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The Human β-Myosin Heavy Chain Gene: Sequence Diversity and Functional Characteristics of the Protein

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Abstract The β-myosin heavy chain gene (MYH7) encodes the motor protein that drives myocardial contraction. It has been proven to be a disease gene for hypertrophic cardiomyopathy (HCM). We analyzed the DNA sequence variation of MYH7 (about 16 kb) of eight individuals: six patients with HCM and two healthy controls. The overall DNA sequence identity was up to 97.2% compared to Jaenicke and coworkers (Jaenicke et al. [1990] Genomics 8:194–206), while the corresponding amino acid sequences revealed 100% identity. In HCM patients, eleven nucleotide substitutions were identified but no causative disease mutation was found: six were detected in coding, four in intronic, and one in 5′ regulatory regions. The average nucleotide diversity across this locus was 0.015% with an average of 0.02% in the coding and 0.012% in the noncoding sequence. Analysis of the kinetic behaviour of β-MHC in the intact contractile structure of normal individuals and HCM patients revealed apparent rate constants of tension development ranging between 1.58 s^{-1} and 1.48 s^{-1}. J. Cell. Biochem. 79:566–575, 2000. © 2000 Wiley-Liss, Inc.

Key words: hypertrophic cardiomyopathy; β-myosin heavy chain gene; molecular genetics; sequence variation; kinetic behaviour

Hypertrophic cardiomyopathy (HCM) is a genetically and clinically heterogeneous disorder that is inherited in an autosomal dominant pattern. A typical manifestation includes hypertrophy of the ventricular septum. Sudden cardiac death is the most severe complication [Spirito et al., 1997]. At the present time, eight HCM loci have been identified on chromosomes 1, 3, 7, 11, 12, 14, 15, and 19. Seven of these have been reported to code proteins of the cardiac sarcomere. The responsible proteins are β-MHC [Geisterfer-Lowrance et al., 1990], cardiac troponin T, α-tropomyosin [Thierfelder et al., 1994], cardiac myosin binding protein C [Bonne et al., 1995; Watkins et al., 1995a], cardiac troponin I [Kimura et al., 1997], and the essential and regulatory light chain [Poetter et al., 1996]. So far, the eighth locus has been mapped on chromosome 7, but the gene has not been detected [Carrier et al., 1993]. The β-MHC, the motor protein in the human ventricle, represents one of the most frequently affected genes [Watkins et al., 1995b, 1995c]. Of these, 45 are missense and three are deletion mutations in the coding sequence. All of these mutations are located in the first 23 exons that encode the globular head of the protein except for two mutations [Fougerousse et al., 1992; Ohsuzu et al., 1997; Warlick et al., 1992; Vosberg and Haberbosch, 1998]. The C-terminus of this protein is essential for tail-to-tail interbinding of the myosin molecules that form the thick filament of the sarcomere. The β-MHC gene (MYH7 or MYHCB) has been assigned to chromosome 14q11.2-q13 [Saez et al., 1987; Cox et al., 1994; Matsuoka et al., 1988].

We focused on the analysis of DNA sequence variability of the human MYH7, considering all

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exonic sequences and those noncoding sequences that include the prominent regulatory and/or supporting motifs with regard to splicing and gene expression. So far, two groups cloned and sequenced the entire gene [Jaenicke et al., 1990; Liew et al., 1990]. Comparison of these data showed several discrepancies in their nucleotide sequences (GenBank/EMBL accession numbers X52889 and M30603, M30604, M30605) as well as their amino acid sequences (Swiss-Prot accession number P12883): eight diverse amino acid sites have been detected, of these, six were detected in the rodlike tail. Furthermore, several investigators have partially sequenced the MYH7 locus and described eight additional divergences in their amino acid sequences [Saez and Leinwand, 1986; Kurabayashi et al., 1988; Yamauchi-Takihara et al., 1989; Bober et al., 1990]. Altogether, 16 variable sites have been found so far in the amino acid sequence.

Here we compared the nucleotide sequence of the entire MYH7 in eight individuals, six of whom were affected by HCM, two of whom were not. In addition, we characterized the kinetic behaviour of the β-MHC in the intact contractile structure of human ventricular muscle of the same normal individuals previously characterized. We used the rate constant of tension development (k_{dev}) as parameter for cross-bridge kinetics at 21°C and maximal Ca^{2+} activation determined from the tension rise following photolytic release of ATP from caged-ATP in rigor [Morano et al., 1995, 1996]. This has been performed to define experimental conditions for the analysis of functional consequences of MYH7 sequence variations.

