Different expressivity of a ventricular essential myosin light chain gene Ala57Gly mutation in familial hypertrophic cardiomyopathy

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Background Familial hypertrophic cardiomyopathy (HCM) is a clinically and genetically heterogeneous disease of the sarcomere. Molecular genetic studies have shown that familial HCM involves mutations in 8 different genes that encode proteins of the myofibrillar apparatus.

Methods We thoroughly searched these genes to find the mutations in 38 probands of unrelated families with familial HCM.

Results We found a novel missense mutation that resulted in Ala57Gly amino acid substitution of the ventricular essential myosin light chain (vMLC1) gene in two unrelated Korean families with familial HCM and one Japanese patient. The mutated site is located in the putative helix-loop-helix region (named EF-hand domain) of the calcium-binding site that is highly conserved in vMLC1 isoforms across the various species. The phenotype of this mutation in the affected families is a classic asymmetric septal hypertrophy, and the disease penetrance in genotyped members older than 18 years is 78%. In one Korean family a 42-year-old woman and two brothers (34 and 38 years old) with the mutation had fully expressed the disease, but two sisters (39 and 29 years old) with the mutation had no phenotypic expression of HCM.

Conclusions Ala57Gly mutation in the vMLC1 gene may exhibit the classic form of familial HCM and widely different penetration of the disease phenotype in the family members with mutation, especially in women. [Am Heart J 2001;141:184-9.]
pedigree showed almost complete disease penetrance in subjects more than 18 years old.

In this study we found a novel point mutation in the vMLC1 gene that correlated with the occurrence of HCM in two unrelated Korean families. The clinical expression of the disease in these families is the usual phenotype of HCM (asymmetric septal hypertrophy) and the mutation is likely to exhibit different penetrance of classic phenotypes in different members of a family, especially in women.

**Material and methods**

**Clinical evaluation of patients**

After giving informed consent, each family member was diagnosed by clinical examination, electrocardiography, and M-mode and two-dimensional echocardiography. The study protocol was approved by internal review board in Samsung Seoul Hospital, Seoul, Korea. Informed consent was obtained from each study subject. In the absence of other known causes associated with left ventricular hypertrophy, a left ventricular septal wall thickness of 13 mm or more was used as a criterion for the diagnosis of HCM. None of family members in the pedigree had a history of systemic hypertension or blood pressure more than 140/90 mm Hg. In addition, blood samples were obtained from each family member for DNA extraction.

**Detection of mutations**

Screening for mutations was performed according to a previously described technique. Individual exons of the eight sarcomeric genes were amplified from genomic DNA and examined for sequence variations by the polymerase chain

![Image](https://example.com/image.png)
reaction–DNA conformation polymorphism (PCR-DCP) method. The primers used to generate the fragment containing exon 3 of the vMLC1 gene were 5’ primer, 5’-TGGGAGTCTGTGGCTCACT-3’ and 3’ primer, 5’-GGGCTCTCGGGCAGGTG-3’. When an abnormal DNA conformation polymorphism (DCP) pattern was observed, the PCR product was cloned by the TA cloning method into the pCRII vector (Invitrogen), and at least 10 independent clones were sequenced on both strands by the dideoxy chain termination methods; a Sequenase ver. 2.0 kit (USB) was used to confirm the sequence variation.

Results
Mutation screening and detection
We have thoroughly searched for mutations in the 8 genes reported to cause familial HCM in 38 probands of unrelated Korean families with familial HCM and found a mutation in the vMLC1 gene in the probands of two families: SM101 and SM325. No sequence variations in other genes beside the vMLC1 gene was found in these probands. DCP analysis of PCR products of the vMLC1 gene exon 3 in the SM101 and SM325 family members showed an abnormal band pattern that cosegregated with the clinical phenotype of HCM (Figure 1, A and B). Subcloning and sequencing revealed that the abnormal fragment had a C-to-G transversion resulting in the replacement of an Alanine (GCC) by a glycine (GGC) in codon 57 in exon 3 (Figure 1, C). The same mutation was also found in one Japanese proband (J0831), who is a 54-year-old man.

We investigated the incidence of this C-to-G transversion in healthy subjects, which indicates that the C-to-G transversion is indeed a functional mutation and not a polymorphism.

