

**Prevalence of *TTR* variants detected by whole-exome sequencing in hypertrophic  
cardiomyopathy**

**Running title:** Whole-exome detected *TTR* variants in HCM

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## Abstract

### Background

A proportion of patients presenting with hypertrophic cardiomyopathy (HCM) have a diagnosis of cardiac amyloidosis. Hereditary transthyretin amyloid cardiomyopathy (hATTR-CM) is caused by more than 100 mutations in the *TTR* gene. Our aim was to study the prevalence of potentially amyloidogenic *TTR* gene variants in a whole-exome sequencing (WES) study of a large cohort of patients with HCM.

### Methods and results

770 consecutive HCM probands underwent WES and clinical characterization. Patients with rare or known pathogenic variants in *TTR* underwent <sup>99m</sup>Techetium labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scintigraphy and were retrospectively re-assessed for the presence of clinical features of amyloidosis.

Two patients had rare *TTR* variants of unknown significance and four had the known pathogenic p.V122I (p.V142I) variant (one homozygous and also heterozygous for a likely pathogenic *MYL3* variant and another double heterozygous for a likely pathogenic *MYBPC3* variant). Four out of 6 patients with *TTR* variants underwent DPD scintigraphy; the only positive study for cardiac amyloidosis was in the patient with the homozygous p.V122I (p.V142I) variant.

### Conclusions

Pathogenic *TTR* variants are rare in carefully assessed patients with HCM and may occur in double heterozygosity with sarcomere protein gene mutations. The lack of evidence for an amyloidosis phenotype in all but one *TTR* mutation carrier illustrates the importance of complete clinical evaluation of patients meeting HCM criteria that harbour pathogenic *TTR* mutations.

**Key words:** hereditary transthyretin amyloidosis; hypertrophic cardiomyopathy; whole-exome sequencing.

## Introduction

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disease that affects 0.2% of the population and which predisposes to sudden cardiac death and heart failure. It is most commonly caused by mutations in genes encoding for sarcomere and related proteins, with a small proportion caused by syndromic diseases and inborn errors of metabolism. [1]

Transthyretin (*TTR*) amyloidosis is a multisystem disorder caused by aggregation of *TTR* amyloid fibrils in various tissues. There are two classical presentations of the disease: transthyretin familial amyloid polyneuropathy (TTR-FAP), which primarily affects the peripheral nerves, and TTR cardiomyopathy (TTR-CM), which presents most commonly as hypertrophic cardiomyopathy. [2] TTR-CM can be an acquired disorder of the elderly or a hereditary condition caused by over 100 mutations in the *TTR* gene, of which at least 22 are associated predominantly with TTR-CM. [2], [3], [4] The point mutation valine 142 to isoleucine (p.V122I (p.V142I)) is the most common TTR-CM variant, with a prevalence of about 3–4% in the African-American population. [2] In a very recent report [5] of a United Kingdom cohort of more than 4000 patients referred due to a diagnostic suspicion of amyloidosis, the most frequent pathogenic variant was p.V122I (p.V142I); nearly 50% of the patients with this mutation were of Afro-Caribbean ancestry, the remaining originating from different regions of Africa, mostly the West.

Recent studies suggest that a substantial number of patients with heart failure with preserved ejection fraction [6], hypertrophic cardiomyopathy [7] and aortic stenosis [8] have evidence for wild type TTR cardiac amyloidosis.

The aim of this study was to study the prevalence of amyloidogenic *TTR* gene variants in a prospective whole-exome sequencing study of nearly 800 patients with hypertrophic cardiomyopathy in whom cardiac amyloidosis was not suspected at first assessment.

## Methods

### *Study population and design, clinical evaluation and sample collection*

This was an observational, prospective single-centre study. All patients gave written informed consent in accordance with the protocol approved by the regional ethics committee (15/LO/0549) and received genetic counselling in accordance with international guidelines. The study population comprised unrelated and consecutively evaluated patients with HCM referred to the Inherited Cardiovascular Disease Department at Barts Heart Centre, London, UK and before 2015 to the Inherited Cardiovascular Disease Unit at The Heart Hospital, UCLH, London, UK. The samples used in this study were collected from 2013 to 2018.

