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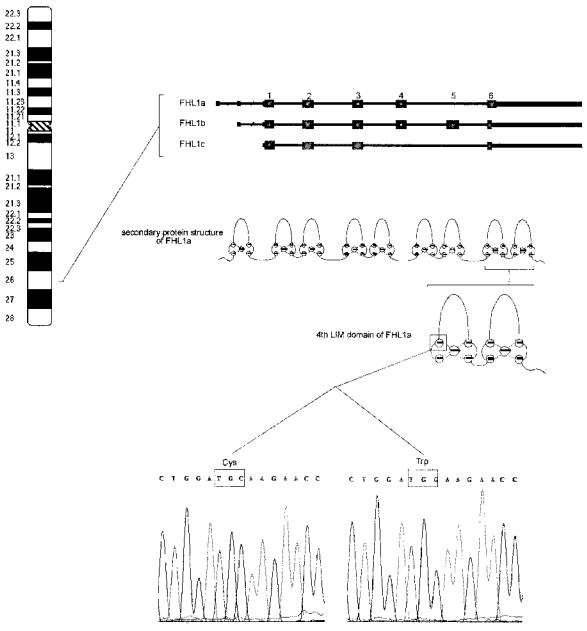
(54) Title: *FHL1* MUTATIONS ASSOCIATED WITH NOVEL X-LINKED MUSCULAR MYOPATHIES

Fig. 6. Ideogrammatic representation of the XMPMA locus on the distal arm of chromosome X, the electropherograms indicating the wild-type and mutation sequence for the Austrian XMPMA family, and the secondary structure of FHL1, indicating the position of the resulting amino acid substitution, C224W, relative to structural features in the protein.



(57) Abstract: Four and a Half LIM domains protein 1 (FHL-1) mutations at positions 128 or 224 that are associated with X-linked muscular myopathy, methods of screening subjects to identify those susceptible to muscular myopathy including muscular dystrophy and cardiomyopathy and kits.

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FHL1 mutations Associated With Novel X-Linked Muscular Myopathies

FIELD OF INVENTION

[0001] The present invention relates to gene mutations. ***FHL1* mutations Associated With a Novel X-Linked Muscular Myopathies.**

BACKGROUND OF THE INVENTION

[0002] Muscular dystrophies (MD) are defined as a group of inherited muscle disorders characterized by the progressive degeneration and weakness of voluntary skeletal muscle (Davies and Nowak, 2006). The various forms of MD vary widely with respect to age of onset, incidence, pattern of inheritance, rate of progression, and distribution and severity of muscle weakness. Certain muscular dystrophies can involve cardiac and smooth muscle tissue. MD most commonly exhibits an X-recessive mode of transmission, and is usually caused by mutations in the DMD gene on Xp21.2. Resulting in deficiencies in dystrophin protein, DMD mutations cause rapidly progressive weakness and wasting of the proximal muscles in the lower body. Duchenne MD (DMD), the most common neuromuscular disorder, is caused by frameshift mutations that result in the complete absence of functional dystrophin, whereas the phenotypically less severe Becker's MD is associated with missense and inframe deletions that result in reduced levels of functional dystrophin or expression of partially functional protein (Davies and Nowak, 2006). This structural protein functions to link the actin cytoskeleton with muscle fibre membranes across the sarcolemma, providing structural support to the muscle cell (Ervasti, 2007). The absence of dystrophin compromises the complex across the muscle, leading to degeneration of muscle tissue. Affecting 1 in 4,000 live male births, DMD is correlated with onset before age 6 and a typical life span of 20-25 years; in contrast, Becker's MD has onset in adolescence or adulthood with symptoms similar to but generally less severe than DMD. These include muscle pseudohypertrophy, proximal muscle atrophy, and rarely, cardiomyopathy and/or mental deficits.

[0003] Emery-Dreifuss MD (EDMD) is another form of late onset X-recessive MD caused by deficiencies in the emerin protein, encoded by the EMD gene on Xq28 (Ellis, 2006). EDMD is phenotypically distinct from other X-linked MDs in that there is humeroperoneal distribution of muscle wasting, absence of muscle pseudohypertrophy, and at very high frequency, cardiomyopathy.

[0004] There is a need in the art to identify FHL-1 mutations, and the proteins encoded therefrom that are associated with muscular myopathies including muscular dystrophy and cardiomyopathy. Further there is a need in the art to be able to screen for such mutations to identify individuals that have or are at risk for developing muscular myopathies, including muscular dystrophy and cardiomyopathy.

SUMMARY OF THE INVENTION

[0005] The present invention relates to gene mutations. More specifically, the present invention relates to gene mutations associated with muscular myopathies.

[0006] According to the present invention there is provided a protein comprising amino acids 1-230 of SEQ ID NO:1, a fragment thereof or a sequence exhibiting at least 70% identity thereto and comprising the amino acid sequence $VAKKCX_1GX_2X_3NPIT$ (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid.

[0007] Preferably X_1 is A or S and X_3 is K, N or Q.

[0008] Also provided is the protein as defined above, wherein X_2 is tryptophan.

[0009] The present invention also provides a protein as defined above, wherein the protein is defined by SEQ ID NO:2 or SEQ ID NO:3.

[0010] Also provided by the present invention is a nucleic acid comprising a sequence

a) encoding the protein as defined above or a fragment thereof;

b) that is the complement of a sequence encoding the protein as defined above, or a fragment thereof;

c) that is capable of hybridizing to a nucleic acid encoding the protein as defined above or fragment thereof under stringent hybridization conditions; or

d) that exhibits greater than about 70% sequence identity with the nucleic acid defined in a) or b).

[0011] Also provided by the present invention is a nucleic acid as defined above wherein the fragment comprises the amino acid sequence GWK.

[0012] Also provided is a nucleic acid as defined above wherein X_2 is tryptophan.

[0013] Also contemplated is the nucleic acid as defined above wherein the protein is defined by SEQ ID NO:2 or SEQ ID NO:3.

[0014] The present invention also provides a method of screening a subject for an X-linked muscular myopathy comprising,

a) obtaining a biological sample from the subject, and;

b) assaying the sample for a nucleic acid encoding the protein as defined above or a fragment thereof comprising the amino acid sequence $VAKKCX_1GX_2X_3NPIT$ (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid, or

c) assaying the sample for the protein as defined above or a fragment thereof comprising the amino acid sequence $VAKKCX_1GX_2X_3NPIT$ (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid.

[0015] Also provided is a method as defined above, wherein the muscular myopathy is a skeletal muscle myopathy, or a cardiomyopathy, for example, but not limited to muscular dystrophy.

[0016] Also provided is a method as defined above, wherein X_2 is tryptophan.

[0017] The invention also provides a method as defined above wherein the protein is defined by SEQ ID NO:2 or SEQ ID NO:3.

[0018] Further provided is the method as defined above, wherein the subject is a human subject.

[0019] Also provided is a method as defined above, wherein the biological sample is a blood sample.

[0020] Also provided is a method as defined above wherein assaying comprises PCR, probe hybridization or sequencing.

[0021] The present invention also provides a kit comprising

i) a protein or fragment thereof that is associated with muscular myopathy as described herein,

ii) an antibody that selectively binds to a protein or fragment thereof associated with muscular myopathy as described herein, rather than a wild-type protein not associated with the muscular myopathy,

iii) one or more nucleic acid primers to amplify a nucleotide sequence encoding a protein or fragment thereof which comprises a mutation associated with an X-linked muscular myopathy as provided herein,

iv) one or more nucleic acid probes of between about 9 and 100 nucleotides that hybridizes to the nucleotide sequence encoding a protein or fragment thereof which comprises a mutation associated with an X-linked muscular myopathy as provided herein,

v) one or more reagents including, but not limited to buffer(s), dATP, dTTP, dCTP, dGTP, or DNA polymerase(s),

vi) instructions for assaying, diagnosing or determining the risk of a subject to muscular myopathy,

vii) instructions for using any component or practicing any method as described herein,

or any combination thereof.

[0022] The present invention also provides a FHL-1 protein comprising an isoleucine insertion at position 128. In a preferred embodiment protein comprises the human isoform a, b or c amino acid sequence or an amino acid sequence which is at least 70% identical thereto.

[0023] The present invention also provides a nucleotide sequence encoding the FHL-1 protein as defined above.

[0024] Also provided by the present invention is an antibody that selectively binds the FHL-1 protein as described above but preferably not a wild type FHL-1 protein.

[0025] The present invention also provides a method of screening a subject for an X-linked muscular myopathy comprising

a) obtaining a biological sample from the subject;

b) assaying the sample for a nucleic acid encoding a FHL-1 protein comprising an isoleucine insertion at position 128, or

c) assaying the sample for the FHL-1 protein comprising an isoleucine insertion at position 128,

wherein the presence of the nucleic acid or protein indicates that the subject has or is at risk of developing a muscular myopathy.

[0026] Also provided by the present invention are kits comprising FHL-1 protein having an isoleucine insertion at position 128, a nucleotide sequence encoding a FHL-1 protein comprising an isoleucine insertion at position 128, a probe that may be employed to identify nucleotide sequences encoding an isoleucine at position 128, primers that can amplify such sequences, antibodies that recognize the proteins as defined above but preferably not wild-type FHL-1 proteins, instructions for screening subjects, one or more reagents that can be used to use one or components of the kit or any combination thereof. Other components as described herein or as would be known in the art can also be included and this list is not meant to be limiting in any manner.

[0027] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0029] **FIGURE 1A** shows the pedigree of the X-linked postural muscular myopathy family. Family members from whom DNA samples were obtained are indicated by arrows (↙). **Figure 1B** shows UK family 2 pedigree members exhibiting muscular myopathies. **Figure 1C** shows UK family 3 pedigree members exhibiting muscular myopathies.

[0030] **FIGURE 2** shows atrophy of the postural back muscles as clinically assessed in a patient in the early stages of disease. Atrophy of the deltoideus muscle. Gluteus maximus, biceps brachii, triceps

brachii, and lower arms appear normal. Biceps femoris (hamstring muscles), adductor magnus (thighs), abductor pollicis brevis and adductor pollicis longus (hand) show signs of atrophy.

[0031] **FIGURE 3** shows muscle biopsy of the vastus lateralis muscle (**A.**) and anterior tibial muscle (**B**). Muscle histology revealed a moderate myopathy with a moderate perimysial and limited endomysial fibrosis. In all biopsies, some round, autophagic vacuoles predominant in type 2 fibers were detectable. These vacuolar changes were most prominent in patient **B**. Additionally, centrally placed myonuclei were increased and rarely single fiber necrosis and granular myofiber degeneration were seen..

[0032] **FIGURE 4** shows muscle biopsy of the vastus lateralis muscle (**A.**) and anterior tibial muscle (**B**). Myosin ATPase staining at acidic pH 4.3/4.6 reveals type I (dark) and type II (light) muscle fibre distribution in patients in the early stages of disease. Variability of fiber size was increased in all specimens, with diameters ranging between 20 to 100μm, and most prominent in type 2 fibers. In NADH and COX histochemistry centrally negative core-like lesions were detected in both patients, without any further mitochondrial alterations.

