THE NEPHROTIC SYNDROME IN THE FIRST YEAR OF LIFE

By

LYDA P JADRESIC

M.B.B.S., M.R.C.P. (Paediatrics)

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Institute of Child Health
30 Guilford Street
LONDON WC1N 1EH
ABSTRACT

Children who develop the nephrotic syndrome during the first year of life have unique characteristics. This thesis comprises clinical and laboratory studies carried out in this group of children.

The clinical, radiological and histological features of 62 children who presented with the nephrotic syndrome under one year of age were analysed. Morbidity and mortality were found to be particularly severe during the first 3 months of life, irrespective of underlying histology. Clinico-histopathological correlations were more clearly established in those presenting over 3 months of age. Complex phenotypes were identified and previously unreported associations were described.

Loss of anionic charge from the glomerular basement membrane in the form of heparan sulphate may lead to the proteinuria in congenital nephrotic syndrome (CNS). In a study of urinary glycosaminoglycan excretion in children with different forms of CNS the ratio of heparan sulphate to chondroitin sulphate was found to be increased. This was not specific to a particular type of CNS and correlated with the degree of proteinuria.

The clinico-pathological features of 12 children with a nephropathy, Wilms' tumour and/or Genital abnormalities (Denys-Drash syndrome) were analysed. Ten progressed to end-stage renal failure, of whom 7 were less than 3 years of age. Seven children had Wilms' tumour, 3 of whom had
bilateral involvement. Ten children had a distinct pelvicalyceal abnormality which might be regarded as the fourth feature of this syndrome. A cytogenetic and molecular study was carried out focussing on the 11p13 region. One patient showed this deletion on karyotype. However, using gene dosage techniques on Southern blots, no microdeletions were demonstrated in any of the other patients studied.

This thesis has helped to define further the clinical description of children with early-onset nephrotic syndrome and has added to the existing knowledge of the pathological changes involved.
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PREFACE

The nephrotic syndrome which occurs during the first year of life (infantile nephrotic syndrome (INS)) should be considered separately from those forms affecting older children for a number of reasons. The main forms of the nephrotic syndrome presenting at this age do not occur in later childhood. They tend to be more severe in their clinical manifestations, are very often unresponsive to treatment and until recently have often been fatal. There is a strong tendency to a familial incidence and some forms are associated with other congenital abnormalities. These disorders constitute a fascinating group of diseases. Little is known about their pathogenesis or their underlying genetic defects. The nephrotic syndrome at this age is conventionally divided into congenital (CNS), when it presents before the age of 3 months, and infantile (INS), when the onset lies between 3 months and the first birthday.

The work undertaken in this thesis can be divided into 4 parts. The first is a retrospective clinical study, in the light of contemporary classification, of 62 children with nephrotic syndrome under the age of 1 year. Within this group, clinical, radiological and histological features of patients with Drash syndrome are described in detail.

Two laboratory studies were carried out. The first was an analysis of heparan sulphate and chondroitin sulphate
urine excretion in the urine in different forms of congenital nephrotic syndrome. It has previously been reported that some children with CNS have a low anionic charge in the glomerular basement membrane (GBM) with a high urinary heparan sulphate excretion. The aim of the present study was to determine whether these findings could be substantiated in a larger sample of patients, to study the relationship with proteinuria and to determine whether heparan sulphate excretion might be useful in differentiating between the various forms of INS.

The second study was a cytogenetic and molecular biological analysis of the 11p13 region in children with Drash syndrome. This region was targetted because of the phenotypic overlap between this syndrome and the WAGR complex (Wilms', aniridia, genito-urinary abnormalities and mental retardation) who may have deletions involving 11p13. This is the first molecular biology study of any group of children with INS.
1. Historical perspective

1.1.1 Early Reports. It has been recognised since the end of the last century, that the nephrotic syndrome was one of the complications of congenital syphilis (1). However, the first description of a child with congenital nephrotic syndrome (CNS) of non-syphilitic origin, by Gautier and Miville in Switzerland, did not appear until 1942 (2). They described a female baby born at term following a normal pregnancy, who had facial oedema at birth which after a few days became generalised. She was oliguric, developed respiratory insufficiency and died on the 19th day of life. Massive albuminuria was detected with a low serum total protein concentration; the serum urea was normal and the cholesterol concentration was high. The Wasserman reaction was negative. The authors concluded that she was a case of lipoid nephrosis of congenital onset. This report was followed by other isolated case reports of the nephrotic syndrome occurring in the neonatal period both in full-term and premature babies (3-9).

It soon became evident that the nephrotic syndrome occurring in the first few months of life constituted a clinical entity distinct from the idiopathic nephrotic syndrome of later childhood (10-12). One variety was reported with an unusually high frequency in Finland (12). Hallman et al were the first to coin the term "congenital
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nephrotic syndrome" (CNS) (12). The principal differences from idiopathic nephrotic syndrome of later childhood were its early onset, unresponsiveness to drug therapy (in particular steroids), very poor outcome, and a strong familial incidence (12).

1.1.2 Recognition of Finnish Type of CNS. During the 1950's and 60's, it became apparent that CNS occurred in Finland with a particularly high incidence (13). This disease soon became known as the CNS of Finnish type (CNSF).

The clinical details of children with CNSF have been extensively studied and the most comprehensive review appeared in 1976, when Huttunen reported the clinical features of 75 affected children (14). He found a high incidence of prematurity. There was a high incidence of breech presentation, and birth asphyxia was frequently reported, probably resulting from the severe hypoproteinaemic state, massive oedema and ascites.

A high placental/foetal weight ratio is one of the hallmarks of CNSF, greater than 25% of body weight (10, 14, 15). A large placenta (16), intrauterine growth retardation and prenatal asphixia (14), together with the presence of a raised amniotic fluid alpha fetoprotein (17), whose molecular size is similar to albumin (18), all indicate that this disease is present in utero. In most infants with CNSF oedema is detected shortly after birth and presentation is rare after 2 months of age (14, 19).
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Hypoproteinaemia and massive proteinurina (18) lead to severe hypovolaemia. Established uraemia is not seen and has not been reported as a cause of death (14). There is a tendency to thrombosis, which, although documented in other types of nephrotic syndrome (20, 21) is particularly severe in CNSF (14, 19). There is a very high incidence of infection and, together with thrombosis and embolic phenomena it is the main cause of death (14, 19).

Common lesions are mesangial hypercellularity and microcystic dilatation of proximal tubules although there is considerable heterogeneity in the histological changes observed (19, 22, 23).

The familial nature of CNSF was first reported by Hallman et al in 1959 (12). Its autosomal recessive inheritance was definitely established by Norio's studies on 57 families in Finland (24). The finding of raised $\alpha$-fetoprotein ($\alpha$FP) in amniotic fluid of affected foetuses has made prenatal diagnosis possible (17).

1.1.3 Diffuse Mesangial Sclerosis. In 1973, Habib and Bois published their experience of the nephrotic syndrome under the age of 1 year and identified the second major form of early onset nephrotic syndrome (25). They described 4 boys and 2 girls who developed nephrotic syndrome between 1 and 11 months of age. All showed signs of renal insufficiency at the time of diagnosis or within 1 to 2 years. Renal function deteriorated rapidly and all of them died in end-stage renal failure (ESRF) during the first 3
years of life. Their renal histology was defined as diffuse mesangial sclerosis (DMS).

In DMS, proteinuria and the nephrotic syndrome develop during the first year of life, occasionally as early as during the neonatal period. There is a gradual decline in renal function with ESRF by the third year of life. Occurrence of DMS in siblings has been reported (23, 25-27), suggesting an autosomal mode of inheritance (28).

This histological lesion with its accompanying clinical course has been described in association with syndromic forms of early onset nephrotic syndrome (29,30).

1.1.4 Syndromic Forms of Nephrotic Syndrome. Over the years it has been recognised the nephrotic syndrome under one year of age may occur in association with other congenital abnormalities. Short descriptions of 2 syndromes are given in this Section as examples of this type of CNS.

a) Denys-Drash syndrome. This syndrome consists of a triad that includes a nephropathy, Wilms' tumour and genital abnormalities (29,31-42). The nephropathy is characterised by proteinuria, which may be severe enough to lead to the nephrotic syndrome, and by the rapid progression into renal failure. Diffuse mesangial sclerosis is the most commonly reported histopathological abnormality (29,33,42).

b) CNS and Central Nervous System abnormalities. Early onset nephrotic syndrome may occur in association with microcephaly. Galloway and Mowat described the association of CNS with microcephaly in 1968 in 2 siblings (43). The
reports on the renal histology have been varied, although most have been associated with diffuse mesangial sclerosis. A fuller description of this syndrome is given later in this chapter.

By the beginning of the 1970's it had become clearly established that CNSF was the most common type of nephrotic syndrome in the first months of life, followed by DMS. Other forms of early onset nephrotic syndrome were also identified (25, 44, 45).

1.2. Classification of Nephrotic Syndrome in the 1st Year of Life

The nephrotic syndrome appearing before the first birthday consists of a heterogenous group of diseases. They have been classified into idiopathic, secondary and syndromic forms. Since the aetiology of neither idiopathic nor syndromic forms are known, the idiopathic group has been subdivided according to histological features and the syndromic group according to their associated clinical features. Most cases belong to the idiopathic group, followed by the syndromic group. Secondary forms are rare.

To some extent, each histological form in the idiopathic group may be correlated to a particular set of clinical features. However, these clinical characteristics as well as their histological features may be indistinct, particularly early in the course of the disease. Most of these forms have a familial incidence. The genetic defect underlying each of these conditions is not known.
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The conditions outlined in the secondary group have an identified aetiological agent, thought to be responsible for the emergence of the nephrotic syndrome. However, the exact mechanisms involved in the production of proteinuria are not known.

In the syndromic group diseases, early onset nephrotic syndrome occurs in association with congenital abnormalities, often involving organs other than the kidney. The histological features of these syndromic forms vary. Diffuse mesangial sclerosis is a frequent finding, and less so are features corresponding to focal glomerulosclerosis. Interestingly, to date, the Finnish type of CNS has not been described as a component of any syndrome.

The classification outlined below (see Table 1.1), was recently suggested by Rapola (46). It is the classification used in this thesis.
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**Table 1.1:** Classification of the Nephrotic Syndrome in the First Year of Life

<table>
<thead>
<tr>
<th>Idiopathic forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital nephrotic syndrome of Finnish type (CNSF)</td>
</tr>
<tr>
<td>Diffuse mesangial sclerosis (DMS)</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis (FSGS)</td>
</tr>
<tr>
<td>Minimal change nephrotic syndrome (MCNS)</td>
</tr>
<tr>
<td>Other glomerular disorders (diffuse mesangial proliferation, mesangiocapillary glomerulonephritis, etc.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secondary forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection: syphilis, toxoplasmosis, cytomegalovirus, rubella, malaria, hepatitis</td>
</tr>
<tr>
<td>Toxic agents: mercury</td>
</tr>
<tr>
<td>Others: systemic lupus erythematosus (SLE)</td>
</tr>
<tr>
<td>membranous glomerulonephritis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Syndromic forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denys-Drash syndrome (associated with either nephroblastoma and/or genital abnormalities)</td>
</tr>
<tr>
<td>Nephrotic syndrome associated with brain malformations (Galloway-Mowat syndrome)</td>
</tr>
<tr>
<td>Nail-patella syndrome</td>
</tr>
</tbody>
</table>
1.3. Important Clinicopathological Correlations in the Idiopathic Group

1.3.1. Congenital Nephrotic Syndrome of the Finnish Type (CNSF). The most important feature of CNSF is the very early onset, often prenatal, of massive proteinuria.

a) Birth characteristics.

Placentomegaly, prematurity, low birth weight, a high incidence of breech presentation are common findings in affected children (12-14).

Infants often showed delayed ossification (12) as well as musculoskeletal abnormalities partly as a result of altered intrauterine posture due to the mechanical effects of a large placenta. Such abnormalities included flexion deformities of the hips, knees and elbows and talipes calcaneovalgus. Other dysmorphic features found in these children are a large fontanelle with wide cranial sutures, small nose, wide set eyes and low ears (47). Umbilical herniae frequently occur due to high hydrostatic pressure exerted by the ascites on a weak musculature. Pyloric stenosis and gastroesophageal reflux have also been reported (19, 48).

A high placental/foetal weight ratio is one of the hallmarks of CNSF, greater than 25% of body weight (10, 14, 15). In a controlled study of 9 CNSF foetal placentae, Autio-Harainen showed a significant decrease in the villous vascularisation and more numerous and longer syncitial microvillous projections compared to normal placenta, but no other differences between normal and CNSF
placentae in villous volume or ramification pattern (16).

b) Characteristics of proteinuria and renal function. Proteinuria is glomerular in origin (49) and is highly selective both in the affected foetus and throughout the course of the disease (18, 49). Tubular proteinuria is not present, but may be seen with advancing tubular atrophy (49). Renal function is normal and increases during the first 6 to 7 months but thereafter it gradually deteriorates (49).

c) Infections and thrombosis. Thrombo-embolic events are particularly severe in CNSF. Hypovolaemia, sepsis, disturbances in coagulation and fibrinolysis all play a part in this phenomenon (14, 50-52). Infections occur as a result of hypogammaglobulinaemia through urinary losses (53, 54) and of failure of opsonisation (55, 56). Children with CNSF are in a permanent state of malnutrition and catabolism resulting from the massive loss of protein in the urine and from malabsorption secondary to gut oedema. This also contributes to their propensity to severe infections, such as peritonitis, pneumonia, septicaemias and meningitis (14, 19). The severe proteinuria may also give rise to secondary hypothyroidism (57, 58).

d) Histology in CNSF. The lesions observed in histology are partly dependant on the age at biopsy and none are pathognomonic (12, 19). Mesangial hypercellularity and microcystic dilatation are often observed. Tubular atrophy with interstitial cellular infiltration and fibrosis occur later. Glomerular sclerosis and tubulointerstitial changes
increase as the disease progresses (12, 19, 59, 60). On electron microscopy there is fusion of epithelial foot processes and microvillus proliferation of epithelial cell surfaces (19, 60). Glomerular basement membrane (GBM) thinning has been reported (61), although it may be within normal limits for age (17, 60). Other GBM changes include splitting and focal duplication (22, 23). Immunofluorescence microscopy is mostly negative (60), although occasionally IgG, IgM and C3 have been reported, occurring mostly in partially or totally sclerotic glomeruli (19, 54).

e) Genetics and prenatal diagnosis in CNSF. The frequency of CNSF in Finland has been estimated to be 1.2/10000 with a gene frequency of 1:200 (24). This disease also appears to occur as an autosomal recessive trait in families of non-Finnish ancestry (25, 19, 26).

The first report of a raised αFP in amniotic fluid dates back to 1967, when Seppala and coworkers reported an abnormally high level in amniotic fluid sampled in the third trimester, where the child had CNSF (62). This was followed by a report of the use of amniotic fluid sampling between 15 to 18 weeks gestation (63). Its reliability was demonstrated when no false positive or negative results were found in 23 high risk families (64). Maternal serum αFP can however be normal and therefore amniocentesis is always necessary for prenatal screening (64). It has been reported that the diagnosis of CNSF on the aborted foetuses may be missed on light microscopy as it may be normal,
therefore it is necessary to perform electron microscopy studies (64, 65).

The more constant features in CNSF are the detection of proteinuria at birth or soon after, a large placenta and a raised amniotic fluid 
afetoprotein. There is no single feature that is pathognomonic for CNSF and the diagnosis is arrived at by the clinical picture that emerges after gathering all the clinical and histological data.

1.3.2 Diffuse Mesangial Sclerosis (DMS). This is the second most important clinico-pathological entity. The most distinctive clinical feature of children with DMS and the major difference with CNSF is the dramatic progression into end-stage renal failure.

In contrast with CNSF, proteinuria may develop rather insiduously and the nephrotic syndrome may appear sometime in the first year of life and in some cases it may present during the second or third year (23, 25, 26, 44). However, some children present during the neonatal period (25, 26). Placental size, birth weight and gestation are normal (25, 26).

The renal lesion is characterised by mesangial matrix expansion without mesangial cell proliferation and progressive sclerosis and contraction of the capillary tuft. This is often accompanied by interstitial fibrosis and tubular atrophy (25, 19, 22, 26, 44). There may be immunofluorescent deposits in either the mesangium or the glomeruli, including IgM, IgA, IgG, C3 and C4 (22, 26, 66).
However, immunofluorescent studies may be completely negative (26, 44). Electron microscopy reveals mesangial cell expansion and irregular thickening of the glomerular basement membrane and fusion of foot process (19, 22, 26, 66).

Occurrence of DMS in siblings has been reported (23, 25, 26, 27, 28), suggesting an autosomal mode of inheritance.

1.3.3 Focal Glomerulosclerosis (FSGS). Children with FSGS may present at any time following birth and throughout childhood, a feature which is not shared by CNSF or DMS (45, 67, 68). Most children have an onset after the age of 1 year and only approximately 10% do so before the first birthday (68, 69). Some children may respond to steroids or to alkylating agents (25, 44, 70). However, this is uncommon in the under one group, particularly so in children with an onset before 3 months of age, who carry a grave prognosis (19, 25). Affected sibships have been described (19, 25). Indeed, it is probable that the earliest report of an infant with a familial form of nephrotic syndrome which appeared in The Lancet in 1893, may have been an example of FSGS (71). In this case, the parents were first cousins. The index child was noted to have "dropsy" or generalised oedema at the age of 12 months, the urine had a high specific gravity and there was gross albuminuria. He developed convulsions and died 4 weeks later. Three other siblings were similarly affected.
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and were diagnosed between 1 and 4 years of age.

On histology FSGS may be seen in association with mesangial proliferation, epithelial crescents and focal global sclerosis with associated tubulo-interstitial atrophy (25, 44). Recurrence of this lesion has been observed after renal grafting (72, 73).

1.3.4 Minimal Change Nephrotic Syndrome (MCNS). This is the most common form of nephrotic syndrome in childhood accounting for approximately 80% of cases (67, 74). However, only around 5% of children with MCNS present in the first year of life (25, 68). There have been only a few reports of infants with MCNS (25, 26, 44). Responsiveness to steroids and alkylating agents has been variable and the younger the onset the less likely the steroid responsiveness (13, 14, 17).

1.4 Controversies on Classification

Bearing in mind that clinico-pathological correlations have been observed, these are not by any means categorical. For example, it is well documented that if the initial biopsy on a child presenting with the nephrotic syndrome is performed early in the course of the disease this may show only minimal changes. Repeat biopsies may later show changes of either focal glomerulosclerosis, CNSF or DMS (25, 75).

Although the idiopathic group has been divided into the histological categories outlined above, some dispute the
extent to which it is possible to be sure that a particular case falls within one or other histological type. Whereas some authors (25, 44, 76) postulate that histology can be used for a working classification of the idiopathic group, others (19, 22) dispute this. They do so on the basis that many histological features are non-specific and are shared among the different types. This, they argue, makes prediction of course and eventual outcome impossible on the basis of histological parameters.

1.5 Features of Secondary Forms of NS under 1 year of Age

1.5.1 NS due to Infections. The nephrotic syndrome may appear in this age group mostly as a result of intrauterine infection, such as syphilis (1, 77-80), toxoplasmosis (81-85), rubella (83), cytomegalovirus (11). The first report of CNS secondary to syphilis dates back to 1871 (1). Only a minority of children with congenital syphilis develop a nephropathy (77-80) and the nephropathy disappears with adequate penicillin therapy alone (78, 79).

In CNS secondary to Toxoplasmosis presentation may occur at birth or it may develop after a latent period which may last between 1 to 12 months (81-85). All cases described have had clinical evidence of systemic toxoplasmosis in the form of chorioretinitis, microphthalmus or intracranial calcifications. There may be haematuria, hypertension and / or renal insufficiency. The renal prognosis is good provided children are treated with steroids. Adequate anti-toxoplasmic therapy alone does not
halt the nephropathy nor it prevents the appearance of the nephrotic syndrome (84, 85).

1.5.2. Toxic Agents. One of the cases reported by Worthen et al developed the nephrotic syndrome at 7 months possibly as a result of exposure to mercury, used to rinse the infants' diapers. Abnormally high urine mercury levels were found and renal biopsy showed a mild degree was glomerular hypercellularity (11).

1.5.3 Others. There has been only one case reported of a child who presented with the nephrotic syndrome at 3 months of age, who was found to have Systemic Lupus Erythematosus (86). She responded to steroids with complete disappearance of haematuria and the nephrotic syndrome.

Membranous nephritis is most common in adults, but it does occur in children although rarely before the age of 1 year (87, 88). It may have no obvious cause but it may follow infections, such as upper respiratory tract infections, acute hepatitis, scarlet fever. Its severity may vary from mild proteinuria to the nephrotic syndrome. It has a variable prognosis and end-stage renal failure tends to occur in children who develop the nephrotic syndrome (87, 88).

1.6 Features of Syndromic Forms of NS under 1 year of Age

1.6.1 Denys-Drash Syndrome. This syndrome was briefly referred to in Section 1.1.4. It consists of a triad that
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includes a nephropathy, Wilms' tumour and genital abnormalities (29,31-34). Incomplete forms also occur where the nephropathy is the common denominator. Thus, children with nephropathy and Wilms' tumour alone have been described (29, 33,35-38). Similarly, there have been numerous reports of the nephropathy and genital abnormalities occurring in the absence of Wilms's tumour (29,33, 39-41). The nephropathy is characterised by the early appearance, usually in the first year of life of proteinurina, which may be severe enough to lead to the nephrotic syndrome. There is a dramatic progression into end-stage renal failure, commonly before the age of 3 years (29,31-41). In those cases where renal histology has been performed, changes of diffuse mesangial sclerosis are commonly described (29,33,34,42). These changes are the same as seen in idiopathic, isolated diffuse mesangial sclerosis, with a similar clinical course as described by Habib and Bois (25). The Wilms' tumour is of early onset, and often bilateral (29). The genital abnormality most often reported is male pseudohermaphroditism, with a sexual phenotype varying from ambiguous genitalia to a normal female phenotype (29,31-34,39,41,42).

1.6.2 CNS and Central Nervous System abnormalities. The association of CNS and microcephaly (Galloway and Mowat syndrome (43)) was briefly described in Section 1.1.4. There have been 14 cases reported to date (43, 89-92). The onset of the nephrotic syndrome has occurred during the first year of life in all but one and half have presented
within the first month of life. Some have developed renal insufficiency. In only one case there seemed to be a partial response to steroids (43). A low brain weight has been observed in all cases examined at autopsy. In all children surviving the first few months of life there have been serious neurological deficit accompanied by mental retardation. Convulsions have been observed in 5. Most have died before 3 years of age due to respiratory infections or renal insufficiency.

The reports on the renal histology have been varied. Some have shown normal glomeruli and a few isolated glomeruli with hyaline change (43), minimal change and subsequently FSGS (30), "microcystic dysplasia" and changes of FSGS (89) and diffuse mesangial sclerosis (30, 90).

A number of dysmorphic features have been described, such as hyper and hypotelorism, lateral iris hypoplasia, large and floppy ears, low foreheads and flat occiput, micrognathia, camptodactyly and club feet (92). The presence of a hiatus hernia has been described in the cases reported by Galloway and Mowat and Shapiro et al (43, 89).

There is a strong family history, with 6 pairs of sibs reported, 9 males and 5 females. No history of parental consanguinity has been reported. Whenever karyotypes have been performed, they have been normal.

1.6.3 Nail-Patella Syndrome. Hereditary onycho-osteodysplasia or nail-patella syndrome is an autosomal
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dominant disease, which consists of multiple osseous abnormalities, including absent or hypoplastic patellae, hypoplasia of radial heads and iliac horns, flexion deformities, nail hypoplasia, ocular abnormalities and a nephropathy (93). There is one report of a neonate who presented with the nephrotic syndrome at birth, which disappeared spontaneously by 2 weeks of age (94).

1.7 Pathogenesis of CNS

Very little is known about the aetiology of the different forms of the idiopathic and syndromic forms of the nephrotic syndrome.

1.7.1 Evolution of Theories on Pathogenesis. From an early date it was believed that the fundamental disorder in CNSF lay in the glomerular basement membrane (GBM) (13). One of the earliest theories postulated that CNS was the result of an inborn error of metabolism. This was based on the observation of crystal formation and proximal tubular dilatation on renal biopsies (8). This crystalisation was later found to be an artifact of the tissue fixation technique (11).

An immune mechanism was postulated by Kouvalainen et al (95) and Lange et al (96) on the basis of the demonstration of IgG on the GBM and early skin graft rejection in nephrotic children receiving a graft from their mothers (97). These observations were not confirmed subsequently (54).
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1.7.2 Genetic Aspects of Early Onset Nephrotic Syndrome

Affected sibships have been described in the idiopathic forms (19, 24, 25) and in some of the syndromic forms of nephrotic syndrome (43, 98). The extensive family studies performed by Norio in the 1950's demonstrated that CNSF was inherited as an autosomal recessive trait (24). Reports of recurrence of DMS in siblings with approximately equal male to female ratio, and parental consanguinity suggest that DMS may also be inherited as an autosomal trait.

These early forms of nephrotic syndrome may arise as a consequence of a genetic defect resulting in the absence or defective synthesis of a protein. This protein might be a constituent of the glomerular filtration barrier.

The process by which the syndromic forms of CNS arise is unknown. They could arise as a result of a missing or dysfunctioning single protein normally present in the organs involved in the syndrome. Alternatively, these diseases might be the result of genetic loss affecting neighbouring loci in the same chromosome.

1.7.3 Alteration of GBM Anionic Charge in Children with CNS. There is considerable evidence that the GBM acts as both a size and charge selectivity barrier (99-101). Research on the pathogenesis of proteinuria in renal disease has focused on alterations in the charge and size filtration characteristics of the GBM (102-106). Based on isolated rat kidney perfusion studies, Rozensweig and Kanwar have shown that removal of heparan sulphate and
other glycosaminoglycans by heparatinase greatly increases the leakage of serum albumin across the glomerular basement membrane (107).

In 1983, Vernier et al published the results of a study of anionic charge in the GBM of children with CNSF. The cationic probe polyethyleneimine, was used to label GBM anionic sites on fixed frozen preparations of kidney tissue. Decreased number of anionic sites were demonstrated on 5 children with CNSF. By using heparatinase, they were able to demonstrate that heparan sulphate was the main provider of negative charge in GBM from normal kidney (108). However, ex-vivo perfusion studies on CNSF nephrectomy specimens, using polyethyleneimine did not reveal differences in anionic charge from normal kidneys (109).

Vermylen et al studied the glycosaminoglycan content in the kidney of a child with DMS. They isolated GBM, separated individual glycosaminoglycans by cellulose acetate electrophoresis and quantitated them by Alcian Blue binding. They found that the heparan sulphate content in the GBM was found to be markedly reduced. However, the urinary content of heparan sulphate was markedly raised in this child and in 3 other children, 2 with CNSF and one with DMS. Thus, they postulated that children with CNS could make heparan sulphate but failed to incorporate it normally to the GBM (110).

However, subsequent studies using monoclonal antibodies to the heparan sulphate core protein did not detect any
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differences in the heparan sulphate content between normal GBM and the GBM from CNSF kidneys (111). The reasons for the differences in these studies is not yet apparent.

1.8 Study

1.8.1 Clinical Review of Children with Early Onset of Nephrotic Syndrome. A review was undertaken of clinical, histological and radiological features of 62 children with onset of the nephrotic syndrome under the age of 1 year, seen at The Hospital for Sick Children from 1963 to 1990.

1.8.2 A Study of Heparan Sulphate and Chondroitin Sulphate Urinary Excretion in Children with Early Onset Nephrotic Syndrome. The observation of increased urinary heparan sulphate (HS) in 2 children with CNSF and 2 with DMS (110) needed to be expanded by studying a greater number of children with different forms of early onset nephrotic syndrome. If these observations were confirmed, a second question arose as to how specific this finding was to a particular histological diagnosis. It was also of interest to study the possible relationship between raised urinary HS and the degree of albuminuria. This study was carried out in children with CNSF, DMS and FSGS and results were compared to normals and to children with steroid sensitive nephrotic syndrome. The relationship of HS and CS excretion with albuminuria was investigated.

1.8.3 Clinical Review of Patients with the Syndrome of
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Nephropathy, Wilms' Tumour and Genital Abnormalities (Denys-Drash Syndrome). A detailed study was undertaken of 12 children with a nephropathy and either Wilms' tumour or genital abnormalities, or both. Clinical, histopathological and radiological features were analysed.

1.8.4 Cytogenetic and Molecular Biological Study of Children with Denys-Drash Syndrome. The Denys-Drash phenotype with its nephropathy, genital abnormalities and Wilms' tumour overlaps with the WAGR complex (Wilms, Aniridia, Genitourinary abnormalities, Mental Retardation). Children with WAGR have been shown to have a deletion involving the 11p13 region (112). This close resemblance to WAGR and its 11p13 deletion presented a genetic "clue" as to where the locus of a form of early onset nephrotic syndrome might lie. In order to investigate this hypothesis a karyotype analysis and a molecular biological study of the p13 region of chromosome 11 was carried out. This section includes the results of a study of the Xq21-22 area in these children.

The thesis is divided into chapters corresponding to the above studies and a discussion of results is included after each one. The final chapter consists of a concluding discussion.
2. CLINICAL REVIEW OF CHILDREN PRESENTING WITH THE NEPHROTIC SYNDROME (NS) UNDER THE AGE OF ONE YEAR

2.1. Introduction

The objective of this chapter is to describe the clinical characteristics, as well as to provide a brief histopathological description of 62 children who presented with the nephrotic syndrome under one year of age. The way in which clinical features correlate with the histological diagnosis is analysed in order to confirm, refute or add to, previously reported clinico-pathological correlations.

This account illustrates the great heterogeneity observed in the nephrotic syndrome in this age group. A number of clinical patterns or syndromes not previously reported are also described.

In this thesis, the histological classification outlined in section 1.2 has been used for the idiopathic group. However, in this chapter, I use some examples to illustrate the pitfalls and shortcomings of this current classification.

2.2. Patients and Methods

2.2.1 Patients. The medical records of all children who presented with the nephrotic syndrome under the age of 1 year between 1963 and 1990 to the Hospital for Sick Children (HSC), Great Ormond Street, were reviewed. The nephrotic syndrome was defined by the presence of
hypoalbuminaemia (plasma albumin concentration less than 25g/l) and proteinuria (urine albumin to urine creatinine ratio of more than 1; normal = less than 0.1 (113), or 3+ or more in dipstick urine testing, or urine protein excretion more than 40 mg/m²SA/hr).

2.2.2 Methods. The following information was obtained from the medical records:

a) Gestation, mode of delivery, placental weight, and maternal α-fetoprotein (α-FP)
b) Age at detection of proteinuria
c) Family history (occurrence of disease in sibs, parental consanguinity)
d) Histological diagnosis
e) State of proteinuria at last follow up
f) Renal outcome and age at end-stage renal failure
g) Clinical complications: infections (site, organism), thrombosis and infarctions (site), fits (and their association with known metabolic disturbances or other cause)
h) Associated congenital abnormalities
i) Outcome

Renal histology was available from biopsy or nephrectomy specimens in 47 and post mortem specimens in 6. A histological review of renal biopsies was carried out. They were classified according to the histological categories outlined in section 1.2.
Chapter 2: NS Clinical Review

2.3 Results

Sixty two children presented with the nephrotic syndrome under one year of age between 1963 and 1990. Table 2.1 on the next page, shows their distribution according to the classification discussed in Chapter 1.
TABLE 2.1: Distribution of children presenting with nephrotic syndrome under 1 yr according to diagnosis

IDIOPATHIC FORMS

Finnish type (CNSF) (17)
Diffuse mesangial sclerosis (DMS) (8)
Focal segmental glomerulosclerosis (FSGS) (8)
Minimal change nephrotic syndrome (MCNS) (3)
Other Glomerular Disorders:
   diffuse mesangial proliferation (2),
   mesangiocapillary glomerulonephritis (1))

SECONDARY FORMS

Infection: syphilis (1), cytomegalovirus (1)
Others: storage disorder (1)

SYNDROMIC FORMS

Denys-Drash syndrome (5)
Galloway-Mowat syndrome) (5)
Others: CNS and ocular abnormalities (1)
   FSGS, VSD and pelvi-calyceal abnormalities (2)
   CNS, epidermolysis bullosa and pulmonary hypertension (1)

UNASSIGNED IDIOPATHIC CASES (no renal biopsy) (6)
2.3.1 Details of Individual Cases According to Diagnosis. Tables 2.2 to 2.6 show details of all children grouped by histological diagnosis.

a) Congenital nephrotic syndrome of Finnish type (Table 2.2). Table 2.2 shows a summary of the clinical details of the 17 children with CNSF. There were 9 males and 8 females. Fifteen were diagnosed during the first month of life, one was diagnosed at 2 months of age and another at 10 months. Six have survived, 4 of whom have had elective bilateral nephrectomies and end-stage renal failure (ESRF) treatment, 1 developed ESRF at 10 years and is now on peritoneal dialysis and another has undergone spontaneous partial remission with a current serum albumin concentration between 25 - 30 g/l. Nine children had musculoskeletal and gastrointestinal abnormalities, which are likely to be the result of massive proteinuria with subsequent poor muscular and skeletal development. Amongst them are flexion contractures, abnormal skull development with wide fontanelles, wide sutures and Wormian bone formation on the vault of the skull. Two had gastro-oesophageal reflux and 2 had patent ductus arteriosus. Patient 7 who had a poor Visual Evoked Response (VER), had had severe birth asphixia and it is unlikely that this abnormality was congenital in origin.
### TABLE 2.2: Clinical details of CNSF group

<table>
<thead>
<tr>
<th>Pt</th>
<th>F.H./consang</th>
<th>Sex</th>
<th>Age at diagnosis (yr)</th>
<th>Age at follow-up</th>
<th>Renal failure</th>
<th>Nephrotic syndrome</th>
<th>Status</th>
<th>Comorbidities</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-/-</td>
<td>M</td>
<td>0.05</td>
<td>0.17</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Septicaemia, hypocalcaemic fits</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-/-</td>
<td>F</td>
<td>0.10</td>
<td>0.23</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>UTI, peritonitis, thrombosis D Pedis artery</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-/-</td>
<td>F</td>
<td>0.01</td>
<td>0.06</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Septicaemia, renal vein thrombosis</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-/-</td>
<td>M</td>
<td>0.01</td>
<td>NK</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>UTI, Flexion deformities</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-/-</td>
<td>F</td>
<td>0.01</td>
<td>0.50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>UTI, Patent ductus arteriosus</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+/-</td>
<td>M</td>
<td>0.03</td>
<td>0.33</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>UTI, hypoglycaemic fits, Flexion contractures</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-/-</td>
<td>F</td>
<td>0.06</td>
<td>0.41</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Renal vein thrombosis, Poor Visual Evoked Response</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+/-</td>
<td>M</td>
<td>0.03</td>
<td>0.13</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Septicaemia, hypocalcaemic fits</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-/-</td>
<td>M</td>
<td>0.06</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Hypocalcaemic fits</td>
<td></td>
</tr>
</tbody>
</table>

**Key to abbreviations used in Tables 2.2. to 2.6:**
- A = alive
- Consang = consanguinity
- Cycloph = cyclophosphamide
- D. Pedis = dorsalis Pedis artery
- F = female
- FH = family history
- IVC = inferior vena cava
- M = male
- MPS = male pseudohermaphroditism
- N = nephrotic
- NK = not known
- PD = peritoneal dialysis
- PDA = patent ductus arteriosus
- PR = partial remission
- Pred = prednisolone
- Pt = patient
- TX = renal transplant
- UTI = urinary tract infection
- + = alive
- - = dead.
TABLE 2.2 (cont.): Clinical details of CNSF group

<table>
<thead>
<tr>
<th>Pt</th>
<th>F.H.</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>THERAPY</th>
<th>OUTCOME</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>consang</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pulmonary embolus. PDA, gastro-oesophageal reflux</td>
</tr>
<tr>
<td>10</td>
<td>-/-</td>
<td>F</td>
<td>0.83</td>
<td>+</td>
<td>1.40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
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</tr>
<tr>
<td>11</td>
<td>-/-</td>
<td>F</td>
<td>0.02</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-/-</td>
<td>M</td>
<td>0.16</td>
<td>-</td>
<td>2.30</td>
<td>PD (1.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>13</td>
<td>+/-</td>
<td>M</td>
<td>0.00</td>
<td>-</td>
<td>5.00</td>
<td>Tx (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>14</td>
<td>+/-</td>
<td>F</td>
<td>0.00</td>
<td>-</td>
<td>2.25</td>
<td>Tx (2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>15</td>
<td>+/-</td>
<td>M</td>
<td>0.07</td>
<td>-</td>
<td>10.00</td>
<td>PD (9.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td>+ PR</td>
</tr>
<tr>
<td>16</td>
<td>+/-</td>
<td>F</td>
<td>0.04</td>
<td>-</td>
<td>0.83</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td>- PR</td>
</tr>
<tr>
<td>17</td>
<td>+/-</td>
<td>M</td>
<td>0.09</td>
<td>-</td>
<td>2.30</td>
<td>Tx (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td>(+)</td>
</tr>
</tbody>
</table>

Hypocalcaemic fits. High frequency deafness, large fontanelle, gastro-oesophageal reflux, hiatus hernia.
b) Diffuse mesangial sclerosis (Table 2.3). There were 11 males and 7 females (in six confirmed by karyotype). Age at presentation spanned the whole of the first year, ranging from the first week to 0.9 yr. There are only 2 survivors, 8 died of renal failure. The other 8 children died of complications attributable to profound hypovolaemia, secondary to the nephrotic state. These complications included cardiovascular collapse and infections. The 2 surviving children presented within the last 4 years and underwent dialysis at an early age (0.9 yr and 2.2 yr respectively).