Clinical evaluation included a personal and family history, physical examination, 12 lead ECG, echocardiography, symptom limited upright exercise testing with simultaneous respiratory gas analysis (cardiopulmonary exercise test) and ambulatory ECG monitoring as previously described [9]. HCM was diagnosed in probands when the maximum left ventricular (LV) wall thickness (MLVWT) on 2D echocardiography measured 15 mm or more in at least one myocardial segment or when MLVWT exceeded 2 standard deviations (SD) corrected for age, size and gender in the absence of other diseases that could explain the hypertrophy [1]. In individuals with unequivocal disease in a first-degree relative, diagnosis was made using extended familial criteria for HCM [1]. Ethnicity was self reported and classified using a modified National Health Service ethnicity questionnaire. Patients were evaluated every 6–12 months or earlier if there was a clinical event. Initial evaluation and follow-up data were collected prospectively and registered in a relational database.

Patients with previously clinically suspected or confirmed inherited metabolic, syndromic or infiltrative diseases including amyloidosis were excluded from the WES study. When a *TTR* variant was detected, hospital records were reanalysed to determine the presence of

phenotypic characteristics of cardiac *TTR*-amyloid. These included, a) low-voltage and/or “pseudoinfarct” pattern, and/or bundle branch block (BBB)/conduction disease on 12 lead ECG; b) concentric left ventricular hypertrophy on echocardiography and/or biventricular hypertrophy and/or base-apical myocardial strain gradient pattern; c) typical late gadolinium enhancement on cardiac magnetic resonance imaging (MRI); d) carpal tunnel syndrome. Patients still under follow-up and with rare/novel variants or the pathogenic p.V122I (p.V142I) variant underwent <sup>99m</sup>Tc-DPD (<sup>99m</sup>Technetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid) scintigraphy to confirm cardiac amyloidosis [10].

#### *DNA Extraction and whole-exome sequencing (WES) library preparation*

DNA was extracted from whole blood using the QIAamp Midi / Mini Kit by Qiagen following the relevant protocol. Sample library preparation from Genomic DNA was done as per the Agilent SureSelectQXT Target Enrichment Illumina multiplexed sequencing protocol. We used Agilent SureSelectXT Human Exon V6 following the manufacturer’s protocol to capture targeted exonic regions. Exome sequencing of the enriched libraries occurred on the Illumina HiSeq4000 platform at a minimum of 30x coverage, using standard manufacturer’s protocols.

#### *Bioinformatic analysis of WES data*

Sequence reads alignment against the reference human genome (UCSC hg19) was performed using NovoAlign (Novocraft Technologies Sdn Bhd). Picard (<http://picard.sourceforge.net/>) was used for duplicate reads removal, format conversion, and indexing. Recalibration of base quality scores and performance of local realignments around possible indels was done with Genome Analysis Toolkit (GATK) (<https://www.broadinstitute.org/gatk/>). HaplotypeCaller 3.1 package in GATK was used for variants calling and generation of a multi-sample joint genotyping. Variant annotation was

done with ANNOVAR [11] and Variant Effect Predictor (VEP) tool from Ensembl [12]. For the *in silico* prediction of pathogenicity of missense variants we used multiple bioinformatics tools, including HumVar-trained PolyPhen-2 model [13], SIFT [14], and MutationTaster [15]. Variants were also annotated with minor allele frequencies reported in the Genome Aggregation Database (gnomAD, <http://gnomad.broadinstitute.org/>) [16]. *TTR* variants were annotated with transcript NM\_000371.

#### *Variant filtering and further downstream annotation*

Variants identified by WES with a minor allele frequency (MAF) higher or equal to 0.0001 (0.01%) in gnomAD were removed from further analysis, except for more common variants known to be pathogenic. Variants outside of the coding and splice-site regions as well as synonymous variants not predicted to alter splicing were also filtered and excluded. Remaining genetic changes were prioritized by their predicted functional effect, including likelihood to result in a loss-of-function (stop gain, frameshift deletion or insertion) or altering splicing. Ingenuity<sup>®</sup> Variant Analysis<sup>™</sup> software (<https://www.qiagenbioinformatics.com/products/ingenuity-variant-analysis>) from QIAGEN, Inc. was used for further downstream annotation, including: presence and classification of the variants in the ClinVar database, American College of Medical Genetics (ACMG) classification and presence in the published literature.

