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The X-Linked Gene G4.5 Is Responsible for Different Infantile Dilated Cardiomyopathies

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Summary

Barth syndrome (BTHS) is an X-linked disorder characterized clinically by the associated features of cardiac and skeletal myopathy, short stature, and neutropenia. The clinical manifestations of the disease are, in general, quite variable, but cardiac failure as a consequence of cardiac dilatation and hypertrophy is a constant finding and is the most common cause of death in the first months of life. X-linked cardiomyopathies with clinical manifestations similar to BTHS have been reported, and it has been proposed that they may be allelic. We have recently identified the gene responsible for BTHS, in one of the Xq28 genes, G4.5. In this paper we report the sequence analysis of 11 additional familial cases: 8 were diagnosed as possibly affected with BTHS, and 3 were affected with X-linked dilated cardiomyopathies. Mutations in the G4.5 gene were found in nine of the patients analyzed. The molecular studies have linked together what were formerly considered different conditions and have shown that the G4.5 gene is responsible for BTHS (OMIM 302060), X-linked endocardial fibroelastosis (OMIM 305300), and severe X-linked cardiomyopathy (OMIM 300069). Our results also suggest that very severe phenotypes may be associated with null mutations in the gene, whereas mutations in alternative portions or missense mutations may give a "less severe" phenotype.

Introduction

The dilated cardiomyopathies are a very heterogeneous group of heart disorders of largely unknown ethiology. Genetic causes have become increasingly evident with mapping of loci and identification of genes responsible for different forms of the disorder (Keating and Sanguinetti 1996). The dilated cardiomyopathies are often idiopathic, and in adults they represent a primary indication for heart transplantation. Less frequent and more severe are the infantile forms, which often cause cardiac failure during the first months of life. One of these severe forms is Barth syndrome (BTHS), an X-linked inherited disorder characterized clinically by the associated features of cardiac and skeletal myopathy, short stature, and neutropenia (Barth et al. 1983). The disease has infantile onset of symptoms and is often fatal in childhood, because of cardiac failure or sepsis: the clinical manifestations of the disease are, in general, quite variable also within families, but cardiac failure as a consequence of cardiac dilatation and hypertrophy is a constant finding. Which of the other manifestations of the disease may also be diagnostic is an open question. X-linked infantile cardiomyopathies with cardiac dilatation but apparently lacking the other symptoms of BTHS have been reported, and it has been proposed that the BTHS may be allelic with both X-linked endocardial fibroelastosis (EFE1; OMIM 305300) and other infantile cardiomyopathies (Gedeon et al. 1995; OMIM 300069).

BTHS has been localized to Xq28 (Bolhuis et al. 1991). We have recently reported the identification of a novel gene, G4.5, which we have proposed as the genetic locus responsible for the disease (Bione et al. 1996). The G4.5 gene is a small single-copy gene with a complex pattern of expression. As many as 10 mRNAs differing either in the region encoded in the central exons (5–7) or in the 5′ end can be synthesized. Two forms, containing all three exons or lacking exon 5 only, were consistently more abundant and ubiquitously expressed. Since the differently spliced mRNAs maintain the same open reading frame (ORF), the tafazzins, the putative proteins encoded by the gene, can differ in the N-terminus and in a central portion. All the mutations found in the four familial cases of BTHS studied so far have introduced a stop codon in the ORF, but in all instances

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they would not interfere with the synthesis of all the possible tafazzins. We now report the study of a larger group, of patients affected with BTHS and of patients affected with severe cardiac disorders compatible with X-linked inheritance. We show that mutations are to be found also in this group of patients and that the G4.5 gene is responsible for most of the X-linked infantile dilated cardiomyopathies.

Material and Methods

Mutation Detection

Genomic DNA preparation, amplification, and direct sequencing of the BTHS gene were as described elsewhere (Bione et al. 1996). Sequences were from the primers described, and they were designed to sequence all exons and exon-intron junctions from both strands. As a control for PCR errors, at least two independent PCR products were sequenced in the region of each mutation. The sequence of each patient was always compared with that of at least one normal individual, in the same gel. As a control for polymorphism, all the mutations were searched in DNA from 100 normal chromosomes randomly selected among different populations available to the laboratory and containing both males and females.

Sequence Analysis

Sequence reactions were run in a Perkin Elmer 373A Automated Sequencer, and they were analyzed by use of SeqEd and Sequence Navigator software. Sequences were compared with the GenBank sequences by use of BLAST, and they were aligned with CLUSTAL.