Fifteen children in this group had other congenital abnormalities affecting various organs. There were 5 children with Denys-Drash syndrome. One had the complete triad (nephropathy, male pseudohermaphroditism and Wilms' tumour) and 4 had incomplete Denys-Drash syndrome: 3 had male pseudohermaphroditism and 1 had Wilms' tumour. Three of the children with Denys-Drash syndrome had pelvi-calyceal abnormalities. These children as well as 7 other children with Denys-Drash syndrome on whom the nephropathy developed after the first year of life, are described in detail in Chapter 4.

Seven children with DMS had neurodevelopmental abnormalities. Four of these had microcephaly, compatible with a diagnosis of Galloway-Mowat syndrome. This group was made up of 2 unrelated sibships. In addition, there were 2 other children with developmental delay, one of whom had incomplete Denys-Drash syndrome and a normal head
TABLE 2.3: Clinical details of DMS group

<table>
<thead>
<tr>
<th>Pt</th>
<th>F.H./ Sex</th>
<th>Age at Sex</th>
<th>Age at</th>
<th>Therpy</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>consang</td>
<td>diagnosis</td>
<td>follow-up</td>
<td>Pred. Cycloph.</td>
<td>Status</td>
<td>Renal failure</td>
</tr>
<tr>
<td>18</td>
<td>-/-</td>
<td>F 0.06</td>
<td>0.15</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>+/+</td>
<td>F 0.29</td>
<td>0.83</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>+/-</td>
<td>F 0.04</td>
<td>0.10</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>+/-</td>
<td>F 0.01</td>
<td>0.16</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>+/-</td>
<td>F 0.03</td>
<td>0.50</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>-/-</td>
<td>M 0.83</td>
<td>1.50</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>-/+</td>
<td>M 0.80</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>-/-</td>
<td>F 0.04</td>
<td>2.74</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>-/-</td>
<td>M 0.09</td>
<td>0.33</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>-/+</td>
<td>M 0.60</td>
<td>NK</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pt</td>
<td>F.H./consang</td>
<td>Sex</td>
<td>Age at diagnosis</td>
<td>THERAPY</td>
<td>Pred. Cycloph.</td>
<td>Age at follow-up</td>
</tr>
<tr>
<td>----</td>
<td>--------------</td>
<td>-----</td>
<td>------------------</td>
<td>---------</td>
<td>----------------</td>
<td>------------------</td>
</tr>
<tr>
<td>28</td>
<td>-/-</td>
<td>M</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
<td>0.09</td>
</tr>
<tr>
<td>29</td>
<td>-/-</td>
<td>M</td>
<td>0.41</td>
<td>-</td>
<td>-</td>
<td>1.60</td>
</tr>
<tr>
<td>30</td>
<td>-/-</td>
<td>M</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>0.24</td>
</tr>
<tr>
<td>31</td>
<td>-/-</td>
<td>M</td>
<td>0.91</td>
<td>-</td>
<td>-</td>
<td>1.16</td>
</tr>
<tr>
<td>32</td>
<td>-/-</td>
<td>M</td>
<td>0.83</td>
<td>-</td>
<td>-</td>
<td>2.30 Tx (2.25)</td>
</tr>
<tr>
<td>33</td>
<td>+/-</td>
<td>M</td>
<td>0.41</td>
<td>-</td>
<td>-</td>
<td>1.16</td>
</tr>
<tr>
<td>34</td>
<td>+/-</td>
<td>F</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>4.00 Tx (4.00)</td>
</tr>
<tr>
<td>35</td>
<td>+/-</td>
<td>M</td>
<td>0.25</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
</tr>
</tbody>
</table>
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circumference; there were no head circumference measurements available in the second child. DMS was also seen in a child with retinal atrophy and cerebral atrophy on computerised tomography with normal head circumference. These cases are described in detail under the heading "Case reports" in Section 2.3.10.

c) Focal segmental glomerulosclerosis (Table 2.4). There were 10 children with FSGS, 7 males and 3 females. Age at presentation ranged from 4 weeks (0.07 yr) to 10 months (0.83 yr). Five presented in the second half of the first year. Four have died, 2 of whom in ESRF with an onset at 2 and 4 yr of age. The cause of death is not known in the other 2 children.

Associated congenital anomalies were observed in this group in 2 patients. Two unrelated children (Pts 39 and 41) had a ventricular-septal defect, ESRF by 4 years of age and abnormal pelvi-calyceal systems detected by intravenous urography. These abnormalities consisted of blunted renal calyceal pelves. An absent renal pelvis was also noted in Pt 41. This constellation of abnormalities has not been reported previously. Their case histories are further described in Section 2.3.10.
**TABLE 2.4: Clinical details of FSGS group**

<table>
<thead>
<tr>
<th>Pt</th>
<th>F.H./M.</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>THERAPY</th>
<th>Age at follow-up</th>
<th>Status</th>
<th>Renal failure</th>
<th>Nephrotic syndrome</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>-/-</td>
<td>M</td>
<td>0.66</td>
<td>+ +</td>
<td>1.24</td>
<td>+</td>
<td>-</td>
<td>PR</td>
<td>UTI, septicaemia. Anaemia, thrombocytopenia, gastrointestinal intolerance</td>
</tr>
<tr>
<td>37</td>
<td>+/-</td>
<td>F</td>
<td>0.58</td>
<td>- -</td>
<td>NK</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>UTI. Patent ductus arteriosus</td>
</tr>
<tr>
<td>38</td>
<td>-/-</td>
<td>M</td>
<td>0.10</td>
<td>+ +</td>
<td>2.16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>UTI.</td>
</tr>
<tr>
<td>39</td>
<td>+/-</td>
<td>M</td>
<td>0.58</td>
<td>+ +</td>
<td>15.16</td>
<td>T x (15.16)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>-/-</td>
<td>M</td>
<td>0.23</td>
<td>- -</td>
<td>4.16</td>
<td>+</td>
<td>+</td>
<td>PR</td>
<td>Blunted calyces, vesico-ureteric reflux, ventriculo-septal defect congenital lung cyst</td>
</tr>
<tr>
<td>41</td>
<td>-/-</td>
<td>M</td>
<td>0.25</td>
<td>- -</td>
<td>6.00</td>
<td>T x (5.00)</td>
<td>+</td>
<td>PR</td>
<td>Meningitis, septicaemia, peritonitis blunted calyces, absent renal pelvis ventriculo-septal defect</td>
</tr>
<tr>
<td>42</td>
<td>-/-</td>
<td>F</td>
<td>0.41</td>
<td>+ -</td>
<td>4.30</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>Wide skull sutures</td>
</tr>
<tr>
<td>43</td>
<td>+/-</td>
<td>M</td>
<td>0.21</td>
<td>+ -</td>
<td>3.16</td>
<td>A</td>
<td>-</td>
<td>+</td>
<td>Pulmonary stenosis</td>
</tr>
<tr>
<td>44</td>
<td>+/-</td>
<td>F</td>
<td>0.83</td>
<td>+ +</td>
<td>6.66</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45</td>
<td>-/-</td>
<td>M</td>
<td>0.66</td>
<td>+ +</td>
<td>8.00</td>
<td>P d (8.00)</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
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d) Miscellaneous (Table 2.5). There were 3 patients with a histological diagnosis of minimal change (MCNS). One presented during the second week of life (Pt 46). Her sister is Pt 19 who had DMS. The other 2 children presented after 6 months of age. All 3 are alive.

There were 2 patients with diffuse mesangial proliferation (DMP), one of whom is well and in remission after treatment with steroids, the other developed ESRF at 4 years of age and died following an unsuccessful renal transplant.

Patient 49 had a histological changes that resembled those observed in Alports' nephropathy. He was born with a severe type of epidermolysis bullosa and subsequently developed a progressive and fatal form of pulmonary hypertension. He is described further in Section 2.3.10.

There was one patient with mesangiocapillary glomerulonephritis who was nephrotic at presentation, had mild impairment of renal function and severe hypertension. At last follow-up she was no longer nephrotic but renal function had deteriorated further.

Patient 53 had a form of storage disorder, the nature of which is not known. He is described further in Section 2.3.9.
### TABLE 2.5: Clinical details of "miscellaneous" group

<table>
<thead>
<tr>
<th>Pt</th>
<th>F.H./consang</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>THERAPY</th>
<th>---</th>
<th>---</th>
<th>OUTCOME</th>
<th>Age at follow-up</th>
<th>Status</th>
<th>Renal failure</th>
<th>Nephrotic syndrome</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>+/+</td>
<td>F</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.16</td>
<td>A</td>
<td>-</td>
<td>PR</td>
<td>-</td>
</tr>
<tr>
<td>47</td>
<td>-/-</td>
<td>F</td>
<td>0.66</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>2.16</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48</td>
<td>-/-</td>
<td>F</td>
<td>0.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14.00</td>
<td>A</td>
<td>-</td>
<td>PR</td>
<td>UTI</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>-/+</td>
<td>M</td>
<td>0.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Septicaemia, Pneumocystis chest inf. Epidermolysis bullosa, pulmonary hypertension</td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>+/-</td>
<td>M</td>
<td>0.33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.50</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>UTI, gut infarcts. Flexion contractures, developmental delay</td>
</tr>
<tr>
<td>51</td>
<td>-/-</td>
<td>F</td>
<td>0.58</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.50</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>Myasthenia gravis (diagnosed at 1.33 yrs)</td>
</tr>
<tr>
<td>MCGB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>+/+</td>
<td>F</td>
<td>0.58</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.25</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>Septicaemia. Cerebral atrophy</td>
</tr>
<tr>
<td>Storage disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>-/-</td>
<td>M</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Hepatomegaly, &quot;gargoyle&quot; face and bone appearances, thickened heart valves</td>
</tr>
</tbody>
</table>
e) Unassigned cases (no renal biopsy) (Table 2.6) There were 9 children who did not have renal biopsy. Five children presented at or before 2 months of age and all died. One of them probably had CNSF as 2 older siblings had had CNSF on biopsy. Of the survivors, Pt 62 presented at 0.27 yr with congenital syphilis. The remaining 3 children (Pts 59, 60 and 61) were diagnosed during the second half of the first year and together with Pts 47 and 48 are probably examples of very early presentation of "idiopathic nephrotic syndrome" (114) usually diagnosed in later childhood. All 3 are in remission.
### TABLE 2.6: Clinical details of unbiopsied group

<table>
<thead>
<tr>
<th>Pt</th>
<th>F.H./consang</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Pred. Cycloph.</th>
<th>THERAPY</th>
<th>Age at follow-up</th>
<th>Status</th>
<th>Renal failure</th>
<th>Nephrotic syndrome</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>+/-</td>
<td>F</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>NK</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Wide skull sutures</td>
</tr>
<tr>
<td>55</td>
<td>-/-</td>
<td>M</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>3.41</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Flexion contractures, odd facies Wormian bones, large fontanelle</td>
</tr>
<tr>
<td>56</td>
<td>+/-</td>
<td>M</td>
<td>0.01</td>
<td>+</td>
<td>+</td>
<td>0.33</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Hyponatraemic fits. Microcephaly, dislocated hip</td>
</tr>
<tr>
<td>57</td>
<td>-/-</td>
<td>M</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>0.03</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>UTI</td>
</tr>
<tr>
<td>58</td>
<td>-/-</td>
<td>M</td>
<td>0.16</td>
<td>-</td>
<td>-</td>
<td>NK</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Septicaemia. Congenital CMV infection</td>
</tr>
<tr>
<td>59</td>
<td>-/-</td>
<td>F</td>
<td>0.91</td>
<td>+</td>
<td>-</td>
<td>1.58</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>UTI</td>
</tr>
<tr>
<td>60</td>
<td>-/-</td>
<td>M</td>
<td>0.91</td>
<td>-</td>
<td>+</td>
<td>3.58</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>UTI</td>
</tr>
<tr>
<td>61</td>
<td>-/-</td>
<td>M</td>
<td>0.66</td>
<td>+</td>
<td>-</td>
<td>0.83</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>-/-</td>
<td>M</td>
<td>0.27</td>
<td>-</td>
<td>-</td>
<td>6.00</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>Congenital syphilis</td>
</tr>
</tbody>
</table>
2.3.2 Age at Presentation According to Diagnosis. In the CNSF group, all but one were diagnosed before 3 months of age. Most presented during the first month of life (Fig 2.1). Approximately half the children with DMS presenting in the first year of life did so before the age of 1 month. The rest were diagnosed throughout the first year. FSGS and MCNS occur much less commonly during the first year and although the figures involved are small, there is the suggestion in the FSGS group of a relatively even spread during this time.

2.3.3 Genetic Factors. Analysis of the family history revealed a number of affected sibships. These were encountered in almost all groups.

There were 4 affected sibships in the CNSF group. Interestingly, patient 6 had 1 sibling with CNS and another with Potters facies and renal dysplasia who died at 12 hrs of age. Patient 13 had a monozygotic twin who was a stillbirth whose post mortem renal histology showed CNSF. Pt 17 had a sib with CNS who died at 40 hrs of age, on whom histology was not available. Pt 54 was not biopsied but had 2 siblings with histologically documented CNSF.

There were 4 affected sibships in the DMS group. Patients 19 and 46 are sisters; pt 19 had DMS on histology but pt 46 had "minimal change" on histology and a milder clinical course. Pts 20 and 21 are sisters and both had a diagnosis of DMS in addition to similar congenital abnormalities. Pt 22, female, had a sibling on whom a
Fig. 2.1: Age at diagnosis in children presenting with the nephrotic syndrome under 1 year of age
probable diagnosis of CNS was made at another hospital; he had severe birth asphyxia and a large placental weight of 900 grammes. Pts 33, 34 and 35 are siblings, 2 males and 1 female. Pt 35 was diagnosed at another hospital but post mortem specimens were studied at HSC. Additional congenital abnormalities were also observed in this sibship.

The karyotype in 6 of the 8 phenotypic females in the DMS group was 46XX. The importance of confirming the gender by karyotype in the females in this group is that it confirms that these cases are unlikely to be missed cases of Denys-Drash syndrome with XY sex chromosomes. In the remaining patient with a female phenotype (Pt 28), the karyotype was 46XY and a diagnosis of incomplete (nephropathy and male pseudohermaphroditism) Denys-Drash syndrome was made. Only 1 patient (Pt 22) with a female phenotype did not have karyotypic analysis.

There were 2 affected sibships in the FSGS group: 2 sisters with FSGS (index Pt 37) and 2 brothers with FSGS (index Pt 43). In both instances the siblings were diagnosed at another hospital.

Pt 52 who has diffuse mesangiocapillary glomerulonephritis (MCGN) had 2 sisters and 1 brother seen at another centre, who had a diagnosis of "glomerulosclerosis". Pt 50, who had mesangioproliferative glomerulonephritis (MPGN) had a brother with a diagnosis of MPGN and FSGS made at another centre.

There were 8 instances of parental consanguinity, 4 in the CNSF group, 3 in DMS and 1 with MCGN.
2.3.4 Obstetric Data.

a) Placental weight to birth weight ratio (Table 2.7).
Records of placental and birth weights were available in 20 children. There was an additional case where the placenta was described as "enormous" but no weight was recorded.

A placenta to birth weight ratio of more than 25% has been described as one of the characteristics of CNSF (16). In the 10 children with CNSF where the data is available, the ratio was greater than 25% in 8 and less than 25% in one. The placenta was described as enormous in another child.

This ratio was also found to be elevated in 3 of 6 children with DMS, 2 children with FSGS, 1 child with a possible storage disorder and in 2 where no biopsy was performed. However, patient 54 with no biopsy, had 2 affected sibs with a histological diagnosis of CNSF.

b) Measurements of α-fetoprotein (α-FP) in maternal serum and amniotic fluid. A record of maternal serum α-FP measurements was obtained only in 4 instances. Maternal serum α-FP was normal when measured at 17 weeks gestation in patients 35 (DMS) and 46 (MCNS). In 2 pregnancies, maternal serum samples had a raised α-FP concentration when measured at 19 and 24 weeks (Pt 15 = CNSF) and at 18 and 27 weeks (Pt20 = DMS) were raised. Antenatal diagnosis was attempted in the case of Pt 20's sib, patient 21. Amniotic α-FP at 18 weeks was normal. The pregnancy was allowed to progress. Unfortunately this offspring was also affected and a diagnosis of DMS was made.
## TABLE 2.7: Placental and Birth Weights

<table>
<thead>
<tr>
<th>Pt</th>
<th>Diagnosis</th>
<th>Plac. Wt (kg)</th>
<th>Birth Wt (kg)</th>
<th>Plac Wt/Birth Wt x100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>CNSF</td>
<td>1.6</td>
<td>2.5</td>
<td>64%</td>
</tr>
<tr>
<td>5</td>
<td>CNSF</td>
<td>1.1</td>
<td>2.4</td>
<td>45%</td>
</tr>
<tr>
<td>6</td>
<td>CNSF</td>
<td>1.0</td>
<td>2.0</td>
<td>51%</td>
</tr>
<tr>
<td>8</td>
<td>CNSF</td>
<td>0.6</td>
<td>1.7</td>
<td>36%</td>
</tr>
<tr>
<td>9</td>
<td>CNSF</td>
<td>0.7</td>
<td>2.5</td>
<td>30%</td>
</tr>
<tr>
<td>11</td>
<td>CNSF</td>
<td>0.9</td>
<td>2.3</td>
<td>40%</td>
</tr>
<tr>
<td>12</td>
<td>CNSF</td>
<td>0.5</td>
<td>3.2</td>
<td>16%</td>
</tr>
<tr>
<td>14</td>
<td>CNSF</td>
<td>&quot;enormous&quot;</td>
<td>2.6</td>
<td>?</td>
</tr>
<tr>
<td>15</td>
<td>CNSF</td>
<td>0.6</td>
<td>1.9</td>
<td>34%</td>
</tr>
<tr>
<td>17</td>
<td>CNSF</td>
<td>1.2</td>
<td>2.8</td>
<td>43%</td>
</tr>
<tr>
<td>18</td>
<td>DMS</td>
<td>0.5</td>
<td>2.5</td>
<td>21%</td>
</tr>
<tr>
<td>19</td>
<td>DMS</td>
<td>0.4</td>
<td>2.7</td>
<td>15%</td>
</tr>
<tr>
<td>21</td>
<td>DMS</td>
<td>0.6</td>
<td>1.9</td>
<td>31%</td>
</tr>
<tr>
<td>27</td>
<td>DMS</td>
<td>1.0</td>
<td>2.3</td>
<td>44%</td>
</tr>
<tr>
<td>33</td>
<td>DMS</td>
<td>0.9</td>
<td>3.1</td>
<td>27%</td>
</tr>
<tr>
<td>35</td>
<td>DMS</td>
<td>0.7</td>
<td>2.8</td>
<td>23%</td>
</tr>
<tr>
<td>37</td>
<td>FSGS</td>
<td>0.7</td>
<td>2.3</td>
<td>29%</td>
</tr>
<tr>
<td>43</td>
<td>FSGS</td>
<td>1.0</td>
<td>2.7</td>
<td>38%</td>
</tr>
<tr>
<td>53</td>
<td>Stdis</td>
<td>0.8</td>
<td>1.8</td>
<td>44%</td>
</tr>
<tr>
<td>54</td>
<td>-</td>
<td>0.7</td>
<td>2.1</td>
<td>35%</td>
</tr>
<tr>
<td>56</td>
<td>-</td>
<td>1.0</td>
<td>2.6</td>
<td>39%</td>
</tr>
</tbody>
</table>

Key: Stdis = storage disorder
2.3.5 Evaluation of Renal Function

a) Nephrotic syndrome. At last review or by the time of death 8 children were in remission following drug therapy (see Section 2.3.7).

Spontaneous remission was observed in 1 child with no biopsy and 3 children (2 MCNS and 1 CNSF) had undergone spontaneous partial remission.

Six children (4 CNSF (Pts 12, 13, 14, 17) and 2 DMS (Pts 34 and 35)) were in remission following elective bilateral nephrectomy. One child (Pt 15 CNSF) underwent full remission and another partial remission (Pt 39 FSGS) as a result of progression into end-stage renal failure.

b) Renal failure. End-stage renal failure was rarely observed in the CNSF group (Fig 2.2). In this group, 2 children developed ESRF. Patient 7 was severely asphyxiated at birth and this may have contributed to the deterioration in renal function. Patient 15 reached ESRF at 10 years of age. Three children experienced a deterioration in renal function, 2 of them as a result of severe hypovolaemia (Pts 3 and 5). In the third child (Pt 17) there was a progressive decline in renal function from the age of 1.8 yr. He underwent bilateral nephrectomies at 2.2 yr and a successful cadaveric renal transplant at 2.3 yr. Three children with normal renal function had bilateral nephrectomies and were dialysed (Pts 12, 13 and 14), 2 of whom have received successful cadaveric transplants (Pts 13 and 14).
Fig. 2.2: Renal function at follow-up in children presenting with the nephrotic syndrome under 1 year of age.
Chapter 2: NS Clinical Review

In the DMS group progression to ESRF is an important feature, seen in 11 of 18 patients (Fig 2.2). Eight of the 11 children reached ESRF within the first year of life. Five children had normal renal function at last follow up. However, 4 of these children died within the first 4 months of life.

Progression to ESRF was observed in 5 of the 10 children with FSGS (Fig 2.2). Deterioration in renal function was seen to occur later than in the DMS group, with all children but one developing ESRF after 4 years of age.

2.3.6 Complications (Table 2.8)

a) Sepsis. Bacterial infections were common and affected 34 of the 62 children. These included life threatening infections such as septicaemias (13 episodes) meningitis (1 episode) and peritonitis (3 episodes). Urinary tract infections were common (14 episodes), as well as bronchopneumonia (10 episodes). Infections were particularly common in the CNSF and DMS group. Gram negative bacteria were the commonest organisms isolated (16 of 26 bacterial infections). There were 2 children with congenital infection-associated nephrotic syndrome, one with congenital syphilis and the other with congenital CMV infection (see Section 2.3.9).

b) Thromboembolic events. These were observed in a significant number of children with CNSF and DMS. There
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>UTI</th>
<th>Bronchopneum</th>
<th>Septicaemia</th>
<th>Meningitis</th>
<th>Peritonitis</th>
<th>Others*</th>
<th>THROMBOSIS</th>
<th>CONVULSIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNSF</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>DMS</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>FSGS</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No Bx</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>OTHER</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*This column includes: Cellulitis (2), bronchiolitis, umbilical (2), Adenovirus diarrhoea and presumed pneumocystis pneumonia.
were no appreciable differences in these two groups in the frequency of this complication. Renal veins were the most common site of thrombus formation (4 patients). Other organs included the inferior vena cava (1 patient) and pulmonary vessels (2 patients). Infarcts were detected in the liver (1 patient), adrenal glands (1 patient) and gut (1 patient).

c) Fits. Fits were a relatively common complication. A metabolic cause was present in most cases. Four children had fits associated with hypocalcaemia, 1 with hypoglycaemia and 2 children in renal failure had fits in association with hyponatraemia. In 2 no obvious biochemical cause was found.

2.3.7 Response to Drug Therapy. Prednisolone was given to 4 children in the CNSF group and to 4 children in the DMS group without response. Two children in the DMS group also received cyclophosphamide without response.

In the children with FSGS there was 1 responder among 6 who received prednisolone and 1 responder among 4 who received cyclophosphamide. Two other children in this group were given vincristine either on its own or in combination with prednisolone and cyclophosphamide children but neither went into remission.

Of the 3 children in the MCNS group, only 1 received drug therapy. Pt 47 received prednisolone with no response but subsequently responded to cyclophosphamide.
One child with DMP has responded to steroids. Among the children with no biopsies, 4 have received steroids and 3 have responded.

One child with congenital syphilis entered remission following treatment with penicillin.

2.3.8 Outcome. Figure 2.3 shows survival according to diagnostic group. Sixty per cent (37/62) of children presented before 3 months of age, mostly during the neonatal period. The mortality in this age group was very high. Only 9 of the 37 babies (24%) survived. Of those who presented between the ages of 3 months and 12 months (25/62), 14 (64%) have survived.
Fig. 2.3: Survival of children presenting with the nephrotic syndrome under 1 year of age
2.3.9 Secondary Forms. Two children had the nephrotic syndrome in association with congenital infections. Patient 58 received an intrauterine blood transfusion for Rhesus incompatibility and developed systemic CMV infection. Patient 62 had congenital syphilis. Neither of these 2 patients had renal histological examinations.

Patient 53 had a probable storage disorder. He was born in 1968 to unrelated parents, at 33 weeks gestation. His birth weight was 1.84 kg, placental weight was 0.8 kg. Slight micrognathia was observed and prominent maxillae. He had low set ears, mild positional talipes, hepatomegaly and a systolic murmur. At 2 weeks of age, oedema, proteinuria and hypoalbuminaemia were noted. A skeletal survey was suggestive of infantile gargoylism. He died at 3 months of age. A post mortem was carried out. This showed widespread foam filled vacuoles in renal tubules and glomeruli, however glomerular sclerosis was not observed (Figs. 2.4a and 2.4b). His heart valves were thichenned and there was fibrinous pericarditis. Kuppfer cells in the liver contained foam filled vacuoles.
Fig. 2.4a: Light microscopy of glomerulus from Patient 53, showing swollen, vacuolated epithelial cells. PAS x 200

Fig. 2.4b: Foam filled vacuoles in glomeruli, renal tubules and interstitial cells (patient 53). PAS x 40. (Illustrations courtesy of the Histology Department, HSC.)
2.3.10 Case Reports. In this section 10 children with significant congenital abnormalities in addition to the nephrotic syndrome are described. Five children had the Galloway-Mowat syndrome (CNS and microcephaly). One child had CNS and retinal atrophy. Two children with the hitherto unreported association of FSGS, Ventriculoseptal defect and Pelvi-calyceal abnormalities are described. Lastly, a child with CNS, a severe unclassified form of Epidermolysis Bullosa and pulmonary hypertension is reported. This latter association has not been described before.

Children with Denys-Drash syndrome form part of a larger group described in Chapter 4.

a) CNS and microcephaly (Galloway-Mowat syndrome). Patient 20 presented in 1979. She was born to unrelated parents at 36 weeks. Maternal serum α-FP was elevated when measured at 18 and 27 weeks gestation. Birth weight was 2.41 kg (10th centile) and head circumference was 30.5 cm. (below the 3rd centile). She had odd facies, low set ears, poor tone and low hair line. She had a petechial rash on legs and buttocks. She developed the nephrotic syndrome during the first week of life. Platelet count was normal initially although it dropped during the fifth week of life. Liver function tests remained within normal limits. She had a 46XX karyotype. She had profuse diarrhoea, became acutely sick and had three cardio-pulmonary arrests and died after the third at 6 weeks of age. Post mortem renal histology showed a mild increase in mesangial matrix with no
corresponding increase in cellularity. Some glomeruli showed advanced mesangial sclerosis. There were small areas of tubular atrophy. A histological diagnosis of diffuse mesangial sclerosis was made. There was a congested fatty liver with focal fibrous scars. The brain weighed 344 g. The folia in the cerebellum looked slightly atrophic.

**Patient 21** (sister of patient 20), presented in 1983. She was born at 35 weeks gestation, Bt Wt. 1.9 kg, Head Circ. 29.5cm (<3rd centile). Amniotic fluid αfetoprotein was in this case normal when tested at 17 weeks gestation. A petechial rash was noted at birth. She developed the nephrotic syndrome during the first week of life. She had odd facies and poor head control. She had a normal female karyotype. A renal biopsy carried out at 3 weeks of age showed diffuse mesangial sclerosis of moderate severity (Fig 2.5). There were a few foci of tubular atrophy with occasional dilated tubules and Bowman's capsules. She died at 2 months of age and a post mortem was performed. Bronchopneumonia and hepatic infarcts were found. Brain weight was low at 255g as was the weight of brain stem and cerebellum. There was poor myelination of the frontal lobes and cerebral and cerebellar heterotopia were found.

**Patient 33** presented in 1979. He was born at term to unrelated parents. His birth Wt was 3.14kg. His head circumference was 36cm (between the 3rd and 10th centile) at 4.5 weeks of age. At 5 months of age he was admitted to another hospital with mild gastroenteritis. Urine microscopy at this time showed 3+ proteinuria. He had
Fig. 2.5: Light microscopy of renal biopsy on Patient 21 showing diffuse mesangial sclerosis. PAS x 100 (Illustration courtesy of Department of Histology, HSC)
congenital bilateral hip dislocations and was referred to the orthopaedic specialist at this hospital, at the age of 11 months. He was not able to sit unsupported and was hypotonic. Oedema was noted. A diagnosis of the nephrotic syndrome was made. At 13 months of age his head circumference was 42.5cm (2.5 cm below the 3rd centile). His renal function was initially normal but deteriorated rapidly into end stage renal failure and he died of uraemia 2 months later. Post mortem renal histology showed diffuse mesangial sclerosis. This child had 2 siblings with CNS (Patients 34 and 35). Patient 34 was microcephalic. Unfortunately there are no head circumference records on Patient 35.

Patient 34 was born in 1987, at 38 weeks, weighing 2.8 kg. She developed proteinuria within the first month of life. She has a long narrow face, strabismus and hypotelorism. She had bilateral hip dislocations. Her head circumference has remained below the 3rd centile. A head CT scan was normal. She is markedly developmentally delayed, has marked trunkal hypotonia and does not walk. She had marked oesophageal reflux and a hiatus hernia. A renal biopsy at 10 months of age showed diffuse mesangial sclerosis. She developed end stage renal failure just after her 2nd birthday. She is now 4 years old and has recently received a successful cadaveric renal transplant.

Patient 35 was born in 1970. His Birth Wt was 2.2 kg. A heart murmur was noted soon after birth and a diagnosis of probable ventricular septal defect was made. He presented
at 3 months of age with loose stools. Oedema was noted. He had proteinuria, a low serum albumin, hyperkalaemia and a high urea. He died in renal failure within days following his admission. At post mortem, there was bronchopneumonia and the findings of a ventricular septal defect were confirmed. Renal histology showed diffuse mesangial sclerosis.

Patient 57 was born in 1974, at 38 weeks gestation, with a Bt Wt. of 2.4 kg. He was seen at this hospital at 10 days of age. On day 5 of life oedema was noted and he had generalised convulsions. He had proteinuria, a low serum albumin, a low serum sodium, high potassium and had mildly impaired renal function. A diagnosis of congenital nephrotic syndrome was made. He had "odd facies" and microcephaly, head circumference at 10 days of age was 30 cm, below the 3rd centile. A hip Xray raised the possibility of congenital hip dislocation. He died at 13 days of age. There was no post mortem.

b) CNS (DMS) and retinal atrophy. Patient 24 was born to consanguinous parents after a normal pregnancy. There is no record of birth weight or placental weight. He was well until 10 months of age. He developed generalised swelling, fever and oliguria apparently after an insect bite.

On admission to hospital, he was oedematous, had a BP of 90/60 mmHg and was noted to have bilateral, oscillatory nystagmus. Initial investigations revealed a Na = 120 mmol/l, K = 7.0 mmol/l, creatinine = 88 μmol/l, albumin of
2.1 G/l and 4+ proteinuria. He was initially treated with steroids but became oligoanuric and hypertensive. He was treated with diazoxide and captopril. He was transferred to the Hospital for Sick Children 2 weeks later.

On arrival, his weight was on the 50th centile and his height was on the 10-25th centiles. His head circumference was on the 50th centile. Nystagmus was noted and retinal examination revealed retinal atrophy. He appeared to fix and follow in spite of the high frequency, low amplitude nystagmus observed. Fundoscopy showed marked retinal attenuation with arteriolar thinning. Retinal and optic atrophy were noted. Ritual burns were noted, his BP was 105/60 mmHg. His Hb was 8.7 g/dl, white cell and platelet count were normal. Serum electrolytes were normal, urea was 48.3 mmol/l, creatinine was 182 μmol/l albumin was 24 g/l, alkaline phosphatase and transaminases were normal. There was 3+ proteinuria, no glycosuria and urine microscopy showed 40 WCC and 15 RBC x 10^6/l, granular casts and a mixed growth on culture. Serum Hepatitis B surface antigen was negative and C3 and C4 were normal. Karyotype was 46XY. Head CT scan showed cerebral atrophy, Electroencephalogram was normal. Electroretinography showed a response of very low amplitude and possibly absent in keeping with a marked loss of function of the outer retinal receptors.

Renal biopsy contained more than 100 glomeruli and showed diffuse mesangial sclerosis. Bowman's capsule and superficial glomeruli showed immature morphology. Approximately half the glomerular tufts showed segmental
sclerosis with increased fibrillary mesangial matrix. There was collapse of peripheral capillary loops with occasional double contours. There were scattered foci of tubular atrophy and interstitial fibrosis. There was IgM deposition in sclerosed glomeruli. He developed ESRF and died shortly afterwards.

c) CNS (FSGS), ventricular septal defect (VSD) and pelvi-calyceal abnormalities. Patient 40 was born to unrelated parents after a normal pregnancy and delivery at term. He weighed 3.62 kg. Oedema was noted at 2 months of age, when he was found to have a low serum albumin, proteinuria and normal renal function. He had a ventricular septal defect and a congenital right middle lobe cyst, from which he was asymptomatic. A micturating cystourethrogram revealed bilateral vesicoureteric reflux into non-dilated ureters. An intravenous urogram showed markedly abnormal, blunted calyces with loss of normal calyceal cupping. The renal pelves were hypoplastic, almost absent.

A renal biopsy at 3 and a half months showed focal glomerulosclerosis. His renal function remained normal until 3 years and 4 months when it started to deteriorate. He reached ESRF just before his 4th birthday and he died shortly afterwards.

