotherapy 114

5.3 Probability of the amyloidogenic class of free light chain remaining below 50% of the pre-treatment values 116

5.4 Probability of survival in 154 patients with systemic AL amyloidosis 117

5.5 Relationship between change in amyloid load and change in serum concentration of the amyloidogenic free light chains 118

5.6 Radiolabelled SAP scintigraphy demonstrating AL amyloid regression 119

6.1 Sections of plasma cell variant Castleman's tumour 128

6.2 Sections of renal biopsy demonstrating AA amyloid 128

6.3 Serial serum IL-6, SAA and CRP values 129

6.4 Radiolabelled SAP scintigraphy demonstrating AA amyloid regression 130

7.1 Distribution of *MEFV* mutations in patients with FMF, their healthy relatives and Turkish controls 142

7.2 Fortnightly SAA measurements in eight asymptomatic FMF patients 145

8.1 Serial SAA measurements in FMF patients 156

8.2 Effect of colchicine compliance of FMF related inflammation 157

LIST OF TABLES

		Page
1.1	Classification of amyloidosis	3
1.2	Conditions associated with systemic AA (secondary) amyloidosis	22
1.3	Tel-Hashomer criteria for the diagnosis of familial Mediterranean fever	24
1.4	Mutations associated with periodic fever syndromes	26
1.5	Conditions associated with systemic AL amyloidosis	36
1.6	Transthyretin mutations associated with FAP	45
1.7	Mutations associated with hereditary non-neuropathic amyloidosis	49
3.1	Clinical features of transthyretin, lysozyme and apolipoprotein AI associated amyloidosis	70
4.1	Clinical features of fibrinogen A α chain Glu526 Val amyloidosis	89
4.2	Outcome of solid organ transplantation	91
4.3	Clinical features and pattern of amyloid deposition in the 4 previously described families	100
5.1	Tissue distribution of localised amyloid deposits and evidence of a systemic plasma cell dyscrasia	113

5.2 Characteristics of patients receiving the different chemotherapy regimes 115

6.1 Details of 5 patients with systemic AA amyloidosis complicating unicentric Castleman's disease 132

6.2 Characteristics of previously reported cases of Castleman's disease complicated by AA amyloidosis 136

7.1 MEFV genotypes in patients with FMF, their asymptomatic first degree relatives and healthy Turkish controls 143

7.2 Grouped results for FMF patients according to MEFV genotype 146

7.3 Grouped results for MEFV wild types and healthy heterozygotes 148

8.1 MEFV mutations, ethnic origins and clinical characteristics of 12 patients with AA amyloidosis 153

8.2 Amyloid distribution and renal function at diagnosis and follow up 155

Chapter One

INTRODUCTION

Amyloidosis is a disorder of protein folding in which normally soluble plasma proteins are deposited in the extracellular space in an abnormal insoluble fibrillar form[1,2]. The underlying molecular abnormalities may be either acquired or hereditary and about 20 different proteins can form clinically or pathologically significant amyloid fibrils *in vivo*[3]. Amyloid is remarkably diverse and can be localised or systemic and rapidly lethal or merely an incidental finding. Localised forms of the disease are associated with several extremely common disorders including type II diabetes mellitus[4] and Alzheimer's disease[5], and small, focal, clinically silent, amyloid deposits in the brain, heart, seminal vesicles, and joints are a universal accompaniment of ageing[6-10]. However, in systemic amyloidosis accumulation of amyloid causes progressive disruption of the structure and function of organs and tissues which can occur at virtually any site except within the brain parenchyma[11-13]. Without treatment, systemic disease is usually fatal, but measures that reduce the supply of amyloid fibril precursor proteins frequently lead to regression of amyloid deposits, to prevention of organ failure and to improved survival[14]. Since the regression of amyloid occurs relatively slowly, early diagnosis has the potential to improve the prognosis.

AMYLOID STRUCTURE

Protein fibrils

Amyloid deposits consist mainly of protein fibrils, the different polypeptide sub-units of which constitute the basis for their classification (Table 1.1)[3, 15]. It has long been known that the ultrastructural morphology and histochemical properties of all amyloid fibrils, regardless of the precursor protein type, are remarkably similar[16-22], and their high component of cross-β secondary structure was identified some 30 years ago[23, 24]. Recent diffraction studies of a number of different *ex vivo* and synthetic amyloid fibrils have confirmed that they all share a common core structure consisting of anti-parallel β-strands forming sheets lying with their long axes perpendicular to the long axis of the fibril[25, 26]. This extremely abnormal, but highly ordered, conformation underlies the distinctive physicochemical properties of amyloid fibrils including their ability to bind molecules of the dye Congo red in a spatially organised manner, their relative resistance to proteolysis, and their capacity to bind serum amyloid P component (SAP)[27, 28]. In some instances the fibrils *in vivo* are composed of intact whole precursor molecules, for example in lysozyme, β_2-microglobulin and some forms of transthyretin amyloidosis[29-31]. More often, amyloid precursor proteins undergo partial cleavage, although it is not known whether this occurs before, during or even after fibril formation[32, 33].

Table 1.1 Classification of amyloidosis

Type	Fibril protein precursor	Clinical syndrome
AA	Serum amyloid A protein	Reactive systemic amyloidosis associated with chronic inflammatory diseases
AL	Monoclonal immunoglobulin light chains	Systemic amyloidosis associated with monoclonal plasma cell dyscrasias
$A\beta_2M$	β_2-microglobulin	Periarticular and, occasionally, systemic amyloidosis associated with long-term dialysis
ATTR	Normal plasma transthyretin	Senile systemic amyloidosis with prominent cardiac involvement
ATTR	Genetically variant transthyretin	Autosomal dominant systemic amyloidosis Familial amyloid polyneuropathy
ACys	Genetically variant cystatin C	Hereditary cerebral haemorrhage with cerebral and systemic amyloidosis
AGel	Genetically variant gelsolin	Autosomal dominant systemic amyloidosis Predominant cranial nerve involvement with lattice corneal dystrophy
ALys	Genetically variant lysozyme	Autosomal dominant systemic amyloidosis Non-neuropathic with prominent visceral involvement
AApoAI	Genetically variant apolipoprotein AI	Autosomal dominant systemic amyloidosis Predominantly non-neuropathic with prominent viscera involvement
AApoAII	Genetically variant apolipoprotein AII	Autosomal dominant systemic amyloidosis Non-neuropathic with prominent renal involvement
AFib	Genetically variant fibrinogen A alpha chain	Autosomal dominant systemic amyloidosis Non-neuropathic with prominent renal involvement
Aβ	β-protein precursor (and rare genetic variants)	Cerebrovascular and intracerebral plaque amyloid in Alzheimer's disease Occasional familial cases

Amyloid composed of peptide hormones, prion protein and unknown proteins not included here

Non fibrillar components

Although most amyloid fibril precursor proteins can form fibrils *in vitro* in the absence of any other moieties[34, 35], there is evidence that the minor non-fibrillar elements that are found in all amyloid deposits contribute significantly to the process *in vivo*[36]. All deposits contain some non-fibrillar common elements[21], most notably restricted subsets of glycosaminoglycans[37, 38] and the normal plasma glycoprotein, serum amyloid P component (SAP)[27]. All amyloid deposits also contain other common constituents including apolipoprotein E, various proteinase inhibitors, complement components, and extracellular matrix constituents[39]. The role, if any, of these molecules in the pathogenesis and/or the effects of amyloid remains to be elucidated.

Glycosaminoglycans

These are the most abundant heteropolysaccharides in the body and are located primarily on the surface of cells or in the extracellular matrix (ECM). They consist of negatively charged long unbranched polysaccharides containing a repeating disaccharide unit. The significance of glycosaminoglycans (GAGs) of the heparan sulphate or chondroitin sulphate types, in amyloid remains unclear, but their universal presence in the deposits, their intimate relationship with the fibrils and their restricted heterogeneity all suggest that they may be important[40]. GAGs are known to participate in the organisation of some normal structural proteins into fibrils and they may have comparable fibrillogenic effects on certain amyloid fibril precursor proteins[38, 41]. Furthermore, low molecular weight polysulphonated compounds that are thought to act *in vivo* as 'GAG-mimetics', substantiallyinhibit experimental induction of AA amyloid deposits in mice[42], presumably by preventing the association of amyloid fibrils with endogenous GAGs[43].

Serum Amyloid P component

The other non fibrillar constituent that is present in relative abundance in all amyloid deposits is the plasma glycoprotein serum amyloid P component (SAP)[44, 45]. SAP has close structural homology with CRP[46, 47], the classical acute phase reactant and, together, these molecules represent the prototypic members of the pentraxin family of plasma proteins[48]. SAP is a pentamer with 5 identical non-covalently associated protomers each bearing a single calcium dependent ligand binding site on one planar face, the binding (B) face of the molecule[49, 50]. The physiological function of SAP has not yet been fully clarified but may be important in host defence[51, 52] and in the handling of chromatin during apoptosis, protecting against the development of autoimmunity[53, 54]. Its universal presence in amyloid deposits[55] reflects its specific calcium dependent binding to an, as yet, uncharacterised ligand(s) shared by all types of amyloid fibrils. SAP is evolutionarily highly conserved and no polymorphisms or deficiencies have been identified[56]. In man it is expressed constitutively by hepatocytes; has a constant plasma concentration of around 30 mg/l[57]; and a half-life in the circulation of 24 hours[58-62].

In amyloidosis, circulating SAP exists in a dynamic equilibrium with the much larger extravascular pool of SAP bound to the amyloid fibrils[63, 64]. Only SAP molecules in the plasma are subject to catabolism, which occurs only in the liver[55]. SAP persists within human amyloid deposits for prolonged periods and shows no modification from circulating SAP[27]. In mice and hamsters, SAP is an acute phase protein and plasma concentrations are closely related to amyloidogenesis[55]. The universal presence and persistence of SAP in amyloid suggests that it might contribute to the pathogenesis of amyloid[27, 65], and there are various mechanisms by which this could be envisaged to occur. On thermodynamic grounds, the pentavalent binding of SAP to amyloid fibrils may have a stabilising effect. SAP may also provide a normal, autologous protein

coat masking the abnormal amyloid fibrils from the scavenging processes that usually clear abnormal material efficiently from the tissues. In addition, SAP itself is highly resistant to proteolysis[66, 67], and the binding of SAP inhibits digestion of amyloid fibrils by phagocytes and proteases *in vitro*[68]. The role of SAP in protecting and promoting amyloid deposition has lately been confirmed in mice with targeted deletion of the SAP gene in whom experimentally induced AA amyloidosis was substantially reduced[69]. The physiological role of SAP is under investigation in SAP knock-out mice, some strains of which have been found spontaneously to develop antinuclear antibodies[70, 71] supporting previous *in vitro* observations suggesting that SAP binds to and regulates the handling and clearance of DNA and chromatin[53, 72].

PATHOGENESIS OF AMYLOID

The pathogenesis of amyloid centres around the aberrant folding of the various fibril precursor proteins[73]. About 20 different extremely heterogeneous proteins are known to form amyloid fibrils *in vivo* and other related and even completely unrelated proteins have lately been manipulated *in vitro* to undergo a similar transformation[74]. Amyloid fibril proteins can evidently exist as two radically different yet stable structures - the normal soluble form and a highly abnormal fibrillar conformation - and thereby refute the traditional dogma that amino acid sequence is the sole determinant of a protein's tertiary form[75, 76]. Amyloidogenic proteins are thought transiently to populate partly unfolded intermediate molecular states[77] that expose β-sheet structure of the requisite type to interact with and bind to similar molecules in a highly ordered fashion[25, 78, 79]. Homozygosity for precursor proteins, such as SAA_1 isotypes[80] in acquired AA amyloidosis and variant TTR[81] or gelsolin[82] in hereditary neuropathic amyloidosis, is recognised to promote the susceptibility to or severity of the amyloidosis. This may reflect lower or absent amyloidogenic potential of certain

isoforms of some proteins, less efficient amyloid aggregation between non-identical precursor molecules. Heterozygosity may confer protection by protein isoforms with low amyloidogenic potential interacting with and stabilising those with a greater propensity to form fibrils[83]. Although amino acid sequence ultimately underlies the propensity for a protein to form amyloid, the propagation from low molecular weight protofilament cores into mature amyloid fibrils and their subsequent progressive accumulation are probably self-perpetuating processes that depend only on a sustained supply of the respective fibril precursor protein[84]

In clinical practice, amyloid deposition occurs in three different circumstances. The first occurs when there is abnormal abundance of a structurally normal precursor protein. This is the case in both dialysis related amyloid, in which the precursor is β_2-microglobulin[85] and in AA type, in which the precursor is the acute phase reactant, SAA.

The second occurs when a normal, but intrinsically amyloidogenic, protein has been present in normal quantities for a very prolonged period of time. An example is senile cardiac amyloidosis in which wild type transthyretin accumulates as amyloid in the myocardium of elderly individuals[86].

The third and commonest with respect to systemic amyloidosis, occurs when amyloid deposition involves a protein of abnormal inherently amyloidogenic structure. Examples include acquired monoclonal immunoglobulin light chain (AL) amyloidosis in patients with clonal plasma cell dyscrasias[87]; and the autosomal dominant hereditary amyloidosis syndromes associated with genetically variant forms of transthyretin[88], fibrinogen A α–chain[89], lysozyme[29], apolipoprotein AI[90], cystatin C[91] and gelsolin[92].

Although all forms of hereditary amyloidosis are rare, they are important in terms of their clinical implications and represent invaluable models for studying the pathogenesis of amyloidosis generally. At the molecular level, genetically variant proteins with a strong predilection to form amyloid can be compared with their wild-type counterparts[34, 93, 94]. Most of these variants differ from the wild type by just a single amino acid substitution or, occasionally, show small deletions or insertions[95]. Variants of transthyretin (TTR) were the first to be discovered and have been studied most widely[96], but a disadvantage of this particular system is that normal TTR is itself inherently amyloidogenic to some degree[86]. However, studies of the two variants of lysozyme, Asp67His and Ile56Thr, associated with hereditary systemic amyloidosis[29, 73] have been informative since, like TTR, lysozyme's complete three-dimensional structure has been resolved[97-99], but wild-type lysozyme is not associated with amyloidosis. Various kinds of *in vitro* manipulation, including heat denaturation and prolonged standing at 4°C, have indicated that the amyloidogenic lysozyme variants are functional but inherently less stable than the wild-type form[73, 100]. The variants much more readily populate partially unfolded states which retain secondary structure typical of 'molten globule' intermediate forms[77, 101, 102], and it is thought that these point mutations destabilise lysozyme sufficiently for this to happen transiently *in vivo*[73, 103]. Remarkably, the whole process of lysozyme amyloid fibril formation has been reversed *in vitro* and soluble functional variant lysozyme has been recovered by denaturing a preparation of isolated *ex vivo* amyloid fibrils[73, 104].

There are still many outstanding questions about amyloid deposition[39]. It is not clear why only a relatively small number of unrelated proteins form amyloid *in vivo* when it is becoming increasingly clear that others can be induced to do so *in vitro*[105, 106]. Little is yet known about the genetic or environmental factors that determine individual susceptibility to amyloid[107, 108]; or those which govern its anatomical distribution and clinical effects[109]. There is huge variation not only between the different types of amyloid but also between (and even within) kindreds with hereditary amyloidosis associated with identical mutations. Experimental studies in mice have shown that parenteral injection of a minute amount of an extract of amyloidotic material primes them for many months to develop AA amyloidosis within 1-2 days of receiving a single acute phase stimulus. In contrast unprimed mice typically require an inflammatory stimulus for about 6 weeks[109-111]. The precise component of amyloid that represents this so-called 'amyloid enhancing factor' (AEF)[84, 112] has now been characterised as the fibrils themselves[113]. There is now evidence from mouse models that amyloid fibrils ingested orally are effective AEF and that this nucleation phenomenon crosses the species barrier[114]. The presence of AEF evidently increases the proportion of precursor proteins that adopt the amyloid conformation, possibly by capturing susceptible transiently unfolded intermediates onto an established 'amyloid template'. Since amyloid itself is extremely rich in AEF, the conditions necessary for amyloid formation and propagation *in vivo* appear to be self-perpetuating once an initial nidus of amyloid material has been laid down, depending thereafter only on a continued supply of the respective fibril precursor protein[84, 113, 115, 116].

PATHOLOGICAL EFFECTS OF AMYLOID

Amyloid deposits accumulate in the extracellular space, progressively disrupting the normal tissue architecture and so impairing organ function. Amyloid deposits can act as both microscopic and macroscopic space occupying lesions. Although, in general, prognosis and organ dysfunction are inversely related to the amyloid load, there is marked individual variation and for any given quantity of amyloid the resulting degree of organ compromise is unpredictable[117]. Although amyloid may be relatively inert in the sense that it fails to stimulate either a local or systemic host inflammatory response, there is some evidence that the fibrils may exert cytotoxic effects, possibly by promoting apoptosis[118, 119]. This is an attractive theory since it could explain how scanty deposits could cause significant functional disruption without any histological evidence of inflammation or tissue necrosis. Another strong clinical impression is that active, progressive deposition of new amyloid is associated with accelerated deterioration of organ function compared to relative stability of function in the presence of even large amounts of stable amyloid deposits. This may reflect better adaptation of the host tissues to amyloid that has accumulated very gradually, or relate to a particular property of newly formed versus mature fibrils. In the cerebral amyloidoses, low molecular amyloid aggregates or protofibrils may be responsible for a substantial proportion of amyloid related toxicity, and the same may apply in the systemic forms of the disease occurring over and above the physical disruption associated with established deposits[120].

Amyloid degradation

The natural history of amyloidosis is usually of relentless progression, leading to organ failure and often death[121]. However, amyloid deposition is not irreversible and clinical progression of the amyloid diseases merely reflects that the deposits are usually being laid down more rapidly than they are "turning over"[122]. The conditions that underlie amyloidosis are, without treatment, typically progressive and unremitting, but there are numerous reports describing regression of amyloid when associated inflammatory and other diseases have been controlled[123-132]. Lately, longitudinal follow up using radiolabelled SAP scans in over 3000 patients with various types of systemic amyloidosis have confirmed that the deposits often regress quite rapidly when the supply of fibril precursors can be reduced. Under favourable circumstances, this is accompanied by stabilisation or recovery of organ function, and much improved patient survival[117, 122, 133, 134].

TYPES OF AMYLOID

Reactive systemic, AA, amyioidosis

This is a potential complication of any disorder associated with a sustained acute phase response (Table 1.2). The amyloid fibrils are derived from the circulating acute phase reactant, SAA; [44, 135-139] and the fibril peptide subunits typically comprise a 76 amino acid N-terminal cleavage fragment known as AA protein[32]. SAA is an apolipoprotein of high density lipoprotein (HDL)[140-142], and is synthesised in hepatocytes under the transcriptional regulation of cytokines including IL1, IL6 and TNF-α[143-145]. There are four SAA genes in man all on chromosome 11. SAA_1 and SAA_2 are responsible for the acute phase response,

SAA_4 is expressed constitutively and SAA_3 is a pseudogene. In health the circulating concentration of SAA is around 1 mg/l, but this can rise by more than a thousand fold in the presence of inflammation. A sustained high plasma level of SAA is a prerequisite for the development of AA amyloidosis, but is clearly not sufficient since this form of amyloid occurs in less than 10% of the overall population at risk[146-148]. There are a number of isoforms at the SAA_1 and SAA_2 loci[149], susceptibility to AA amyloidosis is increased among individuals who are homozygous for their SAA_1 isotype[80, 150], and studies in Japan suggest that $SAA_{1\gamma}$ is more inherently amyloidogenic than the α and β isotypes[151, 152]. Similar results have been reported in SAA_2 where the isotype $SAA_{2\alpha2}$ is significantly over represented in Caucasian patients with AA amyloidosis complicating juvenile inflammatory arthritis[153].

Systemic amyloidosis associated with monoclonal immunocyte dyscrasias, AL amyloidosis

Potentially AL amyloidosis may occur in association with any form of monoclonal B cell dyscrasia[154, 155]. AL proteins are derived from the *N*-terminal region of monoclonal immunoglobulin light chains and consist of the whole or part of the variable (V_L) domain[156-158]. Intact light chains are occasionally found, and the molecular weight therefore varies between about 8000 and 30 000 Da. AL is more commonly derived from λ chains than from κ chains, despite the fact that κ chains predominate among both normal immunoglobulins and the paraprotein products of immunocyte dyscrasias[11]. During early B-cell development, there is rearrangement of the immunoglobulin germ line with retention of selected genes, endowing each B cell with one heavy-chain and one

light-chain variable region gene. These rearranged genes encode subgroups of Vλ and κ and mutate at a much higher rate than the somatic genes, a process that results in unique immunoglobulin variable region gene sequences, that can be used to identify B-cell clones within each subgroup. A new λ chain subgroup, λ_{VI}, was identified first as an AL protein in two cases of amyloidosis before it had been recognised in any other form[159], and it has subsequently been observed in many more cases of AL amyloidosis[160]. Furthermore, there is increasing evidence from sequence analyses of Bence Jones proteins of both κ and λ type from patients with AL amyloidosis, and of AL proteins themselves, that these variable region polypeptides contain unique amino acid replacements or insertions compared to non-amyloidogenic monoclonal light chains[87]. Clones derived from the *6a* $V_{\lambda VI}$ germ line gene have been found to be associated with AL amyloidosis with predominant renal involvement, clones derived from specific other V_λ genes are more likely to present with dominant cardiac and multisystem disease and V_κ clones are over represented in patients with dominant hepatic involvement. $V_{\lambda VI}$ light chains have been found to form amyloid *in vitro* rapidly both with and without amyloid-enhancing factor suggesting that in patients with plasma cell dyscrasias the germ line gene use influences not just the organ tropism of AL deposits but also the tendency to form amyloid at all[161]. The inherent 'amyloidogenicity' of particular monoclonal light chains has been elegantly confirmed in an *in vivo* model in which isolated Bence Jones proteins are injected into mice[159]. Animals receiving light chains from AL amyloid patients developed typical amyloid deposits composed of the human protein whereas animals receiving light chains from myeloma patients without amyloid did not.

Systemic amyloidosis associated with monoclonal immunocyte dyscrasias, AH amyloidosis

A few cases of immunoglobulin heavy chain amyloid deposition have been reported[162, 163].

Dialysis related amyloidosis (DRA)

This is a potential complication of long term renal replacement by either haemodialysis or, less often, chronic peritoneal dialysis[164-168]. The amyloid fibrils are composed of β_2-microglobulin (β_2M)[30, 85]. This is the invariant light chain of Class I MHC antigens and is expressed by all nucleated cells. In health it is catabolised by the proximal renal tubules and it is not adequately cleared by dialysis[169]. Persistently elevated levels of β_2M are universal in dialysed patients and the majority of those receiving dialysis for more than 10 years will develop symptomatic DRA[170].

Senile systemic amyloidosis

This syndrome is not uncommon in the very elderly, and almost never occurs before the age of 60 years[171, 172]. The amyloid fibrils are composed of normal wild-type TTR[86, 173, 174]. The deposits are usually sparse and asymptomatic but occasionally, extensive infiltration of the myocardium causes congestive cardiac failure and may be fatal[175-177].

Hereditary systemic amyloidosis

Hereditary systemic amyloidosis caused by deposition of variant proteins as amyloid fibrils has been reported with the following proteins: transthyretin[88, 178], cystatin C[179], gelsolin[180], apolipoprotein AI[181], apolipoprotein AII[182], lysozyme[29] and fibrinogen α-chain[89]. These diseases are all inherited in an autosomal dominant pattern with variable penetrance, and may present clinically at any time from the teens to old age[183], though usually in adult life. By far the commonest hereditary amyloidosis is caused by transthyretin variants[96] and usually presents as familial amyloid polyneuropathy with peripheral and autonomic neuropathy[88]. Thus far more than 80 amyloidogenic TTR mutations have been described and there are almost certainly many more[88, 95]. Cystatin C amyloidosis presents as cerebral amyloid angiopathy with recurrent cerebral haemorrhage and clinically silent systemic deposits, and has been reported only in Icelandic families[91, 184, 185]. Gelsolin amyloidosis presents with cranial neuropathy but there are also systemic deposits; it is also extremely rare[186]. Hereditary non-neuropathic systemic amyloidosis was first described by Ostertag in 1932[187]. It is now recognised to be caused by mutations in the apolipoprotein AI[181] or AII[182], lysozyme[29] or fibrinogen A α-chain genes[89]. The amyloid deposits in these syndromes can affect any or all the major viscera, with renal involvement usually being prominent, although apolipoprotein AI amyloid occasionally also manifests with neuropathy[188].

Localised amyloidosis

Localised amyloid deposition occurs quite commonly and presumably results either from local production of fibril precursors[189-191], or from properties inherent

to the particular microenvironment, which favour localisation and fibril formation of a more widely distributed precursor protein[192]. The vast majority are AL in type[193, 194] and most symptomatic deposits occur in the eye[195], skin[196], respiratory[197, 198] or urogenital tracts[199, 200]. They are associated with extremely subtle focal monoclonal B cell proliferation and surgical resection of these localised 'amyloidomas' can be curative[199].

Cerebral amyloidosis

The brain and intracerebral blood vessels are usually spared in systemic amyloidosis but are important sites for local deposition of amyloid[201]. The best characterised form of cerebral amyloid is that related to Alzheimer's disease, the commonest form of dementia worldwide[202]. The fibril protein in the intracerebral and cerebrovascular amyloid of Alzheimer's disease, Down's syndrome and hereditary amyloid angiopathy of Dutch type is known as β-protein[203-205]. This 39-43 residue sequence is cleaved from β-amyloid precursor protein (APP). Cerebral amyloid deposition appears to be directly neurotoxic but the exact mechanisms leading to neuronal degeneration have yet to be elucidated[202]. There is mounting evidence that relatively low molecular weight aggregates, as opposed to mature fibrils, may have a major part in the pathogenesis of this disorder[120].

DIAGNOSIS

Diagnosis of amyloid relies on a high index of clinical suspicion and generally requires histological confirmation[206]. Unfortunately amyloid is frequently asymptomatic until a relatively late stage and can then present with highly variable

or non-specific symptoms[11]. The protean manifestations of systemic amyloid depend on the predominant organs affected and can include symptoms and signs referable to any system except the CNS.

Biopsy of a clinically affected visceral organ, e.g. the kidney, liver or heart, is usually diagnostic but gives no information about the total body amyloid load or the distribution of deposits in other organs. Biopsy can be hazardous as there is an increased risk of haemorrhage and significant bleeds have been reported in 5% of liver biopsies[207]. This is attributable to the increased fragility of affected blood vessels, reduced elasticity of severely amyloidotic organs, and, very occasionally in AL type to an acquired deficiency of clotting factors IX and X[208-211]. A less invasive alternative in suspected systemic disease is fine needle aspiration of subcutaneous fat, or rectal or labial salivary gland biopsy[212, 213]. In skilled hands these 'screening' biopsies can produce positive results in up to 80% of cases, but in routine practice sensitivity is only about 50%.

Even after amyloidosis has been confirmed, subsequent identification of amyloid type is not always straightforward[214]. Definitive diagnosis relies on a combination of clinical features, predisposing conditions, immunohistochemistry[28, 215, 216] and, where available, more specialised but highly informative investigations such as SAP scintigraphy[217] and DNA analysis.

GENERAL TREATMENT PRINCIPALS

Although amyloid deposits are not irreversible, as was widely believed until recently, they do turn over relatively slowly and the natural history of amyloidosis is that the rate of fibril deposition usually exceeds that of mobilisation[218]. As a result, the amyloid diseases tend to be progressive. Amyloid would regress if *either* its rate of deposition is slowed or its clearance is enhanced. Although novel therapies with the latter aim are being investigated, at present the treatment of all types of amyloid centres on reducing the supply of the respective amyloid precursor protein and supporting or replacing compromised organ function. Self evidently, treatment depends completely on precise identification of the amyloid fibril type.

Preservation and replacement of organ function

Organs that are extensively infiltrated by amyloid may fail precipitously with little or no warning, and seemingly without provocation, even when organ function has previously been entirely normal. Therapy that retards or halts amyloid deposition is frequently followed by the gradual recovery of organ function[123-132,117, 122, 133, 134] but inexorably progressive organ failure is inevitable, particularly in the case of amyloidotic kidneys, once a certain level of organ dysfunction has occurred[219].

In some cases organs are so severely compromised that, without support or replacement of their function, patients will not survive treatment, or live long enough to derive benefit from it. The most frequent problem encountered in the management of patients with systemic amyloidosis is renal failure[219]. It is

usually feasible to manage this with renal replacement therapy in the form of haemodialysis or peritoneal dialysis until there is clear evidence of a response to treatment of the underlying amyloidogenic condition. Although nephrotic syndrome and, to a lesser extent, moderately impaired renal function frequently improve and can even normalise when amyloid deposits regress[123-132] dialysis or renal transplantation will need to be considered in patients with more advanced renal damage. However, the outcome of amyloidosis patients on long-term dialysis is relatively poor, their 2 year survival being of the order of 80% that of non-amyloidotic patients[220, 221]. By contrast, the outcome of renal transplantation in these patients is more favourable[222], although their early post transplant mortality is increased, due to sepsis and cardiac failure, long-term graft survival and rejection rates compare very well with patients with other systemic diseases, and may even be better[223, 224]. Most amyloid patients have a functioning graft until death[225] and although amyloid deposition in the transplanted organ has been reported in up to 10-20% of patients with AA amyloidosis, this infrequently compromises graft function, and is rarely responsible for graft loss[226-228]. Indeed, clinically significant graft amyloid occurs far less frequently than is widely supposed in all other types of the disease, particularly the hereditary forms which may be very slow to progress generally[229, 230].

A small proportion of patients with systemic amyloidosis present with severely compromised liver or cardiac function but with otherwise well preserved vital organ function[231]. Most of these patients will have AL type and may be suitable for lifesaving liver or heart transplantation before proceeding to chemotherapy[232-236]. Selection of such cases is very difficult, particularly with respect to liver

replacement because all patients with end stage hepatic amyloidosis have substantial amyloid deposits in other organ systems even when those organs appear to retain adequate function[237].

REACTIVE SYSTEMIC, AA, AMYLOIDOSIS

Underlying conditions

AA amyloidosis may occur in any patient who has a sustained acute phase response. The list of chronic inflammatory, infective and neoplastic disorders that can lead to this type of amyloid is almost without limit (Table 1.2)[147, 238-240], but the predominant aetiology varies among different populations[241]. In the Western World the commonest predisposing conditions are adult and juvenile rheumatoid arthritis. The prevalence of AA amyloidosis is not clear as diagnosis is difficult and it is almost certainly under-reported. Biopsy and post-mortem series suggest that the prevalence of AA amyloidosis in patients with chronic arthritides is between 3.6 and 5.8%[146, 147, 242]. Extrapolating from published data on the prevalence and life-time incidence of AA amyloidosis in RA, AS and JIA patients and on the prevalence of these chronic arthritides, an upper limit for the community prevalence of AA amyloidosis in the general European population can be is estimated at 1.8 per 10,000 people. For reasons that are not clear the incidence of AA amyloid is lower in the United States and much higher in parts of central Europe and Scandinavia[243, 244] and, perhaps for the same reasons, its prevalence in Europe is believed to have decreased substantially over the past 40 years[245].

The incidence of AA amyloidosis increases with duration and severity of the underlying inflammatory disorder, and although the median latency between onset of inflammation and diagnosis of amyloid is usually 8 to 14 years, some individuals develop clinically significant amyloid in less than 12 months and others only after many decades[246]. Amyloidosis occurs exceptionally rarely in systemic lupus erythematosus[247] and ulcerative colitis[239], reflecting the unusually modest acute phase response evoked by these particular conditions. AA amyloidosis can complicate cytokine producing tumours particularly Castleman's disease. The inherited fever syndromes, which are significant causes of amyloid in affected populations, will be discussed in greater detail.

Table 1.2

Conditions associated with systemic AA (secondary) amyloidosis

Inflammatory arthritides & vasculitides
Adult Still's disease[248]
Ankylosing spondilitis[148]
Behcet's disease[249]
Giant cell arteritis[250]
Juvenile idiopathic arthritis[147]
Polyarteritis nodosa[251]
Polymyalgia rheumatica[252]
Psoriatic arthropathy[253]
Reiter's syndrome[254]
Rheumatoid arthritis[255]
Systemic lupus erythematosis[247]
Takayasu's arteritis[256]

Hereditary periodic fevers
Familial cold urticaria[257]
Familial Mediterranean fever[258]
Hyperimmunoglobulin D syndrome[259]
Muckle-Wells syndrome[260]
TNF receptor associated periodic syndrome[261]

Chronic infections
Bronchiectasis[262]
Chronic cutaneous ulcers[263]
Chronic pyelonephritis[264]
HIV[265]
Leprosy[266]
Osteomyelitis[267]
Q fever[268]
Subacute bacterial endocarditis[269]
Tuberculosis[241]
Whipples disease[270]

Inflammatory bowel disease
Crohn's disease[238]
Ulcerative colitis[239]

Neoplasia
Adenocarcinoma of the lung, gut, urogenital tract[271]
Basal cell carcinoma[272]
Carcinoid tumour[273]
Castleman's disease[274]
Hairy cell leukaemia[155]
Hepatic adenoma[275]
Hodgkin's disease[276]
Renal cell carcinoma[277]
Sarcoma[278]

Other
Common variable immunodeficiency[279]
Cyclic neutropenia[280]
Cystic fibrosis[281]
Epidermolysis Bullosa[282]
Hyperimmunoglobulin M syndrome[283]
Hypogammaglobulinaemia[284]
IV and subcutaneous drug abuse[265]
Kartagener's syndrome[285]
Paraplegia[264]
SAPHO syndrome[286]
Sarcoidosis[287]
Sex linked agammaglobulinaemia[288]
Sickle cell anaemia[289]

The inherited periodic fever syndromes

A total of five syndromes are recognised; familial Mediterranean fever (FMF), hyper-immunoglobulin D syndrome (HIDS), TNF receptor associated periodic syndrome (TRAPS), previously known as familial Hibernian fever, familial cold urticaria (FCU) and Muckle-Wells syndrome (MWS). Of these FMF and HIDS are autosomal recessive diseases and the latter three are autosomal dominant with variable penetrance. The last 5 years have witnessed striking advances in the understanding of the molecular pathogenesis of the hereditary periodic fevers. The gene defect has been identified in FMF[290, 291], HIDS[259], TRAPS[261] and most recently in both MWS and FCU[292].

Familial Mediterranean fever

Familial Mediterranean fever is an autosomal recessive periodic inflammatory disease characterised by recurrent, self-limiting attacks of fever, serositis, and sometimes arthritis or rash associated with neutrophil migration into the serosal spaces[293, 294]. Most patients will present in childhood or as young adults with attacks typically lasting for 12 to 72 hours and often so intense that they mimic an acute surgical abdomen[258]. Familial Mediterranean fever occurs world wide but predominantly affects the populations arising from the Eastern Mediterranean basin particularly the non-Ashkenazi Jews, Armenians, Turks and Levantine Arabs[293]. Although the gene associated with the disease has been identified the diagnosis remains clinical and a variety of criteria are used to establish the diagnosis (Table 1.3)[294, 295]. Attacks are accompanied by a number of laboratory abnormalities including a low grade neutrophilia and a dramatic elevation of the acute phase proteins. There is considerable heterogeneity in individual disease

susceptibility, severity and frequency of attacks; in clinical manifestations and in predilection to the most serious complication of the disease, which is the development of systemic reactive, AA, amyloidosis. A minority of patients still present with so-called type II phenotype FMF[296]. In this exceptionally rare form of the disease, the initial presentation is with AA amyloidosis without a history of pre-existing clinical FMF. This tends to present in older age group than classical disease but like other FMF patients they respond to long term colchicine and the phenotype does not appear to be limited to any particular genotype (A. Bakkaloğlu, oral communication). Being an inherited chronic inflammatory disease frequently complicated by AA amyloid, FMF affords a rare opportunity for the further investigation of the genetic susceptibilities underlying this form of amyloid deposition.

Table 1.3 Tel-Hashomer criteria for the diagnosis of familial Mediterranean fever

Major Criteria	**Minor Criteria**
1. Recurrent febrile episodes accompanied by serositis or synovitis	1. Recurrent febrile episodes
2. AA amyloidosis without a predisposing cause	2. Erysipelas like erythema
3. Response to continuous colchicine prophylaxis	3. FMF in a first degree relative

Definite diagnosis:
2 major or 1 major and 2 minor criteria

Probable Diagnosis:
1 major and 1 minor criteria

The gene responsible for FMF, *MEFV*, is located on the short arm of chromosome 16 and was identified by linkage studies and positional cloning by two independent consortia in 1997[290, 291]. It is a 10 exon gene encoding an as yet uncharacterised neutrophil protein, pyrin. More than 35 mutations are now recognised, the great majority of these mutations are clustered near the C-terminal in a relatively short section of exon 10 but other mutations have been described in exons 1,2,3 and 5[297, 298]. Pairs of mutations are identifiable in most patients with clinical FMF and these are generally compound heterozygotes consistent with autosomal recessive inheritance. In our experience the majority of patients will have two identifiable mutations within the *MEFV* coding regions but a substantial minority of patients, up to 38% in some series, will have no or only a single identifiable mutation and classical FMF[298].

MEFV is expressed only in cells of the myeloid lineage, predominantly in neutrophils and the gene product, pyrin, is predicted to be a 781 amino acid, 86 Kda, protein but may undergo a degree of post translational modification. Pyrin is as yet poorly characterised but it appears to play a critical role in the auto-regulation of neutrophil function and its expression is upregulated by a number of pro-inflammatory cytokines; interferon γ, interferon α and tumour necrosis factor alpha[299]. It is thought to contribute to a negative feed back process, which is involved in the normal regulation, and perhaps resolution, of inflammation, possibly by promoting apoptosis in a subset of leucocytes involved in the early stages of the inflammatory response.

Table 1.4 Mutations associated with inherited periodic fever syndromes

Mutations associated with FMF	Mutations associated with TRAPS
MEFV Arg42Trp (R42W)	*TNFRSF1A* His22Tyr (H22Y)
MEFV Ser108Arg (S108R)	*TNFRSF1A* Cys29Phe (C29F)
MEFV Leu110Pro (L110P)	*TNFRSF1A* Cys30Ser (C30S)
MEFV Ala138Gly (A138G)	*TNFRSF1A* Cys30Arg (C30R)
MEFV Glu148Gln (E148Q)	*TNFRSF1A* Cys33Tyr (C33Y)
MEFV Glu148Val (E148V)	*TNFRSF1A* Cys33Gly (C33G)
MEFV Glu167Asp (E167D)	*TNFRSF1A* Splice Junction G>A 193-14
MEFV Glu230Lys (E230K)	*TNFRSF1A* Tyr38Cys (Y38C)
MEFV Thr267Ile (T267I)	*TNFRSF1A* Pro46Leu (P46L)
MEFV Pro369Ser (P369S)	*TNFRSF1A* Thr50Met (T50M)
MEFV Arg408Gln (R408Q)	*TNFRSF1A* Cys52Phe (C52F)
MEFV Glu474Lys (E474K)	*TNFRSF1A* Cys55Ser (C55S)
MEFV His478Tyr (H478Y)	*TNFRSF1A* Cys70Arg (C70R)
MEFV Phe479Leu (F479L)	*TNFRSF1A* Cys70Tyr (C70Y)
MEFV Val487Met (V487M)	*TNFRSF1A* Ser86Pro (S86P)
MEFV Arg501Gly (R501G)	*TNFRSF1A* Cys88Arg (C88R)
MEFV Ile591Thr (I591T)	*TNFRSF1A* Cys88Tyr (C88Y)
MEFV Pro646Leu (P646L)	*TNFRSF1A* Arg92Pro (R92P)
MEFV Arg653His (R653H)	*TNFRSF1A* Arg92Gln (R92Q)
MEFV Glu656Ala (E656A)	*TNFRSF1A* Phe112Ile (F112I)
MEFV Ser675Asn (S675N)	
MEFV Gly678Glu (G678E)	**Mutations associated with MWS/FCU**
MEFV Met680Leu (M680L)	
MEFV Met680IleGC (M680IGC)	
MEFV Met680IleGA (M680IGA)	*NALP3/CIAS1* Val198Met (V200M)
MEFV Thr681Ile (T681I)	*NALP3/CIAS1* Arg260Trp (R262W)
MEFV stop codon at Tyr688 (Y688X)	*NALP3/CIAS1* Asp303Asn (D305N)
MEFV deletion Ile692 2074-2076 (ΔI692)	*NALP3/CIAS1* Leu305Pro (L307P)
MEFV deletion Ile692 2076-2078 (ΔI692)	*NALP3/CIAS1* Ala352Val (A354V)
MEFV deletion Met694 (ΔM694)	*NALP3/CIAS1* Ala439Val (A441V)
MEFV Met694Val (M694V)	*NALP3/CIAS1* Ala439Thr (A441T)
MEFV Met694Ile (M694I)	*NALP3/CIAS1* Gly569Arg (G571R)
MEFV Lys695Arg (K695R)	*NALP3/CIAS1* Glu627Gly (E629G)
MEFV Val704Ile (V704I)	
MEFV Ile720Met (I720M)	
MEFV Val726Ala (V726A)	
MEFV Ala744Ser (A744S)	
MEFV Arg761His (R761H)	

An N-terminal domain first identified in pyrin has been recognised in a number of other proteins thought to modulate in inflammatory and apoptotic signalling pathways. These may represent a class of proteins which coordinate apoptotic signalling by homotypic protein protein interactions in an analogous fashion to those of death domain proteins[300]. *MEFV* mutations presumably disrupt pyrin structure, impairing its physiological function sufficiently to allow neutrophil activation and inflammation in response to usually innocuous stimuli. This is compatible with the clinical situation in FMF where the vast majority of attacks appear to be entirely unprovoked with no discernible precipitants.

Systemic AA amyloidosis is the most serious complication of FMF and, although there is a degree of ethnic variation it is remarkably frequent[301]. Before the introduction of colchicine, AA amyloidosis developed in up to 90% of Sephardi Jews of North Africa and 60% of Turks with FMF[301, 302]. The vast majority of these individuals eventually became uraemic and, prior to the widespread availability of renal replacement therapy, 5% died before the age of 10 and 90% before the age of 40 years[301]. Since the introduction of colchicine the prevalence of amyloidosis has fallen and was recently reported at 15% in one large series from Turkey[303]. M694V homozygotes have a more severe disease phenotype in some populations and there is some evidence that they have an increased risk of amyloidosis but this seems to be variable and a wide variety of other *MEFV* genotypes have also been associated with the subsequent development of amyloidosis[297, 303-307]. The risk of AA amyloidosis complicating FMF appears to be greatly increased among first degree family members of a dually affected proband, and even in colchicine treated patients may exceed 50% implying that

additional genetic and environmental factors are involved[308-310]. Historically the genesis of amyloid in FMF has been poorly understood[311]. Most patients will have less than two attacks a month and, as attacks are usually brief, the majority of afflicted individuals will be symptomatic for less than 40 days per year[312] implying substantially less inflammatory activity than is seen in other chronic diseases yet the incidence of AA amyloidosis is higher than that seen complicating rheumatological diseases.

One of the commonest mutations, encoding the pyrin variant E148Q has lately been found to occur in up to 20% of some ethnic groups including Chinese and Indians in whom FMF is not well recognised. Pyrin E148Q causes FMF only when it is associated with other more disruptive *MEFV* mutations, and apparently does not cause overt disease in otherwise healthy homozygotes[313]. However, in patients with non FMF chronic inflammatory arthritis pyrin E148Q has been found to be a major risk factor for developing AA amyloid, suggesting that the inflammatory response in individuals with this variant may be upregulated. The same phenomenon may occur in heterozygous carriers of other pyrin variants which are more pathogenic in FMF, but which are less prevalent globally.

Unusually for a chronic disease, there is a readily available, effective and safe long-term treatment in the form of colchicine. This was serendipitously found to ameliorate the attacks in 1972 by Goldfinger and has transformed patients' quality of life and long-term survival[314]. *In vitro* it is known to bind to tubulin preventing its polymerisation into microtubules[315] but whether this is significant at therapeutic doses is unclear[316]. If used as long-term prophylaxis and in

appropriate dosage, usually 1-2 mg/day it produces total symptom control in up to 65% and substantial benefits in a further 30% of patients[317] and prevents the subsequent development of amyloidosis. Approximately 5% of patients remain symptomatic despite adequate treatment but the vast majority of non-response is due to poor compliance. It is remarkably safe in the long-term at low doses, although it can occasionally cause troublesome diarrhoea[318]. Colchicine therapy can lead to regression of amyloid in FMF, and improvement in renal function so long as renal failure is not too advanced[319, 320].