Results

Mutation Analysis of BTHS Patients

In our previous work we studied patients from four families affected with BTHS. To gain more information on the mutations causing this disorder, we collected eight additional cases. Some were definitely familial. In other families relatives were reported as possibly affected, but the disease could not be well defined before death occurred. Some of the clinical manifestations of the propositi of each family are schematically listed in table 1. Other affected family members were usually studied less thoroughly and are not reported in the table: some of the families were previously published, and more data are available from the literature. In all families, cardiac dilatation and cardiac failure within the 1st year of life were the common feature. Neutropenia (usually cyclic or episodic), myopathy, growth retardation, and alterations in urinary 3-methylglutaconic acid (Kelley et al. 1991) were consistently reported. Life expectancy was very variable, possibly depending on medical treatment. Cardiac transplantation was reported in two of the patients (BS and GW) and seems to have increased life expectancy.

In the search for mutations, PCR products were prepared from genomic DNA of either one patient or an obligate carrier from each family and were sequenced directly, as described elsewhere (Bione et al. 1996). Mutations were found in six of the familial cases, and they are listed in table 1; their localizations with respect to the G4.5 gene are shown in figure 1. Some of the corresponding sequences are shown, aligned with the normal sequence, in figure 2.

Two new mutations were found in exon 2, one was in exon 6, and the remaining three were in exon 8. One of the mutations in exon 2 (family 1) was a deletion causing a frameshift and early stop; all the remaining mutations were missense. As a control for polymorphism, 100 chromosomes from normal individuals were sequenced in the region of the mutations, and they were all normal. On the other hand, additional affected individuals and obligate carriers, when available, were sequenced, and the mutations were all confirmed. In family 1 we sequenced the mother's DNA; in family 6 the mother's and the grandmother's DNAs; in family 8 the mother's and the sister's DNAs; and in family 9 the mother's, the aunt's, and a normal brother's DNAs.

In the remaining two families, published as putative BTHS (families 11 and 12; Örstavik et al. 1993), no mutations were found. Since only the coding region and the splice junctions were studied, and since only the DNAs of obligate carriers were available, we cannot absolutely exclude an involvement of the G4.5 gene. Four sporadic patients were also studied, and no mutations were found.

X-Linked Infantile Cardiomyopathies: Mutations in the BTHS Gene

Gedeon et al. (1995) reported a large family presenting with X-linked inheritance of a fatal infantile cardiomyopathy. The gene was mapped to Xq28. The cardiomyopathy in this family is consistently of congenital onset and is fatal in infancy. The clinical features were insufficient to permit a definite diagnosis, but the possibility that this disease is allelic with BTHS was discussed. We sequenced the G4.5 gene in two affected individuals of the family, and we found deletion of a C at position 919 in exon 8, causing frameshift and a stop codon after 18 nucleotides (patient MH in fig. 1). The mutation may cause the production of a truncated protein or, possibly, a reduced amount of the corresponding mRNAs and therefore no protein at all (Maquat 1995).

We have also studied patients from two families with several affected male relatives (brothers and cousins) who died very early of heart failure. The clinical data were very limited, but after autopsy these patients' hearts showed left-ventricular dilatation and hypertrophy, and they were diagnosed as affected with EFE1.

Table 1

Data on BTHS Patients

Family	Patient	Source Author	Affected Relative(s)	Reference[a]	Year of Birth	Age (years)	Age at Death (mo)	Dilated Cardio-myopathy[b]	Neutropenia[b]	Myopathy[b]	3-Methylglutaconic Aciduria[b]	Growth Retardation[b]	Abnormal Mitochondria[b]	Mutation	Exon	Reference[a]
1	BM	R.M.	Brother	...	1986	10	Living	+	Cyclic	+	+	+	ND	428del13	2	...
2	V.22	P.A.B.	Several (18)	1	1984		10	+	+	+/−	+	+	+	Y51X	2	5
3	BS	M.A.P.	Two brothers	2	1992	5	Living	+[c]	+/−	+/−	+	+	ND	H69Q	2	...
4	K	P.A.B.	Not proved	...	1989		13	+	−	+	+/−	+	ND	527-1 G→A		5
5	GW	A.G.	Nephew	...	1978	19	Living	+[d]	Cyclic	−	+	+	+	527-1 G→C		5
6	FW	K.H.O.	Brother	3	1984		9	+	+	ND	ND	+	ND	F178I[e]	6	...
7	OAT	A.G.	Several	4	1989		>21	+	+	+	ND	ND	ND	868insT	7	5
8	MF	M.A.D.	Not proved	...	1994		14	+	+	ND	+	ND	ND	G197R	8	...
9	FT	P.A.B.	Not proved	...	1991	5	Living	+	Cyclic	+	+	+	ND	G216R	8	...
10	BV	P.A.B.	Not proved	...	1983	16	Living	+	+	+	+	+	ND	G216R	8	...
11	II-1	K.H.O.	Brother	3	1987		1	+	−	+/−	ND	−	+[f]	None[e]		...
12	II-2	K.H.O.	Brother	3	1977		20	+	−	−	ND	−	+[f]	None[e]		...