#### *Copy number variants (CNVs) analysis*

The analysis of large rearrangements in the coding regions of genes was done using a read depth strategy designed to overcome biases associated with sequence capture and high-throughput sequencing. This set of tools is implemented in the package ExomeDepth (freely available at the Comprehensive R Archive Network [17]).

## Results

The study cohort comprised 770 patients, 515 (67%) males, aged 49.3±15.9 years at diagnosis (median 51.6); 64.3% were white, 11% black. Thirty-two percent had variants with a MAF < 1x10<sup>-4</sup> in eight sarcomeric genes which are robustly associated with HCM (*MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *MYL2*, *MYL3*, *TPM1*, *ACTC1*). Twenty-one patients carried multiple rare sarcomere variants, including 9 compound heterozygotes (5 in *MYBPC3*, 3 in *MYH7* and 1 in *TNNI3*) and 12 double (digenic) heterozygotes.

Six patients had candidate *TTR* variants detected. One (p.R21Q (p.R41Q)) was previously described in the literature and is classified as a variant of unknown significance (VUS) in Clinvar [18]. Another (p.R103H (p.R123H)) is also listed as a VUS in Clinvar. The four remaining patients (0.5% of the cohort) had the pathogenic p.V122I (p.V142I) variant. One patient was homozygous for this variant and also carried a rare and likely pathogenic *MYL3* variant (p.R94H) and another was double heterozygous with a rare and likely pathogenic *MYBPC3* variant (p. R495Q).

No copy number variants in *TTR* were detected.

Demographic, electrocardiographic, imaging and genetic data for all 6 patients are shown in table 1. Four were males (67%), one patient was white (17%).

The patient with the homozygous p.V122I (p.V142I) mutation was the only individual to have some clinical features consistent with cardiac amyloidosis on retrospective chart review (table 1). Two other patients with a p.V122I (p.V142I) variant had mild conduction abnormalities (left BBB in one and first degree AV block in another). Low ECG voltages were not present in any patient with a *TTR* variant. In terms of cardiac imaging, only the homozygous p.V122I (p.V142I) patient had an echocardiogram and MRI potentially suggestive of amyloid due to LVH with systolic dysfunction, restrictive pattern and unusual

pattern of late gadolinium enhancement. None had concentric hypertrophy.

Four of the six patients with *TTR* variants underwent DPD scintigraphy (two of the patients still under follow-up in our centre with a p.V122I (p.V142I) variant and those with rare *TTR* variants). Only the patient with a homozygous p.V122I (p.V142I) mutation was positive, with a Perugini grade 3, confirming cardiac amyloidosis.

## Discussion

Transthyretin (*TTR*) is a protein encoded by a single gene (*TTR*) that is synthesized almost exclusively by the liver and which circulates predominantly as a homotetramer, along with a very small amount of dissociated monomers. Amyloidogenesis begins with dissociation of the *TTR* tetramer into dimers and folded monomers. Unfolding of these species leads to the formation of soluble oligomers and polymerisation into amyloid fibrils within tissues. [2]*TTR* mutations destabilise the *TTR* tetramer and in some cases accelerate the rate of dissociation. More than 100 amyloidogenic mutations have been described, all of which are inherited as autosomal dominant traits with incomplete penetrance. The most common *TTR* mutation is V30M, endemic in parts of Portugal, Sweden and Japan where it typically causes polyneuropathy. A number of *TTR* variants including p.V122I (p.V142I) are associated with a predominant cardiac phenotype. [2],[3] The spectrum of cardiac involvement in ATTR ranges from asymptomatic conduction disease to cardiomyopathy and refractory heart failure. [3],[4], [19]

We undertook screening and annotation of the *TTR* gene in an unprecedentedly large cohort of consecutive HCM patients studied with whole-exome sequencing and characterized the cardiac phenotypes of patients who carried rare or known pathogenic variants.

