Clinical research

The 2373insG mutation in the MYBPC3 gene is a founder mutation, which accounts for nearly one-fourth of the HCM cases in the Netherlands

Marielle Alders[a]*, Roselie Jongbloed[b], Wout Deelen[c],
Arthur van den Wijngaard[d], Pieter Doevendans[e], Folkert Ten Cate[f],
Vera Regitz-Zagrosek[g], Hans-Peter Vosberg[h], Irene van Langen[a],
Arthur Wilde[i], Dennis Dooijes[c], Marcel Mannens[a]

[a] Department of Clinical Genetics, Academic Medical Centre, Amsterdam, The Netherlands
[b] Department of Genetics and Cell Biology, University of Maastricht, Maastricht, The Netherlands
[c] Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands
[d] Department of Clinical Genetics, Academic Hospital Maastricht, Maastricht, The Netherlands
[e] Department of Cardiology, Heart Lung Center Utrecht, Utrecht, The Netherlands
[f] Thoraxcenter, Erasmus MC, Rotterdam, The Netherlands
[g] Cardiovascular Disease in Women Charite and DHZB, Berlin, Germany
[h] Department of Experimental Cardiology, Max-Planck-Institute of Physiological and Clinical Research, Bad Nauheim, Germany
[i] Department of Experimental Cardiology, Academic Medical Centre, Amsterdam, The Netherlands

Aims Hypertrophic cardiomyopathy (HCM) is caused by mutations in genes that encode sarcomeric proteins. In this study we investigated the involvement of the sarcomeric myosin binding protein C in the Dutch HCM population.

Methods and results We initially screened 22 Dutch index patients for mutations in the MYBPC3 gene, which revealed four different mutations in 14 patients. The 2373insG mutation was identified in 10 apparently unrelated patients. A subsequent screening for the 2373insG mutation in a group of another 237 unrelated HCM patients revealed 50 additional carriers of the same genetic defect. Genotyping with polymorphic repeat markers and intragenic SNPs of the 60 Dutch as well as two German and five North American 2373insG carriers indicated they all share the same haplotype.

Conclusion The 2373insG mutation accounts for almost one-fourth of all HCM cases in the Netherlands (60/259), which is predominantly present in the northwestern part of the country (22/66) and is a founder mutation probably originating from the Netherlands.

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Introduction

Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular hypertrophy, myocyte hypertrophy and disarray, and interstitial fibrosis. It has a

* Correspondence to: Dr M. Alders, Clinical Genetics, Academic Medical Centre, Meibergdreef 15, 1105AZ Amsterdam, The Netherlands. Tel: +31 205665110; Fax: +31 206918626
 E-mail address: m.alders@amc.uva.nl (M. Alders).

frequency of 0.2% in the adult population and is a major cause of sudden cardiac death.[1] Hypertrophic cardiomyopathy is very diverse in clinical phenotype and in genotype. Mutations in 11 genes that make up the sarcomere are known to cause HCM. Most common are mutations in the B myosin heavy chain (MYH7, 30%), troponin T (TNNT2, 20%) and the myosin binding protein (MYBPC3, 20%). Other genes, mutated in less than 5% of the cases include TPM1, TNNI3, MYL3, MYL2, ACTC, TTN, TNNC1 and MYH6 (reviewed in Fatkin and Graham[2] and Marian and Roberts[3]). An exception is HCM in combination with Wolf–Parkinson–White syndrome, which is caused by mutations in the PRKAG2 gene, encoding the gamma-2 subunit of AMP-activated protein kinase, but this appears to be a glycogen storage disease rather than a structural sarcomere defect.[4]

The cardiac specific myosin binding protein C (MYBPC3) binds myosin and titin and is suggested to contribute to the structural integrity of the sarcomere, but also to be involved in the regulation of myocyte contractility.[5–7] Mutations in this gene are mainly splice junction, nonsense and insertion/deletion mutations leading to truncation of the protein and whereas HCM caused by the other genes is almost completely penetrated by the second or third decade, mutations in MYBPC3 exhibit a delayed penetrance until the fifth decade of life.[8–12]

Here we describe that mutations in the MYBPC3 gene are the most common cause of HCM in the Dutch population due to a high frequency of the 2373insG mutation (23%), and that this insertion is a founder mutation.