Muckle-Wells syndrome and familial cold urticaria

In 1962 Muckle and Wells described a Derbyshire family with an inherited periodic febrile syndrome characterised by recurrent bouts of urticaria, progressive sensorineural deafness and AA amyloidosis[260]. Since their original paper approximately a hundred cases have been reported. The vast majority of these are of Northern European extraction and the mode of inheritance is usually autosomal dominant although both variable penetrance and sporadic cases are recognised. Most affected individuals describe maculopapular rashes associated with malaise from infancy or early childhood[321]. Variable degrees of sensorineural deafness, often of adolescent onset, occur in more than 60% of cases and between a quarter and a third eventually develop reactive systemic, AA type amyloidosis[322, 323]. A number of other clinical features have been described including fever, arthralgia, arthritis, conjunctivitis and episcleritis, abdominal pain and short stature. Symptoms, usually lasting 24 to 48 hours, are accompanied by a raised acute phase response and often leukocytosis and a polyclonal hypergammaglobulinaemia. The long-term prognosis of Muckle-

Wells syndrome (MWS) depends greatly on whether it is complicated by amyloidosis. Treatment of MWS has generally proved unsatisfactory. A variety of drug regimes have been tried, many using potent immunosuppressive combinations with very variable outcomes and there is no consensus on how best to manage these patients in the long-term[321, 324-327].

Familial cold urticaria was first described by Kyle and Rusk in 1940[328]. Approximately 20 families have been described in the literature, and the majority of cases are of northern European ancestry. It shares a number of clinical features with Muckle-Wells syndrome but attacks are characteristically induced by cold exposure. A nonpruritic nonurticarial maculopapular eruption (the urticaria in FCU is a misnomer) is accompanied by myalgia, fever, headache, conjunctivitis and often distal limb swelling within 30 minutes to three hours after contact with a cool or damp environment[329, 330]. Unlike Muckle-Wells sensorineural deafness is not a recognised feature. Most patients with FCU have a normal life expectancy but AA amyloid can develop[257]. Like Muckle–Wells treatment has proved largely unsatisfactory with no response to corticosteroids, colchicine[331] or ketofiten[332]. There are a number of case reports of beneficial responses to anti-histamines or to stanazolol[333], but most patients are advised simply to avoid cold exposure.

Recent linkage studies by different groups in both MWS and FCU have localised the gene to chromosome 1q44. Their phenotypic similarities in combination with a shared linkage site suggested that these two syndromes are due to mutations in either a single or closely related gene(s). Further support for a common genetic

defect was provided by an extensive Indian family with an autosomal dominant periodic febrile syndrome. This family's clinical features overlap with both MWS and FSU with attacks of arthritis, distal swelling, transient erythema and fever often precipitated by cold and a high incidence of AA amyloidosis. Genetic linkage studies have again identified a disease susceptibility locus at 1q44 in this kindred[334]. Subsequently mutations in *NALP3* a previously described gene have been found to underlie the syndrome[335] and as a result a new gene name has been suggested *CIAS1* (for cold induced auto inflammatory syndrome)[292]. *NALP3/CIAS1* has N-terminal structural homologies with pyrin and also appears to have a role in regulating inflammation probably by promoting leukocyte apoptosis[336]. Mutations in the same gene have recently been found to underlie a rare infantile inflammatory disorder, chronic infantile neurological cutaneous and articular syndrome, CINCA, which is characterised by cutaneous symptoms, central-nervous-system involvement with developmental delay, short stature, dysmorphic features and arthropathy[337].

Hyper immunoglobulin D syndrome (HIDS)

This was first described in 1984[338] and disease usually presents in infancy with febrile episodes lasting 3 days to a week usually accompanied by diarrhoea and vomiting, abdominal pain, erythematosus rash and cervical lymphadenopathy. These episodes are accompanied by elevated IgD and frequently IgA levels and transient mevalonic acid urea. Attacks occur every one to two months and are typically precipitated by minor trauma or vaccinations. It is inherited in an autosomal recessive pattern and the majority of affected individuals are northern European Caucasians with 60% of Dutch or French ancestry[339]. HIDS is now

recognised to be caused by a variety of missense mutations in a gene on the short arm of chromosome 12[259], which encodes mevalonate kinase. This enzyme is involved in a number of metabolic pathways including cholesterol, dolichol, farnesyl and isoprenoid synthesis. Complete deficiency of mevalonate kinase is associated with a separate rare disorder, mevalonic aciduria, characterised by severe developmental delay, myopathy and ataxia and death in infancy. Why and how specific mutations in mevalonate kinase produce the characteristic HIDS phenotype is not yet clear.

Tumour necrosis factor receptor associated periodic fever syndrome (TRAPS)

TRAPS was initially reported in 1982 as familial Hibernian fever[340] and subsequently renamed when the underlying genetic defect was identified[261]. It is an autosomal dominant syndrome with onset usually in childhood. Attacks tend to last some weeks and are characterised by fever, variable serositis and arthritis, erythematous rash and frequently gastrointestinal disturbance with diarrhoea and vomiting. It has been reported in a number of ethnic groups including Arabs, and Central Americans but those of Irish and Scots ancestry are over-represented[341, 342]. There are now at least 20 separate mutations recognised, the majority affecting the first two cysteine rich domains of the extracellular portion of the 55-kDa TNF receptor. These mutations appear to result in impaired metalloprotease cleavage of the TNF receptor following TNF induced cellular activation. This impedes shedding of the extracellular portion of the receptor, one of the normal homeostatic pathways by which TNF signalling is terminated. It also reduces the pool of soluble TNF receptors, which contribute to the normal

regulation of inflammation by competing with the membrane bound receptors to bind TNF[343]. TNF receptors have well recognised roles in apoptosis and disruption of this function presumably contributes to the TRAPS phenotype.

Clinical features

AA amyloid deposition can be extensive without causing symptoms[344]. It presents most commonly with non-selective proteinuria or nephrotic syndrome and may be accompanied by renal insufficiency[147]. Approximately 5% of cases will not have proteinuria despite extensive renal amyloid deposition. Haematuria, tubular defects, and diffuse renal calcification occur rarely[345]. End-stage renal failure is the cause of death in 40 to 60% of patients although the clinical course is unpredictable and may be characterised by step-wise deteriorations[319]. A few patients present with organomegaly, e.g., hepatosplenomegaly, sometimes without any accompanying renal dysfunction although, invariably, amyloid deposits are widespread at diagnosis[267]. The spleen is the first site of substantial parenchymal deposition and is infiltrated in almost every case at presentation[344]. The adrenal glands are infiltrated extensively in at least one third of cases[346], and the liver in one quarter but function of both organs is typically well preserved even at a late stage[347]. Microscopic histological involvement of the heart and gut is usual and although clinical sequelae are rare, these may now be encountered more often than previously because patients with AA amyloidosis are surviving for longer with active supportive measures[267].

Prognosis is related to the degree of renal involvement and without treatment the outlook is bleak[348]. Almost three-quarters of patients reach end stage renal failure

within 5 years of diagnosis[255] and once the serum creatinine is elevated beyond the normal range half the patients are dead within 18 months[349]. Although AA amyloid is a relatively unusual complication of JIA it is responsible for 40 to 50 % of all deaths in these patients. 50% of JIA patients with amyloidosis will die within five years and a further 25 % within the following ten years[147, 350, 351].

Treatment

Successful therapy in AA amyloidosis depends on as complete as possible suppression of the acute phase response, and will therefore vary according to the nature of the underlying inflammatory disease[14, 262, 319, 352, 353]. Given that amyloid is potentially fatal, it may be justified to employ aggressive treatment strategies that would otherwise be considered too toxic. Hence, cytotoxic drugs especially alkylating agents have been used to treat patients with AA amyloidosis secondary to rheumatic diseases[352], and limb amputation may be required in cases of osteomyelitis that has not responded to antimicrobial drugs[267]. There have been several case reports describing resolution of nephrotic syndrome following surgical resection of cytokine secreting lesions such as solitary Castleman's disease tumours of the plasma cell type[354-356]. Patients with Crohn's disease have responded to medical therapy, elemental diet and occasionally in resistant disease to colectomy.

In the UK, chlorambucil has been used extensively in patients with AA amyloidosis due to RA and JIA. It was first used in children with JIA in the late 1960's and dramatically improved the prognosis both in terms of survival and renal function[357]. Since then treatment has been extended to adults with a

substantial benefit in about two-thirds of cases. Long term survival is now frequent and reversal of nephrotic syndrome and stabilisation or recovery of renal function are commonly recognised[358]. Among a series of 80 patients with AA amyloidosis under long-term follow up, the 10 year survival of those in whom the plasma SAA had been suppressed to near normal levels was more than 95%, compared with 60% among those whose inflammatory disease did not remit[14]. The majority of these 80 patients had RA or JIA, and most received chlorambucil following the diagnosis of amyloid. However, its use must be considered very carefully in each patient since it is not licensed for this indication, is potentially carcinogenic and causes infertility.

AA amyloid deposits gradually regress in the majority of patients whose inflammatory disease remains in remission, but the rate varies substantially between different individuals[14]. These differences suggest that the amyloid deposits are more stable in some patients than in others, or that individuals simply differ in their capacity to mobilise amyloid. Renal function however, especially proteinuria, often improves even when the amyloid deposits only remain stable, and *vice versa.* Residual amyloid deposits may be present to a substantial extent in some patients who appear to have been in complete clinical remission for decades[359]. In addition, the presence of even a small amount of amyloid may serve as a template for further rapid amyloid accumulation should there be a recrudescence of inflammatory activity[109, 360].

SYSTEMIC AMYLOIDOSIS ASSOCIATED WITH MONOCLONAL IMMUNOCYTE DYSCRASIAS, AL AMYLOIDOSIS

Underlying conditions

AL amyloid fibrils are derived from monoclonal immunoglobulin light chains[158, 361, 362] and AL amyloidosis can complicate most clonal B cell dyscrasias, including myeloma, lymphomas, macroglobulinaemia[11]. Amyloidosis develops in up to 15% of patients with myeloma, and although it occurs at a very much lower frequency among individuals with low grade and otherwise "benign" monoclonal gammopathies, the latter are much more prevalent and underlie the majority (~ 80%) of cases[11]. A monoclonal component can be identified in the serum or urine of 65% and 86% of AL patients respectively provided that very sensitive techniques such as immunofixation of concentrated urine are utilised[11, 363].

Table 1.5

Conditions associated with systemic AL amyloidosis

Low grade plasma cell dyscrasias
Myeloma
Waldenstroms/lymphoplasmacytoid lymphoma
Non-Hodgkin's lymphoma
Hodgkin's lymphoma
Chronic lymphocytic leukaemia (CLL)
Sjögrens Syndrome
Castleman's Disease
POEMS syndrome
Gaucher's disease

Clinical features

AL amyloid usually presents in patients over the age of 50 years, although it can present in very young adults[11]. Clinical manifestations are extremely variable since almost any organ other than the brain can be directly involved[364]. Although certain clinical features are very strongly suggestive of AL amyloidosis, and multiple vital organ dysfunction is common, many patients present with non-specific symptoms such as malaise and weight loss. The outlook of untreated AL amyloid is far worse than AA type, with a 5 year survival of approximately 10% and a 10 year survival of less than 5%[11]. Most affected individuals die of heart failure, uraemia, or autonomic failure within 2 years of diagnosis.

In most cases there is substantial histological cardiac involvement. Restrictive cardiomyopathy is the presenting feature in up to one third of patients and causes death in one half[365]. Renal involvement is frequent in AL amyloidosis and presents in the same manner as renal AA amyloid[366]. Gut involvement can cause motility disturbances (often secondary to autonomic neuropathy), malabsorption, perforation, haemorrhage, or obstruction[367]. Hyposplenism, usually in association with splenomegaly, is an occasional feature[368]. Peripheral neuropathy occurs in one fifth of cases and typically presents with a painful sensory polyneuropathy followed later by motor deficits[11]. Carpal tunnel syndrome is present in 40%[369]. Autonomic neuropathy causing orthostatic hypotension, impotence, and gastrointestinal disturbances may occur in isolation or with a peripheral neuropathy[364].
Involvement of dermal blood vessels results in purpura, characteristically around the eyes. Macroglossia is infrequent, but is almost pathognomonic of AL type. Articular amyloid usually occurs in association with myeloma and may mimic

rheumatoid arthritis or an asymmetric seronegative synovitis. A rare but potentially serious manifestation of AL amyloid is an acquired bleeding diathesis that may be associated with deficiency of factor X and sometimes also factor IX, or with increased fibrinolysis[208-211]. In the extensive Mayo Clinic experience[11, 369], comprising over 400 cases of AL amyloidosis, median survival was only 12-15 months and was even less when associated with multiple myeloma. Median survival is only about 6 months in cases where heart failure is evident at presentation[370, 371]. Other very poor prognostic factors include hyperbilirubinaemia, autonomic neuropathy, and SAP scintigraphic evidence of a large whole body amyloid load[372, 373].

Treatment

The aim of treatment in AL amyloidosis is to suppress proliferation of the underlying B-cell clone and, therefore, production of the amyloid fibril precursor protein. There are, however, many difficulties[374]. Chemotherapy regimes are based on those used in multiple myeloma, but the plasma cell dyscrasias in most AL patients are relatively low grade and may be less chemosensitive. Diagnosis is difficult and can be delayed, and many patients have advanced multi-system disease which limits their options for chemotherapy[374]. Regression of amyloid is a gradual process which may not lead to measurable clinical improvement or recovery of organ function for many months, or even years, after successful suppression of the causative plasma cell dyscrasia[117, 375]. Mobilisation of amyloid from the heart is much slower than from the liver or kidneys, and many patients with cardiac or multi-system dysfunction do not live long enough to benefit from chemotherapy, even when it has suppressed their clonal disease[376]. Moreover,

because of their subtle nature, it has not been possible to know at an early stage whether the underlying plasma cell dyscrasias have responded to treatment. However, despite these problems, many patients with AL amyloidosis do benefit substantially from chemotherapy[376]. Prolonged low intensity cytotoxic regimes such as oral melphalan and prednisolone are beneficial in about 20 per cent of patients and are the only treatment to have been proven in placebo controlled trials[129, 377]. Dose intensive infusional chemotherapy regimes such as vincristine, doxorubicin (Adriamycin) and dexamethasone ('VAD')[378, 379] and autologous peripheral blood stem-cell transplantation show more promising early results[372, 380, 381]. Very rigorous patient selection for high dose chemotherapy is essential because the procedure related mortality is high in individuals with multiple amyloidotic organ involvement, especially patients with autonomic neuropathy, severe cardiac amyloidosis, or a history of gastrointestinal bleeding, and in those aged over 55 years[372, 382]. As a result its apparently more favourable outcome may be biased by selection of fitter patients for this hazardous treatment[383].

DIALYSIS RELATED AMYLOIDOSIS (DRA), β_2-MICROGLOBULIN AMYLOIDOSIS

Underlying conditions

The amyloid fibril precursor protein is β_2-microglobulin which is the invariant chain of the MHC class 1 molecule, and is expressed by all nucleated cells. It is synthesised at an average rate of 150 to 200 mg per day and in normal circumstances is freely filtered at the glomerulus and then reabsorbed and

catabolised by the proximal tubular cells[384]. Decreasing renal function causes a proportionate rise in levels and the 11. 8 KDa protein is poorly cleared by dialysis. β_2-microglobulin amyloidosis was first described in 1980[164] and occurs only in patients who have been on dialysis for several years, or very occasionally in individuals with longstanding severe chronic renal impairment. DRA is better recognised in the haemodialysis population but also occurs in patients on CAPD[168]. Relatively few patients have yet been maintained on peritoneal dialysis for the 5 to 10 years required to develop symptomatic β_2-microglobulin amyloid, but histological studies of early sub-clinical deposits suggests that the incidence of DRA is similar among patients receiving these two modalities of dialysis. Indeed, β_2-microglobulin amyloid deposits are present in 20 to 30% of patients within three years of commencing dialysis for end-stage renal failure[385].

Clinical features

β_2-microglobulin amyloidosis is preferentially deposited in articular and peri-articular structures, and its manifestations are largely confined to the locomotor system[174, 386]. Carpal tunnel syndrome is usually the first clinical manifestation of β_2-microglobulin amyloidosis. Some individuals develop symptoms within 3 to 5 years and by 20 years the prevalence is almost 100%[170, 387]. Older patients appear to be more susceptible to the disease, and tend to exhibit symptoms more rapidly[388]. Amyloid arthropathy tends to occur a little later but eventually affects most patients on dialysis[389]. The arthralgia of β_2-microglobulin amyloidosis affects the shoulders, knees, wrists and small joints of the hand and is associated with joint swelling, chronic tenosynovitis and, occasionally, haemarthroses[390]. Spondylarthropathies are also well recognised, as is cervical cord compression[391].

β_2-microglobulin amyloid deposition within the periarticular bone produces typical appearances of subchondral erosions and cysts which can contribute to pathological fractures particularly of the femoral neck, cervical vertebrae and scaphoid. Manifestation outside the musculoskeletal systemic are rare, but there have been reports of β_2-microglobulin amyloidosis causing congestive cardiac failure, gastrointestinal bleeding[392], perforation[393] or intestinal pseudo-obstruction and macroglossia[394].

Treatment

The only really effective treatment for DRA is successful renal transplantation[395, 396]. Serum levels of β_2-microglobulin fall rapidly following transplantation and this is usually accompanied by a very rapid and substantial improvement in symptoms. Although prospective SAP scintigraphy has shown that β_2-microglobulin amyloid deposits can gradually regress[395], the resolution of DRA symptoms within days or weeks of renal transplantation implicates other factors. These probably include the anti-inflammatory properties of immunosuppression after transplantation, and some effect from discontinuation of the dialysis procedure itself. In contrast to the symptoms, radiological bone cysts heal very slowly indeed[397, 398], and unsurprisingly amyloid can be demonstrated histologically many years after renal transplantation[399]. Symptoms of DRA may reappear very rapidly if the graft is lost, providing further evidence that dialysis is required for the clinical expression of disease associated with β_2-microglobulin amyloid deposits. Possible explanations of this phenomenon are that newly deposited β_2-microglobulin amyloid is more damaging than old, or that the cytokine modulating effects of dialysis are involved. Certainly, β_2-microglobulin

amyloid deposits are unusual in that they are often associated with a degree of inflammation and macrophage infiltration[400].

Attempts have been made to reduce β_2-microglobulin levels and DRA by altering the dialysis prescription. There is some evidence that the risks of DRA are increased in patients dialysed using less 'biocompatible' cuprophane membranes, and that use of the more permeable membrane systems is relatively protective[401]. Greater removal of β_2-microglobulin is attained in patients undergoing high flux haemodiafiltration and in the long-term these patients may be less prone to DRA[388].

Drug treatment of established DRA includes non-steroidal anti-inflammatory analgesics, systemic and intraarticular corticosteroid therapy, but none of these is especially effective and long-term steroid therapy is particularly undesirable in this population of patients[402, 403]. Surgery may be required to relieve carpal tunnel compression, stabilise the cervical spine or to treat bone fractures.

HEREDITARY AMYLOIDOSIS

Familial Amyloid Polyneuropathy (FAP)

This is caused by point mutations in the gene for the plasma protein transthyretin[31] (TTR) and is an autosomal dominant syndrome with peak onset between the third and sixth decades[88, 404]. The disease is characterised by progressive and disabling peripheral and autonomic neuropathy and varying degrees of visceral amyloid involvement[405]. Severe cardiac amyloidosis is common[406-408]. Deposits within the

vitreous of the eye are recognised[409] but renal, thyroid, spleen and adrenals deposits are usually asymptomatic[410]. There are well-recognised foci of the disease in Portugal[404], Japan[411] and Sweden and FAP has been reported in most ethnic groups throughout the world[412-414]. There is considerable phenotypic variation in the age of onset[183], rate of progression, involvement of different systems and disease penetrance[108], although within families the pattern is often quite consistent[415, 416]. More than 80 variant forms of TTR are associated with FAP (Table 1.4), the most frequent of which is the substitution of methionine for valine at residue 30[95, 417, 418].

Treatment

Until recently, the treatment of FAP was limited to supportive measures to help with malnutrition, bladder and bowel dysfunction, hypotension and renal and cardiac complications[404]. Most patients died within 5 to 15 years of diagnosis. However, the situation has improved dramatically following the introduction of orthotopic liver transplantation in 1991[419, 420]. The procedure results in a rapid and near total replacement of the variant protein by donor wild type TTR, since almost all circulating TTR is produced by the liver[419]. Most FAP patients who have liver transplants experience a symptomatic improvement within 6-12 months[420], and successful liver transplantation has now been reported in hundreds of patients with this condition worldwide. Although the peripheral neuropathy usually only stabilises, autonomic function can improve substantially and the associated visceral amyloid deposits have been shown by serial SAP scintigraphy to regress in most cases[421-423].

Disappointingly in a few cases, there is evidence that wild-type *TTR* may continue to be deposited after liver transplantation, on the existing 'template' of amyloid[424]. This may occur to a clinically important extent in the heart[425] and the vitreous[426], but seems to be mutation-specific and fortunately seems to happen rarely in the bulk of FAP patients who have the *TTR* Val30Met variant[427].

An intriguing spin-off from orthotopic hepatic transplantation in FAP has been the use of the explanted livers as 'domino' donor organs[428]. Although the liver produces the amyloidogenic variants of TTR, the organ itself usually remains healthy. Minor vascular amyloid deposits may occur but liver function is never impaired. As donor organ availability becomes increasingly limited, livers explanted from some FAP patients have been re-used in 'domino' transplants. It remains to be seen whether recipients of FAP livers are susceptible to developing FAP themselves in the long-term.

Table 1.6 Transthyretin mutations associated with FAP

TTR Cys10Arg (C10R)	TTR Glu54Gly (E54G)
TTR Leu12Pro (L12P)	TTR Glu54Lys (E54L)
TTR Met13Ile (M13I)	TTR Leu55Pro (L55P)
TTR Asp18Glu (D18E)	TTR Leu55Arg (L55R)
TTR Asp18Gly (D18G)	TTR Leu58His (L58H)
TTR Asp18Asn (D18N)	TTR Leu58Arg (L58R)
TTR Val20Ile (V20I)	TTR Thr59Lys (T59K)
TTR Ser23Asn (S23N)	TTR Thr60Ala (T60A)
TTR Pro24Ser (P24S)	TTR Glu61Lys (E61K)
TTR Ala24Ser (A25S)	TTR Phe64Lys (F64K)
TTR Val28Met (V28M)	TTR Phe64Ser (F64S)
TTR Val30Gly (V30G)	TTR Ile68Leu (I68L)
TTR Val30Ala (V30A)	TTR Tyr69His (Y69H)
TTR Val30Leu (V30L)	TTR Lys70Asn (K70N)
TTR Val30Met (V30M)	TTR Val71Ala (V71A)
TTR Phe33Val (F33V)	TTR Ile73Val (I73V)
TTR Phe33Ile (F33I)	TTR Asp74His (D74H)
TTR Phe33Leu (F33L)	TTR Ser77Phe (S77F)
TTR Phe33Cys (F33C)	TTR Ser77Tyr (S77Y)
TTR Arg34Thr (R34T)	TTR Ile84Thr (I84T)
TTR Lys35Asn (L35N)	TTR Ile84Ser (I84S)
TTR Ala36Pro (A36P)	TTR Ile84Asn (I84N)
TTR Asp38Ala (D38A)	TTR Glu89Lys (E89K)
TTR Asp38Val (D38V	TTR Glu89Gln (E89Q)
TTR Trp41Leu (W41L)	TTR His90Asn (H90N)
TTR Glu42Gly (E42G)	TTR Ala91Ser (A91S)
TTR Glu42Asp (E42D)	TTR Gln92Lys (Q92K)
TTR Phe44Ser (F44S)	TTR Ala97Gly (A97G)
TTR Ala45Thr (A45T)	TTR Ala97Ser (A97S)
TTR Ala45Asp (A45D)	TTR Arg104His (R104H)
TTR Ala45Ser (A45S)	TTR Ile107Val (I107V)
TTR Gly47Ala (G47A)	TTR Ile107Met (I107M)
TTR Gly47Val (G47V)	TTR Ala109Ser (A109S)
TTR Gly47Glu (G47E)	TTR Leu111Met (L111M)
TTR Gly47Arg (G47R)	TTR Ser112Ile (S112I)
TTR Thr49Ala (T49A)	TTR Tyr114His (Y114H)
TTR Thr49Ile (T49I)	TTR Tyr114Cys (Y114C)
TTR Ser50Arg (S50R)	TTR Tyr116Ser (Y116S)
TTR Ser50Ile (S50I)	TTR Thr118Met (T118M)
TTR Glu51Gly (E51G)	TTR Ala120Ser (A120S)
TTR Ser52Pro (S52P)	TTR Val122Ala (V122A)
TTR Gly53Glu (G53E)	TTR Val122Ile (V122I)
TTR Gly53Ala (G53A)	TTR Val122 deletion (ΔV122)
TTR Glu54Gln (E54Q)	

Familial amyloid polyneuropathy with predominant cranial neuropathy

Originally described in Finland[429] but now reported in other ethnic groups[186, 430], this is a very rare autosomal dominant form of hereditary amyloidosis that presents in adult life with cranial neuropathy, corneal lattice dystrophy and a mild distal peripheral neuropathy. There may be skin, renal and cardiac manifestations but these are usually covert and life expectancy approaches normal. The mutant gene responsible encodes a variant form of gelsolin[431], which is an actin-modulating protein. The functional role of circulating gelsolin is unknown but may be related to clearance of actin filaments released by apoptotic cells[432]. There is no specific treatment for this disorder, which is disfiguring due to progressive cranial nerve involvement and very distressing in its late stages.

Hereditary non-neuropathic systemic amyloidosis

Ostertag first described hereditary renal amyloidosis (HRA) in 1932. He reported two families with an autosomal dominant inheritance of renal amyloidosis without neuropathy[187]. This syndrome is now known to be associated with mutations in the genes for lysosyme[29], apolipoprotein AI[90] and fibrinogen A alpha chain[89].

Lysosyme

Hereditary non-neuropathic systemic amyloidosis has been described in association with three lysozyme variants, the substitution of histidine for aspartic acid at position 67[29], threonine for isoleucine at position 56[29] and substitution of

arginine for tryptophane at position 64[433]. Most patients present in middle age with proteinuria, slowly progressive renal impairment and sometimes hepatosplenomegaly with or without purpuric rashes[434]. Virtually all patients have substantial gastrointestinal amyloid deposits, and although these are often asymptomatic, they are important since gastrointestinal haemorrhage or perforation is a frequent cause of death in these patients.

Lysozyme is a ubiquitous protein that is produced diffusely within the body and this type of amyloidosis is not therefore ameliorated by liver transplantation. However, the disease usually runs an extremely slow course, and patients with renal failure merit strong consideration for renal transplantation[434].

Apolipoprotein AI

Apolipoprotein AI is a major constituent of high density lipoprotein (HDL)[435]. Wild type apolipoprotein AI is amyloidogenic and is present as amyloid in human aortic atherosclerotic plaques in 10-20% of autopsies[436]. Ten amyloidogenic variants have been reported, seven single amino acid substitutions[90, 181, 437-441], one deletion/insertion[442] and two deletions[230, 443]. Depending on the mutation, patients can present with massive abdominal visceral amyloid involvement[442], predominant cardiomyopathy[438] or an FAP like syndrome[188]. The majority of patients eventually develop renal failure but despite extensive hepatic amyloid deposition liver function usually remains well preserved. Renal transplantation offers most patients with this disease an excellent quality of life and prolonged survival[444], and some patients have had

renal grafts for over 20 years without evidence of recurrent amyloidosis or any reduction in graft function.

Approximately half of the apolipoprotein AI in the circulation is synthesised in the liver, and only one patient with hereditary apolipoprotein AI amyloidosis has been reported to have undergone liver transplantation[445]. The reduction by 50% in the plasma concentration of variant apolipoprotein AI appeared to be sufficient to facilitate regression of his amyloid deposits generally, and supports the use of liver transplantation in patients with this type of amyloidosis who develop hepatic dysfunction.

Apolipoprotein AII

Two North American families have recently been reported to have hereditary nephropathic amyloidosis due to separate mutations in Apolipoprotein AII[182]. In both kindreds a single base substitution at a stop codon results in extension of translation with a predicted 21 residue C-terminal extension. Apolipoprotein AII is well recognised as an amyloidogenic protein in senescent mice[446] and the mouse strain most susceptible to senile amyloidosis also has a variant form of the protein[447]. The role of apolipoprotein AII in normal human physiology is not clear as total deficiency does not seem to have clinical or biochemical consequences[448].

Table 1.7 Mutations associated with hereditary non-neuropathic amyloidosis

Variant Protein	Organs/tissues predominantly affected by amyloid	Affected Kindreds	Reference
Lysozyme Asp67His	Renal Gastrointestinal tract Liver & spleen	Single British family	Pepys 1993
Lysozyme Ile56Thr	Renal Skin Liver & spleen	Two British families (? related)	Pepys 1993
Lysozyme Try64Arg	Sicca syndrome Gastrointestinal tract Renal	Single French family	Valleix 2002
Apolipoprotein AI wild type	Present as amyloid in human atherosclerotic plaques	In 20-30% of autopsies	Westermark 1995
Apolipoprotein AI Gly26Arg	Renal Gastric mucosa Peripheral nerves Liver & spleen	Multiple families, mostly Northern European	Nichols 1988
Apolipoprotein AI Trp50Arg	Renal Liver & spleen	Single Ashkenazi family	Booth 1995
Apolipoprotein AI Leu60Arg	Renal Liver & spleen Cardiac (rarely)	British & Irish kindreds	Soutar 1992
Apolipoprotein AI deletion60-71/insertion60-61	Liver	Single Spanish family	Booth 1996
Apolipoprotein AI deletion 70-72	Renal Liver & spleen Retina	Single family of British origin	Persey 1997
Apolipoprotein AI Leu75Pro	Liver & spleen Renal	Italy - variable penetrance	Obici 2001
Apolipoprotein AI Leu90Pro	Cardiac Larynx Skin	Single French family	Hamidi 1999
Apolipoprotein AI deletion Lys107	Aortic intima	Single Swedish patient at autopsy	Amarzguioui 1998
Apolipoprotein AI Arg173Pro	Cardiac Larynx Skin	British & American families	Hamidi 1999
Apolipoprotein AI Leu174Ser	Cardiac	Single Italian family	Lachmann 2002
Apolipoprotein AI Ala175Pro	Larynx Testicular	Single British family	Obici 2001
Apolipoprotein AI Leu178His	Cardiac Larynx Skin Peripheral nerves	Single French family	Mendes De Sousa 2000
Apolipoprotein A2 21residue extension at C-terminus	Renal	Two American families	Benson 2001
Fibrinogen A alpha chain Arg554Leu	Renal	Peruvian, French & African American	Benson 1993
Fibrinogen A alpha chain frame shift at codon 522	Renal	Single French family	Hamidi 1997
Fibrinogen A alpha chain frame shift at codon 524	Renal	Single American family	Uemichi 1996
Fibrinogen A alpha chain Glu526Val	Renal Late onset liver (rarely)	Multiple of Northern European extraction	Uemichi 1996
Fibrinogen A alpha chain Gly540Val	Renal	Single German family	

Fibrinogen A alpha chain

Fragments of fibrinogen A α-chain were first isolated from amyloid fibrils in 1993 in a kindred with autosomal dominant systemic amyloidosis[89]. Four amyloidogenic mutations in the fibrinogen A α-chain gene have subsequently been reported, in eight unrelated kindreds, clustered in a relatively small portion of exon 5. Two of these are frame shift mutations that have been recognised in single families, one a single nucleotide deletion in the third base of codon 524[449], and the other a deletion at codon 522[450] both of which result in premature termination of the protein at codon 548. A leucine for arginine substitution at codon 554 has been reported in a Peruvian-Mexican family[89], an African-American family[451] and a French kindred[452] all with systemic amyloidosis. However, much the commonest mutation results in the substitution of valine for glutamic acid at position 526, was first reported in 1994[453] and has been found in five families[133, 454], all with clear autosomal dominant inheritance of systemic amyloidosis and high penetrance. Most patients present in late middle age with proteinuria or hypertension and over the following three to six years progress to end stage renal failure.

GENERIC THERAPIES FOR AMYLOIDOSIS

Treatments that reduce the supply of fibril precursor proteins can lead to regression of amyloid deposits[14, 133, 420, 455], but such an approach is not possible in many forms of acquired and hereditary amyloidosis, and these disorders are usually fatal. Furthermore, amyloid is always associated with the neurodegeneration of Alzheimer's disease and with the islet failure of type 2

diabetes. Although it is not known whether amyloid deposition itself causes the tissue dysfunction in these latter diseases, new treatments, to promote removal of the damaging deposits, are urgently required.

Prevention of fibril formation

Improved understanding of the protein folding mechanisms underlying amyloid fibrillogenesis, and the recognition that relative instability of the precursor molecules is a key factor in amyloidogenesis, strongly support therapeutic strategies based on inhibition of fibrillogenesis. There is active research in this area, exploring small molecules, peptides and glycosaminoglycan analogues that bind to fibril precursors and stabilise their native fold[456], or interfere with refolding and/or aggregation into the cross-β core structure common to amyloid fibrils, or bind to mature amyloid fibrils and promote their refolding back towards the native conformation[42, 457-459]. Some of these agents have been reported to interfere with experimental murine AA amyloidosis and a GAG mimic, fibrillex™ (Neurochem, Quebec, Canada) is currently undergoing clinical trials in human AA amyloidosis.

Enhancement of amyloid regression

Immunotherapy

As amyloid fibrils all share common features there has been considerable interest in producing antibodies not to specific types of amyloid but against conformational epitopes common to all fibrils. Specific monoclonal antibodies raised against Alzheimer's Aβ fibrils have been found to recognise transthyretin,

β_2-microglobulin and islet polypeptide derived fibrils but not the native forms of any of these proteins[460]. In addition mouse monoclonal antibodies raised against human AL fibrils have been reported to result in increased clearance of experimental amyloidomas which were produced by subcutaneous injection of AL amyloid extract in mice. Clearance is produced by an immune mediated mechanism with generation of anti-amyloid antibodies and a subsequent local polymorphonuclear leukocyte infiltration[461]. These antibodies raised against ALλ fibrils also recognised fibrils derived from transthyretin, SAA, lysozyme, Aβ protein and apolipoprotein AI. Clearance of experimental amyloid deposits has been reported in transgenic mice expressing human Aβ, the amyloid fibril protein of Alzheimer's disease, following production or administration of anti-Aβ antibodies[462, 463]. This approach has been tried in man although the development of meningoencephalitis in 15 Alzheimer's patients receiving a vaccine against amyloid β peptide (Elan pharmaceuticals) has resulted in early termination of the trial[464].

Targeting SAP

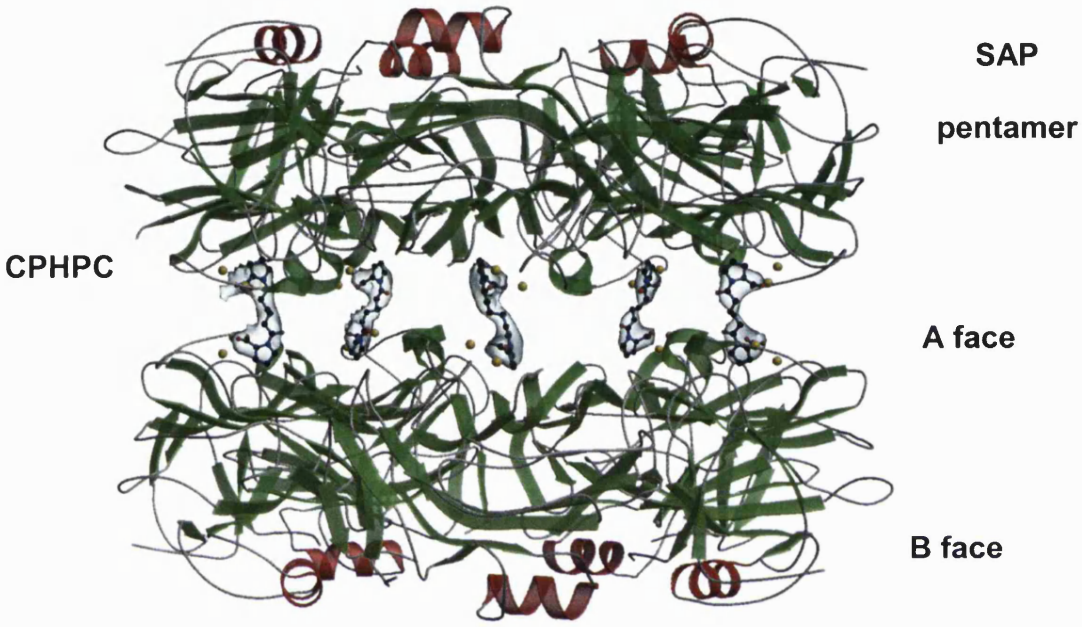
The normal non-fibrillar plasma glycoprotein serum amyloid P component, (SAP), a member of the pentraxin family, is universally present in amyloid deposits[55] and may contribute to the failure to clear amyloid *in vivo*. The evidence that mice with targeted deletion of the SAP gene show retarded and reduced induction of experimental amyloid corroborates the impression that SAP contributes to pathogenesis of amyloidosis[69]. Identification of the first chemically defined low molecular weight ligand for SAP, the cyclic pyruvate acetal of galactose, 4,6-O-(1-carboxyethylidene)-β-D-galactopyranoside

(MOβDG), and demonstration that it could completely dissociate SAP from amyloid deposits *in vitro*, suggested that such molecular dissection of the deposits might be an approach to therapy of amyloidosis[465].

A palindromic compound derived from captopril was serendipitously found to be a potent inhibitor of SAP binding to amyloid fibrils. Further synthesis to optimise the IC_{50} for inhibition of fibril binding *in vitro* produced another palindromic compound, CPHPC[466]. Its structure with two D-proline residues joined by an aliphatic linker chain, suggested that the drug could not only block the ligand binding sites on individual SAP protomers, but could also cross link pairs of pentameric SAP molecules to form B-face to B-face decameric dimmers (Figure 1.1) in whole serum.

Experiments in mice demonstrated that CPHPC was not metabolised and was rapidly excreted, predominantly in the urine, with a small amount in the bile. CPHPC had no effect on the plasma concentrations of mouse SAP in wild type animals, however, injection of CPHPC cleared radiolabelled human SAP tracer from the circulation and inhibited its uptake into experimentally induced mouse AA amyloid deposits. This implies that mouse SAP-CPHPC complexes are formed and/or cleared less efficiently *in vivo* but the human SAP-CPHPC complex is recognised as abnormal and swiftly removed from the circulation, suggesting that drug could be used to selectively deplete SAP from human amyloid.

Figure 1.1 The structure of the CPHPC-SAP complex. Two SAP pentamers are cross linked by their B-faces by five CPHPC molecules (blue). A- face helices are shown in red and the two calcium ions bound to each of the five SAP subunits are shown on the B face in yellow (From Pepys Nature 2002).



Chapter Two

METHODS

PATIENTS

The patients were derived from those seen at the National Amyloidosis Centre. Over the past three years an average of 680 patients have been seen a year of whom 45% are new referrals. Eighty percent of patients are found to have amyloidosis and 9% were referred for investigation of periodic fever syndromes. An access database has been maintained with details on all patients seen over the past decade. All patients with systemic amyloidosis undergo comprehensive clinical assessment with annual or half yearly review. All patients included in this work provided explicit informed consent.

SAP SCINTIGRAPHY

SAP scintigraphy has been used since 1988 as a sensitive, specific and non-invasive means of quantitatively imaging amyloid deposits *in vivo*[467]. Highly purified SAP is labelled with the medium-energy, short half-life, pure gamma emitter ^{123}I and injected intravenously. No localisation or retention of labelled SAP occurs in individuals without amyloidosis, in whom the tracer is rapidly catabolised and excreted. However in patients with amyloidosis, labelled SAP localises rapidly and specifically to the amyloid deposits[217]. It does so in proportion to the amount of amyloid present, and will persist there without breakdown or modification. This

"localisation" of labelled SAP to amyloid is in fact a specific dilution phenomenon since circulating SAP is at all times in a dynamic equilibrium with the far greater quantity of SAP concentrated within amyloid deposits. The technique has 100% diagnostic sensitivity in patients with systemic AA amyloidosis, and approximately 90% in AL type and is the only method available for serial monitoring of the progression or regression of amyloid throughout the body[468]. SAP scintigraphy is also suitable as a screening test in patients thought to be at risk of systemic amyloid deposition, including those with chronic inflammatory conditions, monoclonal gammopathies or known amyloidogenic mutations.

The doses of radioactivity are well within acceptable limits (effective dose equivalent ~3.5 mSv), and more than 4000 patient studies have now been performed without adverse effects at the National Amyloidosis Centre[423]. Anterior and posterior whole body scans and regional images are obtained with an IGE Starcam gamma camera either 6 hours (for AA amyloid) or 24 hours (if any other types are suspected) after intravenous injection of ^{123}I-SAP. Serial comparisons were made between scans obtained at baseline and follow-up. Accumulation of amyloid was defined as an increase in tracer uptake in at least one of the amyloid infiltrated organs compared to the initial scan or involvement of a previously unaffected organ, and regression was defined as a decrease in tracer uptake in at least one affected organ. The amyloid load is defined as steady when the relative amount of tracer in target organs and in the blood-pool background was unchanged between scans.

ECHOCARDIOGRAPHY

The heart is poorly visualised by SAP scintigraphy, probably reflecting cardiac motility, blood pool signal from the cardiac chambers, and relatively slow localisation of labelled SAP across the non fenestrated myocardial endothelium. Cardiac amyloidosis is best evaluated by a combination of echocardiography and ECG[469-471]. Two-dimensional Doppler echocardiography classically reveals concentric biventricular wall thickening, 'sparkling' myocardial echodensity and thickened but pliable valves, with a restrictive filling pattern[472]. Amyloid causes diastolic dysfunction with well preserved contractility until a very late stage[470]. Other frequent findings are biatrial dilatation, pericardial effusions and, in end stage disease right ventricular dilatation with evidence of pulmonary hypertension[365, 473]. The ECG may be normal in patients with substantial cardiac amyloidosis, but in advanced disease commonly shows small voltages, pathological 'Q' waves (pseudo-infarct pattern) in the anterior chest leads and conduction abnormalities. Echocardiographic and ECG studies were repeated at each patient visit and compared. Accumulation of amyloid was defined as an increase in left ventricular wall thickness of at least 2 mm compared to the initial study or new evidence of cardiac involvement, and regression was defined as a decrease in ventricular wall thickness of at least 2 mm.

CRITERIA FOR DIAGNOSIS OF AMYLOID RELATED MAJOR ORGAN INVOLVEMENT

Predominant organ involvement in patients with systemic amyloidosis was defined according to abnormal uptake on SAP scintigraphy alone or any two of the following criteria:

Cardiac

- Echocardiogram showing increased mean ventricular wall thickness and thickened valves with no history of hypertension, HOCM or valvular heart disease
- Electrocardiogram showing unexplained low limb lead voltage, pseudoinfarct pattern and/or non specific conduction abnormalities
- NYHA functional capacity class 2 or higher without ischaemic heart disease

Renal

- Twenty four hour proteinuria greater than 500 mg without other overt cause
- Creatinine clearance less than 60 ml/min without other overt cause

Adrenal

- Biochemical hypoadrenalism

Splenic

- Splenomegaly without other overt cause
- Hyposplenic features on blood film

Hepatic

- Hepatomegaly not attributed to congestive cardiac failure or other cause
- ALP more than 1.5 times the ULN not attributed to congestive cardiac failure and without other overt cause

Peripheral nervous system

- Distal symmetrical sensory or mixed neuropathy without other overt cause

Autonomic nervous system

- Impotence, diarrhoea or constipation, early satiety and/or impaired bladder emptying without other overt cause
- Orthostatic hypotension, defined as a fall of 20 mmHg in systolic BP on standing for 3 minutes, having previously been supine for at least 5 minutes, without other overt cause.

HISTOLOGY

Congo red staining

Amyloid fibrils stained with Congo red give pathognomonic red-green birefringence when viewed under crossed polarised light, and this tinctorial property remains the diagnostic gold standard[19, 474, 475]. The pathognomonic birefringence is dependent on a sufficient density of amyloid so formalin fixed deparaffinised tissue sections were cut at 6-8 μm rather than the usual 2-3 μm. The stain is unstable, working solutions have to be utilised within 20 minutes and solutions prepared fresh at least every 8 weeks. Before staining the sections are rehydrated, counterstained with haematoxylin, 'blued' under running tap water, rinsed in pure water and stained by a modified version of the alkaline-alcoholic

Congo red method described by Puchtler *et al.* (1962). This method gives the most specific and consistent results and reduces any non-specific background staining. The sections are then dehydrated through a series of ascending ethanol concentrations to xylene and mounted. Once dry, slides are viewed under bright field and high-intensity polarised light microscopy and the presence, distribution and extent of amyloid deposition noted.