MATERIAL AND METHODS

Subjects

Blood and tissue samples were obtained from two normal individuals and six patients with HCM. Normal interventricular tissue was obtained from hearts that could not be transplanted due to technical reasons. Patients with HCM were undergoing open heart surgery at the department for thoracic and cardiac surgery at the Heinrich-Heine University Düsseldorf, Germany. All patients had severe hypertrophic obstructive cardiomyopathy predominantly of the interventricular septum with severe obstruction of the left ventricular outflow tract and suffered from dyspnea, angina, and/or syncope. Surgical treatment consisted in all cases of transaortal subvalvular myectomy. The clinical evaluation consisted of physical examination, electrocardiography, and echocardiography. The myocardial tissue was directly prepared for demembranated multicellular fibers. DNA was extracted from 10 ml ethylene diaminetetraacetate (EDTA)-blood as described [Lahiri and Nurnberger, 1991].

Oligonucleotide Design

Polymerase chain reaction (PCR) primers were designed to allow amplification at annealing temperatures of 55°C to 70°C, using the program PrimerSelect from the Dnastar software package.

Polymerase Chain Reaction

Twelve PCR products per individual (363–2297 bp in size) were generated to cover the codogene exons and most of the intronic sequences of the coding and complementary strands, about 16 kb in total. We used PCR primers located in the adjacent intronic sequence, as described in Table I and Figure 1. PCR was performed in a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM of each nucleoside triphosphate, 30 pmoles of each primer, 100 ng genomic DNA, and 3U Taq polymerase in a Perkin Elmer 9600 cycler. Polymerase was added during hot-start following 35 cycles at 94°C (15 s), 60–68°C (15 s) and 72°C (30 s). Routinely, both strands were sequenced using PCR and internal oligonucleotides as sequencing primers (Table I).

DNA Sequence Analysis

Purification of PCR products prior to sequencing was done using an automated procedure based on a magnetic beading technique. Sequencing of these products was performed using the BigDye Terminator kit from PE/ABI and the Thermo Sequenase kit from Amersham with ET-labeled primers; amplification primers and internal oligonucleotides were used as sequencing primers. Parameters for cycling and reaction conditions were always conducted according to the manufacturer's protocols. For data processing and base calling, we used the Macintosh-based Sequencing Analysis Software version 3.2 from PE/ABI.

Sequence Analysis and Variant Identification

For the sequence data comparison, we used the UNIX-based STADEN software package,

which is a specific program for large-scale sequence projects [Bonfield and Staden, 1995; Bonfield et al., 1995]. Electropherograms of the coding and complementary strands were genotyped independently by two expert readers, who were not involved in diagnosis. So, each variant position has been confirmed in both strands using labeled and unlabeled primers, respectively. Genotypic data of both strands and readers were compared and scored. If the genotype could not be resolved, corresponding samples were resequenced.

Genome Sequence Analysis and Database Search

Analysis of the nucleotide variation in the 5′ regulatory region was carried out using the MatInspector program version 2.1 [Quandt et al., 1995] to check the 5′ flanking sequence against the TRANSFAC database [Wingender et al., 1997]. To analyze the genomic sequence variants, we used a software analysis package called NIX evaluated from the human genome mapping project, medical research (http://www.hgmp.mrc.ac.uk). The package included exon prediction programs and Blast analysis of protein and nucleotide sequences including ESTs. To identify and align homologous sequences we used the ClustalX and lAlign programs [Huang et al., 1990].

Kinetic Behaviour of the β-MHC in Human Heart Fibers

All biochemical as well as mechanical experiments were performed with demembranated multicellular heart fibers (skinned fibers) prepared as described previously. In short, ventricular fiber bundles (about 1-mm thick, 1–6 mm length) were incubated in a solution containing 20 mM imidazole, 10 mM NaN_3, 5 mM ATP, 5 mM $MgCl_2$, 4 mM EGTA, 2 mM DTE, 50% glycerol, 1% Triton X-100, pH 7 at 4°C for 18–20 h. Subsequently, the fibers were transferred into the same solution but without Triton X-100 and stored at −20°C.