Several studies have shown that wild-type ATTR is frequently missed as a cause of ventricular dysfunction in patients with heart failure with preserved ejection fraction and aortic stenosis. [6],[8] but most of the published studies focusing on hereditary *TTR*-amyloid (hATTR) were designed to establish the prevalence of cardiac involvement in patients with an already known diagnosis of hATTR or wtATTR. Two previous studies have examined the prevalence of hATTR in patients with HCM and no prior suspicion of TTR-CM, but neither used whole-exome sequencing. [20],[21]. In a Dutch cohort of 345 genotype-negative patients with HCM [21] two individuals were found to harbour a p.V30M (p.V50M) and a p.V122I (p.V142I) mutation. However, no demographic data, scintigraphic or biopsy data were provided. In a second French study, the overall prevalence of *TTR* pathogenic variants in 298 patients with HCM was 5.7%, the majority of which was caused by p.V122I (p.V142I). As anticipated, most patients with this mutation were of African ancestry (6/8).

In our study, two of the identified rare *TTR* variants are variants of unknown significance but likely to be non-pathogenic (both had negative DPD scans). This means that the prevalence of amyloidogenic *TTR* variants in our cohort is only ~0.5%.

The explanation for the dramatic difference in prevalence with the French study is speculative, but is likely related to different ethnic composition (for example, a higher proportion of black patients in the French cohort (23% versus 11%), referral bias and the exclusion of HCM phenocopies in our cohort prior to WES [7]).

A final consideration is the age of recruited patients. The median age of the French cohort was 62 years (74 years in TTR-amyloid and 61 years in other hypertrophic cardiomyopathy patients without *TTR* variants) whilst the median age of our WES cohort was 52 years. Only 9% of our cohort is above 70 years of age and 16% above 65. Furthermore, our patients carrying a p.V122I (p.V142I) mutation were only between 36-61 years. Given that hATTR-cardiac amyloid penetrance is age dependent [22],[5] these differences in age distribution

may also explain the discrepancy with previous studies.

Interestingly, the only patient with a positive DPD scan was homozygous for p.V122I (p.V142I) and also heterozygous for a likely pathogenic mutation in *MYL3*. Another patient with a heterozygous p.V122I (p.V142I) mutation also carried a pathogenic mutation in *MYBPC3*, but this patient had been lost to follow-up and we could not perform a DPD scan. In patients carrying both a pathogenic sarcomere variant and p.V122I (p.V142I), a double hit effect cannot be excluded, but the phenotype of the two patients in whom this was present is not obviously dissimilar to the other HCM patients for the whole cohort. From the clinical point of view, these co-existent sarcomere variants are very relevant to inform family screening.

None of the patients with the pathogenic p.V122I (p.V142I) *TTR* mutation had low voltages. However, low voltages have been reported as infrequent and of low sensitivity in *TTR*-cardiomyopathy and other parameters such as the total QRS score divided by indexed LV mass (g/m<sup>2</sup>) have been proposed as earlier markers of amyloid myocardial infiltration in this context. [23] Similarly, none had a pattern of concentric hypertrophy on imaging. Nevertheless, asymmetric hypertrophy, traditionally described as more typical of sarcomeric hypertrophic cardiomyopathy, was also found to be the most common pattern of hypertrophy in *TTR* cardiomyopathy in a recent publication. [24]

Endemic pathogenic mutations such as p.V30M (p.V50M), p.T60A (p.T80A), p.L111M (p.L131M) and p.I68L (p.I88L) [2],[4],[3] were also not found in our patients, most likely due to differences inherent to the geographical origin of our cohort. In summary, both regional and ethnic bias have contributed to the presence of p.V122I (p.V142I) as the only detected pathogenic *TTR* variant.