Immunohistochemistry

Congo red histology was always followed by immunohistochemical staining of formalin fixed deparaffinised 2 μm sections of amyloidotic tissue to further characterise the amyloid type[216, 476]. Immunohistochemical stains were performed using commercial antisera against SAA protein, κ and λ immunoglobulin light chains, transthyretin, lysozyme, (Dako Ltd, Denmark House, Ely, UK), apolipoprotein AI (Medix, Van Nuys, CA) and fibrinogen (Helena Biosciences, Sunderland, UK). Positive control tissues containing each of these types of amyloid were stained during each run. TPS pH 7.6 with 1% bovine serum albumin was used as diluent and wash buffer. Endogenous peroxidase activity was quenched by 15 minute incubation in aqueous H_2O_2. Sections were pre-treated with 1% trypsin in 0.1% $CaCl_2$ to enhance immunoreactivity. Prior to application of the primary antisera non-specific tissue binding was abolished by 30 min incubation in 10% normal nonimmune serum from the species which provided the secondary antibody. Sections were incubated overnight with primary antisera at 4°C. Unlabelled secondary antibodies, specific for the IgG of the primary antibody source species and PAP complexes (reactive against peroxidase) were incubated for 30 minutes each.

Following washing, bound enzyme-antibody complexes were detected using a metal-enhanced DAB solution (Pierce and Warriner Lt, Chester). After a brief rinse in pure water sections were counterstained in haematoxylin, 'blued' under running tap water, and counter stained with Congo red prior to mounting.

IMMUNOASSAYS

C-reactive protein (CRP)

CRP was measured in serum using a high sensitivity automated microparticle enhanced latex turbidimetric immunoassay (COBAS MIRA; Roche Diagnostics GmbH)[477-479]. The lower limit of detection was 0.2 mg/l with an interassay CV of 4.2% at 4 mg/l and 6.3% at 1 mg/l.

Serum amyloid A protein (SAA)

SAA was measured in serum by latex nephelometry (BNII autoanalyser; Dade Behring, Marburg, Germany)[480]. The lower limit of detection was 0.7 mg/l, with an inter-assay CV of 2.6% at 15 mg/l and 3.7% at 80 mg/l. Standardisation of both CRP and SAA assays is based on the respective WHO International Reference Standards[481, 482].

Serum free immunoglobulin light chain assay

Serum kappa and lambda free immunoglobulin light chains (FLC) were measured using a latex enhanced immunoassay (The Binding Site, Birmingham, UK) on a Behring BNII autoanalyser (Dade Behring, Marburg, Germany)[483-485].

The assay utilises antibodies directed against FLC epitopes that are hidden in whole immunoglobulin molecules, and has a sensitivity of < 5 mg/l. This compares with typical detection limits of 150-500 mg/l by immunofixation, and 500-2000 mg/l by electrophoresis. The reference range was established using 100 healthy blood donor sera in which the mean concentrations of polyclonal free kappa and free lambda light chains were respectively, 11.38 mg/l (95% confidence intervals, 7.41-16.77 mg/l) and 17.36 mg/l (95% confidence intervals, 8.91-29.87 mg/l), with a mean kappa to lambda ratio of 0.7 (95% confidence intervals, 0.37-0.95). Monoclonal FLC were identified as values for kappa or lambda that exceeded the respective reference ranges and produced an abnormal kappa to lambda ratio.

Interleukin 6

IL-6 values were measured by commercial enzyme immunoassay (Biosource International, 542 Flynn Road, Camarillo CA 93012, USA) with a lower detection limit of 10 ng/l.

GENE SEQUENCING

Genomic DNA was isolated by a rapid method[486] from frozen whole blood taken into EDTA and solubilised in 10 mM Tris, pH 7.5/1 mM EDTA. The coding regions of the transthyretin, apolipoprotein AI, fibrinogen A α-chain (part of exon 5), lysozyme (exon 2) and *MEFV* genes were amplified by the polymerase chain reaction (PCR) using *taq* polymerase (Amplitaq, Perkin Elmer Cetus). For

exon 1 of *TTR* the primers were the intron sequences (5' to 3'); CAGCAGGTTTGCAGTCA-GAT and GGTACCCTTGCCCTAGTAAT; for exon 2; CAATTTTGTTAACTTC-TCAGC and CAGATGATGTGAGCCTCTCTC; for exon 3, CCTCCATGCGTAA-CTTAATCC and TAGGACATTTCTGTGGTAAC; and for exon 4, TGGTGGAA-ATGGATCTGTCTG and TGGAAGGGACAATAAG- GGAAT. For apolipoprotein AI three fragments making up the complete coding region were amplified with the following primers: 5'-CACCCTCAGGGAGCCAGGCTCGG (5' end) and 5'-TAGGTGAGGTGC GTCTGG (3' end) (255 base pair fragment of exon 3); 5'-CAGCCCTCAACCCTTCTGTCTCACC (5' end) and 5'-AGATGCG-TGCGCAGCGCGTCCACA (3' end (391 base pair fragment of exon 4); and 5'-AACGTTTATTCTGAGCACCGGGAAG (primer 47, 3' end) (371-base pair fragment of exon 4). Primers were designed to amplify the portion of the fibrinogen A α-chain gene flanking the coding region for the peptide fragment that had previously been identified as the fibril subunit in hereditary fibrinogen A α-chain amyloidosis[89]. The primers were: TGATGACTGCCTTCTTCGA (nucleotides 4817-4837) and CTCATCTGCTTTTATAGCT (nucleotides 5047-5093), which amplified a fragment of 277 base pairs (GenBank accession no. M64982). Haplotype analysis of the fibrinogen α-chain gene utilised the fragment of the gene encoding both the Glu526Val mutation and a polymorphic repeat sequence upstream from it[487] these were amplified by PCR using primers, ATCGGCTTCACTTCCGGC, and CCATAGGTTTTGAACTCACAG, and the Boehringer Mannheim PCR Expand system (Roche, East Sussex, UK). The following primers located in the flanking introns were used to amplify exon 2 of

the lysozyme gene: 5'-AGTACTTAG-TGTTGCGTTT and 5'-ACCAGATTGGTCAAATATTAG. *MEFV* exon 10 was amplified with 5'-TAATACGACTCACTATAGGGCAGAAGAACTACCCT-GTCCC and 5'-ATTTAGGTGACACTATAGAAAGAAGCAGGAAGAG-AGATGC, exon 2 (5' end) with 5'-ATCATTTTGCATC TGGTTGTCCTTCC and 5'-GAGGCTTGCCCTGCGCG, exon 5 5'-TATCGCCTCCTGCTCTGGAATC and 5'-CACTGTGGGTCACCAAGACCAAG and exon 3 5'-TGAGAACTCGCAC-ATCTCAGGC and 5'-GTGTGTCCAAGTGCCTGGCAG.

Cycling conditions were a denaturing step of 94°C for 5 min, then 10 cycles of 94°C for 1 min, 58°C for 30 sec, and 68°C for 2 min, then 10 cycles of 94°C for 1 min, 58°C for 30 sec, and 68°C for 3 min, then 10 cycles of 94°C for 1 min, 58°C for 30 sec, and 68°C for 4 min. This was followed by an elongation step of 68°C for 7 min and PCR products were sequenced using ABI BigDye V3 according to the manufacturer's instructions (AB Applied Biosystems, Warrington, UK). The following sequencing primers were used; *TTR* exon 2 AAGAATAAATCCCT-TTCACTCTG, exon 3 GGTGTATTACTTTGCCATGCC, exon 4 CATCTGTCAC-GTTTTTCGGG, *apoAI* was sequenced using the same primers as for the PCR, the fibrinogen A alpha chain geneCTCATCTGCCATTTTATAGCT, *MEFV* exon 10 5'-AGTGGGAGAGGCTGCCTG, *MEFV* exon2 5'-ATATTCCACACAAGAA-AACGGC, *MEFV* exon 5 5'-GTCCACCCAGCACAGACC and *MEFV* exon 3 5'-CTTTGTGAACCTCTGTGTAAGC.

STATISTICAL ANALYSIS

Statistical tests were performed using SPSS for windows version 10 (SPSS Inc.

Chicago, Illinois).

Chapter Three

FREQUENCY OF HEREDITARY SYSTEMIC AMYLOIDOSIS MASQUERADING AS IMMUNOGLOBULIN LIGHT CHAIN, AL, (PRIMARY) AMYLOIDOSIS

INTRODUCTION

Systemic amyloidosis is the diagnosis in 2.5 per cent of native renal biopsies[488], and is the cause of death in more than 1 in 1500 people in the UK. Acquired monoclonal immunoglobulin light chain (AL) amyloid, formerly known as primary amyloidosis, is the commonest form of systemic amyloidosis, and can respond to chemotherapy[378, 379, 489, 490]. Demonstration of a monoclonal gammopathy supports the diagnosis of AL amyloidosis but may just be an incidental finding and thus does not confirm it. The chief differential diagnosis of AL amyloidosis is reactive systemic AA amyloidosis, but this is always a complication of chronic inflammation and AA deposits can usually be identified immunohistochemically[491]. The only other differential diagnosis is hereditary systemic amyloidosis, in which the amyloid fibrils are usually derived from genetic variants of transthyretin[492], apolipoprotein AI[90, 181], lysozyme[29] or fibrinogen A α-chain[89]. However these autosomal dominant conditions are generally not considered in the absence of a family history and have been thought to be extremely rare.

This is an important issue as the diagnosis of hereditary amyloidosis has major implications for prognosis, genetic counselling and management, which may include orthotopic liver transplantation to correct the underlying metabolic defect. Although most reported mutations causing hereditary amyloidosis display high penetrance, amyloidogenic mutations have occasionally been identified in asymptomatic elderly individuals, and population and haplotype studies have raised the possibility that two particular mutations associated with hereditary amyloidosis, transthyretin Val30Met and fibrinogen A α-chain Glu526Val respectively, may exist more widely[108]. This is the first systematic genetic study of patients with apparent sporadic systemic AL amyloidosis, and demonstrates, surprisingly, that nearly 10 per cent of these cases in fact had hereditary amyloidosis.

PATIENTS

The genes for transthyretin, apolipoprotein AI, lysozyme and fibrinogen A α-chain were studied in all of the 450 patients referred over a 3 year period to the end of 2001 to the UK National Amyloidosis Centre with biopsy proven amyloidosis and a presumptive diagnosis of systemic AL amyloidosis. None had a family history of amyloid or renal disease, and the amyloid was shown immunohistochemically not to be AA type. Monoclonal gammopathy was sought in each case by serum and urine electrophoresis and immunofixation, and was identified in 80 per cent of patients, a proportion comparable with other published AL amyloidosis series[11]. Full clinical assessment of all patients included Doppler echocardiography and ^{123}I serum amyloid P component (SAP)

scintigraphy. Comprehensive immunohistochemical staining for all amyloid fibril proteins was performed on amyloid containing tissue from all patients in whom potentially amyloidogenic mutations were identified. Newly identified mutations in genes for amyloidogenic proteins were sought in 50 anonymous controls from the general British population.

RESULTS

Gene sequencing

Twenty-one patients (4.7 per cent of this series) were heterozygous for a point mutation in the fibrinogen A α-chain gene, encoding the substitution of valine for glutamic acid at codon 526. This mutation was not present in 50 healthy British Caucasian controls. The diagnosis of fibrinogen A α–chain related amyloidosis was supported by the demonstration of a distinctive, relatively homogeneous phenotype shared by all the Glu526Val heterozygotes.

Twenty-one patients were heterozygous for point mutations in the transthyretin gene (Table 3.1), three of which have not been described previously. These patients all presented with cardiac amyloidosis, and variable degrees of autonomic and peripheral neuropathy, but SAP scintigraphy did not demonstrate any hepatic or bone amyloid deposits, both of which are common features in AL amyloidosis but do not occur in transthyretin associated amyloidosis. However, none of the cases had any relevant family history.

Figure 3.1 Partial DNA sequence of the fibrinogen α-chain gene. The sense and antisense strands from a normal control and the patient are shown. The mutation which results in a substitution of GTG (valine) for GAG (glutamic acid) at codon 526 is arrowed.

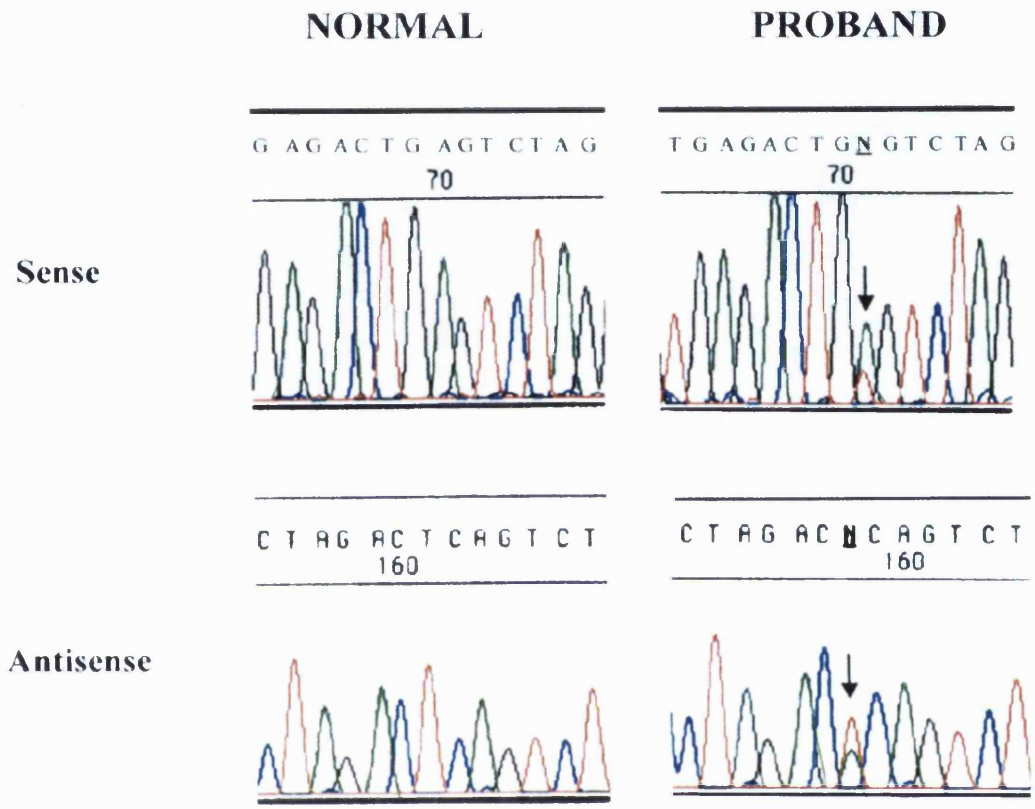


Table 3.1 Clinical features of transthyretin, lysozyme and apolipoprotein AI associated amyloidosis

Variant	Age at presentation	Sex	Predominant clinical features	Ethnic origin	Visceral amyloid involvement by echocardiography and ^{123}I-SAP scintigraphy
Transthyretin					
Val30Met	62	M	Neuropathy	Irish	Cardiac & renal
Val30Met	62	M	Vitreous & neuropathy	English	Cardiac & spleen
Phe33Val	39	M	Vitreous & neuropathy	English	Cardiac, spleen & renal
Phe33Val	60	F	Vitreous & neuropathy	English	Cardiac, spleen & renal
Phe33Leu	57	M	Cardiomyopathy & neuropathy	Polish	Cardiac & renal
Asp38Val	58	M	Neuropathy	Ghana	Cardiac & spleen
Gly47Glu	45	M	Cardiomyopathy & neuropathy	English	Cardiac, spleen & renal
Thr60Ala	54	M	Cardiomyopathy & neuropathy	Scottish	Cardiac & renal
Thr60Ala	65	M	Neuropathy	English	Cardiac & spleen
Thr60Ala	73	F	Cardiomyopathy	Irish	Cardiac, spleen & renal
Thr60Ala	67	M	Neuropathy	Irish	Cardiac
Thr60Ala	63	M	Cardiomyopathy & neuropathy	Irish	Cardiac
Thr60Ala	67	M	Cardiomyopathy & neuropathy	English	Cardiac & renal
Thr60Ala	68	M	Cardiomyopathy	English	Cardiac
Thr60Ala	64	M	Cardiomyopathy & neuropathy	Irish	Cardiac & spleen
Ala120Ser	62	M	Cardiomyopathy & neuropathy	Afro-Caribbean	Cardiac & spleen
Val122Ile	74	M	Cardiomyopathy	Afro-Caribbean	Cardiac
Val122Ile	72	M	Cardiomyopathy	Afro-Caribbean	Cardiac
Val122Ile	75	F	Cardiomyopathy	Afro-Caribbean	Cardiac
Val122Ile	80	M	Cardiomyopathy	Afro-Caribbean	Cardiac
Val122Ile	63	F	Cardiomyopathy & neuropathy	Afro-Caribbean	Cardiac
Lysozyme					
Asp67His	58	F	Proteinuria	English	Spleen & renal
Apolipoprotein A1					
Gly26Arg	28	M	Renal failure	Irish	Liver, spleen, renal
Ala175Pro	35	M	Hoarseness and sterility	English	Renal

Two patients who had presented with slowly progressive renal impairment were heterozygous for the known amyloidogenic mutations encoding lysozyme Asp67His and Apolipoprotein AI Gly26Arg respectively. A man who had presented with hoarseness due to laryngeal amyloid, a long history of sterility with testicular amyloid on biopsy and in whom SAP scintigraphy demonstrated renal deposits despite normal renal function, was found to be heterozygous for a novel mutation encoding apolipoprotein AI Ala175Pro.

Histology

Renal biopsies were available in 20 of the patients with fibrinogen A α-chain Glu526Val, and in all cases the deposits stained immunospecifically with anti-fibrinogen antibodies but there was marked variation in the intensity of staining in different individuals. Staining was positive for lysozyme and apolipoprotein AI respectively in the two patients who had mutations in the corresponding genes, and for transthyretin in each of the 12 patients with a transthyretin variant in whom biopsy material was available. However, robust, reproducible, immunospecific staining of amyloid deposits composed of these various genetically variant proteins required extensive optimisation, including trypsin and microwave pre-treatment of tissues.

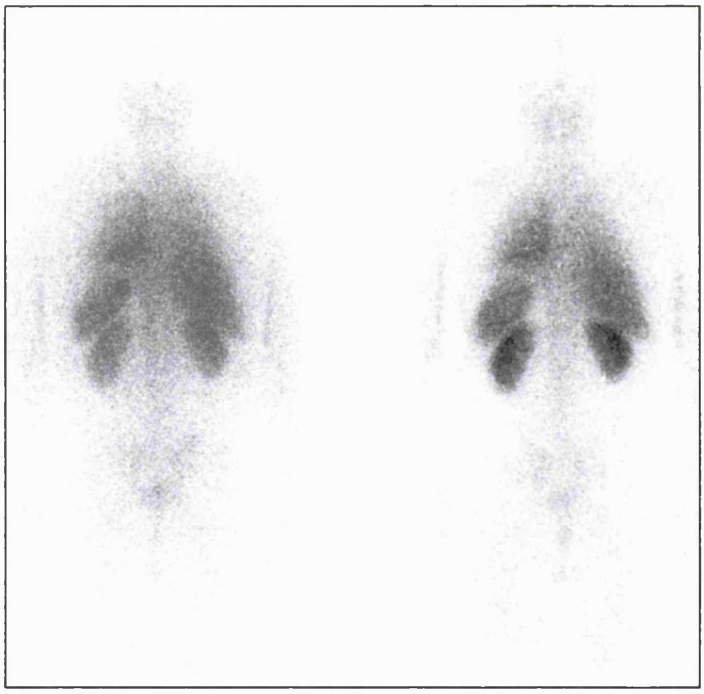
Detection of monoclonal immunoglobulin

Monoclonal immunoglobulins were detected in serum or urine of ten (24 per cent) of the 42 patients with hereditary amyloidosis and 351 (86 per cent) of the 408 patients with AL amyloidosis. However there was no specific immunohistochemical staining of amyloid deposits with antibodies to κ or λ

immunoglobulin light chains in any of the patients with hereditary amyloidosis or, indeed, in 253 (62 per cent) of the 408 patients with AL amyloidosis.

Four of the patients with fibrinogen A α-chain gene mutations had low level paraproteinaemias. This finding had reinforced the initial misdiagnosis of AL amyloidosis and three of them had already received cytotoxic chemotherapy for this presumed condition. Two patients had been given oral melphalan and prednisolone and one had received high dose melphalan followed by autologous stem cell transplantation but amyloid deposition had progressed despite a complete clonal response (Figure 3.2).

Figure 3.2 Progression of amyloid deposits in a patient with fibrinogen A α-chain Glu526Val associated amyloidosis and an incidental monoclonal gammopathy. Serial posterior whole body scintigraphic images following intravenous injection of ^{123}I-human SAP in a 48 year old man with hereditary amyloidosis associated with fibrinogen A α-chain Glu526Val, who was found to have asymptomatic proteinuria. Both parents were alive and well aged over 80 years. He was thought to have AL amyloidosis and received high dose chemotherapy with autologous stem cell rescue with a complete clonal response. The scan at diagnosis (left) shows modest abnormal uptake into renal amyloid deposits. This has increased significantly at follow-up 3 years later (right). The remainder of the image represents the normal distribution of tracer throughout the blood pool.



DISCUSSION

The frequent identification of hereditary amyloidosis in this series of patients apparently suffering from sporadic systemic AL amyloidosis, has major implications for the prognosis, investigation and treatment of such cases. Although hereditary amyloidosis is caused by mutations in single genes, the low penetrance of these mutations observed here indicates that other factors must determine clinical expression. Indeed, the absence of a family history is apparently typical of most patients with fibrinogen A α-chain Glu526Val amyloidosis, which was the commonest form of hereditary amyloid identified in this study. These findings have led to the introduction of DNA analysis into the routine evaluation of patients with systemic amyloidosis, and have already prevented the inappropriate administration of chemotherapy to numerous patients with supposed AL disease, and have enabled potentially curative liver transplants in four patients with previously unsuspected hereditary amyloidosis.

The clinical manifestations of systemic AL amyloidosis are extremely heterogeneous. Although features such as macroglossia or bone involvement are virtually pathognomonic, some of the most characteristic patterns of organ involvement are indistinguishable from those seen among patients with familial amyloid polyneuropathy and hereditary non-neuropathic systemic amyloidosis. All patients with AL amyloidosis have an underlying clonal B cell dyscrasia, but this is not always detectable, and, conversely, subtle monoclonal gammopathies are not infrequent in the general population[11, 493]. The demonstration of a paraprotein may therefore be gravely misleading in a patient with amyloidosis

and it is essential to positively identify the actual amyloid fibril protein whenever possible. The most accessible procedure is immunohistochemical staining of amyloid-containing tissue sections. However, the specificity of any immunostaining must be established by its abolition when the respective antibody has been pre-absorbed with its cognate antigen. Reliable results also require the inclusion in each run of positive and negative control tissues. Furthermore although immunohistochemistry usually yields definitive results in AA amyloidosis, it is frequently not diagnostic with AL deposits[28, 215]. In the present series, AL fibrils were positively identified by immunohistochemical staining in only 32 per cent of the patients who were finally diagnosed as having AL amyloidosis. This reflects the failure of most anti-light chain antibodies to recognise light chain fragments in the abnormal cross-β amyloid fibril conformation, and the problem is exacerbated by the universal presence of non-amyloid background staining due to the ubiquitous presence of normal immunoglobulins in the tissues. Specific fixation procedures or use of unfixed fresh frozen tissue may yield better results, but optimally processed material is often not available following routine biopsy procedures. Expertise in the immunohistochemical typing of hereditary amyloid is restricted, and the problems relating to antibody reactivity and non-amyloid background staining exist for all the relevant fibril proteins. Although the archive biopsy material in this study had not been uniformly processed, extensive optimisation eventually provided immunohistochemical confirmation of the DNA findings in each case.

Hereditary amyloidosis is caused by deposition of genetically variant proteins as amyloid fibrils, and these diseases are inherited in an autosomal dominant

manner. By far the commonest type of hereditary amyloidosis is caused by transthyretin variants, and usually presents as familial amyloid polyneuropathy. This is characterised by progressive peripheral and autonomic neuropathy, and by varying degrees of visceral involvement, especially affecting the heart. Major foci of familial amyloid polyneuropathy exist in Portugal, Japan, and Sweden, but families with the disease have been reported in most ethnic groups throughout the world. More than 80 mutations in the gene for transthyretin are known to cause hereditary amyloidosis[95], and three hitherto unreported amyloidogenic variants, transthyretin Phe33Val, Asp38Val and Ala120Ser, were identified in this series. Transthyretin Val122Ile was detected in five individuals, one of whom had amyloid neuropathy and cardiomyopathy. This transthyretin variant occurs in four per cent of African-Americans[494], in whom it is usually silent or associated with isolated late-onset isolated cardiac amyloidosis. The transthyretin Thr60Ala variant is also typically associated with late onset, decreasing the likelihood of a relevant family history. This variant is well recognised in Western Ireland and four of the eight patients with this mutation in the present series were of Irish ancestry.

Hereditary non-neuropathic systemic amyloidosis was first described by Ostertag in 1932[187] and usually presents with renal dysfunction. It is now known to be caused in different kindreds by mutations in the genes encoding lysosyme[29], apolipoprotein AI[90], fibrinogen A α-chain[89], and most recently apolipoprotein AII[182]. Less than 30 affected families have been reported world-wide. Fibrinogen A α-chain variant, Glu526Val, was first reported in 1994[453] and has been found in five families, all with clear autosomal dominant inheritance of

systemic amyloidosis and high penetrance. The present demonstration that this variant exists in the general population but has low penetrance explains the previously enigmatic finding that haplotype studies in four of the families were consistent with a common ancestor[454].

The patients with lysozyme Asp67His and Apolipoprotein AI Gly26Arg amyloidosis developed slowly progressive renal impairment, a phenotype similar to those of previously described kindreds[434, 495]. The patient with apolipoprotein AI Ala175Pro, a newly identified variant, had hoarseness due to laryngeal amyloid deposits, a feature that commonly occurs in localised AL amyloidosis, but which has also been reported in other patients with mutations that disrupt this region of the apolipoprotein AI molecule[438, 441].

The correct identification of amyloid fibril type has major management implications. AL amyloidosis often responds to chemotherapy that suppresses the underlying clonal plasma cell disorder[378-380, 489], but chemotherapy has no role whatsoever in any type of hereditary amyloidosis, is dangerous and should be avoided. In contrast, forms of hereditary amyloidosis in which the variant amyloidogenic protein is synthesised solely by the liver can be effectively treated by orthotopic liver transplantation. This form of 'surgical gene therapy'[420] has been successfully used in many patients with familial amyloid polyneuropathy associated with variant forms of transthyretin, and was also effective in a patient with fibrinogen A α–chain Glu526Val amyloidosis[133]. However, the progression of hereditary amyloidosis caused by variant lysozyme and apolipoprotein AI is

slow in many patients, in whom supportive measures and renal replacement therapy alone are associated with an excellent outcome.

Chapter Four

CHARACTERISATION OF THE PHENOTYPE OF HEREDITARY AMYLOIDOSIS ASSOCIATED WITH VARIANT FIBRINOGEN A ALPHA CHAIN

INTRODUCTION

Fibrinogen A alpha chain was first isolated from amyloid fibrils in 1993[89]. Fibrinogen itself is a multimeric 340 kD circulating glycoprotein made up of two each of an alpha, beta and gamma chain. The alpha chains are the largest of the three at 66 kDa and are involved in cross-linkage of fibrin. The gene is on chromosome 4q and a large number of variants are recognised, mostly either silent or associated with abnormal haemostasis. A total of four separate amyloidogenic mutations have been described in ten unrelated kindreds. All are towards the carboxyl terminal of the gene, clustered in a relatively small portion of exon 5. Two of these mutations appear to be limited to single families. Both are frame shift mutations resulting in premature termination of the protein at codon 548; one a single nucleotide deletion in the third base of codon 524[449] and the other a deletion at codon 522[450]. A single base transversion resulting in the substitution of leucine for arginine at codon 554 has been reported in a Peruvian-Mexican family[89], an African American family[451] and a French kindred[452]. Fibril extraction and protein sequencing have confirmed the amyloidogenicity of two of these mutations by demonstrating that the fibrils are derived from the variant protein[89, 450].

Much the commonest fibrinogen mutation is the substitution of valine for glutamic acid at codon 526. This variant was first described in 1994[453] and was found in five of the ten reported families, all of whom demonstrate clear-cut autosomal dominant family histories. Although affected individuals now inhabit at least three separate continents they are all of Northern European ancestry; British, Irish, German, French and Polish[133, 454]. Haplotype analysis of four families showed that they shared an unusual haplotype suggesting that they descended from a common ancestor[454]. The combination of a shared progenitor and geographically disparate kindreds suggested that this mutation is considerably more prevalent than reported and we have shown that it occurs relatively frequently in the European population and is easily misdiagnosed as acquired AL amyloid[496].

PATIENTS

Thirty-five patients with confirmed fibrinogen A α chain Glu526Val amyloidosis have been fully assessed at The UK National Amyloidosis Centre and two patients with a previously undescribed mutation Gly540Val. An additional 17 Glu526Val heterozygotes have detected following family screening, 13 of whom have undergone assessment at The UK National Amyloidosis Centre.

RESULTS

Fibrinogen A alpha chain Glu526Val

Family history

Of the 35 patients with confirmed amyloid seen in our unit 13 gave strong family histories of renal failure affecting multiple relatives in several generations. These patients came from three separate kindreds none of whom have been previously reported.

The kindred with the largest number of affected individuals is from North Western Germany (Figure 4.1). A total of seven first cousins have variant fibrinogen, amyloid deposition and renal dysfunction varying from low grade proteinuria to end stage renal failure. Six of these are siblings and their father died at the end of the Second World War aged 47 of uncharacterised renal disease. Three of his sisters had renal problems dying aged 76, 64 and 58 years. The middle sister was said to have glomerulonephritis and her son presented at the age of 50 with proteinuria and was subsequently found to have renal amyloid deposition and fibrinogen A α chain Valine526. The family history can be traced back to the cousins' common grandfather who died in the 1930's at the age of 68 also with renal pathology.

In this kindred nine first cousins, all in their 6^{th} or 7^{th} decades, have been fully assessed. Seven were found to be heterozygous for variant fibrinogen A α chain and all of these had scintigraphic evidence of amyloid deposition and pathological levels of proteinuria. Two siblings were on dialysis. One had

presented 11 years earlier at 58 with hypertension, proteinuria and renal impairment. A renal biopsy demonstrated amyloid deposition. At the time this was diagnosed as AA amyloid and attributed to a 'war wound' and he reached end stage renal failure at the age of 63. His sister presented aged 54 also with hypertension, nephrotic range proteinuria and renal impairment. She became dialysis dependent within three years. Their cousin presented in a very similar fashion at the age of 50 and rapidly required haemodialysis. The two members of the kindred with wild type fibrinogen A α chain had normal renal function.

The second family is of Scottish ancestry (Figure 4.2) and five individuals in two generations are known to have renal amyloid deposition. In generation II out of seven siblings four died in their sixties, one of proven amyloid. He presented aged 65 with heavy proteinuria and near end stage renal failure. A biopsy demonstrated renal amyloidosis and he died of ischaemic heart disease after two years on dialysis. Five of his nephews and nieces have subsequently developed proteinuria between the ages of 48 and 61 and four have renal biopsy proven amyloid deposition. Of these one reached ESRF in just under two years and another four have progressively declining renal function. All five have variant fibrinogen and family screening has detected another seven heterozygotes. Four of these are still under 45 years old, considerably younger than the median age at presentation and may develop symptoms later but the other three are aged from 59 to 66 and have no evidence of renal dysfunction.

Family 3 are New Zealanders of English and Scottish ancestry (Figure 4.3). The proband presented at 59 years with proteinuria but a normal creatinine clearance,

Figure 4.1 German kindred with familial renal amyloidosis due to variant fibrinogen A alpha chain Glu526Val

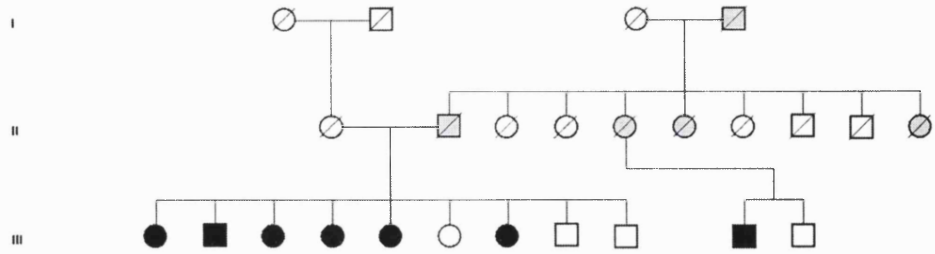
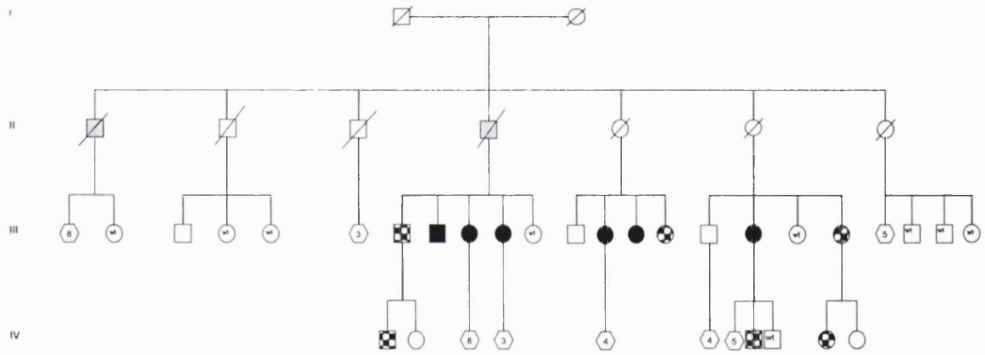


Figure 4.2 Scottish kindred with familial renal amyloidosis due to variant fibrinogen A alpha chain Glu526Val




Died of renal disease


Individual proven to have a fibrinogen A α-chain mutation and amyloid deposition


Fibrinogen A alpha chain Glu526Val but no evidenceof amyloid deposition

a renal biopsy demonstrated amyloid. He was aware of a strong family history of renal disease; his father died at 68 kidney and heart failure and two paternal uncles died in their seventies of renal failure. One had presented with ESRF at the age of 68 years and died eight years later, the other died at 76 years of age. One other brother, an obligate heterozygote died at 64 of a myocardial infarction without other known disease. Eleven of the proband's cousins have been screened and five are heterozygotes for fibrinogen A α chain Glu526Val. Of these two developed proteinuria aged 53 and 56. The latter has an abnormal SAP scan confirming the diagnosis of amyloidosis. Three cousins aged 50 to 54 are asymptomatic heterozygotes as in one individual in the next generation aged 31.

The final kindred is English from the West Midlands (Figure 4.4). Two first cousins were referred with renal amyloidosis resulting in ESRF and the family history only became apparent after they were both found to have fibrinogen amyloidosis and to live in adjacent villages. One cousin presented at the age of 60 with hypertension, heavy proteinuria and advanced renal failure and commenced dialysis ten months later. His father, an obligate heterozygote died aged 78 of pneumonia with no suggestion of renal disease. The other cousin presented at 47 and developed renal failure three years later. His mother who must have carried the variant gene died aged 68 with renal impairment. At the time this had been attributed to longstanding non insulin dependent diabetes mellitus and she never had a renal biopsy. The third child in that generation must also have been a heterozygote and died at 56 of a subarachnoid haemorrhage with no relevant past medical history. Family screening has detected three asymptomatic gene carriers, two in their late 50's and one aged 63. The two former have been comprehensively assessed and have no evidence of amyloid

Figure 4.3 New Zealand kindred with familial renal amyloidosis due to variant fibrinogen A alpha chain Glu526Val

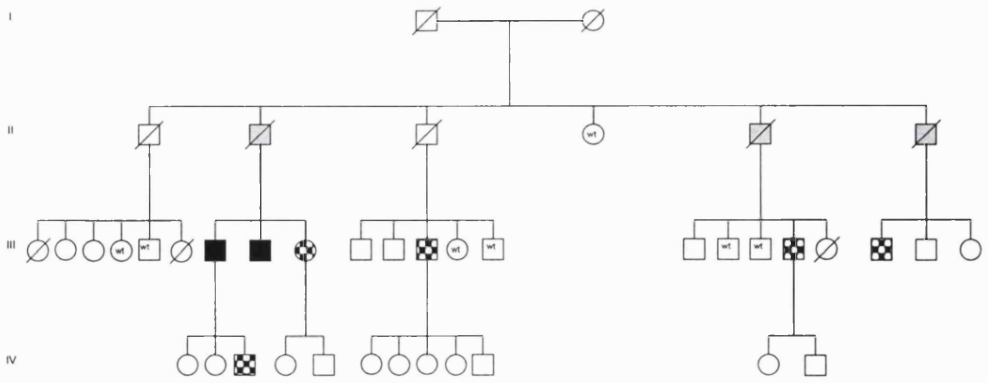
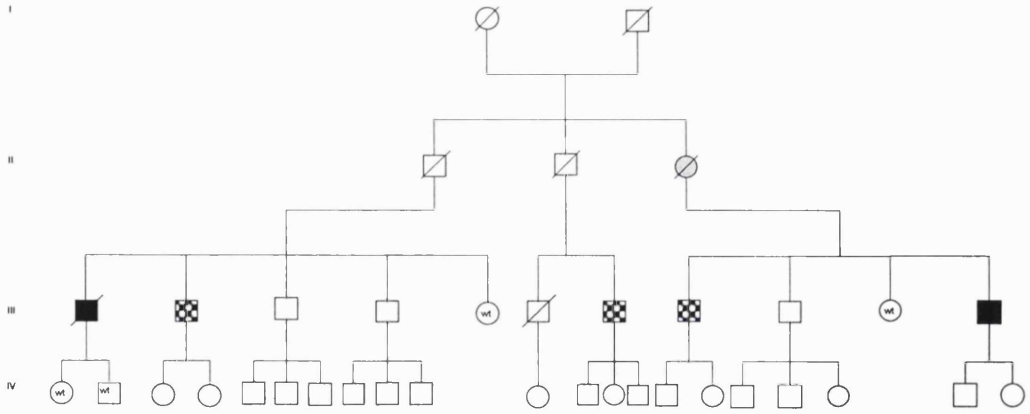


Figure 4.4 English kindred with familial renal amyloidosis due to variant fibrinogen A alpha chain Glu526Val



deposition or renal dysfunction. Two other known cases, who gave no relevant family history originate from the same couple of villages as family three and may be distant relatives, although genealogical studies going back five generations have failed to find a connection.

One patient gave a vague family history with her father dying of possible renal problems aged 66 and two other cases were sisters from the West Midlands. They were diagnosed retrospectively when screening revealed the abnormal gene in one of their daughters, who is in her early 30's and asymptomatic. The elder sister presented with proteinuria in her mid 40's and died ten years later following a colonic perforation after seven years on haemodialysis. The younger was found to have amyloid at post mortem. She was not known to have any renal disease and died aged 67 of aplastic anaemia attributed to carbimazole. One eighty year old patient from North East England reported that her dizygotic twin sister died of renal failure at the age of 76 and two further patients were aware that renal disease had affected first degree family members but knew no further details.

Extensive questioning has failed to produce any relevant family history in the other thirteen patients. It is worth noting that at initial presentation and indeed diagnosis with hereditary amyloid, seventeen, i.e. half the cases were unaware of any family history of renal dysfunction or amyloid.

Clinical phenotype

All of the patients with amyloidosis presented with renal dysfunction and no clinical evidence of extra renal amyloid deposition. Eight patients, approximately a quarter of the patients, had known hypertension, in one case for a decade before her proteinuria was detected. The median age of presentation was 58 years, the youngest patient was in her early 30's and the oldest had presented with proteinuria aged 78 and remains well with impaired but stable renal function at the age of 81 years. Cases from the four kindreds with a high incidence of amyloidosis were not significantly younger at presentation than those with no family history, median age 56 years compared to 58 years. All cases had proteinuria, in the vast majority of cases this was nephrotic range and only one patient had less than 1 g/day of urinary protein loss. Five patients presented with end stage or near end stage renal failure. Out of 35 patients with confirmed fibrinogen A alpha chain amyloidosis and eight of their first degree relatives, one with proven amyloid, the others with renal compromise, 25 are known to have become dialysis dependent or died directly of renal failure. In our cohort of patients seen in a single centre, 19 have reached end stage renal failure with median delay between presentation and dialysis dependence of 2.9 years and a median age of commencing dialysis of 59, youngest 36, oldest 68 years. All individuals who have been followed up for more than 6 years have become dialysis dependent. The median age of the 16 patients still with endogenous renal function is 61.5 and the average follow up since the initial detection of proteinuria is 2.8 years.

Thirty-five patients have had electro and echocardiography performed in a single centre and none has had evidence of cardiac amyloid deposition. There has been no evidence of autonomic neuropathy in any case and although one patient had carpal tunnel syndrome and a three had evidence of mild peripheral neuropathy these have been attributed to long term dialysis and drug side effects. The only clinically relevant extra renal amyloid deposition occurred in one previously reported patient who developed massive hepatomegaly and end stage liver failure due to amyloidosis 13 years after she had started dialysis.
Comprehensive coagulation studies have shown normal coagulation in variant fibrinogen heterozygotes and there has been no clinical evidence of haemorrhagic or thrombotic tendencies. There is some suggestion, particularly in family 3 of an increased risk of ischaemic heart disease. As most patients are hypercholesterolaemic, hypertensive and in advanced renal failure (all independent risk factors for IHD) there is not yet any direct evidence that this is linked to dysfibrinogenaemia.

Five patients with confirmed fibrinogen A alpha chain amyloidosis have died at a median age of 68 years. In only one case was the death a direct complication of amyloidosis or renal failure. This man, who had been dialysis dependent for seven years, died aged 74 of pneumonia and septicaemia following an emergency admission with a splenic haemorrhage which did not require surgery. He had known splenic amyloidosis and presumably a small splenic rupture. Of the other patient deaths, three men were established on dialysis and one woman had advanced renal failure. Of the three men, one died aged 68 of ischaemic heart disease, the second aged 70 of septicaemia and the third aged 64 of severe

Table 4.1 Clinical features of fibrinogen A α Glu526Val amyloidosis

Age at presentation	Sex	Family history	Presenting manifestation	^{123}I-SAP scintigraphy Visceral involvement	Amyloid load	Follow up (years)	ESRF	Age at ESRF
62	F	family 1	proteinuria	spleen	small	1	N	
54	F	family 1	proteinuria	spleen, kidneys & adrenals	small	6	Y	57
70	F	family 1	proteinuria	spleen, kidneys & adrenals	moderate	1	N	
64	F	family 1	hypertension proteinuria	spleen & kidneys	moderate	3	N	
54	F	family 1	hypertension proteinuria	spleen, kidneys & adrenals	small	9	Y	57
58	M	family 1	CRF proteinuria	liver, spleen, kidneys & adrenals	large	11	Y	63
50	M	family 1	ESRF	liver & spleen (renal failure)	large	8	Y	50
48	F	family 2	proteinuria	spleen & kidneys	small	1	N	
61	F	family 2	proteinuria	spleen & kidneys	small	1	N	
52	F	family 2	proteinuria	spleen & kidneys	small	1	N	
50	F	family 2	screening	spleen	small	1	N	
61	F	family 2	proteinuria	spleen, kidneys & adrenals	small	3	N	
59	M	family 3	proteinuria	spleen & kidneys	moderate	3	N	
58	M	family 3	proteinuria	spleen & kidneys	small	2	N	
60	M	family 4	CRF proteinuria	spleen & kidneys	small	3	Y	61
47	M	family 4	proteinuria	spleen & kidneys	small	4	Y	50
50	M	N	proteinuria	spleen & kidneys	small	1	N	
45	M	N	ESRF	liver & spleen (renal failure)	moderate	13	Y	45

				^{123}I-SAP scintigraphy				
Age at presentation	**Sex**	**Family history**	**Presenting manifestation**	**Visceral involvement**	**Amyloid load**	**Follow up (years)**	**ESRF**	**Age at ESRF**
58	F	N	proteinuria	spleen & kidneys	small	2	N	
61	F	N	proteinuria	spleen & kidneys	small	9	Y	63
67	M	N	proteinuria	spleen & kidneys	small	7	Y	68
31	F	father	CRF proteinuria	liver & spleen (renal failure)	large	22	Y	31
62	M	N	proteinuria	spleen & kidneys	moderate	6	Y	67
61	M	N	proteinuria	spleen & kidneys	small	5	Y	65
56	M	N	proteinuria	spleen & kidneys	moderate	6	Y	61
49	M	N	proteinuria	spleen & kidneys	small	4	N	
59	M	N	CRF proteinuria	spleen & kidneys	small	6	Y	64
78	F	sister	CRF proteinuria	spleen & kidneys	small	2	N	
55	M		CRF proteinuria	spleen & kidneys	small	1	Y	56
61	M	father	CRF proteinuria	spleen & kidneys	small	1	N	
71	F	brother	CRF proteinuria	spleen, kidneys, adrenals	moderate	1	N	
69	F	N	CRF proteinuria	spleen & kidneys	small	1	N	
50	M	N	proteinuria	spleen & kidneys	small	3	Y	54
62	M	N	CRF proteinuria	spleen & kidneys	small	8	Y	63
58	M	N	proteinuria	spleen & kidneys	small	1	N	

bullous emphysema, coronary atherosclerosis and tight aortic stenosis. The fourth patient, a woman, died with incipient renal failure at the age of 61 of a previously unsuspected hepatocellular carcinoma.