[0033] **FIGURE 5** shows linkage analysis to the DMD locus using polymorphic STR intragenic markers STR-44, STR-45, STR-48, STR-49, and STR-50 revealed different haplotypes in the affecteds, conclusively excluding the DMD locus. Recombination of markers STR-44, STR-48, STR-49, and STR-50 is evident, as illustrated by haplotypes.

[0034] **FIGURE 6** shows an ideogrammatic representation of the X-linked myopathy with postural muscle atrophy (XMPMA) locus on the distal arm of chromosome X, the electropherograms indicating the wild-type and mutation sequence for the Austrian XMPMA family, and the secondary structure of FHL1, indicating the position of the resulting amino acid substitution, C224W, relative to structural features in the protein.

[0035] **FIGURE 7** shows amino acid and nucleotide sequences as described herein and throughout as well as several wild-type protein sequences known in the art.

[0036] **FIGURE 8** shows a comparative analysis of the 4th LIM binding domain of FHL1 across several species.

DETAILED DESCRIPTION

[0037] The following description is of a preferred embodiment.

[0038] We have identified a large multigenerational Austrian family displaying a novel form of muscular myopathy with an X-recessive mode of inheritance. Affected individuals develop specific atrophy of postural muscles, with histology showing gradual atrophy of type I muscle fibers. Known X-recessive MDs were excluded by immunocytochemical staining, marker analysis and gene sequencing. Marker analysis revealed significant linkage at Xq26-q27. Haplotype analysis based on 250K array SNP chip data of five affected individuals along with three unaffected family members confirmed this linkage region on the distal arm of the X-chromosome (Xq26-q27) and enabled us to narrow down the candidate interval to 26 Mb encompassing approximately 850 consecutive SNPs. Sequencing of functional candidate genes led to the identification of a mutation within the four-and-a-half LIM domain 1 gene (FHL1), which putatively disrupts the 4th LIM domain. FHL1 on Xq27.2, is highly expressed specifically in type I muscle fibers. Thus, we have characterized a new form of myopathy, X-linked myopathy with postural muscle atrophy (XMPMA), and identified FHL1 as the causative gene. Other family studies also confirm FHL1 as the causative gene in X-linked myopathies and cardiomopathies, as described herein.

[0039] **Proteins and Amino Acids**

[0040] According to an embodiment of the present invention there is provided a protein comprising amino acids 1-230 of SEQ ID NO:1, a fragment thereof or an amino acid sequence exhibiting at least 70% identity thereto and comprising the amino acid sequence $VAKKCX_1GX_2X_3NPIT$ (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid. Preferably X_1 is A or S and X_3 is K, N or Q. In a preferred embodiment X_2 is tryptophan, for example, but not limited to as defined by SEQ ID NO:2 or SEQ ID NO:3.

[0041] An amino acid sequence exhibiting at least 70% identity thereto is understood to include sequences that exhibit 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% or 100% identity, or any value therein between to SEQ ID NO:1 or a fragment thereof. Further,

the protein may be defined as comprising a range of sequence identity as defined by any two of the values listed or any values therein between.

[0042] Any method known in the art may be used for determining the degree of identity between polypeptide sequences. For example, but without wishing to be limiting, a sequence search method such as BLAST (Basic Local Alignment Search Tool; (Altschul S F, Gish W, Miller W, Myers E W, Lipman D J (1990) J Mol Biol 215, 403 410) can be used according to default parameters as described by Tatiana et al., FEMS Microbial Lett. 174:247 250 (1999), or on the National Center for Biotechnology Information web page at ncbi.nlm.gov/BLAST/, for searching closely related sequences. BLAST is widely used in routine sequence alignment; modified BLAST algorithms such as Gapped BLAST, which allows gaps (either insertions or deletions) to be introduced into alignments, or PSI-BLAST, a sensitive search for sequence homologs (Altschul et al., Nucleic Acids Res. 25:3389 3402 (1997); or FASTA, which is available on the world wide web at ExPASy (EMBL – European Bioinformatics Institute). Similar methods known in the art may be employed to compare DNA or RNA sequences to determine the degree of sequence identity.

[0043] In an embodiment of the present invention, which is not meant to be considered limiting there is provided a FHL1 protein comprising an amino acid insertion. In a further embodiment, there is provided a FHL1 protein comprising an isoleucine amino acid insertion. In still a further embodiment, there is provided an a FHL1 protein comprising 128InsI. Any isoform, for example, but not meant to be limiting to isoforms a, b or c may comprise this amino acid insertion. Nucleotide sequences encoding such proteins are also encompassed by the invention as described below.

[0044] **Nucleic Acids**

[0045] Also contemplated by the present invention is a nucleic acid comprising a sequence

a) encoding the protein as described above, or a fragment thereof;

b) that is the complement of a sequence encoding the protein as described above, or a fragment thereof;

c) that is capable of hybridizing to a nucleic acid encoding the protein as described above or fragment thereof under stringent hybridization conditions; or

d) that exhibits greater than about 70% sequence identity with the nucleic acid described in a) or b).

[0046] Without wishing to be limiting, representative examples of nucleic acids encoding the proteins as defined above are provided by SEQ ID NOs:5 and 6 wherein X is not cytosine (c) or any other nucleotide that produces cysteine when translated.

[0047] The nucleic acids described above include nucleic acids that may be employed to produce proteins which are associated with X-linked muscular myopathy, probes which may be used to identify or diagnose subjects carrying a mutation which causes or predisposes the subject to muscular myopathy, antisense or short inhibitory RNA that may be used to modulate production of protein from genes associated with muscular myopathy or a combination thereof. The proteins, fragments thereof or nucleic acids as described above also may be used to produce antibodies that selectively recognize the proteins as described above preferably over wild-type proteins known in the art.

[0048] In a preferred embodiment of the nucleic acids as described above, X_2 is tryptophan. In a further embodiment of the method, the protein is defined by SEQ ID NO:2 or SEQ ID NO:3. In still a further embodiment, the protein is a human FHL1 protein comprising an isoleucine amino acid insertion at position 128 (128InsI).

[0049] Stringent hybridization conditions may be, for example but not limited to hybridization overnight (from about 16-20 hours) hybridization in 4 X SSC at 65°C, followed by washing in 0.1 X SSC at 65°C for an hour, or 2 washes in 0.1 X SSC at 65°C each for 20 or 30 minutes. Alternatively, an exemplary stringent hybridization condition could be overnight (16-20 hours) in 50% formamide, 4 X SSC at 42°C, followed by washing in 0.1 X SSC at 65°C for an hour, or 2 washes in 0.1 X SSC at 65°C each for 20 or 30 minutes, or overnight (16-20 hours); or hybridization in Church aqueous phosphate buffer (7% SDS; 0.5M $NaPO_4$ buffer pH 7.2; 10 mM EDTA) at 65°C, with 2 washes either at 50°C in 0.1 X SSC, 0.1% SDS for 20 or 30 minutes each, or 2 washes at 65°C in 2 X SSC, 0.1% SDS for 20 or 30 minutes each for unique sequence regions.

[0050] The present invention is further directed to a nucleotide construct comprising the nucleic acid as described above operatively linked to one or more regulatory elements or regulatory regions. By "regulatory element" or "regulatory region", it is meant a portion of nucleic acid typically, but not always, upstream of a gene, and may be comprised of either DNA or RNA, or both DNA and RNA.

Regulatory elements may include those which are capable of mediating organ specificity, or controlling developmental or temporal gene activation. Furthermore, "regulatory element" includes promoter elements, core promoter elements, elements that are inducible in response to an external stimulus, elements that are activated constitutively, or elements that decrease or increase promoter activity such as negative regulatory elements or transcriptional enhancers, respectively. By a nucleotide sequence exhibiting regulatory element activity it is meant that the nucleotide sequence when operatively linked with a coding sequence of interest functions as a promoter, a core promoter, a constitutive regulatory element, a negative element or silencer (i.e. elements that decrease promoter activity), or a transcriptional or translational enhancer.

[0051] By "operatively linked" it is meant that the particular sequences, for example a regulatory element and a coding region of interest, interact either directly or indirectly to carry out an intended function, such as mediation or modulation of gene expression. The interaction of operatively linked sequences may, for example, be mediated by proteins that interact with the operatively linked sequences.

[0052] Regulatory elements as used herein, also includes elements that are active following transcription initiation or transcription, for example, regulatory elements that modulate gene expression such as translational and transcriptional enhancers, translational and transcriptional repressors, and mRNA stability or instability determinants. In the context of this disclosure, the term "regulatory element" also refers to a sequence of DNA, usually, but not always, upstream (5′) to the coding sequence of a structural gene, which includes sequences which control the expression of the coding region by providing the recognition for RNA polymerase and/or other factors required for transcription to start at a particular site. An example of a regulatory element that provides for the recognition for RNA polymerase or other transcriptional factors to ensure initiation at a particular site is a promoter element. A promoter element comprises a core promoter element, responsible for the initiation of transcription, as well as other regulatory elements that modify gene expression. It is to be understood that nucleotide sequences, located within introns, or 3' of the coding region sequence may also contribute to the regulation of expression of a coding region of interest. A regulatory element may also include those elements located downstream (3′) to the site of transcription initiation, or within transcribed regions, or both. In the context of the present invention a post-transcriptional regulatory element may include elements that are active following transcription initiation, for example

translational and transcriptional enhancers, translational and transcriptional repressors, and mRNA stability determinants.

[0053] The regulatory elements, or fragments thereof, may be operatively associated (operatively linked) with heterologous regulatory elements or promoters in order to modulate the activity of the heterologous regulatory element. Such modulation includes enhancing or repressing transcriptional activity of the heterologous regulatory element, modulating post-transcriptional events, or both enhancing/repressing transcriptional activity of the heterologous regulatory element and modulating post-transcriptional events. For example, one or more regulatory elements, or fragments thereof, may be operatively associated with constitutive, inducible, tissue specific promoters or fragment thereof, or fragments of regulatory elements, for example, but not limited to TATA or GC sequences may be operatively associated with the regulatory elements of the present invention, to modulate the activity of such promoters within plant, insect, fungi, bacterial, yeast, or animal cells.

[0054] There are several types of regulatory elements, including those that are developmentally regulated, inducible and constitutive. A regulatory element that is developmentally regulated, or controls the differential expression of a gene under its control, is activated within certain organs or tissues of an organ at specific times during the development of that organ or tissue. However, some regulatory elements that are developmentally regulated may preferentially be active within certain organs or tissues at specific developmental stages, they may also be active in a developmentally regulated manner, or at a basal level in other organs or tissues within a plant as well.

[0055] By "promoter" it is meant the nucleotide sequences at the 5' end of a coding region, or fragment thereof that contain all the signals essential for the initiation of transcription and for the regulation of the rate of transcription. There are generally two types of promoters, inducible and constitutive promoters.