Material and methods

All patients were diagnosed according to the internationally recognized criteria i.e. left ventrical maximal wall thickness ≥15 mm in the absence of confounding disease, and were referred to one of our three DNA diagnostics centres in the Netherlands for HCM mutation analysis. An initial group of 22 patients was screened for MYBPC3 mutations. Genomic DNA was isolated from blood samples and all coding exons of MYBPC3 were amplified according to Carrier et al.,[8] with some minor modifications. Exons were numbered according to Niimura et al.[9] (coding exons 2 to 35). PCR products were analysed on a WAVE DHPLC system (Transgenomics).

Sequencing was performed using the PE dye terminator kit (applied biosystems) and analysed on an ABI310 sequencer (applied biosystems).

In case a novel mutation was identified a group of 100 Dutch anonymous control individuals was screened to identify common polymorphisms.

Mutation specific amplification of the 2373insG mutation was achieved using the 2373insG specific primer AGGACTCCTGC ACAGTACAGGT in combination with the exon 25 primers. This mutation specific amplification was used to screen 237 index patients to investigate the prevalence of the 2373insG mutation in the Dutch HCM population.

Haplotype analysis

Markers D11S1344, D11S4137, D11S986, D11S4177 and D11S4109, flanking the MYBPC3 gene, were amplified in the presence

Table 1 Mutations and polymorphisms in the MYBPC3 gene identified in a group of 22 apparently unrelated HCM patients. Polymorphisms are considered such when present in a normal control population (200 control chromosomes)

	Number of patients	Polymorphisms
Mutations		
2373insG (fs Q791)	9	V158M
2373insG+I1131T	1	S236G
R298X	2	E248D
E258K+R834W	1	Q820R
IVS23–2 delA+A833T	1	A833V
Unclassified		
P161S	1	
D605N	1	
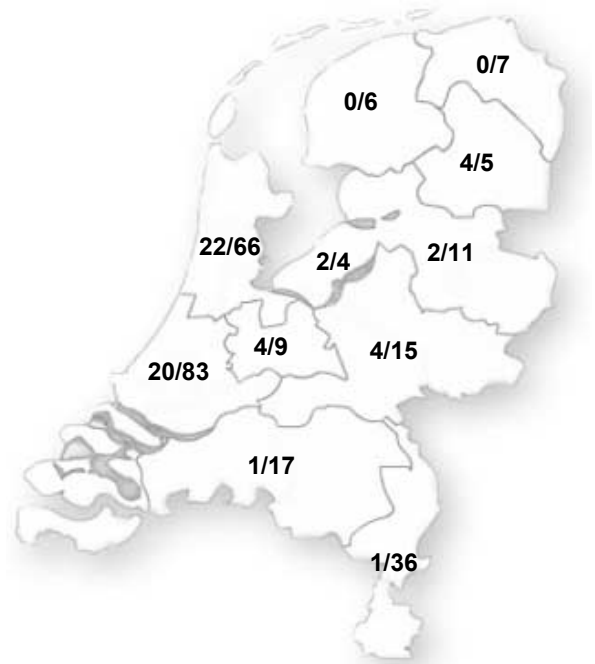
Fig. 1 Distribution of the 2373insG carriers in the Netherlands. This mutation is predominantly present in the northwestern part of the Netherlands.

of Cy5 labelled dCTP. Fragments were analysed on an ALF sequencer (Pharmacia Biotech). In addition, seven single nucleotide polymorphisms (SNPs) within the MYBPC3 gene were genotyped by direct sequence analysis, and most likely haplotypes were constructed. In five probands the phase of the haplotype could be confirmed by family analysis.