Patient 41 was born to nonconsanguinous parents of Indian descent. He was born at 36 weeks gestation with a Bt. Wt. of 2.55 kg. A heart murmur was noted soon after birth. Echocardiography demonstrated a ventricular septal
defect, from which he remained asymptomatic. He presented with oedema at 0.25 yr of age. He had a serum albumin of 18 g/dl and 3+ proteinuria. An initial biopsy, performed at 0.33 yr was unhelpful due to technical problems. Repeat biopsy at 1.5 yr showed focal glomerulosclerosis. A micturating cystourethrogram was normal, but an intravenous urethrogram showed bilateral absent renal pelves and blunt calyces which appeared to drain directly into ureters of normal caliber (Fig. 2.6). These appearances were strikingly similar to those found on Pt 40. During the first 2 years of life, he had several hospital admissions with gastroenteritis and hypovolaemia. At 2 and a half years he developed Strep Pneumoniae peritonitis, meningitis and septicaemia. Fortunately, he responded well to penicillin. His renal function remained normal until the age of 4, after which, it gradually deteriorated reaching ESRF at 4 years 8 months. He was then started on continuous ambulatory peritoneal dialysis.
8) CMS, epidermolysis bullosa and pulmonary hypertension.

Patient 41 was born of consanguineous parents after a normal pregnancy and delivery. His birth weight was 2.76 kg. Bullous skin lesions were noted on day 1. These were present on his face, scalp, trunk and limbs. In the course of time affected areas would heal leaving severe scarring (Fig. 2.7). This process also involved his scalp producing total alopecia.

**Fig. 2.6:** Intravenous urogram of Patient 41 showing bilateral absent renal pelves and blunt calyces.
d) CNS, epidermolysis bullosa and pulmonary hypertension. 

Patient 49 was born of consanguinous parents after a normal pregnancy and delivery. His birth weight was 2.76 kg. Bullous skin lesions were noted on day 1. These were present on his face, scalp, trunk and limbs. In the course of time affected areas would heal leaving severe scarring (Fig. 2.7). This process also involved his scalp producing total alopecia.

He was followed up closely due to poor weight gain. At the age of 6 weeks he developed a chest infection. This was associated with a fourfold rise in Pneumocystis carinii titres and responded to high dose Cotelomoxazole.

Proteinuria was noted at 11 weeks. He had normal electrolytes, a serum albumin of 24 g/l and a creatinine of 31umol/l. Urine albumin to urine creatinine ratio was 7.15 (normal < 0.1). He had a low IgG at 17 u/ml. Immunological investigations showed normal E rosette formation, normal T cell function a normal Phytohaemagglutinin response. A skin biopsy showed separation of epidermis from dermis with no fibrin deposition. The split was located below the basement membrane, which was shown to be attached to epidermal keratinocytes. There was a fluffy appearance to the papillary dermis and there was no lysis in keratinocytes. He was thought to have a severe type of a hitherto undescribed form of dystrophic epidermolysis bullosa. Photographs of this histological material from skin biopsies were unobtainable.

A percutaneous renal biopsy at 3 months of age showed
normal histology or light microscopy. Electron microscopy revealed widespread thickening and "shrink-wrapping" duplication of the glomerular basement membrane (GBM), as seen in Alport's syndrome. Immunohistochemistry studies were normal. Unfortunately, it was not possible to obtain photographs.

A year later, the renal function deteriorated and serum creatinine increased. A repeat renal biopsy showed approximately a third of glomeruli with segmental diffuse thickening and sclerosis, sometimes with "spikes" (i.e., irregular argyrophilic "spikes" [Fig. 2.7]). Patchy tubular atrophy, interstitial thickening, and focal interstitial fibrosis were noted. Interstitial edema was also present. Immunohistochemistry showed staining for 

Fig. 2.7: Dystrophic form of epidermolysis bullosa on Patient 49. (Illustration courtesy of Department of Histology, HSC)
normal histology on light microscopy. Electron microscopy revealed widespread thickening and "basket weaving" duplication of the glomerular basement membrane (GBM), as seen in Alports' syndrome. Immunochemistry studies were normal. Unfortunately, it was not possible to obtain photographs of these findings to include in this report.

A year later his renal function started to deteriorate and serum creatinine climbed to 176 \( \mu \text{mol/l} \). A repeat renal biopsy showed global sclerosis affecting approximately a third of glomeruli. Other glomeruli showed diffuse thickening of the GBM with additional tuft sclerosis, sometimes associated with capsular adhesions. Silver staining demonstrated thickened GBM with irregular argyrophilia producing a "moth-eaten" appearance, but no "spikes" (Fig. 2.8a). There was widespread, patchy tubular atrophy, diffuse interstitial fibrosis and focal interstitial inflammation (Fig. 2.8b). Arterial blood vessels were not included in the biopsy. Immunochemistry showed staining of the thickened GBM for fibrin, IgM, weak staining for IgG and negative staining for IgA, C1q and C3. Unfortunately, electron microscopy studies were not available.

At this time he started to become increasingly breathless and cyanosed. Chest Xray showed increasing heart size and plethoric lungs. His electrocardiogram showed biventricular hypertrophy. Echocardiography showed evidence of pulmonary hypertension and right ventricular hypertrophy. He developed progressive respiratory
Fig. 2.8a: Global sclerosis and tubular atrophy on renal biopsy from Patient 49, taken at 1.9 yr. PAS and methanine silver x 40.

Fig. 2.8b: Light microscopy of glomerulus showing thickenned GBM with "moth-eaten" appearance (Patient 49). PAS and methanine silver x 100. (Illustrations courtesy of Department of Histology, HSC).
insufficiency. He experienced acute pain on swallowing presumed to be due to oesophagitis secondary to epidermolysis bullosa. He died in respiratory failure at the age of 2.16 yr.

2.4. Discussion

Children with the nephrotic syndrome under one year of age, raise a number of very specific considerations. They are a heterogenous and complex group who share some important features.

The following are the most important aspects that emerge after reviewing this clinical group:

a) classification and clinico-pathological correlations.
b) prognosis
c) treatment and supportive management
d) genetic counselling and antenatal diagnosis
e) the recognition of complex phenotypes

2.4.1. Is it Possible to Make a Diagnosis of a Specific Type of Nephrotic Syndrome in the First Year of Life?. This clinical review showed examples from the idiopathic, secondary and syndromic forms of nephrotic syndrome. The most common histological types were CNSF and DMS, followed by FSGS. Other histological forms included examples of MCNS, DMP, Alports' and MCGN. Examples of secondary forms included 2 children with intrauterine infections and 1 child with an unclassified form of storage disease. There were numerous children with associated abnormalities and
the following "syndromic" forms were recognised: Denys-Drash syndrome; Galloway-Mowat syndrome (CNS and microcephaly) with or without congenital hip dislocation; CNS, epidermolysis bullosa and pulmonary hypertension; FSGS, ventricular septal defect and pelvi-calyceal abnormalities, and DMS and retinal atrophy.

The current classification of the idiopathic group, the most common group, relies on histological differentiation. As discussed above, however, there are no pathognomonic characteristics in any of these groups and many features may overlap.

It has been argued that the different histological types can be correlated with a particular clinical course and prognosis (25,44,76). Our study to some extent supports this view. Three main clinicopathological correlates could be identified. The first is the CNSF type which is the earliest presenting one. Children were severely hypoproteinaemic from the onset and they have succumbed within the first 6 months of life to hypovolaemia, infections, thrombotic events and severe cachexia.

The second group is made up by children with DMS on biopsy. Some may present in the neonatal period like the children with CNSF, but most present later during the first year. Some may have an insidious onset of proteinuria. Children with DMS diagnosed in the neonatal period, experience severe hypovolaemia, infections and thrombosis related to their nephrotic state, and at an early stage, they may be clinically indistinct from
children with CNSF. However, the clinical course of children with DMS is further worsened by the relentless progression into ESRF within a year or so after presentation. This occurred in 72% of children in this histological group.

The third clinico-pathological group are children with FSGS. Children in this category had a more varied course. Severe problems with hypovolaemia and the nephrotic state were seen particularly in the children diagnosed within the first 3 months of life. Although the numbers involved are small, the trend appeared to be that in this group, diagnosis after 6 months of age was associated with better prospects for survival and greater likelihood of response to drug therapy. Fifty per cent of children with FSGS developed ESRF, which ensued at a later age than in the DMS group. With one exception, renal failure most commonly developed between the ages of 4 and 8 years. No spontaneous remissions were observed.

Two children in the CNSF group illustrate some of the problems of a histologically based classification. Patient 10 was born at 24 weeks gestation, proteinuria appeared late, at 10 months of age and had a histological diagnosis of CNSF. Her biopsy showed marked mesangial proliferation and very occasional glomeruli showing segmental sclerosis. Two tubules were distended with hyaline casts. Immunofluorescence showed IgM in mesangium and capillary loops. It could be argued that these features, as well as her clinical course, resemble more closely a description of
"diffuse mesangial proliferation" (115). Patient 16 developed proteinuria at 2 weeks of age and had a biopsy that showed CNSF. However, by last follow up at the age of 8 months, she had experienced none of the serious problems of hypovolaemia, infections or thrombosis seen in CNSF and her serum albumin concentration has spontaneously risen to 25 to 30 g/l. Spontaneous partial or full remissions are not described in CNSF. These cases illustrate that a histological diagnosis may be at odds with the clinical state on a given patient. Therefore, a histology based classification may fail on occasions to provide a reliable guide to clinical course and prognosis. This is a problem which has been identified by others (19, 75), particularly in relation to biopsies taken in the neonatal period.

**2.4.2 Prognosis.**

**a) Age at presentation.** With the exception of one child, all children with CNSF presented before one month of age. This is in agreement with other published series (14, 19, 26).

Although most children with DMS presented before the age of 1 month, a few presented later. FSGS and MCNS occur much less commonly during the first year and although the figures involved are small, there is the suggestion of a relatively even spread during this time.

This review showed that regardless of the histological diagnosis, an onset before 3 months, was associated with a very bad prognosis. There was only a 24% survival rate in
this congenital group. An infantile onset, that is after 3 months, significantly improves the chances of survival to 64%. The exception are children with DMS, who had a uniformly bad prognosis independent of age of onset.

In a study of 48 children with nephrotic syndrome under one year of age, Sibley et al concluded that neither specific histopathological features nor histopathological classification had any prognostic value. They showed that only age at presentation gave a reliable indication to prognosis (19).

b) Response to drug therapy. As illustrated in earlier accounts, the response to drug therapy in this group is minimal (14,19,25). None of the children with CNSF or DMS responded to steroids or any other form of therapy. No child with an onset before 3 months in any of the diagnostic categories, responded to drug therapy. Although the numbers are small, children over 6 months at age at presentation were more likely to have drug induced remissions following either steroids or alkylating agents such as cyclophosphamide and chlorambucil. In this age group, remissions occurred in 1 child with MCNS, 2 with FSGS, 1 with DMP and in 3 children on whom biopsies were not carried out.

c) Renal function. End-stage renal failure is not a feature of CNSF, although a decline in renal function may be seen during the 2nd year of life (14, 116,117). In this series,
ESRF was observed in a child at 10 years of age. The 2 other children who experienced a reduction in function, had other complicating factors such as hypovolaemia or severe birth asphixia.

Progression into ESRF, mostly within a year of presentation, is a crucial feature of children with DMS and it is seen in some children with FSGS. ESRF is the main reason for the high mortality observed in this group. It had already developed at time of presentation or it ensued within less than 6 months from diagnosis. Four children died before 4 months of age, of complications of the nephrotic syndrome. Their deaths might have occurred before reduction in renal function could be observed.

2.4.3 Treatment and Supportive Therapy. There were no appreciable differences between the CNSF and DMS groups, in the incidence of complications such as infections, thromboembolic phenomena and convulsions.

Bacterial infections were common in all groups. Gram negative organisms were frequent isolates, and rather more so than gram positive organisms. Interestingly, there were only 2 children who had pneumococcal infections. These figures underline the importance of the use of broad-spectrum antibiotics on suspicion of infection in this group of patients. Hypogammaglobulinaemia, defective opsonisation and impaired specific antibody production against organisms like the pneumococcus contribute to the susceptibility of nephrotic children to bacterial
infections (53,55,56,118).

Supportive treatment, the use of prophylactic penicillin, the aggressive treatment of infections including the use of immunoglobulin infusions, have been tried with some success (116, 117).

Renal veins were the most common site for thrombus formation. Other organs included adrenals and lungs. Thromboembolic events were seen with similar frequencies in the CNSF and DMS groups. Frequent albumin infusions, together with avoidance of overuse of diuretics are indicated. Antiplatelet therapy has been used in children who have had thrombotic episodes (116, 119).

Convulsions were another common complication. A metabolic cause was usually present; the most common was hypocalcaemia, followed by hyponatraemia. However, all children with hyponatraemia associated convulsions were in renal failure at the time.

Over the last decade, there has been a much more aggressive approach to management of children with CNS. This consists of daily albumin infusions, optimal nutrition often through nasogastric or gastrostomy tubes, and aggressive treatment of infections. Bilateral nephrectomies are then carried out within the first few months of life. With the advent of improved dialysis techniques and the ability to perform renal transplants in young children, this approach has radically altered the prognosis of these young children (116,117,119). With the exception of FSGS no recurrences of the original disease in the graft have been
2.4.4 Genetic Counselling and Antenatal Diagnosis.

a) Familial incidence. In this review, familial instances of nephrotic syndrome were observed in the CNSF, DMS and FSGS groups. In addition, 2 siblings with DMP and 2 with MCGN were encountered. Two siblings had CNS where the index case did not have a biopsy and the sibling was reported as having CNS with no more information regarding the type.

The familial nature of CNSF was first reported by Hallman et al in 1959 (12) and definitely established as an autosomal recessive disease by Norio' studies on 57 families (24). The frequency of CNSF in Finland has been estimated to be 1.2/10000 with a gene frequency of 1:200 (24). This disease also appears to occur as an autosomal recessive trait in families of non-Finnish ancestry (25,19,26).

An increased incidence of familial cases of DMS has been reported where sibships have been affected (25,26,66) with some instances of parental consanguinity (26). This points to an autosomal recessive mode of inheritance (28).

This series includes a pair of siblings with FSGS. Familial cases with affected sibships have been reported (19,25).

Reliable prenatal diagnosis is only possible, in the CNSF group, through measurement of amniotic fluid αFP.

b) α-fetoprotein. As discussed in the Introduction, a
raised α-FP is a well established feature of CNSF. It is a reflection of the intrauterine onset of proteinuria and it has been extensively used in Finland for prenatal diagnosis of CNSF (62-64). Maternal serum α-FP can however be normal and therefore amniocentesis is always necessary for prenatal screening (64).

To date, there is only one report on the use of amniotic fluid α-FP for prenatal diagnosis in other forms of CNS other than CNSF. Schneller reported a normal amniotic fluid α-FP where the offspring developed DMS (23).

In our study, rather surprisingly, maternal serum α-FP was found to be raised in a case of DMS. As stated above, α-FP is the manifestation of intrauterine onset of foetal proteinuria. In this child, oedema and proteinuria were documented at birth, which raises the possibility of an intrauterine onset of nephrotic syndrome. Therefore, it is possible that occasionally α-FP may be raised in other forms of CNS with intrauterine onset. However, this is a rarity and it cannot be used for prenatal diagnosis in forms of nephrotic syndrome other than CNSF.

2.4.5 Recognition of Secondary Forms and Complex Phenotypes.

a) Secondary forms. Amongst this group of children with early onset nephrotic syndrome, 2 children had the nephrotic syndrome most probably as a result of intrauterine infections. One of them had congenital syphilis and the other had congenital CMV infection,
acquired as a result of intrauterine CMV positive blood transfusion for Rhesus incompatibility.

Patient 53 was described in the "Secondary forms" in Section 2.3.9. He probably had a form of storage disorder. Review of the histopathological material showed foamy cells in glomeruli and tubular epithelial cells, but no sclerosis. The liver showed foamy cell appearance in the Kupffer cells but not in the hepatocytes. He had a normal blood film as well as a normal bone marrow histology. Thin layer chromatography of kidney, liver and brain tissue showed normal phospholipid, ganglioside and ceramide patterns, making the diagnosis of storage diseases such as Gauchers', Niemann-Pick, GM1 and GM2 gangliosidosis and Fabry's unlikely.

Renal changes have been reported to occur in a number of storage disorders. These include sialidosis (120-125), Hurler's syndrome (126), GM₁-gangliosidosis type 1 (127), I-cell disease (128), Gaucher's disease, Niemann-Pick, and Fabry's disease (129). With the exception of sialidosis (120, 124, 125) and Hurler's syndrome (126), none of the above diseases have resulted in the development of a clinically apparent nephropathy. However, none has been reported to occur in the first year of life.

b) Associated abnormalities and malformation syndromes in children with early onset nephrotic syndrome. Congenital abnormalities were seen most commonly in children with DMS. Fifteen of 18 children with this histological diagnosis had
other congenital defects. In some cases the entire complex phenotype was also transmitted to a sibling. Congenital anomalies were described in 2 children with FSGS. Interestingly, children with CNSF did not have other congenital abnormalities other than musculo-skeletal abnormalities likely to arise as a result of severe protein loss of intrauterine onset.

Some of the syndromic associations seen in this review are already known and have been well described in the literature.

The best known syndrome in this context is Denys-Drash syndrome, where a nephropathy occurs in association with either Wilms' tumour and or genital abnormalities (29,31,32,34). Complete and incomplete forms may exist with the nephropathy as the common denominator. This syndrome forms the subject of Chapter 4, where the detailed clinical features of a series of 12 children are described and discussed.

The recognition of this syndrome has 2 important practical implications. Firstly, it is important to seek karyotypic confirmation of female infants presenting with DMS, as otherwise the diagnosis may be missed in affected children with a female phenotype. Secondly, it is important to consider prophylactic nephrectomies and gonadectomies in children with the sexual abnormalities and the nephropathy, due to the risk of Wilms' tumour and gonadoblastoma.

Less clearly defined is the association of CNS and microcephaly also known as the Galloway-Mowat syndrome
I have given a detailed description of 5 such children in Section 2.3.10. All but one were biopsied and showed DMS on histology. As discussed in the Introduction the nephropathy appears to be heterogenous, although the onset of the nephrotic syndrome has occurred during the first year of life in all cases but one. Some have had renal insufficiency. Renal histology has been varied.

None of the cases reported to date have had congenital hip dislocation, a feature observed in siblings Pts 33 and 35. It is possible that Pt 57 who died at 12 days of age without biopsy and had microcephaly and dislocated hip may have had this syndrome too.

The high proportion of affected sibships was also a feature in our cases where there were 2 pairs of affected siblings.

One of the children in this review had DMS and ocular abnormalities which consisted of retinal atrophy with associated nystagmus. To date there have been 5 children where the association of eye abnormalities and DMS has been observed (22,130,131). These changes were described in 2 pairs of siblings (130,131). The abnormalities described include nystagmus alone (130), strabismus, nystagmus and hypertelorism (22), miosis, nystagmus and cataracts (131). Retinal atrophy as in our case has been documented clearly in only one of these children (131).

Although our case had evidence of cerebral atrophy on brain CT scan, there was no neurological deficit and he was too ill to accurately assess his developmental progress.
Chapter 2: NS Clinical Review

The variety of congenital abnormalities seen in association with DMS, raises the possibility that this form of histology is the result of a number of different pathogenic mechanisms, probably involving different genes. DMS has also occurred in association with hypoadrenalism (132).

Two children in this review had FSGS, VSD and abnormal calyceal systems. This constellation of abnormalities has not been reported previously. Both were male and presented with the nephrotic syndrome at about 3 months of age and both developed ESRF in the fourth year of life. FSGS was demonstrated on their biopsies. Both children had a ventriculo-septal defect but were asymptomatic. The pelvi-calyceal abnormalities were of particular interest. Similar pelvi-calyceal appearances were also seen in children with Denys-Drash syndrome. They have also been described in the Laurence Moon Biedle syndrome (133).

Patient 49 had a severe form of CNS that progressed to ESRF, a dystrophic but unclassified form of epidermolysis bullosa and severe lung disease with pulmonary hypertension of unknown aetiology. It is possible that all 3 organs were affected by a common single defect affecting perhaps a constituent of connective tissue or basement membrane. There is one report of 2 siblings (a boy and a girl), who had hereditary nephritis and pretibial or type VII epidermolysis bullosa, in association with B-thalassaemia minor in Oriental Jewish children (134). They presented at a later age and the EB was confined to the pretibial areas.
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The relatively high frequency of syndromic forms of CNS, particularly in the DMS group should lead the physician to search for possible associated congenital abnormalities, as these may have a bearing on management and prognosis. Karyotypic examinations, particularly in the young female infant with CNS, should form part of the routine assessment in this group of children.

2.4.6 Conclusion. This review has dealt with the major characteristics and problems seen in children with an infantile or congenital onset of nephrotic syndrome. Prognosis has improved greatly in recent years and there has been a shift towards a more vigorous plan of supportive therapy and early renal transplantation. However, we are a long way from clearly understanding this group of diseases and the pathogenesis of the proteinuria. Currently, a histology based classification system is used which has limitations, not least because histological change is probably the last stage in a series of events that have their origin at molecular level. The aim now should be to try and identify their molecular basis and search for the genetic abnormalities that underlie them.
3. URINE HEPARAN SULPHATE AND CHONDROITIN SULPHATE EXCRETION IN CHILDREN WITH CONGENITAL AND ACQUIRED NEPHROTIC SYNDROME

3.1. Introduction

The first step in the formation of urine consists of the transcapillary passage of a largely protein-free ultrafiltrate of plasma across the glomerular capillary wall (GCW). The GCW is made up of three layers: from inside the glomerulus outwards there lies the endothelium with fenestrated cytoplasm, the glomerular basement membrane (GBM) and the epithelial cell foot processes with the intervening slit-pore diaphragms (101).

3.1.1 Evidence for Size and Charge Selectivity Properties of the GBM. During the past 20 years, considerable evidence has been gathered which suggests that the GCW acts as both a size (135-137) and charge selectivity barrier to filtration (99,100,138-141). Using dextran molecules of different molecular charge, Bohrer et al (141) and Brenner (100) demonstrated that the clearance of anionic dextrans was less and cationic dextrans greater than that of neutral dextrans of similar molecular size. Experiments by Renkke et al (99) showed that the passage of modified ferritin molecules through the GBM was influenced by their charge.

Heparan sulphate (HS) is a proteoglycan made up of glycosaminoglycans (GAG) chains attached to a protein core.
(142). HS is found predominantly in the lamina rarae of the GBM (143), and is the major component of the fixed anionic groups responsible for the charge selectivity of filtration (108,101,143). There is also evidence that other anionic groups, such as carboxyl residues linked to GBM glycoproteins, are important (144). The contribution to the charge barrier of other glycoproteins such as chondroitin sulphate (CS) (101,107,145) is less clear. Sialic acid residues linked to podocalyxin line the epithelial cell foot processes; it is believed that their main function is to maintain the space between them open for the passage of filtrate. However, they may also play a role in charge selectivity (101).

3.1.2 Pathophysiology of Proteinuria in Glomerular Diseases. Research on the pathogenesis of proteinuria in renal disease has focussed on alterations in the charge and size filtration characteristics of the GBM (102-106,108,110). Clearance studies in experimentally induced glomerulonephritis and in patients with minimal change nephrotic syndrome have demonstrated a loss of charge selectivity of filtration (103,104,146).

3.1.3 Loss of Anionic Charge from the GBM in Congenital Nephrotic Syndrome. Vernier et al reported that the concentration of anionic sites in the GBM was decreased in children with congenital nephrotic syndrome (CNS) (108). In their study the cationic probe polyethyleneimine (PEI)
Chapter 3: Urinary GAGs

was used to identify anionic sites and normal kidney tissue was incubated with heparatinase to demonstrate that HS was indeed the major anionic group labelled by PEI in the GBM. Vermylen et al (110), using Alcian Blue staining of GBM GAGs, separated by electrophoresis, found a marked reduction in the GBM content of HS in a child with CNS histologically characterised as diffuse mesangial sclerosis (DMS). They also found an increased excretion of HS relative to CS in the urine of this case and 3 other children with CNS, two of whom had Finnish-type histology (CNSF) and the third DMS. They suggested that the basic defect was a failure to incorporate HS into the macromolecular structure of the GBM with subsequent loss of HS into the urine.

3.1.4 Chemical Features of GAGs. With the exception of keratan sulphate (KS), GAGs are unbranched chains of repeating disaccharide units. The disaccharide units are composed of a hexosamine (glucosamine or galactosamine) and an uronide (glucuronic or iduronic acid). Most GAGs found in tissues are linked to a core protein, forming proteoglycans. GAGS are anionic molecules, their negative charge being conferred principally by sulphate groups and to a lesser extent by carboxyl groups (142).

The purpose of the present study was to investigate HS excretion in children with various forms of congenital and acquired nephrotic syndrome, and to compare it to normal children. In particular, we wished to study whether an
increased urinary excretion of HS was specific for a particular form of CNS and thus provide some insight into the pathogenesis of the disease as well as a simple diagnostic investigation. The relationship of HS excretion and the severity of proteinuria was also studied.

3.2 Patients

Twenty-three children with early onset nephrotic syndrome (NS) were studied: 7 with CNSF aged less than 0.1 years at time of diagnosis; 7 with diffuse mesangial sclerosis (DMS) aged <0.1-0.8 years and 9 with focal segmental glomerulosclerosis (FSGS) aged 0.3-2.7 years, all of whom were steroid resistant. None of the FSGS children were on steroids at the time of study. Fifteen children with acquired steroid sensitive nephrotic syndrome (SSNS) in relapse were studied. They were aged 1.3-10.4 years at time of diagnosis. Twelve were receiving steroids at the time of study, in doses ranging from 0.05 mg/kg on alternate days to 2 mg/kg/day. Eight had biopsy evidence confirming minimal change histology. The controls consisted of 17 normal children aged 0.4-9.1 years, who either attended the Hospital crèche or were the children of laboratory staff.

Although most of the samples were taken from children attending the Hospital for Sick Children, some came from children under the care of paediatric nephrologists at other centers in Great Britain and Europe.
3.3 Methods.

Random morning urine samples were collected in merthiolate 1:10,000 as preservative, coded and stored at -70°C until analysed. The code was not broken until the end of the study.

3.3.1 Quantitation of Total Urine GAGS with Alcian Blue. The highly cationic dye Alcian Blue 8GX is a copper phthalocyanin compound with up to four S-methylene tetramethylisothiouronium chloride side chains per molecule and one atom of copper. It is chemically unstable in alkaline solution (147). Polyanions such as acidic GAGs are precipitated from aqueous solutions by Alcian Blue. This interaction is greatly influenced by the salt concentration, and the salt molarity at which there is a sharp decrease in binding is termed the "critical electrolyte concentration" (CEC). The CEC is a function of the molecular weight of the polyanion and the nature of its ionic charges and chemical properties (148). Scott et al (149) reported that Alcian Blue formed insoluble complexes with acidic GAGs. Magnesium chloride was found to be the most effective salt in differentiating the CEC's of polyanions, polycarboxylates and polysulphates. Proteins may form salt links with GAGs in acidic solutions, blocking the interaction with Alcian Blue. To overcome this the reaction should be carried out at pH 5.8. Urinary GAG precipitation by Alcian Blue was extensively studied by Whiteman (150) who found the greatest specificity of the
reaction to be ensured by the presence of 50mM MgCl$_2$ at pH 5.8. More specifically, Whiteman's studies showed that under these conditions there was no interaction between Alcian Blue and albumin.

Quantitation of total urine GAGs was undertaken by the method developed by Whiteman (150). After a period of equilibration of one hour, GAG precipitation by Alcian Blue in 50mM MgCl$_2$ is nearly at a maximum, with only a slight increase in precipitation after 2 hours. The individual GAGs chondroitin-4-sulphate (C$_4$S), chondroitin-6-sulphate (C$_6$S) and HS have a nearly identical and almost linear calibration curves (148). Because of its commercial availability the sodium salt of C$_4$S was used as reference standard for the total GAG determination.

a) Procedure. Fifty µl of centrifuged urine or standard solution was added to one ml Alcian Blue reagent (Alcian Blue 0.05% w/v in 50mM sodium acetate buffer pH 5.8 and 50mM MgCl$_2$). The Alcian Blue was a gift from Imperial Chemical Industries Ltd, Blackly, Manchester, UK. Standards were made by mixing 0, 5, 10, 20, 30, 40 and 50 µl of a 200 mg/l solution of C$_4$S standard (Sigma Chemicals Ltd) with deionised water to a total volume of 50 µl. These standards correspond to CS concentrations of 0, 20, 40, 80, 120, 160 and 200 mg/l. Urine and standards were allowed to equilibrate for 4 hours at room temperature. The Alcian Blue-GAG complex was centrifuged at 10,000g for 3 minutes. The supernatant was carefully decanted.

The Alcian Blue-GAG complex was washed with 2 ml
absolute ethanol in a "Vortex" mixer. The precipitate was isolated again by centrifugation as above. The ethanolic supernatant was decanted and the tube containing the precipitate at the bottom was left standing at room temperature overnight to allow the alcohol to evaporate. The washed precipitate was dissolved in 1.0 ml of 7.5% sodium dodecyl sulphate solution and left at room temperature for 30 min. Its absorbance was read at 678 nm using a one cm light path semi-micro plastic cuvette in a Pye Unicam PU8610 spectrophotometer. After subtracting the reagent blank value, a calibration curve was made from which urine total GAG values were determined (Fig 3.1).

b) Validation of the Method. Recovery: Total GAG was determined in a 50μl sample of normal urine before and after the addition of 3 μg/μl of a GAG standard containing C4S and HS in equal proportions. The expected increment in total GAG concentration was 60 mg/l. The total GAG concentration in the original urine was 32 mg/l and in the augmented urine was 91 mg/l: the GAG recovery was thus 59 mg/l (98.3 %).

Inter-assay reproducibility: The coefficient of variation of 5 total GAG assays on the same urine sample was 11%.

Intra-assay reproducibility: The average difference in a series of duplicate determinations of 10 urine samples was 5.4 % (median = 3.6 %, range 0 – 17 %).
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Fig. 3.1: Total GAG assay calibration curve using C4S
3.3.2 Extraction and Quantitation of Individual GAGs from Urine. a) Procedure. Urinary GAGs were extracted with the cationic dye Alcian Blue as described by Whiteman et al (148,151). Two ml centrifuged urine was added to 20 ml of the Alcian Blue reagent. The resulting GAG precipitate was dissociated with 0.2 ml 4M sodium chloride solution and 0.1 ml methanol. The Alcian Blue was denatured by alkalinising the mixture with 0.1 ml 0.1 M sodium carbonate and 0.4 ml water and separated by centrifugation at 10,000g for 3 minutes. The GAGs were precipitated from the remaining mixture by the addition of 2.4 ml ethanol and subsequent centrifugation. The supernatant was discarded, the GAGs were dried overnight at room temperature, and dissolved in 20 µl water. Aliquots of 0.5 or 1.0 µl were applied to a cellulose acetate strip adjacent to standards containing both HS and CS at concentrations ranging from 0.12 mg/ml to 0.75 mg/ml. Electrophoresis was performed in 0.1 M barium acetate pH 6.0 for 3 hours with a current of 7.5 volts/cm. The individual GAGs separated into discrete bands which were visualised by staining with the Alcian Blue reagent. Excess Alcian Blue was removed by washing with a solution containing 50 mM MgCl₂ and 50 mM sodium acetate pH 5.8. The individual bands were cut out and eluted with 1 ml of dimethyl sulfoxide reagent, which was prepared by dissolving 0.51 G anhydrous sodium acetate, 1.27 G MgCl₂·6H₂O and 1.56 ml glacial acetic acid in 250 dimethyl sulfoxide (Sigma Chemicals Ltd, spectroscopic grade). The optical density was read at 678 nm and C4S and HS standards.
(Sigma Chemicals Ltd) were run on the same cellulose acetate strip (Fig 3.2). Calibration curves were obtained from these standards from which individual GAG values were determined (Fig 3.3). Although almost linear curves were obtained, there was loss of linearity at concentrations above 1.5 mg/ml (Fig 3.4).

b) Validation of individual GAG measurements. Recovery: A normal urine sample was divided into two 2 ml aliquots, A and B. Ten μl of C4S 1 mg/ml was added to B, giving an increment in C4S concentration of 5 mg/l. GAGs were precipitated from A and B with Alcian Blue, as described in Section 3.3.2, and were reconstituted in 20 μl of water. Five μl aliquots of the reconstituted GAGs were diluted to 50 μl with water. The final Total GAG concentration in A was 14.7 mg/l and in B 45.5 mg/l, indicating concentrations of 1.47 mg/l and 4.58 mg/l in the original urine samples. The recovery of C4S was thus \[(4.55-1.47)/5\]x100 = 62 %. Similar steps were taken to calculate the recovery rate of HS; this was \[(4.16-1.47)/5\]x100 = 54 %.

Intra-assay reproducibility of method for quantitation of CS and HS: On the basis of 6 determinations on the same urine sample, the intra-assay coefficient of variation was 7% for HS and 9% for CS.

Inter-assay reproducibility of quantitation method for CS and HS: The inter-assay reproducibility of the HS/CS ratio
Fig. 3.2: Cellulose acetate strip showing separation of urinary GAGs after electrophoresis. HS and CS standards were applied in increasing concentrations.
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Fig 3.3: Calibration curve for C4S and HS after electrophoresis
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**Fig. 3.4:** Loss of linearity on C4S and HS calibration curves at concentrations above 1.5 mg/ml
was 13%. This figure represents the average of the coefficient of variation of different urine samples, assayed between 5 and 12 times.

3.3.3 Measurement of Urinary Albumin and Urine Creatinine. The urinary albumin concentration was measured by a single radial immunodiffusion assay (152). A 2% solution of anti-human serum albumin antibody (RaHu/Alb Ref 3283, Nordic, Tilburg, Netherlands) in 1.5% agarose gel was used and the concentrations of the samples were calculated by reference to commercial standards (Sigma Chemicals Ltd). Urine creatinine concentration was measured using a Beckman analyser utilising the Jaffé reaction, and results were expressed as the urinary albumin/creatinine ratio (Alb/Cr, mg/mg).

The non-parametric Mann Whitney test and Spearman rank correlation were used in the statistical analysis.