Disease penetrance and clinical features of the Ala57Gly mutation
The phenotype of the Ala57Gly mutation consisted of the classic asymmetric septal hypertrophy (ASH). The expressivity of the mutation varied widely among family members carrying the mutation (Table I). Fourteen of 29 genotyped members of the two unrelated Korean families carried the Ala57Gly mutation. Four members of the SM101 family and two members of the SM325 family exhibited the clinical phenotype. Among the 14 family members carrying the mutation, 7 are more than 18 years old. The seven family members who were below age 18 years did not show any phenotypic manifestation of the disease.

In the SM-101 family the proband (II-3) is a 42-year-old woman. When she was 28 years old she had a sensation of chest pressure and syncope. She was diagnosed as having HCM at the time. The echocardiogram showed a classic type of ASH. The interventricular septal thickness in diastole (IVSd) was 32 mm and the left ventricular posterior wall thickness in diastole (LVPWd) was 8 mm. The left ventricular global systolic function was normal. She has led an almost normal life and has few symptoms now. The echocardiographic findings in 1998 had barely progressed from the findings in 1990. There was a mild systolic anterior movement (SAM) of the anterior mitral leaflet during systole and a 30 mm Hg pressure gradient at the left ventricular outflow tract. The left ventricular ejection fraction was 60%.

The proband’s brother (II-2) died suddenly at age 34.
years. He collapsed at age 29 years while climbing stairs. He was diagnosed as HCM at the time. At the age of 34 years, after drinking alcohol, he collapsed and died suddenly. Autopsy was done and the diagnosis of HCM was confirmed.

The proband’s younger brother (II-7) is 38 years old. He has few symptoms. His echocardiogram shows findings to those of the proband. ASH was present and a mild degree of left ventricular outflow pressure gradient (36 mm Hg) was present. Mild SAM was also present.

The proband’s two younger sisters carry the same mutation as the proband. They both do not express any abnormality in terms of clinical phenotype of HCM. II-5 is 39 years old. She is healthy. Her electrocardiogram and echocardiogram are completely normal. II-14 is 29 years old. She is also not symptomatic. Her electrocardiogram and echocardiogram are completely normal.

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The son (III-1, age 16 years) and the daughter (III-2, age 14 years) of II-2 both carry the mutation but are completely free of clinical phenotypic expression. The two sons of II-7 (III-6, III-7) are 9 and 6 years old. They both carry the mutation but are free of clinical symptoms. III-10 is a 4-year-old boy carrying the mutation and does not show the clinical phenotype.

In the SM-325 family the proband (II-1) was a 28-year-old man. He was not symptomatic. He died suddenly at age 28 years after dining with office colleagues. The proband’s father (I-1) is 60 years old. He had an episode of syncope at age 58 years. The echocardiogram showed a thickened interventricular septum and left ventricular posterior wall. SAM was not present. There was no sign of left ventricular obstruction. Mild mitral regurgitation was present. The proband’s grandfather died at age 72 years, 3 years after he had a cerebrovascular accident. The grandfather’s two brothers died suddenly at young ages.

The Japanese patient (JO831) is 54 years old and is not at all related to the Korean families. In his family there was no information available about other family members. This patient carries the same mutation as the Korean families. His electrocardiogram showed left ventricular hypertrophy (LVH). Two-dimensional echocardiography showed ASH. The IVSd was 16 mm and the LVPWd was 10 mm. The left ventricular dimension was 46 mm in end-diastole and 25 mm in end-systole.

### Discussion

The ventricular myosin essential (or alkali) light chain (vMLC1) gene is located on chromosome 3p21.2-p21.3. It is composed of 7 exons, 6 exons of which encode a polypeptide of 195 amino acids. Six putative functional domains have been characterized: an actin-binding site, a proline-rich region, and four helix-loop-helix regions. The vMLC1 is expressed both in the ventricular myocardium and in the slow-twitch muscles. The protein belongs to the superfamily of EF-hand proteins, which includes calmodulin and troponin C. VanBuren et al. reported that myosin light chains affect the ability of the myosin motor to produce force. vMLC1 removal from the skeletal myosin caused a 2-fold reduction in force, which could be reversed by vMLC1 readdition. Thus vMLC1 may play a specific role in the generation of maximum force.

Amino acid alignment across species and isoforms have shown that the Ala57 amino acid in vMLC1 is highly conserved (Figure 2). Moreover, it is localized in the putative helix-loop-helix region (named EF-hand domain) of the calcium binding site, which is important in the structure.
or function of the proteins in the cardiac sarcomere. These observations indicate that the change caused by Ala57Gly substitution is likely to affect essential function or structure of vMLC1 protein.