[0056] An inducible promoter is a promoter that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor that binds specifically to an inducible promoter to activate transcription is present in an inactive form which is then directly or indirectly converted to the active form by the inducer. The inducer can be a chemical agent such as a protein, metabolite, growth regulator, or a physiological stress imposed directly by heat, cold, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus.

[0057] A constitutive promoter directs the expression of a gene throughout the various parts of an organism and/or continuously throughout development of an organism. Any suitable constitutive promoter may be used to drive the expression of the proteins or fragments thereof as described herein. Examples of known constitutive promoters include but are not limited to those associated with the CaMV 35S transcript. (Odell et al., 1985, *Nature*, 313: 810-812).

[0058] The term "constitutive" as used herein does not necessarily indicate that a gene is expressed at the same level in all cell types, but that the gene is expressed in a wide range of cell types, although some variation in abundance is often observed.

[0059] The gene construct of the present invention can further comprise a 3′ untranslated region. A 3′ untranslated region refers to that portion of a gene comprising a DNA segment that contains a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by effecting the addition of polyadenylic acid tracks to the 3 prime end of the mRNA precursor.

[0060] The gene construct of the present invention can also include further enhancers, either translation or transcription enhancers, as may be required. These enhancer regions are well known to persons skilled in the art, and can include the ATG initiation codon and adjacent sequences. The initiation codon must be in phase with the reading frame of the coding sequence to ensure translation of the entire sequence. The translation control signals and initiation codons can be from a variety of origins, both natural and synthetic. Translational initiation regions may be provided from the source of the transcriptional initiation region, or from the structural gene. The sequence can also be derived from the regulatory element selected to express the gene, and can be specifically modified so as to increase translation of the mRNA.

[0061] The present invention further includes vectors comprising the nucleic acids as described above. Suitable expression vectors for use with the nucleic acid sequences of the present invention include, but are not limited to, plasmids, phagemids, viral particles and vectors, phage and the like. For insect cells, baculovirus expression vectors are suitable. For plant cells, viral expression vectors (such as cauliflower mosaic virus and tobacco mosaic virus) and plasmid expression vectors (such as the Ti plasmid) are suitable. The entire expression vector, or a part thereof, can be integrated into the host cell genome.

[0062] Those skilled in the art will understand that a wide variety of expression systems can be used to produce the proteins or fragments thereof as defined herein. With respect to the in vitro production, the precise host cell used is not critical to the invention. The proteins or fragments thereof can be produced in a prokaryotic host (e.g., *E. coli* or *B. subtilis*) or in a eukaryotic host (e.g., *Saccharomyces* or *Pichia*; mammalian cells, such as COS, NIH 3T3, CHO, BHK, 293, or HeLa cells; insect cells; or plant cells). The methods of transformation or transfection and the choice of expression vector will depend on the host system selected and can be readily determined by one skilled in the art. Transformation and transfection methods are described, for example, in Ausubel et al. (1994) Current Protocols in Molecular Biology, John Wiley & Sons, New York; and various expression vectors may be chosen from those provided, e.g., in Cloning Vectors: A Laboratory Manual (Pouwels et al., 1985, Supp. 1987) and by various commercial suppliers.

In addition, a host cell may be chosen which modulates the expression of the inserted sequences, or modifies / processes the gene product in a specific, desired fashion. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the activity of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen by one skilled in the art to ensure the correct modification and processing of the expressed cardiac stem cell proliferation protein.

[0063] **Methods of Screening**

[0064] The present invention also provides a method of screening a subject for an X-linked muscular myopathy comprising,

a) obtaining a biological sample from the subject, the biological sample comprising DNA or RNA if the sample is assayed for nucleic acid, or FHL-1 protein if the sample is assayed for protein, and;

b) assaying the sample for a nucleic acid encoding the protein as defined above or a fragment thereof comprising the amino acid sequence $VAKKCX_1GX_2X_3NPIT$ (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid, or

c) assaying the sample for the protein as defined above or a fragment thereof comprising the amino acid sequence VAKKCX_1GX_2X_3NPIT (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid.

[0065] The present invention also provides a method of screening a subject for an X-linked muscular myopathy comprising,

a) obtaining a biological sample from the subject, the biological sample comprising DNA or RNA if the sample is assayed for nucleic acid, or FHL-1 protein if the sample is assayed for protein, and;

b) assaying the sample for a nucleic acid encoding a FHL-1 protein comprising an isoleucine insertion at position 128 (128InsI), or

c) assaying the sample for the FHL-1 protein comprising an isoleucine insertion at position 128 (128InsI).

The FHL protein may be identical or substantially identical to human FHL-1 protein isoform a, b or c, as described herein or it may be substantially identical meaning comprising at least 70% identity, more preferably at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identity thereto.

[0066] Also provided is a method as defined above, wherein the muscular myopathy is a skeletal muscle myopathy, for example, but not limited to muscular dystrophy. Alternatively, but not wishing to be limiting, the muscular myopathy may be a cardiomyopathy. Cardiomyopathies are specifically contemplated as the affected individuals studied herein appear to exhibit symptoms of such and/or die of heart related disease.

[0067] In the embodiment described above, it is to be understood that identifying the target nucleic acid, protein or both in the biological sample obtained from the subject, may be employed to identify a subject having or being at risk for developing a muscular myopathy, for example, but not limited to an X-linked muscular dystrophy or cardiomyopathy

[0068] By the terms "assaying the sample for a nucleic acid" it is meant testing and/or characterizing the sample provided by the subject for a nucleic acid that encodes a protein as defined above and is

meant to include without limitation hybridization assays, nucleotide sequencing, nucleotide PCR including, but not limited to RT-PCR, etc or any combination thereof.

[0069] In a preferred embodiment of the method of screening as defined above, X_2 is tryptophan. In a further embodiment, which is not meant to be limiting, the protein is defined by SEQ ID NO:2 or SEQ ID NO:3. Also, while the method of screening may be practiced on a variety of subjects, preferably, the subject is a human subject.

[0070] The sample obtained from the subject may comprise any tissue or biological fluid sample from which DNA or RNA may be obtained. For example, but not wishing to be limiting, DNA may be obtained from blood, hair follicle cells, skin cells, cheek cells, tissue biopsy, or the like. In a preferred embodiment, the sample is blood.

[0071] The present invention also contemplates screening methods which identify and/or characterize the proteins as defined above within biological samples from subjects. Such samples may or may not comprise DNA or RNA. For example, such screening methods may employ immunological methods, for example, but not limited to antibody binding assays such as ELISAs or the like, protein sequencing, electrophoretic separations to identify the proteins as described above in a sample. As will be evident to a person of skill in the art, the screening methods allow for the differentiation of the proteins as defined herein from wild type proteins known in the art.

[0072] **Kits**

[0073] Also provided by the present invention is a kit comprising one or more proteins or fragments thereof that is associated with muscular myopathy, for example, but not limited to, a muscular dystrophy or cardiomyopathy as described herein, an antibody that selectively binds to a protein or fragment thereof associated with muscular myopathy, dystrophy, or cardiomyopathy as described herein, rather than a wild-type protein not associated with muscular myopathy, dystrophy, or cardiomyopathy, one or more nucleic acid primers to amplify a nucleotide sequence encoding a protein or fragment thereof which comprises a mutation associated with an X-linked muscular myopathy, dystrophy or cardiomyopathy as described herein, one or more nucleic acid probes of between about 9 and 100 nucleotides that hybridizes to the nucleotide sequence encoding a protein or fragment thereof which comprises a mutation or insertion associated with an X-linked muscular myopathy, dystrophy or cardiomyopathy as described herein, one or more reagents including, but not limited to buffer(s),

dATP, dTTP, dCTP, dGTP, DNA polymerase(s), instructions for assaying, diagnosing or determining the risk of a subject to a muscular myopathy, dystrophy, or cardiomyopathy, instructions for using any component or practicing any method as described herein, or any combination thereof.

[0074] In a further embodiment, which is not meant to be considered limiting in any manner, there is provided a method of producing a non-human animal that comprises the protein as defined herein and throughout, the method comprising,

transforming the non-human animal with a nucleotide construct that encodes the protein as defined above, preferably in the absence of the wild type FHL-1 protein, more preferably in the absence of all isoforms of the FHL-1 protein. As human subjects exhibit hypertrophy of specific muscles, the method as defined above may be employed in animals, for example, in beef, horses, poultry, swine or any other non-human animal to produce animals that may exhibit increased muscle mass in various body areas.

[0075] The present invention will be further illustrated in the following examples.

Examples

[0076] **EXAMPLE 1: Materials and Methods**

[0077] **Clinical assessment**

[0078] Probands are from a multigenerational Austrian family displaying clinical features suggesting MD, but with clinical differences from previously described muscular dystrophies (Fig. 1). We identified living 6 patients (all males). Neurological examination was performed by a neurologist trained in neuromuscular disorders (S.Q.). First-degree relatives were examined when possible. Serum creatine kinase (CK) levels were measured in all affected individuals and their family members.

[0079] **Myosin ATPase staining**

[0080] Standard histological protocols were employed to stain for myosin ATPase at acidic pH 4.3/4.6 and assess the distribution of type I (slow twitch) and type II (fast twitch) muscle fibre types. Procedures were performed on adductor, biceps, deltoideus, erector, extensor, flexor, frontalis, gastrocnemius, gluteus, latissimus, pectoralis, peronaeus, rectus, sartorius, soleus, tibialis, triceps, vastus muscles, etc.

[0081] **Muscle immunocytochemistry**

[0082] Standard immunocytochemistry protocols were utilized to perform staining for dystrophin, adhalin, merosin, dysferlin, caveolin, α-dystroglycan, emerin, lamin A/C, desmin, β-slow myosin heavy chain, spectrin, and α-sarcoglycan following muscle biopsies of patient 50. Monoclonal antibodies were obtained from Novocastra Laboratories Ltd. (Vision BioSystems, U.K.) for spectrin (NCL-SPEC1), dysferlin (NCL-Hamlet), emerin (NCL-Emerin), and α-sarcoglycan (NCL-α-SARC). Additional Novocastra antibodies were used for dystrophin staining, specific to the dystrophin rod-like domain (NCL-DYS1), C-terminus (NCL-DYS2), and N-terminus (NCL-DYS3). Monoclonal antibodies were employed for merosin (MAB 1922; Chemicon, Germany), caveolin (Caveolin3; Transduction Laboratories, BD Biosciences, Europe), α-dystroglycan (KlonVIA4-1; Upstate Biotechnology, Europe), lamin A/C (Mouse Hybridoma Supernatant), desmin (M0760, Klon D33; Dako, Europe), and myosin (805-502-L001, Lot L02279, Klon A4.951; Alexis Biochemicals, Europe) staining procedures.