3.4 Results
3.4.1 Urine Creatinine Concentration in Controls and Nephrotic Children. There was no difference in the urine creatinine concentration of children with DMS, FSGS or SSNS groups (Table 3.1b-d) and age matched controls (Table 3.2). However, urine creatinine in the CNSF group (Table 3.1a) was significantly lower than in age matched controls (p < 0.05).
TABLE 3.1a: Urinary GAG excretion in CNSF

<table>
<thead>
<tr>
<th>PT</th>
<th>SEX</th>
<th>AGE AT DIAGNOSIS (years)</th>
<th>AGE AT STUDY (years)</th>
<th>PCR µmol/l</th>
<th>UCR mg%</th>
<th>UA/UC</th>
<th>TGAG/CR mg/µmol</th>
<th>HS/CR mg/mg</th>
<th>CS/CR</th>
<th>HS/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>&lt;0.1</td>
<td>0.9</td>
<td>141</td>
<td>15.4</td>
<td>89.0</td>
<td>0.22</td>
<td>0.33</td>
<td>0.26</td>
<td>1.30</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>&lt;0.1</td>
<td>1.8</td>
<td>42</td>
<td>43.0</td>
<td>158.1</td>
<td>0.04</td>
<td>0.06</td>
<td>0.06</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>&lt;0.1</td>
<td>0.8</td>
<td>NA</td>
<td>15.4</td>
<td>19.7</td>
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<td>0.25</td>
<td>0.58</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>&lt;0.1</td>
<td>1.09</td>
<td>NA</td>
<td>25.8</td>
<td>27.4</td>
<td>0.18</td>
<td>0.10</td>
<td>0.10</td>
<td>0.81</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>&lt;0.1</td>
<td>0.32</td>
<td>32</td>
<td>6.6</td>
<td>57.9</td>
<td>0.33</td>
<td>0.13</td>
<td>0.13</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>&lt;0.1</td>
<td>0.11</td>
<td>40</td>
<td>22.8</td>
<td>1.5</td>
<td>0.45</td>
<td>0.37</td>
<td>0.95</td>
<td>0.47</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>&lt;0.1</td>
<td>0.35</td>
<td>35</td>
<td>11.2</td>
<td>11.7</td>
<td>0.28</td>
<td>0.25</td>
<td>0.32</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>median</td>
<td></td>
<td>&lt;0.1</td>
<td>0.80</td>
<td>40ns</td>
<td>15.4*</td>
<td>27.4***</td>
<td>0.22</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>range</td>
<td></td>
<td>(0.11-1.80)</td>
<td>(32-141)</td>
<td>(6.6-43)</td>
<td>(1.50-158.1)</td>
<td>(0.04-0.45)</td>
<td>(0.06-0.37)</td>
<td>(0.06-0.95)</td>
</tr>
</tbody>
</table>

Key to Table 3.1a:
NA = not available, ns = not significant, alt = alternate
*** = p < 0.0005, ** = p < 0.001, * = p < 0.01
(cont.)
### TABLE 3.1b: Urinary GAG excretion in DMS

<table>
<thead>
<tr>
<th>PT SEX</th>
<th>AGE AT DIAGNOSIS</th>
<th>AGE AT STUDY (years)</th>
<th>PCR (μmol/l)</th>
<th>UCR (mg%)</th>
<th>UA/UC</th>
<th>TGAG/CR</th>
<th>HS/CR mg/mg</th>
<th>CS/CR</th>
<th>HS/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 M</td>
<td>0.1</td>
<td>0.13</td>
<td>14</td>
<td>7.7</td>
<td>107.9</td>
<td>0.39</td>
<td>0.50</td>
<td>0.51</td>
<td>1.06</td>
</tr>
<tr>
<td>9 M</td>
<td>0.1</td>
<td>0.52</td>
<td>36</td>
<td>62</td>
<td>8.7</td>
<td>NA</td>
<td>0.08</td>
<td>0.09</td>
<td>0.81</td>
</tr>
<tr>
<td>10 M</td>
<td>0.8</td>
<td>0.91</td>
<td>268</td>
<td>46.8</td>
<td>19.2</td>
<td>0.06</td>
<td>0.07</td>
<td>0.15</td>
<td>0.49</td>
</tr>
<tr>
<td>11 M</td>
<td>0.4</td>
<td>0.83</td>
<td>40</td>
<td>34.2</td>
<td>101.8</td>
<td>0.36</td>
<td>0.17</td>
<td>0.23</td>
<td>0.76</td>
</tr>
<tr>
<td>12 M</td>
<td>&lt;0.1</td>
<td>1.22</td>
<td>31</td>
<td>26.8</td>
<td>15.8</td>
<td>0.15</td>
<td>0.08</td>
<td>0.08</td>
<td>0.99</td>
</tr>
<tr>
<td>13 M</td>
<td>0.4</td>
<td>0.72</td>
<td>73</td>
<td>23</td>
<td>6.8</td>
<td>0.21</td>
<td>0.37</td>
<td>0.33</td>
<td>1.13</td>
</tr>
<tr>
<td>14 M</td>
<td>0.3</td>
<td>1.90</td>
<td>230</td>
<td>13.4</td>
<td>5.6</td>
<td>0.24</td>
<td>0.16</td>
<td>0.29</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**Median:**
- 0.3
- 0.83
- 57<sup>ns</sup>
- 26.8<sup>ns</sup>
- 15.8***
- 0.23
- 0.16
- 0.23
- 0.81***

**Range:**
- (<0.1-0.8)
- (0.13-1.90)
- (14-268)
- (7.7-62)
- (5.6-107.9)
- (0.06-0.39)
- (0.07-0.50)
- (0.08-0.51)
- (0.49-1.13)

**Key to Table 3.1b:**
- NA = not available, ns = not significant, alt = alternate
- *** = p < 0.0005, ** = p < 0.001, * = p < 0.01

(cont.)
### TABLE 3.1c: Urinary GAG excretion in FSGS

<table>
<thead>
<tr>
<th>PT</th>
<th>SEX</th>
<th>AGE AT DIAGNOSIS (years)</th>
<th>AGE AT STUDY (years)</th>
<th>PCR $\mu$mol/l</th>
<th>UCR mg%</th>
<th>UA/UC</th>
<th>TGAG/CR</th>
<th>HS/CR</th>
<th>CS/CR</th>
<th>HS/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>M</td>
<td>0.3</td>
<td>2.2</td>
<td>39</td>
<td>20.2</td>
<td>30.0</td>
<td>0.08</td>
<td>0.19</td>
<td>0.30</td>
<td>0.57</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>0.4</td>
<td>0.9</td>
<td>87</td>
<td>68.8</td>
<td>26.0</td>
<td>0.09</td>
<td>0.06</td>
<td>0.12</td>
<td>0.50</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>0.7</td>
<td>1.4</td>
<td>21</td>
<td>36</td>
<td>16.2</td>
<td>0.24</td>
<td>0.63</td>
<td>0.96</td>
<td>0.66</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>1.9</td>
<td>2.3</td>
<td>50</td>
<td>41.8</td>
<td>25.6</td>
<td>0.09</td>
<td>0.04</td>
<td>0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
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<td>2.8</td>
<td>34</td>
<td>49.8</td>
<td>4.0</td>
<td>0.11</td>
<td>0.07</td>
<td>0.17</td>
<td>0.43</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>2.7</td>
<td>3.8</td>
<td>175</td>
<td>10.8</td>
<td>37.5</td>
<td>0.49</td>
<td>0.48</td>
<td>0.30</td>
<td>1.60</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>2.5</td>
<td>7.7</td>
<td>51</td>
<td>67.6</td>
<td>1.6</td>
<td>0.04</td>
<td>0.04</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>1.3</td>
<td>8.4</td>
<td>125</td>
<td>43.6</td>
<td>3.8</td>
<td>0.06</td>
<td>0.07</td>
<td>0.10</td>
<td>0.70</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>0.6</td>
<td>14.8</td>
<td>105</td>
<td>7.3</td>
<td>10.1</td>
<td>0.11</td>
<td>0.36</td>
<td>0.32</td>
<td>1.13</td>
</tr>
</tbody>
</table>

**Median:** 1.2 2.8 51 ns 41.8 ns 16.1 *** 0.09 0.07 0.17 0.66*

**Range:** (0.3–2.7) (0.9–14.8) (21–175) (7.3–68.8) (1.6–37.5) (0.04–0.49) (0.04–0.63) (0.05–0.96) (0.38–1.60)

**Key to Table 3.1c:**
NA = not available, ns = not significant, alt = alternate
*** = p < 0.0005, ** = p < 0.001, * = p < 0.01 (cont.)
<table>
<thead>
<tr>
<th>PT</th>
<th>SEX</th>
<th>AGE AT DIAGNOSIS (years)</th>
<th>AGE AT STUDY (years)</th>
<th>PCR μmol/l</th>
<th>UCR mg%</th>
<th>UA/UC</th>
<th>TGAG/CR mg/mg</th>
<th>HS/CR</th>
<th>CS/CR</th>
<th>HS/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>M</td>
<td>2.1</td>
<td>10.7</td>
<td>51</td>
<td>98</td>
<td>3.6</td>
<td>0.09</td>
<td>0.06</td>
<td>0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>2.5</td>
<td>3.6</td>
<td>34</td>
<td>23</td>
<td>3.2</td>
<td>0.09</td>
<td>0.09</td>
<td>0.24</td>
<td>0.38</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>10.4</td>
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<td>67</td>
<td>94</td>
<td>1.2</td>
<td>0.05</td>
<td>0.05</td>
<td>0.07</td>
<td>0.71</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>1.6</td>
<td>16.4</td>
<td>61</td>
<td>206</td>
<td>4.9</td>
<td>0.02</td>
<td>0.04</td>
<td>0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>1.5</td>
<td>7.0</td>
<td>44</td>
<td>372</td>
<td>8.4</td>
<td>0.05</td>
<td>0.02</td>
<td>0.04</td>
<td>0.45</td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>1.5</td>
<td>12.4</td>
<td>96</td>
<td>48</td>
<td>2.9</td>
<td>0.38</td>
<td>0.03</td>
<td>0.06</td>
<td>0.44</td>
</tr>
<tr>
<td>30</td>
<td>M</td>
<td>1.5</td>
<td>9.7</td>
<td>NA</td>
<td>135</td>
<td>3.0</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.49</td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>7.6</td>
<td>17.7</td>
<td>58</td>
<td>376</td>
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<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
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</tr>
</tbody>
</table>

(cont)
### TABLE 3.1d (cont): Urinary GAG excretion in SSNS

<table>
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<tr>
<th>PT</th>
<th>SEX</th>
<th>AGE AT DIAGNOSIS (years)</th>
<th>AGE AT STUDY (years)</th>
<th>PCR/UCR μmol/L/mg%</th>
<th>UA/UC mg/L</th>
<th>TGAG/CR mg/mg</th>
<th>HS/CR mg/mg</th>
<th>CS/CR mg/mg</th>
<th>HS/CS mg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>M</td>
<td>1.3</td>
<td>8.0</td>
<td>42</td>
<td>133</td>
<td>2.7</td>
<td>0.1</td>
<td>0.10</td>
<td>0.30</td>
</tr>
<tr>
<td>33</td>
<td>F</td>
<td>2.5</td>
<td>6.6</td>
<td>46</td>
<td>206</td>
<td>6.4</td>
<td>0.06</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>34</td>
<td>M</td>
<td>4.6</td>
<td>11.0</td>
<td>52</td>
<td>113</td>
<td>9.9</td>
<td>0.08</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>35</td>
<td>M</td>
<td>1.9</td>
<td>10.3</td>
<td>37</td>
<td>104</td>
<td>1.1</td>
<td>0.05</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>36</td>
<td>M</td>
<td>1.7</td>
<td>3.7</td>
<td>53</td>
<td>92</td>
<td>1.8</td>
<td>0.09</td>
<td>0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>37</td>
<td>F</td>
<td>1.4</td>
<td>4.9</td>
<td>45</td>
<td>19</td>
<td>2.2</td>
<td>0.16</td>
<td>0.15</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>median</td>
<td></td>
<td>1.7</td>
<td>10.3</td>
<td>51&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>133&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.2***</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>range</td>
<td></td>
<td>(1.3-10.4)</td>
<td>(3.6-17.7)</td>
<td>(34-96)</td>
<td>(19-372)</td>
<td>(1.1-9.9)</td>
<td>(0.02-0.16)</td>
</tr>
</tbody>
</table>

(MCNS confirmed on biopsy on Patients 24 to 31)

**Key to Table 3.1d:**

NA = not available, ns = not significant, alt = alternate

*** = p < 0.0005, ** = p < 0.001, * = p < 0.01
### TABLE 3.2: Urinary GAG excretion in controls

<table>
<thead>
<tr>
<th>PT</th>
<th>AGE AT STUDY (years)</th>
<th>UCR mg%</th>
<th>UA/UC</th>
<th>TGAG/CR</th>
<th>HS/CR</th>
<th>CS/CR</th>
<th>HS/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0.4</td>
<td>8</td>
<td>0.2</td>
<td>0.10</td>
<td>0.32</td>
<td>0.88</td>
<td>0.36</td>
</tr>
<tr>
<td>b</td>
<td>0.4</td>
<td>19</td>
<td>0.1</td>
<td>0.19</td>
<td>0.26</td>
<td>0.60</td>
<td>0.42</td>
</tr>
<tr>
<td>c</td>
<td>0.7</td>
<td>29</td>
<td>0.1</td>
<td>0.13</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>d</td>
<td>0.7</td>
<td>26</td>
<td>0.1</td>
<td>0.16</td>
<td>0.09</td>
<td>0.17</td>
<td>0.53</td>
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<tr>
<td>e</td>
<td>0.9</td>
<td>52</td>
<td>0.0</td>
<td>0.13</td>
<td>0.03</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td>f</td>
<td>1.2</td>
<td>36</td>
<td>0.0</td>
<td>0.15</td>
<td>0.10</td>
<td>0.37</td>
<td>0.27</td>
</tr>
<tr>
<td>g</td>
<td>1.6</td>
<td>47</td>
<td>0.1</td>
<td>0.05</td>
<td>0.06</td>
<td>0.23</td>
<td>0.28</td>
</tr>
<tr>
<td>h</td>
<td>1.7</td>
<td>59</td>
<td>0.0</td>
<td>0.15</td>
<td>0.07</td>
<td>0.27</td>
<td>0.26</td>
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<td>0.07</td>
<td>0.33</td>
<td>0.22</td>
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<tr>
<td>j</td>
<td>2.0</td>
<td>13</td>
<td>0.0</td>
<td>0.21</td>
<td>0.12</td>
<td>0.22</td>
<td>0.54</td>
</tr>
<tr>
<td>k</td>
<td>2.1</td>
<td>30</td>
<td>0.1</td>
<td>0.12</td>
<td>0.13</td>
<td>0.37</td>
<td>0.36</td>
</tr>
<tr>
<td>l</td>
<td>2.2</td>
<td>30</td>
<td>0.0</td>
<td>0.11</td>
<td>0.08</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>m</td>
<td>2.5</td>
<td>18</td>
<td>0.0</td>
<td>0.13</td>
<td>0.11</td>
<td>0.26</td>
<td>0.43</td>
</tr>
<tr>
<td>n</td>
<td>4.0</td>
<td>120</td>
<td>0.0</td>
<td>0.08</td>
<td>0.05</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>o</td>
<td>7.0</td>
<td>69</td>
<td>0.0</td>
<td>NA</td>
<td>0.04</td>
<td>0.06</td>
<td>0.68</td>
</tr>
<tr>
<td>p</td>
<td>7.7</td>
<td>45</td>
<td>0.0</td>
<td>0.15</td>
<td>0.07</td>
<td>0.17</td>
<td>0.44</td>
</tr>
<tr>
<td>q</td>
<td>8.1</td>
<td>56</td>
<td>0.0</td>
<td>0.05</td>
<td>0.08</td>
<td>0.19</td>
<td>0.41</td>
</tr>
<tr>
<td>r</td>
<td>9.1</td>
<td>38</td>
<td>0.0</td>
<td>0.14</td>
<td>0.07</td>
<td>0.16</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Median: 1.9 (33) 0.0 (0.0-0.2) 0.13 (0.05-0.21) 0.08 (0.03-0.32) 0.23 (0.06-0.88) 0.36 (0.21-0.68)

Range: (0.4-9.1) (8-120) (0.05-0.21) (0.03-0.32) (0.06-0.88) (0.21-0.68)
3.4.2 Total GAG Excretion in Controls and Nephrotic Children. The median urinary GAG/creatinine (total GAG/Cr) in the 17 controls was 0.13 mg/mg, range 0.05 - 0.21 (Table 3.2). Total GAG/Cr decreased with age, as observed by others (110, 148).

In the nephrotic children, total GAG/Cr had a median of 0.22 mg/mg, range 0.04 - 0.45 in the CNSF group; median of 0.22 mg/mg, range of 0.06 - 0.39 in the DMS group; median of 0.17, range 0.06 - 0.49 in the FSGS group, and a median of 0.09, range of 0.05 - 0.21 in the SSNS group (see Tables 3.1a-d). There were no significant differences between total GAG/Cr in children from the DMS, FSGS and SSNS groups and age matched controls. Total GAG/Cr was significantly higher in the CNSF group than in age-matched controls (p<0.05). However, urine creatinine was much lower in the CNSF group than in age-matched controls. The higher total GAG/Cr in CNSF children may therefore be partly accounted for by their lower urine creatinine.

3.4.3 Urinary Heparan sulphate/chondroitin sulphate (HS/CS) Ratios in Controls and Nephrotic Children. The urinary heparan sulphate/creatinine (HS/Cr) ratio (Table 3.2) in the 17 control children had a median of 0.08, range 0.03-0.32 mg/mg and the chondroitin sulphate/creatinine (CS/Cr) ratio had a median 0.23, range 0.06-0.88 mg/mg. The urinary HS/CS ratio had a median of 0.36, range 0.21-0.68. Both HS/Cr and CS/Cr decreased with age (Fig 3.5), but HS/CS did not ($r = 0.39$, p >0.1) (Fig. 3.6).
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Fig. 3.5: Relationship between CS/Cr and HS/Cr and age in normal children
Chapter 3: Urinary GAGs

Fig. 3.6: Urinary HS/CS ratio in normal children
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The HS/CS ratios in the CNSF, DMS and FSGS groups (Tables 3.1a-d) were all significantly greater than the control values, but that for SSNS was not (CNSF median: 0.80, range 0.43-1.28, p <0.001; DMS median: 0.81, range 0.49-1.13, p <0.0005; FSGS median: 0.66, range 0.38-1.6, p <0.01; SSNS median: 0.44, range 0.28-0.70, p >0.05, (Fig. 3.7).

3.4.4 The HS/CS Ratio and Proteinuria. The HS/CS ratio in the CNSF, DMS, FSGS and SSNS groups when taken together was strongly correlated with the urine Alb/Cr ($r_S = 0.62$, $p <0.0001$) (Fig. 3.8). However Alb/Cr in the SSNS group was significantly lower than that in the CNSF, DMS or FSGS groups ($p<0.001$), but the correlation between HS/CS and Alb/Cr persisted even when SSNS patients were excluded from the regression analysis ($r = 0.45$, $p <0.05$). Within the individual groups only the CNSF group showed a significant correlation between HS/CS and Alb/Cr ($r_S = 0.8$, $p< 0.05$).

There was no correlation between HS/CS and plasma creatinine concentration, nor were there any differences in plasma creatinine concentration between any of the subgroups of nephrotic children studied. There was no correlation between steroid dose and the HS/CS ratio in the SSNS group.

3.5 Discussion

I discuss first the methodology then the findings of the study.
Chapter 3: Urinary GAGs

**Fig 3.7:** The urinary HS/CS ratio in controls, CNSF, DMS, FSGS and SSNS groups. Median values are indicated by horizontal bars.
Fig. 3.8: Relationship between Alb/Cr and the HS/CS ratio. Correlation coefficient is 0.62 (p<0.0001)
3.5.1 Methodology. The recovery rate of 98.4% obtained with the total GAG assay was good and is comparable to the figures quoted by Whiteman (148). Recovery rates for CS and HS following Alcian Blue extraction and electrophoresis have not been previously been available. In our assay these were 54% for HS and 62% for CS.

Although theoretically, measurements on 24 hour urine collections would have been preferable random urine samples were used for practical reasons. Obtaining accurate timed collections from young children and babies is difficult. It has been shown that if early morning urine samples are avoided the ratio of uronic acid to creatinine are as discriminating as 24-hour urine collections (153). Urine samples were collected in merthiolate preservative as GAGs may be degraded in heavily infected urines (154).

We elected to study the urinary HS/CS ratio for a number of reasons. It corrects for GAG recovery and is independent of age in healthy children. In addition, the use of HS/Cr would have been unreliable as the urinary creatinine concentration was particularly low in children with CNSF. It was also low in some children in the other nephrotic groups, and is probably due to their poor nutritional state resulting in a low muscle mass. Therefore the use of HS/Cr would have led to artificially elevated values, specially in the CNSF group.

We refined the quantitative assay for HS and CS in urine used previously (110), introducing separate standard curves in each analysis. The reason for this, was the
observation of non-linearity at high concentrations of GAGs. Moreover, Alcian Blue precipitates other macromolecules such as Tamm-Horsfall protein (150), and therefore the calculation of individual GAG concentration from the total GAG measurement and the HS/CS optical density ratio, as in the previous study, is less accurate than direct measurement.

3.5.2 Study findings. The observation of Vermylen et al (110) of increased HS excretion in the urine of 4 children with CNS was of interest when taken in the context of the virtual absence of HS in the GBM of one them, suggesting failure of incorporation of HS into the GBM. We investigated children with different forms of CNS in order to ascertain whether abnormalities of the HS/CS ratio were characteristic of a particular type of CNS. It also prompted the speculation that urinary HS measurement might be of diagnostic value in the assessment of CNS.

Our results, based on a larger sample of patients, confirm Vermylen et al's findings of an increased HS/CS ratio in the urine of children with CNSF and DMS. However, we also observed a raised HS/CS ratio in some children with FSGS. We found that total urine GAGs were elevated in the CNSF group compared to controls.

The observation by Vermylen et al, of decreased HS content in the GBM of children with CNS lent support to Vernier et al's previous findings of decreased anionic sites in the GBM in this group of children (108). Similar
findings have been described in other proteinuric states where the GBM charge barrier seems to be damaged; examples include diabetic nephropathy (155-157), puromycin aminonucleoside nephrosis (PAN) (102,158,159) and in an animal model of cationic albumin-induced membranous nephropathy, changes in the sulphation of heparan sulphate glycoprotein correlated with the level of proteinuria (104).

However, the findings by different groups working in this area are not consistent. For example, some workers have found unaltered anionic charge in PAN nephrosis (160, 161). These differences are partly accountable by the variety of techniques used (102). For example, in a study of the HS content in the GBM of CNSF kidneys, using a monoclonal antibody to HS core protein, van den Heuvel et al found no difference in the GBM content of HS in children with CNSF compared to normals or other glomerulonephritis (111). However, these observations do not necessarily contradict the findings of decreased HS reported by Vernier and Vermylen et al who, by the use of a cationic probe or cationic dye were assaying the anionic GAG side chains (108,110). It is conceivable that the core protein may be intact, but that there might be a loss of anionic sites, which are conferred by the negatively charged side GAG chains and not the core protein.

We found that the HS/CS ratio correlated strongly with albuminuria in the nephrotic groups taken as a whole. This finding however, would have been strengthened if it had
been confirmed in each nephrotic group. This was confirmed when testing the CNSF group but not the others. None of the other groups has as wide a range of Alb/Cr values or HS/CS values as the CNSF group, and this may obscure any positive correlation which may exist between these two variables in any of the other groups.

The explanation for the relationship between the HS/CS ratio and proteinuria is not clear. It may be that increased losses of HS from the GBM lead to a greater degree of proteinuria as result of loss of GBM anionic charge. Alternatively, a raised HS/CS ratio might be the non-specific consequence of high levels of proteinuria.

The origin of urinary GAGs is not fully understood. GAGs are found widely distributed in tissues, such as connective tissue, synovial fluid and hyaline cartilage (162). CS is found in the mesangial matrix and HS is an important component of the fixed anionic barrier at the GBM (101).

In normal plasma, the predominant GAG is CS with a much smaller amount of HS, and both are protein bound. Urinary GAGs represent a heterogenous mixture of small size polymers, where CS is also the major component, accounting for more than 80% (163). There is also a small amount of HS and keratan sulphate. Urinary GAGs contain virtually no protein (148,163,164,165).

It would be expected that if in the nephrotic state, plasma GAGs were being lost in the urine, their qualitative composition should reflect that of plasma. This, however is
not the case as we found a relative increase of HS in relation to CS.

In order to explain the findings of this study further studies are needed into the degradation and synthesis of HS in tissues and the in the GBM, in normals and in children with nephrotic syndrome.

We confirmed that children with SSNS have a normal urinary HS/CS ratio. However, they had lower levels of proteinuria. Interpretation of the results in the SSNS group is made difficult as this group was receiving steroids at the time of study. Selective proteinuria is unlikely to account for the low urinary HS excretion seen in the SSNS group. In CNSF there is also selective proteinuria (18), but they had the highest urinary HS excretion.

3.6 Conclusions

In summary, we have shown that children with CNSF, DMS and some children with FSGS, but not children with SSNS, have an increased HS content in the urine compared to controls. There was, however, a positive correlation of the HS/CS ratio with Alb/Cr, suggesting that raised HS/CS ratios are related to states of heavy albuminuria rather than characteristic of specific histological categories.
4. CLINICO-PATHOLOGICAL REVIEW OF CHILDREN WITH NEPHROPATHY, WILMS' TUMOUR AND GENITAL ABNORMALITIES (DENYS-DRASH SYNDROME)

4.1 Introduction

Denys-Drash syndrome was briefly discussed in Chapter 1 as an example of a syndromic form of nephrotic syndrome presenting under one year of age. In this chapter, the results of a detailed clinico-pathological study carried out in 12 children with this syndrome are described.

In recent years there has been increasing awareness within paediatric nephrology that Denys-Drash syndrome is an important cause of end-stage renal failure (ESRF) in children below 5 years of age. It accounts for approximately 15% of children entering the ESRF programme in this age group (166, 167). General paediatricians, and in particular paediatric oncologists and endocrinologists, should be alerted to the existence of this disorder in order that early screening for the onset of the nephropathy is undertaken in those children presenting with unaccounted genital abnormalities and/or early onset Wilms' tumour.

A clinico-pathological analysis of their presenting features and subsequent clinical evolution is given in this chapter. It includes the description of a hitherto unreported pelvicalyceal abnormality, which may constitute a fourth feature of the syndrome and which, in some instances, may also provide a clue to the diagnosis.

The first report of a child with nephropathy, genital
abnormalities and Wilms tumour was published by Denys et al in 1967 (31). Their patient had XY/XX mosaicism with ambiguous genitalia, presented at the age of 3.5 months with nephrotic syndrome, hypertension and Wilms tumour, and died of renal failure at 15 months. Three years later Drash et al reported 2 further examples (32), and since then over 60 cases have been described. Although the eponym of Drash became accepted, this syndrome should perhaps be known as the Denys-Drash syndrome (168).

Some of the reported cases do not display the full spectrum of the syndrome. The nephropathy may be associated with either genital abnormalities (29,33,39-41,169-171) or Wilms' tumour (35-38,172,173); others have had all 3 features (29,31-34,42,166,174-181). The occurrence of nephropathy has become generally accepted as the common denominator of the syndrome (29,33). The association of Wilms' tumour with urogenital abnormalities has long been recognised (36,179,182-184), but in the absence of nephropathy these cases fall outside the definition of Denys-Drash syndrome. Such cases are not included in this review, but this does not exclude the possibility that there is a common aetiologic link among all 3 features which might present in any combination, as suggested by Barakat et al and Gallo et al (39,181).

4.2 Patients and Methods

The 12 patients were seen at the Hospital for Sick Children, between the years 1972 and 1987. Their clinical
presentation, laboratory investigations and radiological findings were reviewed. The names were obtained from computerised records of all children who have presented since 1970 to the department of Paediatric Nephrology at this hospital. The children studied were those recorded as having presented with a "glomerulonephritis", and either Wilms' tumour or genital abnormalities, or both. Five of these children, who presented with the nephrotic syndrome under one year of age, were briefly mentioned in the CNS review in Chapter 2.

Renal histology was available in all patients. There were 12 nephrectomy specimens from 8 patients, 5 needle biopsy specimens and one open wedge biopsy. Gonadal histology was available in 5 cases. For light microscopy tissues were fixed in 10% buffered formalin and processed routinely; 5 μm paraffin sections were stained with haematoxylin and eosin. Renal biopsy specimens and non-tumorous kidney from nephrectomy specimens were also stained with periodic-acid-Schiff, Masson trichrome, hexamine silver, Martius scarlet blue and elastic van Gieson. For immunohistochemistry studies, paraffin sections were cut at 3 μm and stained by a peroxidase-antiperoxidase technique with pronase digestion using polyclonal antibodies to IgG, IgM, IgA, C1q, C3 and fibrinogen. For electron microscopy tissue was fixed in 2.5% glutaraldehyde; ultrathin sections stained with lead citrate and uranyl acetate were examined and photographed using a Joel CX 100 electron microscope. Glomerular and
tubulo-interstitial damage was graded in order of severity from + to +++.

Radiological studies of the genito-urinary system were reviewed. Eight children had an ultrasound (U/S) examination, 9 had intravenous urography (IVU), 7 had a micturating cystourethrogram (MCU), 2 had Technetium 99m-Dimercaptosuccinic Acid (DMSA) scans and 3 had abdominal computerised tomography (CT scan).

4.3 Results

4.3.1 Case Descriptions. Individual case descriptions are given in this section. A summary of their clinical features is provided on Tables 4.1.

The children were divided into 3 groups. Group 1 (patients 1-4) consists of the children with all 3 abnormalities - nephropathy, Wilms' tumour and genital abnormalities. Group 2 (patients 5-9) consists of children who have the nephropathy and genital abnormalities, and Group 3 (patients 10-12) consists of children with nephropathy and Wilms' tumour.

a) Group 1 (Nephropathy, Genital Abnormalities and Wilms' tumour) (patients 1-4).

Patient 1: This child presented to The Hospital for Sick Children at the age of 12 days for investigations of ambiguous genitalia consisting of penoscrotal hypospadias and cryptorchidism. He had been delivered at term after an uncomplicated pregnancy with a birth
### TABLE 4.1: Summary of clinical features

<table>
<thead>
<tr>
<th>Pt No</th>
<th>NEPHROPATHY</th>
<th>WILMS' TUMOUR</th>
<th>GENITAL ABNORMALITIES/KARYOTYPE</th>
<th>PELVI-CALYCEAL ABNORMALITIES</th>
<th>OTHER FEATURES</th>
<th>OUTCOME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age at diagnosis</td>
<td>Age at diagnosis</td>
<td>(years)</td>
<td>(years)</td>
<td>Pelvi-calyceal</td>
<td>Other Features</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>0.9</td>
<td>+/46XY</td>
<td>+</td>
<td>-</td>
<td>Alive, Tx</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>4.8</td>
<td>+/46XY</td>
<td>+</td>
<td>-</td>
<td>Died in ESRF</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>0.4(R)</td>
<td>+/46XY</td>
<td>+</td>
<td>-</td>
<td>CAPD</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>1.6</td>
<td>+/46XX</td>
<td>+</td>
<td>-</td>
<td>Died in ESRF</td>
</tr>
</tbody>
</table>

**Group 1 (Nephropathy, Wilms' tumour and Genital abnormalities)**

<table>
<thead>
<tr>
<th>Pt No</th>
<th>NEPHROPATHY</th>
<th>WILMS' TUMOUR</th>
<th>GENITAL ABNORMALITIES/KARYOTYPE</th>
<th>PELVI-CALYCEAL ABNORMALITIES</th>
<th>OTHER FEATURES</th>
<th>OUTCOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.1</td>
<td>-</td>
<td>+/46XY</td>
<td>+</td>
<td>Sensorineural deafness. Adrenal insufficiency</td>
<td>Alive, low GFR</td>
</tr>
<tr>
<td>6</td>
<td>0.1</td>
<td>-</td>
<td>+/46XY</td>
<td>+</td>
<td>-</td>
<td>Died in ESRF</td>
</tr>
<tr>
<td>7</td>
<td>1.7</td>
<td>-</td>
<td>+/46XY</td>
<td>-</td>
<td>-</td>
<td>Alive, Tx</td>
</tr>
<tr>
<td>8</td>
<td>0.4</td>
<td>-</td>
<td>+/46XY</td>
<td>-</td>
<td>MR* nystagmus, cleft palate</td>
<td>Died in ESRF</td>
</tr>
<tr>
<td>9</td>
<td>0.0</td>
<td>-</td>
<td>+/46XY</td>
<td>+</td>
<td>Flexion contractures at birth</td>
<td>Died in ESRF</td>
</tr>
</tbody>
</table>

**Group 2 (Nephropathy and Genital abnormalities)**

<table>
<thead>
<tr>
<th>Pt No</th>
<th>NEPHROPATHY</th>
<th>WILMS' TUMOUR</th>
<th>GENITAL ABNORMALITIES/KARYOTYPE</th>
<th>PELVI-CALYCEAL ABNORMALITIES</th>
<th>OTHER FEATURES</th>
<th>OUTCOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.9</td>
<td>0.9</td>
<td>-/ND</td>
<td>+</td>
<td>-</td>
<td>Died of tumour</td>
</tr>
<tr>
<td>11</td>
<td>2.2</td>
<td>1.0</td>
<td>-/46XX</td>
<td>obstructed calyces</td>
<td>MR*, aniridia</td>
<td>Alive, Tx at 7 yr</td>
</tr>
<tr>
<td>12</td>
<td>1.5</td>
<td>1.7</td>
<td>-/46XY</td>
<td>+</td>
<td>-</td>
<td>Alive, Tx</td>
</tr>
</tbody>
</table>

* = mental retardation
weight of 3 kg. His parents were unrelated. His karyotype was 46XY and he had a positive testosterone response to the 3 day Human Chorionic Gonadotrophin test (HCG test), performed at 1 month of age. A diagnosis of male pseudohermaphroditism was made and he was reared as a male. At this stage his plasma electrolytes, urea and creatinine were normal. Unfortunately his urine was not tested for protein at this time. He was admitted at the age of 0.90 yr with a 4 month history of facial and periorbital swelling. Clinical examination revealed ambiguous genitalia, generalised oedema and a blood pressure of 110/60 mmHg. His plasma albumin was 20 g/l, serum creatinine 185 μmol/l and he had 4+ proteinuria. There was no haematuria. Over the following 2 weeks his renal function continued to deteriorate rapidly. CT scan of his abdomen revealed a mass in the upper pole of the left kidney. He underwent bilateral nephrectomies and was started on Continuous Ambulatory Peritoneal Dialysis (CAPD). Histology revealed a left sided Wilms' tumour. He was maintained on CAPD until he received a successful cadaveric renal transplant at the age of 2.3 yr.

**Patient 2:** This child was born with severe penoscrotal hypospadias, bifid scrotum and cryptorchidism. Chromosomal analysis revealed a normal male 46XY pattern. He was seen at The Hospital for Sick Children for endocrinological assessment at the age of 0.5 yr. He had a positive testosterone response to HCG and a diagnosis of
male pseudohermaphroditism was made. He was reared as a male. At this stage he had 1+ proteinuria, no haematuria and normal blood pressure and renal function. During the following 4 years he underwent a series of corrective procedures to his hypospadias. During this period his proteinuria worsened to 3+, he became hypertensive and developed progressive renal insufficiency. At the age of 4.8 yr he presented with a palpable abdominal mass. An IVU showed a left renal tumour. On the right there was poor concentration of contrast in the pelvicalyceal system as well as a small pelvis. At this time he had a serum creatinine of 268 \( \mu \)mol/l. He had a nephrectomy for a left Wilms' tumour and a biopsy of the right kidney. Vincristine and adriamycin were given post-operatively. He died 5 months later in ESRF.

**Patient 3:** This boy was seen at The Hospital for Sick Children at 2 yr of age and had initially been looked after at another hospital. He was born with penoscrotal hypospadias, chordee and cryptorchidism. A right sided Wilms' tumour was diagnosed at the age of 0.41 yr when he presented with a palpable mass. There was no proteinuria at the time. He underwent a right nephrectomy and post-operatively received a 10 week course of weekly Vincristine injections. At the age of 1.5 yr he developed a left sided Wilms' tumour. He received cyclophosphamide, vincristine, Adriamycin and Actinomycin D. He also received radiotherapy totalling
1500 cCy in 9 fractions. As there was no reduction in tumour mass, he received further courses of Actinomycin and Adriamycin together with Ifosfamide. He was seen at this hospital at the age of 2.1 yr for consideration of further management of his chemotherapy and radiotherapy-resistant tumour. For the first time, proteinuria was noted at this stage. He had a urine albumin to urine creatinine ratio (UA/UC) of 2.63 and his blood pressure was 130/70. He had a serum creatinine of 65 μmol/l. A laparotomy was performed in which a massive tumour was found with only a very thin rim of normal looking renal tissue. A total nephrectomy was performed and he was started on CAPD with the plan to wait for a tumour-free period of 2 years before entering him in the renal transplant programme. He remained tumour-free during this period and now awaits a renal transplant.

**Patient 4:** This girl presented at 1.6 yr with haematuria and an abdominal mass. She had been born at 38 weeks, the second of dizygotic twins. The first twin died at sixteen hours of age, of a diaphragmatic hernia. An IVU showed bilateral renal masses sparing the left lower pole. She was normotensive, had a plasma albumin of 22g/l and an elevated serum creatinine of 173 μmol/l. She had 3+ proteinuria but no haematuria. Her karyotype was 46XX. She underwent a total right nephrectomy and a partial left nephrectomy. Following this operation her creatinine continued to rise and she died a year later of renal
failure.

Group 2 (Nephropathy and Genital abnormalities) (patients 5-9).