Results

Mutation analysis

Mutation detection analysis of the complete coding region of the MYBPC3 gene was performed in a group of

22 unrelated HCM index patients. The results are summarized in Table 1. We identified four clear pathogenic mutations in 14 patients. A one basepair (G) insertion at position 2373 of the MYBPC3 cDNA (2373insG) was found in 10 patients, a nonsense mutation R298X was found in two patients and splice site mutation IVS23-2delA was found in one. These mutations all give a truncated protein product. Missense mutation E258K was found once and is a known pathogenic mutation.[9]

Two additional patients carried a missense mutation, P161S and D605N. Both these mutations were absent in 200 control chromosomes. The amino acid Proline at position 161 is highly conserved and the transition to Serine changes polarity, suggesting this mutation likely to be pathogenic. The D605N transition on the other hand, is less drastic and Aspartic acid (N) at position 605 is present in the Axolotl MYBPC and human skeletal muscle specific isoforms, which makes the pathogenicity of this mutation questionable. Involvement of the latter mutations in hypertrophic cardiomyopathy remains to be determined.

Three patients carried a second mutation in addition to their known pathogenic mutation (see Table 1). These include the amino acid substitutions A833T, R834W and I1131T. Although these mutations were not present in 200 control chromosomes, they occur at less conserved positions and, although some might seem drastic, their contribution to the HCM phenotype remains uncertain. The Alanine to Threonine substitution (at position 833) can be regarded as a relatively mild amino acid change. In addition, the variant A833V is found in the normal population (Table 1) and therefore A833T is more likely to be a polymorphism. It should be noted that the other two cases presented a relatively early onset (17 years, 2373insG+I1131T) or a severe clinical phenotype (E258K+R834W, SCD 16 years). Whether the severity of the phenotype is influenced by the second mutation is uncertain.

We found a remarkably high frequency of the 2373insG mutation in this initial patient sample (10/22). An additional 237 unrelated patients originating from all parts of the Netherlands were examined exclusively for this mutation, which yielded an additional number of 50 carriers. Thus, the overall frequency of the 2373insG mutation in the Dutch HCM population (60/259) is 23% (95% CI: ±5.1%).

Fig. 1 shows the distribution of carriers over the 11 out of 12 provinces of the Netherlands. Most carriers live in the northwestern part of the country.

This specific insertion has been described previously in two German and four North American families (where it was reported as insG791).[9,12,13] Haplotype analysis was performed on 23 carriers and the German (2) and North American (5) probands by using 5 polymorphic CA repeat markers and 7 intragenic SNPs. A shared haplotype for the MYBPC3 SNPs and D11S4117 and D11S4109 could be constructed from all carriers as shown in Table 2. The legitimacy of the constructed haplotype was confirmed in five patients whose family members were available for analysis thus enabling the determination of phase (see example Fig. 2). Testing for the 492T, 3488A and IVS33-66T variants, which are linked with the 2373insG mutation and are uncommon in the normal population confirmed sharing of this haplotype in all remaining carriers.

At least 10 of the 30 genotyped patients did not share the insG-associated alleles for D11S986, D11S1344 or D11S4137. These 10 represented at least six different haplotypes, indicating that at least six recombination's must have occurred between the MYBPC3 gene and the markers D11S986, D11S1344 or D11S4137 (distance <0.9 cm). The high frequency of the mutation and the frequent recombination that occurred (20%) in this small 0.9 cm region indicate it represents a founder mutation that must have arisen at least 25 generations ago (calculated according to Bergman et al.).[14]

Discussion

In this paper we describe the identification of novel mutations in MYBPC3 and a remarkably high incidence of the 2373insG mutation in the Dutch HCM population. Haplotype analysis of all Dutch probands and those of North American and German families described previously shows that this mutation is a founder mutation originating from the Netherlands. The high prevalence of this mutation and the number of recombination's that have occurred in the <0.9 cm region between MYBPC3 and distal CA repeat markers suggest that this mutation arose many centuries ago, and was introduced into North America by European (Dutch) immigrants. Given the prevalence of HCM, i.e. 0.2% in the general population,[1] and the frequency of this mutation in the Dutch HCM patient group (23%), one can very roughly estimate that there must be 7360 2373insG carriers in the Netherlands (population 16 million people).

Most of the HCM causing mutations are unique per family and founder effects are described in only a few cases, usually concerning only a few families with the same mutation.[12,15–18] Only mutations that do not exhibit a high mortality rate before reproductive age can be transmitted and become founder mutations. The strong founder effect of the 2373insG mutation suggests this mutation has mild effects in the first three decades of life, which was indeed noticed in larger families carrying the 2373insG mutation and which has been reported for other MYBPC3 mutations.[9–11] When symptoms develop however, the phenotype is not mild; at least 29 of the probands had family members that died of SCD.