To obtain a direct correlation and to prevent the influence of possible regional inhomogeneities of atrial myofin light chain 1 (ALC-1) expression, myosin light chains (MLCs) of the same skinned fiber previously investigated mechanically were analyzed by a high-resolution two-dimensional gel electrophoresis (2D PAGE) technique. Isoelectric focussing (first dimension) was performed in glass capillaries (12.5 cm length, 1 mm inner diameter) using the pH gradient 4.5–5.4 (Pharmalytes Pharmacia, Sweden). The gels were run overnight at 600 V constant for the first dimensional separation. The second dimension was a sodium dodecylsulfate-electrophoresis, using slab gels 10.5 × 9.5 cm, 1 mm thick. The gels were stained with Coomassie Blue, and the MLCs were evaluated by computer-assisted scanner densitometry (ScanPack, Biometra, Germany).

For all mechanical experiments, fibers were dissected into bundles of 150–200 μm diameters and 3–6 mm length under a preparation microscope. Fibers were mounted isometrically between a force transducer and a length-step generator with microsyringes in relaxation solution. Length was adjusted such that resting tension was threshold, using a micrometer screw. Sarcomere length at resting tension was always between 1.95 and 2.0 μm as detected by laser diffraction analysis. Relaxation solution contained 25 mM imidazol, 10 mM ATP, 10 mM creatinphosphate, 12.5 mM $MgCl_2$, 5 mM NaN_3, 1 mM DTE, 5 mM EGTA, 12.5 mM KCl, 380 U/ml creatine kinase, pH 7. Contraction solution was the same as relaxation solution except that EGTA was substituted by 5 mM CaEGTA. Ca^{2+}-free rigor solution contained 2.5 mM $MgCl_2$, 5 mM EGTA, 2 mM DTE, 100 mM 2,3-butanedione monoxime (BDM), pH 7. Ca^{2+} rigor contained 2.5 mM $MgCl_2$, 5 mM CaEGTA, 2 mM DTE, pH 7. Photolysis solution was made by adding 5 mM P_3-1-(2-nitrophenyl)-ethyladenosine-5′-triphosphate (caged-ATP; Calbiochem, San Diego, CA) and 10 mM DTE to the Ca^{2+}-rigor solution. Ionic strength was adjusted with KCl to 130 mM. Free Ca^{2+} concentrations were calculated according to a commercially available computer program [Fabiato and Fabiato, 1979].

Dissected fiber bundles were mounted isometrically in a 40-μl quartz cuvette (Scientific Instruments, Heidelberg, Germany). Photolysis of caged-ATP was achieved using a xenon flash lamp system (G. Rapp Optoelectronic, Hamburg, Germany). The lamp delivered ultraviolet light pulses with a duration of about 1 ms and was focused through a UG-11 filter. Force signals were displayed on a storage digital oscilloscope (HM 408) and analyzed with an IBM-compatible personal computer. The experimental design was the same as elaborated previously [Morano et al., 1995, 1996]. In short, the fibers were mounted isometrically and in-

TABLE I. Oligonucleotides Defining the 12 Target Segments and Sequencing Reactions of the Human MYH7[a]