**Patient 5:** This child was noted to have abnormal genitalia at birth. She had partially fused labia, an enlarged clitoris and impalpable gonads. She had been born at 37 weeks with a birth weight of 2.5 kg. and the pregnancy had been complicated by a threatened miscarriage at 10 weeks. Her karyotype was 46XY. She showed a poor testosterone, plasma androstenedione, and plasma dihydroandrostenedione response to the 3 day HCG stimulation test, performed at 2 months of age. No uterus was visualised on U/S and a MCU showed vaginal filling corresponding to a cloaca. She underwent a laparotomy. Mullerian and Wolffian remnants were found, she had absent gonads and she was reared as a girl. At the age of 4 weeks she was noted to have hyperkalaemia, acidosis and a mildly elevated creatinine. A Synacthen stimulation test showed a flat response with no rise in 17 ketosteroids or 17 OH steroids. She had a low plasma renin and a low plasma aldosterone. Adrenal biopsy showed hypoplasia. She was started on hydrocortisone replacement therapy. Serum creatinine remained elevated and a renal biopsy was performed at 2 years of age. Her glomerular filtration rate (GFR) using chromium EDTA, at the age of 9 yr was 32 mls/min/1.73m². Her urine has remained free of protein and red cells. Her blood pressure is mildly elevated. She also
has sensori-neural deafness.

**Patient 6:** This child was born at 42 weeks gestation with a birth weight of 2.9 kg. Penoscrotal hypospadias and cryptorchidism were noted at birth. He had a normal 17-hydroxy progesterone and his karyotype was 46XY. At the age of 2 weeks he developed oedema. His plasma albumin was 17 g/l and his serum creatinine was 107 \( \mu \text{mol/l} \). His urine had an UA/UC ratio of 31 and there was no haematuria. He had evidence of tubular dysfunction with glycosuria and a maximum urine osmolarity of 279 mosmols/kg after pitressin stimulation. An IVU was performed but there was very poor uptake of contrast. A MCU demonstrated right vesicoureteric reflux (VUR), an absent renal pelvis and an abnormal calyceal system. A retrograde pyelogram revealed blunted calyces, confirmed the absence of a renal pelvis but demonstrated a normal calibre ureter. A renal biopsy was performed when he was 1 month old. His renal failure progressed and he died in ESRF at the age of 6 weeks.

**Patient 7:** This phenotypically normal girl was referred to the Gastroenterology Unit of this hospital at the age of 1.7 yr with a one year history of loose stools. She had been born at term weighing 3.7 kg. A jejunal biopsy performed at 1.2 years had been normal. At this time her plasma albumin was slightly low at 32 g/l. and serum creatinine was 44 \( \mu \text{mol/l} \). On examination she had generalised oedema and a blood pressure of 120/80 mmHg. She
had a plasma albumin of 11g/l and an elevated creatinine of 106 μmol/l. The UA/UC was 7. Urine microscopy was normal. She was initially treated with steroids. Her condition deteriorated rapidly with the development of increasing hypertension and progressive renal insufficiency and pulmonary oedema, requiring ventilation and peritoneal dialysis. Steroids were stopped and there was a transient improvement in renal function. A renal biopsy was performed. Her karyotype revealed 46XY and there was no rise in testosterone following a 3 day HCG stimulation test, performed at 2.5 yr. Over the subsequent 5 months, her renal function continued to deteriorate and she was started on CAPD. A diagnosis of incomplete Denys-Drash syndrome was made and bilateral nephrectomies were performed as prophylaxis against the possible development of Wilms' tumour. No evidence of tumour was found in either nephrectomy specimen. She was transplanted successfully at the age of 3.2 yr.

Patient 8: This child was born with abnormal genitalia consisting of penoscrotal hypospadias, bifid scrotum and micropenis. He had a cleft palate, was hyperteloric and had bilateral nystagmus. His irides were normal. He had been born at 36 weeks' gestation after a normal pregnancy with a birth weight of 1.94 kg. He was mentally subnormal, showing gross motor and socio-developmental delay. His karyotype was 46XY. He had a positive testosterone response to a 3 day HCG stimulation test, performed at the age of 0.9
yr. Visual evoked responses and electroretinogram were normal. A brain CT scan at 0.33 yr showed a slightly prominent inter-hemispheric fissure but was otherwise normal. A skeletal survey was normal, apart from slight asymmetry of the skull vault. From the age of 0.41 yr to 0.75 yr he had numerous hospital admissions with gastroenteritis and on all occasions his urine showed 3+ proteinuria. At the age of 0.83 yr, he was admitted with another episode of gastroenteritis. On examination he was grossly oedematous and his blood pressure was 140/90 mmHg. Plasma albumin was 10 g/l and UA/UC was 110. There were 2+ red cells in the urine. Serum creatinine was 49 µmol/l. A MCU showed no vesicoureteric reflux. An abdominal CT scan was normal and showed no evidence of Wilms' tumour. Over the following 2 weeks renal function continued to deteriorate rapidly. A renal biopsy was performed. He was started on CAPD but died in pulmonary oedema at 1.7 yr. Autopsy was refused.

**Patient 9:** This child was born at 41 weeks gestation with a birth weight of 2.45 kg. There had been oligohydramnios during pregnancy. Dysmorphic features were noted at birth. She had an upturned nose, a wide fontanelle and flexion contractures affecting elbows, hips and knees. She had normal female genitalia. She required oxygen and intermittent pressure ventilation for the first day of life. At 4 days of age she developed generalised oedema. When 17 days old her urine grew
Pseudomonas aeruginosa for which she was treated with gentamicin. Her plasma albumin was 22 g/l and there was 3+ proteinuria, microscopic haematuria and glycosuria. Serum creatinine was 396 µmol/l. A MCU showed bilateral VUR and normal calibre ureters. She had bilateral hypoplastic renal pelves and blunted calyces. Her karyotype was 46XY. A renal biopsy was performed. She continued to deteriorate, becoming progressively oliguric and hypertensive. She died in ESRF at 6 weeks of age.

Group 3 (Nephropathy and Wilms' tumour) (patients 10-12).

**Patient 10:** This boy was born at term weighing 3.5 kg. He presented at the age of 0.9 yr with a right sided abdominal mass and hypertension. He had normal male genitalia. Plasma albumin was 18g/l and serum creatinine was 44 µmol/l. There was 3+ proteinuria and glycosuria but no haematuria. His UA/UC was 16. An IVU revealed a renal tumour on the right and, on the left, absent renal pelvis with blunted non-dilated calyces. A right nephrectomy was performed. He remained nephrotic and hypertensive. At 1.2 yr he underwent a laparotomy for recurrence of the tumour on the contralateral kidney and paraaortic nodes. Resection was attempted but he died on the first post-operative day.

**Patient 11:** This child was noted at birth to have aniridia. She had normal female genitalia. She was kept under review and at 1 yr of age, bilateral renal masses were detected on ultrasonography. An IVU confirmed the presence
of a left renal mass and a right lower pole mass compatible with Wilms' tumour. Chromosomal analysis revealed a 46XX karyotype and an 11p13 deletion. She received a course of Vincristine, Adriamycin and Actinomycin D and 500 rads in 8 fractions over a 2 week period shielding the upper, normal half of the right kidney. At the age of 1.16 yr she underwent a left nephrectomy and a partial right nephrectomy. A year later she had a GFR of 61 mls./min./1.73m2 and had developed significant proteinuria, with a UA/UC of 6. Her renal function remained stable although her proteinuria persisted. At the age of 6 yr her serum creatinine started to rise and she became progressively hypertensive. Six months later she had reached ESRF and was subsequently successfully transplanted. Her intellectual development is impaired. She had a normal onset of menarche at the age of 14 yr.

**Patient 12:** This boy was born at term weighing 3.7 kg. His parents were from Iran and were unrelated. At the age of 1.5 yr he underwent an emergency bronchoscopy to remove an inhaled nut. Routine urine testing at the time showed 3+ proteinuria. He was admitted a month later with haematuria, periorbital oedema and hypertension. On admission he had a low serum albumin (29 g/l), a serum creatinine of 116 μmol/l. There was haematuria, glycosuria and 3+ proteinuria. An IVU showed a left renal mass and a normal calyceal system in the right, but an absent renal pelvis. His karyotype was 46XY. A left
nephrectomy and a biopsy of the right kidney were performed. His renal function deteriorated rapidly and he was started on CAPD in the post-operative period. A right nephrectomy was performed at 1.7 yr prophylactically, against the risk of tumour recurrence on the contralateral side. He received a successful renal transplant at 3 yr of age.

4.3.2 Nephropathy

a) Clinical evolution. The nephropathy was characterised by the early onset of proteinuria, by the presence of hypertension, and by the rapid development of ESRF (Table 4.2). Proteinuria was present in 11 of the 12 patients and resulted in the nephrotic syndrome in 8. The age at detection of proteinuria ranged from birth to 2 yr, 6 being less than 1 yr of age. Hypertension was present in 10 of the 12 patients ranging from mild in 3 to severe and requiring treatment in 7. Haematuria was detected in only 3 patients. Glycosuria was detected on at least one occasion in 5 patients.

Progression to ESRF was seen in 9 of the 12 patients, 7 of whom were younger than 3 years of age. Five died in renal failure, one whilst on CAPD, and 4 have had successful transplants. An additional patient (pt. 3), who had mild proteinuria and hypertension, had an initial nephrectomy for a right sided nephroblastoma and later had a left nephrectomy for tumour recurrence, which proved to be drug- and radiotherapy-resistant. He is on CAPD. One child
has diminished but stable renal function.

b) Renal histology. Renal histology was available in all 12 patients (Table 4.2). Except for one child who showed changes of focal glomerular sclerosis, the rest had varying degrees of mesangial sclerosis, either diffuse (7 cases) or focal (4 cases).

In the 7 children with diffuse mesangial sclerosis, the superficial subcapsular cortex was atrophic and contained small, immature glomeruli, with a prominent layer of epithelial cells enveloping the tuft and resembling those usually encountered in the foetal kidney. Many of these glomeruli were sclerosed and crowded together, with atrophy and loss of intervening tubules, and marked interstitial fibrosis. In the middle cortex glomeruli showed expansion of the mesangial matrix by fibrillary PAS positive, argyrophilic material (Fig. 4.1). Preserved nuclei were present but there was no increase in cellularity. Silver staining revealed double contours in the glomerular basement membrane in a proportion of glomeruli in most cases (Fig. 4.2). Crescents and adhesions were rarely encountered and glomerular abnormalities were less severe in the inner cortex.

Of the 4 renal histology specimens exhibiting focal mesangial sclerosis the changes were mild in two. One case (pt. 3) lacked the distinctive subcapsular zone, but in the others this feature was well developed. Abnormal glomeruli in the middle and outer cortex were similar in appearance
<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>PROTEINURIA (onset)</th>
<th>TENSION</th>
<th>HISTOLOGY</th>
<th>RADILOGICAL FEATURES</th>
<th>OUTCOME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>glomerulo-pathy</td>
<td>% sclerosed tubulo-interst. Immunocyto-</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>glomeruli damage</td>
<td>chemistry</td>
<td></td>
</tr>
<tr>
<td>Group 1 (Nephropathy, Wilms' tumour and Genital abnormalities)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+ (0.9 yr)</td>
<td>+</td>
<td>DMS</td>
<td>42%</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>+ (0.5 yr)</td>
<td>+</td>
<td>FMS</td>
<td>27%</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>+ (2.0 yr)</td>
<td>+</td>
<td>FMS</td>
<td>0%</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+ (1.6 yr)</td>
<td>-</td>
<td>FMS</td>
<td>16%</td>
<td>++</td>
</tr>
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</table>

(cont.)
### TABLE 4.2 (cont.): Nephrological features of children with Drash syndrome

<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>PROTEINURIA</th>
<th>HYPERTENSION</th>
<th>glomerulopathy</th>
<th>% sclerosed glomeruli</th>
<th>tubulo-interstitial damage</th>
<th>Immunocytochemistry</th>
<th>RADIOLOGICAL FEATURES</th>
<th>OUTCOME</th>
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<tr>
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<tr>
<td><strong>Group 2 (Nephropathy and Genital abnormalities)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
<td>FGS</td>
<td>28%</td>
<td>+</td>
<td>Focal IgG, C1q, C3</td>
<td>Blunt calyces, absent pelves</td>
<td>Low GFR, hypertensive, no proteinuria at 9 yr</td>
</tr>
<tr>
<td>6</td>
<td>+ (0.1 yr)</td>
<td>-</td>
<td>DMS</td>
<td>20%</td>
<td>+++</td>
<td>C3</td>
<td>(R) VUR, blunt calyces absent pelves</td>
<td>NS at 0.1 yr. Died in ESRF at 0.3 yr</td>
</tr>
<tr>
<td>7</td>
<td>+ (1.7 yr)</td>
<td>+</td>
<td>DMS</td>
<td>67%</td>
<td>+++</td>
<td>IgM, C1q, C3</td>
<td>-</td>
<td>NS at 1.7 yr. ESRF at 2 yr. 1x at 2.5 yr</td>
</tr>
<tr>
<td>8</td>
<td>+ (0.4 yr)</td>
<td>+</td>
<td>DMS</td>
<td>0%</td>
<td>++</td>
<td>IgM, C1q, C3</td>
<td>-</td>
<td>NS at 0.8 yr. ESRF at 0.8 yr. Died at 1.7 yr</td>
</tr>
<tr>
<td>9</td>
<td>+ (0.0 yr)</td>
<td>+</td>
<td>DMS</td>
<td>67%</td>
<td>+++</td>
<td>IgM, C3</td>
<td>Bilateral VUR, blunt calyces, absent pelves</td>
<td>NS at birth. Died in ESRF at 0.1 yr</td>
</tr>
</tbody>
</table>

(cont.)
### TABLE 4.2 (cont.): Nephrological features of children with Drash syndrome

<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>PROTEINURIA (onset)</th>
<th>HYPERTENSION</th>
<th>glomerulopathy</th>
<th>% sclerosed glomeruli</th>
<th>tubulo-interst. damage</th>
<th>Immunochemistry</th>
<th>RADIOLOGICAL FEATURES</th>
<th>OUTCOME</th>
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<tr>
<td><strong>Group 3 (Nephropathy and Wilms' tumour)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+ (0.9 yr)</td>
<td>+</td>
<td>DMS</td>
<td>11%</td>
<td>+++</td>
<td>IgM</td>
<td>Blunt calyces, (L) absent pelvis</td>
<td>NS at 0.9 yr. Died of tumour recurrence at 1.2 yr</td>
</tr>
<tr>
<td>11</td>
<td>+ (2.2 yr)</td>
<td>+</td>
<td>FMS</td>
<td>28%</td>
<td>+++</td>
<td>Neg</td>
<td>Bilateral obstructed calyces</td>
<td>ESRF at 6.5 yr. Tx at 7 yr</td>
</tr>
<tr>
<td>12</td>
<td>+ (1.5 yr)</td>
<td>+</td>
<td>DMS</td>
<td>71%</td>
<td>+++</td>
<td>IgM, IgG, C1q, C3</td>
<td>Normal calyces, absent (R) pelvis</td>
<td>NS at 1.5 yr. ESRF at 1.8 yr</td>
</tr>
</tbody>
</table>

**Key to abbreviations used in Table 4.2:**
- **CAPD** = continuous ambulatory peritoneal dialysis
- **DMS** = diffuse mesangial sclerosis
- **ESRF** = end-stage renal failure
- **FMS** = focal mesangial sclerosis
- (L) = left, neg = negative
- **NS** = nephrotic syndrome
- Pt. No. = patient number
- (R) = right
- tubulo-interstitial
- **Tx** = transplant
- **VUR** = vesico-ureteric reflux
- yr = year
Fig. 4.1: Diffuse expansion of the mesangial matrix by fibrillary PAS positive material (patient 4). PAS x 40
to those seen in the diffuse cases, but a proportion of these glomeruli, as well as those in the inner cortex, were morphologically normal. Tubulo-interstitial damage was invariably present and was of a similar nature to that seen in patients with diffuse mesangial sclerosis, but the severity was commensurate with that of the associated glomerular changes. Diagnosed tubular atrophy with microcystic dilatation was accompanied by interstitial fibrosis.

Electron microscopy was available in 6 patients, of whom 5 had increased mesangial matrix and podocyte hypertrophy and foot process fusion. The GBM contained electron dense linear and particulate formations in the lamina rara externa and lamina densa in two cases (1, 7) and wrinkling of the subepithelial GBM was seen. Subendothelial and intramembranous deposits were present in one case; in

Fig. 4.2: Silver staining of GBM showing double contours (Patient 10). x 100. (Illustration courtesy of Department of Histology, HSC)
to those seen in the diffuse cases, but a proportion of these glomeruli, as well as those in the inner cortex, were morphologically normal. Tubulo-interstitial damage was invariably present and was of a similar nature to that seen in patients with diffuse mesangial sclerosis, but the severity was commensurate with that of the associated glomerular changes. Widespread tubular atrophy with microcystic dilatation was accompanied by interstitial fibrosis and patchy chronic inflammation.

Immunocytochemistry studies were positive in 9 of 12 patients with granular deposition of IgG, IgM, C1q and C3, principally along peripheral capillary walls and sometimes in the mesangium (Fig 4.3). IgA and fibrinogen were consistently absent. Positive immunostaining was confined to morphologically abnormal glomeruli. Entirely negative results were obtained in 3 of the earlier cases in the series. However, the tissue had been embedded in paraffin wax for 12-15 years, and the results may be spurious for technical reasons.

Electron microscopy was available in 6 patients, of whom 5 had increased mesangial matrix and podocyte hypertrophy and foot process fusion. The GBM contained electron dense linear and reticular formations in the lamina rara externa and lamina densa in two cases (1,7) and wrinkling of the subepithelial GBM was seen. Subendothelial and intramembranous deposits were present in one case; in another, mesangial interposition was seen.
Chapter 4: Demyë-Brush review

a) Radiological features (Table 4.3). On contrast imaging of the urinary tract, 7 patients were found to have abnormal calyceal systems characterised by blunted, non-dilated calyces with no evidence of obstruction (Fig 4.4). Five of these patients also had either absent or small renal pelvis, and two had VUR (Figs. 4.4 and 4.5).

Two additional patients had normal calyceal systems but their renal pelvis were either small or absent. It was not possible to confirm whether these were normal or abnormal cranial or renal development. A non-clear cell renal tumour was an incidental finding with the presence of ambiguous genitalia or of aniridia. Six children were less than 2 years of age (median 1.25 yr) at diagnosis. One child was diagnosed at 3.9 yr. Two children had bilateral disease at presentation. One child developed a recurrence in the contralateral kidney 13 months after the diagnosis of the first tumour. None had extrarenal involvement. All had normal benign histological features of Wilms' tumour, at

**Fig. 4.3:** Immunoperoxidase staining for IgM reveals granular positivity predominantly in peripheral capillary loops (Patient 7). x 400. (Illustration courtesy of Department of Histology, HSC)
c) Radiological features (Table 4.2). On contrast imaging of the urinary tract, 7 patients were found to have abnormal calyceal systems characterised by blunted, non-dilated calyces with no evidence of obstruction (Fig 4.4). Five of these patients also had either absent or small renal pelves, and two also had VUR (Figs. 4.4 and 4.5).

Two additional patients had normal calyceal systems but their renal pelves were either small or absent. It was not possible to study two patients with IVU as they had advanced renal failure at presentation, but their MCUs were normal. Eight patients had renal U/S scans and in all of them there was loss of cortico-medullary differentiation, a non-specific finding.

4.3.3 Wilms' tumour. Seven children developed Wilms' tumour (Table 4.1). Three children were found to have an abdominal mass as an incidental finding after presenting with minor symptoms. Two children presented with haematuria and in 2 the diagnosis was systematically sought because of the presence of ambiguous genitalia or of aniridia. Six children were less than 2 years of age (median 1.25 yr) at diagnosis. One child was diagnosed at 4.8 yr. Two children had bilateral disease at presentation. One child developed a recurrence in the contralateral kidney 13 months after the diagnosis of the first tumour. None had extrarenal involvement. All 10 tumours were of "favourable" triphasic histologic type (187) (Figs. 4.6 and 4.7).

Five children had no evidence of Wilms' tumour, at
Fig. 4.4: Micturating cystourethrogram of Patient 9 showing vesico-ureteric reflux, hypoplastic renal pelves, blunt calyceal systems and non-dilated ureters
Fig. 4.5: Micturating cystourethrogram of Patient 6 showing right sided vesico-ureteric reflux, absent renal pelvis and blunt calyceal systems
Fig. 4.6 and 4.7: Haematoxylin and eosin staining of "favourable" triphasic Wilm's tumour (Patient 4). x 40 and x 100. (Illustration courtesy of Department of Histology, HSC)
autopsy (2 patients), bilateral nephrectomy (1 patient) or on repeated abdominal ultrasound or computed tomography (2 patients). One of the 2 patients followed up with sequential imaging was 9 yr of age at last review (pt. 5). The other died at 1.7 yr (pt. 8). The diagnosis of Wilms' tumour preceded the diagnosis of nephropathy in 2 children by 1.6 yr and 1.2 yr respectively. Wilms' tumour was diagnosed at the same time as the nephropathy in 3 children, and after the onset of the nephropathy in 2 patients.

4.3.4 Genital Abnormalities.

a) Clinical features. Nine children had genital abnormalities (Table 4.3). Patients 1, 2, 3 in Group 1 had 46 XY karyotypes and ambiguous external genitalia consisting of penoscrotal hypospadias and cryptorchidism (Fig. 4.8). Patient 4 was a phenotypically normal female with a 46XX karyotype, but was found to have Müllerian and Wolffian structures and a streak ovary at autopsy. All patients in Group 2 had 46XY karyotype. Patient 5 had a large clitoris with fused labia (Fig 4.9). Patient 6 had penoscrotal hypospadias and cryptorchidism. Patients 7 and 9 had female external genitalia and patient 8 had penoscrotal hypospadias and bifid scrotum with palpable gonads.

b) Sex Hormones Profile (Table 4.3). The testosterone response to a 3 day HCG stimulation test was studied in 4 children (pts 1, 5, 7 and 8). Two children had a rise in
### Table 4.3: Sexual and endocrine features of children with Denys-Drash syndrome

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Karyotype</th>
<th>Genital Abnormalities</th>
<th>Testosterone response to HCG</th>
<th>17αH progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anatomy</td>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (Nephropathy, Wilms' tumour and Genital abnormalities)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46XY</td>
<td>Peno-scrotal hypospadias</td>
<td>Cryptorchidism</td>
<td>Normal rise</td>
</tr>
<tr>
<td>2</td>
<td>46XY</td>
<td>Peno-scrotal hypospadias</td>
<td>Cryptorchidism</td>
<td>Dysgenetic testes</td>
</tr>
<tr>
<td>3</td>
<td>46XY</td>
<td>Peno-scrotal hypospadias</td>
<td>Cryptorchidism</td>
<td>L dysgenetic testis, R absent</td>
</tr>
<tr>
<td>4</td>
<td>46XX</td>
<td>Normal female external genitalia</td>
<td>M &amp; W ducts</td>
<td>Streak ovary</td>
</tr>
<tr>
<td>Group 2 (Nephropathy and Genital abnormalities)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>46XY</td>
<td>Large clitoris, Labial fusion</td>
<td>No gonads found at laparotomy</td>
<td>Flat response</td>
</tr>
<tr>
<td>6</td>
<td>46XY</td>
<td>Peno-scrotal hypospadias</td>
<td>Cryptorchidism</td>
<td>Dysgenetic testes</td>
</tr>
<tr>
<td>7</td>
<td>46XY</td>
<td>Normal female external genitalia</td>
<td>-</td>
<td>Flat response</td>
</tr>
<tr>
<td>8</td>
<td>46XY</td>
<td>Peno-scrotal hypospadias</td>
<td>Cryptorchidism</td>
<td>Normal rise</td>
</tr>
<tr>
<td>9</td>
<td>46XY</td>
<td>Normal female M &amp; W ducts</td>
<td>Bicornuate uterus</td>
<td>R streak gonad, L absent</td>
</tr>
<tr>
<td>Group 3 (Nephropathy and Wilms' tumour)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>Normal male</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>46XX</td>
<td>Normal female del 11(p13)</td>
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<td>-</td>
</tr>
<tr>
<td>12</td>
<td>46XY</td>
<td>Normal male</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

Key to abbreviations: del = deletion, HCG = human chorionic gonadotrophin
Fig. 4.8: Penoscrotal hypospadias and cryptorchidism (Patient 6).
Male pseudohermaphroditism. Enlarged clitoris (Patient 5)
testosterone (pts. 1 and 8). Both children had ambiguous genitalia. The other 2 children showed no increase in testosterone secretion (pts. 5 and 7); one of them (pt. 5) had a large clitoris and labial fusion, the other (pt 7) had a normal female external phenotype.

c) Histology of Gonads and Genital Tract (Table 4.3). Gonads and/or genital duct structures were examined histologically in 5 cases. Atrophic changes attributable to cryptorchidism were present in patients 2 and 3. In patient 4 a streak ovary containing primordial follicles was accompanied by Müllerian and Wolffian ducts. Dysgenetic intrabdominal testes were present in patient 6; histologic examination showed severe architectural derangement of the cortex and focal invasion of the tunica albuginea by tubules. In patient 9 a bicornuate uterus (Fig 4.10) was accompanied by bilateral Müllerian and Wolffian duct structures. A single streak gonad was identified at postmortem. This comprised ovarian type stroma lacking primordial follicles. Poorly differentiated sex cords were present and there were occasional tubules suggestive of primitive canalicular seminiferous tubules. No gonadal tumours were identified in any of the specimens examined.

4.4 Discussion

The basic characteristics of the children in this series are similar to those reported previously. However, this review revealed some features which have not hitherto
4.4.1 Nephropathy. The nephropathy is characterized by the presence of proteinuria at an early age and in most children it evolves into the nephrotic syndrome, and eventually progresses to FSGS. Of 64 reported cases, 78% developed FSGS, 60% of whom did so before the age of 2.77 years (33-34, 38-42, 174-181, 182). With one exception, the histological findings consisted of varying degrees of focal or segmental glomerulosclerosis. These patterns are often accompanied by diffuse interstitial fibrosis involving cortical and medullary segments, which is characteristic of the evolution of a glomerular process.

Classifications of the renal abnormalities have included diffuse mesangial sclerosis (29,33,42,172), focal glomerulosclerosis (33), membranoproliferative glomerulonephritis (175,177), and membranoproliferative glomerulonephritis with focal segmental glomerulosclerosis (173). There are numerous reports without adequate information on renal immuncytolysis in these cases.

**Fig. 4.10:** Bicornuate uterus (Patient 9). (Illustration courtesy of Department of Histology, HSC)
been reported.

4.4.1 Nephropathy. The nephropathy is characterized by the presence of proteinuria at an early age and in most children it evolves into the nephrotic syndrome, and eventually progresses to ESRF. Of 64 reported cases, 76% developed ESRF, 60% of whom did so before the age of 2 yr (29, 31-33, 35-42, 174-181, 186). With one exception the histological findings consisted of varying degrees of focal or diffuse mesangial sclerosis as described by Habib et al (29), Waldherr et al (35) and Gallo et al (181). Those patients in this series who had focal mesangial sclerosis tended to have less severe disease than those with diffuse involvement. The distribution of lesions was in striking contrast with that of focal segmental glomerulosclerosis, which tends to involve the juxtamedullary cortex first and most severely. The characteristic major involvement of the outer cortex, previously reported by others, was present in both focal and diffuse cases. The two groups appear to represent a morphologic continuum possibly reflecting evolution of a common process.

Classifications of the renal abnormalities have included diffuse mesangial sclerosis (29, 33, 42, 172), focal sclerosis (36), membrano-proliferative glomerulonephritis (175, 177) and membrano-proliferative glomerulonephritis with focal segmental glomerulosclerosis (173). There are numerous reports without adequate information on renal histology. The presence of complement and immunoglobulins in
the glomeruli has been reported \((29,35,36,166,174,175)\) and was common amongst the patients in this series. Deposition was confined to morphologically abnormal glomeruli suggesting non-specific trapping rather than an immunologically mediated process, as observed by Habib et al \((29)\). In any case, an immune mechanism related to the presence of tumour-derived antigens seems unlikely, since in 5 of the 7 patients with Wilms' tumour, the nephropathy coincided or preceded the development of nephroblastoma. In addition, such a mechanism would not explain the nephropathy in children with genital abnormalities alone.

Several reports have described a nephropathy in association with XY gonadal dysgenesis in phenotypic females presenting with primary amenorrhoea in adolescence \((187-190)\). They all reached ESRF but at a later onset than in the typical child with Denys-Drash syndrome. The age of onset of ESRF in the patients described in these four reports varied between 14 to 23 yr. Renal histology was described in one report as focal glomerular sclerosis with mesangial cell matrix expansion \((188)\), as showing the changes of end-stage disease in another \((189)\), and was not provided in two others \((187,190)\). Thus there may well be different forms of nephropathy seen in association with genital abnormalities.

The features of the nephropathy seen in two patients merit special attention. The first is patient 5. The course of her nephropathy differs somewhat from that of the majority of the other children in this series. To date she
has not developed proteinuria and her renal biopsy showed focal glomerular sclerosis. The basic features of the nephropathy in Denys-Drash syndrome are the presence of proteinuria, often leading to the nephrotic syndrome, and a rapid deterioration of renal function into ESRF. Her renal disease appears to be different as she has not developed proteinuria and she continues to have a reduced but stable GFR. In addition, her renal biopsy showed focal glomerular sclerosis without evidence of either focal or diffuse mesangial sclerosis. She meets the clinical criteria for inclusion in this series as an example of incomplete Denys-Drash syndrome, in view of the presence of male pseudohermaphroditism and a nephropathy of early onset. However, it is possible that her constellation of congenital abnormalities, which include sensori-neural deafness and adrenal insufficiency, could represent a different syndrome.

The second patient presenting a dilemma is patient 11. She developed proteinuria at 2 yr of age. At 6 yr she became hypertensive and showed signs of renal insufficiency. This progressed rapidly over the ensuing 6 months into ESRF. Renal histology, at the end of 6 weeks of chemotherapy and radiotherapy, showed focal mesangial sclerosis. Proteinuria and the subsequent onset of hypertension are the main features of radiation nephritis (191). Histological changes consisting of mesangial sclerosis with mesangiolysis, are usually not seen until 6 months or more following radiotherapy. Mesangiolysis was
not observed in our patient. However, it is conceivable that her nephropathy was due, at least in part, to the treatment for her Wilms' tumour.

Other forms of nephropathy have been reported in association with Wilms' tumour. Minimal change nephrotic syndrome that responded to steroids was reported by Lines (192), and membranous nephropathy has been reported by Row et al (193). Focal segmental glomerulosclerosis has developed in the remaining kidney 10 to 20 years after unilateral nephrectomy for treatment of Wilms' tumour, believed to be a consequence of hyperfiltration in the remaining kidney (193,195).

4.4.2 Wilms' Tumour. Three of the 7 patients with Wilms' tumour had bilateral involvement. In the published cases there is also a high incidence of bilaterality. In contrast, the incidence of bilateral Wilms tumour among all cases is 4-5%. The mean age at diagnosis in our group was 1.6 yr, which is earlier than the mean age of diagnosis in children with sporadic Wilms' tumour (3.8 yr) (196).

The histology was of the "favourable" type in all our patients. There was only one death related to Wilms' tumour, due to extensive abdominal recurrence. In agreement with other reports, within this group of children the prognosis for Wilms' tumour is good. None of the children in this study, with a diagnosis of Wilms' tumour and who are alive at present, has had a recurrence of their tumour.
4.4.3 Genital Abnormalities. The most commonly reported sexual abnormality including our series, is male pseudohermaphroditism. Children have a 46XY karyotype and incompletely virilised external genitalia, either ambiguous or completely female. Gonadal histology in these cases reveals testes which vary from rudimentary to normal. There was, in our patients, a wide range of response in testosterone secretion to the Human Chorionic Gonadotrophin stimulation test. This varied from almost no rise to a normal response. There is only one reference in the published reports to the results of a Human Chorionic Gonadotrophin stimulation test. Testosterone and dihydrotestosterone were at the lower normal level in the case reported (170). One of our cases with male pseudohermaphroditism fits the diagnosis of pure gonadal dysgenesis. Pt. 9 had a 46 XY karyotype, female external genitalia and a right streak gonad with agonadism on the left.

Therefore, the genital abnormalities seen in Denys-Drash syndrome are heterogenous. Other genital abnormalities reported include the case reported by Eddy et al (178), which although designated a male pseudohermaphrodite, was in fact a true hermaphrodite, having both testicular and ovarian tissue (197). Fisher et al (180) reported a child with 46XX karyotype, normal female phenotype, Wilms' tumour and congenital nephrosis who had hypoplastic ovaries and bilateral gonadoblastomas; this case strongly resembles patient 4 in this series who
had a 46XX karyotype, female external genitalia and a streak ovary. It is possible that female children with XX karyotype, nephropathy and Wilms' tumour have an under-diagnosed incidence of gonadal abnormalities. Gonadal biopsies have not been routinely performed in this group of children. Gonadal biopsies should be carried out for complete diagnosis and, if indicated, gonadectomies should be performed in order to prevent the development of gonadoblastomas. As suggested by Eddy et al (198), the definition of this syndrome should be expanded to include all abnormalities of gonadal differentiation.

It is conceivable that some of the karyotypically female (XX) children with diffuse mesangial sclerosis might carry a gonadal abnormality that remains undiagnosed. Until very recently, most of these children have succumbed to their renal disease much before puberty when abnormalities might have become apparent.

4.4.4 Pelvi-calyceal Abnormalities. A consistent, previously undescribed, pelvi-calyceal abnormality was observed on contrast imaging. The calyceal system appeared blunted, without evidence of obstruction or dilatation. The renal pelves were in many cases either small or absent. Two of the 3 children reported by Barakat et al (39) had hydronephrosis on one side and calyceal blunting on the other non-hydronephrotic side.

This is likely to be a congenital rather than an acquired abnormality and is probably another manifestation
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of the abnormal development of the genito-urinary system seen in this syndrome. It is unlikely to be the result of vesicoureteric reflux which was detected only in 2 of the 8 patients studied by micturating cystourethrogram. In these 2 patients the size of the ureters was normal. Unfortunately there was only one gross specimen available for comparison which showed a normal pelvi-calyceal system (patient 9). This was a surprising finding as the micturating cystourethrogram appearances in this patient were striking. These pelvicalyceal abnormalities may not only form part of the Denys-Drash syndrome but also, in some cases, may provide a clue to the diagnosis.

There are 3 patients in this series who had other congenital abnormalities besides those belonging to the Denys-Drash triad. Patient 5 had sensorineural deafness and adrenal insufficiency and she has already been discussed in this chapter. Patient 8 had a cleft palate, nystagmus and mental retardation. Although nystagmus and mental retardation have been reported in siblings in association with diffuse mesangial sclerosis, they have not been described in children with Denys-Drash syndrome (199).

Patient 11 had aniridia and mental retardation and a constitutional deletion involving chromosome region 11p13. None of these features has previously been reported in patients with Denys-Drash syndrome. There is clinical overlap between this syndrome and the WAGR complex (Wilms' tumour, aniridia, ambiguous genitalia and mental retardation), in which there is a deletion involving
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chromosome region 11p13 (112). Turleau et al reported a child with incomplete Denys-Drash syndrome, who had ambiguous genitalia, a nephropathy with diffuse mesangial sclerosis and low androgen receptors (170). Low androgen receptors have also been reported by Malpuech in a child with the WAGR complex and a deletion in 11p13 (200). Turleau et al draw attention to the phenotypic overlap between both syndromes, and suggest that there may be a common genetic background to Denys-Drash syndrome and WAGR complex (170). Thus it is possible that Denys-Drash syndrome could arise as a consequence of a deletion of one or more genes located within the p13 region in the short arm of chromosome 11.

Alternatively, the genetic origin of this syndrome could lie on the X chromosome. Most reported patients have had an XY karyotype which, together with the presence in this syndrome of a substantial disorder of gonadal differentiation, could point to a genetic abnormality in the sex chromosomes.