Solid organ transplantation

Five patients have received renal transplants (Table 4.2). One man without any relevant family history lost his transplant after 2 years due to chronic allograft rejection without biopsy evidence of recurrent amyloidosis. One member of the German family had a transplant aged 54 after 4 years on dialysis. This was functioning normally without scintigraphic evidence of amyloid deposition at 4 year follow up.

Table 4.2 Outcome of solid organ transplantation in the 5 patients who have received grafts

Age at presentation	Sex	Solid organ transplant and outcome
50	M	RTx good function with no evidence of recurrent amyloid deposition after 4 years
45	M	First RTx failed after 5 years due to recurrent amyloid deposition Second RTx failing after 5½ years due to recurrent amyloid deposition
61	F	RTx good function but appearance of subclinical amyloid deposition at 4 year follow up
31	F	First RTx failed after 6 years due to recurrent amyloid deposition Combined OLTx/RTx good graft function and no evidence of amyloid deposition after 3½ years
62	M	RTx failed after 2 years due to chronic rejection with no evidence of amyloid deposition

A third patient, again with apparently sporadic amyloidosis, had received a renal transplant four years prior to referral to our centre. She had excellent graft function and no proteinuria despite the development of scintigraphic evidence of early amyloid deposition at 4½ years of follow up (Figure 4.5). The forth and fifth patients have lost transplants due to amyloid reaccumulation.

Figure 4.5 Asymptomatic progressive amyloid accumulation in a renal transplant. A 54 year old woman received a renal transplant 4 years after presentation with nephrotic syndrome and 2 years after commencing dialysis. 5½ years later she has excellent graft function and no proteinuria despite scintigraphic evidence of amyloid deposition in the graft. Serial anterior whole body scintigraphic images following intravenous injection of ^{123}I-human SAP. The image on the left taken a year after a successful transplant demonstrates a small amount of amyloid in the spleen and normal blood pool signal from her right renal transplant. The middle image taken a year later shows modest amyloid accumulation in the spleen and renal transplant. This is more marked in the image on the right taken 5½ years after her transplant.

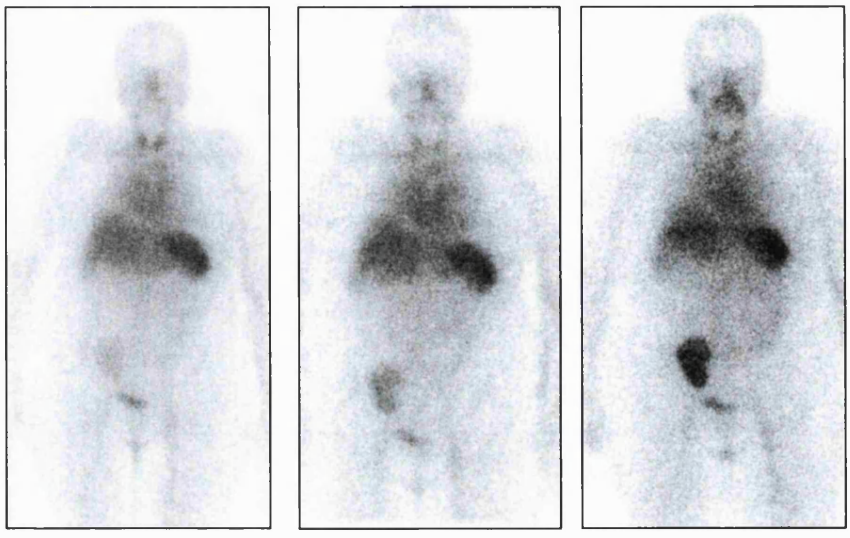
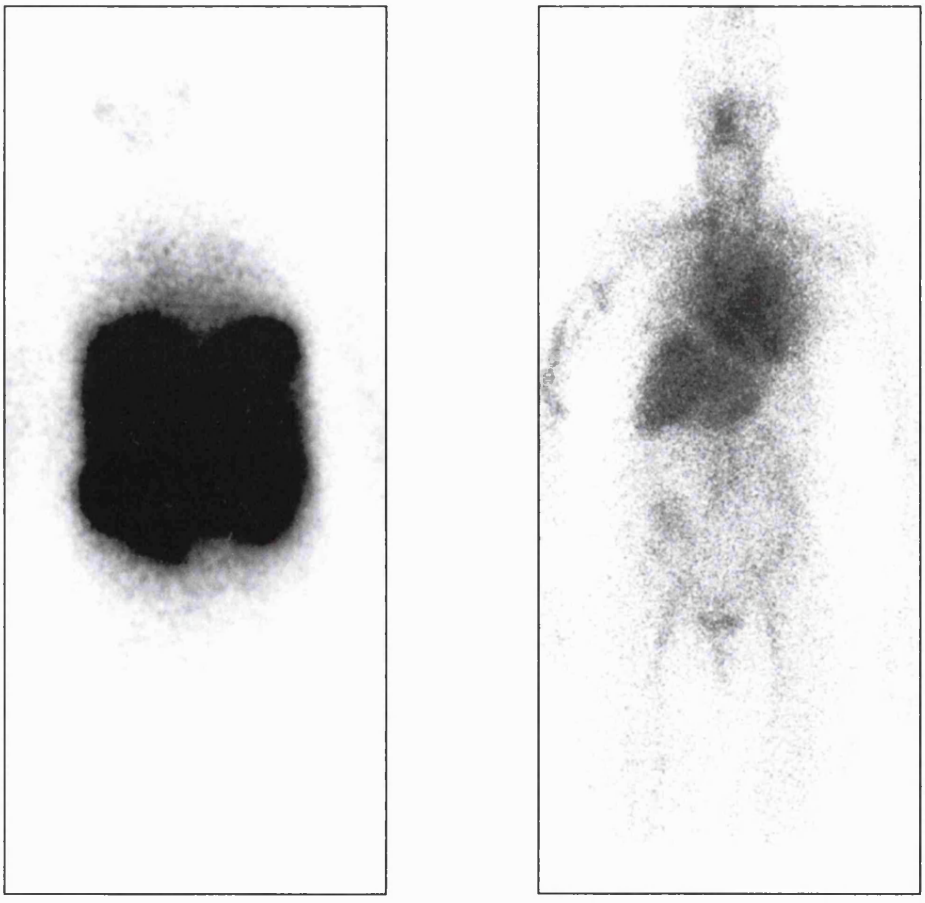


Figure 4.6 Complete regression of fibrinogen A α chain Glu526Val associated amyloidosis following hepatorenal transplantation. Serial anterior whole body scintigraphic images following intravenous injection of ^{123}I-human SAP in a patient with amyloidosis associated with fibrinogen A α chain Glu526Val. Prior to hepatorenal transplantation (left) there was heavy amyloid deposition in an enlarged liver and spleen. No amyloid deposits were identified in a follow-up study obtained 42 months after hepatorenal transplantation (right), which shows only a normal distribution of tracer throughout the blood pool.



The forth was a previously reported patient[133] with an usually aggressive phenotype; she presented in her early thirties with ESRF and a renal transplant failed within 6 years due to amyloid deposition. Progressive hepatic amyloidosis eventually caused liver failure although the function of other organs was well preserved. She therefore received combined hepatorenal transplants and after 5 years has normal organ function with no evidence of systemic amyloid deposition (Figure 4.6). The final patient also presented relatively early with ESRF aged 45. His initial renal transplant failed due to biopsy proven amyloidosis within 5 years and a second graft is now severely compromised with marked scintigraphic evidence of amyloid deposition.

Asymptomatic Fibrinogen A alpha chain Glu526Val heterozygotes detected by family screening

Forty-two family members have been screened and 17 have been found to be heterozygotes for the abnormal fibrinogen allele. None of these 17 carriers have proteinuria or renal impairment, three are known to be hypertensive. Their median age is 50, eight years below the peak age at presentation. Thirteen carriers have been fully assessed including SAP scintigraphy, echocardiograms and laboratory assessment of liver and renal function and coagulation profiles. None had evidence of amyloid deposition or renal compromise. Twelve of them were aged over 50 years and three were over 65 years.

SAP scintigraphy

Forty-eight fibrinogen A alpha chain Glu526Val heterozygotes have had SAP scans of whom 35 had clinical evidence of amyloidosis. Thirteen asymptomatic

carriers had normal scans, all of the other patients had scintigraphic evidence of amyloidosis. Scan appearances were remarkably consistent, all 35 patients had evidence of splenic amyloid deposition and all but two had evidence of significant renal amyloid deposition (Table 4.1). In the German family four of seven individuals had adrenal uptake and two had liver uptake. These two patients had presented in their sixth decade and had been followed up for eight and 11 years respectively. The only other patients who had evidence of hepatic amyloidosis had both become symptomatic fairly young, in their mid thirties and forties, and were assessed 13 and 15 years post presentation.

All but three patients had small or moderate amyloid loads and in those who had serial scans there was little evidence of disease progression from year to year. Only one patient had definite evidence of amyloid progression over three years, this has recently been accompanied by a sudden increase in his proteinuria. As described above in one patient there has been scintigraphic evidence of amyloid accumulation in a renal graft.

Biopsy appearances

Histological examination of the 29 available renal biopsies showed very similar appearances and suggested a number of characteristic features, indeed two patients were initially diagnosed purely on the basis of their histological appearances and the diagnosis was subsequently confirmed by DNA analysis. The glomeruli were strikingly enlarged and the normal architecture was almost entirely obliterated by extensive amyloid deposition (Figure 3A-C). In contrast the vessels and tubular interstitium contained remarkably little amyloid. It has

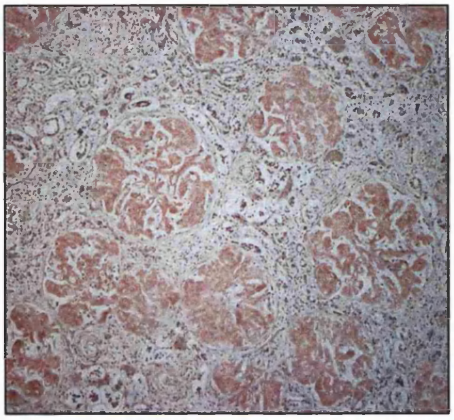
proved possible to demonstrate the presence of fibrinogen within the deposits but the staining is weak and immunohistochemistry remains technically difficult and highly operator dependent. The only renal transplant biopsy examined showed identical appearances.

Haplotype evidence

A DNA microsatellite (TCTT) repeat and two RFLP sites, *Rsal* and *Taql* are recognised with the fibrinogen gene locus. Haplotype analysis of a number of published kindreds with the Glu526Val fibrinogen α-chain variant, has shown that all share the same haplotype, B5-*Rasl*(+) -*Taql* (-)[454]. The allelic frequencies of *Rasl*(+) and *Taql* (-) are 0.759 and 0.73 respectively and the B5 allele is less common with a frequency of 0.15. We have confirmed that a patient who received a liver transplant and multiple members of a large German kindred assessed in our unit also shared this rare haplotype.

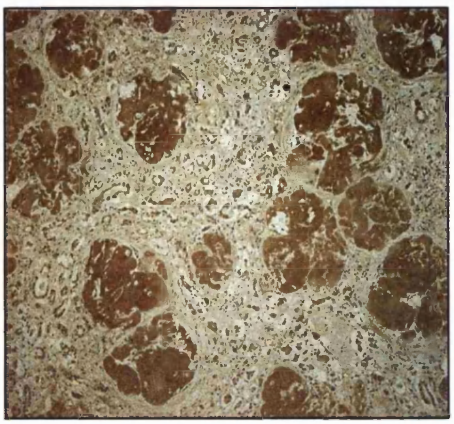
Figure 4.7 Characteristic renal histology in fibrinogen A α–chain Glu526Val amyloidosis; **A.** Congo Red stain. The glomeruli are strikingly enlarged and the normal architecture is almost entirely obliterated by amyloid deposition. In contrast the vessels and tubular interstitium contain remarkably little amyloid. **B.** Same section viewed under cross polarised light demonstrating apple green birefringence. **C.** Immunohistochemical staining with sheep polyclonal anti-fibrinogen antibodies confirming the presence of fibrinogen within the deposits.

A.



B.

C.



Fibrinogen A alpha chain Gly540Val

Two sisters of German origin were referred with biopsy proven renal amyloidosis. The proband was found to have incidental proteinuria at the age of 49 when under investigation for presumed early rheumatoid arthritis. She was normotensive with no organomegaly and investigations demonstrated a normal creatinine clearance and less than a gram of proteinuria. Her father had died at the age of 47 of end stage renal failure and had been known to have renal disease from his mid-forties. He was one of nine children, three of whom had died in childhood and one died in his mid-seventies of cancer. Four siblings remain alive with no evidence of renal disease in their late seventies and early eighties. A subsequent renal biopsy demonstrated extensive amyloid deposition within the glomeruli and was strikingly similar to the appearances noted in fibrinogen A alpha chain Glu526ValGlu amyloidosis. Her sister was found to have proteinuria at the age of 47 as part of family screening and also had amyloidosis on renal biopsy. She was hypertensive with nephrotic range proteinuria. SAP scan appearances were similar in both sisters demonstrating amyloid in the spleen, kidneys and adrenal glands. There were no features of amyloid cardiomyopathy and coagulation studies were entirely normal. Genetic sequencing demonstrated a novel missense mutation in the fibrinogen A alpha chain gene with substitution of valine for glycine at position 540.

DISCUSSION

This is the first description of fibrinogen A alpha chain amyloidosis Glu526Val in a large cohort of patients and the clinical features are much like those of the four previously reported families[453, 454] (Table 4.3). Unlike all of the other known types of hereditary amyloidosis this phenotype demonstrates remarkably variable penetrance. We have described one family with 100% penetrance, two families with high but incomplete penetrance, and one family which appears to be intermediate with three confirmed asymptomatic gene carriers in their late 50's and early 60's. Almost 50% of cases give no family history of renal dysfunction and in the majority careful exploration of the pedigree supports this. We know of at least eight obligate heterozygotes who survived beyond their late 70's and one has normal renal function at the age of 100. None of the 17 heterozygotes picked up following family screening have evidence of renal dysfunction. In some of these this may be because they are one or two decades younger than the median age of presentation but in the seven individuals who are older than 56 years of age this may reflect true asymptomatic carriage. The additional genetic and environmental factors responsible for the observed highly variable penetrance remain to be elucidated.

Table 4.3 Clinical features and pattern of amyloid deposition in the 4 previously described families with fibrinogen A alpha chain valine 526 amyloidosis

Number affected in family	Ancestry	Age at presentation (years)	Clinical features	Biopsy evidence of amyloid deposition	Reference
6	Irish	43-61	Proteinuria Hypertension Renal failure	Kidney Spleen Liver	Uemichi 1994
2	Irish	60, 66	Proteinuria Hypertension Renal failure	Kidney	Uemichi 1994
4	Irish	49-55	Proteinuria Hypertension Renal failure	Kidney Lung	Uemichi 1996
6	Polish	42-58	Proteinuria Hypertension	Kidney Spleen Adrenals	Uemichi 1996

Aside from the variable penetrance, the presentation and the disease course appear unusually constant. This type of amyloidosis presents much later than most other types of hereditary amyloid and displays a remarkable predilection for the spleen and kidneys. Once amyloid deposition has been initiated, progression to renal failure is probably inevitable. We are aware of no patients who still have native renal function more than six years after presentation. Otherwise the disease is relatively benign with excellent survival with renal replacement therapy. Symptomatic liver involvement has occurred in only one case and there is no evidence of cardiac or neurological involvement.

It may be that the variant fibrinogen A alpha chains have a relatively stable configuration and a low propensity populate to the intermediate forms necessary for amyloid genesis. Fibrinogen is present in relatively high circulating concentrations compared with other soluble amyloid precursors and once an initial nidus of amyloid has formed there could be relatively rapid progression with deposition onto a pre-existing amyloid template and presentation with clinical renal disease within a short time frame. This might explain the unusual feature of recurrent amyloid deposition within renal grafts, which although not unique to this type of genetic amyloidosis appears to be much more frequent and destructive than in hereditary apolipoprotein AI or lysozyme related amyloidosis.

The relatively late onset, often absent family history and lack of any pathognomonic clinical features has frequently resulted in diagnostic confusion. Much the commonest type of systemic disease is AL amyloid, in which the fibrils are derived from circulating monoclonal immunoglobulin light chains.

A correct diagnosis is vital as chemotherapy is beneficial only in AL type and carries significant morbidity and mortality[489, 497]. Twenty-two patients who were eventually found to have fibrinogen A alpha chain amyloidosis were initially thought to have AL amyloidosis secondary to an underlying monoclonal gammopathy and three of them had detectable but incidental paraproteins contributing to the misdiagnosis. Three patients received inappropriate chemotherapy, fortunately without serious long-term side effects. In addition as AL amyloidosis is perceived as a disease with a poor long-term prognosis, in which nephrologists have historically been reluctant to consider renal transplantation, six patients on long-term renal replacement were never considered to be suitable candidates for transplantation.

Worldwide experience suggests that the vast majority of patients with hereditary renal amyloidosis do remarkably well with renal replacement and that, despite continued production of the variant protein, recurrent amyloid deposition within renal grafts is slow and often clinically silent. The role of isolated renal transplantation in this particular form of hereditary amyloidosis is not entirely clear although it probably remains the treatment of choice, particularly in older patients with other medical problems. We are now aware of renal graft amyloid reaccumulation within six years in at least four individuals (three reported here and one from the USA) and with increasing experience we may find that this is a common problem. In addition long-term follow up suggests that hepatic amyloid deposition may eventually become clinically relevant. There is limited data on very long term follow up but there is a suggestion that significant hepatic deposits may appear 10 to 20 years after initial renal presentation. Fibrinogen is

synthesised solely by the liver and as there is no evidence that wild-type fibrinogen A α-chain is amyloidogenic, orthotopic liver transplantation is potentially curative as it completely halts the production of the circulating amyloid precursor. This in combination with renal transplantation may be the treatment of choice for individuals with unusually aggressive disease. So far only three individuals have received combined hepatorenal transplants. Two American patients with fibrinogen A α-chain amyloidosis have been recently reported to have had successful combined transplant[498]. The other was a 49 year old woman, previously reported by our unit[133], who demonstrated dramatic amyloid regression following the procedure and after 42 months of follow up continues to have normal organ function and no evidence of recurrent amyloidosis. One patient who has evidence of asymptomatic hepatic amyloid and incipient failure of his second renal transplant is also being considered for the procedure.

Previous haplotype analysis of kindreds with the Glu526Val fibrinogen α-chain variant, including English, Irish, German and Polish families has shown that all share the same rare haplotype and thus may have a common founder[454]. We have confirmed that our patient who received a liver transplant and multiple members of a large German kindred assessed in our unit also shared this haplotype. This is consistent with a relatively common gene and highly variable penetrance and suggests that this condition has been significantly under diagnosed. Indeed this form of hereditary amyloidosis accounts for 6% of the new patients referred to our centre in the past three years with systemic amyloidosis which was neither AA nor TTR in type. In the absence of generic

anti-amyloid therapy it is essential to fully characterise the amyloid fibril type in every patient in order to provide suitable treatment. Misdiagnosis as AL exposes patients to considerable iatrogenic morbidity as well as lessening their chances of appropriate treatment in the form of solid organ transplantation. Consequently DNA analysis is mandatory, regardless of the family history, in all patients with systemic amyloidosis in whom the amyloid type has not been positively confirmed by immunohistochemistry.

Chapter Five

MEASUREMENT OF CIRCULATING FREE IMMUNOGLOBULIN LIGHT CHAINS IN PATIENTS WITH AL AMYLOIDOSIS

INTRODUCTION

As discussed earlier primary AL amyloid is the most common form of systemic amyloidosis, but its diagnosis and treatment remains difficult and unsatisfactory. Although AL fibrils are derived from circulating monoclonal immunoglobulin light chains[158], the underlying clonal plasma cell dyscrasias are often impossible to quantitate[11, 361]. Patients may benefit from cytotoxic chemotherapy although symptomatic improvement and functional recovery of amyloidotic organs is usually slow[117, 375]. Intermediate dose infusional regimens such as vincristine, Adriamycin and dexamethasone (VAD)[499], cyclophosphamide, vincristine, Adriamycin and methylprednisolone (C-VAMP)[500] and intermediate dose melphalan (IDM)[501] have been widely used in the United Kingdom, but the results have not hitherto been analysed systematically.

A circulating whole paraprotein cannot be identified by serum electrophoresis at the time of diagnosis in more than half of all patients with AL amyloidosis[11], and in many others the concentration is so low that quantification is either

imprecise or impossible. Immunofixation is more sensitive, and can detect free monoclonal immunoglobulin light chains in the urine in up to 90% of patients, but results are not quantitative and quality control surveys indicate poor laboratory reliability[363]. Also, urine is a poor medium for testing, since the amount of FLC excreted is strongly influenced by the reabsorptive capacity of the renal tubules. Studies indicate that between 10 and 30 g of FLC can be metabolised per day by the kidneys compared with normal plasma cell production of 0.5-1 g per day[502]. Therefore, large amounts of FLC do not appear in the urine until FLC production is considerably elevated, making urine tests potentially unreliable when the clone is small or FLC synthesis is inefficient. Furthermore, 24-hour urine collections may be difficult to obtain in elderly, frail individuals.

The introduction of a new highly sensitive and quantitative nephelometric immunoassay by Bradwell and his colleges[483-485] has enabled identification of FLC in the serum of virtually all patients with AL amyloidosis. Use of this assay in conjunction with serial serum amyloid P component (SAP) scintigraphy, has allowed us to study the relationships between the abundance of the light chain precursors of the AL amyloid fibril protein, the whole body amyloid load, and the clinical outcome in a series of patients with AL amyloidosis.

METHODS

Patients

Three hundred and forty five patients with systemic AL amyloidosis were studied and 46 patients who had concurrent multiple myeloma were excluded. One hundred and fifty six patients with localised amyloid deposits were also studied. All had biopsy evidence of amyloid deposition in a single site with no evidence of visceral organ dysfunction or systemic amyloid deposition by SAP scintigraphy or echocardiography. Thirty-three patients with MGUS but no evidence of systemic or localised amyloidosis were also studied. A bone marrow examination including immunophenotyping was performed at baseline in all patients. A normal range of FLC results in renal failure was established using 32 patients with dialysis dependent renal failure due to confirmed AA amyloidosis with no detectable M-protein in serum or urine on 3 separate assays.

Of the patients with systemic AL amyloidosis, 168 cases who had been referred since the introduction of the FLC assay, were available for assessment on only a single occasion, at presentation. The remaining 186 patients had initially been evaluated from 1992 onwards and 154 of them had been followed-up on at least one occasion after undergoing infusional chemotherapy in the form of VAD/C-VAMP, IDM or HDM. Recommendations for cytotoxic chemotherapy, favouring infusional approaches, were offered on an individual basis. Diagnosis of AL amyloidosis was confirmed immunohistochemically[28], and when staining for AL amyloid was not definitive, the genes encoding transthyretin, fibrinogen

A α-chain, apolipoprotein AI and lysozyme were sequenced to exclude all known amyloidogenic mutations[503]. Baseline and biannual follow-up evaluations included assessment of monoclonal immunoglobulin production and quantitative whole body ^{123}I-labelled SAP scintigraphy to monitor amyloid load. Serum samples obtained at each visit were stored at minus 30°C in order to prospectively investigate the relationship between the circulating monoclonal immunoglobulin light chain concentration and amyloid fibril deposition.

Chemotherapy

Among the 154 patients in the study, 89 received monthly courses of VAD[499] or C-VAMP[500] on three to six occasions; 26 were given two to six courses of intravenous IDM at 25 mg/m^2 and 39 had HDM. Most patients were treated in local Haematology centres. Eighty-five other patients who had received similar treatments were excluded; 32 because they died within six months of commencing treatment prior to re-assessment (9 VAD/C-VAMP, 7 IDM, 16 HDM) and 53 because they were referred following treatment (30 VAD/C-VAMP, 5 IDM, 18 HDM).

Clinical assessment

Patients underwent comprehensive clinical assessment and biannual review at the UK National Amyloidosis Centre. Monoclonal immunoglobulin was sought by electrophoresis and immunofixation of serum and urine, and quantitative assessment of the distribution and extent of visceral amyloid deposits was monitored by whole body ^{123}I-labelled SAP scintigraphy[217]. Blinded comparisons were made between the scans obtained at baseline and one year

after undergoing chemotherapy. Accumulation of amyloid was defined as an increase in tracer uptake in at least one of the amyloid infiltrated organs compared to the initial scan or involvement of a previously unaffected organ, and regression was defined as a decrease in tracer uptake in a least one affected organ. The amyloid load was defined as steady when the relative amount of tracer in target organs and in the blood-pool background was unchanged between scans. Echocardiography was used to evaluate cardiac amyloid.

Serum free immunoglobulin light chain assay

Serum kappa and lambda FLC were measured using a latex enhanced immunoassay. Monoclonal FLC were identified as values for kappa or lambda that exceeded their respective reference ranges and produced an abnormal kappa to lambda ratio. In each patient the serum concentration of the monoclonal class of FLC six months after undergoing chemotherapy was expressed as a percentage of the pre-treatment value.

Statistical analysis

Patient survival from baseline visit was estimated by Kaplan-Meier analysis and groups were compared by log-rank tests. Cox regression analysis was used to assess the impact on survival of age, initial serum concentration of the monoclonal class of FLC, cardiac involvement, class of FLC and renal function at presentation. The responses to IDM, VAD/C-VAMP and HDM were compared by the Kruskal-Wallis and Fisher's exact tests. Patients were classified according to whether their amyloid load had accumulated, remained

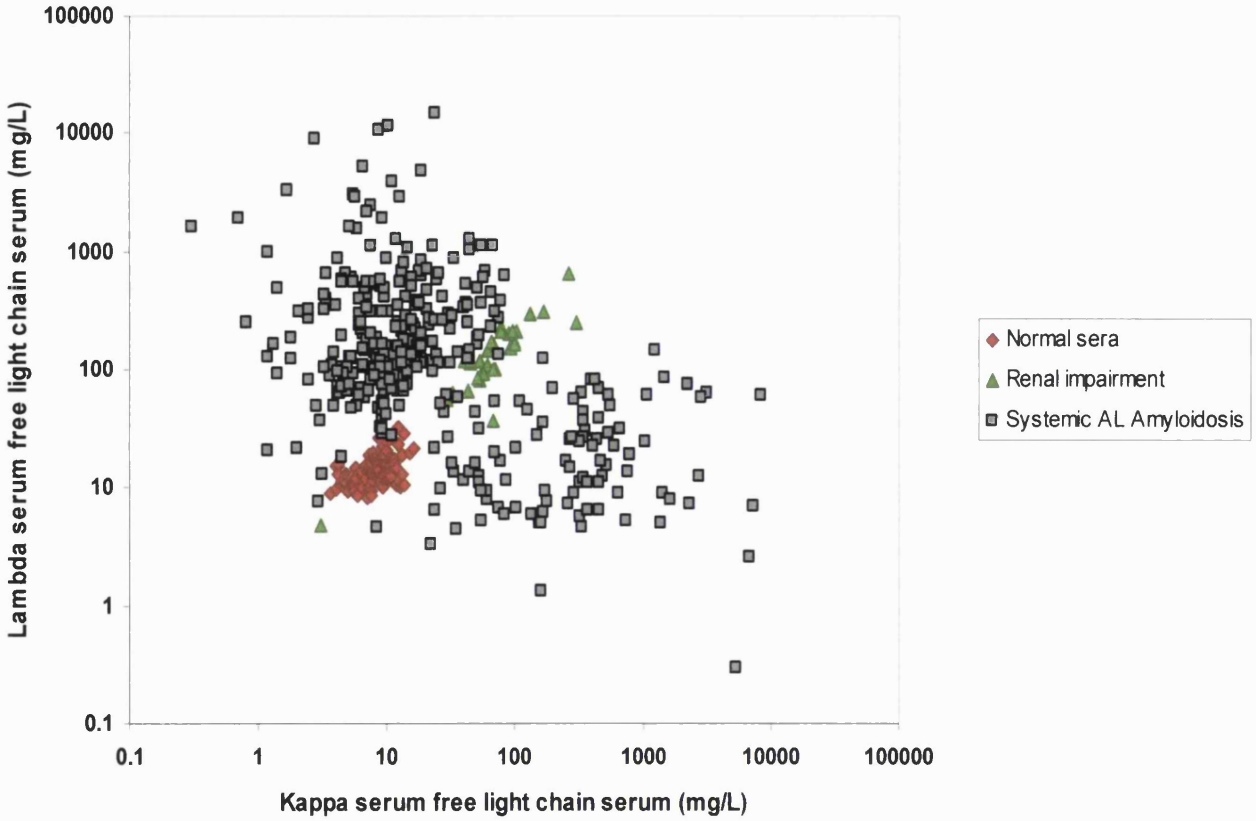
unchanged or diminished between the initial SAP scan and the follow-up scan one year after undergoing chemotherapy. Relationships between changes in amyloid load and the effect of chemotherapy on the serum concentration of the monoclonal class of FLC were sought by the Kruskal-Wallis test.

RESULTS

Detection of excess FLC in patients with systemic AL prior to cytotoxic treatment

Monoclonal immunoglobulin could not be detected at presentation, by electrophoresis or immunofixation in either serum or urine, in 67 of the 345 patients (19%). In a further 72 patients (21%) monoclonal light chains could only be detected qualitatively by immunofixation of serum or urine. A quantifiable circulating monoclonal immunoglobulin was identified in 209 patients (60%), in each case at a concentration of less than 3 g/dl. In contrast, circulating monoclonal FLC was identified by the nephelometric assay in 332 (97%) patients at presentation (Figure 5.1). The kappa or lambda class of monoclonal immunoglobulin demonstrated by the FLC assay in 96 and 237 patients respectively was corroborated by immunofixation or bone marrow studies in each of 270 cases in which these other investigations gave positive results. In most cases the concentration of the monoclonal class of FLC was within the range of 30-500 mg/l, and these values did not correlate with the concentration of whole monoclonal immunoglobulin when this was present.

Figure 5.1 Serum concentration of free kappa and lambda immunoglobulin light chains in 345 patients with systemic AL amyloidosis prior to treatment. The normal ranges derived from 100 healthy blood donor controls and in renal failure, derived from 32 patients with ESRF are marked.



Detection of excess FLC in patients with localised AL amyloidosis

Of the 156 patients, 20 had evidence of a monoclonal gammopathy by electrophoresis or immunofixation of serum or urine. In 19 cases there was evidence of a serum M-protein and three of these were detectable by immunofixation only. An excess of one or other class of FLC was detected in 29 patients (19%), kappa in 18 and lambda in the remaining 11 cases (Table 5.1). Excess FLC were detected in six of the seven patients with localised amyloid deposits in bone and further investigations demonstrated underlying lymphoma in one patient, myeloma in two cases, and two other patients had amyloid in association with a localised plasmacytoma. Localised bowel amyloid was associated with serum and bone marrow evidence of MGUS in three cases and the fourth had smouldering myeloma with 20% plasma cells on bone marrow.

Detection of excess FLC in patients with uncomplicated MGUS

Of the 33 patients with uncomplicated MGUS 20 had kappa and 13 lambda secreting clones. Six patients had associated Bence Jones proteinuria, four kappa and two lambda. Thirteen patients (39%) had an excess of one class of FLC, seven kappa (35% of the kappa MGUS) and six lambda (46% of the lambda MGUS). All of the Bence Jones positive patients had evidence of free monoclonal immunoglobulin light chains in the circulation.

Table 5.1 Tissue distribution of localised amyloid deposits and evidence of a systemic plasma cell dyscrasia by conventional and FLC assays

Site of amyloid deposits	Number of patients	Serum M-protein or Bence-Jones Proteinuria	Kappa or lambda FLC excess
Bone	7	3 (43%)	6 (88%)
Bladder	25	1 (4%)	3 (12%)
Bowel	10	4 (40%)	3 (30%)
Bronchial	13	2 (15%)	1 (8%)
Nodular pulmonary	13	3 (23%)	3 (23%)
Laryngeal	22	0	1 (4.5%)
Nasopharynx	16	1 (6%)	2 (13%)
Skin	18	2 (11%)	2 (11%)
Occular	10	2 (20%)	2 (20%)
Lymph node	16	3 (19%)	5 (31%)
Miscellaneous	6	0	1 (17%)

Definition of a normal range of serum FLC in renal failure

There was a polyclonal increase in both kappa and lambda FLC in renal failure with preservation of the normal kappa to lambda ratio. The mean concentrations of polyclonal free kappa and free lambda light chains in 32 patients with dialysis dependent renal failure were; kappa, 82.9 mg/l (95% confidence intervals, 63.5-102.3 mg/l) and lambda, 160 mg/l (95% confidence intervals, 122.3-198.9 mg/l), with a mean kappa to lambda ratio of 0.56 (95% confidence intervals, 0.47-0.64). The range is marked in green on Figure 5.1.

Serial assays of serum FLC after cytotoxic treatment in patients with systemic AL amyloidosis

Each of the 154 patients who were followed-up after chemotherapy had an excess of one or other class of FLC by nephelometric assay at presentation. The serum concentration of the clonal FLC fell by more than 50% in 96 (62%) of these patients, whose characteristics are shown in Table 5.2. There was no significant difference in FLC response or survival among the patients who had been treated with IDM, VAD/C-VAMP and HDM (Figure 5.2).

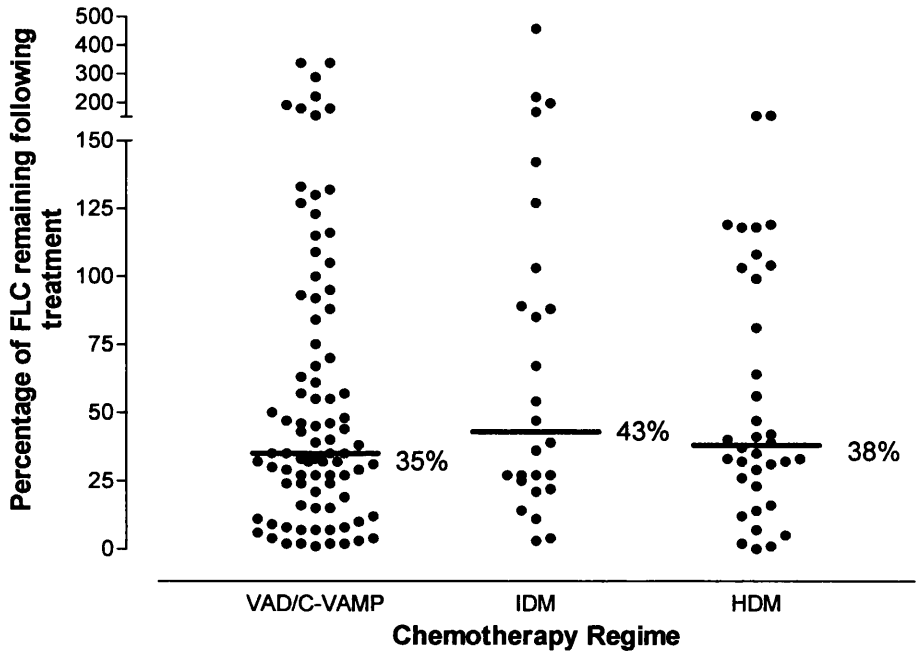


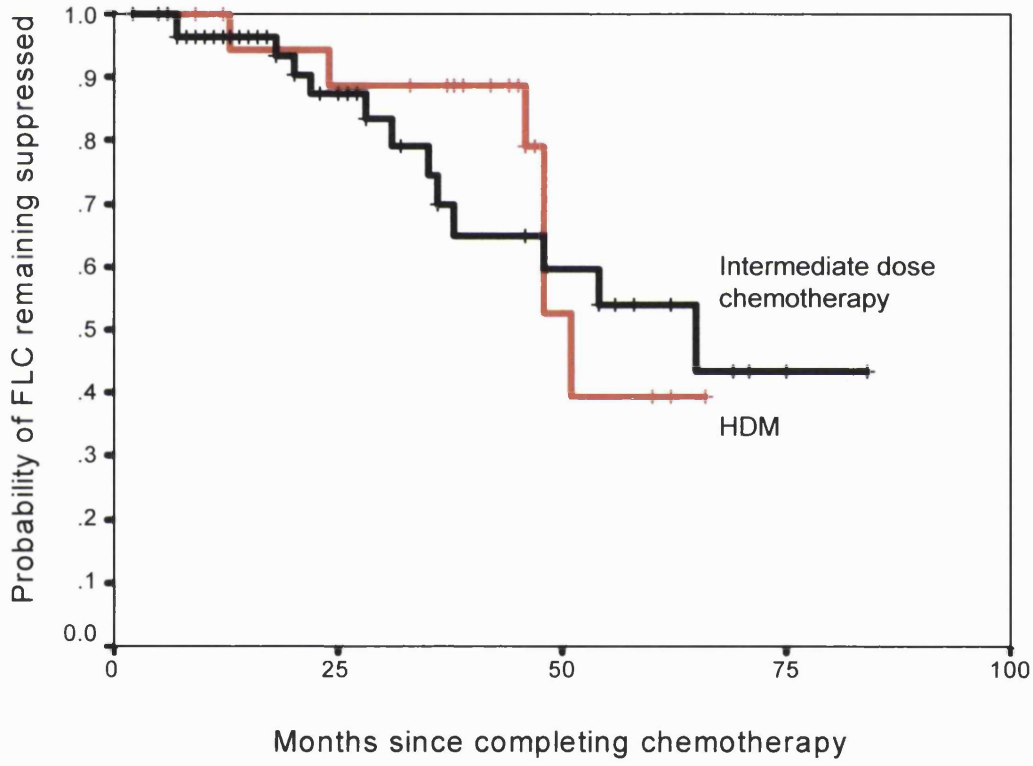
Figure 5.2 Concentration of the amyloidogenic class of free light chain after 6 months of chemotherapy expressed as a percentage of the value immediately before treatment. The median for each group is marked. There was no significant difference between patients who received VAD/C-VAMP (n = 89), IDM (n = 26) and those who completed HDM (n = 39) (Kruskal-Wallis test, $P = 0.547$).

Table 5.2 Characteristics of patients receiving different chemotherapy regimes

Chemotherapy regime	Number of patients	Median age	Echocardiographic features of amyloid cardiomyopathy	Dialysis at time of chemotherapy	Median follow-up post chemotherapy	Deaths	Change in amyloid load by SAP scintigraphy at 12-month follow-up		
							Regression	Stable	Progression
VAD	98	55.5 years (range 29-77)	36 (37%)	10 (10%)	21 months (range 0-85)	42 (43%)	34 (41%)	25 (30%)	24 (29%)
IDM	33	64 years (range 46-77)	17 (51%)	5 (15%)	8 months (range 0-62)	13 (39%)	9 (39%)	6 (23%)	8 (35%)
HDM	55	54 years (range 32-67)	20 (36%)	3 (5%)	20 months (range 0-78)	24 (44%)	16 (55%)	7 (24%)	6 (21%)

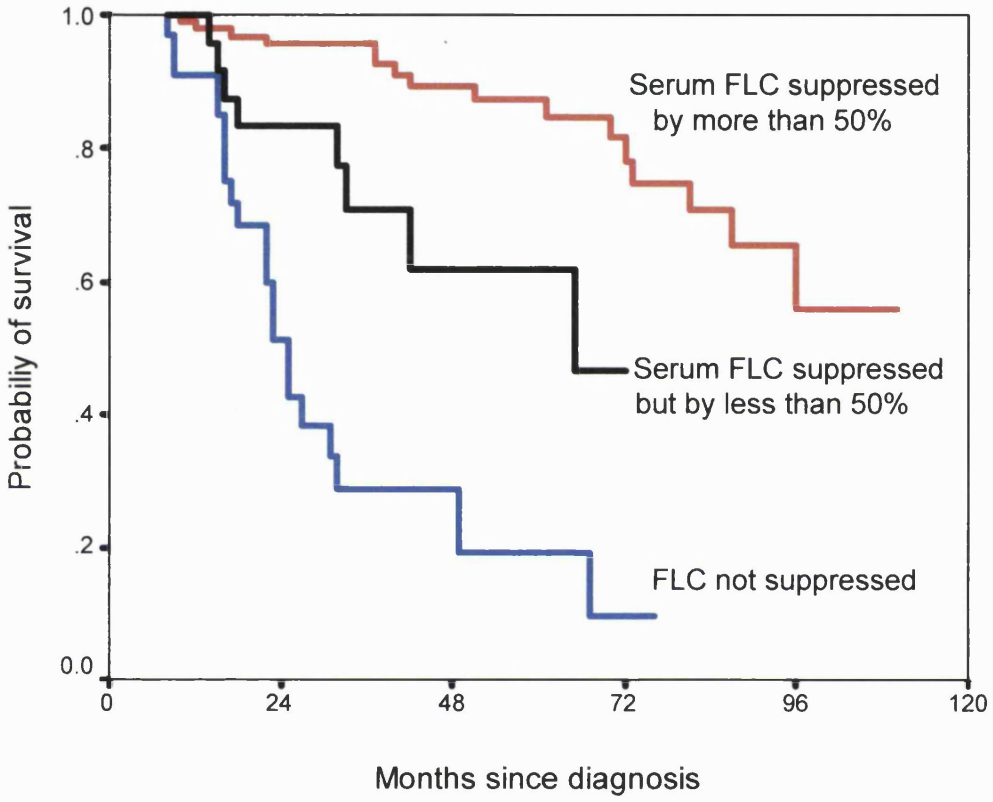
The probability of serum FLC values remaining below 50% of pre-treatment values after five years was also the same in the HDM and infusional (VAD/C-VAMP or IDM) chemotherapy groups.

Figure 5.3 Kaplan-Meier estimate of the probability of the concentration of the amyloidogenic class of free light chain remaining below 50% of the pre-treatment values in 96 patients who had responded to intermediate dose chemotherapy (VAD, C-VAMP or IDM) or HDM. There was no significant difference between the groups (p=0.78).



Survival at five years was 85% among patients whose FLC concentration fell by more than half, and 39% among patients whose FLC concentration remained above this value ($P < 0.001$)

Figure 5.4 Kaplan-Meier estimate of survival in 154 patients with systemic AL amyloidosis following initial assessment. Ninety-six patients (62%) had a greater than 50% fall in the concentration of the amyloidogenic class of free light chains. Their survival was significantly greater than among the 24 patients whose free light chain concentration fell by less than 50% after completing chemotherapy and the 34 whose plasma cell clones were refractory to chemotherapy ($P < 0.0001$).



Median survival was 25 months in the 34 patients whose FLC showed no response at all ($P < 0.0001$). Suppression of the amyloidogenic FLC concentration by greater than 75% or 90% or into the normal polyclonal range was not associated with better survival than a fall of more than just 50%. Using Cox regression analysis the relative risk of death was 10 fold higher in patients whose FLC values were suppressed by less than 50% ($P < 0.0001$, 95% confidence intervals: 4.15-24.12). In this post-treatment study period survival was not influenced by age, renal failure, or by class or initial concentration of

serum FLC, but there was a trend towards increased mortality in patients with echocardiographic evidence of amyloid.

Follow-up SAP scintigraphy one year after baseline in the 135 survivors showed accumulation of amyloid in 38 patients (28%), no change in 37 (27%) and regression in 60 (44%) cases. Changes in the amyloid load were associated with changes in serum FLC concentration ($P < 0.0001$), and did not differ significantly between the three treatment groups.

Figure 5.5 Relationship between change in amyloid load at 12-month assessment and percentage change in serum concentration of the amyloidogenic class of free light chains following chemotherapy in the 135 surviving patients. The median percentage change in free light chain concentration in each group is indicated (Kruskal-Wallis test, $P < 0.0001$).