Fragment	Oligo	Sequence (5′–3′)	Oligo	Sequence (5′–3′)	PCR Size
1	**F A**	**GCCTCACTCCTTCCAAGCTGACAG**	R A	ACTCACGTTCCTGCTCTGCCCCAC	1,613 bp
	F B	GTGGGGCAGAGCAGGAACGTGAGT	R B	AGCCAGGTGGCAAGAACAATAACC	
	F C	TTCTTGCCACCTGGCTAGGAAAT	R C	TCGTCATTGTTATGGCATGGACTG	
	F caat	CAGTCCATGCCATAACAATGACGA	**R caat**	**ACATACGCCCATGTTTAGACCTG**	
2	**F 3a**	**CATGGTGCTAGGTTTTGGGGCTCC**	R 3a	CAGGTCTGCATGGGCATGGGGCAT	1,652 bp
	F 4	ATCCCTTCTGTCTTCCCTGTGAGAT	R 4	ATGTTGGGACGAGTTAGAGTGTAA	
	F 5	TTACACTCTAACTCGTCCCAACAT	R 5	GCCTTCTCCCTTCCTTCTCCCTCTCC	
	F 6a	AGAAGCCCCACGAGAGCATCCTGT	**R 6a**	**GGGAAAGAAAATGCAGAGGTGAAGC**	
3	**F 7**	**GAGGCTTGTCGGTCTCCAGTAGTA**	R 7	CATATCTGAGACCATTCCTCCACCAG	1,570 bp
	F 9	CTGGTGGAGGAATGGTCTCAGATATG	R 9	GAGAGATAGAACGAGACCCGAGGAA	
	F 10	CTCTCGTCGCTCTTTGTCGTCT	R 10	GCTCGCCACAGCACATGCCTGAGGAA	
	F 11	TCCTCAGGCATGTGCTGTGGCGAG	**R 11**	**TTTGATAGAACACCAGATACTG**	
4	**F 11**	**TCCTCAGGCATGTGCTGTGGCGAG**	R 11	TTTGATAGAACACCAGATACTG	2,323 bp
	F 12	GAACCCCACAGGATTAAGGATAC	R 12	TGGGCAACAGAGCAAGAGCAAGACT	
	F 13	GAACCCCACAGGATTAAGGATAC	R 13	TGAACTTGAAAACTCTCATCCC	
	F 14	GGGATGAGAGTTTTCAAGTTCA	R 14	GAAGACAGAGTAAATGACTGCCTC	
	F 15	GAGGCAGTCATTTACTCTGTCTTCA	**R 15**	**GGGAGAGGGGCTGCTATTTTGTCTAT**	
5	**F 16**	**CAGAATCCATGTCACCTGTGTG**	R 16	TGGCTCAGAACCTTGGCAGAATCCC	1,267 bp
	F 17	GGATGGAACTGTGTGAAGAAACTGA	R 17	GAGAGAAGGGAGATGGGAAGTAA	
	F 18	TTCTCTCCTCTTTCCCTTCTGTCT	**R 18**	**TGTGGTGGTACGTAGGGAGATGTC**	
6	**F 19**	**GCTAGAAGAAAATGAAACAAATGAT**	R 19	CAGTGAGTCTGCTCTGCCCCATAG	2,727 bp
	F 20	CCTATGGGGCAGAGCAGACTCACTG	R 20	CTAGAAAAGGATTGCAGGAGAGGTCA	
	F 21	TGACCTCTCCTGCAATCCTTTTCTA	R 21	CTTCATCCCTCCCACCCTTCCTGAG	
	F 22	CTCAGGAAGGGTGGGAGGGATGAAG	R 22	GGAGGTGCAGGGTCTGTGGGAAGT	
	F 23	CCCTCCTATTTGAGTGATGTGC	R 23	TCAAGGTCAGTATGGTCTGAGAGT	
	F 24	ACTCTCAGACCATCATGACCTTGA	**R 24**	**CACAGAGCTCTGGGCACAGATAGAC**	
7	**F 25**	**CCAAGTCCTGAACACAAGATTTAC**	**R 25**	**CCACTTGTGGAGGCTGCGTGAG**	363 bp
8	**F 26**	**CACTCCTCCCATTACATTCTTGT**	R 26	ATTAATCATTCCTGTAATGCTGT	1,448 bp
	F 27	ATTCCAGTGGAGGGGTCCAGGCG	**R 27**	**AAGGGGAGGTGGGAGGAGGAAGTT**	

TABLE I. Oligonucleotides Defining the 12 Target Segments and Sequencing Reactions of the Human MYH7[a] (continued)

Fragment	Oligo	Sequence (5′–3′)	Oligo	Sequence (5′–3′)	PCR Size
9	**F 28**	**CTTTCTGTCCATGTAGTGTCTCTT**	R28	TCTGTGTGTGCGTGTATTGGCTT	2,374bp
	F 29	AAGCCAATACACGCACACACAGA	R29	TGTTGGTCCACAGCCAGCCTTAT	
	F 30	ATAAGGCTGGCTGTGGACCAACA	R30	CCGGGGCAGAGTCCTCCTGTGT	
	F 31	CTTCTGAGGTTTCAAGGATTGTCT	**R31**	**ATGGCTCTGGCCTCTCACTGA**	
10	**F 32**	**TCAGTGAGAGGCCAGAGCCAT**	R32	CCAGTGGAGTTGGAGGGACACG	1,499bp
	F 33	GGCGTGTCCCTCCAACTCCACTG	R33	GTCATTCATTCACTCCACAAATCT	
	F34b	CTTCTTTGAACCACTTACACCACTCT	**R34**	**TCAAGACACTACTGCTTACGCCAG**	
11	**F 35**	**GATTCCTCCTGCCCCTAGGTTACT**	R35	TAGGTTAAGCATTGAGCATCTATG	1,003bp
	F 36	CATAGATGCTCAATGCTTAACCTA	R36	ACACTTGCTGCCCAGCCCACGG	
	F 37	CCGTGGGCTGGGCAGCAAGTGTG	**R37**	**TCAGCTGGTTGTCACTGTGGCTAT**	
12	**F 38**	**ACCTTCTATGACTGTGCCATCTTCA**	R38	GTATGCCTGCTGTGGGGGTGACTA	1,868bp
	F 40	CTCAGCCTCCCAAAGTACGGATTA	**R40**	**GGTTTTCAGTGCTGCTCCACGAA**	