4.5 Conclusions

It is important to consider the diagnosis of Denys-Drash syndrome in any infant who presents with an unexplained nephropathy, particularly in young phenotypic females, in children with ambiguous genitalia or with early presenting Wilms' tumour. A vigorous and optimistic approach to treatment should be pursued, including prophylactic bilateral nephrectomies even in the absence of
Wilms' tumour. The practice at this hospital has been to take this step once ESRF has occurred but it could be argued that there is a case for nephrectomy before ESRF ensues because of the risk of early development of Wilms' tumour. However, since most deaths have been related to the renal disease and the onset of ESRF, better dialysis and transplantation programmes for young children constitute the major factor in the improved prognosis of these children. Children who have the nephropathy and Wilms tumour should be rendered anephric once ESRF ensues but should be observed for one and a half to two years for possible metastases before undergoing renal transplantation.
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5. MOLECULAR AND CYTOGENETIC STUDY IN CHILDREN WITH DENYS-DRASH SYNDROME

5.1 Introduction

The clinical features of the three main components of Denys-Drash syndrome (DDS), namely the nephropathy, Wilms' tumour (WT) and genital abnormalities, have been well defined (see Chapter 4) which make its clinical recognition relatively easy. However, the aetiology of the syndrome remains obscure. In this chapter, I describe the cytogenetic and molecular biology analysis of children with DDS.

5.1.1 Renal and Gonadal Embryogenesis and DDS. From the earliest descriptions several authors have pointed out that the abnormalities seen in DDS are found in two organ systems which share a common embryonic origin, namely the renal and genital organs which arise from the mesoderm or urogenital ridge (29,32,33,39). During the sixth to ninth week of gestation the urogenital ridge differentiates into what will eventually become the ureteric bud and nephrogenic cap as well as into germ cell-containing gonads and Wolffian and Müllerian ducts which are precursors of the internal genital organs (201,202).

As discussed in Chapter 4, one of the key characteristics of DDS is the early onset of each of the associated abnormalities. The nephropathy is very often evident during the first year of life with some cases
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presenting during the first month of life. This degenerative change culminates in end-stage renal failure (ESRF), usually within the first 3 years. The genital abnormalities are evident at birth. The two embryonic cancers, Wilms' tumour and gonadoblastoma (33,186,178,180) also have a significantly early onset in DDS patients. Therefore, it is likely that the features seen in this syndrome result from an insult on embryonic tissue during intrauterine life (29,33).

5.1.2 Aetiology of DDS. To date there has been only one report of DDS involving siblings. Zunin and Soave described 2 sisters who both developed Wilms' tumour and the nephrotic syndrome (203). In one sister, however, the nephropathy appeared to recover. Since the nephropathy in DDS syndrome progresses relentlessly into ESRF, it is unlikely that she had the typical pathology associated with the syndrome.

In the absence of any reports of patients with a family history, it might be argued that DDS syndrome is not a genetically determined and might instead arise as a result of a teratogenic agent. However, to date, no environmental agents have been implicated in the aetiology of this syndrome, nor have there been reports of factors in pregnancy which could be of aetiological significance, although there has not been a systematic search. No environmental agents (in the form of chemicals, radiation, drugs or intrauterine infections) that might play a part in
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the intrauterine development of these children have been identified and pregnancies have almost invariably been uneventful.

It was recently suggested that paternal occupation was important in the development of leukaemia (204). The implication was that exposure to carcinogens or radiation might have caused mutations in the paternal genes. Similar information on parental occupation in the case of DDS is not available.

5.1.3 Development of the "Two-hit" Hypothesis". The concept of hereditary predisposition to cancer was first outlined by Armitage and Doll (205) and later refined by Knudson (206). The early age of onset and high incidence of bilaterality seen in hereditary forms of cancers, in particular retinoblastoma and later Wilms' tumour, led Knudson and Strong (207) to suggest that these children inherited a genetic defect from one of the parents (the "first hit"), which would be present in all cells. A second event ("hit") would then be required at the homologous gene locus to initiate tumour formation. This second step would occur as a somatic event. In this theory the "events " or "hits" could be any disturbance of the genetic material such as point mutations or chromosomal deletions, inversions or translocations. In sporadic cancers, which tend to be unilateral and present later, both hits are postzygotic, somatic events which accounts for their later onset.
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Given that all the features in DDS have an early onset, it is possible that they might result from a genetic predisposing abnormality affecting the urogenital ridge during embryogenesis, followed by a second "event" restricted to the organ in question.

5.1.4 WAGR syndrome and Localization of a Wilm's Tumour Gene to Chromosome Region 11p13. Wilms' tumour and genital abnormalities are not only seen in DDS but also in the phenotypically related WAGR complex, where children have Wilms' tumour, Aniridia, Genitourinary abnormalities and mental Retardation. Children with this syndrome have been shown to have a deletion on the short arm of chromosome 11 (112). Chromosome analysis in these cases has demonstrated consistent loss of a subregion of band 11p13 (208-212). Although only 50% of AGR patients develop WT (208), this consistent constitutional chromosome abnormality implicates region 11p13 as the site of a WT-predisposition gene.

5.1.5 11p13 as a Possible Site for Kidney Development Genes. A neonate, with Potter's facies (213) and kidney agenesis, has been shown to have a constitutional chromosome translocation with one breakpoint in 11p13 (214). Molecular studies have shown that this breakpoint is distinct from the Wilms' tumour locus in that region (215) but, if the abnormality is related to the kidney agenesis, the finding may point to the site of another gene important in kidney development. These observations
suggest that there may be a "cluster" of genes in 11p13 that are important in both kidney and genito-urinary development. The coincidence of a nephropathy in patient 11 of our series (see Chapter 4), early onset bilateral Wilms' tumour, aniridia and mental retardation and a deletion involving 11p13 was a strong reason for looking for deletions in this region in the other DDS patients.

Both cytogenetic analysis and molecular probes from region 11p13 were used in this study to determine whether submicroscopic deletions or rearrangements could be detected.

5.1.6 X-chromosome Probes. The close association during embryogenesis, between the development of the reproductive and urinary systems and the severe disorders in the development of gonadal, internal and external genitalia seen in DDS syndrome, might implicate a defect on the sex chromosomes. A limited study of selected gene loci in the X chromosome was therefore performed. Uniparental disomy study: this phenomenon is introduced, together with the results and discussion of a limited study carried out in DDS families, at the end of this Chapter.

5.2. Materials and Methods

5.2.1 Cytogenetic Analysis. In order to carry out the molecular and cytogenetic analysis of patients without repeated venepunctures, lymphoblastoid cell lines were generated wherever possible. This was achieved using
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Epstein Barr virus (EBV).

a) Preparation of lymphoblastoid cell lines. Peripheral blood was diluted 1:1 with unsupplemented RPMI culture medium and then carefully layered on top of 8 ml of lymphocyte separation medium ("Lymphoprep." Flow Laboratories). The gradient was centrifuged at 1500g for 20 minutes at 4°C in a Jouan CR412 centrifuge. White cells collect at the Fycoll/medium interphase and were removed with a 1ml pipette, taking care not to disturb the red cell layer, and were then washed twice with PBS. Cells prepared in this way were frozen in liquid nitrogen until transformation. For this the freezing cocktail contained 90% foetal calf serum and 10% DMSO.

b) Cell Transformation with EBV. Approximately $5 \times 10^6$ cells were washed and mixed with EBV for 2 hr before the virus was removed by centrifugation and the cells resuspended in 1 ml of RPMI with 20% foetal calf serum. One μg/ml cyclosporin was added for the first 2 weeks. Progressively growing cultures were available after 6 to 8 weeks.

c) Chromosome harvest. Six drops of peripheral blood (working in quadruplets) were added to 10 mls of RPMI culture medium containing 20% foetal calf serum, 1% glutamine and 6.25mg% of penicillin and streptomycin. One hundred and fifty μl of phytohaemagglutinin (Welcome) were
then added and the cells incubated at 37°C for 72 hr. After
72 hr 0.01 mg/ml vinblastine sulphate was added to the
cultures for 15 min. Aliquots of lymphoblastoid cells were
treated similarly. Cells were recovered at 1200g and the
supernatant was discarded. The cells were then resuspended
in a hypotonic solution of 10 ml of 0.07M potassium
chloride for 10 min and recovered by centrifugation at
1200g for 2-5 minutes. The white cell pellet was then fixed
in three changes of 10 ml of a 3:1 methanol/acetic acid
solution.

Chromosomes were prepared using standard air-drying
techniques. Chromosome preparations were allowed to age for
7 days before analysis by a modified trypsin-Giemsa banding
protocol (216). For trypsin-Giemsa banding the slides were
first treated for 10-40 sec with a 0.25% trypsin solution
made up in unsupplemented E4 tissue culture medium,
adjusted to pH 7.0 with 5% acetic acid. They were then
stained with 3% Giemsa (Gurr Ltd.) for 20 min. After
satisfactory staining these preparations were photographed
with an Olympus camera on UHT microfilm (Kodak).

5.2.2 Concepts in DNA Analysis.
a) Restriction enzymes and "polymorphic" and "non-
polymorphic" gene probes. DNA "restriction enzymes" are
able to cut double stranded DNA at specific sequence-
dependent sites yielding millions of DNA fragments of
der differing lengths. Fig 5.1 shows gene X, flanked by
cleavage sites for restriction enzymes A and B. The 2
Enzyme A

Enzyme B

non-polymerism

polymorphism

*Fig. 5.1:* Restriction fragment length polymorphism
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genes, or alleles, have identical cleavage sites for enzyme A; gene X is "non-polymorphic" with respect to the cleavage site for enzyme A. The 2 alleles, however, differ from each other by the position of the cleavage sites for enzyme B. Gene X is "polymorphic" or exhibits a "restriction fragment length polymorphism" (RFLP) with respect to enzyme B. Inheritance of identical alleles of a given gene is seen in homozygotes. An individual with a pair of different alleles is called heterozygote. Fig. 5.2 shows the banding pattern that would be obtained in each case.

The frequency with which a particular RFLP occurs in a given population has been studied for numerous markers or "probes". The observation that particular RFLP's co-segregate with certain genetic diseases has made the genetic localization or mapping of these conditions possible. Polymorphisms are often independent of the gene in question and may arise as a consequence of individual base variation, resulting in loss of a cleavage site or the formation of a new one. Insertion or deletion of blocks of DNA within a fragment could also alter its site.

b) Southern Blotting. After cleavage with DNA restriction enzymes, the DNA fragments can be separated according to their molecular weight by agarose gel electrophoresis (Fig 5.3). The DNA fragments can be rendered single stranded by a process called "denaturation", where the hydrogen bonds between complementary base pairs are broken. This can be achieved by exposing double stranded DNA to highly alkaline
**Fig 5.2:** RFLPs: Schematic representation of "homozygous" and "heterozygous" states
Fig 5.3: Steps involved in Southern blotting
conditions.

The DNA fragments in the agarose gel are then transferred by capillary action onto a nitrocellulose filter achieved by setting up a flow of appropriate buffer solution through the thickness of the gel. The agarose gel is mounted on a tray containing buffer solution and a piece of nitrocellulose filter paper is laid over the gel. Sufficient filter paper is laid on top of the nitrocellulose filter as well as a moderately heavy weight to ensure that the buffer solution travels up through these layers. This causes the DNA fragments to flow out of the agarose gel onto the nitrocellulose filter where they associate. The DNA fragments are then covalently linked by baking the filter. The filter can then be hybridized to a suitable radioactive DNA probe or known gene. The specific DNA fragments that hybridize to the probe produce a signal following autoradiography.

5.2.3 Practical Methods for DNA Extraction and Southern Blotting.

a) DNA extraction. DNA was prepared from lymphoblastoid cell lines using conventional phenol/chloroform extraction procedures (217). Cells growing in cell culture medium (see Section 5.2.1.b) were spun at 1300 rpm for 10 minutes at 4°C and the supernatant discarded. The cells were then washed with PBSA and centrifuged again. Cells were resuspended in "cell lysis buffer" (100 mM Tris pH 8.0, 100 mM NaCl and 10 mM EDTA) and then lysed by the addition of
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0.5% lauryl sulphate. Proteolysis was carried out by the addition of 300 μl of a 200 mg/ml solution of Proteinase K (BRL) at 55°C for 2-3 hr. Protein was extracted by the addition of an equal volume of phenol, mixed and centrifuged. Phenol layer was discarded and any remains of phenol were removed by the addition of phenol/chloroform 50:50 vol/vol and finally chloroform alone, mixed and centrifuged. The DNA was precipitated by the addition of 1/10 vol of 3 M sodium acetate pH 4.8 and 2 vol of cold absolute ethanol and left at -20°C for 30 min. High molecular weight DNA could be removed with a sealed pasteur pipette and washed in 70% ethanol. DNA was then dissolved in TE buffer (10 mM Tris, 1 mM EDTA), pH 7.8. RNA was removed with RNAase at a concentration of 100 mg/ml of DNA solution, incubated for 30-60 min at 37°C. The phenol/chloroform extraction procedure was repeated and the DNA resuspended in TE.

b) Southern Blotting. The high molecular weight DNA extracted by the method described in Section 5.2.1b was digested with the appropriate restriction enzymes. These were used in combinations with probes so as to result in a single, non-polymorphic band. The conditions recommended by the manufacturers (Anglian Biotech) regarding temperature and specific buffers were followed. After digestion DNA fragments were separated by agarose gel electrophoresis. One per cent agarose gels were used. It was necessary to load approximately equal amounts of DNA in each lane of the agarose gel for this procedure. We found
that quantitation using ultraviolet light absorbance was more accurate if measurements were taken after digestion of the DNA with the appropriate restriction enzyme. This procedure largely overcame quantitation problems associated with incompletely dissolved DNA samples and pipetting errors due to the high molecular weight of the DNA. In all experiments we used 10 ug of DNA per lane.

Electrophoresis was carried out in a tank containing "E" buffer (Tris Base 242 g/l, glacial acetic acid 57.1 ml/l, 0.5 EDTA (pH 8.0) 100 ml/l) and 100 μl of 10 mg/ml ethidium bromide, at 4 volts per cm overnight. Denaturation was carried out with "denaturing solution" containing 0.5 M NaOH and 1.5 M NaCl, pH 14.0. The gel was then washed with "neutralising" solution (Na citrate and Tris buffer, pH 5.5). DNA fragments were transferred to nitrocellulose filters (Hybond-N membranes, Amersham) as described by Southern (218). 20 x SSC was used as buffer. The Hybond-N membranes were washed in 2 x SSC and then fixed by heating at 80°C for 2 hr.

Southern blots included the following control samples for 100% and 50% 11p13 genetic content: (1) a patient with no evidence of any phenotypic or chromosomal abnormality, representing 100% normal genomic content; (2) a patient with a large 11p deletion (GOS 157), spanning the region covered by the 11p13 probes (210). He had a 46XY karyotype, the WAGR complex and no evidence of nephropathy and represents a 50% gene content for 11p13.
c) Oligolabelling DNA probes. DNA probes were labelled to high-specific activity using \(^{32}\text{P}\)-CTP in the oligo-primer extension procedure described by Feinberg and Vogelstein (219).

The first step of this procedure involves the preparation of the labelling solution (LS): TM solution: 250 mM Tris-HCl, 25 mM MgCl\(_2\), 50 mM 2-mercaptoethanol, pH 8.0 \(\mu\)l. DTM solution: 100\(\mu\)M deoxyadenine triphosphate, 100 \(\mu\)M deoxythymine triphosphate and 100 \(\mu\)M deoxyguanine triphosphate in TM solution. OLigolabelling solution (OL): 1 mM Tris, 1 mM EDTA, pH 7.5, to which 90 optical density units per milliliter of oligodeoxyribonucleotides was added. LS is then made by the addition of: 1 M Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid pH 6.6)/ solution DTM/solution OL in a 25:25:7 ratio, stored at -20°C. An aliquot containing 50-100 ng/ml (1-5 \(\mu\)l) of DNA probe to be labelled was mixed with water to a total volume of 22.5 \(\mu\)l in a sealed tube. This was boiled for 5 minutes to 95-100°C, immediately cooled to 0°C, centrifuged and returned to ice. Three \(\mu\)l of LS solution, 1.5 \(\mu\)l of bovine serum albumin (nucleic acid enzyme grade) (10 mg/ml), 2.5 \(\mu\)l of \(^{32}\text{P}\) dCTP (3000 Ci/mmol) and 1 \(\mu\)l (5u) of Klenow fragment (large fragment of DNA polymerase I) were then added. The contents were mixed gently, centrifuged at room temperature to get all the liquid to the bottom of the tube and left to incubate at room temperature overnight. After labelling the probes were filtered through a Sephadex G-50 column using 150 \(\mu\)l
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aliquots of 2 x SSC. Label incorporation was counted in a desk top "Bioscan" β counter. Counts of $1 \times 10^7$ to $10^8/\mu g$ DNA were obtained with this method.

d) Hybridization. The nitrocellulose filters were initially prehybridized using 10 ml of hybridization solution. This contained 0.75 M NaCl/0.075 M sodium citrate, 4 x concentrated Denhardt's solution (Denhardt's solution: 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 10% dextran sulphate, 0.1% NaDodSO₄, 0.1% sodium pyrophosphate, and 100 μg of denatured salmon sperm DNA per ml. Filters were hybridized by adding the labelled DNA probe to 10 ml hybridizing solution in a sealed plastic bag and left at 65°C overnight. After hybridization the filters were washed at 65°C in 2 x SSC and 0.1% NaDodSO₄ and exposed to Kodak XAR5 Xray film for 24 to 72 hr producing an autoradiograph. In order to reuse the filters the first probe was removed. Probes were removed from the filters by washing in 50% formamide, 10mM phosphate, pH 7.5, for 30 min at 65°C.

Probes, band size and enzymes used are listed in Table 5.1.

5.2.4 Gene Dosage Analysis by Densitometry. Figure 5.4 illustrates an example of DNA/gene dosage analysis. DNA has been cut with enzyme H which recognises 2 cleavage sites at either side of radioactively labelled probe C on the 2 alleles. Binding of this probe to the 2 alleles may be
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**TABLE 5.1: List of probes, enzymes, band sizes in 11p13 gene dosage study**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Enzyme</th>
<th>Band size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT800</td>
<td>Xba I</td>
<td>3.0 kb</td>
<td>(220)</td>
</tr>
<tr>
<td>p9RH0.65</td>
<td>HindIII</td>
<td>4.0 kb</td>
<td>(221)</td>
</tr>
<tr>
<td>D11S87</td>
<td>HindIII</td>
<td>3.5 kb</td>
<td>(222)</td>
</tr>
<tr>
<td>p56H2.4</td>
<td>HindIII</td>
<td>2.5 kb</td>
<td>(223)</td>
</tr>
<tr>
<td>JM115</td>
<td>PstI</td>
<td>5.1 kb</td>
<td>Van Heyningen, (pers. comm)</td>
</tr>
<tr>
<td>FSHB</td>
<td>EcoRl</td>
<td>9.4 kb</td>
<td>(224)</td>
</tr>
</tbody>
</table>

**Control probe:**

<table>
<thead>
<tr>
<th>p123M1.8 (13q14)</th>
<th>XbaI</th>
<th>9.4 kb</th>
<th>Dryja (pers. comm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HindIII</td>
<td>18.0 kb</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PstI</td>
<td>11.0 kb, 2.3 kb &amp; 2.1 kb</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EcoRl</td>
<td>9.4 kb &amp; 6.2 kb</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 5.4: Scanning densitometry in gene dosage studies
taken to represent 100% hybridization. Scanning densitometry tracing of this signal would reveal a full deflection. In Fig 5.4b one of the alleles has a deletion involving the DNA sequence that hybridizes with probe C. Hybridisation, therefore is only 50% and would represented by a half scale deflection on densitometry.

Probes from the 11p13 region were used in gene dosage studies (209) (Fig 5.5) and were used in combination with restriction enzymes chosen to produce single, non-polymorphic bands, making quantitation of the amount of hybridization simpler. After hybridization with the 11p13 gene probes, the blots were stripped and rehybridized with a control gene probe, p123M1.8, derived from chromosome 13 (TP Dryja, personal communication). The intensity of the band on the autoradiographs obtained with the 11p13 probes and with the chromosome 13 control probe was measured using scanning laser densitometry. As there is a degree of inequality of band intensity accounted for by unavoidable uneven lane loading, this was corrected by expressing each patient band as a factor of the most intense band on the control probe autoradiograph and multiplying the signals from the 11p13 autoradiographs by this factor. The signal derived from the DNA from the DDS patients was then compared to that from the normal (100%) control on the same filter.

Densitometry profiles were obtained using an Ultroscan XL (Pharmacia) with a fixed wavelength of 633 nm. The X-width was set to 10 giving a scanning beam width of 8 mm,
Fig 5.5: Diagrammatic illustration of the relative order of the 11p13 DNA sequences used in gene dosage studies
effectively integrating absorbance over the width of the band on the autoradiograph.

5.3 Patients.

The patients studied in this genetic survey form part of the group of 12 children with DDS syndrome seen at the Hospital for Sick Children, London, between the years 1972 and 1987. They were reviewed in Chapter 4. At the time of the study there were 7 surviving children with DDS syndrome who were available for study. These were Patients 1, 3, 5, 7, 8, 11 and 12. Informed parental consent was obtained and venepunctures were performed which coincided with the children's routine blood investigations. Unfortunately, since attempts at transformation of blood from Pt 5 were unsuccessful this patient was not included in the molecular biology study. Therefore this study was performed using the 6 other children, from whom lymphoblastoid cell lines were established. Karyotypes from three of the deceased children (Pts 2, 4 and 9) and Pt 5 were performed as part of their clinical investigations in the Cytogenetics Laboratory at the Hospital for Sick Children. The results of these cytogenetic investigations have been included. As these were old preparations it was not possible to draw definite conclusions on the presence or absence of small interstitial deletions.

5.4 Results

5.4.1 Genetic and Cytogenetic Analysis. A summary of
**TABLE 5.2: Genetic and cytogenetic characteristics of Drash patients**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Nephropathy</th>
<th>Wilms' tumour</th>
<th>Genital Abnormalities</th>
<th>Other Abnormalities</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>MR, AN</td>
<td>46XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>del(11)(p12p13)</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>46XY</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>MR, CP</td>
<td>46XY</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>46XY</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>46XY</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>46XY</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>DF</td>
<td>46XY</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>46XY</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>46XY</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>46XX</td>
</tr>
</tbody>
</table>

**Key to abbreviations:** MR = mental retardation, AN = aniridia, CP = cleft palate, DF = deafness.
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The karyotypic and phenotypic data for the 10 DDS patients is given in Table 5.2. Eight of the 10 patients had a normal 46XY karyotype (Fig 5.6a and 5.6b), but only one (Pt 12) had normal male genitalia. Four patients had ambiguous genitalia and 3 were phenotypically female. Two patients (Pt 11 and Pt 4) had a 46XX karyotype with apparently normal external genitalia. At autopsy, however, Pt 4 was shown to have a streak gonad and both Mullerian and Wolffian duct structures. There is no information regarding the internal genitalia for Pt 11 but she has undergone normal female pubertal changes. She has an interstitial deletion, (11)(p12;p13) (Fig. 5.7). She also has aniridia, mental retardation and is short in stature.

5.4.2 Molecular Analysis of 11p13 in DDS Children. Although cytogenetic analysis excluded chromosome translocations or large deletions in 9/10 patients, it was still possible that more subtle chromosome deletions were present but not detected by conventional karyotype analysis.

A typical result using probe p9RH0.65 is shown in Fig 5.8. As expected, the signal intensity from lanes 2 and 10, which contained DNA from deletion control patient GOS 157, is clearly lower than in the control lanes. When the same blot was reprobed with the control sequence, p123M1.8, from chromosome 13, the relatively even loading of the lanes was demonstrated. The overall ratios for each probe in each patient is given in Table 5.3. Only Pt 11, who
Fig 5.7: Trypsin-Giemsa banded chromosome 11 pair from patient 11 showing del (11) (p12,p13)
GOS 400 = Pt 11
GOS 368 = Pt 7
GOS 374 = Pt 8
GOS 372 = Pt 1
GOS 378 = Pt 12
GOS 389 = Pt 3

Fig 5.8: Comparison between hybrid band intensities following hybridization with control probe pl23M1.8 (above) and 11p13 probe p9RH0.65 (below).
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**TABLE 5.3: Summary of ratios of absorbance between a normal control and six Drash patients for various probes from region 11p13**

<table>
<thead>
<tr>
<th>Patient</th>
<th>FSHB</th>
<th>JM115</th>
<th>p56H2.4</th>
<th>D11S87</th>
<th>P9RH0.65</th>
<th>CAT800</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Control</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GOS 157</td>
<td>0.41</td>
<td>0.49</td>
<td>0.27</td>
<td>0.40</td>
<td>0.36</td>
<td>0.42</td>
</tr>
<tr>
<td>11</td>
<td>0.96</td>
<td>1.29</td>
<td>0.65</td>
<td>0.30</td>
<td>0.40</td>
<td>0.46</td>
</tr>
<tr>
<td>7</td>
<td>1.04</td>
<td>1.01</td>
<td>0.91</td>
<td>0.90</td>
<td>0.84</td>
<td>1.26</td>
</tr>
<tr>
<td>8</td>
<td>0.67</td>
<td>1.25</td>
<td>0.93</td>
<td>1.07</td>
<td>1.08</td>
<td>1.00</td>
</tr>
<tr>
<td>12</td>
<td>1.02</td>
<td>1.7</td>
<td>0.89</td>
<td>0.97</td>
<td>1.08</td>
<td>0.85</td>
</tr>
<tr>
<td>3</td>
<td>0.95</td>
<td>1.5</td>
<td>1.18</td>
<td>1.22</td>
<td>1.4</td>
<td>0.91</td>
</tr>
<tr>
<td>N Control</td>
<td>0.90</td>
<td>1.74</td>
<td>1.1</td>
<td>0.85</td>
<td>1.16</td>
<td>0.96</td>
</tr>
<tr>
<td>GOS 157</td>
<td>0.28</td>
<td>0.64</td>
<td>0.32</td>
<td>0.25</td>
<td>0.4</td>
<td>0.33</td>
</tr>
</tbody>
</table>
carried a visible 11p13 deletion, showed any reduction in relative band intensity for any of the probes. Thus, none of the other DDS patients could be shown to have deletions within the 11p13 region identified by the probes used. There was one equivocal result; using the FSHB probe a ratio of 0.67 was seen in Pt 8. Since the ratio for the deletion control in this experiment was also low this result is probably normal. In Pt 11 normal band intensities were found with FSHB, but only 50% levels were observed with p56, thus defining the distal breakpoint of her 11 chromosome deletion between these probes (see Fig 5.5).

5.4.3 X Chromosome Probes. A limited study of selected loci in the X chromosome was carried out because of the severe disruption of normal sexual differentiation seen in DDS children. In addition, the association of diffuse mesangial sclerosis, as seen in DDS and Duchenne muscular dystrophy, mapped to Xp22 has been described (199). Three X chromosome probes that hybridise to Xp22 were used and are listed on Table 5.4. The presence/dosage of Xp22 probes DXS84 and DXS103, which lie distal to the DMD locus, did not identify deletions in any of the XY or XX patients. We also used the cDNA probe, ZFY (225), until recently a candidate gene for the testis determining factor (226). This gene probe recognises homologous sequences on both the X and Y chromosomes each of which produces characteristic bands on Southern blots following autoradiography (227). No rearrangements or deletions of the gene were detected.
TABLE 5.4: Name of X chromosome probes, enzymes, band sizes, and references

<table>
<thead>
<tr>
<th>Probe</th>
<th>Name</th>
<th>Location</th>
<th>Enzyme</th>
<th>Band size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIXS84</td>
<td>754</td>
<td>Xp21.1</td>
<td>EcoR1</td>
<td>9.4 kb</td>
<td>(228)</td>
</tr>
<tr>
<td>DIXS103</td>
<td>KY6</td>
<td>Xp22.1</td>
<td>EcoR1</td>
<td>2.5 kb</td>
<td>(229)</td>
</tr>
<tr>
<td>ZFY</td>
<td>pMF01</td>
<td>Yp</td>
<td>EcoR1</td>
<td>δ = 4.0, 1.8 kb</td>
<td>(225)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Φ = 1.8 kb</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 5: Molecular Genetic Analysis

5A. 11 Chromosome Uniparental Disomy Study

5A.1 Introduction

The concept of uniparental disomy refers to the inheritance of both copies of a gene, cluster of genes, segments of chromosome or a whole chromosome from the same parent (230,231). The first evidence that this phenomenon could be a mechanism for human disease was provided by Spence et al, in 1988 (232). Inheritance of two identical copies of mutant maternal sequences for the cystic fibrosis locus and much of chromosome 7 was demonstrated in a child with cystic fibrosis (CF), whose father was not a CF carrier. A similar observation in another child with CF has been made by Voss et al (233).

Uniparental disomy has been described in Prader-Willi syndrome (234). This syndrome is associated with deletions involving 15q11q13, present in around 60% of cases (235). The deletion has been shown to be paternally derived in most cases (236). Nicholls et al demonstrated in 2 non-deletion PWS cases from different families the inheritance of 2 intact maternal chromosome 15q11q13 regions (234). They suggested that this syndrome may arise from the absence of a paternal contribution to region 15q11q13, either by paternal deletion or maternal uniparental disomy. Differences in gene expression that depend on the sex of the transmitting parent is known as "genetic imprinting" (237,238) and uniparental disomy is an example of this phenomenon.
5A.1.1 Molecular Biology Techniques. The study of these phenomena has been possible particularly as a result of the development of hypervariable DNA markers which have allowed the identification of the parental origin of a given genetic region (239). These probes have a highly polymorphic character because a core DNA sequence is repeated tandemly at a given locus such that many alleles are present in the population. Their high degree of heterozygosity is conferred by a variable number of tandem nucleic acid repeat sequences (VNTRs) (239). The polymorphism arises from allelic differences in the number of repeats.

5A.2 Methods

DNA was extracted from lymphoblastoid cell lines as described above. The study was carried out in Patients 1, 3, 7, 8 and 12. DNA from patients and parents was digested with Taq I under conditions described by the manufacturers. Southern blots were made and probed with calcitonin, catalase 800 and MS51 which hybridise to 11p15, 11p13 and 11q13 respectively. Band size and probes characteristics are shown on Table 5.5. Blood for DNA analysis was not available from all parents. In Tables 5.6 to 5.8, the sign "-" means result unavailable.

5A.3 Results

The results obtained with calcitonin, MS51 and catalase 800 are summarised below, in Tables 5.6, 5.7 and 5.8.
Chapter 5: Molecular Genetic Analysis

**TABLE 5.5: Probes used in uniparental disomy study**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Location</th>
<th>Reference</th>
<th>Enzyme</th>
<th>Band size</th>
<th>Reference**</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALC</td>
<td>11p15.4</td>
<td>(240)</td>
<td>TaqI</td>
<td>9kb or 10kb</td>
<td>(241)</td>
</tr>
<tr>
<td>CAT800</td>
<td>11p13</td>
<td>(220)</td>
<td>TaqI</td>
<td>3.5kb or 2.5 &amp; 1.0kb</td>
<td>(220)</td>
</tr>
<tr>
<td>MS51</td>
<td>11q13</td>
<td>(242)</td>
<td>TaqI</td>
<td>1.3-4.3kb</td>
<td>(243)</td>
</tr>
</tbody>
</table>

* = Reference for probe
** = Reference for polymorphism
respectively. In most patients uniparental disomy could not be ruled out or confirmed. The results of this study were limited to excluding uniparental disomy at certain loci in some patients.

5A.3.1 Parental and Child Alleles Obtained with a Taq I Southern Blot Probed with Calcitonin (11p15.4). Allele A was 10.0 kb and allele B was 9.0 kb in size. Results are shown on Table 5.6. The results were uninformative:

<table>
<thead>
<tr>
<th>Index patient</th>
<th>Mother</th>
<th>Father</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt 1</td>
<td>AB</td>
<td>-</td>
<td>AB</td>
</tr>
<tr>
<td>Pt 3</td>
<td>AB</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Pt 7</td>
<td>B</td>
<td>-</td>
<td>B</td>
</tr>
<tr>
<td>Pt 8</td>
<td>B</td>
<td>-</td>
<td>B</td>
</tr>
<tr>
<td>Pt 12</td>
<td>AB</td>
<td>AB</td>
<td>B</td>
</tr>
</tbody>
</table>

5A.3.2 Parental and Child Alleles Obtained with a Taq I Southern Blot Probed with Hypervariable Probe MS51 (11q13). Fig 5.9 shows the autoradiograph obtained on a TaqI Southern blot hybridized with MS51. Five alleles (A to E) were obtained, ranging in size from 1.3 to 4.3 kb. From Table 5.7, uniparental disomy involving the MS51 locus in
### Table 5.3: Parental and child alleles using MS51 and TaqI

<table>
<thead>
<tr>
<th>GOS 368</th>
<th>GOS 389</th>
<th>GOS 378</th>
<th>GOS 372</th>
<th>GOS 374</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td></td>
</tr>
</tbody>
</table>

### Fig 5.9: Uniparental disomy study. Autoradiograph shows MS51 probe hybridization pattern after DNA digestion with TaqI

- GOS 368 = Pt 7
- GOS 389 = Pt 3
- GOS 378 = Pt 12
- GOS 372 = Pt 1
- GOS 374 = Pt 8
11q13 can be excluded in Patient 3, who inherited B allele from the father and D allele from the mother.

Table 5.7: Parental and child alleles using MS51 and TaqI

<table>
<thead>
<tr>
<th>Index patient</th>
<th>Mother</th>
<th>Father</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt 1</td>
<td>CD</td>
<td>-</td>
<td>CD</td>
</tr>
<tr>
<td>Pt 3</td>
<td>CD</td>
<td>AB</td>
<td>BD</td>
</tr>
<tr>
<td>Pt 7</td>
<td>BC</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>Pt 8</td>
<td>AC</td>
<td>-</td>
<td>AE</td>
</tr>
<tr>
<td>Pt 12</td>
<td>CD</td>
<td>CC</td>
<td>C</td>
</tr>
</tbody>
</table>

5A.3.3 Parental and Child Alleles Obtained on a TaqI Southern Blot Probed with CAT 800 (11p13). Allele A was 3.5 kb in size and allele B had a 2.5 kb and a 1.0 kb fragments. Maternal uniparental disomy for the catalase locus is ruled out for Pt 7 as she is homozygous for allele A and therefore she must have inherited an A allele from her father. No other conclusions can be derived from the results obtained with catalase. The pattern of inheritance is not informative for the other incomplete or complete families: in the incomplete families (Pt 1 and Pt 8) the children are homozygous for alleles present in the available parent, which happens to be the mother. As the fathers' DNA is not available it is not known whether the children are homozygous because they inherited the same.
allele from each parent or only from the mother. In the complete families (Pts. 3 and 12) the child is homozygous for an allele which is shared by both parents. On the basis of these data it is not possible to ascertain whether the child has inherited a copy from each parent or only from one parent.

Table 5.8: Parental and child alleles using CAT 800 and Taq1

<table>
<thead>
<tr>
<th>Index patient</th>
<th>Mother</th>
<th>Father</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt 1</td>
<td>A</td>
<td>-</td>
<td>A</td>
</tr>
<tr>
<td>Pt 3</td>
<td>B</td>
<td>AB</td>
<td>B</td>
</tr>
<tr>
<td>Pt 7</td>
<td>AB</td>
<td>-</td>
<td>A</td>
</tr>
<tr>
<td>Pt 8</td>
<td>AB</td>
<td>-</td>
<td>AB</td>
</tr>
<tr>
<td>Pt 12</td>
<td>B</td>
<td>AB</td>
<td>B</td>
</tr>
</tbody>
</table>

In summary, uniparental disomy was ruled out at the MS51 locus (11q13) in Patient 3. Maternal disomy was excluded at the catalase locus (11p13) in Patient 7.

5A.4 Discussion of uniparental disomy study

Given that, on the basis of our study, no 11p13 deletions were found in children with DDS syndrome, we
investigated the possibility that DDS syndrome might arise instead, as a result of uniparental disomy of the whole or part of chromosome 11, in a manner analogous to the non-deletion Prader Willi patients and chromosome 15 (234).