This insertion at position 2373 in the MYBPC3 gene has been extensively analysed by Moolman et al.[13] The insertion creates an alternative splice donor site and leads to alternative splicing, skipping of exon 25 and a frameshift after Q791. However, expression of a truncated protein was not detected in affected heart tissue, suggesting that, in this case, HCM is caused by haploinsufficiency. Hypertrophic cardiomyopathy exhibits a wide spectrum in disease onset, manifestation and progression, even between subjects in the same family. Disease development is thought to be influenced by lifestyle, environmental and also genetic influences. Study in a large

Table 2 The haplotype associated with the 2373insG mutation as determined in 30 probands[a,b,c]

	10		11		12		13		14		15		16		17		18		19		bl14		fe111		bw11		bl261		bw115	
D11S986	7	7	7	8	7	4	7	2	7	9	7	7	7	8	7	3	7	3	7	8	7	7	7	7	7	8	7	10	7	6
D11S4137	1	4	1	4	1	2	1	4	1	4	1	4	1	4	1	1	1	5	1	1	1	10	1	5	1	1	1	1	1	1
D11S1344	1	6	1	6	1	3	1	1	1	7	1	1	1	1	1	2	1	1	1	6	1	6	1	6	1	1	1	6	1	6
472	G	G	G	G	G	A	G	G	G	G	G	G	G	A	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
492	T	C	T	C	T	C	T	T	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	T	T	C	T	C
IVS5-15	3	4	3	3	3	3	3	3	3	4	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
537	C	C	C	C	C	C	C	C	C	T	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
706	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A
3288	A	A	A	A	A	G	A	G	A	A	A	A	A	G	A	A	A	G	A	G	A	A	A	G	A	G	A	A	A	G
IVS34-66	T	C	T	T	T	C	T	C	T	T	T	T	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
D11S4117	6	4	6	4	6	4	6	6	6	3	6	7	6	4	6	4	6	4	6	6	6	4	6	6	6	7	6	6	6	6
D11S4109	1	4	1	3	1	4	1	1	1	2	1	5	1	10	1	8	1	6	1	4	1	5	1	14	1	4	1	5	1	2

	20		21		22		23		M		1		8		2		7		3		6		9		4		5		E	
D11S986	7	7	7	7	7	7	7	5	7	7	7	1	7	7	4	9	4	10	7	7	7	5	7	7	7	2	4	7	7	7
D11S4137	1	9	1	3	1	3	1	3	1	1	3	4	3	7	5	3	1	5	1	4	1	1	1	5	5	10	10	9	1	6
D11S1344	1	1	1	1	1	1	1	5	1	7	1	1	1	5	1	6	1	6	6	4	6	5	6	5	1	7	1	6	2	4
472	G	G	G	G	G	A	G	G	G	G	G	A	G	G	G	G	G	G	G	A	G	G	G	G	G	G	G	G	G	G
492	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
IVS5-15	3	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	4
537	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
706	A	G	A	A	A	A	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
3288	A	A	A	G	A	G	A	G	A	A	A	G	A	G	A	G	A	A	A	G	A	G	A	G	A	G	A	G	A	A
IVS34-66	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	T	T	C	T	C	T	C	T	C	T	C	T	T
D11S4117	6	6	6	4	6	7	6	5	6	3	6	6	6	4	6	4	6	4	6	6	6	5	6	1	6	6	6	3	6	3
D11S4109	1	6	1	1	1	8	1	7	1	13	1	2	1	5	1	3	1	9	1	2	1	8	1	2	1	2	1	12	1	12

[a]Numbers 1-23 are dutch carriers, bl14, fe111, bw11, bl261 and bw115 are five North American probands described by Niimura et al.,[9] M is the German proband described by Moolman et al.,[13] and E is the German proband described by Erdmann et al.[12]

[b]All 2373insG carriers shared the MYBPC3 intragenic SNPs and markers D11S4117 and D11S4109 alleles. Ten probands shared no alleles for D11S1344, D11S4137 or D11S986. The minimal number of haplotypes that can be constructed adds up to 7, indicating that at least 6 recombination's must have occurred.