[a]Oligonucleotides of the coding (F) and complementary strands (R) were used as amplification as well as sequencing primers (bold type) or internal sequencing primers (regular type).

cubated in relaxation solution and subsequently in Ca^{2+}-free rigor solution containing 100 mM BDM for 30 min. In the presence of BDM, a "low-tension rigor" developed. Subsequently, the fibers were transferred in Ca^{2+}-containing rigor solution (pCa 4.5) for 4 min and then in Ca^{2+} containing rigor plus 5 mM caged-ATP for 5 min. Force signals were displayed on a storage oscilloscope (HM 408) and analyzed with an IBM compatible personal computer using the Proscope Hameg software SP91. Since the tension rise followed a monoexponential function [Morano et al., 1995], rate constant of force development (k_{dev}) was calculated from the half-time of tension development (t1/2) using the formula $t1/2 = \ln 2/k_{dev}$. Temperature was always 21°C.

RESULTS

Analysis of Nucleotide Variation in the Human MYH7 Locus

We have completely analyzed the DNA sequence variation of the human MYH7 locus in eight individuals, six of whom were affected by HCM. MYH7 consists of 40 exons distributed across 23 kb of genomic DNA. For this study, we focused on the entire gene that includes all 38 coding exons as well as most of the intermediate intronic sequence, and a part of the 5′ regulatory region. The gene-coding region involves the exons 3–40 of 5,805 bp in length, approximately 36% of the target sequence and the noncoding sequences upstream and between the gene-coding sequences of about 10 kb, approximately 64% of the target sequence.

As to DNA baseline sequence, two different types of genomic sequences have been published [Jaenicke et al., 1990; Liew et al., 1990]. Both encode the same protein but indicate eight (with regard to the known partial MYH7 DNA sequences) and 16 diverse amino acid sites [Saez and Leinwand, 1986; Kurabayashi et al., 1988; Yamauchi-Takihara et al., 1989; Bober et al., 1990] in total.

We amplified this target locus by dissection into twelve specific long fragments (Fig. 1) and detected the DNA variants by DNA sequencing, using PCR and internal oligonucleotides as labeled and nonlabeled primers, followed by the contemporary UNIX-based STADEN software package analysis [Bonfield and Staden, 1995; Bonfield et al., 1995].

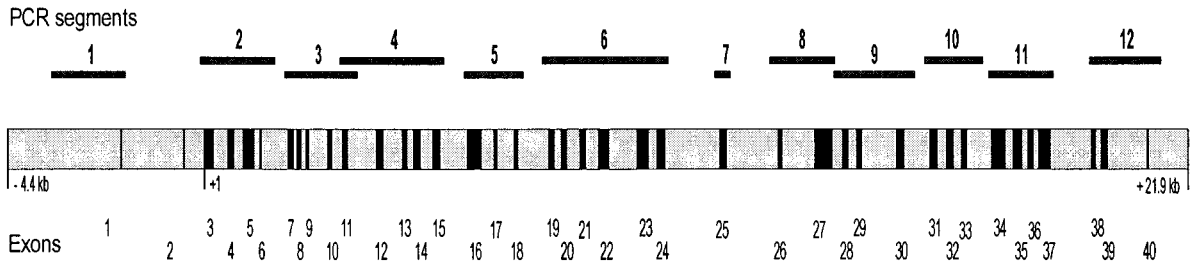

Fig. 1. Dissection of the human MYH7 locus into 12 target segments. DNA segments are identical for the coding and complementary strands. Base pair coordinates are given relative to the translation start in the codogene sequence (+1) and in the 5′ regulatory region (−1), with the exception of intronic regions 1–40 (IVS1+n to IVS40+n). Segments as well as baseline are drawn to scale.