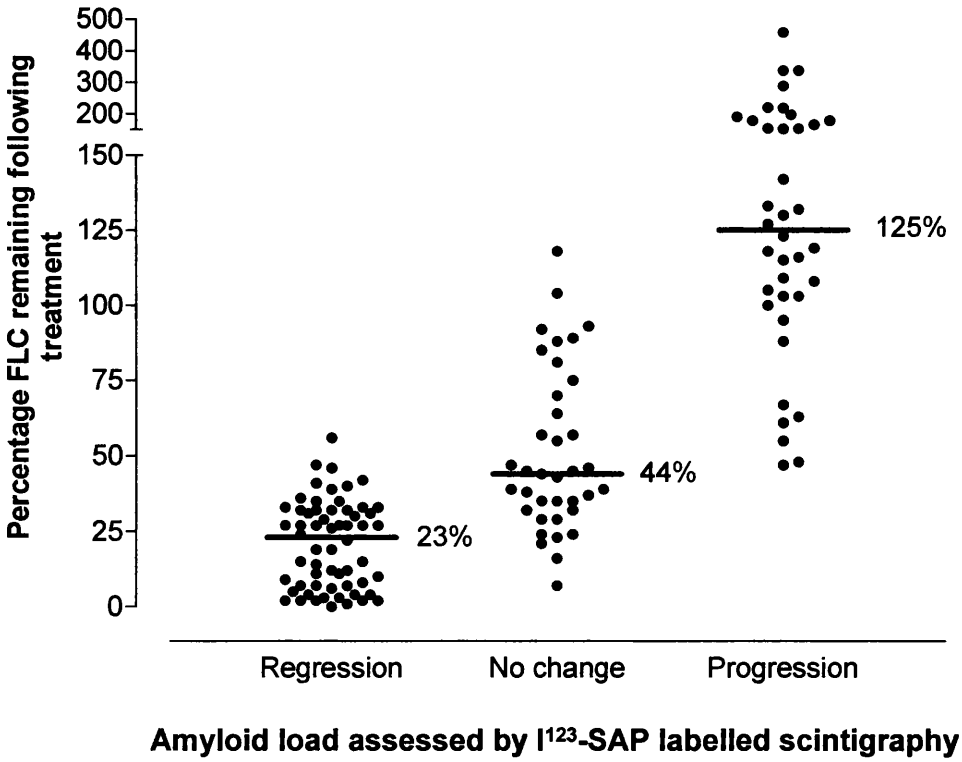
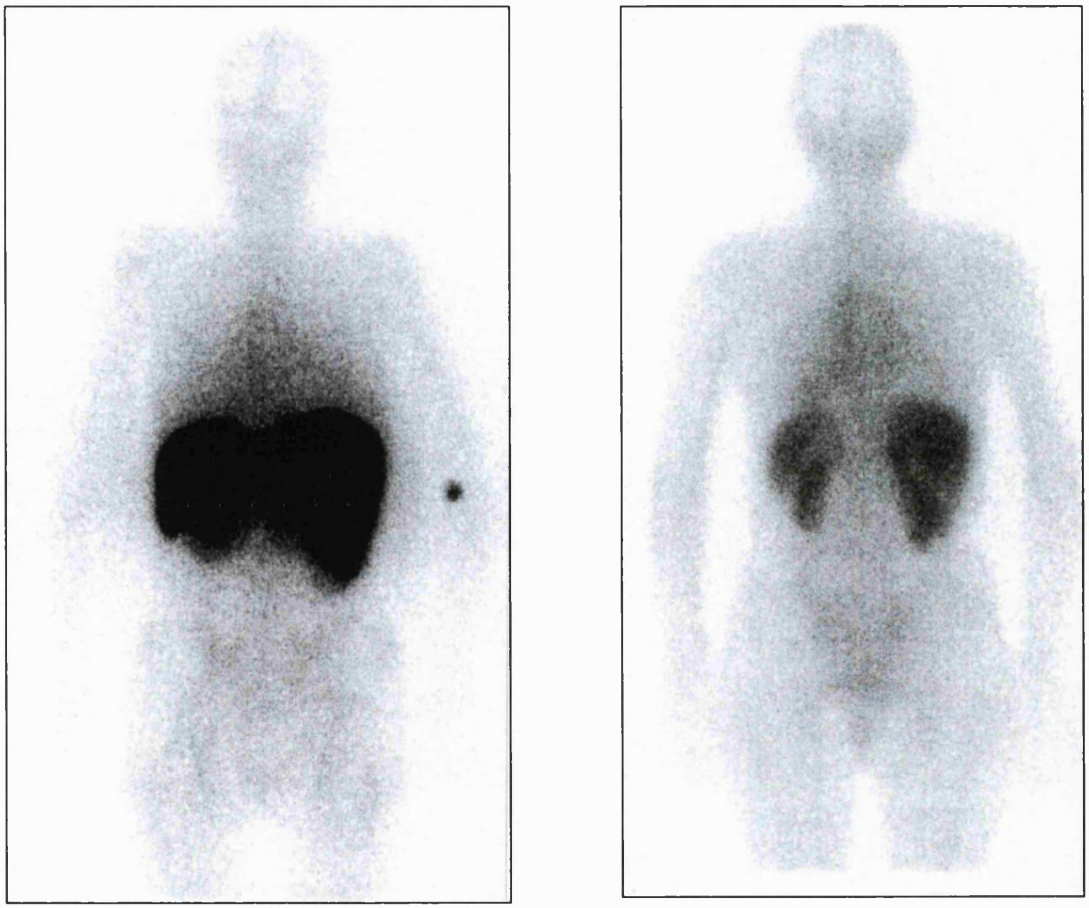


Figure 5.6 Radiolabelled SAP scintigraphy. Posterior images of a 52-year old woman with systemic AL kappa amyloidosis, before (left) and one year after (right) high dose melphalan chemotherapy, demonstrating regression of amyloid deposits in the liver, spleen and bone marrow. The serum concentration of free kappa light chains had fallen from 551 mg/l to 52 mg/l and in the 12 months between the scans she regained 10 kg.



DISCUSSION

AL amyloid deposits are derived from circulating monoclonal immunoglobulin light chains, but it has not previously been possible to investigate the relationship between abundance of the AL amyloid precursor protein and its fibril product. The combination of serum FLC quantification and serial SAP scintigraphy indicates that AL amyloid deposits exist in a state of turnover, and that reduction in amyloidogenic FLC concentration by more than 50% is frequently associated with regression of amyloid and prolonged survival. This demonstration of the clear relationship between the serum concentration of FLC and clinical outcome provides a robust objective criterion for monitoring chemotherapy in AL amyloidosis.

Conventional techniques were unable to identify monoclonal immunoglobulin in serum or urine in one fifth of cases in this series of patients with systemic AL, and only detected it qualitatively in a further 20%. In most other cases quantification was imprecise due to the low level of paraprotein, or because a monoclonal immunoglobulin could be measured only in the urine. In contrast, monoclonal serum FLC were identified in more than 97% of patients at diagnosis, and could be monitored quantitatively following chemotherapy. Although the antibodies in the assay do not distinguish monoclonal FLC from low level polyclonal FLC that exists in health, a relative excess of kappa or lambda FLC correctly identified the amyloidogenic FLC class in every case in which it could be verified. Demonstration of free clonal light chains in virtually

all patients with systemic AL amyloidosis supports *in vitro* observations that their free state is associated with amyloidogenicity[156, 361]. In addition, since 60% of patients with uncomplicated low grade monoclonal gammopathies have normal values in the FLC assay, this test may help to support or argue against a diagnosis of AL amyloidosis. In contrast 80% of patients with localised amyloidosis and 89% of patients whose local deposits are in sites other than bone or lymph nodes have no evidence of an underlying plasma cell dyscrasia. These deposits are almost always AL in type[193, 194], but the majority appear to derive from monoclonal light chains produced by clonal plasma cells within the local environment[190, 191, 200] rather than the bone marrow. The FLC results reported here would be consistent with this view and certainly the over representation of monoclonal FLC in patients with bone or lymph node associated deposits is consistent with the association of amyloid in these sites with myeloma and plasmacytoma in the former and low grade lymphomas in the latter. The finding of a higher incidence of monoclonal FLC in a small series of patients with bowel amyloid is intriguing. The bowel has extensive mucosal surfaces and might provide an environment for a plasma cell clone large enough for locally produced FLC to appear in the serum, or there may be a degree of tissue tropism with systemically produced monoclonal FLC demonstrating a predilection for forming amyloid within the bowel. The long-term prognosis of localised amyloidosis appears to be excellent with progression to systemic amyloid deposition seemingly very rare[197, 199], perhaps consistent with the rather indolent behaviour of well differentiated and sparse mucosal clones.

Rational treatment of systemic amyloidosis centres on reduction of the supply of amyloid fibril precursor proteins, but in AL amyloidosis only low dose chemotherapy has been subject to controlled studies. Moreover, in these and all other open chemotherapy studies, the response of the underlying clonal disease has been assessed using methods and criteria developed in multiple myeloma. These have insufficient sensitivity and quantitative capacity to effectively monitor the very low levels of monoclonal proteins that typically occur in AL amyloidosis. The high sensitivity and quantitative nature of the nephelometric FLC assay address these shortcomings. In some individuals the plasma concentration of FLC may increase in association with age or reduced glomerular filtration, but the kappa to lambda ratio that indicates monoclonality is unaffected[504]. Renal failure did develop during the study in five patients, and therefore the response to chemotherapy may have been underestimated in these few cases. Conversely, retention of amyloidogenic FLC in the circulation due to renal impairment, which would not be reflected by conventional measurements of whole monoclonal immunoglobulin, might promote amyloid deposition and this possibility merits further investigation.

Although complete suppression of the clonal plasma cell disorder would seem desirable, the present results show that reduction of the amyloidogenic serum FLC concentration by greater than just 50% is associated with a favourable clinical outcome in patients with AL amyloidosis. Reduction of amyloidogenic FLC production by this degree is usually sufficient to prevent further accumulation of amyloid, can lead to regression of existing amyloid deposits and substantially improves survival. In contrast to the situation in multiple myeloma,

there were no significant differences between the degree of suppression of the clonal disease or the durability of this response among patients who were treated with intermediate dose or high dose chemotherapy regimens. The efficacy of VAD/C-VAMP, IDM and HDM in the non-randomised and selected patients in this study was similar to the efficacy of HDM in other reported series[381]. Interestingly, substantial suppression of FLC production was achieved in some patients who, for various reasons, had received only one or two courses of VAD/C-VAMP or IDM. These intermediate dose regimens are given monthly in up to six or more cycles, and, in contrast with HDM, the cumulative risks are therefore reduced in patients who require fewer courses of treatment. These findings may reflect differences in the biology of the plasma cell clones that typically underlie AL amyloidosis compared with those in multiple myeloma, and have implications regarding the choice of chemotherapy in individuals and in future randomised controlled studies in AL amyloidosis. Although the present study necessarily comprised patients who survived 6 months after baseline evaluation, our experience has been that 7, 18 and 22% of patients treated respectively with VAD/C-VAMP, IDM and HDM died within 6 months of commencing therapy. This lends further support to the desirability of the intermediate dose chemotherapy approach.

The beneficial effect of a reduction in serum FLC concentration, even by only 50%, in terms of regression of amyloid and improved survival reflects natural turnover of the deposits, uniquely demonstrated here by SAP scintigraphy. This specific and safe technique overcomes the sampling error inherent in biopsy

histology, and provides a readily repeatable quantitative survey of whole body amyloid load.

In conclusion, this study demonstrates the utility of the nephelometric serum FLC assay in AL amyloidosis, and indicates that treatment strategies in AL amyloid may be guided by their effect on reducing the amyloidogenic serum FLC concentration. Although many factors contribute to outcome in AL amyloidosis, including age, sex, early diagnosis, pattern of organ involvement, and supportive measures including organ transplantation, therapy that lowers the amyloidogenic FLC concentration by more than 50% usually stops accumulation of AL amyloid deposits, can lead to their regression and greatly improves survival. Unlike whole immunoglobulins, FLC have a circulating half-life of 2-4 hours rather than many weeks (IgG half-life 20-25 days), and their measurement enables response to chemotherapy to be evaluated rapidly. Frequent FLC analysis may enable exposure to chemotherapy to be minimised, and help define patients with refractory disease at an early stage. Finally, quantitative monitoring of serum FLC will contribute importantly to systematic studies comparing different chemotherapy regimens in AL amyloidosis.

Chapter Six

SUCCESSFUL TREATMENT OF FIVE CASES OF UNICENTRIC CASTLEMAN'S DISEASE COMPLICATED BY SYSTEMIC AA AMYLOIDOSIS

INTRODUCTION

Castleman's disease[505] or angiofollicular lymph node hyperplasia is a rare B cell lymphoproliferative disorder characterised by giant hyperplastic lymph node follicles, capillary proliferation and plasma cell infiltration, and is often associated with marked constitutional symptoms. It is a disease spectrum, comprising anatomically unicentric and multicentric forms, and hyaline vascular and plasma cell histological variants[506]. Multicentric disease, commonly of the plasma cell type, usually follows an aggressive and rapidly fatal course. Unicentric disease tends to occur in younger patients and is of hyaline vascular type in more than 70% of cases, with plasma cell or mixed histology in the remainder[507, 508]. Most tumours occur within the mesenteries or mediastinum and are complex consisting of a dominant mass surrounded by multiple enlarged lymph nodes reaching 15 cm or more in diameter. Constitutional symptoms including night sweats, fever and weight loss are common and laboratory abnormalities including anaemia, elevation of the erythrocyte sedimentation rate (ESR) and polyclonal hypergammaglobulinaemia are almost universal. Acquired systemic amyloidosis is a recognised rare complication of all forms of angiofollicular lymph node hyperplasia, and is usually of reactive systemic, AA,

type resulting from a persistent acute phase response. More rarely it is of immunoglobulin light chain, AL, type associated with the presence of a monoclonal gammopathy. The treatment of localised Castleman's disease is complete surgical excision[509]. This is almost always curative resulting in rapid resolution of systemic symptoms and laboratory abnormalities. In patients with multicentric disease or in whom complete resection is not possible partial resection, radiotherapy, combination chemotherapy and anti-cytokine therapies have all been used with variable responses[507, 510].

The aetiology of Castleman's disease is poorly understood. It is possible that reactive lymphoid hyperplasia arises in response to chronic antigen stimulation although there may be a contribution from primary developmental abnormalities[506]. However greatly increased production of the pleiotropic cytokine IL-6, which enhances B-cell proliferation and survival possibly by interfering with normal apoptotic mechanisms[511], has been demonstrated in the germinal centres of plasma cell variants of Castleman's disease[512] and is clearly responsible for the marked constitutional and systemic manifestations of disease. Exogenous administration of pharmacological doses of IL-6 mimics many of the disease features, including upregulation of the acute phase response proteins[506], and mice genetically modified to over express IL-6 are an excellent model of Castleman's disease in man[513]. A further association is suggested by evidence of human herpes virus 8 infection in aggressive multicentric tumours expressing a virus derived IL-6 cytokine homologue[514, 515]. Treatment with monoclonal anti IL-6 antibody has been reported to alleviate systemic manifestations[510, 516] and

after successful resection of unicentric disease, the resolution of systemic inflammation closely parallels the fall in circulating IL-6 values.

Here are described a series of five patients demonstrating that resection of the affect lymph nodes leads to clinical improvement and amyloid regression.

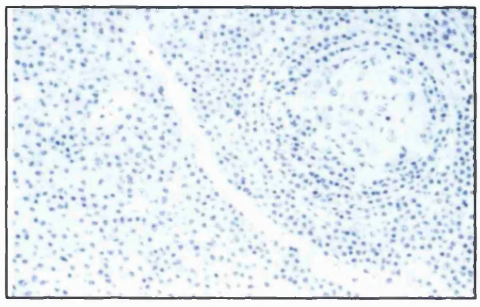
CASE HISTORIES

Case 1

A 26-year-old woman with a three-year history of severe iron resistant anaemia presented with right sided supraclavicular lymphadenopathy and a right hilar mass on chest X-ray. She had a low serum albumin value of 34 g/l and ESR of 100 mm. Biopsy of a supraclavicular lymph node was not diagnostic, showing reactive changes only. At mediastinoscopy she was found to have a large group of lymph nodes lying anterior to the superior vena cava. Excision biopsy of one of these revealed Castleman's disease of the plasma cell type (Figure 6.1). Surgical resection of the whole mass was deemed too hazardous and she was managed conservatively. Despite a median haemoglobin of less than 8 g/dl she remained relatively asymptomatic over the following 15 years until she presented, aged 41 years, with advanced renal failure. Renal biopsy demonstrated AA amyloid, and ^{123}I-SAP scintigraphy revealed a moderate amount of amyloid in the tissue distribution

Figure 6.1 A. Sections of plasma cell variant Castleman's tumour from Case 1 stained with H & E demonstrating a lymphoid follicle with marked plasma cell infiltration.

B. Immunohistochemistry using anti IL-6 antibody demonstrating localised cytokine production within the tumour.

A. 

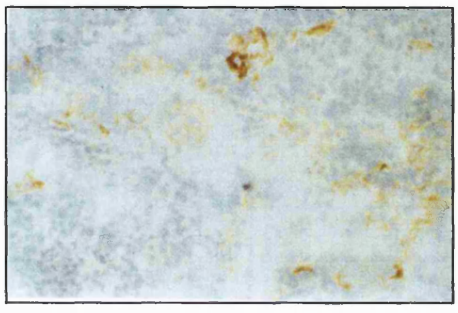
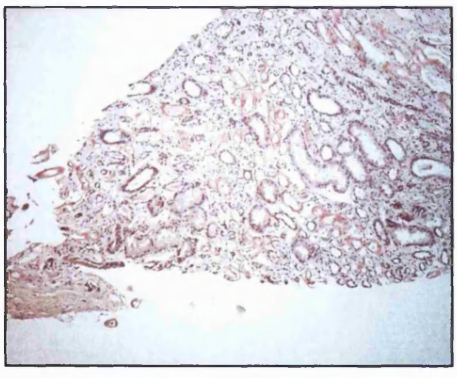
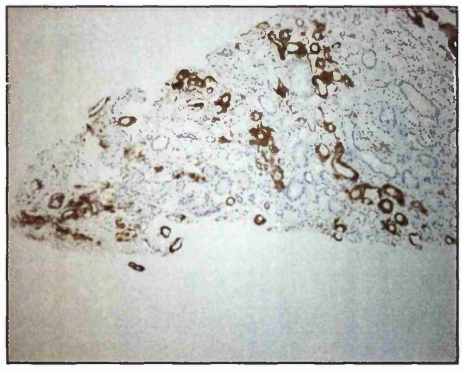
B. 

Figure 6.2 A. .Sections of renal biopsy from case 1.stained with Congo red demonstrating amorphous eosinophilic amyloid deposits.

B. Immunohistochemistry using Dako mouse antibodies directed against human amyloid A protein demonstrating the amyloid is of AA type.

A. 

B. 

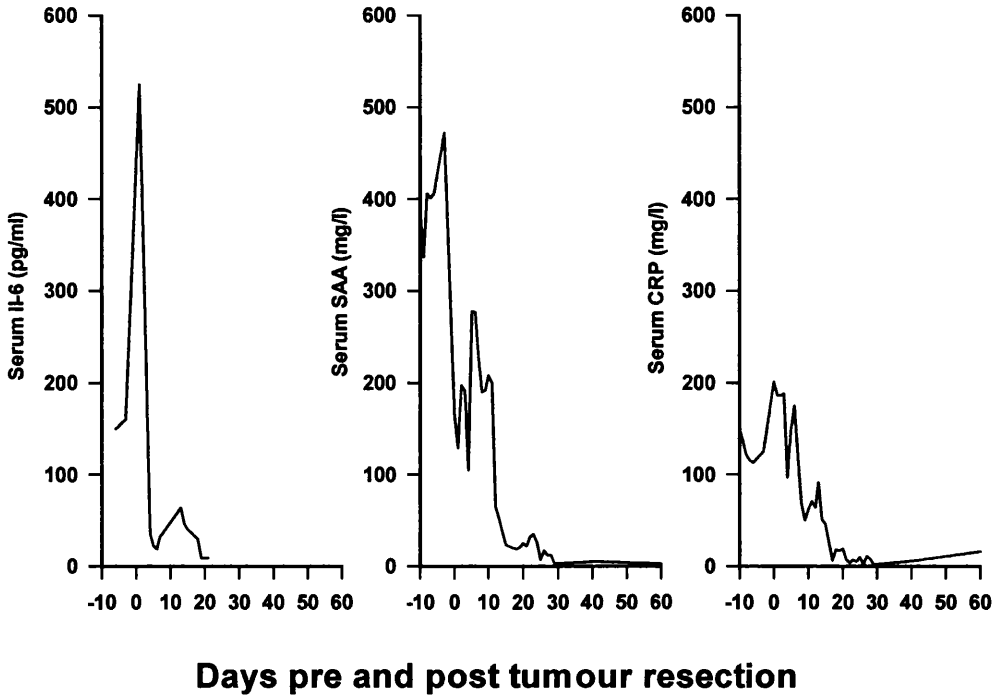
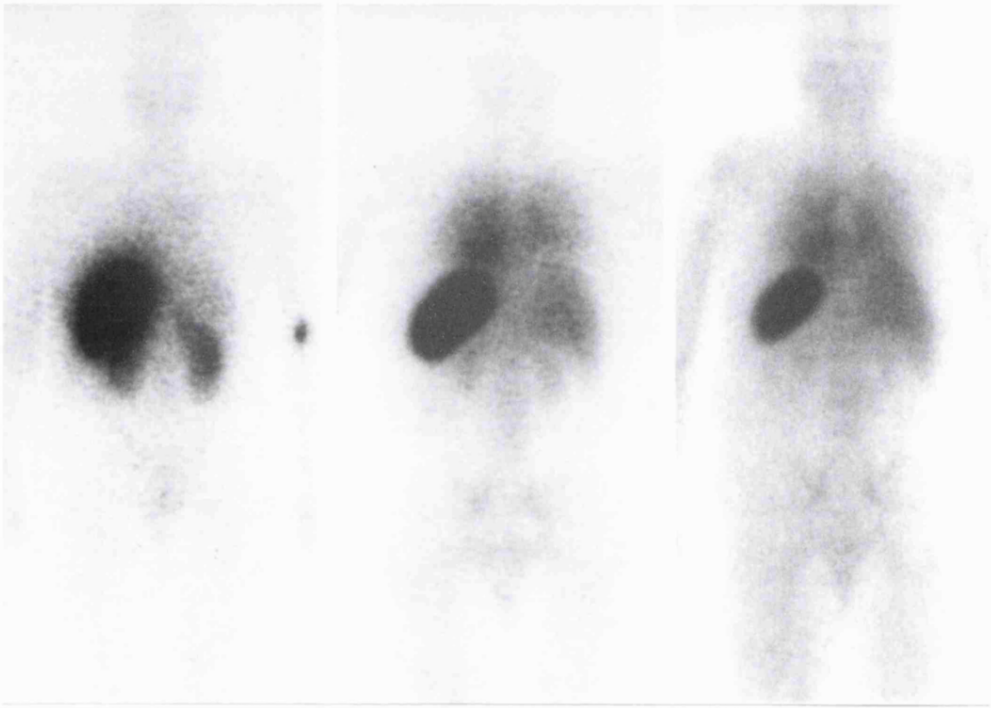


Figure 6.3 Graphs showing, from left to right, serial serum IL-6, SAA and CRP values from 10 days prior to tumour resection to 4 weeks post procedure showing a sustained fall in circulating levels post tumour resection to within the normal range. There is a brief rise between the fifth and twelfth postoperative days associated with systemic sepsis that precipitated end stage renal failure.

typical of AA amyloidosis. The mediastinal mass had enlarged but was nonetheless completely resected at thoracotomy. Unfortunately a septicaemic episode in the immediate post-operative period caused hypotension and precipitated end stage renal failure. However, after resection of the Castleman's lesion the serum values of IL-6, CRP and SAA fell rapidly to within the normal range (Figure 6.3), and serial ^{123}I-SAP scintigraphy demonstrated amyloid regression over the following year (Figure 6.4). Five years later she remains dialysis dependent but otherwise well.

Figure 6.4 Serial posterior whole body scintigraphic images after intravenous injection of ^{123}I-human SAP. Prior to resection of the tumour (left) there is heavy amyloid deposition in the spleen, kidneys and adrenal glands. The image in the middle was taken 1 year later and shows a marked diminution in her amyloid with abnormal uptake only in the spleen, the rest of the image represents normal blood pool signal. The image on the right was obtained 6½ years after surgery and shows sustained amyloid regression.



Case 2

A 30-year-old man presented with hepatomegaly, nephrotic syndrome and renal impairment. A renal biopsy demonstrated amyloidosis, which was presumed to be AL type secondary to an occult plasma cell dyscrasia. He received no treatment and remained well until end stage renal failure supervened 10 years later. He had a successful renal transplant but within four years his renal function had deteriorated and he was increasingly symptomatic from massive hepatomegaly. Scintigraphy with ^{123}I-SAP demonstrated a large amount of amyloid in the liver, spleen and renal graft. He had a persistent acute phase response, with CRP 68 mg/l and SAA 79 mg/l. Direct sequencing of amyloid fibrils extracted from a transjugular liver biopsy identified AA protein. Further investigation revealed a mesenteric mass which was incompletely resected with the adjacent 25 cm of small bowel. The mass comprised a plasma cell variant of Castleman's disease with widespread amyloid deposition in normal and tumour tissue. The acute phase response subsided after surgery and over the ensuing three years his general condition, renal and liver function have consistently improved with partial amyloid regression on serial SAP scans.

Case 3

A 43-year-old man with a six-year history of arthralgia, intermittent diarrhoea, and weight loss presented with hepatosplenomegaly and low grade proteinuria.

Table 6.1 Details of 5 patients with biopsy proven systemic AA amyloidosis complicating unicentric Castleman's disease

Sex	Age at presentation (years)	Age at Castleman's diagnosis (years)	Age at diagnosis of amyloid (years)	Laboratory abnormalities at presentation	Variant of Castleman's tumour	Site of tumour	Change in amyloid load post resection assessed by SAP scintigraphy, laboratory tests and clinical evaluation
F	23	26	41	Anaemia Polyclonal gammopathy Acute phase response	Plasma cell	Mediastinum	Complete regression over 5 years
M	30	45	30	Anaemia Acute phase response	Plasma cell	Mesentery	Partial regression with improvement in renal and liver function over 2 years
M	37	43	43	Anaemia Polyclonal gammopathy Acute phase response	Plasma cell	Mediastinum	Complete regression from liver, spleen & kidneys over 5 years
F	30	52	52	Anaemia Acute phase response	Plasma cell	Mesentery	Complete regression from liver, spleen & kidneys over 8 years
M	22	39	31	Anaemia Acute phase response	Mixed	Retroperitoneal	Symptomatic improvement in the 3 months since surgery

He had a normochromic normocytic anaemia, ESR persistently >150 mm, and a polyclonal hypergammaglobulinaemia. Rectal and liver biopsies contained extensive AA amyloid deposits, and SAP scintigraphy showed amyloid deposition in the grossly enlarged liver and spleen. A mediastinal shadow in the chest X-ray was found to be a circumscribed mass of lymph nodes with histology of the plasma cell variant of Castleman's disease. The tumour was resected completely and his acute phase response rapidly subsided, all laboratory indices returned to normal, and the organomegaly gradually resolved. An SAP scan five years post-operatively showed complete regression of the amyloid deposits.

Case 4

A 52-year-old woman with a 20-year history of intermittent fevers, normocytic anaemia and reactive thrombocytosis was found to have an epigastric mass. A 5 cm plasma cell variant Castleman's tumour was excised from the small bowel mesentery and an intra-operative liver biopsy contained extensive AA amyloid deposits. An SAP scan performed two months post operatively confirmed that she had extensive amyloid deposits in the liver, spleen and kidneys. Her symptoms and laboratory abnormalities all resolved rapidly after the operation and she has remained well over the next 7 years with normal organ function and evidence of substantial amyloid regression.

Case 5

Following the finding of a mild persistent rise in ESR (17-38 mm in first hour) during routine military medical examinations in his early twenties, which had not been investigated, a man presented aged 30 with abdominal pain, diarrhoea and

weight loss. He was found to have retroperitoneal and mesenteric lymphadenopathy with mildly deranged liver function and biopsies were reported to show non-specific inflammatory changes. Over the following eight years no diagnosis was made despite extensive investigations, and his signs and symptoms were not affected by systemic anti-inflammatory and immunosuppressive treatment with methyl prednisolone and cyclophosphamide. Duodenal biopsies taken at the age of 39 years and re-examination of the earlier liver and lymph node biopsies demonstrated AA amyloid deposition. Further investigation, specifically seeking Castleman's disease, revealed an 8 cm mass of lymph nodes behind the head of the pancreas, and this was resected. Histology was consistent with Castleman's of the mixed cell type with reactive changes in the surrounding lymph nodes and marked amyloid deposition. Postoperatively his acute phase response, anaemia and diarrhoea resolved, and he gained weight.

DISCUSSION

Reactive systemic, AA, amyloidosis is a potential complication of any disorder associated with a sustained inflammatory response. Il-6 is a potent multi-functional cytokine with effects on differentiation of B and T cells and haematopoiesis, and it is one of the major stimulants of acute phase protein synthesis by hepatocytes[517]. The germinal centres of hyperplastic lymph nodes in the plasma cell variant of Castleman's disease produce large amounts of IL-6, and the high circulating concentration of tumour derived IL-6 is evidently a major contributor to the systemic manifestations of Castleman's disease,

including a sustained overproduction of SAA leading in some patients to AA amyloidosis.

Twenty six cases of systemic amyloidosis associated with Castleman's disease have been reported previously. Seventeen of these had confirmed reactive systemic, AA, amyloidosis (Table 6.2)[240, 353, 356, 510, 518-527]. Two were confirmed as AL type derived from monoclonal immunoglobulins, and in the remaining seven cases the amyloid was not characterised[528-530]. The characteristics of all these patients are broadly similar to those of our present series. The median age at tumour resection or medical treatment of the 19 cases with confirmed AA amyloid was 37 years with a male to female ratio of 1:2. Half of the tumours were intra-abdominal, six mesenteric, one retroperitoneal and one peri-hepatic; two were located in the mediastinum; one in axillary lymph nodes and six were multicentric. Twelve tumours were of the plasma cell type, two were classified as hyaline vascular and three had mixed histological features. Three patients whose disease was multicentric, and thus not amenable to surgery, were treated with humanised anti-interleukin-6 receptor antibodies. This led to improvement in their constitutional symptoms, a fall in their acute phase response and, in one case, improvement in renal function suggesting amyloid regression[510]. One patient with multicentric disease had an incomplete surgical resection combined with corticosteroids with a fall in his acute phase response and partial amelioration of his nephrotic syndrome[527].

Table 6.2 Characteristics of 17 previously reported patients with Castleman's disease complicated by AA amyloidosis.

Age	Sex	Site of Castleman's tumour	Variant	Outcome	Reference
49	M	multicentric	plasma cell	Improvement in anaemia and renal function with cyclophosphamide and prednisolone	Funabiki 1998
46	F	mesentery	plasma cell	Resected Complete response	Ordi 1992
23	F	mesentery	plasma cell	Resected Complete response	Ordi 1992
37	M	mesentery	mixed	Resected Complete response	Keven 2000
44	M	axilla	plasma cell	Resected Complete response	Perfetti 1994
31	F	mediastinum	hyaline vascular	Resected ESRF on dialysis	Montoli 1993
21	F	perihepatic	plasma cell	Resected Complete response	Ikeda 1997
53	F	retroperitoneum	plasma cell	Resected progressed to ESRF but fall in acute phase	Tanaka 1995
28	F	mesenteric	mixed	Resected Complete response	Paydas 1995
48	F	mesenteric	plasma cell	Resected Complete response	Kazes 1995
29	F	mediastinum	plasma cell	Cyclophosphamide and prednisolone Died	Chan 1992
36	M	mesentery	plasma cell	Resected Complete response	Bonneau 1982
39	M	multicentric	Hyaline vascular	Partial resection Prednisolone	Curioni 2001
39	M	multicentric	plasma cell	Not described	Kanoh 1989
59	F	multicentric	plasma cell	Anti IL-6 receptor antibody Improved symptoms, fall in acute phase proteins	Nishimoto 2000
51	M	multicentric	plasma cell	Anti IL-6 receptor antibody Improved symptoms and fall in acute phase	Nishimoto 2000
23	M	multicentric	mixed	Anti IL-6 receptor antibody Improved symptoms and fall in acute phase	Nishimoto 2000

Following complete resection of the Castleman's tumours one patient was already in end stage renal failure and remained on dialysis and another became dialysis dependent in the peri-operative period. The other eleven patients all showed clinical improvement and this was sustained in those cases in whom follow up was reported.

As demonstrated by the five cases in the present series, AA amyloid deposits can regress quite rapidly when the supply of fibril precursors is reduced and, under favourable circumstances, this is accompanied by clinical improvement and stabilisation or improvement in organ function. Clearly the control of systemic inflammation is greatly facilitated when the nature of underlying inflammatory condition is known but in a significant proportion of patients diagnosis may be very difficult. Among 228 patients with confirmed systemic AA amyloidosis referred to our centre the cause of the underlying chronic inflammation was not apparent in 21 cases, and many of these individuals had received potent immunosuppressive treatment often with considerable morbidity and limited clinical benefit. As shown here almost 20% of this group were found to have a resectable Castleman's tumour and have done well after surgery. Unicentric Castleman's disease is a potentially fully remediable cause of AA amyloidosis, which often affects an unusually young age group, and the diagnosis should be actively sought in patients with unexplained chronic inflammation.

Chapter Seven

SERIAL MONITORING OF THE ACUTE PHASE RESPONSE IN HEALTHY INDIVIDUALS WITH WILD TYPE *MEFV*, HETEROZYGOUS CARRIERS OF THE FMF TRAIT AND PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER

INTRODUCTION

Familial Mediterranean fever is an autosomal recessive periodic inflammatory disease characterised by recurrent, self-limiting attacks of fever and serositis and a high[293, 294]. Attacks are accompanied by dramatic elevations of the acute phase proteins, SAA and CRP. As previously discussed the gene responsible for FMF, *MEFV* has been identified by linkage studies and multiple mutations have been found in association with clinical FMF.

Historically the genesis of amyloid in FMF has been poorly understood[311]. Most patients will have less than two attacks a month and, as attacks are usually brief, the majority of afflicted individuals will be symptomatic for less than 40 days per year[312]. This implies substantially less inflammatory activity than is seen in other chronic diseases yet the incidence of AA amyloidosis is higher than that seen complicating rheumatological diseases. These observations led us to look prospectively over several months at the acute phase proteins, SAA and CRP, in

FMF patients prescribed colchicine prophylaxis but without amyloid to see whether these correlated with the clinical assessment of disease activity.

METHODS

Patients

Serial blood samples were collected, by our Turkish collaborators, from ethnic Turks living in the western coastal region of Turkey in an area served by the Dokuz Eylül University School of Medicine, Izmir. The group included 43 patients from 35 families with a clinical diagnosis of familial Mediterranean fever all prescribed colchicine, 75 first degree relatives and 50 unrelated healthy controls. Individuals from the 35 families were followed up with home visits every fortnight for 5 months. Between 7 and 10 serum samples were collected from each patient and all the subjects were asked to keep a symptom diary during the study period. The probands with FMF were particularly asked to note any disease attacks and all participants were asked to document any intercurrent illness. The 50 controls were all overtly healthy adults and provided a minimum of three serum samples each at fortnightly intervals.

Gene sequencing

Exon 10 was fully sequenced in all cases and the mutation encoding pyrin E148Q was sought by gel electrophoresis of the 5 prime exon 2 amplicon after MvaI digestion. Exons 2, 3 and 5 were sequenced in probands with clinical FMF but without paired mutations in exon 10. *MEFV* exons 10 and 5 were sequenced, and E148Q and P369S status determined by RFLP for all control samples.

Statistical Analysis

The median and maximum CRP and SAA values were determined for each participant and the results were compared in patients with a clinical diagnosis of FMF and healthy controls. Within the FMF group inflammatory indices were compared during and between clinical attacks. Comparisons were made between *MEFV* genotypes both within FMF patients and overtly well heterozygotes. The results were analysed using non-parametric statistics and the Mann Whitney test.

RESULTS

MEFV genotypes

Controls

Forty nine healthy Turkish controls provided samples suitable for DNA sequencing and 38 (77.6%) had no identified *MEFV* mutations in exons 2, 3, 5 or 10. There were 2 apparently well individuals with paired *MEFV* mutations, one V726A/E148Q compound heterozygote and the other M694V/E148Q. Ten other controls (20.4%) were heterozygotes, the majority for E148Q, and one had a recognised mutation in exon 3, P369S.

FMF patients

Of the 42 patients with a clinical diagnosis of FMF, 36 (84%) had two identified *MEFV* mutations and 30 patients had both mutations in exon 10. Six individuals (14%) had an exon 10 mutation in conjunction with the common exon 2 polymorphism, E148Q. In five patients (11.6%) only a single mutation was detected and in two individuals (4.7%) no mutations were detected within the

coding region. No FMF patients had mutations detected only in exon 2 and in the whole group a total of six point mutations were detected, four in exon 10 and two separate mutations at position 148 in exon 2, including a previously unreported mutation E148V (manuscript in preparation). The majority of symptomatic individuals were compound heterozygotes but there were nine homozygotes; six M694V (14%), two M680I and one V726A.

First degree relatives

Seventy-three asymptomatic parents or siblings of FMF cases were analysed. Of these nine (12.3%) had two identified *MEFV* mutations and in four of these (5.5%) both the mutations were in exon 10. There were a substantial number of heterozygotes, 57 in total (78.1%), and just over 8% of individuals had no identified mutations.

Figure 7.1 Distribution of *MEFV* mutations in patients with FMF, their healthy relatives and Turkish controls

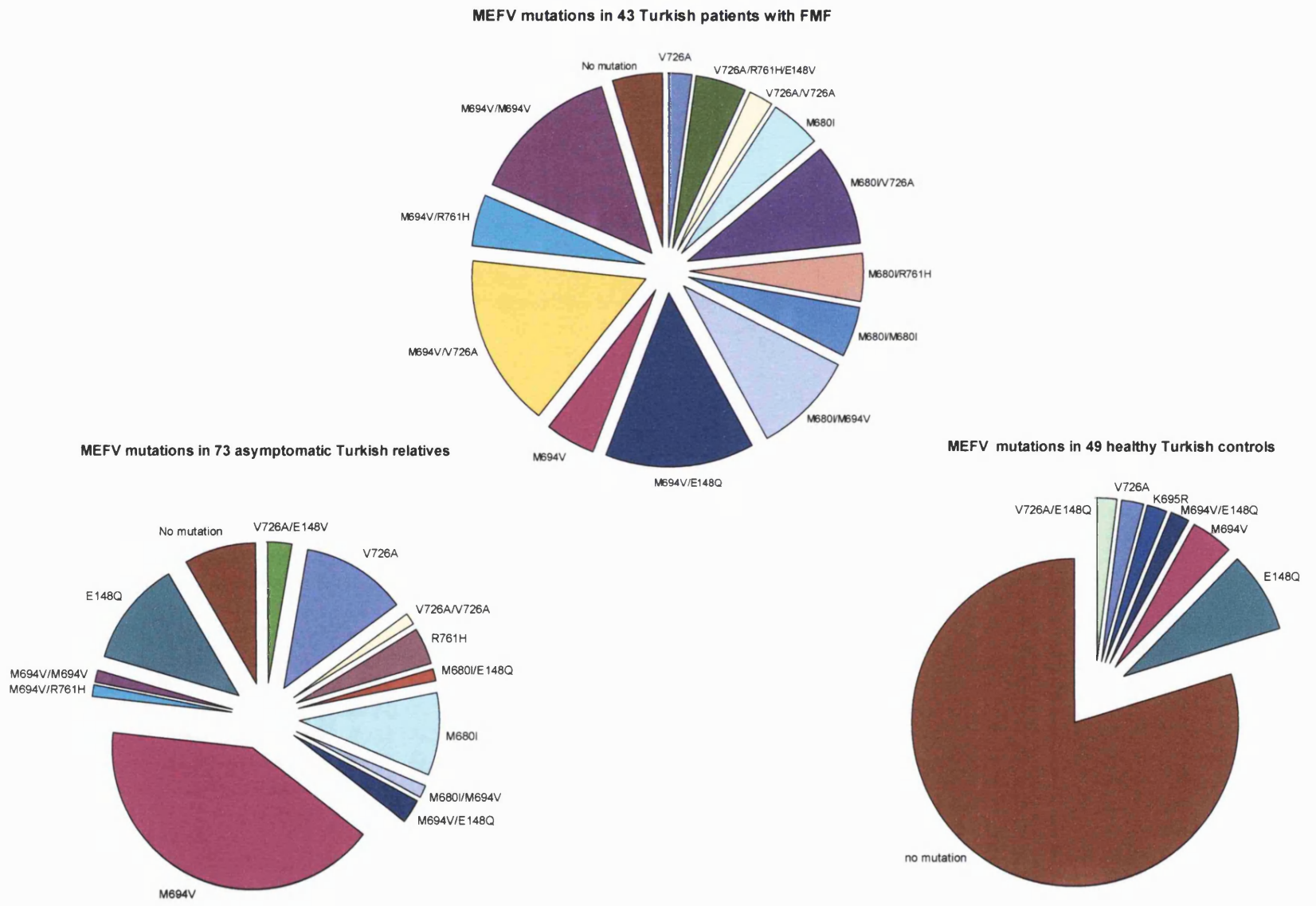


Table 7.1 *MEFV* genotyping in patients with familial Mediterranean fever, their asymptomatic first degree relatives and healthy Turkish controls

MEFV mutation Exon 10	Exon 2	Clinical FMF n=43	Asymptomatic relatives n=73	Healthy controls n=49
	E148Q		9 (12%)	4 (8.2%)
V726A	**E148V**	2 (4.7%)	2 (3%)	
M680I	**E148Q**		1 (1.4%)	
M680I		2 (4.7%)	7 (9.6%)	
M680I/M680I		2 (4.7%)		
M680I/M694V		4 (9.3%)	1 (1.4%)	
M680I/V726A		4 (9.3%)		
M680I/R761H		2 (4.7%)		
M694V	**E148Q**	6 (14%)	2 (2.7%)	1 (2%)
M694V		2 (4.7%)	30 (41%)	2 (4.1%)
M694V/M694V		6 (14%)	1 (1.4%)	
M694V/V726A		7 (16.3%)		
M694V/R761H		2 (4.7%)	1 (1.4%)	
K695R				1 (2%)
V726A	**E148Q**			1 (2%)
V726A	**E148V**		2 (2.7%)	
V726A		1 (2.3%)	9 (12.3%)	1 (2%)
V726A/V726A		1 (2.3%)	1 (1.4%)	
V726A/R761H	**E148V**	2 (4.7%)		
R761H			3 (4.1%)	
no mutation identified		2 (4.7%)	6 (8.2%)	38 (77.6%)
single mutation identified		5 (11.6%)	58 (79.4%)	9 (18.4%)
2 or more mutations identified		36 (83.7%)	9 (12.3%)	2 (4.1%)
2 mutations identified in exon 10		30 (69.8%)	4 (5.5%)	

Serial measurements of the acute phase response proteins; serum amyloid A protein (SAA) and C-reactive protein (CRP)

MEFV wild type, healthy controls

The 38 healthy controls without *MEFV* mutations had acute phase response profiles which were very similar to the normal profile described in other populations where 90% of normal healthy individuals have values of <3 mg/l[144, 479, 531, 532]. The baseline SAA and CRP were low with self limiting spikes associated with intercurrent upsets, median SAA 2.2 mg/l (minimum 0.7 - maximum 277 mg/l), median CRP 1.3 mg/l (minimum 0.1 – maximum 24.4 mg/l). None of these controls had raised acute phase proteins without an overt systemic cause, usually symptomatic upper respiratory tract infections, and none had sustained supra-normal values.

FMF patients

The acute phase response was highly variable over time in all the patients studied. As expected levels of both proteins were grossly elevated during clinical attacks of FMF with a dramatic bias of five to ten fold towards SAA; median CRP 115 mg/l (range: minimum 26 – maximum 296 mg/l) and a median SAA 639 mg/l (range: minimum 140 – maximum 1330 mg/l). Between attacks, when the patients were apparently well. their acute phase response fell considerably but remained two to three fold higher than that of the control group, median CRP 4.0 mg/l (range: minimum 2.7 – maximum 262 mg/l) and SAA 6.0 mg/l (range: minimum 0.7 – maximum 1230 mg/l). Although the majority of

readings were reduced to near the normal range (<3 mg/l), there was considerable asymptomatic inflammatory activity and 13% of the SAA measurements taken in apparently well patients were greater than 50 mg/l. This was the case both in patients who had a number of attacks during the study period and in the eight individuals who remained clinically well throughout (Figure 7.2).

Figure 7.2 Fortnightly SAA measurements plotted on a logarithmic scale, in eight FMF patients who remained asymptomatic throughout the study period, showing substantial subclinical inflammatory activity with 15% of SAA values greater than 50 mg/l.