[0083] **Exclusion of the DMD locus**

[0084] Genomic DNA was extracted from blood samples using standard procedures. DNA was amplified by PCR with conditions for thermal cycling adapted from the protocol set out by ABI Prism® Linkage Mapping Set v2.5. Denaturation was performed at 95°C for 15 min, followed by 10 cycles of 94°C for 15 min, 55°C for 15 sec, 72°C for 30 sec. This was followed by 20 cycles of 89°C for 15 sec, 55°C for 15 sec, and 72°C for 30 sec, with a final extension step of 72°C for 10 min. Reaction mix consisted of 50ng genomic DNA, 0.1μmol of each primer, and HotStart Taq Master Mix (Qiagen, Europe) in a reaction mix of 10μL. Linkage analysis to the DMD locus was performed using standard techniques as will be described under 'Linkage analysis.' Five polymorphic STR microsatellite markers surrounding the DMD gene, STR-44 (DXS1238; 180-210bp), STR-45 (DXS1237; 160-185bp), STR-48 (DXS997; 105-120bp), STR-49 (DXS1236; 230-260bp), and STR-50 (DXS1235; 230-260bp), were selected for this purpose. Forward primers were labelled at their 5' ends with either 5-carboxyfluorescein (FAM) or NED fluorochromes. STR-44 (forward primer: TCC AAC ATT GGA AAT CAC ATT TCA A; reverse primer: TCA TCA CAA ATA GAT GTT TCA CAG), STR-45 (forward primer: GAG GCT ATA ATT CTT TAA CTT TGG C; reverse primer: CTC TTT CCC TCT TTA TTC ATG TTA C), STR-48 (forward primer: GCT GGC TTT ATT TTA AGA GGA; reverse primer: GGT TTT CAG TTT CCT GGG TA), STR-49 (forward primer: CGT TTA CCA GCT CAA

AAT CTC AAC; reverse primer: CAT ATG ATA CGA TTC GTG TTT TGC), and STR-50 (forward primer: AAG GTT CCT CCA GTA ACA GAT TTG G; reverse primer: TAT GCT ACA TAG TAT GTC CTC AGA C).

[0085] **Genome-wide SNP analysis: Mapping of a new locus to Xq26-q27**

[0086] A genome-wide 250K NspI Affymetrix SNP microarray was performed on five affected cases (individuals 20, 29, 50, 11, and 45) and three unaffected relatives at the Microarray Facility at The Centre for Applied Genomics (Toronto, Canada). Capable of genotyping on average 250,000 SNPs, the single nucleotide polymorphisms are separated by a median physical distance of 2.5Kb and an average distance of 5.8Kb between SNPs (Affymetrix, CA, USA). The average heterozygosity of these SNPs is 0.30, with approximately 85% of the human genome found within 10Kb of a SNP. SNP microarray gene chip data was subsequently analyzed using dCHIP software.

[0087] **Linkage analysis**

[0088] Multipoint X-recessive nonparametric linkage was computed using easyLINKAGE plus v5.02. Allele frequencies were considered equal. One cM was assumed to be equivalent to 1Mb.

[0089] **Sequencing and mutation analysis of candidate genes (MBNL3, VGLL1, FGF13)**

[0090] The National Center for Biotechnology Information Entrez Genome Map Viewer, Ensembl Human Genome Server and GenBank databases were employed to locate known genes, expressed-sequence tags and putative new genes that map to Xq26-q27. Exon-intron boundaries of the candidate sequences were determined by BLAST searches against the human genome sequence database at the National Center for Biotechnology Information. Intronic primers (primer sequences available on request) were used to amplify all exons of the functional candidate genes by PCR. PCR products were sequenced using the BigDye® Terminator 3.1 Cycle Sequencing Kit (Perkin-Elmer, Applied Biosystems). Sequencing reactions were loaded on the ABI Prism® 3100 DNA Analyzer (Perkin-Elmer, Applied Biosystems) and generated data was collected using the ABI® DATA COLLECTION version 1.1, and subsequently analyzed using the DNA SEQUENCING ANALYSIS version 3.6 software. Sequencing and mutation analysis were performed at the Centre for Addiction and Mental Health (Toronto, Canada).

[0091] **EXAMPLE 2: Identification and Characterization of a Novel X-linked Muscular Myopathy**

[0092] This current study is the first to describe a family affected by a mild X-linked MD that specifically features atrophy that is limited mainly to type I muscle fibers in postural muscles. This large multigenerational Austrian family originates from the Czech republic, and six living affected members have been ascertained and examined to date. Pedigree analysis (Figure 1) shows an X-linked pattern of inheritance. Clinical assessment in all six patients as well as two now-deceased patients from this family revealed a fairly uniform and characteristic phenotype (See Table 1). All subjects appeared to show an athletic stature (Fig. 2), however more detailed examination revealed an almost selective atrophy and wasting of postural muscles, while other muscles were hypertrophic. Predominantly weak and atrophic muscles include the soleus, peroneus longus, tibialis anterior, vastus medialis, erector spinae, lower part of the latissimus dorsi, and abductor pollicis muscles. Additionally, all patients had significant contractures of the Achilles tendon and hamstrings, a short neck and also a mechanically limited range of neck flexion and extension. Tendon reflexes, sensory examination and mental status were normal. In all affected individuals scoliosis, back pain, gait problems and elevated creatine kinase levels were noted. The pseudo-athletic musculature is likely to be a compensatory response to the atrophy of the postural muscles. Cases were asymptomatic until the age of 30, and in six deceased family members who had suffered from the disease there was a wide range in age of death (45-72 years), typically from heart failure but of unknown mechanism. It appears that family members with more active lifestyles show less severe phenotypes and slower progression of disease.

[0093] Muscle biopsies from affected individuals revealed dystrophic changes in postural muscles with variation in fiber sizes, degeneration of muscle endurance type I fibers, increased fatty and connective tissue, and multinucleated sarcomeres (Fig.3). Immunocytochemical staining of biopsied muscle tissue revealed no deficiencies of proteins associated with either autosomal or X-linked forms of MD, including dystrophin and emerin. This is consistent with the clinical and apparent epidemiological differences that distinguish and typify this new type of MD. Myosin ATPase staining revealed a gradual atrophy of high-oxidative, low-glycolytic, endurance type I muscle fibers in postural muscles. While patients in the early stages of the disease show a relatively normal distribution of type I and type II fibers, as the disease progresses there are decreased numbers of type I fibers, which appear atrophied (Fig. 4). Non-postural muscles, including, among others, the gluteus medius, gluteus maximus, biceps

brachii, triceps brachii, lower arms, latissimus dorsi, and extensor muscles, appear normal with respect to muscle fiber distribution and function (Table 2).

[0094] Three different antibodies were used to detect distinct domains of the dystrophin protein. Staining was faint, but not significantly different than unaffected individuals, suggesting this family does not display a variant form of DMD or Becker's MD. Adhalin staining was performed, which excluded autosomal-recessive limb-girdle MD 2C (LGMD2C), LDMD2D, LGMD2E, and LGMD2F. Normal merosin staining excluded congenital MD. Staining for dysferlin and caveolin allowed for exclusion of LGMD2B and LGMD1C, respectively. LGMD1I was excluded following α-dystroglycan staining. The likelihood of this postural MD representing a variant form of X-recessive EDMD was diminished following normal emerin staining. LGMD2D (Duchenne-like autosomal-recessive MD) and spinocerebellar ataxia type 5 (SCA5) were excluded following α-sarcoglycan (LGMD2D) and spectrin (SCA5) staining. Normal staining for lamin A/C, desmin, and β-slow myosin heavy chain excluded autosomal-dominant EDMD2 and LGMD1B (lamin A/C), desminopathies (desmin), and distal myopathy MPD1 (myosin), respectively. Myotonic dystrophy 2 (DM2) and proximal myotonic myopathy (PROMM) were also suggested as possible causative factors, but molecular genetic analysis revealed no mutations.

[0095] Immunocytochemical data and pedigree analysis suggested that this family displays an unsevere myopathy with multinucleated sarcomeres and a pattern of recessive X-chromosome inheritance. To exclude the possibility that the phenotype in this family is a variant form of DMD or Becker's MD, we performed linkage analysis to the DMD locus using five selected polymorphic STR microsatellite markers surrounding the DMD gene; STR-44 (DXS1238), STR-45 (DXS1237), STR-48 (DXS997), STR-49 (DXS1236), and STR-50 (DXS1235). Different haplotypes were revealed in the affecteds across the DMD locus, excluding this locus as the causative gene in this family. Recombination of the intragenic markers STR-44, STR-48, STR-49 and STR-50 was evident (Fig. 5). Subsequent screening for mutations in the DMD gene was conducted by sequencing cDNA proximal to the area spanned by the intragenic markers, which ruled out intragenic recombination. Genotypes for markers across the X-chromosome were analyzed. Multipoint lod scores were found to be significant for the Xq26-q27 region (lod > 3), giving further confirmation for exclusion of the DMD locus. Multipoint lod scores revealed positive, non-significant results for areas surrounding the candidate interval that was later specified by SNP analysis (Fig. 5). A genome-wide SNP genotype analysis was performed on the five affected individuals along with three unaffected family members at The Centre for Applied Genomics

(Toronto, Canada). A ~250K NspI Affymetrix SNP micorarray was used, and subsequent analysis using dCHIP implicated a candidate region on Xq26-q27, the candidate region encompasses approximately 850 consecutive SNPs.

[0096] Three candidate genes from the Xq26-q27 critical region that encode structural proteins expressed in muscle were screened. The muscleblind-like protein 3 (MBNL3), vestigial-like 1 (VGLL1) gene fibroblast growth factor 13 (FGF13) were all sequenced from genomic DNA, but no coding mutations were identified.

[0097] Sequencing of the coding and 5'UTR region of FHL1 (NM_001449) resulted in a transversion at position 672 C to G leading to the amino acid substitution C224W. This mutation co-segregated with disease status within the family, all 6 affected subjects were hemizygous and all obligate carriers were heterozygous for the mutated allele. The mutation was not detected in mixed Caucasian and Austrian control chromosomes.

[0098] FHL1 is a member of LIM-only proteins, containing four and a half LIM domains with a common consensus sequence C-X2-C-X16-21-H-X2-C-X2-C-X2-C-X17-C-X2-C. LIM only proteins are zinc-binding proteins that are known to be play a role in cell signaling and transcriptional regulation. So far, 5 FHL proteins have been identified: FHL1-5 are known to act as transcription regulators.

[0099] The C224W mutation replaces a highly conserved cysteine of the fourth LIM domain of FHL1 which is one of the four cysteines needed for the central binding of a zinc ion. Mutations of conserved cysteines that are part involved in zinc binding have been shown to have a highly deleterious effect on the tertiary structure of the protein (Taira et al, 1994). Furthermore, the C224W mutation also is located in the first nuclear localization signal (NLS1) of the alternatively expressed isoform FHL1b (SLIMMER), which might lead to impaired FHL1b protein from shuttle between the cytoplasm and the nucleus (Brown S et al; J Biol Chem. 1999 Sep 17;274(38):27083-91

[00100] FHL1 has at least 3 different isoforms (a, b and c), each with different tissue specificities. The C224W mutation affects FHL1 isoforms a (the most prevalent isoform) and b, but not isoform c. Hence, mutations within different regions of the gene may affect specific isoforms, with other isoforms unaffected, and thus may have different phenotypic consequences. Furthermore, FHL1 has a number of protein binding partners that bind to different LIM domains within the protein, and

thus a mutation affecting the conformation of one LIM domain may have different phenotypic consequences to a mutation affecting a different LIM domain.