In this study, we identified a number of variations relative to the published sequence [Jaenicke et al., 1990; Liew et al., 1990] (Fig. 2). The genomic sequence published by Jaenicke and coworkers [1990] demonstrated a higher identity with our DNA sequence, compared to the one reported by Liew and coworkers [1990]. The overall DNA sequence identity with Jaenicke et al. [1990] was up to 97.2%, and the corresponding amino acid sequences of our and the MYH7 protein of Jaenicke et al. [1990] were identical. However, amino acid sequence of MYH7 was different from the amino acid sequence described by Liew et al. [1990] at eight positions: D107, CII672, R858, KL942, A1124, C1159, insL1681, and EQ1703-1704. Moreover, we did not detect any of the eight variable amino acid residues that have previously been published in other partial sequences [Saez and Leinwand, 1986; Kurabayashi et al., 1988; Yamauchi-Takihara et al., 1989; Bober et al., 1990].

Distribution of Nucleotide Variation of the Human MYH7 Locus

We identified 11 new biallelic variants in the MYH7 locus, covering about 16 kb, none of which had been described before, in eight individuals (Fig. 3, Table II). In total, we obtained data from 128 kb of genomic DNA. The average nucleotide diversity across this region was 0.015% (or an average of one variable site every 6.65 kb) with an average of 0.02% in the coding and 0.012% in the noncoding region.

Most of the analyzed sequence was noncoding. In the coding region, six sites of 5,805 bp varied and in the noncoding regions, five sites of about 10 kb varied. Therefore, the relative number of varying sites in coding and noncoding regions were different. One consequence of the observed sequence variation is that each individual is heterozygous at an average of 11 sites in the analyzed DNA sequence of the MHY7 gene (Table II). Single-base substitutions were the only form of DNA variation that could be observed. Among these, transition substitutions were more prevalent (eight of 11, 73%) than transversions (three of 11, 27%).

The six coding variants were silent, third-base substitutions (189C>T, 1128C>T, 2967T>C, 4282T>C, 4566C>T, 5106G>A). Four substitutions were observed in the intronic sequences (IVS19+91A>G, IVS28+27T>A, IVS29+204A>G, IVS29+347C>A). Consensus criteria AG/GT were met at all 5′ splice sites, criteria AG/NN at all 3′ splice sites, and criteria NPyPyPuAPy at all branch sites. Also, splice-assistance sites that involve alternative splicing, in addition to the classical consensus sites, were not detected in the intronic sequences. Furthermore, we used the software analysis package called NIX for the analysis of ESTs that could be generated as a result of one of these 11 variants detected in the DNA sequence of the MYH. In fact, no additional EST could be detected. One single nucleotide substitution was located in the 5′ regulatory region 1,967 bp upstream from the translation start site. Further analysis, using the MatInspector program package, failed to reveal putative transcription regulatory motifs in the variable site.

Five of these variants, three in the codogene and two in intronic regions, occur in the patient group as well as in the control group (189C>T, IVS28+27T>A, IVS29+347C>A, 4282T>C, 4566C>T). The remaining six variants were detected only in the patient group (−1967G>A, 1128C>T, IVS19+91A>G, 2967T>C, IVS29+

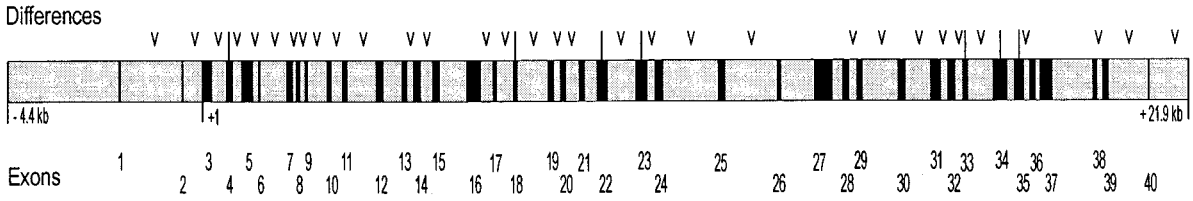

Fig. 2. Single nucleotide polymorphisms (SNPs) in the MYH7 locus, when comparing the sequence to those previously described [Jaenicke et al., 1990; Liew et al., 1990]. SNPs in the nucleotide sequence of exons (|) and introns (V) are indicated. Base pair coordinates are given relative to the translation start in the codogene sequence (+1) and in the 5′ regulatory region (−1), with the exception of intronic regions 1–40 (IVS1+n to IVS40+n). Segments as well as baseline are drawn to scale.