[a] 1 = Barth et al. (1983); 2 = Patton et al. (1994); 3 = Örstavik et al. (1993); 4 = Àdes et al. (1993); and 5 = Bione et al. (1996).
[b] + = Present; − = absent; +/− = uncertain status; and ND = not done.
[c] Cardiac transplant at age 4 mo.
[d] Cardiac transplant at age 14 mo.
[e] Sequence was determined on the basis of the mother's DNA.
[f] Only in myocardium.

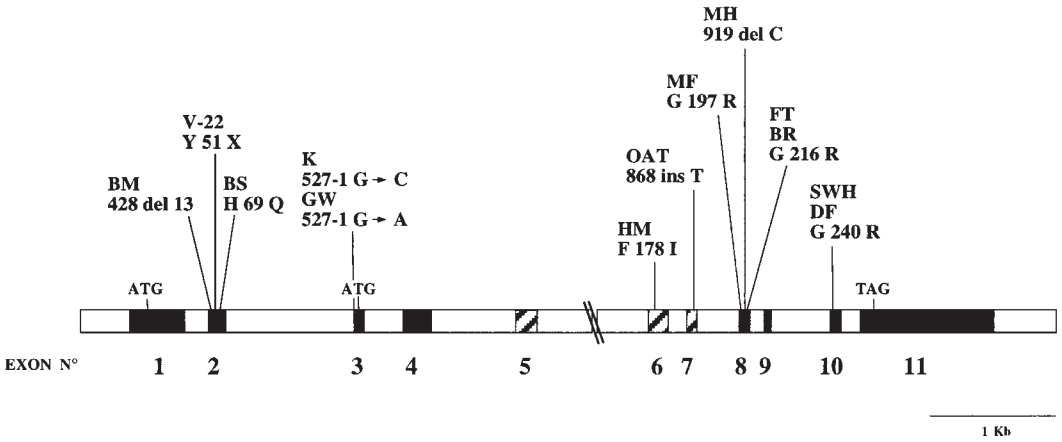

Figure 1 Mutations in G4.5 gene, and their localization in a schematic representation of the gene. Blackened boxes are invariant exons; and diagonally striped boxes are alternatively spliced exons.

One family was published by Lindenbaum et al. in 1973 (Lindenbaum et al. 1973). In the second large and yet-unpublished family (fig. 3), four affected males were described: three (IV-1, IV-16, and V-5) died within the first 3–4 mo of life of cardiac failure. The fourth, SWH (IV-4), had cardiac failure at age 5 wk, survived, and now, at age 25 years, is normal. In both families we found the same mutation (a G→A change at nucleotide 1006) in exon 10, causing a G→R change in the sequence of the protein (G240R). The mutation was not found in 100 normal chromosomes. In the family studied by Lindenbaum et al., the mutation was found in three obligate carriers. In the family of SWH, all the patients and the mother (II-1) of SWH were sequenced and shown to carry the mutation.