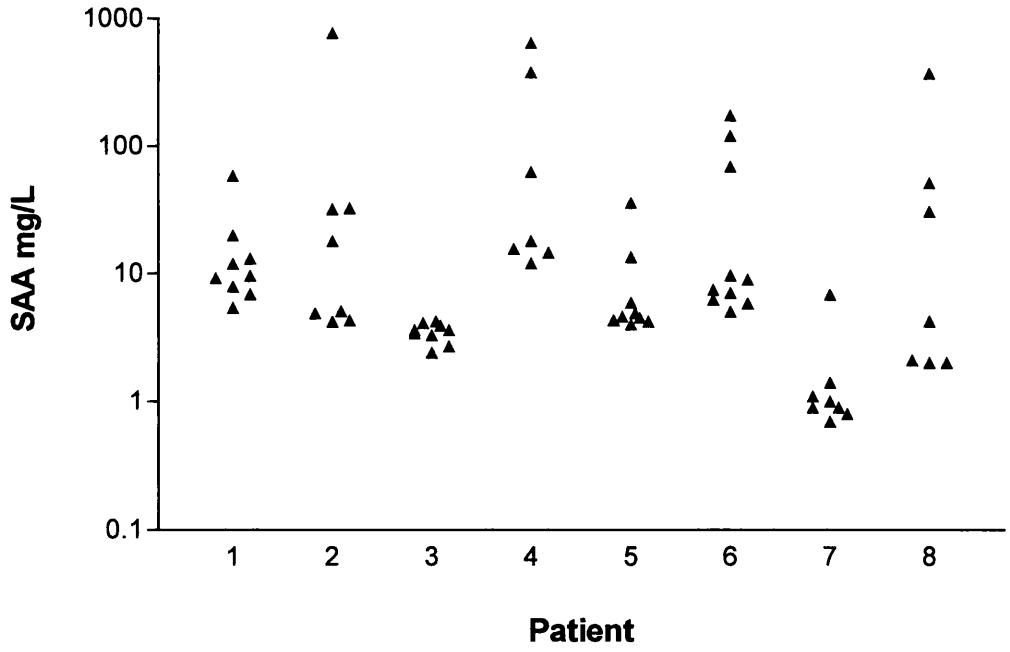


Table 7.2 Summary table of grouped results for FMF patients according to *MEFV* genotype showing the median values and range of each parameter of the acute phase response during the study period. Man Whitney analysis was performed on the acute phase parameters of each individual. $^{\otimes}p<0.05$, $^{\otimes\otimes}p<0.01$ compared with wild type controls, $^{\clubsuit}p<0.05$, $^{\clubsuit\clubsuit}p<0.01$ compared with asymptomatic *MEFV* heterozygotes and $^{*}p<0.05$ comparing the all other FMF patient genotypes with M694V homozygotes.

***MEFV* mutation**	**Median CRP mg/l**	**Median SAA mg/l**	**Maximum CRP mg/l**	**Maximum SAA mg/l**	**Median number of attacks per patient**
M694V/E148Q N=6	$3.0^{\otimes}$ (5.7-644)	3.0 (1.0-18.2)	15.4 (1.6-82)	$42.9^{\otimes}$ (5.7-644)	0 (0-2)
Single exon 10 & an exon 2 mutation N=11	$2.7^{\otimes\otimes}$ (1.1-11.3)	2.4 (1.0-18.2)	$9.4^{\otimes}$ (1.6-82)	$7.9^{\otimes\otimes}$ (2.0-644)	1 (0-2)
Two exon 10 mutations N=30	$4.9^{\otimes\otimes\clubsuit}$ (1.2-36.6)	$7.0^{\otimes\otimes}$ (1.0-162)	$41.8^{\otimes\otimes\clubsuit}$ (1.8-262.3)	$152^{\otimes\otimes\clubsuit}$ (1.7-1330)	1 (0-3)
M694V & additional exon 10 mutation N=13	$3.0^{\otimes\otimes\clubsuit}$ (1.2-11.3)	$4.6^{\otimes}$ (1.0-23.2)	$48.9^{\otimes\otimes\clubsuit\clubsuit}$ (1.8-129.7)	$91.3^{\otimes\otimes\clubsuit\clubsuit}$ (1.7-1230)	1 (0-2)
M694V homozygotes N=6	$16.0^{*\otimes\otimes\clubsuit\clubsuit}$ (11.4-36.6)	$31.3^{*\otimes\otimes\clubsuit\clubsuit}$ (15.1-162)	$51.0^{\otimes\otimes\clubsuit\clubsuit}$ (26.6-152)	$279^{\otimes\otimes\clubsuit\clubsuit}$ (136-1030)	1 (0-3)

Previous studies have suggested that M694V homozygotes have a more severe disease phenotype in some populations but not in the Turks]. We found that the median SAA and CRP ($p<0.05$) were significantly higher in the six M694V homozygotes than in patients with any other combination of mutations. No CRP and SAA parameters were significantly elevated in the 30 patients with two exon

10 mutations compared with the 11 patients with only a single identified mutation (values summarised in Table 7.2.).

MEFV Heterozygotes

Among the healthy relatives and the control group there were a total of 66 asymptomatic individuals with a single detected *MEFV* mutation. Three E148Q carriers were omitted from the study as they were first degree relatives of patients with clinical FMF and only one identified *MEFV* mutation. These families were excluded as they were presumed to carry other, as yet unidentified, genetic variants. Overtly well, asymptomatic carriers of these four mutations had evidence of subtly upregulated acute phase responsiveness compared to the healthy controls with elevated basal and peak values of SAA and CRP (Table 7.3). Comparisons between carriers of the 4 major mutations revealed few statistically significant differences in their SAA and CRP measurements. The median SAA was lower in the E148Q heterozygotes than M680I ($p<0.01$) but higher than V726A heterozygotes ($p<0.01$) and peak SAA's were lower in the V726A group than in the M680I carriers ($p<0.01$ respectively).

Table 7.3 Summary table of grouped results for *MEFV* wild types and healthy heterozygotes showing the median values and range (in brackets) of each parameter of the acute phase response. * $p<0.05$, ** $p<0.01$ for Man Whitney analysis comparing the wild type controls and *MEFV* heterozygotes performed on the acute phase parameters of each individual

***MEFV* mutation**	**Median CRP mg/l**	**Median SAA mg/l**	**Maximum CRP mg/i**	**Maximum SAA mg/i**
Wild Type N=38	1.2 (0.2-6.3)	2.2 (0.7-17)	2.2 (0.2-24.4)	3.5 (1.0-277)
E148Q N=11	2.0* (0.4-9.0)	4.2* (1.3-8.7)	3.4 (0.4-26.8)	11.0* (1.4-187.0)
M694V N=32	1.5** (0.6-5.2)	2.7 (1.1-13.2)	4.4** (1.3-38.1)	11.1** (1.6-536)
V726A N=10	1.3** (0.3-6.2)	2.0 (1.2-5.9)	4.4 (0.8-15.8)	15.4** (3.4-49.9)
M680I N=7	2.0** (1.3-3.1)	4.7** (1.8-5.9)	4.8* (3.0-26.5)	10.5* (4.1-509)

DISCUSSION

The results of this study provide, for the first time, evidence of substantial subclinical inflammation in patients with familial Mediterranean fever and suggest that clinically overt attacks represent the 'tip of the iceberg' of persistent inflammatory activity in FMF.

It has long been recognised that acute attacks of FMF are accompanied by a dramatic elevation in the acute phase response[293]. The two acute phase response

proteins, SAA and CRP rise and fall together and are of similar clinical and diagnostic value in inflammatory conditions[533]. The bias towards SAA is very marked in FMF and may simply reflect, that with an extreme inflammatory response, SAA synthesis has five to ten fold higher ceiling than CRP.

The significance of SAA values in patients is two fold. Firstly it is helpful in the initial diagnosis. In our experience, few conditions produce episodic SAA values of more than one thousand milligrams per litre and, in the absence of a plausible alternative, such results should prompt consideration of a diagnosis of FMF. Secondly SAA is the circulating protein precursor of AA amyloidosis and sustained elevated levels are necessary for its genesis and maintenance[135, 137, 138, 140, 146, 534]. Clearly not all individuals with elevated SAA values go on to develop AA amyloid. Indeed, for most chronic inflammatory conditions the risk is approximately 10%. These results suggest that in the case of FMF, the remarkably high prevalence of amyloidosis in untreated patients reflects the unprecedented peaks of SAA during attacks on a background of lower grade near-continuous inflammation over many years or decades.

It is well recognised that AA amyloidosis complicating both classical and type II FMF can regress with long term colchicine therapy[131, 319, 320]. This is entirely consistent with previous work showing that amyloid deposition is not irreversible, and clinical progression of amyloid diseases merely reflects that the deposits are usually laid down more rapidly than they are turned over. Previous work has demonstrated that in patients with AA type and well controlled underlying inflammatory disease (median SAA <50 mg/l) long-term survival is

excellent, greater than 95% 10 year survival, whereas in those with poorly controlled disease the 10 year survival is less than 50%[134]. It is worth noting that in this series of FMF patients, none of whom had amyloid, 15% of SAA measurements taken between attacks when the patients were entirely well, were greater than 50 mg/l. Analogy with the above data in amyloid suggests that this may reflect a critical threshold in the genesis of amyloid fibrils.

Worldwide there must be substantial numbers of asymptomatic *MEFV* heterozygotes, as there were two out of 50 controls in this study. In such individuals, particularly those with a family history of amyloid, serial SAA measurements could be used to select those at highest risk of subsequent amyloidosis and thus allow targeted prophylactic colchicine treatment.

The evidence for a subtle up regulation of the basal and peak acute phase response in healthy heterozygote carries of *MEFV* mutations is the first evidence of a heterozygote phenotype for pyrin variants. This data, in combination with the high carriage rate of *MEFV* mutations - up to one in three in the Armenian population - supports the hypothesis that the heterozygote state confers or, more likely, conferred in the past a survival advantage[535]. The nature of advantage is unclear but one plausible candidate is potentiation of the immune response to endemic pathogens. Higher levels of mediators of innate immunity, such as CRP and SAA, may be helpful in resistance to microbial disease particularly early in infection before specific antibodies are synthesised and in infants during the physiological gap between the loss of maternally acquired antibodies and the

development of a competent immune system a period with high mortality due to gastro-enteritis.

Chapter Eight

CHARACTERISTICS AND OUTCOME OF PATIENTS WITH AA AMYLOIDOSIS COMPLICATING FMF

INTRODUCTION

As pointed out in the previous chapter patients with FMF are at high risk of AA amyloidosis and despite the availability of treatment in the form of colchicine the prevalence of amyloidosis remains high. Pyrin M694V homozygotes have a more severe disease phenotype and there is some evidence that they have an increased risk of amyloidosis but a wide variety of other *MEFV* genotypes have also been associated with amyloidosis.

PATIENTS

The characteristics and outcome of the 12 patients with secondary reactive, AA amyloidosis complicating FMF who have been followed up in the UK National Amyloidosis Centre are reported here.

Table 8.1 *MEFV* mutations, ethnic origins and clinical characteristics of 12 patients with AA amyloidosis complicating their FMF

MEFV mutation	Sex	Ethnicity	Age at onset of FMF years	Age at diagnosis of AA amyloid years	FH of amyloidosis
M694V/M694V	M	Turkish Kurd	16	25	No
M694V/M694V	F	Turkish	5-10	30	No
M694V/V726A	M	Egyptian	5-10	34	No
M694V/V726A	F	Syrian	30	66	No
M694V/V726A	F	Armenian	5	12	No
M694V/T681I	F	Turkish	5-10	37	No
M680I/M680I	M	Egyptian	27	41	No
M680I/V726A	F	Armenian	5-10	45	No
M694I/V726A	F	Turkish Cypriot	5-10	36	No
M694I/E148Q	M	Jordanian	38	58	No
V726A/F479L	M	Greek Cypriot	11	19	No
ΔM694	M	British	5-10	45	No

RESULTS

Gene sequencing

The distribution of *MEFV* mutations and clinical characteristics are summarised in Table 8.1. Only one of the patients, who was heterozygous for pyrin deletion M694, had British ancestry, the remainder having Mediterranean roots. Two patients were homozygous for pyrin M694V, and one for M680I. Each of the remaining eight patients was a compound heterozygote, the V726A allele represented in six cases, M694V in four, M694I in two, M680I in two, and F479L and E148Q in one case each.

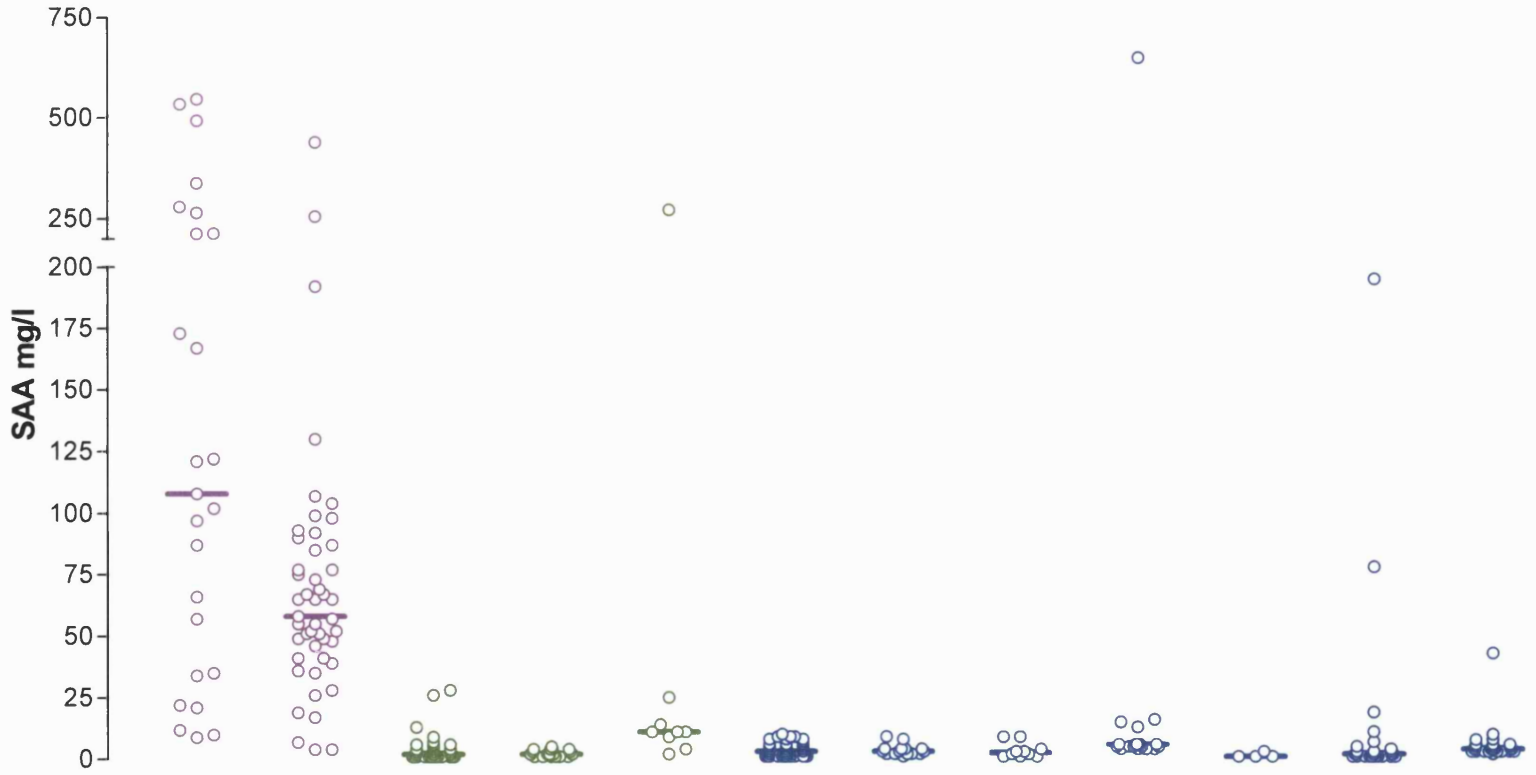
Clinical features

Seven patients presented in childhood and two in adolescence but in another two cases the diagnosis of FMF was not made until after the age of 30 years. A diagnosis of AA amyloidosis was made at a median of 22.5 years (range 7-40 years) after presentation with FMF and was after the age of 40 years in five cases. The six patients with at least one M649V allele were not significantly younger than the patients with other pyrin mutations either at presentation or at diagnosis with amyloidosis. At diagnosis with AA amyloidosis two patients had severe renal impairment (creatinine clearance $\leq$ 30 ml/min), five moderate renal impairment (creatinine clearance 30 - 60 ml/min) and five patients had a creatinine clearance of less than 60 ml/min. Two of the patients did not have significant proteinuria, six patients had between one and 3 g proteinuria/day and four patients had frank nephrotic syndrome (Table 8.2).

Table 8.2 Amyloid distribution and renal function at diagnosis of amyloid and at most recent follow up

At diagnosis			Follow up months	Response to colchicine		At follow up		
Amyloid load and distribution by ^{123}I-SAP scintigraphy	**Creatinine Clearance ml/min**	**Proteinuria g/24 hours**		**Symptomatic**	**Median SAA mg/l**	**Change in amyloid by ^{123}I-SAP scan**	**Creatinine Clearance ml/min**	**Proteinuria g/24 hours**
Moderate-spleen, kidneys & adrenals	11	16.2	18	Partial - poor compliance	11.2	Stable	ESRF	anuric
Moderate-spleen, kidneys & adrenals	96	2.3	71	Complete	2.7	Stable	ESRF-polycystic kidney disease	anuric
Large-spleen, kidneys & adrenals	69	2.2	122	Partial - poor compliance	58	Progression	21	9
Moderate-liver, spleen & kidneys	31	0.9	62	Complete	4.1	Regression	54	0.4
Moderate-spleen, kidneys & adrenals	98	2.6	37	Complete	0.7	Regression	157	0.59
Moderate-spleen, kidneys & adrenals	60	0.2	48 - died	Refractory	108	Progression	35	7
Small - spleen & kidneys	45	1.2	44	Complete	2.9	Regression	99	0.19
Small - spleen & kidneys	73	1.5	80	Complete	6.4	Regression	82	0.17
Moderate-spleen, kidneys & adrenals	21	5.1	108	Complete	1.6	Stable	17.5	4.33
Moderate-spleen, kidneys & adrenals	105	5	71	Complete	2.4	Regression	97.2	4.32
Moderate-liver, spleen, kidneys & adrenals	96	14	65	Complete	2.4	Regression	106	0.33
Small - spleen & kidneys	32	2.8	46	Complete	3.4	Regression	60	0.2

Figure 8.1 Serial SAA measurements for each patient. The two patients with evidence of amyloid progression on SAP scintigraphy (marked in purple) had significantly higher median SAA levels than those seen in the three patients with stable deposits (marked in green) or the seven with amyloid regression (marked in blue)($p < 0.001$).

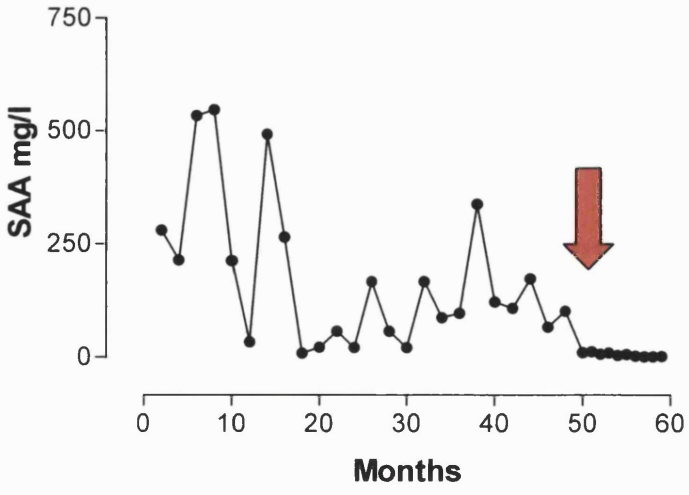


All of the patients were treated with colchicine following the diagnosis of amyloid, Four patients had been prescribed the drug for up to 20 years beforehand but had been poorly compliant.

Serial measurements of serum amyloid A protein (SAA)

Serum SAA concentration was monitored monthly in all patients, and was maintained with a median of less than 10 mg/l in all but 2 patients (Figure 8.1). SAA levels were significantly higher in the patients with progressive amyloid deposits ($P<0.001$). One patient with progressive disease proved refractory to colchicine at the maximum tolerated dose of 1.5 mg/day and died with 4 years. The other patient was poorly compliant but subsequent patient education has been effective (Figure 8.2).

Figure 8.2 Effect of colchicine compliance of FMF related inflammation measured by SAA levels. The patient had been prescribed colchicine 1.5 mg/day for many years; following strenuous attempts to increase drug compliance there have been no further symptomatic attacks and a sustained suppression of SAA levels.



Changes in ^{123}I-labelled SAP scintigraphy and renal function

Radiolabelled SAP scintigraphy quantitatively imaged visceral amyloid deposits in each patient, and demonstrated widely varying amyloid loads at diagnosis. Serial SAP scans demonstrated regression of amyloid in seven cases, and stable deposits in all but two of the remainder (Table 8.2). Renal function improved significantly (defined as improvement in creatinine clearance of more than 25% and/or decline in proteinuria of more than 2 g, without concomitant deterioration in creatinine clearance, or urinary protein levels falling into the normal range) in six of the seven patients with scintigraphic evidence of amyloid regression. Renal function remained stable in one patient with amyloid regression and one with stable deposits on imaging. Two patients with stable deposits have become dialysis dependent, one presented with near ESRF and one other has required chronic dialysis for a second unrelated disorder, adult polycystic kidney disease. Renal function deteriorated in both patients with progressive amyloid deposition.

DISCUSSION

Although this series is small, it nevertheless demonstrates substantial heterogeneity in these patients' underlying *MEFV* mutations, clinical presentations and outcomes. It has systematically confirmed that AA amyloid deposits frequently regress in patients with FMF treated with colchicine whose SAA levels are maintained with a median value below 10 mg/l. The findings also emphasize that amyloid can develop in FMF late, after the age of 40 years, and that patients' histories of colchicine prophylaxis must be regarded with caution and vigorous efforts made to encourage compliance. These findings are

consistent with results in AA amyloidosis complicating other chronic inflammatory diseases and remarkably even relatively advanced renal impairment can be reversed or stabilise if long-term suppression of SAA production can be achieved.

GENERAL CONCLUSIONS

These studies in a large population of patients have produced a number of novel findings concerning the diagnosis and management of amyloidosis. The finding that systemic amyloidosis due to genetic variation in the precursor proteins is remarkably common and can not always be distinguished clinically from AL amyloidosis, even the presence of a monoclonal gammopathy, demonstrates that gene analysis is frequently required to make a definitive diagnosis of amyloid type. Accurate diagnosis is of fundamental importance in the management of patients with amyloidosis as current treatments are largely directed at reducing the supply of circulating amyloid precursor proteins. Treatment of AL amyloidosis has previously been difficult to assess because of the lack of robust short-term parameters for measuring efficacy. Measurement of free light chains by the ultra sensitive assay introduced by Bradwell and his colleagues is now meeting this need. Reduction in circulating levels of FLC following treatment appears to provide a valuable prognostic indicator for clinical outcome in the longer term. FLC assays are therefore a valuable method for monitoring and tailoring cytotoxic treatment in patients with the commonest type of systemic amyloidosis - the AL type.

Work on a large cohort of patients with familial Mediterranean fever has provided possible explanations for the high risk of AA amyloidosis in this disease. Even clinically well patients show a substantial acute phase response suggesting that they have persisting background inflammation.

Finally follow-up studies in patients with AA amyloidosis complicating both FMF and Castleman's disease have provided further evidence that a sustained reduction in circulating SAA levels, the amyloid precursor, can substantially improve clinical outcome and that turnover of existing deposits eventually permits significant amyloid regression if new amyloid formation is inhibited.

PUBICATIONS ARISING FROM STUDIES IN THIS THESIS

Booth, D.R., Gillmore, J.D., Lachmann, H.J., Booth, S.E., Bybee, A., Soyturk, M., Akar, S., Pepys, M.B., Tunca, M., Hawkins, P.N. (2000) The genetic basis of autosomal dominant familial Mediterranean fever. Q. J. Med.; **93:** 217-21.

Lachmann, H.J., Booth, D.R., Bybee, A., Hawkins, P.N. (2000) Transthyretin Ala97Ser is associated with familial amyloidotic polyneuropathy in a Chinese-Taiwanese family. Hum Mutat. **16:**180.

Sinha, M.K.; Lachmann, H.J.; Kuriakose, B.; Abdulla, A.K., Aggarwal, R.K. (2001) An unusual cause of progressive heart failure. Lancet **357:** 1498

Lachmann H.J., Hawkins P.N. Amyloidosis: Pathogenesis & diagnosis (Chapter 19)
Amyloidosis: Rheumatic disease & amyloid; clinical features and treatment (Chapter 20) Rheumatology and the Kidney 2001 OUP Ed Adu, Emery and Madaio p305-348

Lachmann H.J., Hawkins P.N. Amyloidosis, Familial Renal (2001) www.emedicine.com/emerg/topic169.htm.

Nardin, R. and Lachmann, H.J. (2001) Familial Amyloid Polyneuropathy: Presentation, Diagnosis and Treatment. J. Clin. Neuromusc Dis. **2:** 221-226
Booth, D.R., Lachmann, H.J., Gillmore, J.D., Booth, S.E. and Hawkins, P.N. (2001) Prevalence and significance of the familial Mediterranean fever gene mutation encoding pyrin Q148. Q. J. Med.; **94:** 527-531

Lachmann, H.J., Gilbertson, J.A., Gillmore, J.D., Hawkins, P.N., and Pepys, M.B. (2002) Unicentric Castleman's disease complicated by systemic AA amyloidosis: a curable disease. Q. J. Med.; **95:**211-218

Harrison, C.J., Mazzullo, H., Ross, F.M., Cheung, K.L., Gerrard, G., Harewood, L., Mehta, M., Lachmann, H.J., Hawkins, P.N., Orchard, K.H. (2002) Translocations of 14q32 and deletions of 13q14 are common chromosomal abnormalities in systemic amyloidosis. Br.J.Haematol., **117:**427-35.

Pepys, M.B, Herbert, J., Hutchinson, W.L., Tennent, G.A., Lachmann, H.J., Gallimore, J.R., Lovat, L.B., Bartfai, T., Alanine, A., Hertel, C., Hoffmann, T., Jakob-Roetne, T., Norcross, R.D., Kemp, J.A., Yamamura, K., Suzuki, M., Taylor, G.W., Murray, S., Thompson, D., Purvis, A., Kolstoe, S., Wood, S.P. and Hawkins, P.N. (2002) Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. Nature, **417:**254-259.

Lachmann, H.J., Booth, D.R., Booth, S.E., Bybee, A., Gilbertson, J.A., Gillmore, J.D., Pepys, M.B., Hawkins, P.N. (2002) Misdiagnosis of Hereditary Amyloidosis as AL (primary) Amyloidosis. N.Engl.J.Med.,**346:**1786-1891.

Aganna, E., Martinon, F., Hawkins, P.N., Ross, J.B., Swan, D.C., Booth, D.R., Lachmann, H.J., Gaudet, R., Woo, P., Feighery, C., Cotter, F.E., Thome, M., Hitman, G.A., Tschopp, J., McDermott, M.F. (2002) Mutations in the NALP3/CIAS1/PYPAF1 gene are associated with a broad phenotype including recurrent fevers, cold sensitivity, sensorineural deafness and AA amyloidosis. Arthritis and Rheumatism., **46:**2445-2452.

Elkayam O, Hawkins PN, Lachmann H, Yaron M, Caspi D (2003)Rapid and complete resolution of proteinuria due to renal amyloidosis in a patient with rheumatoid arthritis treated with infliximab. Arthritis and Rheumatism., **46:**2571-2573

Lachmann HJ, Gallimore R, Gillmore JD, Carr-Smith HD, Bradwell AR, Pepys MB, Hawkins PN (2003) Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. British Journal of Haematology (in press).

Hawkins P.N., Lachmann H.J, McDermott M.F. (2003) Efficacy of interleukin-1 receptor antagonist in Muckle-Wells syndrome. N.Engl.J.Med.(in press).

Lachmann H.J., Hawkins P.N. (2003) Diagnosis and management of the periodic fever syndromes. CPD Bulletin Immunology and Allergy, **3**:16-19.

Lachmann H.J, Hawkins P.N., McDermott M.F. (2003) Familial Mediterranean Fever PIER: (The Physicians' Information and Education Resource) http://pier.acponline.org/index.html?hp (in press).

REFERENCES

1. Pepys MB. Amyloidosis. In: Frank MM, Austen KF, Claman HN, Unanue ER, eds. Samter's Immunologic Diseases. Boston: Little, Brown and Company, 1994:637-655.

2. Pepys MB. Amyloidosis. In: Weatherall DJ, Ledingham JGG, Warrell DA, eds. Oxford Textbook of Medicine. Vol. 2. Oxford: Oxford University Press, 1996:1512-1524.

3. Westermark P, Araki S, Benson MD, et al. Nomenclature of amyloid fibril proteins. Amyloid: Int. J. Exp. Clin. Invest. 1999; 6:63-66.

4. Hayden MR, Tyagi SC. "A" is for amylin and amyloid in type 2 diabetes mellitus. JOP 2001; 2:124-139.

5. Gorevic PD, Castano EM, Sarma R, Frangione B. Ten to fourteen residue peptides of Alzheimer's disease protein are sufficient for amyloid fibril formation and its characteristic X-ray diffraction pattern. Biochem. Biophys. Res. Commun. 1987; 147:854-862.

6. Cornwell GG, Westermark P. Senile amyloidosis: a protean manifestation of the aging process. J. Clin. Pathol. 1980; 33:1146-1152.

7. Pitkanen P, Westermark P, Cornwell GG, Murdoch W. Amyloid of the seminal vesicles. A distinctive and common localised form of senile amyloidosis. Am. J. Pathol. 1983; 110:64-69.

8. Takeda T, Sanada H, Ishii M, et al. Age-associated amyloid deposition in surgically-removed herniated intervertebral discs. Arthritis Rheum. 1984; 27:1063-1065.

9. Bartley CJ, Orford CR, Gardner DL. Amyloid in ageing articular cartilage. J. Pathol. 1985; 145:107A.

10. Athanasou NA, Sallie B. Localized deposition of amyloid in articular cartilage. Histopathology 1992; 20:41-46.

11. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Semin. Hematol. 1995; 32:45-59.

12. Wester AL, Vatn MH, Fausa O. Secondary amyloidosis in inflammatory bowel disease: a study of 18 patients admitted to Rikshospitalet University Hospital, Oslo, from 1962 to 1998. Inflamm. Bowel Dis. 2001; 7:295-300.

13. David J. Amyloidosis in juvenile chronic arthritis. Clin. Exp. Rheumatol. 1991; 9:73-78.

14. Gillmore JD, Lovat LB, Persey MR, Pepys MB, Hawkins PN. Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. Lancet 2001; 358:24-29.

15. Husby G. Nomenclature and classification of amyloid and amyloidoses. J. Intern. Med. 1992; 232:511-512.

16. Perlmann GE, Longsworth LG. The specific refractive increment of some purified proteins. J. Am. Chem. Soc. 1948; 70:2719-2724.

17. Cohen AS, Calkins E. Electron microscopic observations on a fibrous component in amyloid of diverse origins. Nature 1959; 183:1202-1203.

18. Kidd M. Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 1963; 197:192-193.

19. Puchtler H, Sweat F, Levine M. On the binding of Congo red by amyloid. J. Histochem. Cytochem. 1962; 10:355-364.

20. Bladen HA, Nylen MU, Glenner GG. The ultrastructure of human amyloid as revealed by the negative staining technique. J. Ultrastruct. Res. 1966; 14:449-459.

21. Cathcart ES, Cohen AS. The identification of two new components common to a variety of amyloid tissues. J. Clin. Invest. 1966; 45:995.

22. Harada M, Isersky C, Cuatrecasas P, et al. Human amyloid protein: chemical variability and homogeneity. J. Histochem. Cytochem. 1971; 19:1-15.

23. Eanes ED, Glenner GG. X-ray diffraction studies on amyloid filaments. J. Histochem. Cytochem. 1968; 16:673-677.

24. Burke MJ, Rougvie MA. Cross-β protein structures. I. Insulin fibrils. Biochemistry 1972; 11:2435-2439.

25. Blake CCF, Serpell LC. Synchrotron X-ray studies suggest that the core of the transthyretin amyloid fibril is a continuous β-sheet helix. Structure 1996; 4:989-998.

26. Sunde M, Serpell LC, Bartlam M, Fraser PE, Pepys MB, Blake CCF. Common core structure of amyloid fibrils by synchrotron X-ray diffraction. J. Mol. Biol. 1997; 273:729-739.

27. Pepys MB, Rademacher TW, Amatayakul-Chantler S, et al. Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. Proc. Natl. Acad. Sci. USA 1994; 91:5602-5606.

28. Tan SY, Pepys MB. Amyloidosis. Histopathology 1994; 25:403-414.

29. Pepys MB, Hawkins PN, Booth DR, et al. Human lysozyme gene mutations cause hereditary systemic amyloidosis. Nature 1993; 362:553-557.

30. Gorevic PD, Casey TT, Stone WJ, DiRaimondo CR, Prelli FL, Frangione B. Beta-2 microglobulin is an amyloidogenic protein in man. J. Clin. Invest. 1985; 76:2425-2429.

31. Saraiva MJM, Birken S, Costa PP, Goodman DS. Amyloid fibril protein in familial amyloid polyneuropathy, Portuguese type. Definition of molecular abnormality in transthyretin (prealbumin). J. Clin. Invest. 1984; 74:104-119.

32. Husebekk A, Skogen B, Husby G, Marhaug G. Transformation of amyloid precursor SAA to protein AA and incorporation in amyloid fibrils *in vivo*. Scand. J. Immunol. 1985; 21:283-287.

33. Mori H, Takio K, Ogawara M, Selkoe DJ. Mass spectrometry of purified amyloid β protein in Alzheimer's disease. J. Biol. Chem. 1992; 267:17082-17086.

34. Goldfarb LG, Brown P, Haltia M, Ghiso J, Frangione B. Synthetic peptides corresponding to different mutated regions of the amyloid gene in familial Creutzfeldt-Jakob disease show enhanced *in vitro* formation of morphologically different amyloid fibrils. Proc. Natl. Acad. Sci. USA 1993; 90:4451-4454.

35. Yamada T, Kluve-Beckerman B, Liepnieks JJ, Benson MD. Fibril formation from recombinant human serum amyloid A. Biochim. Biophys. Acta 1994; 1226:323-329.

36. Holck M, Husby G, Sletten K, Natvig JB. The amyloid P-component (Protein AP): an integral part of the amyloid substance? Scand. J. Immunol. 1979; 10:55-60.

37. Dalferes ER, Radhakrishnamurthy B, Berenson GS. Glycosaminoglycans in experimental amyloidosis. Proc. Soc. Exp. Biol. Med. 1968; 127:925-929.

38. Nelson SR, Lyon M, Gallagher JT, Johnson EA, Pepys MB. Isolation and characterization of the integral glycosaminoglycan constituents of human amyloid A and monoclonal light-chain amyloid fibrils. Biochem. J. 1991; 275:67-73.

39. Kisilevsky R. Review: amyloidogenesis-unquestioned answers and unanswered questions. J Struct Biol 2000; 130:99-108.

40. Magnus JH, Stenstad T. Proteoglycands and the extracellular matrix in amyloidosis. Amyloid: Int J Exp Clin Invest 1997; 4:121-134.

41. McCubbin WD, Kay CM, Narindrasorasak S, Kisilevsky R. Circular-dichroism studies on two murine serum amyloid A proteins. Biochem J 1988; 256:775-783.

42. Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, Hultin PG, Szarek WA. Arresting amyloidosis *in vivo* using small molecule anionic sulphonates or sulphates: implications for Alzheimer's disease. Nature Med. 1995; 1:143-148.

43. Ancsin JB, Kisilevsky R. The heparin/heparan sulfate-binding site on apo-serum amyloid A. Implications for the therapeutic intervention of amyloidosis. J Biol Chem 1999; 274:7172-7181.

44. Cathcart ES, Comerford FR, Cohen AS. Immunologic studies on a protein extracted from human secondary amyloid. N. Engl. J. Med. 1965; 273:143-146.

45. Binette JP, Calkins E. The purification of the P-component. Arthritis Rheum. 1973; 16:536.

46. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv. Immunol. 1983; 34:141-212.

47. Srinivasan N, White HE, Emsley J, Wood SP, Pepys MB, Blundell TL. Comparative analyses of pentraxins: implications for protomer assembly and ligand binding. Structure 1994; 2:1017-1027.

48. Osmand AP, Friedenson B, Gewurz H, Painter RH, Hofmann T, Shelton E. Characterisation of C-reactive protein and the complement subcomponent Clt as homologous proteins displaying cyclic pentameric symmetry (pentraxins). Proc. Natl. Acad. Sci. USA 1977; 74:739-743.

49. Emsley J, White HE, O'Hara BP, et al. Structure of pentameric human serum amyloid P component. Nature 1994; 367:338-345.

50. Hutchinson WL, Hohenester E, Pepys MB. Human serum amyloid P component is a single uncomplexed pentamer in whole serum. Mol. Med. 2000; 6:482-493.

51. Noursadeghi M, Bickerstaff MCM, Gallimore JR, Herbert J, Cohen J, Pepys MB. Role of serum amyloid P component in bacterial infection: protection of the host or protection of the pathogen. Proc. Natl. Acad. Sci. USA 2000; 97:14584-14589.

52. Hind CRK, Collins PM, Baltz ML, Pepys MB. Human serum amyloid P component, a circulating lectin with specificity for the cyclic 4,6-pyruvate acetal of galactose. Interactions with various bacteria. Biochem. J. 1985; 225:107-111.

53. Breathnach SM, Kofler H, Sepp N, et al. Serum amyloid P component binds to cell nuclei *in vitro* and to *in vivo* deposits of extracellular chromatin in systemic lupus erythematosus. J. Exp. Med. 1989; 170:1433-1438.

54. Hintner H, Booker J, Ashworth J, Auböck J, Pepys MB, Breathnach SM. Amyloid P component binds to keratin bodies in human skin and to isolated keratin filament aggregates *in vitro*. J. Invest. Dermatol. 1988; 91:22-28.

55. Pepys MB, Booth DR, Hutchinson WL, Gallimore JR, Collins PM, Hohenester E. Amyloid P component. A critical review. Amyloid: Int. J. Exp. Clin. Invest. 1997; 4:274-295.

56. Pepys MB, Dash AC, Richardson N, Munn EA, Feinstein A, Fletcher TC. Stable evolutionary conservation of complement-activating and related pentagonal serum proteins: C-reactive protein and protein SAP. J. Immunol. 1978; 120:1792.

57. Nelson SR, Tennent GA, Sethi D, et al. Serum amyloid P component in chronic renal failure and dialysis. Clin. Chim. Acta 1991; 200:191-200.

58. Hutchinson WL, Noble GE, Hawkins PN, Pepys MB. The pentraxins, C-reactive protein and serum amyloid P component, are cleared and catabolized by hepatocytes *in vivo*. J. Clin. Invest. 1994; 94:1390-1396.

59. Pepys MB, Baltz M, de Beer FC, et al. Biology of serum amyloid P component. Ann. N.Y. Acad. Sci. 1982; 389:286-297.

60. Skinner M, Sipe JD, Yood RA, Shirahama T, Cohen AS. Characterization of P-component (AP) isolated from amyloidotic tissue: half-life studies of human and murine AP. Ann. N.Y. Acad. Sci. 1982:190-198.

61. Baltz ML, Dyck RF, Pepys MB. Studies of the *in vivo* synthesis and catabolism of serum amyloid P component (SAP) in the mouse. Clin. Exp. Immunol. 1985; 59:235-242.

62. Hawkins PN, Wootton R, Pepys MB. Metabolic studies of radioiodinated serum amyloid P component in normal subjects and patients with systemic amyloidosis. In: Natvig JB, Forre O, Husby G, et al., eds. Amyloid and Amyloidosis 1990. Dordrecht: Kluwer Academic Publishers, 1991:254-257.

63. Hawkins PN, Myers MJ, Lavender JP, Pepys MB. Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component. Lancet 1988; i:1413-1418.

64. Hawkins PN, Tyrrell P, Jones T, et al. Metabolic and scintigraphic studies with radiolabeled serum amyloid P component in amyloidosis: applications to cerebral deposits and Alzheimer disease with positron emission tomography. Bull. Clin. Neurosci. 1991; 56:178-190.

65. Hutchinson WL, Herbert J, Hawkins PN, Pepys MB. Is serum amyloid P component required for *in vivo* amyloid deposition? Clin. Sci. 1996; 90 (Suppl. 34):33P.

66. Pepys MB. Amyloid P component: structure and properties. In: Marrink J, van Rijswijk MH, eds. Amyloidosis. Dordrecht: Martinus Nijhoff, 1986:43-50.

67. Kinoshita CM, Gewurz AT, Siegel JN, et al. A protease-sensitive site in the proposed Ca^{2+}-binding region of human serum amyloid P component and other pentraxins. Protein Sc. 1992; 1:700-709.

68. Tennent GA, Lovat LB, Pepys MB. Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer's disease and systemic amyloidosis. Proc. Natl. Acad. Sci. USA 1995; 92:4299-4303.

69. Botto M, Hawkins PN, Bickerstaff MCM, et al. Amyloid deposition is delayed in mice with targeted deletion of the serum amyloid P component gene. Nature Med. 1997; 3:855-859.

70. Bickerstaff MCM, Botto M, Hutchinson WL, et al. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. Nature Med. 1999; 5:694-697.

71. Bickerstaff MCM. Physiological and pathophysiological roles of serum amyloid P component and the acute phase response. London: London, 1999.

72. Butler PJG, Tennent GA, Pepys MB. Pentraxin-chromatin interactions: serum amyloid P component specifically displaces H1-type histones and solubilizes native long chromatin. J. Exp. Med. 1990; 172:13-18.

73. Booth DR, Sunde M, Bellotti V, et al. Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. Nature 1997; 385:787-793.

74. Jiménez JL, Guijarro JI, Orlova E, et al. Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing. EMBO J. 1999; 18:815-821.

75. Kelly JW. Alternative conformations of amyloidogenic proteins govern their behaviour. Curr. Opin. Struct. Biol. 1996; 6:11-17.

76. Dobson CM, Ellis RJ, Fersht AR. Protein Misfolding and Disease. In: Zeki S, ed. Phil. Trans. R. Soc. Lond. B. Vol. 356. London: The Royal Society, 2001:127-227.

77. Kuwajima K. The molten globule state as a clue for understanding the folding and cooperativity of globular-protein structure. Proteins: Struct. Funct. Genetics 1989; 6:87-103.

78. Glenner GG. Amyloid deposits and amyloidosis - the β-fibrilloses. I. N. Engl. J. Med. 1980; 302:1283-1292.

79. Glenner GG. Amyloid deposits and amyloidosis - the β-fibrilloses. II. N. Engl. J. Med. 1980; 302:1333-1343.

80. Booth DR, Booth SE, Gillmore JD, Hawkins PN, Pepys MB. SAA_1 alleles as risk factors in reactive systemic AA amyloidosis. Amyloid: Int. J. Exp. Clin. Invest. 1998; 5:262-265.

81. Holmgren G, Haettner E, Nordensen I, Sandgren O, Steen L, Lundgren E. Homozygosity for the transthyretin-met30-gene in two Swedish sibs with familial amyloidotic polyneuropathy. Clin. Genet. 1988; 34:333-338.

82. Maury CPJ, Kere J, Tolvanen R, de la Chapelle A. Homozygosity for Finnish type familial amyloidosis is associated with severe nephropathy. J. Rheumatol. 1993; 20:191.

83. McCutchen SL, Lai Z, Miroy GJ, Kelly JW, Colon W. Comparison of lethal and nonlethal transthyretin variants and their relationship to amyloid disease. Biochemistry 1995; 34:13527-13536.

84. Axelrad MA, Kisilevsky R, Willmer J, Chen SJ, Skinner M. Further characterisation of amyloid-enhancing factor. Lab. Invest. 1982; 47:139-146.

85. Gejyo F, Yamada T, Odani S, et al. A new form of amyloid protein associated with chronic hemodialysis was identified as β_2-microglobulin. Biochem. Biophys. Res. Commun. 1985; 129:701-706.

86. Westermark P, Sletten K, Johansson B, Cornwell GG. Fibril in senile systemic amyloidosis is derived from normal transthyretin. Proc. Natl. Acad. Sci. USA 1990; 87:2843-2845.

87. Hurle MR, Helms LR, Li L, Chan W, Wetzel R. A role for destabilizing amino acid replacements in light-chain amyloidosis. Proc. Natl. Acad. Sci. USA 1994; 91:5446-5450.