Our data show that in a large HCM cohort where cardiac amyloidosis has been previously

excluded after careful clinical assessment, TTR-amyloid is a rare finding. We do not suggest WES should be applied to HCM patients as a clinical screening strategy to exclude TTR-amyloid. However, it is very reasonable to include *TTR* gene as part of the initial genetic testing panel used in these patients, as it is current standard practice in most referral centres, given the relevant implications of an amyloid diagnosis and the overlap in terms of history, ECG and imaging that can exist between sarcomere and amyloid cardiomyopathy, precluding a strong *a priori* clinical suspicion (i.e. no obvious clinical “red-flags” in some patients). Additionally, this genetic sequencing strategy also allows the detection of the rare patients where pathogenic sarcomere variants are co-inherited with *TTR* mutations, with relevant implications in terms of genetic counseling and cascade/predictive genetic testing.

## Conclusions

In this series of carefully phenotyped patients with HCM, pathogenic *TTR* variants occurred in less than 1% of all individuals. In accord with the published literature, the only mutation to be identified, p.V122I (p.V142I), was predominantly found in black individuals and was often non-penetrant.

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## Tables

Table 1. Genetics, demographics and extra-cardiac red-flags.

Patient	TTR variant	MAF GnomAD	MAF matched for ethnicity	Clinvar classification	Ethnicity	Age first assessment	Sex	Last follow-up	Extra cardiac "red-flags"	Other variants
1	R41Q	8.12E-06	0.00006497	VUS	Asian	38	m	2018	no	no
2	R123H	7.218e-5	0.00066583	VUS	Black	42	f	2018	no	no
3	V122I (V142I)	0.001505	0.00006497	pathogenic	Asian	45	m	2018	no	no
4	V122I (V142I)	0.001505	0.00003948	pathogenic	White	36	m	2014	no	<i>MYBPC3</i> <i>p.R495Q</i>
5	V122I (V142I) (homozygous)	0.001505	0.01601897	pathogenic	Black	61	m	2018	yes – carpal tunnel syndrome	<i>MYL3</i> p.R94H
6	V122I (V142I)	0.001505	0.01601897	pathogenic	Black	43	f	2018	no	no

MAF: minor allele frequency; VUS: variant of uncertain significance; M: male, F: female.

Table 2. Main features of the ECG, imaging and clinical data.

Patient	ECG	Echocardiogram	MRI	DPD scan	Holter
1	SR, LBBB (post-miectomy)	ASH and apical HCM, MLVWT 19mm	NA (ICD)	Neg	NSVT
2	SR, LVH; Deep inverted T waves laterally	Apical HCM, MLVWT 18mm; mid-cavity obstruction	Apical predominant hypertrophic cardiomyopathy, MLVWT 18mm, minor LGE	Neg	NSVT
3	SR, Prolonged PR; LVH; Deep inverted T waves laterally	ASH and apical, MLVWT 22mm; LVOTO	NA (declined)	NA (declined)	no
4	SR, bi-atrial enlargement	ASH, MLVWT 20 mm; LVOTO	NA (lost to follow-up)	NA (lost to follow-up)	NA
5	SR, left axis deviation, LBBB-like, no progression R waves V1-V3	ASH with low EF (45%), MLVWT 17mm; low GLS (-13%); Diastolic dysfunction type 3	ASH MLVWT 17mm; LA severely dilated; moderate systolic impairment; extensive sub-endocardial LGE	Pos	NSVT
6	SR, LVH; Deep inverted T waves laterally	ASH, MLVWT 25; low GLS (-6%); LVOTO	ASH MLVWT 25mm; patchy extensive LGE	Neg	NSVT

ASH: asymmetrical septal hypertrophy; DPD: 99mTechnetium labeled 3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy; EF: ejection fraction; GLS: global longitudinal strain; ICD: implantable cardioverter defibrillator; LA: left atria; LBBB: left bundle branch block; LGE: late gadolinium enhancement; LVH: left ventricular hypertrophy; LVOTO: left ventricular outflow tract obstruction; MLVWT: maximal left ventricular wall thickness; MRI: Magnetic resonance imaging; NSVT: non-sustained ventricular tachycardia; NA: not available; SR: sinus rhythm.