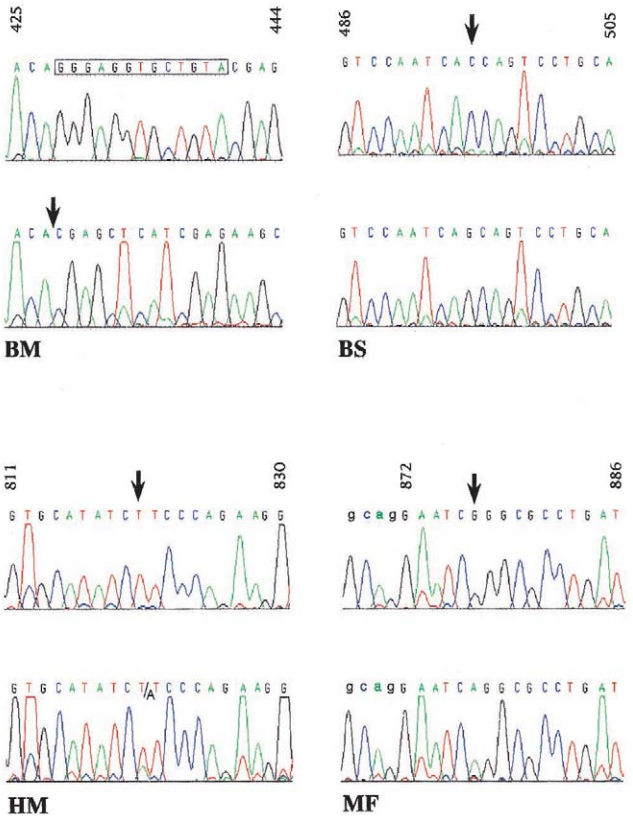

Figure 2 Portions of chromatograms showing mutations in some patients (BM, BS, and MF [*bottom sections of panels*]) or in an obligate carrier (HM [*bottom section of panel*]), compared with the normal sequence (*top sections of panels*). Lowercase letters denote intron sequences. Numbers correspond to nucleotide positions in the cDNA (Bione et al. 1996). Arrowheads point to mutations. A 13-base deletion is boxed.

Missense Mutations: Change of Conserved Amino Acids

The G4.5 gene is conserved in evolution. A BLAST search of GenBank showed that ORFs encoding very similar protein sequences exist in *Caenorhabditis elegans* and *Saccharomyces cerevisiae* genomes. The alignment of the tafazzins from the three organisms is shown in figure 4. The region corresponding to the alternative exon 5 in human is missing in the *C. elegans* gene; in *S. cerevisiae* the corresponding region is present, but it is not conserved. Striking conservation was observed in most of the rest of the protein. The four regions boxed in figure 4 are the most conserved: >50% of the amino acids are identical between the three species, and >80% are conservative substitutions. The missense mutations found in the patients are indicated in figure 4, and in all instances they correspond to residues conserved between two or all three organisms. No function is known for the *C. elegans* or *S. cerevisiae* tafazzins, since they correspond to ORFs identified by genomic sequencing.

Discussion

In this paper we have presented the results of a study of mutations in the BTHS gene, G4.5. We show that mutations in this gene are found in BTHS and in other

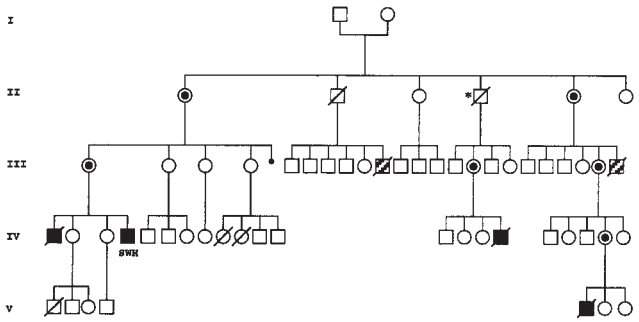

Figure 3 Pedigree of family SWH. The asterisk (*) indicates the "normal" male (II-4) carrying the mutation. Blackene boxes denote affected males; and diagonally striped boxes denote males who died of heart disorder different from BTHS.

X-linked dilated cardiomyopathies, previously considered different conditions and listed with different OMIM numbers (305300 and 300069). The patients diagnosed as affected with BTHS were often well characterized, and at least one patient in each family was thoroughly studied. We have tried to summarize the most common features of the disease, and the data in table 1 demonstrate that, in addition to cardiac failure in the 1st year of life, growth arrest, cyclic neutropenia, and methylglutaconic aciduria appear to be reliable diagnostic signs of BTHS. Patients affected with either EFE1 or severe cardiomyopathy have not been as thoroughly studied, and they were just described as affected with dilated cardiomyopathy. Whether the other symptoms of BTHS were present cannot be established with the available clinical data, but our findings suggest that mutations in the G4.5 gene have to be considered as a possible cause of infantile dilated cardiomyopathies affecting males, even in the absence of the typical BTHS signs.