88. Benson MD, Uemichi T. Transthyretin amyloidosis. Amyloid: Int. J. Exp. Clin. Invest. 1996; 3:44-56.

89. Benson MD, Liepnieks J, Uemichi T, Wheeler G, Correa R. Hereditary renal amyloidosis associated with a mutant fibrinogen α-chain. Nature Genetics 1993; 3:252-255.

90. Soutar AK, Hawkins PN, Vigushin DM, et al. Apolipoprotein AI mutation Arg-60 causes autosomal dominant amyloidosis. Proc. Natl. Acad. Sci. USA 1992; 89:7389-7393.

91. Jensson O, Palsdottir A, Thorsteinsson L, Arnason A. The saga of cystatin C gene mutation causing amyloid angiopathy and brain hemorrhage - clinical genetics in Iceland. Clin. Genet. 1989; 36:368-377.

92. Maury CPJ. Gelsolin-related amyloidosis. Identification of the amyloid protein in Finnish hereditary amyloidosis as a fragment of variant gelsolin. J. Clin. Invest. 1991; 87:1195-1199.

93. Abrahamson M, Grubb A. Increased body temperature accelerates aggregation of the Leu-68-Gln mutant cystatin C, the amyloid-forming protein in hereditary cystatin C amyloid angiopathy. Proc. Natl. Acad. Sci. USA 1994; 91:1416-1420.

94. Maury CPJ, Nurmiaho-Lassila E-L, Rossi H. Amyloid fibril formation in gelsolin-derived amyloidosis. Definition of the amyloidogenic region and evidence of accelerated amyloid formation of mutant Asn-187 and Tyr-187 gelsolin peptides. Lab. Invest. 1994; 70:558-564.

95. Connors LH, Richardson AM, Théberge R, Costello CE. Tabulation of transthyretin (TTR) variants as of 1/1/2000. Amyloid 2000; 7:54-69.

96. Costa PP, Figueira AS, Bravo FR. Amyloid fibril protein related to prealbumin in familial amyloidotic polyneuropathy. Proc. Natl. Acad. Sci. USA 1978; 75:4499-4503.

97. Blake CCF, Koenig DF, Mair GA, North ACT, Phillips DC, Sarma VR. Structure of hen egg-white lysozyme. Nature 1965; 206:757-761.

98. Rupley JA, Banerjee SK, Kregar I, Lapanje S, Shrake AF, Turk V. Structure and chemistry of lysozyme: pH-rate profile, calorimetric studies, and computations on exposure to solvent. In: Osserman EF, Canfield RE, Beychok S, eds. Lysozyme. New York: Academic Press, 1974:251-267.

99. Artymiuk PJ, Blake CCF. Refinement of human lysozyme at 1.5Å resolution analysis of non-bonded and hydrogen-bond interactions. J. Mol. Biol. 1981; 152:737-762.

100. Radford SE, Dobson CM. Insights into protein folding using physical techniques: studies of lysozyme and α-lactalbumin. Phil. Trans. R. Soc. Lond. B 1995; 348:17-25.

101. Martinez JC, Pisabarro MT, Serrano L. Obligatory steps in protein folding and the conformational diversity of the transition state. Nature Struct. Biol. 1998; 5:721-729.

102. Dobson CM. Finding the right fold. Nature Struct. Biol. 1995; 2:513-517.

103. Funahashi J, Takano K, Ogasahara K, Yamagata Y, Yutani K. The structure, stability and folding process of amyloidogenic mutant human lysozyme. J. Biochem. 1996; 120:1216-1223.

104. Booth DR, Bellotti V, Sunde M, et al. Molecular mechanisms of amyloid fibril formation: the lysozyme model. Clin. Sci. 1996; 90 (Suppl. 34):1P.

105. Glenner GG, Eanes ED, Bladen HA, Linke RP, Termine JD. β-pleated sheet fibrils. A comparison of native amyloid with synthetic protein fibrils. Prog. Histochem. Cytochem. 1974; 22:1141-1158.

106. Dobson CM. Protein misfolding, evolution and disease. Trends Biochem. Sci. 1999; 24:329-332.

107. Buck FS, Koss MN, Sherrod AE, Wu A, Takahasi M. Ethnic distribution of amyloidosis: an autopsy study. Mod Pathol 1989; 2:372-377.

108. Holmgren G, Costa PM, Andersson C, et al. Geographical distribution of TTR met30 carriers in northern Sweden: discrepancy between carrier frequency and prevalence rate. J. Med. Genet. 1994; 31:351-354.

109. Kisilevsky R, Boudreau L. Kinetics of amyloid deposition. I. The effects of amyloid-enhancing factor and splenectomy. Lab. Invest. 1983; 48:53.

110. Ranløv P. The adoptive transfer of experimental mouse amyloidosis by intravenous injections of spleen cell extracts from casein-treated syngeneic donor mice. Acta Path. Microbiol. Scand. 1967; 70:321-335.

111. Kisilevsky R, Axelrad M, Corbett W, Brunet S, Scott F. The role of inflammatory cells in the pathogenesis of amyloidosis. Lab. Invest. 1977; 37:544-553.

112. Baltz ML, Caspi D, Hind CRK, Feinstein A, Pepys MB. Isolation and characterisation of amyloid enhancing factor. In: Glenner GG, Osserman EF, Benditt EP, Calkins E, Cohen AS, Zucker-Franklin D, eds. Amyloidosis. New York: Plenum Press, 1986:115-121.

113. Lundmark K, Westermark GT, Nystrom S, Murphy CL, Soloman A, Westermark P. Transmissibility of systemic amyloidosis by a prion-like mechanism. Proc Natl Acad Sci USA 2002; 99:6979-84.

114. Cui D, Hiroo K, Takahashi M, Hoshii Y, Setoguchi M. Acceleration of murine AA amyloidosis by oral administration of amyloid fibrils extracted from different species. Pathol int 2002; 52:40-45.

115. Janigan DT, Druet RL. Experimental amyloidosis. Role of antigenicity and rapid induction. Am. J. Pathol. 1966; 48:1013-1025.

116. Hawkins PN, Pepys MB. A primed state exists *in vivo* following histological regression of amyloidosis. Clin. Exp. Immunol. 1990; 81:325-328.

117. Hawkins PN. The diagnosis, natural history and treatment of amyloidosis. The Goulstonian Lecture 1995. J. R. Coll. Physicians Lond. 1997; 31:552-560.

118. Lorenzo A, Razzaboni B, Weir GC, Yankner BA. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. Nature 1994; 368:756-760.

119. Simmons LK, May PC, Tomaselli KJ, et al. Secondary structure of amyloid beta peptide correlates with neurotoxic activity *in vivo*. Mol. Pharmacol. 1994; 45:373-379.

120. Bucciantini M, Giannoni E, Chitti F, et al. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 2002; 416:483-484.

121. Pepys MB, Hawkins PN. Amyloidosis. In: Warrell DA, Cox TM, Firth JD, eds. Oxford Textbook of Medicine. Oxford: Oxford University Press, 2001:(in press).

122. Hawkins PN. Studies with radiolabelled serum amyloid P component provide evidence for turnover and regression of amyloid deposits *in vivo*. Clin. Sci. 1994; 87:289-295.

123. Lowenstein J, Gallo G. Remission of the nephrotic syndrome in renal amyloidosis. N. Engl. J. Med. 1970; 282:128-132.

124. Paraf A, Caste T, Rautureau J. La regression de l'amylose. Disparition d'une amylose hepatique massive apres nephrectomie pour cancer. Presse Med. 1970; 78:547-548.

125. Cohen HJ, Lessin LS, Hallal J, Burkholder P. Resolution of primary amyloidosis during chemotherapy. Ann. Intern. Med. 1975; 82:466-473.

126. Dikman SH, Kahn T, Gribetz D, Churg J. Resolution of renal amyloidosis. Am. J. Med. 1977; 63:430-433.

127. Falck HM, Törnroth T, Skrifvars B, Wegelius O. Resolution of renal amyloidosis secondary to rheumatoid arthritis. Acta Med. Scand. 1979; 205:651-656.

128. Kyle RA, Wagoner RD, Holley KE. Primary systemic amyloidosis. Resolution of the nephrotic syndrome with melphalan and prednisolone. Arch. Intern. Med. 1982; 142:1445-1447.

129. Gertz MA, Kyle RA. Response of primary hepatic amyloidosis to melphalan and prednisone: a case report and review of the literature. Mayo Clin. Proc. 1986; 61:218-223.

130. Schattner A, Varon D, Green L, Hurwitz N, Bentwich Z. Primary amyloidosis with unusual bone involvement: reversibility with melphalan, prednisone, and colchicine. Am. J. Med. 1986; 86:347-348.

131. Zemer D, Pras M, Sohar E, Modan M, Cabili S, Gafni J. Colchicine in the prevention and treatment of amyloidosis of familial Mediterranean fever. N. Engl. J. Med. 1986; 314:1001-1005.

132. Sezer O, Schmid P, Shweigert M, et al. Rapid reversal of nephrotic syndrome due to primary systemic AL amyloidosis after VAD and subsequent high-dose

chemotherapy with autologous stem cell support. Bone Marrow Transplant 1999; 23:967-969.

133. Gillmore JD, Booth DR, Rela M, et al. Curative hepatorenal transplantation in systemic amyloidosis caused by the Glu526Val fibrinogen α-chain variant in an English family. Q.J. Med. 2000; 93:269-275.

134. Gillmore JD, Lovat LB, Persey MR, et al. Cumulative SAA production and outcome of AA amyloidosis. In: Kyle RA, Gertz MA, eds. Amyloid and Amyloidosis 1998. Pearl River, New York: Parthenon Publishing, 1999:402-404.

135. Husby G, Sletten K, Michaelsen TE, Natvig JB. Antigenic and chemical characterization of non-immunoglobulin amyloid fibril proteins. Scand. J. Immunol. 1972; 1:393-400.

136. Levin M, Franklin EC, Frangione B, Pras M. The amino acid sequence of a major non-immunoglobulin component of some amyloid fibrils. J. Clin. Invest. 1972; 51:2773-2776.

137. Pras M, Schubert M, Zucker-Franklin D, Rimon A, Franklin EC. The characterisation of soluble amyloid prepared in water. J. Clin. Invest. 1968; 47:924-933.

138. Pras M, Zucker-Franklin D, Rimon A, Franklin EC. Physical, chemical, and ultrastructural studies of water-soluble human amyloid fibrils. Comparative analyses of nine amyloid preparations. J. Exp. Med. 1969; 130:777-784.

139. Benditt EP, Eriksen N. Amyloid protein SAA is associated with high density lipoprotein from human serum. Proc. Natl. Acad. Sci. USA 1977; 74:4025-4028.

140. Anders RF, Natvig J, Michaelsen TE, Husby G. Isolation and characterisation of amyloid related SAA as a small molecular weight protein. Scand. J. Immunol. 1975; 4:397-401.

141. Eriksen N, Benditt EP. Isolation and characterisation of the amyloid-related apoprotein (SAA) from human high density lipoprotein. Proc. Natl. Acad. Sci. USA 1980; 77:6860-6864.

142. Parmelee DC, Titani K, Ericsson LH, Eriksen N, Benditt EP, Walsh KA. Amino acid sequence of amyloid-related apoprotein (apoSAA$_1$) from human high density lipoprotein. Biochemistry 1982; 21:3298-3303.

143. Pepys MB. Acute phase proteins. In: Delves PJ, Roitt IM, eds. Encyclopedia of Immunology. London: Academic Press Ltd, 1998:18-20.

144. Yamada T. Serum amyloid A (SAA): a concise review of biology, assay methods and clinical usefulness. Clin. Chem. Lab. Med. 1999; 37:381-388.

145. Morrow JF, Stearman RS, Peltzman CG, Potter DA. Induction of hepatic synthesis of serum amyloid A protein and actin. Proc. Natl. Acad. Sci. USA 1981; 78:4718-4722.

146. de Beer FC, Mallya RK, Fagan EA, Lanham JG, Hughes GRV, Pepys MB. Serum amyloid A protein (SAA) concentration in inflammatory diseases and its

relationship to the incidence of reactive systemic amyloidosis. Lancet 1982; ii:231-234.

147. Schnitzer TJ, Ansell BM. Amyloidosis in juvenile chronic polyarthritis. Arthritis Rheum. 1977; 20:245-252.

148. Gratacos J, Orellana C, Sanmarti R, et al. Secondary amyloidosis in ankylosing spondylitis. A systematic survey of 137 patients using abdominal fat aspiration. J. Rheumatol. 1997; 24:912-915.

149. Faulkes DJ, Betts JC, Woo P. Characterization of five human serum amyloid A_1 alleles. Amyloid: Int. J. Exp. Clin. Invest. 1994; 1:255-262.

150. Cazeneuve C, Ajrapetyan H, Papin S, et al. Identification of *MEFV*-independent modifying genetic factors for familial Mediterranean fever. Am. J. Hum. Genet. 2000; 67:1136-1143.

151. Baba S, Masago SA, Takahashi T, et al. A novel allelic variant of serum amyloid A, SAA1γ: genomic evidence, evolution, frequency, and implication as a risk factor for reactive systemic AA-amyloidosis. Hum. Med. Genet. 1995; 4:1083-1087.

152. Yamada T, Okunda Y, Takasugi K, Igari J. Relative serum amyloid A (SAA) values: the influence of SAA1 genotypes and corticosteroid treatment in japanese patients with rheumatoid arthritis. Ann Rheum Dis 2001; 60:124-127.

153. Faulkes DJ, Woo P. Do alleles at the serum amyloid A locus influence susceptibility to reactive amyloidosis in systemic onset juvenile chronic arthritis? Amyloid: Int. J. Exp. Clin. Invest. 1997; 4:75-79.

154. Osserman EF, Takatsuki K, Talal N. The pathogenesis of "amyloidosis". Studies on the role of abnormal gammaglobulins and gammaglobulin fragments of the Bence Jones (L-polypeptide) types in the pathogenesis of "primary" and "secondary" amyloidosis associated with plasma cell myeloma. Semin. Hematol. 1964; 1:3.

155. Linder J, Silberman HR, Croher BP. Amyloidosis complicating hairy cell leukaemia. Am. J. Clin. Pathol. 1982; 78:864-867.

156. Glenner GG, Ein D, Eaves ED, Bladen HA, Terry W, Page DL. Creation of "amyloid" fibrils from Bence Jones protein *in vitro*. Science 1971; 174:712-714.

157. Glenner GG, Terry W, Harada M, Isersky C, Page D. Amyloid fibril proteins: proof of homology with immunoglobulin light chains by sequence analyses. Science 1971; 172:1150-1151.

158. Glenner GG, Ein D, Terry WD. The immunoglobulin origin of amyloid. Am. J. Med. 1972; 52:141-147.

159. Solomon A, Frangione B, Franklin EC. Bence Jones proteins and light chains of immunoglobulins. Preferential association of the $V_{\gamma VI}$ subgroup of human light chains with amyloidosis AL(γ). J. Clin. Invest. 1982; 70:453-460.

160. Ozaki S, Abe M, Wolfenbarger D, Weiss DT, Solomon A. Preferential expression of human lambda-light-chain variable-region subgroups in multiple

myeloma, AL amyloidosis, and Waldenstrom's macroglobulinemia. Clin Immunol Immunopathol 1994; 71:183-189.

161. Comenzo RL, Zhang Y, Martinez, Osman CK, Herrera GA. The tropism of organ involvement in primary systemic amyloidosis: contributions of Ig VL germ line gene use and clonal plasma cell burden. Blood 2001; 98:714-720.

162. Pruzanski W, Katz A, Nyburg SC, Freedman MH. *In vitro* production of an amyloid-like substance from γ3 heavy chain disease protein. Immunol. Commun. 1974; 3:469-476.

163. Eulitz M, Weiss DT, Solomon A. Immunoglobulin heavy-chain-associated amyloidosis. Proc. Natl. Acad. Sci. USA 1990; 87:6542-6546.

164. Assenat H, Calemard E, Charra B, Laurent G, Terrat JC, Vanel T. Hémodialyse, syndrome du canal carpien et substance amyloide. Nouv. Presse. Med. 1980; 9:1715.

165. Charra B, Calemard E, Uzan M, Terrat JC, Vanel T, Laurent G. Carpal tunnel syndrome, shoulder pain and amyloid deposits in long-term haemodialysis patients. Proc. EDTA-ERA 1984; 21:291-295.

166. Hillion D, Villeboeuf J, Hillion Y, Nakamura S, Bruet A. Appearance of systemic amyloidosis in a chronic hemodialysis patient. Nephron 1985; 41:127-128.

167. Miguel Alonso JL, Cruz A, Lopez Revuelta L, et al. Continuous ambulatory peritoneal dialysis does not prevent the development of dialysis-associated amyloidosis. Nephron 1989; 53:389-390.

168. Jadoul M, Noel H, van Ypersele de Strihou C. β_2-Microglobulin amyloidosis in a patient treated exclusively by continuous ambulatory peritoneal dialysis. Am. J. Kidney Dis. 1990; 15:86-88.

169. Floege J, Ketteler M. Beta2-microglobulin-derived amyloidosis: an update. Kidney Int Suppl 2001; 78:S164-171.

170. Bardin T, Zingraff J, Kuntz D, Drüeke T. Dialysis-related amyloidosis. Nephrol. Dial. Transplant. 1986; 1:151-154.

171. Cornwell GG, Murdoch WL, Kyle RA, Westermark P, P. P. Frequency and distribution of senile cardiovascular amyloid. A clinicopathologic correlation. Am J Med 1983; 75:618-623.

172. Kyle RA, Spittell PC, Gertz MA, et al. The premortem recognition of systemic senile amyloidosis with cardiac involvement. Am J Med 1996; 101:395-400.

173. Cornwell GG, Westermark P, Natvig JB, Murdoch W. Senile cardiac amyloid: evidence that fibrils contain a protein immunologically related to prealbumin. Immunology 1981; 44:447-452.

174. Bardin T, Kuntz D, Zingraff J, Voidin M-C, Zelmar A, Lansamer J. Synovial amyloidosis in patients undergoing long-term hemodialysis. Arthritis Rheum. 1985; 28:1052-1058.

175. Johansson B, Westermark P. Senile systemic amyloidosis: a clinico-pathological study of twelve patients with massive amyloid infiltration. Int J Cardiol 1991 Jul;32(1):83-92 1991; 32:83-92.

176. Pomerance A. The pathology of senile cardiac amyloidosis. J. Pathol. Bacteriol. 1966; 91:357.

177. Pitkanen P, Westermark P, Cornwell GG. Senile systemic amyloidosis. Am. J. Pathol. 1984; 117:391-399.

178. Van Allen M, Frohlich J, Davis J. Inherited predisposition to generalized amyloidosis. Neurology 1969; 19:10-25.

179. Ghiso J, Jensson O, Frangione B. Amyloid fibrils in hereditary cerebral hemorrhage with amyloidosis of Icelandic type is a variant of γ-trace basic protein (cystatin C). Proc. Natl. Acad. Sci. USA 1986; 83:2974-2978.

180. Maury CPJ, Alli K, Baumann M. Finnish hereditary amyloidosis. Amino acid sequence homology between the amyloid fibril protein and human plasma gelsoline. FEBS Lett. 1990; 260:85-87.

181. Nichols WC, Dwulet FE, Liepnieks J, Benson MD. Variant apolipoprotein AI as a major constituent of a human hereditary amyloid. Biochem. Biophys. Res. Commun. 1988; 156:762-768.

182. Benson MD, Liepnieks JJ, Yazaki M, et al. A new human hereditary amyloidosis: the result of a stop-codon mutation in the apolipoprotein AII gene. Genomics 2001; 72:272-277.

183. Sequeiros J, Saraiva MJM. Onset in the seventh decade and lack of symptoms in heterozygotes for the TTR^{Met30} mutations in hereditary amyloid neuropathy-type I (Portuguese, Andrade). Am. J. Med. Genet. 1987; 27:345-357.

184. Gudmundsson G, Hallgrimsson J, Jonasson TA, Bjarnason O. Hereditary cerebral haemorrhage with amyloidosis. Brain 1972; 95:387-404.

185. Wattendorf AR, Bots GTAM, Went LN, Erdtz LJ. Familial cerebral amyloid angiopathy presenting as recurrent cerebral haemorrhage. J. Neurol. Sci. 1982; 55:121-135.

186. Kiuru S. Gelsolin-related familial amyloidosis, Finnish type (FAF), and its variants found worldwide. Amyloid: Int. J. Exp. Clin. Invest. 1998; 5:55-66.

187. Ostertag B. Demonstration einer eigenartigen familiaren paraamyloidose. Zentralbl. Aug. Pathol. 1932; 56:253-254.

188. Nichols WC, Gregg RE, Brewer HBJ, Benson MD. A mutation in apolipoprotein A-I in the Iowa type of familial amyloidotic polyneuropathy. Genomics 1990; 8:318-323.

189. Pasternak S, White VA, Gascoyne RD, Perry SR, L. JR, Rootman J. Monoclonal origin of localised orbital amyloidosis detected by molecular analysis. Br J Ophthalmol 1996; 80:1013-1017.

190. Hamidi Asl K, Liepnieks JJ, Bihrle R, Benson MD. Local synthesis of amyloid fibril precursors in AL amyloidosis of the urinary tract. Amyloid: Int J Exp clin 1998; 5:49-54.

191. Hamidi Asl K, Leipnieks JJ, Nakamura A, Benson MD. Organ-specific (localized) synthesis of Ig light chain amyloid. J Immunol 1999; 162:5556-5560.

192. Livneh A, Shtrasburg S, Martin BM, Baniel J, Gal R, Pras M. Light chain amyloidosis of the urinary bladder. A site restricted deposition of an externally produced immunoglobulin. J Clin Pathol 2001; 54:920-923.

193. Westermark P, Sletten K, Pitkanen P, Natvig J, Lindholm C. Localised laryngeal amyloidosis: partial characterization of an amyloid fibril protein AL. Mol Immunol 1982; 19:447-450.

194. Lewis JE, Olsen KD, Kurtin PJ, Kyle RA. Laryngeal amyloidosis: a clinicopathologic and immunohistochemical review. Otolaryngol Head Neck Surg 1992; 106:372-377.

195. Murdoch IE ST, Moseley I, Hawkins PN, Pepys MB, Tan SY, Garner A, Wright JE. Primary localised amyloidosis of the orbit. Br J Ophthalmol 1996; 80:1083-1086.

196. Woollons A, Black MM. Nodular localized primary cutaneous amyloidosis: a long-term follow-up study. Br J Dermatol 2001; 145:105-109.

197. Thompson LD, Derringer GA, Wenig BM. Amyloidosis of the larynx: a clinicopathologic study of 11 cases. Mod pathol 2000; 13:528-535.

198. Utz JP, Swensen SJ, Gertz MA. Pulmonary amyloidosis. The Mayo Clinic experience from 1980 to 1993. Ann Intern Med 1996; 124:07-413.

199. Tirzaman O, Wahner-Roedler DL, Malek RS, Sebo TJ, Li CY, Kyle RA. Primary localized amyloidosis of the urinary bladder: a case series of 31 patients. Mayo Clin Proc 2000; 75:1264-1268.

200. Berg AM, Troxler RF, Grillone G, et al. Localized amyloidosis of the larynx: evidence for light chain composition. Ann Otol Rhinol Laryngol 1993; 102:884.

201. Duchen LW. Current status review: cerebral amyloid. Int. J. Exp. Pathol. 1992; 73:535-550.

202. Small DH, Mok SS, Bornstein JC. Alzheimer's disease and Abeta toxicity: from top to bottom. Nat Rev Neurosci 2001; 2:595-598.

203. Glenner GG, Wong CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochem. Biophys. Res. Commun. 1984; 122:1131-1135.

204. Coria F, Castano EM, Frangione B. Brain amyloid in normal ageing and cerebral amyloid angiopathy is antigenically related to Alzheimer's disease β-protein. Am. J. Pathol. 1987; 129:422-428.

205. Delabar J-M, Goldgaber D, Lamour Y, et al. β amyloid gene duplication in Alzheimer's disease and karyotypically normal Down syndrome. Science 1987; 235:1390-1392.

206. Hawkins PN. Diagnosis and monitoring of amyloidosis. In: Husby G, ed. Baillière's Clinical Rheumatology: Reactive Amyloidosis and the Acute Phase Response. Vol. 8. London: Baillière Tindall, 1994:635-659.

207. Gertz MA, Kyle RA. Hepatic amyloidosis (primary [AL], immunoglobulin light chain): the natural history in 80 patients. Am. J. Med. 1988; 85:73-80.

208. McPherson RA, Orstad JW, Ugoretz RJ, Wolf PL. Coagulation in amyloidosis: combined deficiency of factors IX and X. Am. J. Hematol. 1977; 3:225-235.

209. Triplett DA, Bang NU, Harms CS, Benson MD, Miletich JP. Mechanism of acquired factor X deficiency in primary amyloidosis. Blood 1977; 50 (Suppl. 1):285.

210. Griepp PR, Kyle RA, Bowie EJW. Factor X deficiency in amyloidosis: a critical review. Am. J. Hematol. 1981; 11:443-450.

211. Mumford AD, O'Donnell J, Gillmore JD, Manning RA, Hawkins PN, Laffan M. Bleeding symptoms and coagulation abnormalities in 337 patients with AL amyloidosis. Br. J. Haematol. 2000; 110:454-460.

212. Westermark P, Stenkvist B. A new method for the diagnosis of systemic amyloidosis. Arch. Intern. Med. 1973; 132:522-523.

213. Delgado AW, Mosqueda A. A highly sensitive method for diagnosis of secondary amyloidosis by labial salivary gland biopsy. J. Oral Pathol. Med. 1989; 18:310-314.

214. Shah PL, Gillmore JD, Copley SJ, et al. The importance of complete screening for amyloid fibril type and systemic disease in patients with amyloidosis in the respiratory tract. Sarcoidosis Vasc Diffuse Lung Dis 2002; 19:134-142.

215. Linke RP, Gärtner HV, Michels H. High-sensitivity diagnosis of AA amyloidosis using Congo red and immunohistochemistry detects missed amyloid deposits. J. Histochem. Cytochem. 1995; 43:863-869.

216. Tennent GA, Cafferty KD, Pepys MB, Hawkins PN. Congo red overlay immunohistochemistry aids classification of amyloid deposits. In: Kyle RA, Gertz MA, eds. Amyloid and Amyloidosis 1998. Pearl River, New York: Parthenon Publishing, 1999:160-162.

217. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with ^{123}I-labeled serum amyloid P component. N. Engl. J. Med. 1990; 323:508-513.

218. Hawkins PN, Vigushin DM, Richardson S, et al. Natural history and regression of amyloidosis. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. Amyloid and Amyloidosis 1993. Pearl River, New York: Parthenon Publishing, 1994:638-641.

219. Gertz MA, Kyle RA, O'Fallon WM. Dialysis support of patients with primary systemic amyloidosis. A study of 211 patients. Arch Intern Med 1992; 152:2245-2250.

220. Moroni G, Banfi G, Montoli A, et al. Chronic dialysis in patients with systemic amyloidosis: the experience in Northern Italy. Clin. Nephrol. 1992; 38:81-85.

221. Martinez-Vea A, Garcia C, Carreras M, Revert L, Oliver JA. End-stage renal disease in systemic amyloidosis: clinical course and outcome on dialysis. Am J Nephrol 1990; 10:283-289.

222. Gurland HJ, Chantler C. Combined report on regular dialysis and transplantation in Europe, VI. Proc Eur Dail Transplant Assoc 1976; 13.

223. Kilicturgay S, Haberal M. Transplantation for renal amyloidosis. Ren Fail 1993; 15:629-633.

224. Isoniemi H, Eklund B, Hockestedt K, Salmela K, Ahonen J. Renal transplantation in amyloidosis. Transplant Proc 1989; 21:2039-2040.

225. Jacob ET, Bar-Nathan N, Shapira Z, Gafni J. Renal transplantation in the amyloidosis of familial Mediterranean fever. Experience of ten cases. Arch. Intern. Med. 1979; 139:1135-1138.

226. Hartmann A, Holdaas H, Fauchald P, et al. Fifteen years' experience with renal transplantation in systemic amyloidosis. Transpl Int 1992; 5:15-18.

227. Shmueli D, Lustig S, Nakache R, et al. Renal transplantation in patients with amyloidosis due to familial Mediterranean fever. Transplant Proc 1992; 24:1783-1784.

228. Pasternack A, Ahonen J, Kuhlback B. Renal transplantation in 45 patients with amyloidosis. Transplantation 1986; 42:598-601.

229. Gillmore JD, Madhoo S, Pepys MB, Hawkins PN. Renal transplantation for amyloid end-stage renal failure - insights from serial serum amyloid P component scintigraphy. Nucl. Med. Commun. 2000; 21:735-740.

230. Persey MR, Booth DR, Booth SE, van Zyl-Smit R, Hawkins PN, Pepys MB. A new deletion mutation of the apolipoprotein AI gene causing hereditary amyloidosis. Clin. Sci. 1996; 90 (Suppl. 34):33P.

231. Dubrey SW, Burke MM, Khaghani A, Hawkins PN, Yacoub MH, Banner NR. Long term results of heart transplantation in patients with amyloid heart disease. Heart 2001; 85:202-207.

232. Merlini G. Symposium on Amyloid Cardiomyopathy: Diagnosis and Treatment, Pavia, September 21, 1996 [Meeting Report]. Amyloid: Int. J. Exp. Clin. Invest. 1997; 4:296-299.

233. Hosenpud JD, DeMarco T, Frazier OH, et al. Progression of systemic disease and reduced long-term survival in patients with cardiac amyloidosis undergoing heart transplantation. Circulation 1991; 84:338-343.

234. Hall R, Hawkins PN. Cardiac transplantation for AL amyloidosis: a personal account. B.M.J. 1994; 309:1135-1137.

235. Hawkins PN, Pepys MB. Role of heart transplantation in systemic amyloidosis. J. Heart Lung Transplant. 1996; 15:321-322.

236. Nowak G, Westermark P, Wernerson A, Herlenius G, Sletten K, Ericzon BG. Liver transplantation as rescue treatment in a patient with primary AL kappa amyloidosis. Transpl Int 2000; 13:92-97.

237. Stangou A, Heaton ND, Rela M, et al. Liver transplantation for end stage hepatic amyloidosis. Amyloid: Int. J. Exp. Clin. Invest. 2001; 8:151.

238. Lovat LB, Madhoo S, Pepys MB, Hawkins PN. Long term survival in systemic AA amyloidosis complicating Crohn's disease. Gastroenterology 1997; 112:1362-1365.

239. Greenstein AJ, Sachar DB, Panday AK, et al. Amyloidosis and inflammatory bowel disease. A 50-year experience with 25 patients. Medicine(Baltimore) 1992; 71:261-270.

240. Kazes I, Deray G, Jacobs C. Castleman disease and renal amyloidosis. Ann. Intern. Med. 1995; 122:395-396.

241. Mehta HJ, Talwalkar NC, Merchant MR, et al. Pattern of renal amyloidosis in western India. A study of 104 cases. J Assoc Physicians India 1990; 38:407-410.

242. Myllykangas-Luosujärvi R, Aho K, Kautiainen H, Hakala M. Amyloidosis in a nationwide series of 1666 subjects with rheumatoid arthritis who died during 1989 in Finland. Rheumatology 1999; 38:499-503.

243. Filipowicz-Sosnowska AM, Roztropowicz-Denisiewicz K, Rosenthal CJ, Baum J. The amyloidosis of juvenile rheumatoid arthritis - comparative studies in Polish and American children. I. Levels of serum SAA protein. Arthritis Rheum. 1978; 21:699-703.

244. Svantesson H, Akesson A, Eberhardt K, Elborgh R. Prognosis in juvenile rheumatoid arthritis with systemic onset. A follow-up study. Scand. J. Rheumatol. 1983; 12:139-144.

245. Laiho K, Tiitinen S, Kaarela K, Helin H, Isomaki H. Secondary amyloidosis has decreased in patients with inflammatory joint disease in Finland. Clin Rheumatol 1999; 18:122-123.

246. Persey MR, Lovat LB, Madhoo S, Gallimore JR, Pepys MB, Hawkins PN. Dynamics of AA amyloidosis in RA and JCA. Br. J. Rheumatol. 1996; 35 (Suppl. 1):76.

247. Queffeulou G, Berenbaum F, Michel C, Mougenot B, Mignon F, Berentbaum F. AA amyloidosis in systemic lupus erythematosus: an unusual complication. Nephrol Dial Transplant 1998; 13:1846-1848.

248. Rivera F, Gil CM, Gil MT, Batlle-Gualda E, Trigueros M, Olivares J. Vascular renal AA amyloidosis in adult Still's disease. Nephrol Dial Transplant 1997; 12:1714-1716.

249. Rosenthal T, Bank H, Aladjem M, David R, Gafni J. Systemic amyloidosis in Behcet's disease. Ann Intern Med 1975; 83:220-223.

250. Monteagudo M, Vidal G, Andreu J, et al. Giant cell (temporal) arteritis and secondary renal amyloidosis: report of 2 cases. J Rheumatol 1997; 24:605-607.

251. Yorioka N, Taniguchi Y, Okushin S, Amimoto D, Kataoka K, Taguchi T. Classic polyarteritis nodosa associated with renal amyloidosis. Nephron 1999; 82:93-94.

252. Escriba A, Morales E, Albizua E, et al. Secondary (AA-type) amyloidosis in patients with polymyalgia rheumatica. Am J Kidney Dis 2000; 35:137-140.

253. Ahmed Q, Chung-Park M, Mustafa K, Khan MA. Psoriatic spondyloarthropathy with secondary amyloidosis. J Rheumatol 1996; 23:1107-1110.

254. Wakefield D, Charlesworth J, Pussell B, Wenman J. Reiter's syndrome and amyloidosis. Br J Rheumatol 1987; 26:156-158.

255. Ahlmen M, Ahlmen J, Svalander C, Bucht H. Cytotoxic drug treatment of reactive amyloidosis in rheumatoid arthritis with special reference to renal insufficiency. Clin. Rheumatol. 1987; 6:27-38.

256. Graham AN, Delahunt B, Renouf JJ, Austad WI. Takayasu's disease associated with generalised amyloidosis. Aust N Z J Med 1985; 15:343-345.

257. Shepard MK. Cold Hypersensitivity. Birth Defects: Original Article Series 1971; 7:352.

258. Ben-Chetrit E, Levy M. Familial Mediterranean fever. Lancet 1998; 351:659-664.

259. Houten SM, Kuis W, Duran M, et al. Mutations in MVK, encoding mevalonate kinase, cause hyperimmunoglobulinaemia D and periodic fever syndrome. Nat Genet 1999; 22:175-177.

260. Muckle TJ, Wells MV. Urticaria, deafness and amyloidosis: a new heredo-familial syndrome. Q.J. Med. 1962; 31:235-248.

261. McDermott MF, Aksentijevich I, Galon J, et al. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. Cell 1999; 97:133-144.

262. Goldsmith DJA, Roberts ISP, Short CD, Mallick NP. Complete clinical remission and subsequent relapse of bronchiectasis-related (AA) amyloid induced nephrotic syndrome. Nephron 1996; 74:572-576.

263. Landau M, Ophir J, Gal R, Pras M, Brenner S. Systemic amyloidosis secondary to chronic leg ulcers. Cutis 1992; 50:47-49.

264. Barton CH, Vaziri ND, Gordon S, Tilles S. Renal pathology in end-stage renal disease associated with paraplegia. Paraplegia 1984; 22:31-41.

265. Osick LA, Lee TP, Pedemonte MB, et al. Hepatic amyloidosis in intravenous drug abusers and AIDS patients. J Hepatol 1993; 19:79-84.

266. Nakayama EE, Ura S, Fleury RN, Soares V. Renal lesions in leprosy: a retrospective study of 199 autopsies. Am J Kidney Dis 2001; 38:26-30.

267. Gertz MA, Kyle RA. Secondary systemic amyloidosis: response and survival in 64 patients. Medicine 1991; 70:246-256.

268. Kayser K, Wiebel M, Schulz V, Gabius HJ. Necrotizing bronchitis, angiitis, and amyloidosis associated with chronic Q fever. Respiration 1995; 62:114-116.

269. Herbert MA, Milford DV, Silove ED, Raafat F. Secondary amyloidosis from long-standing bacterial endocarditis. Pediatr Nephrol 1995; 9:33-35.

270. Farr M, Hollywell CA, Walton KW, Morris C, Scott DL, Bacon PA. Amyloidosis in Whipple's arthritis. J. Royal Soc. Med. 1983; 76:963-966.

271. Melato M, Manconi R, Falconieri G. Amyloidosis and lung cancer. A morphological and histochemical study. Morphol Embryol (Bucur) 1981; 27:137-142.

272. Beck HI, Andersen JA, Birkler NE, Ottosen PD. Giant basal cell carcinoma with metastasis and secondary amyloidosis: report of case. Acta Derm Venereol 1983; 63:564-567.

273. el-Gatit A, al-Kaisi N, Moftah S, et al. Atypical bronchial carcinoid tumour with amyloid deposition. Eur J Surg Oncol 1994; 20:586-587.

274. Lachmann HJ, Gilbertson JA, Gillmore JD, Hawkins PN, Pepys MB. Unicentric Castleman's disease complicated by systemic AA amyloidosis: a curable disease. Q.J. Med. 2002; 95:211-218.

275. Cosme A, Horcajada JP, Vidaur F, Ojeda E, Torrado J, Arenas JI. Systemic AA amyloidosis induced by oral contraceptive-associated hepatocellular adenoma: a 13-year follow up. Liver 1995; 15:164-167.

276. Barista I, Benekli M, Tekuzman G. Amyloidosis in Hodgkin's and Castleman's diseases. Ann Oncol 1999; 10:998-999.

277. Pras M, Franklin EC, Shibolet S, Frangione B. Amyloidosis associated with renal cell carcinoma of the AA type. Am. J. Med. 1982; 73:426-428.

278. Agha I, Mahoney R, Beardslee M, H. L, Cowart RG, Juknevicius I. Systemic amyloidosis associated with pleomorphic sarcoma of the spleen and remission of nephrotic syndrome after removal of the tumor. Am J Kidney Dis 2002; 40:411-415.

279. Lee J, Cunningham-Rundles C. Thyroid amyloidois in a patient with common variable immunodeficiency. J. Allergy Clin. Immunol 2000; 105:S216.

280. Metin A, Fugen E, Tinaztepe K, Besbas N, Tezcan I, Sandal O. Cyclic neutropenia complicated by renal AA amyloidosis. Turk. J. Pediatr. 2000; 42:61-64.

281. Alvarez-Sala R, Prados C, Sastre Marcos J, Garcia Rio F, Vincandi B, de Ramon A. Amyloid goitre and hypothyroidism secondary to cystic fibrosis. Post Med J 1995; 71:307-308.

282. Gunduz K, Vatansevr S, Turel A, Sen S. Recessive dystrophic epidermolysis bullosa complicated with nephrotic syndrome due to secondary amyloidosis. Int. J. Derm. 2000; 39:151-153.

283. Oner A, Demircin G, Erdogan O, et al. A family with hyperimmunoglobulin M syndrome and systemic amyloidosis. Nephrol Dial Transplant 2000; 15:1480-1482.

284. Kotilainen P, Vuori K, Kainulainen L, et al. Systemic amyloidosis in a patient with hypogammaglobulinaemia. J Intern Med 1996; 240:103-6.

285. Osman EM, Abboud OI, Sulaiman SM, Musa A, Beleil OM, Sharfi AA. End-stage renal failure in Kartagener's syndrome. Nephrol Dial Transplant 1991; 6:747.

286. Valentin R, Gurtler KF, Schaker A. Renal amyloidosis and renal failure--a novel complication of the SAPHO syndrome. Nephrol Dial Transplant 1997; 12:2420-2423.

287. Tchenio X, Bertocchi M, McGregor B, Daoud S, Mornex JF, Cordier JF. Type AA renal amyloidosis in sarcoidosis. Rev Mal Respir 1996; 13:601-602.

288. Tezcan I, Ersoy F, Sanal O, Gonc EN, Arici M, Berkel I. A case of X linked agammaglobulinaemia complicated with systemic amyloidosis. Arch Dis Child 1998; 79:94.

289. Win N, Brozovic M, Gabriel R. Secondary amyloidosis accompanying multiple sickle cell crises. Trop Doct 1993; 23:45-46.

290. Consortium TFF. A candidate gene for familial Mediterranean fever. Nat. Genet. 1997; 17:25-31.

291. Consortium TIF. Ancient missense mutations in a new member of the *RoRet* gene family are likely to cause familial Mediterranean fever. Cell 1997; 90:797-807.

292. Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. Nat Genet 2001; 29:301-305.

293. Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. Am. J. Med. 1967; 43:227-253.

294. Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum. 1997; 40:1879-1885.

295. Grateau G, Pêcheux C, Cazeneuve C, et al. Clinical versus genetic diagnosis of familial Mediterranean fever. Q.J. Med. 2000; 93:223-229.

296. Blum A, Gafni J, Sohar E, Shibolet S, Heller S. Amyloidosis as the sole manifestation of familial Mediterranean fever (FMF). Further evidence of its genetic nature. Ann. Intern. Med. 1962; 57:795-799.

297. Booth DR, Gillmore JD, Booth SE, Pepys MB, Hawkins PN. Pyrin/marenostrin mutations in familial Mediterranean fever. Q.J. Med. 1998; 91:603-606.

298. Dodé C, Pêcheux C, Cazeneuve C, et al. Mutations in the *MEFV* gene in a large series of patients with a clinical diagnosis of familial Mediterranean fever. Am. J. Med. Genet. 2000; 92:241-246.

299. Centola M, Wood G, Frucht DM, et al. The gene for familial Mediterranean fever, *MEFV*, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. Blood 2000; 95:3223-3231.

300. Manji GA, Wang L, Geddes BJ, et al. PYPAF1, a PYRIN-containing Apaf1-like protein that assembles with ASC and regulates activation of NF-κB. J Biol Chem 2002; 277:11570-11575.

301. Pras M, Bronshpigel N, Zemer D, Gafni J. Variable incidence of amyloidosis in familial Mediterranean fever among different ethnic groups. Johns Hopkins Med. J. 1982; 150:22-26.

302. Gafni J, Ravid M, Sohar E. The role of amyloidosis in familial mediterranean fever. A population study. Isr. J. Med. Sci. 1968; 4:995-999.

303. Yalçinkaya F, Çakar N, Misirlioglu M, et al. Genotype-phenotype correlation in a large group of Turkish patients with familial Mediterranean fever: evidence for mutation-independent amyloidosis. Rheumatology 2000; 39:67-72.

304. Livneh A, Langevitz P, Shinar Y, et al. *MEFV* mutation analysis in patients suffering from amyloidosis of familial Mediterranean fever. Amyloid: Int. J. Exp. Clin. Invest. 1999; 6:1-6.

305. Cazeneuve C, Sarkisian T, Pêcheux C, et al. MEFV-gene analysis in Armenian patients with familial Mediterranean fever: diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype - genetic and therapeutic implications. Am. J. Hum. Genet. 1999; 65:88-97.

306. Shohat M, Magal N, Shohat T, et al. Phenotype-genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis. Eur. J. Hum. Genet. 1999; 7:287-292.

307. Yalçinkaya F, Tekin M, Çakar N, Akar E, Akar N, Tümer N. Familial Mediterranean fever and systemic amyloidosis in untreated Turkish patients. Q.J. Med. 2000; 93:681-684.

308. Saatci Ü, Ozen S, Özdemir S, et al. Familial Mediterranean fever in children: report of a large series and discussion of the risk and prognostic factors of amyloidosis. Eur. J. Pediatr. 1997; 156:619-623.

309. Mimouni A, Magal N, Stoffman N, et al. Familial Mediterranean fever: effects of genotype and ethnicity on inflammatory attacks and amyloidosis. Pediatrics 2000; 105:E70.

310. Majeed HA, Rawashdeh M, El-Shanti H, Qubain H, Khuri-Bulos N, Shahin HM. Familial Mediterranean fever in children: the expanded clinical profile. Q.J. Med. 1999; 92:309-318.

311. Grateau G. The relation between familial Mediterranean fever and amyloidosis. Curr. Opin. Rheumatol. 2000; 12:61-64.

312. Pras M. Familial Mediterranean fever: from the clinical syndrome to the cloning of the pyrin gene [editorial]. Scand. J. Rheumatol. 1998; 27:92-97.

313. Booth DR, Lachmann HJ, Gillmore JD, Booth SE, Hawkins PN. Prevalence and significance of the familial Mediterranean fever gene mutation encoding pyrin Q148. Q.J. Med. 2001; 94:527-531.

314. Goldfinger WE. Colchicine for familial Mediterranean fever. N. Engl. J. Med. 1972; 287:1302.

315. Fitzgerald TJ. Molecular features of colchicine associated with antimitotic activity and inhibition of tubulin polymerization. Biochem. Pharmacol. 1976; 25:1383-1387.