[00101] In summary, we have identified the gene FHL1, and its encoded protein, as responsible for a new form of muscular myopathy, XMPMA. The phenotypic features described in the Austrian family, in particular the specific atrophy of postural muscles and pseudo athleticism, may be specific for mutations within the SRF and MyBPC1 (muscle fiber type 1-specific isoform) and ERK2 binding regions of FHL1. Mutations elsewhere in the gene may result in a much more heterogeneous myopathic phenotype. This has considerable implications for diagnostic evaluation, screening and genetic counseling for patients (also carriers) with muscular or myotonic dystrophy of unknown genetic cause, in particular where the familial nature indicates X-linked inheritance and where the Becker's/Duchenne's MD and Emery-Dreifuss MD loci have been excluded, but also for sporadic cases. Additional information concerning this example may be obtained from Windpassinger et al., The American Journal of Human Genetics 82, 88-99, January 2008 which is herein incorporated by reference.

[00102] Example 3: UK pedigrees (Families 2 an 3) exhibiting muscular myopathies

[00103] Four 4 male individuals in 3 consecutive generations presented with slowly progressive hip and arm weakness with onset in the 3rd-4th decades. The index patient showed prominent shoulder girdle and arm hypertrophy, with CK levels elevated to 1300 U/l. Respiratory failure was reported in two patients who died in their 50s. The UK family 2 pedigree is shown in Figure 1B.

[00104] A third family, with a putative diagnosis of Becker muscular dystrophy was identified, where 6 females and 6 males, spread over 5 generations, were affected. The UK family 3 pedigree is shown in Figure 1C. In male patients, age at onset was in the late teens-3rd decade, and presenting clinical symptoms were progressive limb-girdle weakness with prominent scapular winging. Muscle hypertrophy was not a prominent feature, while neck/cervical rigidity or weakness and Achilles tendon contractures were reported in three patients. CK levels were around 1500-2200 U/l. Two patients were wheelchair bound from their 30s. Respiratory and heart failure in the late 40s-50s were the causes of death in 2 patients. Female mutation carriers presented with a similar but milder clinical picture with onset in the 5th decade or later and CK levels only slightly elevated at 300 U/l. One female patient died at the age of 88 years due to congestive heart failure. The index patient presented with first symptoms of hip flexor weakness (MRC 4) and elevated serum CK levels of around 1300 U/l at the age of 35

years. At that time he was playing competitive football and showed a very athletic habitus. Muscle hypertrophy was most prominent in his shoulder girdle and arm muscles. Neck flexion was compromised by spinal rigidity. His lung function showed a FVC of 4.6 l (90%) in a sitting position and dropped to 4.0 l (78%) in a lying position. There were no additional clinical signs or symptoms of an underlying skeletal muscle or heart disease. Nerve conduction studies and an EMG were normal. A muscle biopsy from the vastus lateralis showed type I fibre atrophy, variation in fibre size, with some measuring up to 125 μm in diameter, and a few necrotic fibres. Immunohistochemical and Western blot analysis for proteins of the dystrophin glycoprotein complex, emerin, dysferlin, caveolin and calpain were normal. Mutation analysis of the genes for dystrophin and emerin did not reveal any abnormalities. The maternal grandfather of the index patient started to experience difficulties with walking at 42 years of age and used a wheelchair for the last years of his life. He died of respiratory failure at 52 with the label of Becker muscular dystrophy. Two nephews of the grandfather were also labeled with Becker muscular dystrophy and experienced slowly progressive muscle weakness in legs and arms from their early 40ies. One of them died in his 50's of respiratory failure.

[00105] Data for the index patient, Family 2:

Age of onset: 35

CK: 1342 U/L

EMG: normal

Muscle MRI: N.D.

Athletic habitus in early stages: yes

Muscle biopsy: myopathic

Cardiac involvement: normal heart evaluation

Neck and Achilles tendons: short (AT)

[00106] The mutation c.381_382insATC (leading to p.Phe127_Thr128insIle) was identified in the index patients of both families and segregates with the phenotype. The F127_T128InsI mutation occurs within the second LIM domain, and thus is present in all three isoforms of FHL1, a, b and c. In

conclusion, the data presented herein shows that the same FHL1 mutation may give rise to heterogeneous phenotypes, with X-linked recessive or dominant inheritance.

[00107] Example 4: Study of Cardiomyopathies in the Austrian XMPMA family

[00108] Patients with the clinical diagnosis of XMPMA and their immediate relatives were invited to participate in a study for cardiovascular investigation of XMPMA. Standard 12 lead ECGs were recorded in the recumbent position. The echocardiographic studies were all performed by one operator using a GE Vivid 7 scanner. Measurements were made according to the standards of the American Society of Echocardiography and analyses were performed using the software programs of the scanner. The doppler variables measured were the peak aortic and LVOT velocities, and transmitral flow for assessing the diastoly. Strain and strain rate measurements were obtained by the non-Doppler 2D strain imaging technique as well as with TDI technique. Genomic DNA and serum profile (enzymes) were extracted from blood samples with standard procedures. Also used were: Magnet Resonance Imaging; Intracardiac catheter with biopsy of the left ventricle; Treadmill testing; ECG Holter monitoring.

[00109] The most common abnormality was T-wave inversion in V4-V6 and other ST-T wave changes, partly signs of left ventricular hypertrophy. All affected family members had pathological treadmill tests with ST wave changes and arrhythmia with extrasystoles (whereas Holter ECG has not been done yet). Left ventricular hypertrophy with thickening confined to the apex as well as involvement of the right ventricle was present in all affected family members. The left ventricle was normal in size with normal systolic but impaired diastolic function. No abnormalities of the mitral valve and its supporting structures were seen, and no LVOT gradient. All affected patients had a dilated left atrium and increased left atrial volume. Tissue velocities, strain rate and strain are also reduced. All affected male members had elevated levels of serum creatinine kinase, CK-MB, LDH, NT-pro BNP, Trop T and liver enzymes. Without wishing to be limiting in any manner, important clinical findings included symptoms from Dyspnoe New York Heart association class II.

[00110] All citations are hereby incorporated by reference.

[00111] The present invention has been described with regard to one or more embodiments. However, it will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

[00112] References:

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[00113] **URLs**

The National Center for Biotechnology Information Entrez Genome Map Viewer is available at http://www.ncbi.nlm.nih.gov/mapview/. Ensembl Human Genome Server is available at http://www.ensembl.org/index.html. GenBank database is available at http://www.ncbi.nlm.nih.gov/Genbank/index.html.

Table 1. Clinical evaluations for members of the XMPMA family from Austria, including electromyogram, NCV, muscle MRI, histological examination of biopsied tissue, and involvement of heart, and of tendons in neck and Achilles heel.

Patients ID	Age of onset	CK level	EMG	NCV studies	Muscle MRI	Athletic habitus at onset	Muscle biopsie	Heart affection	Neck and Achilles tendon
SK060666	26	620	myopathic	normal	Nd	yes	nd	?	
FM240432	30	500-900	myopathic	normal	Nd	yes	myopathic	Cardio myopathy with arrhythmia	short
AJ020657	32	620		normal	Selective muscle atrophie, bent spine	yes	myopathic	Dialtativ cardio myopathy hypertrophic	short
AA030554	32	400-1774	myopathic	normal	Selectiv muscle atrophy, bent spine	yes	myopathic	Normal heart evaluation	short
AF061160	30	780	myopathic	normal	-	yes	nd	Unkown	short
MF250358	30	700	myopathic	normal	Selective muscle atrophy bent spine	yes	myopathic	Hypertrophic cardiomyopathy	short
MW211168	31	550	myopathic	normal	Nd	unkown	myopathic	Hypertrophic cardiomyopathy	short
BJ180830	30	800-1200	myopathic	normal	-nd	yes	myopathic	Respiratory failure	short

Table 2. Type I and type II muscle fibre distribution in several muscles in a patient in progressed stages of disease. Muscles represented in bold display significantly high portion of type I muscle fibres. There is a pronounced decrease in the proportion of type I muscle fibres in postural muscles; adductor magnus, biceps femoris, deltoideus, peronaeus longus, soleus, tibialis anterior, and vastus medialis muscles showed gradual atrophy of type I slow-twitch muscle fibres, whereas many muscles with a high percentage of fiber type II show mild to pronounced hypertrophy.

Muscle	Average muscle fiber composition				
	Typ I	Typ II	atrophic	hypertrophic	normal
Abductor digiti minimi	51,8	48,2			X
Abductor pollicis brevis	**63.0**	**37.0**			X
Abductor hallucis					X
Adductor magnus (surface)	53.5	46,5			
Adductor magnus (deep)	**63.3**	**36.7**	**X**		
Adductor pollicis	**80,4**	**19.6**			?
Biceps brachii (surface)	42,3	57,7		X	
Biceps brachii (Deep)	50,5	49,5		X	
Biceps fernoris	**66.9**	**33.1**	**X**		
Brachioradialis	39.8	60.2			X
Deltoideus (Surface)	53.3	46.7	X		
Deltoideus (Deep)	**61,0**	**39,0**	**X**		
I dorsal interosseus	57,4	42.6			X
Erector spinae (Surface)	58,4	41,6	X		

Erector spinae (Deep)	54,9	45,1	X		
Extensor digitorum	47,3	52.7		X	
Extensor digitorum brevis	45,3	54,7		X	X
Flexor digitorum brevis	44.5	*55,5*		X	
Flexor digitorum profundus	47,3	52,7		X	
Frontalis	**64,1**	**35,9**			?
Gastrocnemius (lat. head. Surface)	43.5	56.5	X		
Gastrocnemius (lat. head. Deep)	*50,3*	49,7	X		
Gastrocnemius (medial head)	50,8	49.2	X		
Gluteus medius					X
Gluteus maximus	52,4	47.6			X
Iliopsoas	49,2	50,8			?
Iliocostalis			X		
Interspinales cervicis			X		
Infraspinatus	45,3	54,7		X	
Longus capitis					X
Longus colli					X
Longisimus dorsi			X		
Latissimus dorsi	50,5	49.5			X
multifidus			X		
Orbicularis oculi	15.4	84,6			X
Obliqus capitis			X		
Pectoralis major (clavic. head)	42,3	57,7			?
Pectoralis major (sternal head)	43.1	56,9			?
Peronaeus longus	**62,5**	**37,5**	**X**		

Psoas					X
Rectus abdominis	46.1	53,9		X	
Rectus femoris (lat. head. Surface)	29.5	10,5			X
Rectus femoris (lat. head. Deep)	42,0	58,0			X
Rectus femoris (medial head)	42,8	57,2			X
Rhomboideus	44,6	55.4		X	X
Sartorius	49,6	*50,4*			
Semimembranosus			X		
semispinalis			**X**		
Soleus (Surface)	**86,4**	**13.6**	**X**		
Soleus (Deep)	**89.0**	**11.0**	**X**		
Splenius					X
Sternocleidomastoideus	35,2	64.8		X	X
Supraspinatus	59.3	40,7	X		
Temporalis	*46,5*	53,5			X
Tibialis anterior (Surface)	**73,4**	**26.6**	**X**		
Tibialis anterior (Deep)	**72.7**	**27.3**	**X**		
Trapezius	53,7	46.2	X		X
Transversus occipitalis					
Triceps surae			X		
Triceps (Surface)	32.5	67,5		X	
Triccps (Deep)	32,7	67,3		X	
Vastus lateralis (Surface)	37,8	62,2		X	
Vastus lateralis (Deep)	46,9	53.1	X		
Vastus medialis (surface)	43,7	56,3			X

Vastus medialis (Deep)		**61,5**		**38,5**	**X**	
	JOHNSON et al. (1973).					