The loci studied flanked the region of interest, namely 11p13. Unfortunately, the probes used proved to be largely uninformative in most of the patients studied. In addition, the study suffered from the high proportion of only a single parent being available for study due to parental separation where the parents were no longer in contact.

It is not possible to reach any definite conclusions regarding uniparental disomy, on the basis of our very limited results. They do, however suggest that uniparental disomy, involving the whole of chromosome 11 or a large part of it, is unlikely to be a very common occurrence in children with DDS. It must be stressed, however that in order to study this phenomenon in depth, many more patients as well as other informative 11p13 probes would have to be tried.
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5.5 Discussion

The DDS phenotype is most probably due to a genetic abnormality which could take different forms. Given that the phenotype is a complex one, involving more than one organ, it could be argued that it may be due to a deletion involving a number of closely juxtaposed genes. Alternatively, since the organs involved arise from an early common embryonic origin a defect involving a single developmental gene controlling normal differentiation of these tissues may be responsible.

The association between compound phenotypes and highly specific chromosome abnormalities have often been used to pinpoint the position of the genes responsible for the associated syndrome. Where constitutional chromosome translocations have been identified they are sometimes associated with the loss of genetic material at the breakpoint or, in the case of single disease the translocation may interrupt the disease gene. Examples of the latter include Duchenne muscular dystrophy (244), aniridia (245) and retinoblastoma (246) and the expectation is that, in many other cases, the cloning of breakpoints in predisposing translocations will yield the "culprit" gene, although this will not be possible in those cases where large deletions are associated with the rearrangement.

Chromosome deletions, often identified by association of other congenital abnormalities, also pinpoint disease loci but the variability of the position of the breakpoints
means that many overlapping deletions must be analysed. This approach has been used successfully in the analysis of retinoblastoma and Wilms' tumour (for review see ref 247), as well as in Duchenne muscular dystrophy (248).

The term "contiguous gene syndromes" was coined by Schmikel in 1986, to denote syndromes involving a number of malformations, associated with small chromosomal deletions or duplications, often not detected by high banding resolution (249). These syndromes also tend to be sporadic in nature (250). Examples include deletions of 17p13 in Miller-Dieker syndrome (MDS) (251), deletions of 22q11 in Di George syndrome (DGS) (252) and deletions of 11p13 in the WAGR complex (112). The wide phenotypic spectrum results from the variability in the size of the deletion reflecting the loss of different numbers of contiguous genes. This has clearly been shown in the case of 11p13 deletions and the WAGR complex (209,253,254).

The cytogenetic and molecular analysis of DDS patients focussed on the 11p13 region because of its known association with a predisposition, in AGR-triad patients, to Wilms' tumour and genital abnormalities, which are also features of DDS syndrome. A second embryonic tumour, gonadoblastoma may also occur in both, and at a young age (178,180,186,255).

We have used a series of DNA sequences spaced regularly throughout the 11p13 region but could not demonstrate any heterozygous chromosome deletions in the children studied, with the exception of Pt 11, who has a microscopically
visible chromosome deletion involving 11p12-11p13.

Pt 11 had Wilms' tumour and nephropathy together with two other features of the AGR triad – aniridia and mental retardation. Although a formal endocrinological assessment or gonadal histology have not been performed, she is unlikely to have a major gonadal disorder as she has undergone normal female pubertal changes. As discussed in chapter 4, her nephropathy may have been due partly to her Wilms' tumour treatment. However, the non-tumorous area showed changes of mesangial sclerosis, as seen in the other DDS children. In spite of the occurrence of a nephropathy in this child, our failure to demonstrate microdeletions in any of the other children studied, would suggest that the locus for DDS syndrome is unlikely to be on the 11p13 region.

Of course, in the absence of a candidate gene small chromosome deletions confined to that region would not be detected by this method. Clearly if mutations are restricted to the gene responsible it will be difficult to identify. However, a systematic survey in 11p13 was worthwhile since even the smallest deletions often include adjacent regions as seen in children with WAGR.

Because of the severe disorder in sexual differentiation seen in most DDS children, which might implicate the sex chromosomes in the aetiology of this condition, selected loci on the X chromosome were studied. Both kidneys and internal genitalia derive from the urogenital ridge and it is likely that this differentiation
Chapter 5: Molecular Genetic Analysis

process is under genetic control arising from one or both sex chromosomes and that in this condition, one or more of these "developmental" genes may be either deleted or abnormally expressed. The report of a Duchenne phenotype in a patient with the type of nephropathy seen in DDS syndrome, but no other features of the triad (199), possibly implicated the Xp21/22 region as a potential site for genetic mutation. None of the 3 probes used were deleted. Since carrying out this study ZFY (225), the putative sex determining gene has been superseded as the Testis Determining Factor by the finding of SRY, a highly conserved sequence in the Y chromosome (256).

5.6 Conclusion

A molecular analysis of chromosome region 11p13 was carried out in patients with DDS syndrome. The studies performed showed that there are no submicroscopic deletions within this region. The possible involvement of this area however, cannot be completely ruled out as the techniques used in this study would not detect very small rearrangements or point mutations.

Postscript

Since finishing this work a candidate Wilm's tumour gene (WT1) was isolated (257,258). Shortly afterwards, Pritchards-Jones et al found that its expression was highly specific to renal and gonadal tissue in the developing fetus (259). The exon/intron structure of WT1 was
determined by Haber et al (260). This together with the development of Single-Stranded Conformational Polymorphism Analysis (261), a new technique to look for single base pair mutations, enabled Pelletier et al to demonstrate point mutations in exons 8 and 9 of the Wilm's tumour gene in an elegant study of 10 children with DDS (262). Using these techniques, the presence of similar mutations has since been confirmed in patients 1, 3, 7 and 12 (263).
6. CONCLUSION

6.1 Clinical Features of CNS

This thesis began with a review of the clinical features of children who developed the nephrotic syndrome in the first year of life. Problems common to all cases, arising directly from their nephrotic state, were described. These included infections, hypovolaemia, thromboembolic phenomena and metabolic disturbances. The high incidence of familial cases, a feature common to all types, was also emphasised.

Although children with CNSF and DMS had the worst outcome, the prognosis was also related to the age at presentation. Those children who presented below 3 months of age had the most severe problems, did not respond to drug therapy, and had the highest mortality rate, irrespective of the underlying histology.

At the same time, this review of children with CNS showed the condition to have several heterogeneous features. Clinico-histopathological correlations were more clearly established in those presenting over 3 months of age, with renal outcome and survival, as well as response to drug therapy, being more specifically linked to histological type.

The range of associated congenital abnormalities was striking. These occurred particularly with DMS, but were seen in all other types with the exception of CNSF. Children with DMS stood out as having a more complicated
clinical course due to their inexorable progress into early ESRF and the common association with congenital abnormalities as in the Denys-Drash syndrome.

6.2 Histological Review

The pitfalls of the present classification of idiopathic CNS, which is largely based on histology, were discussed in Chapter 2. Biopsies taken early in CNS are sometimes reported as showing minimal change histology. The clinical presentation and type of clinical problems seen in children presenting within the first 3 months of life are common to all histological types. Moreover, on the basis of this review, even features which have been considered specific to a particular type of nephrotic syndrome, such as a big placenta or raised α-fetoprotein in CNSF, are occasionally seen in other types.

6.3 Pathogenesis of Proteinuria

The present theories on the pathogenesis of proteinuria were discussed with particular reference to the work of Vernier et al (108) and Vermylen et al (110) on the CNS. They produced evidence of loss of anionic charge, in the form of heparan sulphate, from the GBM of affected children. This work provided the background for the study on urinary glycosaminoglycan excretion described in Chapter 3. The ratio of heparan sulphate to chondroitin sulphate was shown to be increased in the urine of children with CNS. However, the phenomenon was not specific to any
Chapter 6: Conclusions

particular type of CNS but was related to the degree of proteinuria.

These observations raised the question of whether the loss of heparan sulphate preceded the development of proteinuria, or was a secondary phenomenon. Further work is needed on the synthesis and degradation of plasma and GBM heparan sulphate and other glycosaminoglycans, and on how these change in states of heavy proteinuria.

6.4 Associated Congenital Abnormalities

The detailed study of children with more complicated phenotypes has added to our awareness of the clinical spectrum of CNS. Children were identified who had presented with similar features. Some of these associations had not been reported previously and might constitute new syndromes. These include FSGS, ventricular septal defect and pelvicalyceal abnormalities, and also the association of a nephropathy with the ultrastructural features of Alport's syndrome with a severe dystrophic form of epidermolysis bullosa and pulmonary hypertension.

The newly reported pelvicalyceal abnormalities, observed in the majority of children with Denys-Drash syndrome, should perhaps be regarded as the fourth feature of this syndrome. Interestingly, these abnormalities are similar to those found on the 2 children with FSGS and VSD. The nature of these defects is not known. They are unlikely to be a consequence of heavy proteinuria as they were found in some children with Denys-Drash syndrome with only mild
proteinuria, and not at all in children with CNSF.

Descriptions of children with CNS and microcephaly (Galloway syndrome), as well as of one child with CNS and retinal dystrophy, add to those of the few children with similar associations that have been already reported in the literature.

6.5 Molecular Genetic Analysis

The value of highlighting the syndromic forms of CNS is manyfold. Firstly, it is useful in alerting the paediatric nephrologist to look for abnormalities in other systems. This is particularly relevant in the case of Denys-Drash syndrome with its associated risk of Wilms' tumour and the possibility of gonadal abnormalities, especially in the phenotypic female infant with DMS, who may turn out to have a male karyotype.

Secondly, analysis of these patients' karyotypes may provide the first clues as to where the genetic defect associated with these early forms of nephrotic syndrome is located.

The association of the clinically overlapping WAGR syndrome with an 11p13 deletion was the basis for studying this particular region in children with Denys-Drash syndrome. However, with the exception of one case, we did not show any such deletions in affected children with the techniques used. The observations made at the time were that point mutations would not have been detected and that perhaps, in addition, a more extensive study was needed to
look at the possibility of uniparental disomy. As discussed in Chapter 5, this phenomenon has been described in a number of non-familial syndromes.

The discovery that children with Denys-Drash syndrome have point mutations of the WT1 gene opens up further areas for research. The classic renal histological abnormality in this syndrome is DMS. It would be of great interest to study patients affected with the idiopathic and other syndromic forms of DMS looking for abnormalities in this important gene. Similarly, it would be of value to study patients with ambiguous genitalia of unknown aetiology, given that this gene appears to play an important role in sexual differentiation. Defining the clinical phenotypes that arise as a result of mutations in this gene will help to elucidate further its exact developmental role.

Recent years have seen a major improvement in the survival of children with early-onset nephrotic syndrome. This has resulted from the advances made in the programmes for dialysis and transplantation in very young children. However, there is still very little knowledge of the molecular genetics of these diseases and the exact pathophysiology of the proteinuria has not yet been fully unravelled. Understanding these mechanisms would also help our understanding of the normal kidney and the functioning of the healthy GBM. Knowledge of the molecular genetics would also be invaluable in giving accurate prenatal diagnosis and genetic counselling to the families of affected children.
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Molecular analysis of chromosome region 11p13 in patients with Drash syndrome

L. Jadresic¹, R.B. Wadey², B. Buckle³, T.M. Barratt¹, C.D. Mitchell², and J.K. Cowell²

¹Department of Paediatric Nephrology and ²ICRF Laboratory of Molecular Genetics, Department of Haematology/Oncology, Institute of Child Health, 30 Guilford Street, London WC1N 3EH, UK

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Summary. The association of nephropathy, Wilms' tumour and genital abnormalities is known as Drash syndrome. Two of these features are also seen in the WAGR (Wilms' tumour, aniridia, genito-urinary abnormalities, mental retardation) complex, known to be associated with deletions of chromosome region 11p13. We have carried out karyotypic and molecular studies in 10 Drash patients, 5 males and 5 females. All the males had a 46XY karyotype as did 3/5 of the phenotypic females, the other two having a 46XX karyotype. One of the 46XX females also had a deletion of region 11p13-p12, the only detectable autosomal chromosome abnormality in any of the patients studied. Lymphoblastoid cell lines were prepared from 6 of the Drash patients and were used in dosage studies using a variety of DNA probes from the 11p13 region. There was no evidence of microdeletions in any patient with a normal karyotype. Because of the 46XY karyotype in phenotypic females, selected X and Y chromosome loci were analysed and all found to be normal. Although Drash syndrome is likely to be of genetic origin, there are no readily detected deletions within the 11p13 region.

Introduction

The clinical triad comprising a nephropathy, Wilms' tumour and genital abnormalities is known by the eponym of Drash syndrome (Drash et al. 1970). The first report of this rare syndrome was published in 1967 (Denys et al. 1967). Since then, over 60 cases have been described, mostly as isolated case reports. Although some children exhibit all three features of the syndrome, others only have two. The nephropathy is the common denominator (Habib et al. 1985), occurring with either Wilms' tumour or genital abnormalities or both. Wilms' tumour may be associated with urogenital abnormalities (Angstrom 1965; Bond 1977; Miller et al. 1964; Riccardi et al. 1978). Children showing this association but without a nephropathy are not generally considered to be examples of the Drash syndrome (Habib et al. 1985; Manivel et al. 1987).

There are well-defined features to each component of the Drash triad. The nephropathy is characterised by the early onset of proteinuria, which, in most cases, is sufficiently severe to cause nephrotic syndrome. There is early impairment of renal function, which progresses to end-stage renal failure, commonly before the age of 3 years. Histology of the kidneys shows varying degrees of focal or diffuse mesangial sclerosis. Deposition of fibrillar material in the cytoplasm leads to mesangial cell expansion. The severity of tubulo-interstitial damage corresponds to the degree of glomerular changes (Habib et al. 1985; Gallo and Chemes 1987; Jadresic et al. 1990). There is also non-specific deposition of immunoreactants. Drash patients who develop Wilms' tumour usually have bilateral tumours and present earlier (mean age of approximately 18 months; Gallo and Chemes 1987; Manivel et al. 1987; Jadresic et al. 1990) than sporadic patients with Wilms' tumour (median age 45 months; Breslow and Beckwith 1982). Tumour histology almost invariably demonstrates a triphasic appearance (epithelium/fibroblastic/blastemal) of the favourable type. The genital abnormality most often described is male pseudohermaphroditism with children having either ambiguous external genitalia or a normal female phenotype with an XY karyotype. Other forms of genital abnormalities have been described including true hermaphroditism (Edidin 1985; Eddy and Mauer 1985a,b) and hyperplastic (Fisher et al. 1983) or streak gonads. In some cases, children have developed gonadoblastomas at a young age (Eddy and Mauer 1985a,b; Fisher et al. 1983; Frasier et al. 1964), an observation also made in children with chromosome deletions known to predispose to Wilms' tumour (Andersen et al. 1978).

Predisposition to Wilms' tumour and the occurrence of genital abnormalities in Drash patients suggests a genetic defect of the type reported for patients with deletions on the short arm of chromosome 11 (Riccardi et al. 1978), a location usually associated with aniridia, genitourinary abnormalities, mental retardation and Wilms'
tumour, the so-called WAGR complex (Ricardi et al. 1978; Narahara et al. 1984; Cowell 1990). Chromosome analysis in these cases has demonstrated consistent loss of a subregion of band 11p13 (Van Heyningen et al. 1985; Cowell et al. 1989; Coullin et al. 1989). Although only 50% of AGR patients develop Wilms' tumour (Narahara et al. 1984), this consistent constitutional chromosome abnormality implicates region 11p13 as the site of a Wilms' tumour predisposition gene. One neonate, with Potter's facies (Potter 1946) and kidney agenesis, was shown to have a constitutional chromosome translocation with one breakpoint in 11p13 (Porteous et al. 1987). Molecular studies have shown that this breakpoint in distinct from the Wilms' tumour locus in that region (Compton et al. 1988) but, if it is related to the kidney agenesis, this implies the presence of another gene important in kidney development. These observations suggest that there is a "cluster" of genes in 11p13 that are important in both kidney and genitourinary development. We therefore chose to use molecular probes from region 11p13, to determine whether deletions/rearrangements could be detected in this region in patients with Drash syndrome.

Materials and methods

Cytogenetic analysis

Chromosomes were prepared, using standard air-drying techniques, from either phytohaemagglutinin-stimulated peripheral blood lymphocytes or lymphoblastoid cell lines established using Epstein-Barr virus. In either case, cells were treated with vinblastine sulphate (0.01 mg/ml) for 15 min prior to harvesting followed by a hypotonic shock of 0.07 M KCl for 10 min. Fixation was in methanol:acetic acid (3:1). Chromosome preparations were allowed to age for 7 days before analysis by a modified trypsin-Giemsa banding protocol (Cowell 1980).

DNA extraction and analysis

DNA was extracted from lymphoblastoid cell lines using conventional phenol/chloroform procedures (Maniatis et al. 1982) and digested with the appropriate restriction enzymes using the conditions described by the manufacturers (Anglian Biotech, Colchester, UK). DNA was transferred to Hybond-N membranes (Amersham International, Aylesbury, UK) as described by Southern (1975). DNA probes were labelled to high-specific activity using 32P-CTP in the oligo-primer extension procedure described by Feinberg and Vogelstein (1983). Probes were removed from the filters by washing in 50% formamide, 10 mM phosphate, pH 7.5, for 30 min at 65°C.

Scanning densitometry

Densitometry profiles were obtained using an Ultrascan XL (Pharmacia LKB Biotechnology, Uppsala, Sweden) with a fixed wavelength of 633 nm. The X-width was set to 10 giving a scanning beam width of 8 mm, effectively integrating absorbance over the width of the band on the autoradiograph. The band intensities were measured on the autoradiograph probed first with the control DNA sequence; the intensity of the bands on autoradiographs probed with 11p13 probes were expressed as a ratio of the corresponding control value.

Results

Patients

The 10 patients studied in this survey form part of a group of 12 children with Drash syndrome seen at the Hospital for Sick Children, London, between the years 1972 and 1987. They were selected because it had been possible to analyse karyotypes in constitutional cells. Their clinical, radiological and histological features have been described elsewhere (Jadresic et al. 1990).

Genetic and cytogenetic analysis

A summary of the karyotypic and phenotypic data for the 10 Drash patients is given in Table 1. Of the 10 patients, 8 had a normal 46XY karyotype, but only one (GOS 378) had normal male genitalia. Four patients had ambiguous genitalia and 3 were phenotypically female. Only 2 patients (GOS 400 and LJ4) had a 46XX karyotype and apparently normal external genitalia.

At autopsy, however, LJ4 was shown to have a streak gonad and both Mullerian and Wolffian duct structures. There is no information regarding the internal genitalia for GOS 400. She has an interstitial deletion, (11) (p12;p13) (Fig. 1). She also has aniridia, mental retardation and is short in stature.

Molecular analysis

Although cytogenetic analysis excluded chromosome translocations or large deletions in 9/10 patients, it was still possible that more subtle chromosome deletions

![Fig. 1. Trypsin-Giemsa banded chromosome 11 pair from patient GOS 400 showing del (11) (p12;p13)](image)

Table 1. Genetic and cytogenetic characteristics of Drash patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Neophropy</th>
<th>WT</th>
<th>GA</th>
<th>OA</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOS 400</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>MR, AN</td>
<td>46XXdel(11)(p12p13)</td>
</tr>
<tr>
<td>GOS 368</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td>46XY</td>
</tr>
<tr>
<td>GOS 374</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>MR, CP</td>
<td>46XY</td>
</tr>
<tr>
<td>GOS 372</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>46XY</td>
</tr>
<tr>
<td>GOS 378</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>46XY</td>
</tr>
<tr>
<td>GOS 389</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>46XY</td>
</tr>
<tr>
<td>LJ1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>DF</td>
<td>46XY</td>
</tr>
<tr>
<td>LJ2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td>46XY</td>
</tr>
<tr>
<td>LJ3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>46XY</td>
</tr>
<tr>
<td>LJ4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>46XY</td>
</tr>
</tbody>
</table>
The intensity of the band on the autoradiographs was then stripped and reprobed with other llp l3 probes. Loading of the lanes was demonstrated (Fig. 3). This blot sequence, pl23M1.8, derived from chromosome 13 (T. P. Dryja, personal communication), the relatively even absorbance was more accurate if measurements were taken after digestion of the DNA with the appropriate restriction enzyme. This procedure largely overcame quantitation problems associated with incompletely dissolved DNA samples and pipetting errors arising because of the high molecular weight of the DNA. In all experiments, we used 10 μg DNA per lane.

Southern blots included the following control samples: 1) a patient with no evidence of any phenotypic chromosomal abnormality, 2) a patient (GOS 157) with a large 11p deletion (Cowell et al. 1989) spanning the region covered by the 11p13 probes. Lymphoblastoid cell lines from 6 Drash patients were available for this study. The p9RH0.65 probe is a unique DNA sequence located at the 11p-breakpoint in a T-ALL patient described by Boehm et al. (1988). A typical result using this probe is shown in Fig. 3. As expected, the signal intensity from lanes 2 and 10, which contained DNA from deletion patient GOS 157, was clearly lower than in the control lanes. When the same blot was reprobed with a control sequence, p123M1.8, derived from chromosome 13 (T. P. Dryja, personal communication), the relatively even loading of the lanes was demonstrated (Fig. 3). This blot was then stripped and reprobed with other 11p13 probes. The intensity of the band on the autoradiographs was measured using scanning laser densitometry, and the signal produced in DNA from Drash patients was compared with controls on the same filter. The overall ratios for each probe in each patient is given in Table 2. Only GOS 400, who carried a visible 11p13 deletion, showed any reduction in relative band intensity for any of the probes. Thus, none of the other Drash patients could be shown to have deletions within the 11p13 region. There was one equivocal result; using the FSHB probe, a ratio of 0.67 was seen in GOS 374. Since the ratio for the deletion control in this experiment was also low, this result is probably normal. In patient GOS 400, normal band intensities were found with FSHB and JM115, but only 50% levels were observed with p56, thus defining the distal breakpoint between these probes (see Fig. 2).

### X chromosome probes

Because of the XY phenotype in our patients, we studied selected loci on the X chromosome. The report of a Duchenne phenotype in a patient with the type of nephropathy seen in Drash syndrome, but no other features of the triad (Habib et al. 1988), implicated the Xp21/22 region as a potential site for a genetic mutation. The presence/dosage of Xp22 probes XUT and 754, which lie distal to the DMD locus, did not identify deletions in any of the XY or XX patients (data not shown). We also used the cDNA probe, ZFY, until recently a candidate gene for the testis determining factor (Palmer et al. 1989). This gene probe recognises homologous sequences on both the X and Y chromosomes, each of which gives a characteristic banding pattern on Southern blots following autoradiography. No rearrangements or deletions of the gene were detected.

### Discussion

The association between compound phenotypes and highly specific chromosome abnormalities has often been used to pinpoint the position of the genes responsible for the associated syndrome. Where constitutional chromosome translocations have been identified, they sometimes interrupt the disease gene. Examples of this principle in-

---

**Table 2.** Summary of ratios of absorbance between a normal control and 6 Drash patients for various probes from region 11p13

<table>
<thead>
<tr>
<th>Probe</th>
<th>GOS 157</th>
<th>GOS 400</th>
<th>GOS 368</th>
<th>GOS 374</th>
<th>GOS 372</th>
<th>GOS 378</th>
<th>GOS 389</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSHB</td>
<td>0.41</td>
<td>0.96</td>
<td>1.04</td>
<td>0.67</td>
<td>1.06</td>
<td>1.02</td>
<td>0.95</td>
<td>0.90</td>
</tr>
<tr>
<td>JM115</td>
<td>0.49</td>
<td>1.29</td>
<td>1.01</td>
<td>1.25</td>
<td>1.43</td>
<td>1.7</td>
<td>1.5</td>
<td>1.74</td>
</tr>
<tr>
<td>p56H2.4</td>
<td>0.27</td>
<td>0.65</td>
<td>0.91</td>
<td>0.93</td>
<td>1.07</td>
<td>0.89</td>
<td>0.89</td>
<td>1.1</td>
</tr>
<tr>
<td>D11S87</td>
<td>0.40</td>
<td>0.30</td>
<td>0.90</td>
<td>1.07</td>
<td>0.95</td>
<td>0.97</td>
<td>1.22</td>
<td>0.85</td>
</tr>
<tr>
<td>p9RH0.65</td>
<td>0.36</td>
<td>0.40</td>
<td>0.84</td>
<td>1.08</td>
<td>1.00</td>
<td>1.08</td>
<td>1.4</td>
<td>0.91</td>
</tr>
<tr>
<td>CAT980</td>
<td>0.42</td>
<td>1.26</td>
<td>1.26</td>
<td>1.00</td>
<td>1.02</td>
<td>0.85</td>
<td>0.96</td>
<td>0.96</td>
</tr>
</tbody>
</table>

---

**Fig. 2.** Diagrammatic illustration of the relative order of the 11p13 DNA sequences used in DNA quantitation studies.

**Fig. 3.** Comparison between hybrid band intensities in Drash patients and control samples following hybridisation with the chromosome 13 probe p123M1.8 (right) and the 11p13 probe p9RH0.65 (left). Reduced hybridisation can be seen in control sample GOS 157 and in Drash patient GOS 400 in the left autoradiograph.
clude Duchenne muscular dystrophy (Ray et al. 1985), aniridia (Moore et al. 1986) and retinoblastoma (Mitchell and Cowell 1989); the expectation is that, in many other cases, the cloning of breakpoints in predisposing translocations will yield the “culprit” gene, although this is not possible in those cases where large deletions are associated with the rearrangement. Chromosome deletions, often identified by association of other congenital abnormalities, also pinpoint disease loci, but the variability of the position of the breakpoints means that many overlapping deletions must be analysed. This approach has been used successfully in the analysis of retinoblastoma, Wilms’ tumour, Duchenne muscular dystrophy and Prader-Willi syndrome.

Our cytogenetic and molecular analysis of Drash patients focussed on the 11p13 region because of its known association with a predisposition, in AGR-triad patients, to Wilms’ tumour, which is also a feature of Drash syndrome. Analysis of retinoblastoma tumours (Friend et al. 1986) has given rise to the expectation that small homozygous deletions that include the critical gene will occur in tumour cells at a detectable frequency. The search for the Wilms’ tumour predisposition gene has generated a variety of probes from region 11p13, only one of which (D11S87) has, so far, shown evidence of homozygous deletion in a tumour (Lewis et al. 1988) indicating its proximity to the predisposition gene. We have used a series of DNA sequences spaced regularly throughout the 11p13 region, but have not been able to demonstrate any heterozygous chromosome deletions. Of course, in the absence of a candidate gene, small chromosome deletions confined to that region would not be detected by this method. Loss of heterozygosity has also been an indication of the site of recessive cancer predisposition genes (Sager 1989). Tumours from two of our Drash patients (GOS 378 and GOS 389), however, did not show allele loss on the short arm of chromosome 11 (Wadey et al. 1990). Recently, a candidate Wilms’ tumour gene, WT33, has been isolated from 11p13 (Call et al. 1990). Using an almost complete length cDNA probe (a kind gift of Dr. D. Housman), we did not find any structural rearrangements of this gene in our Drash patients or their tumours, but, because of the complex hybridisation pattern of the gene in enzyme restricted DNA, we could not evaluate whether partial internal heterozygous deletions were present. Careful sequencing of this gene in tumour and normal tissue should clarify this problem.

The contention that 11p13 may not be the only site of genes important in the development of Wilms’ tumour arises from the analysis of loss of heterozygosity in tumour cells (Reeve et al. 1989; Mannens et al. 1988; Wadey et al. 1990) and family linkage data (Huff et al. 1988; Grundy et al. 1988) where linkage with 11p13 probes was positively excluded.

One of our patients has a deletion involving 11p12–11p13. She had Wilms’ tumour and nephropathy together with two other features of the AGR triad, VIZ, aniridia and mental retardation. She developed proteinuria in 2 years of age and reached end-stage renal failure at 6.5 years, somewhat later than the mean of 2.5 years for the other children in this study. Following presentation with bilateral Wilms’ tumour at the age of 1 year, she had a short course of vincristine, adriamycin and actinomycin-D and radiotherapy with shielding of the non-involved right upper pole. Six weeks later, she underwent a left nephrectomy and partial right nephrectomy and her non-tumorous area showed changes of mesangial sclerosis, as seen in the other Drash children. In spite of the occurrence of a nephropathy in this patient, our failure to demonstrate microdeletions in any of the other children studied suggests that the locus for Drash syndrome may not be on the 11p13 region.

To date, there has been only one report of Drash syndrome involving siblings. Zunin and Soave (1964) described 2 sisters who developed Wilms’ tumour and the nephrotic syndrome. In one of them, the nephropathy appeared to recover, which makes it unlikely to have been the nephropathy as seen in Drash syndrome, which progresses relentlessly into end-stage renal failure. In the absence of a familial incidence, it could be argued that Drash syndrome could arise as a result of a teratogenic agent. However, no environmental agents have ever been implicated in the aetiology of this syndrome.

Another factor in favour of an underlying genetic cause of this disease is the overwhelming preponderance (8/10 in this series) of patients with XY karyotypes. There is a close association, during embryogenesis, between the development of the reproductive and urinary systems, both arising from the mesonephros. It is likely that this differentiation process is under genetic control arising from one or both sex chromosomes and that, in this condition, one or more of these “developmental” genes may be either deleted or abnormally expressed.

When the genes responsible for Wilms’ tumour and sexual differentiation are identified, it will be of great interest to investigate their possible role in the formation of the complex phenotype seen in this syndrome.

Acknowledgements. We would like to thank Dr. L. Butler who performed the initial karyotypes of several of the patients reported here. We are grateful to Dr. V. van Heyningen for providing the JM115 probe, Dr. V. Huff for p56H24.1, and Dr. P. Goodfellow for ZDY. Our special thanks go to Dr. J. Pritchard for his critical reading of the manuscript. L. J. was supported by a grant from the Kidney Research Aid Foundation.

References


Autosomal dominant retinitis pigmentosa: a new multi-allelic marker (D3S621) genetically linked to the disease locus (RP4)

Rajendra Kumar-Singh, Daniel G. Bradley, G. Jane Farrar, Mark Lawler, Siobhan A. Jordan, and Peter Humphries
Department of Genetics, Trinity College, Dublin 2, Ireland

Summary. We report the characterization of a new eight-allele microsatellite (D3S621) isolated from a human chromosome 3 library. Two-point and multi-locus genetic linkage analysis have shown D3S621 to co-segregate with the previously mapped RP4 chromosome, including D3S14 (R208) (θm = 0.12, Zm = 4.34) and with other genetic markers on the long arm of the chromosome, including D3S14 (R208) (θm = 0.00, Zm = 15.10), D3S47 (C17) (θm = 0.11, Zm = 4.95), Rh0 (θm = 0.07, Zm = 1.37), D3S21 (L182) (θm = 0.07, Zm = 2.40) and D3S19 (U1) (θm = 0.13, Zm = 2.78). This highly informative marker, with a polymorphic information content of 0.78, should be of considerable value in the extension of linkage data for autosomal dominant retinitis pigmentosa with respect to loci on the long arm of chromosome 3.

Materials and methods

A hamster/human chromosome 3 library (LA03NS02) inserted in the vector Charon 21A was obtained from the American Type Culture Collection. This library was amplified, and plaques lifted from nylon membranes (Hybond-N) were hybridized with synthetic poly(dA-dC).poly(dG-dT) (Pharmacia) that had been nick-translated with α32P-dCTP as described previously (Litt and Luty 1989). Filters were autoradiographed for 1 day and then rehybridised to nick-translated human genomic DNA. DNA from reactive plaques was digested with the restriction nuclease Sau3A and ligated into the BamHI site of the vector pUC 19. Poly(dA-dC).poly(dG-dT)-positive clones in E. coli TG1 were sequenced using the T7 DNA polymerase sequencing system (Promega Corporation) and oligodeoxynucleotide primers (20 nt) were generated to regions flanking the CAGT repeat (Fig. 1) using an Applied Biosystems 391 DNA synthesizer.

DNA was prepared from peripheral blood as described elsewhere (Humphries et al. 1987). Polymerase chain reactions were carried out in a 25 μl volume containing 200 ng genomic DNA template, 50 pmol each oligodeoxynucleotide primer, 200 μM each dATP, dTTP, and dGTP, 2.5 μM dCTP, 1 μCi α32P-dCTP, 10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 1.25 mM MgCl2 and 0.75 units of Taq polymerase (Perkin Elmer Cetus). Samples were overlaid with 7 μl paraffin oil and were heated at 94°C for 3 min; they were then processed through 39 temperature cycles consisting of 20 s at 94°C (denaturation), 20 s at 55°C (annealing), and 30 s at 72°C (elongation) on a programmable DNA Thermal Cycler (Perkin Elmer Cetus). Aliquots (1–2 μl) of the amplified DNA were mixed with 3 μl formamide buffer and were electrophoresed on 8% denaturing polyacrylamide gels. Gels were dried and autoradiographed for 12–15 h.

TCMD1 and TCDG1 are two large pedigrees of Irish origin that segregate with ADRP; they have been described previously (McWilliam et al. 1989; Bradley et al. 1989; Farrar et al. 1990a). Members of TCDM1 and TCDG1 exhibit type 1 and type 2 ADRP, respectively.
Urine glycosaminoglycans in congenital and acquired nephrotic syndrome

LYDA P. JADRESIC, GUIDO FILLER, and T. MARTIN BARRATT

Institute of Child Health, University of London, London, England, United Kingdom

Research on the pathogenesis of proteinuria in renal disease is focused on alterations in the charge and size filtration characteristics of the glomerular basement membrane (GBM) [4]. Much of this work has centered around the components of the GBM that are responsible for its charge [7-12]. Heparan sulphate (HS), a proteoglycan found predominantly in the lamina rarae of the GBM [11], is the major component of the anionic charge in the GBM [4, 9, 11]. There is also evidence that other anionic groups, such as carboxyl residues added to GBM glycoproteins, are important [10]. The contribution to the charge barrier of other glycoproteins such as dermatan sulphate (CS) is less clear [7-9].

Vernier et al reported that the concentration of anionic sites on the GBM was decreased in children with congenital nephrotic syndrome (CNS) [4]. In their study the cationic probe polyethylenimine (PEI) was used to identify anionic sites, and normal glomeruli tissue was incubated with heparitinase to demonstrate at HS was indeed the major anionic group labelled by PEI in the GBM. Vermylen et al [5] found a marked reduction in the GBM content of HS in a child with CNS histologically characterized as diffuse mesangial sclerosis (DMS). They also found an increased excretion of HS relative to CS in the urine of this case and three other children with CNS, two of whom had Finnish-type histology (CNSF) and the third DMS. They suggested that the basic defect was a failure to incorporate HS into the macromolecular structure of the GBM with subsequent loss of HS into the urine.

The purpose of the present study was to investigate HS and CS excretion in children with various forms of CNS together with age-matched controls and children with later onset, acquired nephrotic syndrome. In particular, we wanted to find out whether an increased urine HS/CS ratio was specific for a particular form of CNS.

Methods

Patients

Twenty-three children with early onset NS were studied: seven with CNSF, seven with DMS, nine children with focal glomerulosclerosis (FSGS), three of whom were under six months of age and all steroid resistant. None were on steroids at the time of study. Fourteen children with acquired steroid-sensitive nephrotic syndrome (SSNS) were studied, 10 of whom were receiving steroids at the time of study, at doses ranging from 0.05 mg/kg alternate day to 2 mg/kg/day. Eight had biopsy evidence confirming minimal change histology. The controls consisted of 17 normal children who either attended the hospital crèche or were the children of laboratory staff.

Methods

Random urine samples were collected with merthiolate 1:10,000 as preservative, coded, and stored at −70°C until analyzed. The code was not broken until the end of the study.