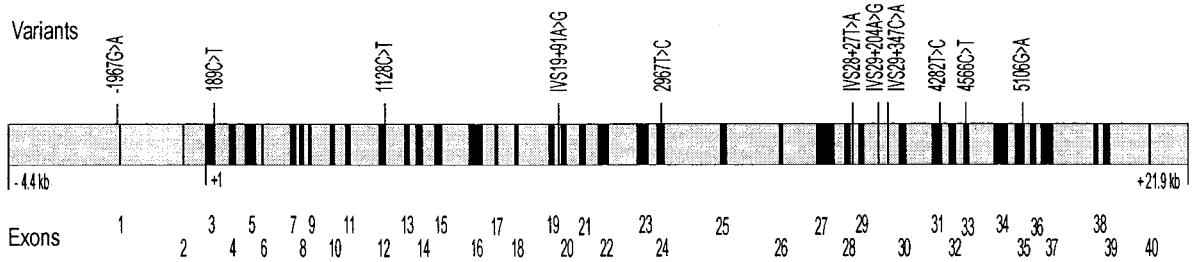

Fig. 3. Variant spectrum of the human MYH7 sequence. All gene variants are specified by position, number, and substitution. Base pair coordinates are given relative to the translation start in the codogene sequence (+1) and in the 5′ regulatory region (−1), with the exception of intronic regions 1–40 (IVS1+n to IVS40+n). Segments as well as baseline are drawn to scale.

204A>G, 5106G>A). Among these, three were single nucleotide variants (−1967G>A, 1128C>T, IVS19+91A>G), of which two were identified in the same patient (−1967G>A, 1128C>T). Remarkably, the variable intronic site IVS29+204A>G was exclusively observed in homozygous conditions.

ALC-1 Expression Analysis by 2D-PAGE

Since the level of ALC-1 expressed in the heart strongly influenced the cross-bridge kinetics [Morano et al., 1996], we studied ALC-1 content in the skinned fibers subsequent to the mechanical experiment by 2D-PAGE. Ventricular fibers from normal individuals contained no detectable amounts of ALC-1. However, four of the six patients with HCM expressed high levels of ventricular ALC-1 (not shown).

Rate Constant of Tension Development (k_{dev})

Figure 4 shows an original registration of tension development after photolytic release of ATP from caged-ATP in low tension rigor of a demembranated fiber from a normal human ventricle with high time resolution. Half time of tension development of control fibers (i.e., without superfusate incubation) was 466 ± 122 ms, equivalent a rate constant of 1.58 ± 0.4 s^{-1} (two normal individuals, four different fibers per individual). Those HCM patients without ventricular ALC-1 expression and normal primary sequence of their β-MHC gene revealed apparent k_{dev} of 1.48 ± 0.42 s^{-1}, and 1.54 ± 0.34 s^{-1}, (patient numbers 1211 and 1212, four different fibers per patient), i.e., not statistically significant to the normal ventricular fibers.

DISCUSSION

We have identified a genomic sequence of the MYH7 that differs from the previously published DNA sequences. The overall DNA sequence identity was up to 97.2%, whereby the sequence published by Jaenicke and coworkers [1990] demonstrated a higher homology with our DNA sequence, compared to the one of Liew and coworkers [1990]. In comparison, the pairwise alignment of our sequences to both published sequences demonstrated an identity of 97.2% and 93.8%, respectively. The amino acid sequences corresponding to our sequence and that of Jaenicke and coworkers [1990] were identical, whereas the amino acid se-

TABLE II. Genotype Variation of the Human MYH7[a]