Mutations in the gene of vMLC1 and vMLC2 have been reported.7 By screening DNA isolated from representatives of 383 unrelated families with familial HCM, we found 2 missense mutations (Met149Val, Arg154His) in exon 4 of the vMLC1 gene, one in all affected members of a single family and the other in an unrelated individual. Half these patients (6/13) exhibited a rare phenotype involving mid left ventricular chamber thickening and a ragged red fiber pattern in histochemical staining of deltoid muscle biopsy specimens. On the other hand, the Ala57Gly mutation in the vMLC1 gene in the two Korean families appear to be associated with the classic form of familial HCM and no midventricular obstruction was found in any members of these families. The results suggest that the phenotype of familial HCM can vary widely depending on the site of the mutation in the same gene. We also found a vMLC2 mutation in two Japanese HCM families. The clinical phenotype of patients of these families also had the classic form of HCM (unpublished observations). In addition, we have searched for mutations in the 8 known disease genes in 5 patients with the midventricular obstruction phenotype of HCM. We found a cMBPC mutation in one patient but no vMLC1 or vMLC2 mutation was found in any of these patients. These observations suggest that the midventricular obstruction phenotype of HCM reported by Poetter et al7 is not a feature specific to myosin light chain mutations.

The penetrance of the disease also vary widely according to the type of sarcomeric protein that is mutated. The Met149Val mutation and Arg154His mutation in vMLC1 had 100% disease penetrance in subjects above the age of 18 years.7 We previously reported that a Gly716Arg mutation in β-MHC caused early expression of a malignant phenotype and complete disease penetrance.18 Clinical phenotypes caused by mutations in cMBPC and cTnT were characterized by low disease penetrance of familial HCM.10-13 Comparisons of the clinical phenotype and genetic status of 16 families harboring a mutation in the gene for cMBPC have shown that the disease starts to develop predominantly after middle age.12 The disease caused by cTnT mutations is usually associated with a 20% incidence of nonpenetrance, a relatively mild and sometimes subclinical hypertrophy but a high incidence of sudden death that can occur even in the absence of significant clinical LVH.10,11

In the Korean families carrying the Ala57Gly mutation in the vMLC1 gene, all genotyped members below the age of 18 years had normal clinical phenotypes, whereas the disease penetrance of this mutation in the genotyped subjects older than 18 years was 78% (7/9, Table I). The Ala57Gly mutation of vMLC1 is associated with a markedly different penetrance in affected individuals. In one family a 42-year-old sister and a 38-year-old brother fully expressed the clinical phenotype, whereas two younger sisters (38 and 30 years old) were completely free of the disease, although they carried the same mutation (Table I). Two affected members in two different families had sudden death, whereas other affected members had benign clinical course with mild symptoms. These marked differences in the penetrance of the disease among affected members in the same family provoke questions about possi-
ble roles of modifying factors affecting the manifestation of the disease.

Genetic studies have also revealed the existence of clinically healthy individuals carrying the mutant allele. In some cases, as many as one fourth of the genetically affected individuals do not express the disease.

Several mechanisms could account for the large variability in the phenotypic expression of the mutations, such as variations in the degree of functional impairment that is caused in the sarcomere by the mutation (it may vary markedly with the position of the mutation in the molecule and the type of protein involved), environmental factors and acquired traits (e.g., lifestyle, risk factors, exercise), and the existence of modifier genes or polymorphisms that could modulate the phenotypic expression of the disease.

Marian and Roberts reported that I/D polymorphism in the angiotensin-converting enzyme gene was associated with disease expression in a family with mutations of the Arg103 codon in the c-MHC gene. We therefore analyzed several possible modifier gene polymorphisms such as angiotensin-converting enzyme gene I/D polymorphism, angiotensinogen gene polymorphism, and endothelin-1 gene polymorphism in the family members of the current study. We did not find any significant differences between clinically expressed and unexpressed members carrying the Ala57Gly mutation (data not shown).

Our results indicate that it is highly likely that the Arg57Gly mutation is the cause of HCM in the study subjects. But it is necessary to analyze more families for this mutation before we can definitely state that the Arg57Gly mutation is responsible for the disease.

References