Urine glycosaminoglycans (GAGs) were isolated with the cationic dye Alcian Blue as described by Whitman [13]. Alcian Blue in a 0.05% solution in 50 mM sodium acetate buffer pH 5.8 with 50 mM magnesium chloride has been shown to precipitate GAGs specifically [13]. The Alcian Blue was used from Imperial Chemical Industries Ltd. Two milliliters of centrifuged urine was added to 20 ml of the Alcian Blue solution. The resulting GAG precipitate was dissociated with 0.2 ml 4 M sodium chloride solution and 0.1 ml methanol. The Alcian Blue was denatured by alkalinizing the mixture with 0.1 ml 0.1 M sodium carbonate and 0.4 ml water, and separated by centrifugation. The GAGs were precipitated from the remaining mixture by the addition of 3 volumes of ethanol and subsequent centrifugation. The supernatant was discarded, the GAGs were dried overnight at room temperature, and dissolved in 20 μl water. Aliquots of 0.5 or 1.0 μl were applied to a cellulose acetate strip adjacent to
Table 1. Urinary GAG excretion in normal controls

<table>
<thead>
<tr>
<th>Controls (N = 17)</th>
<th>Age at study years</th>
<th>UA/UC</th>
<th>HS/UC</th>
<th>CS/UC</th>
<th>HS/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian mean</td>
<td>1.9</td>
<td>0.04</td>
<td>0.08</td>
<td>0.23</td>
<td>0.36</td>
</tr>
<tr>
<td>Served range</td>
<td>0.4-9.1</td>
<td>0.00-0.10</td>
<td>0.03-0.32</td>
<td>0.06-0.88</td>
<td>0.21-0.68</td>
</tr>
</tbody>
</table>

The urinary heparan sulphate/chondroitin sulphate ratio in normal children—no significant relationship with age ($r = 0.39, P > 0.1$).

**Results**

The urinary heparan sulphate/creatinine (HS/Cr) ratio (Table 1) in the 17 control children had a median of 0.08, observed range 0.03 to 0.32 mg/mg, and the chondroitin sulphate/creatinine (CS/Cr) ratio had a median 0.23, range 0.06 to 0.88 mg/mg. The urinary HS/CS ratio had a median of 0.36, range 0.21 to 0.68 mg/mg. Both HS/Cr and CS/Cr decreased with age, but HS/CS did not ($r = 0.39, P > 0.1$) (Fig. 1).

The HS/CS ratios (Table 2) in the CNSF, DMS and FSGS groups were all significantly greater than the control values, but that for SSNS was not (CNSF median: 0.80, range 0.43 to 1.28, $P < 0.001$; DMS median: 0.81, range 0.49 to 1.13, $P < 0.0005$; FSGS median: 0.66, range 0.38 to 1.6, $P < 0.01$; SSNS median: 0.44, range 0.28 to 0.70, $P > 0.05$). There was no difference in the HS/CS ratio within the SSNS group, between those children with confirmed MCNS histology and those without histology (Table 2, Fig. 2).

The HS/Cr ratio in both CNSF and DMS was greater than in controls, although it only reached significance level in the CNSF group ($P < 0.005$). However, there was no significant difference between the CS/Cr ratio in the controls and the CNSF, DMS and FSGS groups, indicating that the raised HS/CS ratio is due to an elevation in urine HS concentration rather than a decrease in the CS concentration (Table 2).

The HS/CS ratio in the CNSF, DMS, FSGS and SSNS groups was strongly correlated with the urine Alb/Cr ($r = 0.66, P < 0.0001$) (Fig. 3). However, Alb/Cr in the SSNS group was significantly lower than that in the CNSF, DMS or FSGS groups ($P < 0.001$), but the correlation between HS/CS and Alb/Cr persisted even when SSNS patients were excluded from the regression analysis ($r = 0.45, P < 0.05$).

There was no correlation between HS/CS and plasma creatinine concentration, nor were there any differences in plasma creatinine concentration between any of the nephrotic subgroups studied.

**Discussion**

The observation of Vermyleen et al [5] of increased HS excretion in the urine of four children with CNS was of interest.
**Table 2. Urinary GAG excretion in CNSF, DMS, FSGS and SSNS**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Sex</th>
<th>Histology</th>
<th>Age at diagnosis</th>
<th>Age at study</th>
<th>PCR μmol/liter</th>
<th>UA/UC</th>
<th>HS/CR</th>
<th>CS/CR</th>
<th>HS/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>years</td>
<td></td>
<td></td>
<td>µg/mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>CNSF</td>
<td>&lt;0.1</td>
<td>0.9</td>
<td>141</td>
<td>89.0</td>
<td>0.33</td>
<td>0.26</td>
<td>1.30</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>CNSF</td>
<td>&lt;0.1</td>
<td>1.8</td>
<td>42</td>
<td>158.1</td>
<td>0.06</td>
<td>0.06</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>CNSF</td>
<td>&lt;0.1</td>
<td>0.8</td>
<td>NA</td>
<td>19.7</td>
<td>0.25</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>CNSF</td>
<td>&lt;0.1</td>
<td>1.09</td>
<td>NA</td>
<td>27.4</td>
<td>0.10</td>
<td>0.10</td>
<td>0.81</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>CNSF</td>
<td>&lt;0.1</td>
<td>0.32</td>
<td>NA</td>
<td>57.9</td>
<td>0.13</td>
<td>0.13</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>CNSF</td>
<td>&lt;0.1</td>
<td>0.11</td>
<td>40</td>
<td>1.5</td>
<td>0.37</td>
<td>0.95</td>
<td>0.47</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>CNSF</td>
<td>&lt;0.1</td>
<td>0.35</td>
<td>35</td>
<td>11.7</td>
<td>0.25</td>
<td>0.32</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>median</td>
<td>0.1</td>
<td>0.80</td>
<td>40</td>
<td>27.4</td>
<td>0.17</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>range</td>
<td>0.11-1.80</td>
<td>32-141</td>
<td>1.50-158.1</td>
<td>0.06-0.37</td>
<td>0.06-0.95</td>
<td>0.42-1.30</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>DMS</td>
<td>0.1</td>
<td>0.13</td>
<td>14</td>
<td>107.9</td>
<td>0.50</td>
<td>0.51</td>
<td>1.06</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>DMS</td>
<td>0.1</td>
<td>0.52</td>
<td>36</td>
<td>8.7</td>
<td>0.08</td>
<td>0.09</td>
<td>0.81</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>DMS</td>
<td>0.8</td>
<td>0.91</td>
<td>268</td>
<td>19.2</td>
<td>0.07</td>
<td>0.15</td>
<td>0.49</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>DMS</td>
<td>0.4</td>
<td>0.83</td>
<td>40</td>
<td>101.8</td>
<td>0.17</td>
<td>0.23</td>
<td>0.76</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>DMS</td>
<td>&lt;0.1</td>
<td>1.22</td>
<td>31</td>
<td>15.8</td>
<td>0.08</td>
<td>0.08</td>
<td>0.99</td>
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<tr>
<td>13</td>
<td>M</td>
<td>DMS</td>
<td>0.4</td>
<td>0.72</td>
<td>73</td>
<td>6.8</td>
<td>0.37</td>
<td>0.33</td>
<td>1.13</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>DMS</td>
<td>0.3</td>
<td>1.90</td>
<td>230</td>
<td>5.6</td>
<td>0.16</td>
<td>0.29</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>median</td>
<td>0.83</td>
<td>37</td>
<td>15.8</td>
<td>0.16</td>
<td>0.23</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>range</td>
<td>&lt;0.1-0.8</td>
<td>0.13-1.90</td>
<td>14-268</td>
<td>5.6-107.9</td>
<td>0.07-0.50</td>
<td>0.08-0.51</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>FSGS</td>
<td>0.3</td>
<td>2.2</td>
<td>39</td>
<td>30.0</td>
<td>0.19</td>
<td>0.30</td>
<td>0.57</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>FSGS</td>
<td>0.4</td>
<td>0.9</td>
<td>87</td>
<td>26.0</td>
<td>0.06</td>
<td>0.12</td>
<td>0.50</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>FSGS</td>
<td>0.7</td>
<td>1.4</td>
<td>21</td>
<td>16.2</td>
<td>0.63</td>
<td>0.96</td>
<td>0.66</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>FSGS</td>
<td>1.9</td>
<td>2.3</td>
<td>50</td>
<td>25.6</td>
<td>0.04</td>
<td>0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>FSGS</td>
<td>1.2</td>
<td>2.8</td>
<td>34</td>
<td>4.0</td>
<td>0.07</td>
<td>0.17</td>
<td>0.43</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>FSGS</td>
<td>2.7</td>
<td>3.8</td>
<td>175</td>
<td>37.5</td>
<td>0.48</td>
<td>0.30</td>
<td>1.60</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>FSGS</td>
<td>2.5</td>
<td>7.7</td>
<td>51</td>
<td>1.6</td>
<td>0.04</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>FSGS</td>
<td>1.3</td>
<td>8.4</td>
<td>63</td>
<td>3.8</td>
<td>0.07</td>
<td>0.10</td>
<td>0.70</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>FSGS</td>
<td>0.6</td>
<td>14.8</td>
<td>105</td>
<td>10.1</td>
<td>0.36</td>
<td>0.32</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>median</td>
<td>2.8</td>
<td>51</td>
<td>16.1</td>
<td>0.07</td>
<td>0.17</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>range</td>
<td>0.3-2.7</td>
<td>0.9-14.8</td>
<td>21-175</td>
<td>1.6-37.5</td>
<td>0.04-0.63</td>
<td>0.05-0.96</td>
</tr>
</tbody>
</table>

**Abbreviations are:** NA = not available, alt = alternate

*P < 0.01, **P < 0.001, ***P < 0.0005, Not significant

when taken in the context of the virtual absence of HS in the GBM of one them, suggesting failure of incorporation of HS into the GBM. We investigated children with different forms of congenital nephrotic syndrome in order to ascertain whether abnormalities of the HS/CS ratio were characteristic of a particular type of CNS. It also prompted the speculation that urinary HS measurement might be of diagnostic value in the assessment of CNS.

We refined the quantitative assay for HS and CS in urine used previously [5], introducing separate standard curves in each analysis. The reaction for this was the observation of non-linearity at high concentrations of GAGs. Moreover, Alcian
blue precipitates other macromolecules such as Tamm-Horsfall protein, and therefore the calculation of individual GAG concentration from the total GAG measurement and the HS/CS optical density ratio, as in the previous study, is less accurate than direct measurement.

We elected to study the urinary HS/CS ratio. The use of IS/Cr would have been unreliable as the urinary creatinine excretion may be low in children in poor nutritional state. It would have led to artificially elevated values, especially in the CNSF group. In addition, we observed that the HS/CS ratio is independent of age.

Our results, based on a larger sample of patients, confirm Vermylen et al's findings of an increased HS/CS ratio in the urine of children with CNSF and DMS. We also observed a raised HS/CS ratio in some children with FSGS.

We found that the HS/CS ratio correlated strongly with albuminuria. It might be that increased losses of HS from the GBM leads to a greater degree of proteinuria as result of loss of GBM anionic charge. Alternatively, a raised HS/CS ratio might be the non-specific consequence of high levels of proteinuria. It might also be that the loss of HS in the urine is related to the extent of structural damage of the glomeruli, which occurs to a varying degree in the different forms of nephrotic syndrome in which we found a raised HS/CS ratio. However, it is not possible on the basis of this study to explain these differences or their underlying mechanisms. Further studies are needed into degradation and synthesis of HS in the GBM in this group of disorders.

We confirmed that children with SSNS have a normal urinary HS/CS ratio. However, they had lower levels of proteinuria. Selective proteinuria is unlikely to account for the low urinary HS excretion seen in the SSNS group. In CNSF there is also selective proteinuria [15], but they had the highest urinary HS excretion. Most of the children in the SSNS group were on steroids at the time of study. However, there was no significant difference in the HS/CS ratios between those children on steroids and those children off steroids.

In summary, we have shown that children with CNSF, DMS and some children with FSGS, but not children with SSNS, have an increased HS content in the urine compared to controls. There was, however, a positive correlation of the HS/CS ratio with Alb/Cr, suggesting that raised HS/CS ratios are related to states of heavy albuminuria rather than characteristic of specific histological categories.

Acknowledgments

LJ was supported by a grant from the Kidney Research Aid Fund. We thank the pediatric nephrologists who made samples from their patients available for this study. Alcian Blue used in this study was a gift from Imperial Chemical Industries, Ltd., United Kingdom.

Reprint requests to Dr. Lyda Jadresic, Department of Paediatric Nephrology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom.
Fig. 3. The relationship between the urinary albumin/creatinine and heparan sulphate/creatinine ratio (r = 0.66, P < 0.0001). Symbols are: (●) CNSF; (△) DMS, (○) FSGS; (*) SSNS.

References

8. ROSENZWEIG LJ, KANWAR YS: Removal of sulfated (heparan sulfate) or nonsulfated (hyaluronic acid) glycosaminoglycans results in increased permeability of the glomerular basement membrane to 125I-bovine serum albumin. Lab Invest 47:177–184, 1982
Clinicopathologic review of twelve children with nephropathy, Wilms tumor, and genital abnormalities (Drash syndrome)


From the Departments of Nephrology, Endocrinology, Haematology and Oncology, Pathology, and Radiology, Institute of Child Health and Hospital for Sick Children, Great Ormond Street, London, United Kingdom

The clinicopathologic and radiologic features of 12 children with complete and incomplete forms of Drash syndrome are reported. Their common denominator was a nephropathy. Four had the full triad, consisting of nephropathy, Wilms tumor, and genital abnormalities; five had nephropathy and genital abnormalities, and three had nephropathy and Wilms tumor. Of the 11 children who had proteinuria, eight had the nephrotic syndrome. Of the 10 whose condition progressed to end-stage renal failure, seven were less than 3 years of age. The histologic features of Wilms tumors were favorable in all seven children, and the tumor was bilateral in three. Of the nine patients who had genital abnormalities, eight had 46,XY karyotype and either ambiguous genitalia (six patients) or normal female phenotype (two). One other patient had a normal 46,XX female karyotype and phenotype but had both mullerian and wolffian structures and a streak ovary. Nine patients had a distinct pelviccalceal abnormality not previously reported as a feature of this syndrome. Other congenital abnormalities were aniridia, mental retardation, deafness, nystagmus, and cleft palate. This syndrome must be considered in any infant with unexplained nephropathy, particularly in young phenotypic female infants and in those children with ambiguous genitalia or Wilms tumor with an early presentation. (J Pediatr 1990;117:717-25)

The first report of a child with nephropathy, genital abnormalities, and Wilms tumor was published by Denys et al. in 1967. Their patient had XY/XX mosaicism with ambiguous genitalia, was found, at the age of 3½ months, to have nephrotic syndrome, hypertension, and Wilms tumor, and died of renal failure at 15 months of age. Three years later, Drash et al. reported two further examples, and since then over 60 cases have been described. Although the Drash eponym has continued in use, this syndrome should perhaps be known as the Denys-Drash syndrome, as previously suggested.

Some of the reported cases do not express the full spectrum of the syndrome. The nephropathy may be associated with either genital abnormalities or Wilms tumor; others have had all three features. The occurrence of nephropathy has become generally accepted as the

<table>
<thead>
<tr>
<th>ESRF</th>
<th>End-stage renal failure</th>
</tr>
</thead>
</table>

Drash et al. reported two further examples, and since then over 60 cases have been described. Although the Drash eponym has continued in use, this syndrome should perhaps be known as the Denys-Drash syndrome, as previously suggested.

References 1, 2, 6, 10, 11, 17, 26-34.

Supported by a grant from the Kidney Research Aid Fund (Dr. Jadresic) and by the Imperial Cancer Research Fund (Dr. Pritchard).

Submitted for publication Jan. 22, 1990; accepted June 18, 1990.

Reprint requests: Lyda Jadresic, MRCP, Department of Paediatric Nephrology, Institute of Child Health, 30 Guilford St., London WC1N 1EH, United Kingdom.

9/20/23203

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Renal histologic findings were available for all patients. There were 12 nephrectomy specimens from eight patients, five needle biopsy specimens, and one open-wedge biopsy. Gonadal histologic findings were available in five cases. For light microscopy, tissues were fixed in 10% buffered formalin and processed routinely; 3 μm paraffin sections were stained with hematoxylin and eosin. Renal biopsy specimens and nonmalignant kidney from nephrectomy specimens were also stained with periodic acid–Schiff, Masson trichrome, hexamine silver, Martius scarlet blue, and elastic van Gieson stains. For immunohistochemistry studies, paraffin sections were cut at 3 μm and stained by a peroxidase-antiperoxidase technique with pronase digestion using polyclonal antibodies to IgG, IgM, IgA, C1q, C3, and fibrinogen. For electron microscopy, tissue was fixed in 2.5% glutaraldehyde; ultrathin sections stained with lead citrate and uranyl acetate were examined and photographed with a model CX 100 electron microscope (Joel, Tokyo, Japan). Glomerular and tubulointerstitial damage was graded in order of severity on a scale of 1 to 3 (+ to +++). All radiologic studies of the genitourinary system were reviewed. Eight children had an ultrasound examination, nine had intravenous urography, and seven had micturating cystourethrogram.

RESULTS

Clinical features. The children were divided into three groups (Table). Group 1 (patients 1 to 4) consisted of patients with all three abnormalities—nephropathy, Wilms tumor, and genital abnormalities. Group 2 (patients 5 to 9) consisted of the children who had only a nephropathy and genital abnormalities, and group 3 (patients 10 to 12) consisted of children with nephropathy and Wilms tumor.

Nephropathy. The nephropathy in our patients was characterized by the early onset of proteinuria, by the presence of hypertension, and by the rapid development of end-stage renal failure (Table). Proteinuria was present in 11 of the 12 patients and resulted in the nephrotic syndrome in eight. The age at detection of proteinuria ranged from birth to 2 years, six patients being less than 1 year of age. Hypertension was present in 10 of the 12 patients, ranging from mild in three to severe and requiring treatment in seven. Hematuria was detected in only two patients: one had hematuria at the time of a Candida tropicalis urine infection caused by a urine catheter in situ, and one had hematuria initially at the time of presentation with a nephroblastoma. Glycosuria was detected on at least one occasion in five patients.

On contrast imaging of the urinary tract, seven patients were found to have abnormal caliceal systems characterized by blunted, nondilated calices with no evidence of obstruction. Five of these patients also had either absent or small renal pelves, and two also had vesicoureteral reflux. Two

common denominator of the syndrome.

The association of Wilms tumor with urogenital abnormalities has long been recognized but in the absence of nephropathy these cases fall outside the definition of Drash syndrome. We have not included such cases in this review, but this does not exclude the possibility that there is a common etiologic link among all three features that might occur in any combination, as suggested by Barakat et al.5 and by Gallo and Chemes.25

The purpose of this report is to add our experience to the published descriptions of this condition, to draw attention to its veiled presentation in young phenotypic female children with nephrosis, and to describe a previously unreported pelvicaliceal abnormality that may constitute a fourth feature of the syndrome.

METHODS

The 12 patients were seen at the Hospital for Sick Children, London, between the years 1972 and 1987. Their clinical presentation, results of laboratory investigations, and radiologic findings were reviewed.
Table. Clinicopathologic and radiologic features of children enrolled in study

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at diagnosis of nephropathy (yr)</th>
<th>Age at diagnosis of Wilms tumor (yr)</th>
<th>Genital abnormalities/ karyotype</th>
<th>Renal histologic features</th>
<th>Glomerulopathy; Immunocytochemistry</th>
<th>Tubulo-interstitial damage</th>
<th>IVU and MCU findings</th>
<th>Outcome at specific age (yr)</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>0.9</td>
<td>+/46,XY</td>
<td>DMS; IgM, Clq, C3</td>
<td>+</td>
<td>Blunt calices; no reflux</td>
<td>NeS at 0.9; ESRF at 1.0; renal transplant at 2.3</td>
<td>Died in ESRF at 5.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>4.8</td>
<td>+/46,XY</td>
<td>FMS; negative</td>
<td>+</td>
<td>Normal calices; (R) small pelvis</td>
<td>Bilateral nephrectomy and CAPD at 2.1</td>
<td>NeS at 1.6; died in ESRF at 2.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>0.4 (R) 1.5 (L)</td>
<td>+/46,XY</td>
<td>Focal IgM</td>
<td>+</td>
<td>Blunt calices; absent (L) pelvis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>1.6</td>
<td>+/46,XX</td>
<td>FMS; negative</td>
<td>++</td>
<td>Blunt (L) calices</td>
<td></td>
<td></td>
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<tr>
<td>Group 2†</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>0.1</td>
<td>—</td>
<td>+/46,XY</td>
<td>DMS; C3</td>
<td>+++</td>
<td>Blunt calices; absent pelvis</td>
<td>GFR 32 ml/min/1.73 m²; hypertensive; no proteinuria at 9</td>
<td>Sensorineural deafness</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.1</td>
<td>—</td>
<td>+/46,XY</td>
<td>DMS; C3</td>
<td>+++</td>
<td>(R) VUR; blunt calices; absent pelvis</td>
<td>NeS at 0.1; died in ESRF at 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.7</td>
<td>—</td>
<td>+/46,XY</td>
<td>DMS; IgM, Clq, C3</td>
<td>+++</td>
<td>—</td>
<td>NeS at 1.7; ESRF at 2; renal transplant at 2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.4</td>
<td>—</td>
<td>+/46,XY</td>
<td>DMS; IgM, Clq, C3</td>
<td>++</td>
<td>—</td>
<td>NeS at 0.8; ESRF at 0.8; died at 1.7</td>
<td>Mental retardation; nystagmus; cleft palate</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.0</td>
<td>—</td>
<td>+/46,XY</td>
<td>DMS; IgM, C3</td>
<td>+++</td>
<td>Bilateral VUR; blunt calices; absent pelvis</td>
<td>NeS at birth; died in ESRF at 0.1</td>
<td>Flexion contractures at birth</td>
<td></td>
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<tr>
<td>Group 3‡</td>
<td></td>
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<td></td>
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<tr>
<td>10</td>
<td>0.9</td>
<td>0.9</td>
<td>−/ND</td>
<td>DMS; IgM</td>
<td>+++</td>
<td>Blunt calices; (L) absent pelvis</td>
<td>NeS at 0.9; died of tumor at 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2.2</td>
<td>1.0</td>
<td>−/46,XX with del 11(p13)</td>
<td>FMS; negative</td>
<td>+++</td>
<td>Bilateral obstructed calices</td>
<td>ESRF at 6.5; renal transplant at 7</td>
<td>Mental retardation; aniridia</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.5</td>
<td>1.7</td>
<td>−/46,XY</td>
<td>DMS; IgM, Clq, C3</td>
<td>+++</td>
<td>Normal calices; absent (R) pelvis</td>
<td>NeS at 1.5; ESRF at 1.8; renal transplant at 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

—, Absent; +, present (genital abnormalities); +, +, or ++, degree of severity of tubulointerstitial damage; IVU, intravenous urogram; MCU, micturating cystourethrogram; DMS, diffuse mesangial sclerosis; FMS, focal mesangial sclerosis; NeS, nephrotic syndrome; R, right; L, left; CAPD, continuous ambulatory peritoneal dialysis; FGS, focal glomerulosclerosis; GFR, glomerular filtration rate; VUR, vesicoureteral reflux; ND, not done; del, deletion.

*Group 1 had nephropathy, Wilms tumor, and genital abnormalities.
†Group 2 had nephropathy and genital abnormalities.
‡Group 3 had nephropathy and Wilms tumor.
additional patients had normal caliceal systems, but their renal pelves were either small or absent (Fig. 1; Table). We were unable to study two patients with intravenous urography because they had advanced renal failure at the time of presentation, but their micturating cystourethrograms were normal. Eight patients studied by renal ultrasonography, and in all there was loss of corticomedullary differentiation, a nonspecific finding.

Renal histologic findings were available for all 12 patients (Table). Except for one child who had changes characteristic of focal glomerular sclerosis, the remainder had varying degrees of mesangial sclerosis, either diffuse (seven children) or focal (four). In the seven children with diffuse mesangial sclerosis the superficial subcapsular cortex was atrophic and contained small, immature glomeruli, with a prominent layer of epithelial cells enveloping the tuft and resembling those usually encountered in the fetal kidney. Many of these glomeruli were sclerosed and crowded together, with atrophy and loss of intervening tubules and marked interstitial fibrosis. In the middle cortex the glomeruli showed expansion of the mesangial matrix by fibrillar, argyrophilic material with a positive reaction to periodic acid–Schiff reagent. Preserved nuclei were present, but there was no increase in cellularity (Fig. 2). Silver staining revealed double contours in the glomerular basement membrane in a proportion of glomeruli in most cases. Crescents and adhesions were rarely encountered, and glomerular abnormalities were less severe in the inner cortex. Of the four specimens exhibiting focal mesangial sclerosis, the changes were mild in two. One case (No. 3) lacked the distinctive subcapsular zone, but in the others this feature was well developed. Abnormal glomeruli in the middle and outer cortex were similar in appearance to those seen in the diffuse cases, but a proportion of these glomeruli, as well as those in the inner cortex, were morphologically normal. Tubulointerstitial damage was invariably present and was of a similar nature to that seen in patients with diffuse mesangial sclerosis, but the severity was commensurate with that of the associated glomerular changes. Widespread tubular atrophy with microcystic dilation was accompanied by interstitial fibrosis and patchy chronic inflammation. Results of immunocytochemistry studies were positive in 9 of 12 patients, showing granular deposition of IgG, IgM, C1q, and C3, principally along peripheral capillary walls and sometimes in the mesangium (Fig. 3). Fibrinogen and IgA were consistently absent. Positive immunostaining was confined to morphologically abnormal glomeruli. Entirely negative results were obtained in three of the earlier cases in the series; however, the tissue had been embedded in paraffin for 12 to 15 years, and the results may be spurious for technical reasons. Electron microscopy was available for six patients, of whom five had increased mesangial matrix and podocyte hypertrophy and foot process fusion. The glomerular basement membrane contained electron-dense linear and reticular formations in the lamina rara externa and lamina densa in two cases, and wrinkling of the subepithelial glomerular basement membrane was seen (Fig. 4). Subendothelial and intramembranous deposits were

Fig. 2. Patient 6. Photomicrograph of diffuse expansion of mesangial matrix by fibrillar material. There is no increase in mesangial cellularity. (Periodic acid–Schiff stain; X250.)
Fig. 3. Patient 7. Photomicrograph of glomerulus stained for Clq reveals granular positivity predominantly in peripheral capillary loops. (Immunoperoxidase staining; X400.)

present in one case; in another, mesangial interposition was seen.

Progression to ESRF was seen in 9 of the 12 patients, 7 of whom were younger than 3 years of age. Five died in renal failure, one while receiving continuous ambulatory peritoneal dialysis, and four have had successful transplants. An additional patient, who had mild proteinuria and hypertension, had an initial nephrectomy for a right-sided nephroblastoma and later a left-sided nephrectomy for tumor recurrence, which proved to be drug and radiotherapy resistant; he is now receiving CAPD. One child has diminished but stable renal function.

Wilms tumor. Wilms tumor developed in seven children. Three children were found to have an abdominal mass as an incidental finding after seeking medical care for minor symptoms. Two children had hematuria, and in two the diagnosis was systematically sought because of the presence of ambiguous genitalia in one child and aniridia in the other. Six children were less than 2 years of age (median 1.25 years) at diagnosis. One child's tumor was diagnosed at 4.8 years. Two had bilateral disease at presentation; one had a recurrence in the contralateral kidney 13 months after the diagnosis of the first tumor. None had extrarenal involvement. All 10 tumors were of a "favorable" triphasic histologic type.

Five children had no evidence of Wilms tumor, at autopsy (two patients), at bilateral nephrectomy (one patient), or on repeated abdominal ultrasonography or computed tomography (two patients). One of the two patients followed up with sequential imaging was 9 years of age at last review. The other died at 1.7 years; autopsy on this patient was refused. The diagnosis of Wilms tumor preceded the diagnosis of nephropathy in two children by 1.6 years and 1.2 years, respectively. Wilms tumor was diagnosed at the same time as the nephropathy in three children, and after the onset of nephropathy in two patients.

Genital abnormalities. Nine children had genital abnormalities. Patients 1, 2, and 3 had 46,XY karyotypes and ambiguous external genitalia consisting of penoscrotal hypospadias and cryptorchidism. Patient 4 was a phenotypically normal female with a 46,XX karyotype but was found to have müllerian and wolffian structures and a streak ovary at autopsy. All patients in group 2 had 46,XY karyotype. Patient 5 had a large clitoris with fused labia. Patient 6 had penoscrotal hypospadias and cryptorchidism. Patients 7 and 9 had female external genitalia, and patient 8 had penoscrotal hypospadias and bifid scrotum with palpable gonads.

Gonads and genital duct structures were examined histologically in five cases. Atrophic changes attributable to cryptorchidism were present in patients 2 and 3. In patient 4 a streak ovary containing primordial follicles was accompanied by müllerian and wolffian ducts. Dysgenetic intraabdominal testes were present in patient 6; histologic examination showed severe architectural derangement of the cortex and focal invasion of the tunica albuginea by tubules. In patient 9 a bicornuate uterus was accompanied by bilateral müllerian and wolffian duct structures; a single streak gonad was identified at postmortem examination. This go-
necrosis comprised an ovarian type of stroma lacking primordial follicles. Poorly differentiated sex cords were present, and there were occasional tubules suggestive of primitive canalicular seminiferous tubules. No gonadal tumors were identified in any of the specimens examined.

DISCUSSION

The basic features of the children in this series are similar to those reported previously. The nephropathy is characterized by the presence of proteinuria at an early age, and in most children it evolves into the nephrotic syndrome and eventually progresses to ESRF. The ESRF developed in 76% of the 64 reported patients, in 60% of them before the age of 2 years. With one exception the histologic findings consisted of varying degrees of focal or diffuse mesangial sclerosis, as described by Habib et al., Walderherr and Ostertag-Korner, and Gallo and Chemes. Our patients with focal mesangial sclerosis tended to have less severe disease than did those with diffuse involvement. The distribution of lesions was in striking contrast with that of focal segmental glomerulosclerosis, which tends to involve the juxtamedullary cortex first and most severely. The characteristic major involvement of the outer cortex, previously reported by others, was present in both focal and diffuse cases. The two groups of patients appear to represent a morphologic continuum possibly reflecting evolution of a common process.

Classifications of the renal abnormalities have included diffuse mesangial sclerosis, focal sclerosis, membranoproliferative glomerulonephritis, and membranoproliferative glomerulonephritis with focal segmental glomerulosclerosis. There are numerous reports without adequate information on renal histologic features. The presence of complement and immunoglobulins in the glomeruli has been reported and was common among our patients. Deposition was confined to morphologically abnormal glomeruli, suggesting nonspecific trapping rather than an immunologically mediated process, as observed by Habib et al. An immune mechanism related to the presence of tumor-derived antigens seems unlikely, because in five of our seven patients with Wilms tumor the nephropathy coincided with or preceded the development of nephroblastoma. In addition, such a mechanism would not explain the presence of nephropathy in children with genital abnormalities alone.

Several reports have described a nephropathy in association with XY gonadal dysgenesis in phenotypic female patients with primary amenorrhea in adolescence. All of these patients reached ESRF but at a later stage than in the typical child with Drash syndrome. The age at onset of ESRF in the patients described in these four reports varied from 14 to 23 years. Renal histologic features were de-
scribed in one report as focal glomerular sclerosis with mesangial cell matrix expansion\textsuperscript{11} and as showing the changes of end-stage disease in another\textsuperscript{12}; such features were not described in the two other reports.\textsuperscript{9,16} Thus different forms of nephropathy may well be associated with genital abnormalities. Neither proteinuria nor ESRF has yet developed in one of the children in our review, even though she is 9 years of age.

Other forms of nephropathy have been reported in association with Wilms tumor. Minimal-change nephrotic syndrome that responded to steroids was reported by Lines,\textsuperscript{40} and membranous nephropathy has been reported by Row et al.\textsuperscript{41} Focal segmental glomerulosclerosis has developed in the remaining kidney 10 to 20 years after unilateral nephrectomy for treatment of Wilms tumor, believed to be a consequence of hyperfiltration in the remaining kidney.\textsuperscript{42,43}

Bilateral Wilms tumor developed in three of our seven patients. In the published cases there is also a high incidence of bilaterality. In contrast, the incidence of bilateral Wilms tumor among all cases is 4% to 5%. The mean age at diagnosis in our group was 1.6 years, which is earlier than the mean age at diagnosis in children with sporadic Wilms tumor (45.1 months).\textsuperscript{44}

Male pseudohermaphroditism is the commonest genital abnormality in this syndrome, although other abnormalities are seen. The patient reported by Eddy and Mauer,\textsuperscript{32} although designated a male pseudohermaphrodite, was in fact a true hermaphrodite, having both testicular and ovarian tissue.\textsuperscript{45} Fisher et al.\textsuperscript{34} reported the case of a child with a 46,XX karyotype, normal female phenotype, Wilms tumor, and congenital nephrosis who had hypoplastic ovaries and bilateral gonadoblastomas; this case strongly resembles that of patient 4 in our series, who had a 46,XX karyotype, female external genitalia, and a streak ovary. It is conceivable that female children with an XX karyotype, nephropathy, and Wilms tumor have an underdiagnosed incidence of gonadal abnormalities. Gonadal biopsies have not been routinely performed in this group of children. We suggest that gonadal biopsies be carried out for complete diagnosis and that gonadectomy be performed, if indicated, to prevent the development of gonadoblastomas. We agree with Eddy and Mauer\textsuperscript{46} that this syndrome should be expanded to include all abnormalities of gonadal differentiation.

There is clinical overlap between this syndrome and the WAGR complex (Wilms tumor, aniridia, ambiguous genitalia, and mental retardation), in which there is a deletion involving chromosome region 11p13.\textsuperscript{47} Our patient 11 has a constitutional deletion of this band. She had nephropathy, bilateral Wilms tumor, aniridia, and mental retardation.

Hers is the only reported case of Drash syndrome involving such a deletion. It is possible that this syndrome could arise as consequence of a deletion of one or more genes located within this region in the short arm of chromosome 11. In a study of six patients from this series, we looked for evidence of microdeletions in this region by using DNA probes mapping to 11p13. No deletions were found in any of the patients studied, apart from the deletion found in patient 11, which is sufficiently large to be detectable also on karyotyping (manuscript submitted for publication). Alternatively the genetic origin of this syndrome could lie on the X chromosome. In most reported cases, the patients have had an XY karyotype, which, together with the presence in this syndrome of a substantial disorder of gonadal differentiation, could point to a genetic abnormality in the sex chromosomes.

A consistent, previously undescribed caliceal abnormality was observed on contrast imaging. The caliceal system appeared blunted, without evidence of obstruction or dilation. The renal pelvis were in many cases either small or absent. Two of the three children described by Barakat et al.\textsuperscript{4} had hydronephrosis on one side and caliceal blunting on the other, nonhydronephrotic side. This is likely to be a congenital rather than an acquired abnormality and is probably another manifestation of the abnormal development of the genitourinary system associated with this syndrome. It is unlikely to be the result of vesicoureteral reflux, which was detected only in two of the eight patients studied by micturating cystourethrography. In these two patients the size of the ureters was normal. Unfortunately there was only one gross specimen available for comparison; it showed a normal pelviccaliceal system (patient 9). This was a surprising finding because this patient's micturating cystourethrogram was striking. These pelviccaliceal abnormalities may not only form part of the Drash syndrome but also may provide a clue to the diagnosis in some cases.

It is important to consider the diagnosis of Drash syndrome for any infant with an unexplained nephropathy, particularly for young phenotypic girls and for children with ambiguous genitalia or with an early Wilms tumor. We advocate a virgorous and optimistic approach to treatment, including prophylactic bilateral nephrectomies, even in the absence of Wilms tumor. We have taken this step once ESRF has occurred, but it could be argued that there is a case for nephrectomy before ESRF ensues because of the risk of early development of Wilms tumor. However, most deaths have been related to the renal disease and the onset of ESRF, so better dialysis and transplantation programs for young children constitute the major factor in the improved prognosis of these children.
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