WHAT IS CLAIMED IS:

1. A protein comprising amino acids 1-230 of SEQ ID NO:1, a fragment thereof or a sequence exhibiting at least 70% identity thereto and comprising the amino acid sequence $VAKKCX_1GX_2X_3NPIT$ (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid.

2. The protein as defined in claim 1, wherein X_2 is tryptophan.

3. The protein of claim 1, defined by SEQ ID NO:2 or SEQ ID NO:3.

4. A nucleic acid comprising a sequence

a) encoding the protein of claim 1 or a fragment thereof;

b) that is the complement of a sequence encoding the protein of claim1, or a fragment thereof;

c) that is capable of hybridizing to a nucleic acid encoding the protein of claim 1 or fragment thereof under stringent hybridization conditions; or

d) that exhibits greater than about 70% sequence identity with the nucleic acid defined in a) or b).

5. The nucleic acid of claim 4, wherein the fragment comprises the amino acids sequence GWK.

6. The nucleic acid of claim 4, wherein X_2 is tryptophan.

7. The nucleic acid of claim 4, wherein the protein is defined by SEQ ID NO:2 or SEQ ID NO:3.

8. A method of screening a subject for an X-linked muscular myopathy comprising

a) obtaining a biological sample from the subject, and;

b) assaying the sample for a nucleic acid encoding the protein as defined in claim 1 or a fragment thereof comprising the amino acid sequence $VAKKCX_1GX_2X_3NPIT$ (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid, or

c) assaying the sample for the protein as defined in claim 1 or a fragment thereof comprising the amino acid sequence VAKKCX_1GX_2X_3NPIT (SEQ ID NO:4) wherein X_2 is any amino acid except C; and X_1 and X_3 are independently any amino acid.

9. The method as defined in claim 8 wherein X_2 is tryptophan.

10. The method as defined in claim 8 wherein the protein is defined by SEQ ID NO:2 or SEQ ID NO:3.

11. The method of claim 8, wherein the subject is a human subject.

12. The method of claim 8, wherein the biological sample is a blood sample.

13. The method of claim 8, wherein assaying comprises PCR, probe hybridization, immunohistochemistry, nucleotide sequencing or protein sequencing.

14. A kit comprising

i) a protein or fragment thereof that is associated with muscular myopathy as defined herein,

ii) an antibody that selectively binds to a protein or fragment thereof associated with muscular myopathy as defined herein, as compared to a wild-type protein not associated with muscular myopathy,

iii) one or more nucleic acid primers to amplify a nucleotide sequence encoding a protein or fragment thereof which comprises a mutation associated with an X-linked muscular myopathy as provided herein,

iv) one or more nucleic acid probes of between about 9 and 100 nucleotides that hybridizes nucleotide sequence encoding a protein or fragment thereof which comprises a mutation associated with an X-linked muscular myopathy as provided herein,

v) one or more reagents including, but not limited to buffer(s), dATP, dTTP, dCTP, dGTP, or DNA polymerase(s),

vi) instructions for assaying, diagnosing or determining the risk of a subject to muscular myopathy,

vii) instructions for using any component or practicing any method as described herein,

or any combination thereof.

15. The method of claim 8, wherein the muscular myopathy is a skeletal muscle myopathy or a cardiomyopathy.

16. The method of claim 15, wherein the muscular myopathy is muscular dystrophy.

17. A FHL-1 protein comprising an isoleucine insertion at position 128.

18. The protein of claim 17 wherein said protein has the human isoform a, b or c amino acid sequence or an amino acid sequence which is at least 70% identical thereto.

19. A nucleotide sequence encoding the FHL-1 protein of claim 17.

20. An antibody that selectively binds the FHL-1 protein of claim 17 but not a wild type FHL-1 protein.

21. A method of screening a subject for an X-linked muscular myopathy comprising

a) obtaining a biological sample from the subject;

b) assaying the sample for a nucleic acid encoding a FHL-1 protein comprising an isoleucine insertion at position 128, or

c) assaying the sample for the FHL-1 protein comprising an isoleucine insertion at position 128,

wherein the presence of the nucleic acid or protein indicates that the subject has or is at risk of developing a muscular myopathy.

Fig. 1A. Pedigree of the X-recessive postural muscular myopathy family. Family members from whom DNA samples were obtained are indicated by arrows (↙).

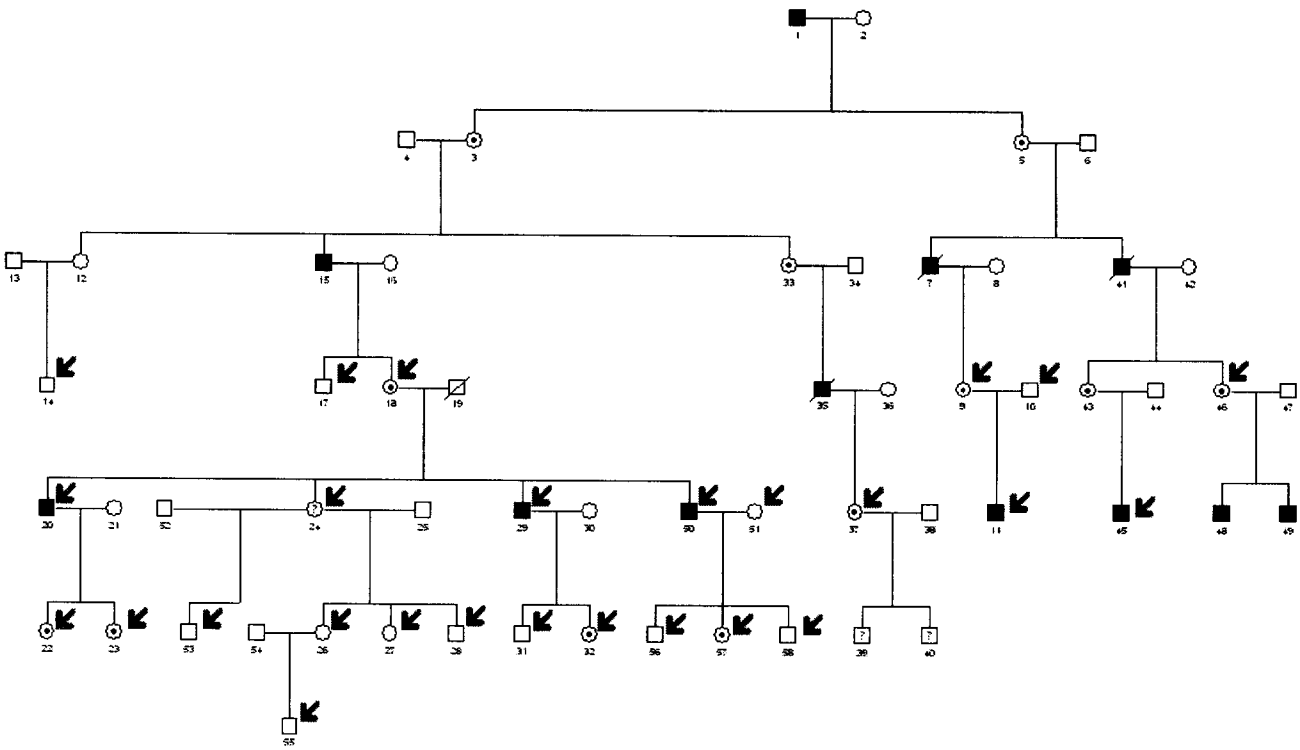


Figure 1B. UK family 2 pedigree.