316. Bar-Eli M, Wilson L, Peters RS, Schwabe AD, Territo MC. Microtubules in PMNs from patients with familial Mediterranean fever. Am. J. Med. Sci. 1982; 284:2-7.

317. Zemer D, Livneh A, Danon YL, Pras M, Sohar E. Long-term colchicine treatment in children with familial Mediterranean fever. Arthritis Rheum. 1991; 34:973-977.

318. Ben-Chetrit E, Levy M. Colchicine: 1998 update. Semin. Arthritis Rheum. 1998; 28:48-59.

319. Livneh A, Zemer D, Langevitz P, Laor A, Sohar E, Pras M. Colchicine treatment of AA amyloidosis of familial Mediterranean fever. An analysis of factors affecting outcome. Arthritis Rheum. 1994; 37:1804-1811.

320. Livneh A, Zemer D, Siegal B, Laor A, Sohar E, Pras M. Colchicine prevents kidney transplant amyloidosis in familial Mediterranean fever. Nephron 1992; 60:418-422.

321. Lieberman A, Grossman ME, Silvers DN. Muckle-Wells syndrome: case report and review of cutaneous pathology. J. Am. Acad. Dermatol. 1998; 39:290-291.

322. Muckle TJ. The 'Muckle-Wells' syndrome. Br. J. Dermatol. 1979; 100:87-92.

323. Berthelot J-M, Maugars Y, Robillard N, et al. Autosomal dominant Muckle-Wells syndrome associated with cystinuria, ichthyosis, and aphthosis in a four-generation family. Am. J. Med. Genet. 1994; 53:72-74.

324. Watts RA, Nicholls A, Scott DGI. The arthropathy of the Muckle-Wells syndrome. Br. J. Rheumatol. 1994; 33:1184-1187.

325. Fuger K, Fleischmann E, Weber M, Mann J. Complications in the course of the Muckle-Wells syndrome. Dtsch. Med. Wochenschr. 1992; 117:256-260.

326. Schwarz RE, Dralle H, Linke RP, Nathrath WBJ, Neumann KH. Amyloid goiter and arthritides after kidney transplantation in a patient with systemic amyloidosis and Muckle-Wells syndrome. Am. J. Clin. Pathol. 1989; 92:821-825.

327. Bustorff MM, Oliveria JP, Moura C, Carvalho E, Faria V, Guerra L. Muckle-Wells syndrome nephropathy: lack of response to colchicine therapy. Nephrol. Dial. Transplant. 1995; 10:709-710.

328. Kile RL, Rusk HA. A case of cold urticaria with an unusual family history. J.A.M.A. 1940; 114:1067-1068.

329. Doeglas HMG, Bleumink E. Familial cold urticaria. Clinical findings. Arch. Dermatol. 1974; 110:382-388.

330. Commerford PJ, Meyers OL. Arthropathy associated with familial cold urticaria. S. Afr. Med. J. 1977; 51:105-108.

331. Vlagopoulos T, Townley R, Villacorte G. Familial cold urticaria. Ann. Alergy 1975; 34:366-369.

332. Zip CM, Ross JB, Greaves MW, Scriver CR, Mitchell JJ, Zoar S. Familial cold urticaria. Clin. Exp. Dermatol. 1993; 18:338-341.

333. Ormerod AD, Smart L, Reid TM, Milford-Ward A. Familial cold urticaria. Investigation of a family and response to stanozolol. Arch Dermatol. 1993; 129:343-346.

334. McDermott MF, Aganna E, Hitman GA, Ogunkolade BW, Booth DR, Hawkins PN. An autosomal dominant periodic fever associated with AA amyloidosis in a north Indian family maps to distal chromosome 1q. Arthritis Rheum. 2000; 43:2034-2040.

335. Aganna E, Martinon F, Hawkins PN, et al. Mutations in the NALP3/CIAS1/PYPAF1 gene are associated with a broad phenotype including recurrent fevers, cold sensitivity, sensorineural deafness and AA amyloidosis. Arthritis Rheum. 2002; (in press).

336. Kastner D, O'Shea JJ. A fever gene comes in from the cold. Nat Genetics 2001; 29:241-242.

337. Feldmann J, Prieur AM, Quartier P, et al. Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in CIAS1, a gene highly expressed in polymorphonuclear cells and chondrocytes. Am J Hum Genet 2002; 71:198-203.

338. van der Meer JW, Vossen JM, Radl J. Hyperimmunoglobulin D and periodic fever: a new syndrome. Lancet 1984; 1:1087-1090.

339. Derenth JPH, van der Meer JW. Hereditary periodic fever. N Engl J med 2001; 345:1748-1757.

340. Williamson LM, Hull D, Mehta R, Reeves WG, Robinson BH, Toghill PJ. Familial Hibernian fever. Q J Med 1982; 51:469-480.

341. McDermott MF. Autosomal dominant recurrent fevers. Clinical and genetic aspects. Rev. Rhum. [Engl. Ed.] 1999; 66:484-491.

342. Aksentijevich I, Galon J, Soares m, et al. The tumor-necrosis-factor receptor-associated periodic syndrome: new mutations in TNFRSF1A, ancestral origins,

genotype-phenotype studies, and evidence for further genetic heterogeneity of periodic fevers. Am J Hum Genet 2001 Nov;69(5):1160 2001; 69:301-314.

343. Engelmann H, Holtmann H, Brakebusch C, et al. Antibodies to a soluble form of a tumor necrosis factor (TNF) receptor have TNF-like activity. J Biol Chem 1990; 265:14497-14504.

344. Hawkins PN, Richardson S, Vigushin DM, et al. Serum amyloid P component scintigraphy and turnover studies for diagnosis and quantitative monitoring of AA amyloidosis in juvenile rheumatoid arthritis. Arthritis Rheum. 1993; 36:842-851.

345. Luke RG, Allison ME, Davidson JF, Duguid WP. Hyperkalemia and renal tubular acidosis due to renal amyloidosis. Ann. Intern. Med. 1969; 70:1211-1217.

346. Persey MR, Lovat LB, Madhoo S, Pepys MB, Hawkins PN. Adrenal function in AA amyloidosis. Br. J. Rheumatol. 1996; 35 (Suppl. 1):76.

347. Lovat LB, Persey MR, Madhoo S, Pepys MB, Hawkins PN. The liver in systemic amyloidosis: insights from ^{123}I serum amyloid P component scintigraphy in 484 patients. Gut 1998; 42:727-734.

348. Joss N, McLaughlin K, Simpson K, Boulton-Jones JM. Presentation, survival and prognostic markers in AA amyloidosis. Q J Med 2000; 93:535-542.

349. Lindqvist B, Anderson S, Isacsson B, Lundberg E. The course of renal amyloidosis with uraemia. Int. Urol. Nephrol. 1989; 21:555-559.

350. Smith ME, Ansell BM, Bywaters EG. Mortality and prognosis related to the amyloidosis of Still's disease. Ann. Rheum. Dis. 1968; 27:137-145.

351. Baum J, Gutowska G. Death in juvenile rheumatoid arthritis. Arthritis Rheum. 1977; 20 (Suppl. 2):253-255.

352. Berglund K, Thysell H, Keller C. Results, principles and pitfalls in the management of renal AA-amyloidosis; a 10-21 year followup of 16 patients with rheumatic disease treated with alkylating cytostatics. J. Rheumatol. 1993; 20:2051-2057.

353. Keven K, Nergizoglu G, Ates K, et al. Remission of nephrotic syndrome after removal of localized Castleman's disease. Am. J. Kidney Dis. 2000; 35:1207-1211.

354. Vigushin DM, Pepys MB, Hawkins PN. Rapid regression of AA amyloidosis following surgery for Castleman's disease. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. Amyloid and Amyloidosis 1993. Pearl River, New York: Parthenon Publishing, 1994:48-50.

355. Perfetti V, Bellotti V, Maggi A, et al. Reversal of nephrotic syndrome due to reactive amyloidosis (AA-type) after excision of localized Castleman's disease. Am. J. Hematol. 1994; 46:189-193.

356. Ordi J, Grau JM, Junque A, Nomdedeu B, Palacin A, Cardesa A. Secondary (AA) amyloidosis associated with Castleman's disease. Report of two cases and review of the literature. Am. J. Clin. Pathol. 1993; 100:394-397.

357. Ansell BM, Eghtedari A, Bywaters EG. Chlorambucil in the management of juvenile chronic polyarthritis complicated by amyloidosis. Ann. Rheum. Dis. 1971; 30:331.

358. Berglund K, Keller C, Thysell H. Alkylating cytostatic treatment in renal amyloidosis secondary to rheumatic disease. Ann. Rheum. Dis. 1987; 46:757-762.

359. Hawkins PN, Peters AM, Vigushin DM, et al. SAP scintigraphy and turnover studies demonstrate regression of amyloidosis. Nucl. Med. Commun. 1993; 14:259-260.

360. Hawkins PN, Pepys MB. A primed state exists *in vivo* following regression of murine AA amyloidosis. In: Natvig JB, Forre O, Husby G, et al., eds. Amyloid and Amyloidosis 1990. Dordrecht: Kluwer Academic Publishers, 1991:264-267.

361. Buxbaum J. Aberrant immunoglobulin synthesis in light chain amyloidosis. Free light chain and light chain fragment production by human bone marrow cells in short-term tissue culture. J. Clin. Invest. 1986; 78:798-806.

362. Buxbaum J. Mechanisms of disease: monoclonal immunoglobulin deposition. Amyloidosis, light chain deposition disease, and light and heavy chain deposition disease. Hematol. Oncol. Clin. North Am. 1992; 6:323-346.

363. Keren DF. Procedures for the evaluation of monoclonal immunoglobulins. Arch. Pathol. Lab. Med. 1999; 123:126-132.

364. Gertz MA, Kyle RA. Primary systemic amyloidosis - a diagnostic primer. Mayo Clin. Proc. 1989; 64:1505-1519.

365. Dubrey SW, Cha K, Anderson J, et al. The clinical features of immunoglobulin light-chain (AL) amyloidosis with heart involvement. Q.J. Med. 1998; 91:141-157.

366. Gertz MA, Kyle RA. Prognostic value of urinary protein in primary systemic amyloidosis (AL). Am. J. Clin. Pathol. 1990; 94:313-317.

367. Lovat LB, Pepys MB, Hawkins PN. Amyloid and the gut. Dig. Dis. 1997; 15:155-171.

368. Gertz MA, Kyle RA, Greipp PR. Hyposplenism in primary systemic amyloidosis. Ann. Intern. Med. 1983; 98:475-477.

369. Kyle RA, Bayrd ED. Amyloidosis: review of 236 cases. Medicine 1975; 54:271-299.

370. Kyle RA, Gertz MA. Cardiac amyloidosis. Int. J. Cardiol. 1990; 28:139-141.

371. Kyle RA, Greipp RR. Primary systemic amyloidosis: comparison of melphalan and prednisone versus placebo. Blood 1978; 52:818-827.

372. Gillmore JD, Apperley JF, Pepys MB, Hawkins PN. High dose chemotherapy for systemic AL amyloidosis. Kidney Int. 1999; 55:2103.

373. Gillmore JD, Lovat LB, Hawkins PN. Amyloidosis and the liver. J. Hepatol. 1999; 30 (Suppl. 1):17-33.

374. Comenzo RL, Gertz MA. Autologous stem cell transplantation for primary systemic amyloidosis. Blood 2002 2002; 99:4276-4282.

375. Gertz MA, Kyle RA, Greipp PR. Response rates and survival in primary systemic amyloidosis. Blood 1991; 77:257-262.

376. Kyle RA, Gertz MA, Greipp PR, et al. Long-term survival (10 years or more) in 30 patients with primary amyloidosis. Blood 1999; 93:1062-1066.

377. Kyle RA, Gertz MA, Greipp PR, Witzig TE, Lust JA, Lacy MQ. A trial of three regimens for primary amyloidosis: colchicine alone, melphalan and prednisone, and melphalan, prednisone, and colchicine. N. Engl. J. Med. 1997; 336:1202-1207.

378. Wardley AM, Jayson GC, Goldsmith DJ, Venning MC, Ackrill P, Scarffe JH. The treatment of nephrotic syndrome caused by primary (light chain) amyloid with vincristine, doxorubicin and dexamethasone. Br. J. Cancer 1998; 78:774-776.

379. Persey MR, Lovat LB, Apperley JF, Madhoo S, Pepys MB, Hawkins PN. Intensive chemotherapy for AL amyloidosis. Br. J. Rheumatol. 1996; 35 (Suppl. 1):12.

380. Gertz MA, Lacy MQ, Gastineau DA, et al. Blood stem cell transplantation as therapy for primary systemic amyloidosis (AL). Bone Marrow Transplant. 2000; 26:963-969.

381. Sanchorawala V, Wright DG, Seldin DC, et al. An overview of the use of high-dose melphalan with autologous stem cell transplantation for the treatment of AL amyloidosis. Bone Marrow Transplant. 2001; 28:637-642.

382. Moreau P, Leblond V, Bourquelot P, et al. Prognostic factors for survival and response after high-dose therapy and autologous stem cell transplantation in systemic AL amyloidosis: a report on 21 patients. Br. J. Haematol. 1998; 101:766-769.

383. Dispenzieri A, Lacy MQ, Kyle RA, et al. Eligibility for hematopoietic stem-cell transplantation for primary systemic amyloidosis is a favorable prognostic factor for survival. J. Clin. Oncol. 2001; 19:3350-3356.

384. Karlsson FA, Groth T, Sege K, Wibell L, Peterson PA. Turnover in humans of β_2-microglobulin : the constant chain of HLA-antigens. Eur. J. Clin. Invest. 1980; 10:293-300.

385. Jadoul M, Garbar C, Vanholder R, et al. Prevalence of histological β_2-microglobulin amyloidosis in CAPD patients compared with hemodialysis patients. Kidney Int. 1998; 54:956-959.

386. Drueke TB. Beta2-microglobulin and amyloidosis. Nephrol Dial Transplant 2000; 15:17-24.

387. Bardin T. Dialysis related amyloidosis (Editorial). J. Rheumatol. 1987; 14:647-648.

388. Jadoul M. Dialysis-related amyloidosis: importance of biocompatibility and age. Nephrol. Dial. Transplant. 1998; 13 (Suppl.):61-64.

389. Drüeke TB. Dialysis-related amyloidosis. Nephrol. Dial. Transplant. 1998; 13:58-64.

390. Noel LK, Zingraff J, Bardin T, Atienza C, Kuntz D, Drueke T. Tissue distribution of dialysis amyloid. Clin. Nephrol. 1987; 27:175-178.

391. Ito M, Abumi K, Takeda N, Satoh S, Hasegawa K, Kaneda K. Pathologic features of spinal disorders in patients treated with long-term hemodialysis. Spine 1998; 23:127-133.

392. Maher ER, Hamilton Dutoit S, Baillod RA, Sweny P, Moorhead JF. Gastrointestinal complications of dialysis related amyloidosis. B.M.J. 1988; 297:265-266.

393. Zhou H, Pfeifer U, Linke R. Generalized amyloidosis from β_2-microglobulin, with caecal perforation after long-term haemodialysis. Virchows Arch. A. Pathol. Anat. Histopathol. 1991; 419:349-353.

394. Sethi D, Cary NR, Brown EA, Woodrow DF, Gower PE. Dialysis-associated amyloid: systemic or local? Nephrol. Dial. Transplant. 1989; 4:1054-1059.

395. Tan SY, Irish A, Winearls CG, et al. Long term effect of renal transplantation on dialysis-related amyloid deposits and symptomatology. Kidney Int. 1996; 50:282-289.

396. Jadoul M, Drueke T, Zingraff J, van Ypersele de Strihou C. Does dialysis-related amyloidosis regress after transplantation? Nephrol Dial Transplant 1997; 12:655-657.

397. Tan SY, Madhoo S, Brown E, et al. Effect of renal transplantation on dialysis-related amyloid deposits: Prospective evaluation by ^{123}I-SAP scintigraphy (abstract). Nephrol. Dial. Transplant. 1994; 9:1017.

398. Jadoul M, Malghem J, Pirson Y, Maldague B, van Ypersele de Strihou C. Effect of renal transplantation on the radiological signs of dialysis amyloid osteoarthropathy. Clin Nephrol 1998; 32:184-7.

399. Jadoul M, Noel H, Malghem J, Galant C, van Ypersele de Strihou C. Histological beta-2-microglobulin amyloidosis 10 years after a successful renal transplantation. Am J Kidney Dis 1996; 27:880-890.

400. Hou FF, Reddan DN, Seng WK, Owen WFJ. Pathogenesis of beta(2)-microglobulin amyloidosis: role of monocytes/macrophages. Semin Dial 2001; 14:135-139.

401. Miyata T, Jadoul M, Kurokawa K, van Ypersele de Strihou C. Beta-2 microglobulin in renal disease. J. Am. Soc. Nephrol. 1998; 9:1723-1735.

402. Bardin T. Low-dose prednisone in dialysis-related amyloid arthropathy. Rev. Rhum. [Engl. Ed.] 1994; 61 (Suppl.):97S-100S.

403. Nangaku M, Miyata T, Kurokawa K. Pathogenesis and management of dialysis-related amyloid bone disease. Am J Med Sci 1999; 317:410-415.

404. Saraiva MJM, Costa PP, Goodman DS. Transthyretin and familial amyloidotic polyneuropathy. In: Rosenberg RN, Prusiner SB, DiMauro S, Barchi RL, Kunkel LM, eds. The Molecular and Genetic Basis of Neurological Disease. Boston: Butterworths, 1993:889-894.

405. Nardin R, Lachmann H. Familial amyloid polyneuropathy: presentation, diagnosis, and treatment. J. Clin. Neuromusc. Dis. 2001; 2:221-226.

406. Hesse A, Altland K, Linke RP, et al. Cardiac amyloidosis: a review and report of a new transthyretin (prealbumin) variant. Br. Heart J. 1993; 70:111-115.

407. Booth DR, Gillmore JD, Pepys MB, Hawkins PN. Transthyretin mutations in patients with apparent senile cardiac amyloidosis. In: Kyle RA, Gertz MA, eds. Amyloid and Amyloidosis 1998. Pearl River, New York: Parthenon Publishing, 1999:554-555.

408. Gillmore JD, Booth DR, Pepys MB, Hawkins PN. Hereditary cardiac amyloidosis associated with the transthyretin Ile122 mutation in a white man. Heart 1999; 82:e2.

409. Ando Y, Ando E, Ohlsson PI, et al. Analysis of transthyretin amyloid fibrils from vitreous samples in familial amyloidotic polyneuropathy (Val30Met). Amyloid: Int J Exp clin 1999; 6:119-123.

410. Hawkins PN, Rydh A, Persey MR, et al. SAP scintigraphy in 43 patients with TTR-associated FAP. Proceedings of the Third International Symposium on Familial Amyloidotic Polyneuropathy and Other TTR Related Disorders, Lisbon, 27-29 October 1995. Neuromusc. Disord. 1996; 6 (Suppl. 1):S54.

411. Ikeda S, Nakazato M, Ando Y, Sobue G. Familial transthyretin-type amyloid polyneuropathy in Japan: clinical and genetic heterogeneity. Neurology 2002; 58:1001-1007.

412. Reilly MM, Adams D, Booth DR, et al. Transthyretin gene analysis in European patients with suspected familial amyloid polyneuropathy. Brain 1995; 118:849-856.

413. Booth DR, Gillmore JD, Persey MR, et al. Transthyretin Ile73Val is associated with familial amyloidotic polyneuropathy in a Bangladeshi family. Hum. Mutat. 1998; 12:135.

414. Lachmann HJ, Booth DR, Bybee A, Hawkins PN. Transthyretin Ala97Ser is associated with familial amyloidotic polyneuropathy in a Chinese-Taiwanese family. Hum. Mutat. 2000; 16:180.

415. Vigushin DM, Hawkins PN, Pepys MB. Clinical phenotypes in familial amyloid polyneuropathy associated with new transthyretin variants Val47 and Pro52. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. Amyloid and Amyloidosis 1993. Pearl River, New York: Parthenon Publishing, 1994:483-485.

416. Saraiva MJM, Sherman W, Marboe C, et al. Cardiac amyloidosis: report of a patient heterozygous for the transthyretin isoleucine 122 variant. Scand. J. Immunol. 1990; 32:341-346.

417. Serpell L, Blake CCF. Frequency analysis and structural correlation of FAP mutations in transthyretin. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. Amyloid and Amyloidosis 1993. Pearl River, New York: Parthenon Publishing, 1994:447-449.

418. Terry CJ, Damas AM, Oliveira P, et al. Structure of Met30 variant of transthyretin and its amyloidogenic implications. EMBO J. 1993; 12:735-741.

419. Holmgren G, Steen L, Ekstedt J, et al. Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met^{30}). Clin. Genet. 1991; 40:242-246.

420. Holmgren G, Ericzon B-G, Groth C-G, et al. Clinical improvement and amyloid regression after liver transplantation in hereditary transthyretin amyloidosis. Lancet 1993; 341:1113-1116.

421. Hawkins PN, Booth DR, Booth SE, et al. Clinical features, diagnosis and treatment of familial amyloid polyneuropathy. Clin. Sci. 1996; 90 (Suppl. 34):19P.

422. Hawkins PN, Rydh A, Danielsson Å, et al. SAP scintigraphy in familial amyloid polyneuropathy: regression of visceral amyloid following liver transplantation. In: Kyle RA, Gertz MA, eds. Amyloid and Amyloidosis 1998. Pearl River, New York: Parthenon Publishing, 1999:333-335.

423. Rydh A, Suhr O, Hietala S-O, Åhlström KR, Pepys MB, Hawkins PN. Serum amyloid P component scintigraphy in familial amyloid polyneuropathy: regression of visceral amyloid following liver transplantation. Eur. J. Nucl. Med. 1998; 25:709-713.

424. Yazaki M, Tokuda T, Nakamura A, et al. Cardiac amyloid in patients with familial amyloid polyneuropathy consists of abundant wild-type transthyretin. Biochem Biophys Res Commun 2000; 11:274.

425. Stangou AJ, Hawkins PN, Heaton ND, et al. Progressive cardiac amyloidosis following liver transplantation for familial amyloid polyneuropathy: implications for amyloid fibrillogenesis. Transplantation 1998; 66:229-233.

426. Munar-Ques M, Salva-Ladaria L, Mulet-Perera P, Sole M, Lopez-Andreu FR, Saraiva MJ. Vitreous amyloidosis after liver transplantation in patients with familial amyloid polyneuropathy: ocular synthesis of mutant transthyretin. Amyloid: Int J Exp clin 2000; 7:266-269.

427. Olofsson BO, Backman C, Karp K, Suhr OB. Progression of cardiomyopathy after liver transplantation in patients with familial amyloidotic polyneuropathy, Portuguese type. Transplantation 2002; 73:745-751.

428. Stangou AJ, Heaton ND, Rela M, Pepys MB, Hawkins PN, Williams R. Domino hepatic transplantation using the liver from a patient with familial amyloid polyneuropathy. Transplantation 1998; 65:1496-1498.

429. Maury CPJ, Kere J, Tolvanen R, de la Chapelle A. Finnish hereditary amyloidosis is caused by a single nucleotide substitution in the gelsolin gene. FEBS Lett. 1990; 276:75-77.

430. Gorevic PD, Munoz PC, Gorgone G, et al. Amyloidosis due to a mutation of the gelsolin gene in an American family with lattice corneal dystrophy type II. N. Engl. J. Med. 1991; 325:1780-1785.

431. de la Chapelle A, Tolvanen R, Boysen G, et al. Gelsolin-derived familial amyloidosis caused by asparagine or tyrosine substitution for aspartic acid at residue 187. Nature Genetics 1992; 2:157-160.

432. Sun HQ, Yamamoto M, Mejillano M, Yin HL. Gelsolin, a multifunctional actin regulatory protein. J Biol Chem 1999; 274:33179-33182.

433. Valleix S, Drunat S, Philit JB, et al. Hereditary renal amyloidosis caused by a new variant lysozyme W64R in a French family. Kidney Int 2002; 61:907-912.

434. Gillmore JD, Booth DR, Madhoo S, Pepys MB, Hawkins PN. Hereditary renal amyloidosis associated with variant lysozyme in a large English family. Nephrol. Dial. Transplant. 1999; 14:2639-2644.

435. Assmann G, Schmitz G, Funke H, von Eckardstein A. Apolipoprotein A-I and HDL deficiency. Curr. Opin. Lipidol. 1990; 1:110-115.

436. Westermark P, Mucchiano G, Marthin T, Johnson KH, Sletten K. Apolipoprotein A1-derived amyloid in human aortic atherosclerotic plaques. Am. J. Pathol. 1995; 147:1186-1192..

437. Booth DR, Tan SY, Booth SE, et al. A new apolipoprotein AI variant, Trp50Arg, causes hereditary amyloidosis. Q.J. Med. 1995; 88:695-702.

438. Hamidi Asl L, Liepnieks JJ, Hamidi Asl K, et al. Hereditary amyloid cardiomyopathy caused by a variant apolipoprotein A1. Am. J. Pathol. 1999; 154:221-227.

439. Hamidi Asl K, Liepnieks JJ, Nakamura M, Parker F, Benson MD. A novel apolipoprotein A-1 variant, Arg173Pro, associated with cardiac and cutaneous amyloidosis. Biochem. Biophys. Res. Commun. 1999; 257:584-588.

440. Obici L, Bellotti V, Mangione P, et al. The new apolipoprotein A-I variant $Leu^{174} \rightarrow$ Ser causes hereditary cardiac amyloidosis, and the amyloid fibrils are constituted by the 93-residue N-terminal polypeptide. Am. J. Pathol. 1999; 155:695-702.

441. de Sousa MM, Vital C, Ostler D, et al. Apolipoprotein AI and transthyretin as components of amyloid fibrils in a kindred with apoAI Leu178His amyloidosis. Am. J. Pathol. 2000; 156:1911-1917.

442. Booth DR, Tan SY, Booth SE, et al. Hereditary hepatic and systemic amyloidosis caused by a new deletion/insertion mutation in the apolipoprotein AI gene. J. Clin. Invest. 1996; 97:2714-2721.

443. Amarzguioui M, Mucchiano G, Häggqvist B, et al. Extensive intimal apolipoprotein A1-derived amyloid deposits in a patient with an apolipoprotein A1 mutation. Biochem. Biophys. Res. Commun. 1998; 242:534-539.

444. Persey MR, Booth DR, Booth SE, et al. Hereditary nephropathic systemic amyloidosis caused by a novel variant apolipoprotein A-I. Kidney Int. 1998; 53:276-281.

445. Gillmore JD, Stangou AJ, Tennent GA, et al. Clinical and biochemical outcome of hepatorenal transplantation for hereditary systemic amyloidosis associated with apolipoprotein AI Gly26Arg. Transplantation 2001; 71:986-992.

446. Higuchi K, Naiki H, Kitagawa K, Hosokawa M, Takeda T. Mouse senile amyloidosis. AS_{SAM} amyloidosis in mice presents universally as a systemic age-associated amyloidosis. Virchows Arch. B. Cell Pathol. 1991; 60:231-238.

447. Higuchi K, Kitagawa K, Naiki H, Hanada K, Hosokawa M, Takeda T. Polymorphism of apolipoprotein A-II (apoA-II) among inbred strains of mice. Relationship between the molecular type of apoA-II and mouse senile amyloidosis. Biochem. J. 1991; 279:427-433.

448. Deeb SS, Takata K, Peng R, Kajiyama G, Albers JJ. A splice-junction mutation responsible for familial apolipoprotein A-II deficiency. Am J Hum Genet 1990; 46:822-827.

449. Uemichi T, Liepnieks JJ, Yamada T, Gertz MA, Bang N, Benson MD. A frame shift mutation in the fibrinogen A α-chain gene in a kindred with renal amyloidosis. Blood 1996; 87:4197-4203.

450. Hamidi Asl L, Liepnieks JJ, Uemichi T, et al. Renal amyloidosis with a frame shift mutation in fibrinogen a α-chain gene producing a novel amyloid protein. Blood 1997; 90:4799-4805.

451. Uemichi T, Liepnieks JJ, Gertz MA, Benson MD. Fibrinogen A α chain Leu554: an African-American kindred with late onset renal amyloidosis. Amyloid: Int. J. Exp. Clin. Invest. 1998; 5:188-192.

452. Hamidi Asl L, Fournier V, Billerey C, et al. Fibrinogen A α chain mutation (Arg554Leu) associated with hereditary renal amyloidosis in a French family. Amyloid: Int. J. Exp. Clin. Invest. 1998; 5:279-284.

453. Uemichi T, Liepnieks JJ, Benson MD. Hereditary renal amyloidosis with a novel variant fibrinogen. J. Clin. Invest. 1994; 93:731-736.

454. Uemichi T, Liepnieks JJ, Alexander F, Benson MD. The molecular basis of renal amyloidosis in Irish-American and Polish-Canadian kindreds. Q.J. Med. 1996; 89:745-750.

455. Hawkins PN, Hall M, Hall R, et al. Regression of AL amyloidosis and prolonged survival following cardiac transplantation and chemotherapy. N. Engl. J. Med. 1993:(submitted for publication).

456. Klabunde T, Petrassi HM, Oza VB, Raman P, Kelly JW, Sacchettini JC. Rational design of potent human transthyretin amyloid disease inhibitors. Nature Struct. Biol. 2000; 7:312-321.

457. Merlini G, Ascari E, Amboldi N, et al. Interaction of the anthracycline 4′-iodo-4′-deoxydoxorubicin with amyloid fibrils: inhibition of amyloidogenesis. Proc. Natl. Acad. Sci. USA 1995; 92:2959-2963.

458. Peterson SA, Klabunde T, Lashuel HA, Purkey H, Sacchettini JC, Kelly JW. Inhibiting transthyretin conformational changes that lead to amyloid fibril formation. Proc. Natl. Acad. Sci. USA 1998; 95:12956-12960.

459. Soto C. Plaque busters: strategies to inhibit amyloid formation in Alzheimer's disease. Mol. Med. Today 1999; 5:343-350.

460. O'Nuallain B, Wetzel R. Conformational Abs recognizing a generic amyloid fibril epitope. PNAS 2002; 99:1485-1490.

461. Hrncic R, Wall J, Wolfenbarger DA, et al. Antibody-mediated resolution of light chain-associated amyloid deposits. Am J Pathol 2000; 157:1239-1246.

462. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999; 400:173-177.

463. Bard F, Cannon C, Barbour R, et al. Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nature Med. 2000; 6:916-919.

464. Bishop GM, Robinson SR, Smith MA, Perry G, Atwood CS. Call for Elan to publish Alzheimer's trial details (letter). Nature 2002; 416:667.

465. Hind CRK, Collins PM, Caspi D, Baltz ML, Pepys MB. Specific chemical dissociation of fibrillar and non-fibrillar components of amyloid deposits. Lancet 1984; ii:376-378.

466. Pepys MB, Herbert J, Hutchinson WL, et al. Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. Nature 2002; 417:254-259.

467. Hawkins PN, Myers MJ, Epenetos AA, Caspi D, Pepys MB. Specific localization and imaging of amyloid deposits *in vivo* using ^{123}I-labeled serum amyloid P component. J. Exp. Med. 1988; 167:903-913.

468. Hawkins PN, Pepys MB. Imaging amyloidosis with radiolabelled SAP. Eur. J. Nucl. Med. 1995; 22:595-599.

469. Hind CRK, Gibson DG, Lavender JP, Pepys MB. Non-invasive demonstration of cardiac involvement in acquired forms of systemic amyloidosis. Lancet 1984; i:1417.

470. Klein AL, Hatle LK, Burstow DJ, et al. Doppler characterization of left ventricular diastolic function in cardiac amyloidosis. J. Am. Coll. Cardiol. 1989; 13:1017-1026.

471. Klein AL, Hatle LK, Burstow DJ, et al. Comprehensive Doppler assessment of right ventricular diastolic function in cardiac amyloidosis. J. Am. Coll. Cardiol. 1990; 15:99-108.

472. Vigushin DM, Clesham G, Hawkins PN, et al. Echocardiography in systemic AL amyloidosis. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. Amyloid and Amyloidosis 1993. Pearl River, New York: Parthenon Publishing, 1994:268-270.

473. Cueto-Garcia L, Reeder GS, Kyle RA, et al. Echocardiographic findings in systemic amyloidosis: spectrum of cardiac involvement and relation to survival. J. Am. Coll. Cardiol. 1985; 6:737-743.

474. Ruinen L, Smit-Van Dijk W, Pras M, Scholten JH, Mandema E. The binding of congo red and toluidine blue to isolated amyloid. Acta Path. Microbiol. Scand. 1972; 80:122-127.

475. Glenner GG. The basis of the staining of amyloid fibers: their physico-chemical nature and the mechanisms of their dye-substrate interaction. Prog. Histochem. Cytochem. 1981; 13:1-37.

476. Pepys MB, Breathnach SM, Black MM, et al. Diagnosis and characterisation of amyloid deposits by immunohistochemistry staining. In: Tribe CR, Bacon PA, eds. Amyloidosis E.A.R.S. Bristol: John Wright & Sons, 1983:189-191.

477. Eda S, Kaufmann J, Roos W, Pohl S. Development of a new microparticle-enhanced turbidometric assay for C-reactive protein with superior features in analytical sensitivity and dynamic range. J. Clin. Lab. Anal. 1998; 12:137-144.

478. Eda S, Kaufmann J, Molwitz M, Vorberg E. A new method of measuring C-reactive protein, with a low limit of detection, suitable for risk assessment of coronary heart disease. Scand. J. Clin. Lab. Invest. 1999; 230:32-35.

479. Wilkins J, Gallimore JR, Moore EG, Pepys MB. Rapid automated high sensitivity enzyme immunoassay of C-reactive protein. Clin. Chem. 1998; 44:1358-1361.

480. Ledue TB, Weiner DL, Sipe JD, Poulin SE, Collins MF, Rifai N. Analytical evaluation of particle-enhanced immunonephelometric assays for C-reactive protein, serum amyloid A and mannose-binding protein in human serum. Ann. Clin. Biochem. 1998; 35:745-753.

481. WHO Expert Committee on Biological Standardization tR. WHO Technical Report Series 760. In: WHO, ed. Geneva, 1987:pp. 21-22.

482. Poole S, Walker D, Gaines Das RE, Gallimore JR, Pepys MB. The first international standard for serum amyloid A protein (SAA). Evaluation in an international collaborative study. J. Immunol. Methods 1998; 214:1-10.

483. Bradwell AR, Carr-Smith HD, Mead GP, et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. Clin. Chem. 2001; 47:673-680.

484. Drayson M, Tang LX, Drew R, Mead GP, Carr-Smith H, Bradwell AR. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. Blood 2001; 97:2900-2902.

485. Abraham RS, Clark RJ, Bryant SC, et al. Correlation of serum immunoglobulin free light chain quantification with urinary Bence Jones protein in light chain myeloma. Clin. Chem. 2002; 48:655-657.

486. Talmud P, Tybjaerg-Hansen A, Bhatnagar D, et al. Rapid screening for specific mutations in patients with a clinical diagnosis of familial hypercholesterolaemia. Atherosclerosis 1991; 89:137-141.

487. Mills KA, Even D, Murray JC. Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum. Mol. Genet. 1992; 1:779.

488. Davison AM. The United Kingdom Medical Research Council's glomerulonephritis registry. Contrib. Nephrol. 1985; 48:24-35.

489. Gillmore JD, Davies J, Iqbal A, Madhoo S, Russell NH, Hawkins PN. Allogeneic bone marrow transplantation for systemic AL amyloidosis. Brit. J. Haematol. 1998; 100:226-228.

490. Gertz MA, Lacy MQ, Dispenzieri A. Myeloablative chemotherapy with stem cell rescue for the treatment of primary systemic amyloidosis: a status report. Bone Marrow Transplant. 2000; 25:465-470.

491. Shirahama T, Skinner M, Cohen AS. Immunocytochemical identification of amyloid in formalin-fixed paraffin sections. Histochemistry 1981; 72:161-171.

492. Herbert J, Younger D, Latov N, Martone RL. Clinical spectrum of familial amyloidotic polyneuropathy. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, eds. Amyloid and Amyloidosis 1993. Pearl River, New York: Parthenon Publishing, 1994:486-488.

493. Kyle RA, Greipp PR. Amyloidosis (AL): clinical and laboratory features in 229 cases. Mayo Clin. Proc. 1983; 58:665-683.

494. Jacobson DR, Pastore RD, Yaghoubian R, et al. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. N. Engl. J. Med. 1997; 336:466-473.

495. Jones LA, Harding JA, Cohen AS, Skinner M. New USA family has apolipoprotein AI (Arg26) variant. In: Natvig JB, Førre Ø, Husby G, et al., eds. Amyloid and Amyloidosis 1990. Dordrecht: Kluwer Academic Publishers, 1991:385-388.

496. Lachmann HJ, Booth DR, Booth SE, Bybee A, Gillmore JD, Hawkins PN. Hereditary systemic amyloidosis is unexpectedly frequent in Britain. Q.J. Med. 2000; 93:773.

497. Comenzo RL, Vosburgh E, Falk RH, et al. Dose-intensive melphalan with blood stem-cell support for the treatment of AL (amyloid light-chain) amyloidosis: survival and responses in 25 patients. Blood 1998; 91:3662-3670.

498. Zeldenrust S, Gertz MA, Uemichi T, et al. Orthotopic liver transplantation for hereditary fibrinogen amyloidosis. Transplantation 2003; 75:560-561.

499. Samson D, Gaminara E, Newland A, et al. Infusion of vincristine and doxorubicin with oral dexamethasone as first-line therapy for multiple myeloma. Lancet 1989; ii:882-885.

500. Raje N, Powles R, Kulkarni S, et al. A comparison of vincristine and doxorubicin infusional chemotherapy with methylprednisolone (VAMP) with the addition of weekly cyclophosphamide (C-VAMP) as induction treatment followed by autografting in previously untreated myeloma. Br. J. Haematol. 1997; 97:153-160.

501. Schey SA, Kazmi M, Ireland R, Lakhani A. The use of intravenous intermediate dose melphalan and dexamethasone as induction treatment in the management of *de novo* multiple myeloma. Eur. J. Haematol. 1998; 61:306-310.

502. Maack T, Johnson V, Kau ST, Figueredo J, Sigulem D. Renal filtration, transport and metabolism of low-molecular-weight proteins - A review. Kid Int 1979; 16:251-270.

503. Lachmann HJ, Booth DR, Booth SE, et al. Misdiagnosis of hereditary amyloidosis as AL (primary) amyloidosis. N. Engl. J. Med. 2002; 346:1786-1791.

504. Clark RJ, Katzmann JA, Abraham RS, Lymph JF, Kyle RA, Bradwell AR. Detection of monoclonal free light chains by nephelometry: normal ranges and relative sensitivity. Clin. Chem. 2001; 47:A27 (abstract).

505. Castleman B, Iverson L, Menendez P. Localized mediastinal lymph-node hyperplasia resembling thymoma. Cancer 1956; 9:822-830.

506. Palestro G, Turrini F, Pagano M, Chiusa L. Castleman's disease. Adv. Clin. Path. 1999; 3:11-22.

507. McCarty MJ, Vukelja SJ, Banks PM, Weiss RB. Angiofollicular lymph node hyperplasia (Castleman's disease). Cancer Treat. Rev. 1995; 21:291-310.

508. Parez N, Bader-Meunier B, Roy CC, Dommergues JP. Paediatric Castleman disease: report of seven cases and review of the literature. Eur. J. Pediatr. 1999; 158:631-637.

509. Bowne WB, Lewis JJ, Filippa DA, et al. The management of unicentric and multicentric Castleman's disease: a report of 16 cases and a review of the literature. Cancer 1999; 85:706-717.

510. Nishimoto N, Sasai M, Shima Y, et al. Improvement in Castleman's disease by humanized anti-interleukin-6 receptor antibody therapy. Blood 2000; 95:56-61.

511. Brouet JC, Levy Y. IL6 and lymphoproliferative disorders. Nouv. Rev. Fr. Hematol. 1991; 33:433-436.

512. Leger-Ravet MB, Peuchmaur M, Devergne O, et al. Interleukin-6 gene expression in Castleman's disease. Blood 1991; 78:2923-2930.

513. Brandt SJ, Bodine DM, Dunbar CE, Nienhuis AW. Dysregulated interleukin 6 expression produces a syndrome resembling Castleman's disease in mice. J. Clin. Invest. 1990; 86:592-599.

514. Kikuta H, Itakura O, Taneichi K, Kohno M. Tropism of human herpesvirus 8 for peripheral blood lymphocytes in patients with Castleman's disease. Br. J. Haematol. 1997; 99:790-793.

515. Teruya-Feldstein J, Zauber P, Setsuda JE, et al. Expression of human herpesvirus-8 oncogene and cytokine homologues in an HIV-seronegative patient with multicentric Castleman's disease and primary effusion lymphoma. Lab. Invest. 1998; 78:1637-1642.

516. Beck JT, Hsu SM, Wijdenes J, et al. Brief report: alleviation of systemic manifestations of Castleman's disease by monoclonal anti-interleukin-6 antibody. N. Engl. J. Med. 1994; 330:602-605.

517. Papanicolaou DA, Vgontzas AN. Interleukin-6: the endocrine cytokine. J. Clin. Endocrinol. Metab. 2000; 85:1331-1333.

518. Ikeda S, Chisuwa H, Kawasaki S, et al. Systemic reactive amyloidosis associated with Castleman's disease: serial changes of the concentrations of acute phase serum amyloid A and interleukin 6 in serum. J. Clin. Pathol. 1997; 50:965-967.

519. Montoli A, Minola E, Stabile F, et al. End-stage renal failure from renal amyloidosis of the AA type associated with giant lymph node hyperplasia (Castleman's disease). Am. J. Nephrol. 1995; 15:142-146.

520. Arinsoy T, Yilmaz M, Ataoglu O, Bali M, Sindel S. Castleman's disease with renal amyloidosis and nephrotic syndrome. Nephrol. Dial. Transplant. 1997; 12:2713-2716.

521. Kanoh T, Shimada H, Uchino H, Matsumura K. Amyloid goiter with hypothyroidism. Arch. Pathol. Lab. Med. 1989; 113:542-544.

522. Funabiki K, Kaneko S, Terajima M, Tomita H, Kawano Y, Tomino Y. A case of multicentric Castleman's disease associated with renal amyloidosis and pure red cell aplasia. Am. J. Nephrol. 1998; 18:247-250.

523. Paydas S, Gonlusen G, Sagliker Y. Regression of nephrotic syndrome with colchicine therapy secondary to amyloidosis with associated Castleman's disease. Nephron 1995; 71:463-464.

524. Pilon VA, Gomez LG, Butler JJ. Systemic amyloidosis associated with a benign mesenteric lymphoid mass. Am. J. Clin. Pathol. 1982; 78:112-116.

525. Chan KW, Lo WK, Cheng IK. Nephrotic syndrome associated with angiofollicular lymph node hyperplasia, and subsequent amyloidosis. Pathology 1992; 24:229-230.

526. Tanaka K, Horita M, Shibayama H, et al. Secondary amyloidosis associated with Castleman's disease. Intern. Med. 1995; 34:122-126.

527. Curioni S, D'Amico M, Quartagno R, Martino S, Dell'Antonio G, Cusi D. Castleman's disease with nephrotic syndrome, amyloidosis and autoimmune manifestations. Nephrol. Dial. Transplant. 2001; 16:1475-1478.

528. Chan WC, Hargreaves H, Keller J. Giant lymph node hyperplasia with unusual clinicopathologic features. Cancer 1984; 53:2135-2139.

529. Miura A, Sato I, Suzuki C. Fatal diarrhea in a patient with Castleman's disease associated with intestinal amyloidosis. Intern. Med. 1995; 34:1106-1109.

530. West KP, Morgan DR, Lauder I. Angiofollicular lymph node hyperplasia with amyloidosis. Postgrad. Med. J. 1989; 65:108-111.

531. Wilkins J, Gallimore JR, Tennent GA, et al. Rapid automated enzyme immunoassay of serum amyloid A. Clin. Chem. 1994; 40:1284-1290.

532. Yamada T, Kazuaki U, Yakat M, Gejyo F. Sandwich enzyme immunoassay for serum amyloid A protein (SAA). Clin. Chim. Acta 1989; 179:169-176.

533. Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. Immunol. Today 1994; 15:81-88.

534. Levin M, Pras M, Franklin EC. Immunologic studies of the major non-immunoglobulin protein of amyloid. I. Identification and partial characterization of a related serum component. J. Exp. Med. 1973; 138:373-380.

535. Langevitz P, Livneh A, Padeh S, et al. Familial Mediterranean fever: new aspects and prospects at the end of the millenium. Isr. Med. Assoc. J. 1999; 1:31-36.

UNIV.