[c]The presence of this haplotype in the remaining 42 patients was confirmed by testing for the in the normal population relatively uncommon 492T (0.05), 3288A (0.36) and IVS33-66T (0.16) alleles. One patient was homozygous for the 492C allele, suggesting an intragenic recombination.
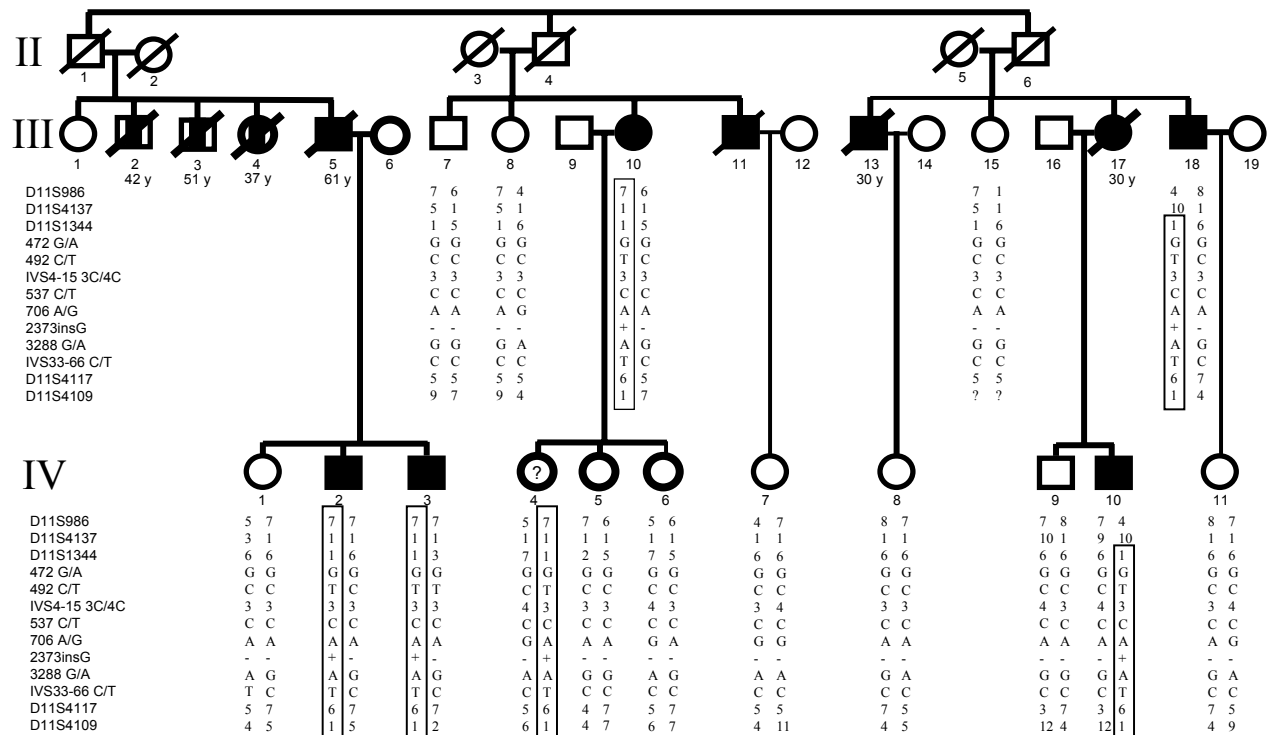
Fig. 2 A pedigree showing the haplotype segregating with the 2373insG mutation. Note that in II-6 a recombination between the MYBPC3 gene and D11S986 must have occurred. Filled symbols indicate affected persons. The age when SCD occurred is indicated when available. III-2, II-3 and III-4 died suddenly, but the cause was never examined.

family with 26 2373insG carriers indicates that poly-morphisms in the renin-angiotensin-aldosterone system influence expression of the mutation[19] and it is likely that additional genes that contribute to phenotypic varia-bility, penetrance and age of onset await identification.

The discovery of a founder mutation with such a high frequency facilitates DNA diagnostics, making cascade screening possible in the near future. A multidisciplinary approach, strict monitoring of pros and cons of this screening and the development of clinical guidelines for testing and follow up of carriers is prerequisites for this large-scale screening.

Acknowledgements

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