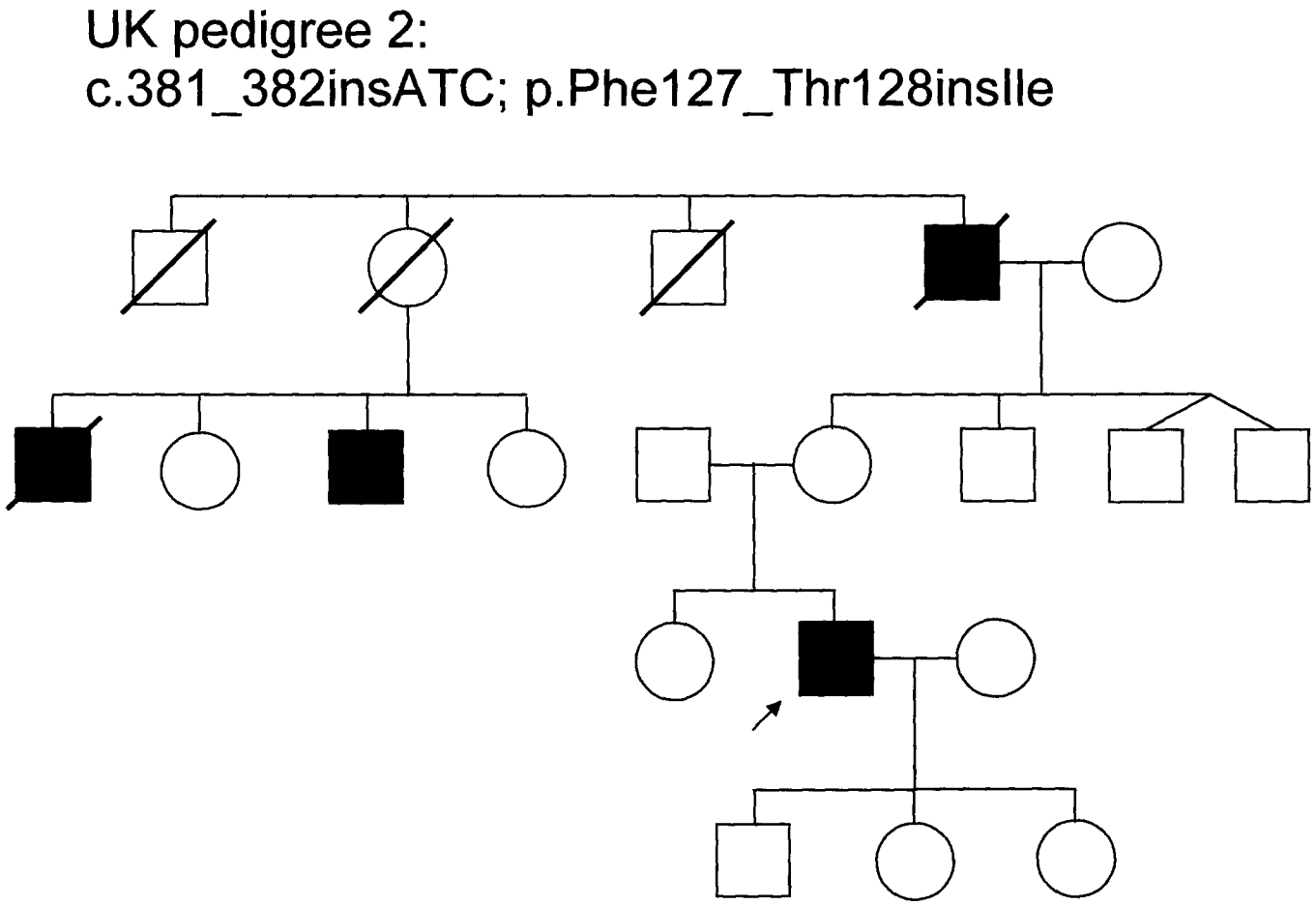


Figure 1C. UK family 3 pedigree: c.381_382insATC; p.Phe127_Thr128insIle

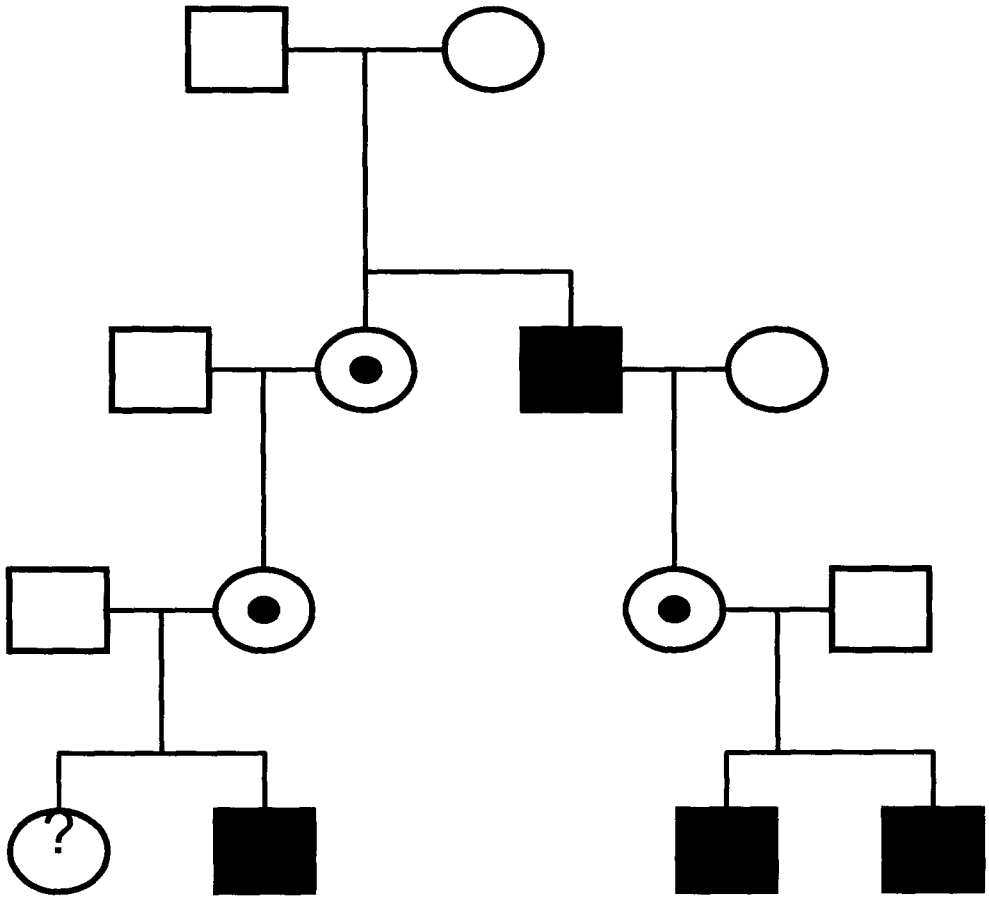


Fig 2. Atrophy of the postural back muscles as clinically assessed in a patient in the early stages of disease. Atrophy of the deltoideus muscle. Gluteus maximus, biceps brachii, triceps brachii, and lower arms appear normal. Biceps femoris (hamstring muscles), adductor magnus (thighs), abductor pollicis brevis and adductor pollicis longus (hand) show signs of atrophy.

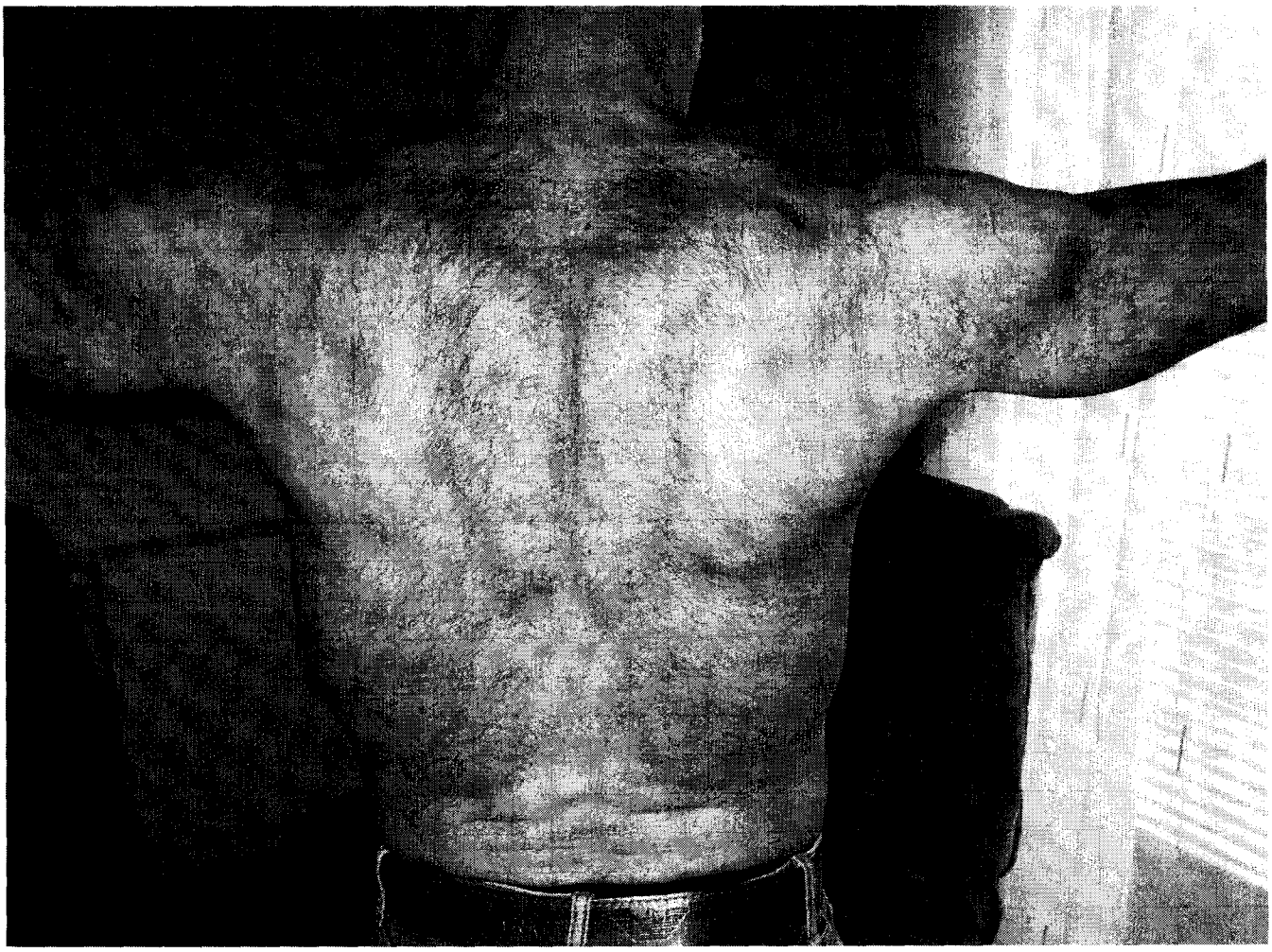
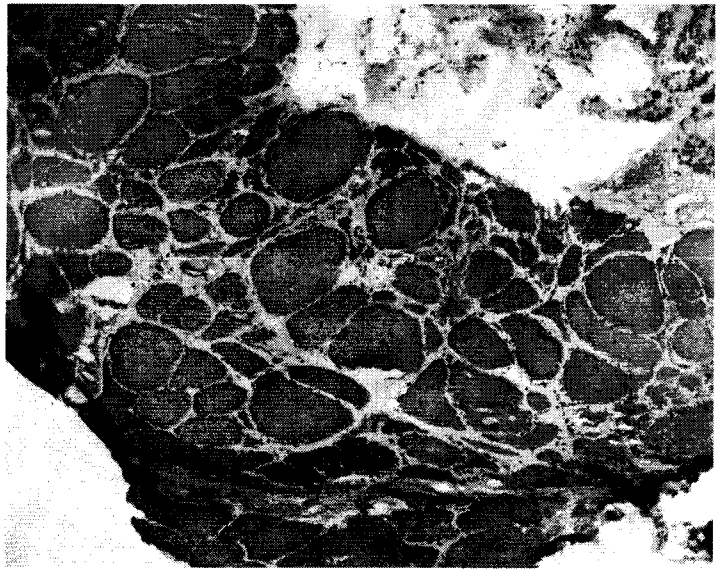


Fig. 3. Muscle biopsy of the vastus lateralis muscle (**A.**) and anterior tibial muscle (**B**). Muscle histology revealed a moderate myopathy with a moderate perimysial and limited endomysial fibrosis. In all biopsies, some round, autophagic vacuoles predominant in type 2 fibers were detectable. These vacuolar changes were most prominent in patient **B**. Additionally, centrally placed myonuclei were increased and rarely single fiber necrosis and granular myofiber degeneration were seen.

A.