	−1967G>A	189C>T	1128C>T	IVS19+ 91A>G	2967T>C	IVS28+ 27T>A	IVS29+ 204A>G	IVS29+ 347C>A	4282T>C	4566C>T	5106G>A
N123	11	12	11	11	11	22	11	22	12	12	11
N136	11	12	11	11	11	11	11	11	11	11	11
1092	11	12	11	11	11	11	22	11	11	11	11
1093	11	12	11	11	12	22	11	22	22	11	12
1097	12	12	12	11	11	12	11	12	11	12	11
1211	11	11	11	11	12	11	11	11	11	12	11
1212	11	12	11	12	12	12	22	12	11	12	12
1230	11	11	11	11	11	11	22	11	11	11	11

[a]Genotypes in eight individuals, two controls (N), and six patients. All variants are specified by position numbers and nucleotide substitution. Base pair coordinates are given relative to the translation start in the codogene sequence (+1) and in the 5′ regulatory region (−1), and those in the intronic regions relative to the start of intervening sequences (IVSx+1), respectively. Coding: 11, homozygous identical with the reference sequence; 12, heterozygous; and 22, homozygous diverse from the reference sequence.

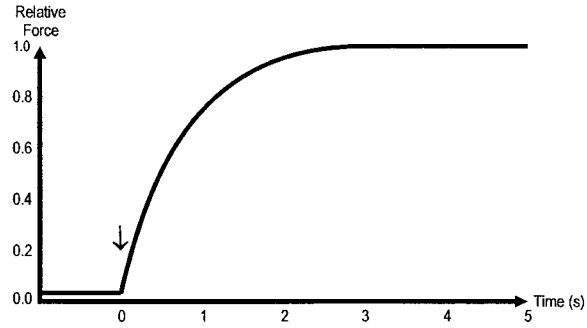

Fig. 4. Isometric force development of a demembranated multicellular human ventricular fiber obtained from a normal human heart after photolytical release of ATP from caged-ATP at low-tension rigor. Light flash is indicated as arrow.

quences of Liew et al. [1990] showed a 99.6% identity.

We have analyzed the entire MYH7 locus in eight individuals, six HCM-affected and two unaffected. In this context, we detected 11 biallelic variants in the 16 kb sequence, including exonic, intronic, and 5′ regulatory regions of each individual, whereby three of these were single-nucleotide substitutions. None of these have been previously reported. In total, we sequenced 128kb of all eight individuals. Of these variants, six are in coding sequences. The most striking feature of these data, however, is the distribution of variant sites. The total sequence diversity was (0.015), and the diversity was twofold more in coding (0.02) than in noncoding DNA (0.012). By sequence comparison of the MYH7, we detected a similar level of variation in a common analysis of allelic variation obtained from a scan of the human genome sequence database [Li and Sadler, 1991]. A larger number of individuals has been analyzed across 3 kb of the human β-globin gene and result in a value of 0.002 [Harding et al., 1997], that is similar to the smaller number of individuals across 9.7 kb of the human LPL [Nickerson et al., 1998]. One consequence of the observed sequence variation is that each individual is heterozygous at an average of 13 sites across 16 kb in the MHY7 gene, which is less in the average of 17 sites across 9.7 kb in the LPL region [Nickerson et al., 1998]. This finding shows a varying distribution of variable sites across different regions of each gene.

To characterize the kinetic behaviour of the β-MHC of those normal individuals with reference nucleotide and protein sequence, we studied the rate constant of force development (k_{dev}) of chemically demembranated fibers of

the same controls. k_{dev} at 21°C and maximal Ca^{2+} activation level was 1.58 s^{-1}. This is in line with previous determinations of k_{dev} in the porcine ventricle [Morano et al., 1995] as well as in the right human ventricle with very low ALC-1 expression [Morano et al., 1996]. This was not statistically significant from the k_{dev} values obtained from two HCM patients with normal primary sequence of the β-MHC. We could mechanically evaluate only two of the six HCM patients sequenced in this study because four of them expressed considerable amounts of ALC-1 in the ventricle [Ritter et al., 1999]. These patients had to be excluded in the characterization of β-MHC function because ALC-1 is a major determinant of k_{dev} [Morano et al., 1996] and could falsify the mechanical experiments.

In summary, we detected a genomic sequence of the MYH7 that contained considerable difference in both the nucleotide and amino acid sequence compared to already published sequences. This referenced β-MHC revealed a rate constant of force development of 1.58 s^{-1}. Although we detected nucleotide variations, mutations in the β-MHC affecting the amino acid sequence could not be identified.

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