Seven different mutations have been reported in this work. They were searched and not found in 100 normal chromosomes, and, when DNA of family members was available, they were shown to segregate with the disease. The 11 mutations described thus far in the BTHS gene are null or missense mutations localized in alternative, as well as in invariant, parts of the gene. Both missense and null mutations seem to be responsible for a similar disease, since the clinical characteristics and life expectancy of the patients in each BTHS family do not appear to profoundly differ. This is unlike the situation in many other disorders, where missense often causes a phenotype less severe than that caused by null mutations. However, since comparison with similar sequences in distant species has indicated that the amino acids changed by the mutations are highly conserved residues, the very severe effect of missense mutations could be ascribed to a drastic structural modification of the pro-

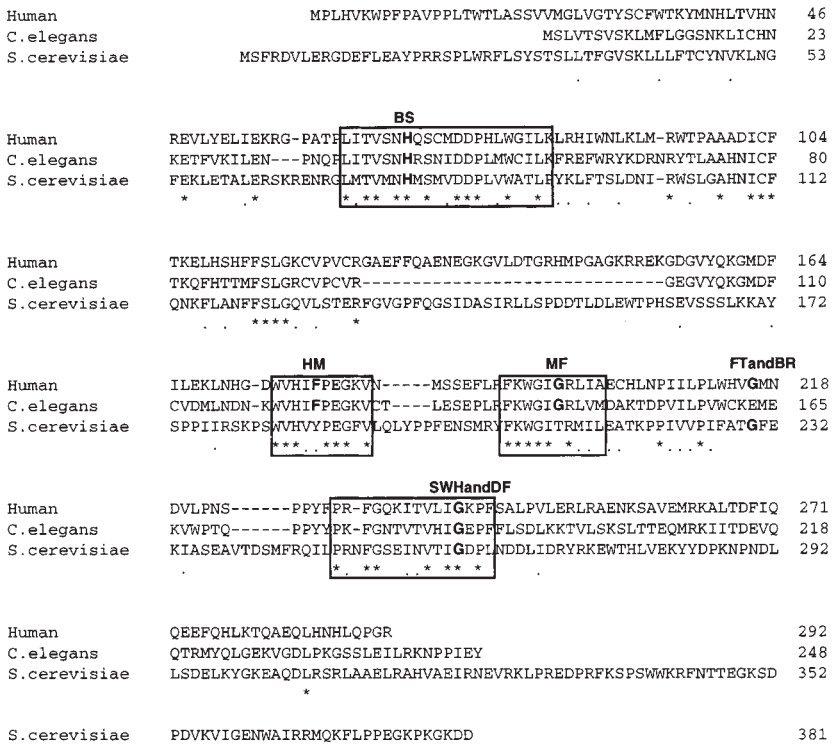

Figure 4 Alignment of amino acid sequence of tafazzins with *C. elegans* and *S. cerevisiae* homologues, done by use of CLUSTAL. Asterisks (*) indicate identical amino acids; and dots indicate conserved amino acids. Highly conserved regions are boxed; and amino acids changed in the patients indicated are in boldface.

teins, as a consequence of their substitution with different amino acid.

In the family affected with X-linked fatal infantile cardiomyopathy (Gedeon et al. 1995), the mutation is a 1-base deletion in exon 8, causing a frameshift and eventually a stop codon after 18 nucleotides (patient MH), and it is unique among the 13 patients studied, since it causes knockout of all the putative tafazzins. The very severe clinical manifestations of the disorder in this family (described in six affected males and in eight males suspected of being affected) could thus be related to the severity of the mutation. More patients should be studied to confirm this observation, but the results suggest that null mutations in the invariant part of the tafazzins, near the C-terminus of the protein, may be rare and may have a more severe phenotypic effect. The direct study of the tafazzins, by determination of which are the proteins present in each affected cell type, will help to further clarify this point.

The function of the G4.5 gene is presently unknown, nor did the gene product show similarity to known proteins. The sequence of the tafazzins is very conserved in evolution: ORFs encoding proteins highly homologous to the tafazzins have been found in *S. cerevisiae* and *C. elegans*. The conservation, together with the very severe phenotype associated with mutations in the gene, suggests that the role of the tafazzins must be of great importance for the correct function of the heart and other organs during fetal and neonatal life. In this paper, we have presented a family in which at least one individual (SWH) carrying a mutation causing a severe phenotype had a very severe heart failure but survived to live a normal life. In the same family, another male (II-4) also must have carried the mutation: one of his sisters (II-5) and his daughter (III-15) are obligate carriers, and his grandson was affected. Despite the mutation, he was able to reproduce and to transmit the disease. We do not know much about and cannot study II-4, but the finding of the SWH patient and the characteristics of his family suggest that some "protecting" factor(s) could act in fetal life and early after birth. Accordingly, some other patients seem to be able to survive to age >10 years (see table 1 and one patient reported by Christodoulou et al. [1994] who is suspected to have BTHS). These findings suggest that the role of the tafazzin(s) may be very important early in life and for a limited time after birth and that in later life it may be substituted by other functions. Such function(s) may be responsible also for the very large phenotypic heterogeneity of the symptoms within families.

Acknowledgments

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