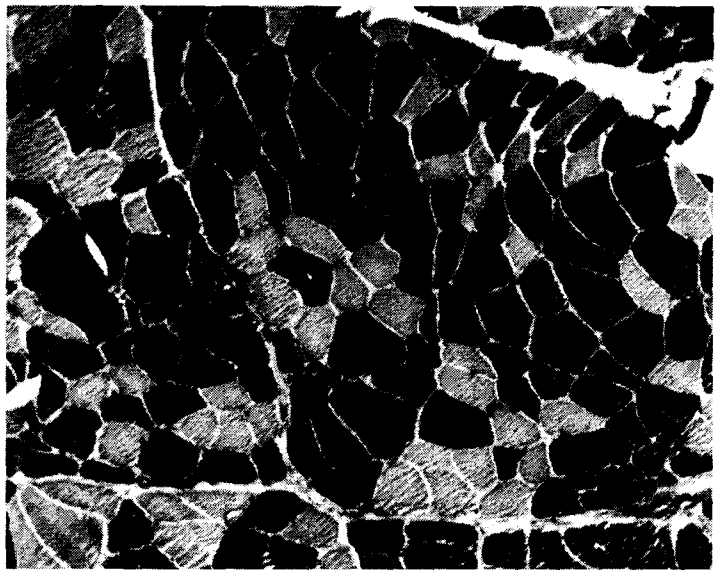
B.



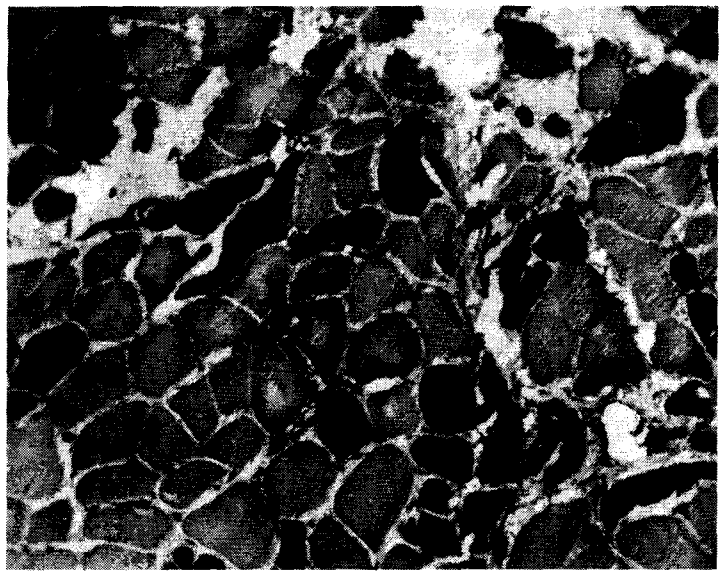
PCT/CA2008/001062

Fig 4. Muscle biopsy of the vastus lateralis muscle (**A.**) and anterior tibial muscle (**B**). Myosin ATPase staining at acidic pH 4.3/4.6 reveals type I (dark) and type II (light) muscle fibre distribution in patients in the early stages of disease. Variability of fiber size was increased in all specimens, with diameters ranging between 20 to 100μm, and most prominent in type 2 fibers. In NADH and COX histochemistry centrally negative core-like lesions were detected in both patients, without any further mitochondrial alterations.

A.



B.



PCT/CA2008/001062

Fig. 5. Linkage analysis to the *DMD* locus using polymorphic STR intragenic markers STR-44, STR-45, STR-48, STR-49, and STR-50 revealed different haplotypes in the affecteds, conclusively excluding the *DMD* locus. Recombination of markers STR-44, STR-48, STR-49, and STR-50 is evident, as illustrated by haplotypes.

Error!

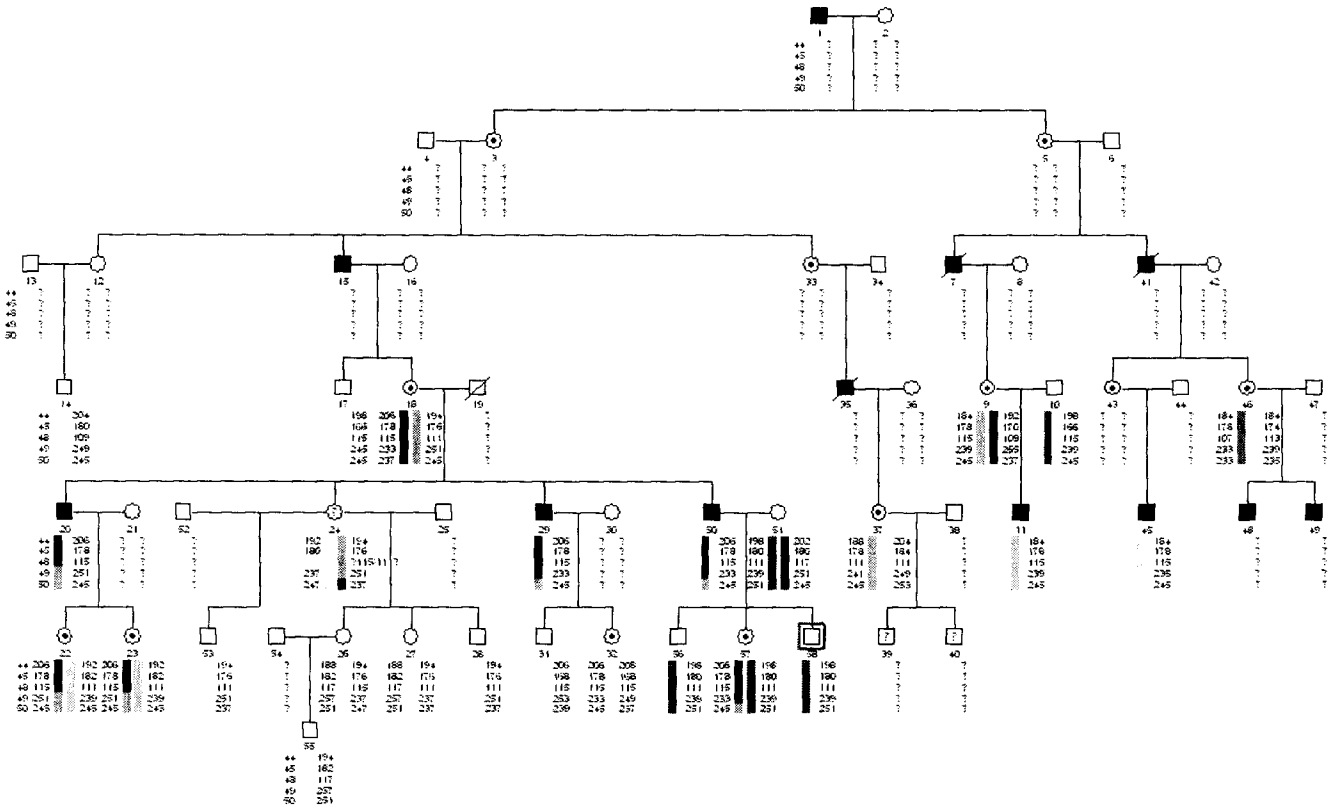
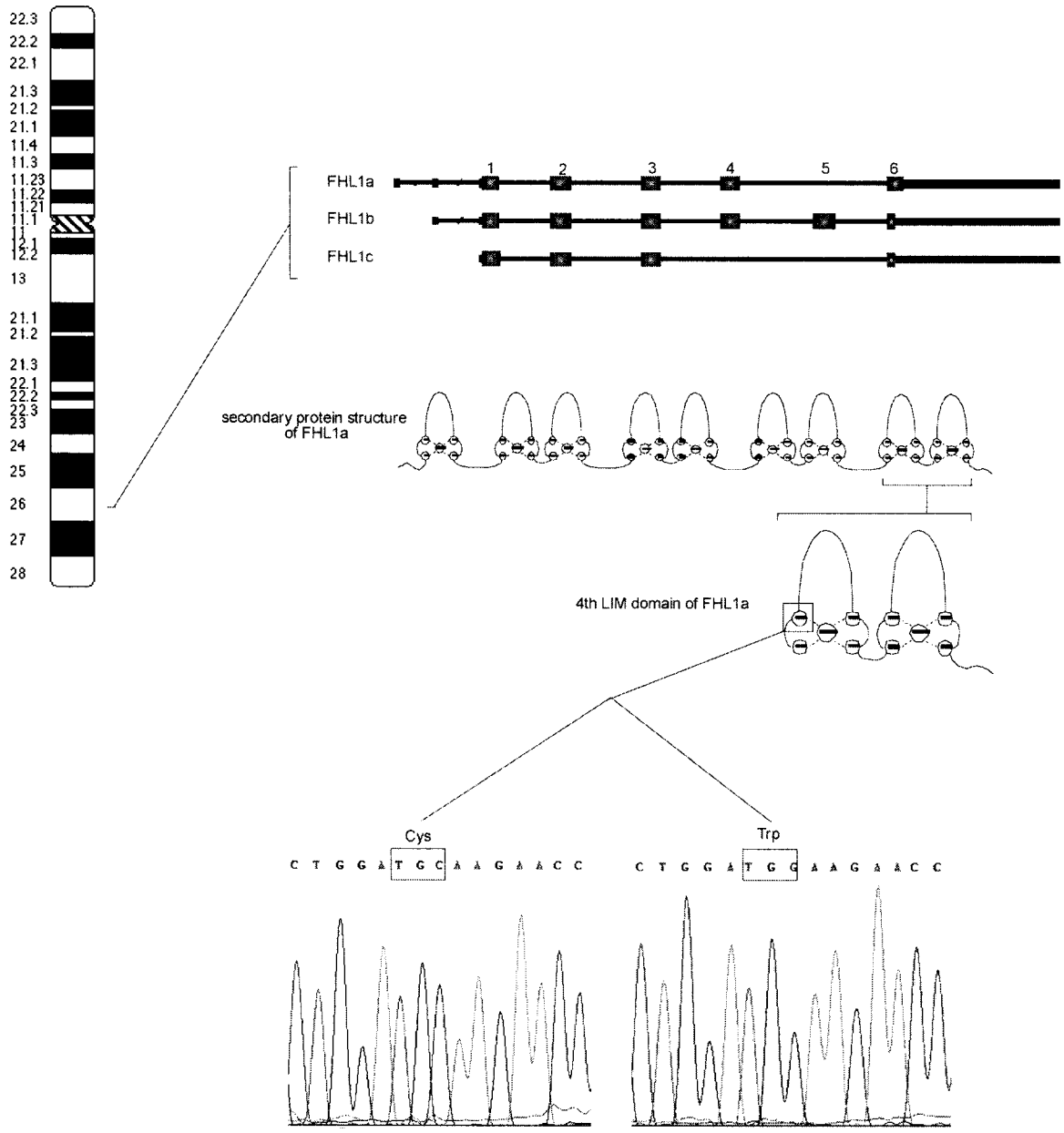


Fig. 6. Ideogrammatic representation of the XMPMA locus on the distal arm of chromosome X, the electropherograms indicating the wild-type and mutation sequence for the Austrian XMPMA family, and the secondary structure of FHL1, indicating the position of the resulting amino acid substitution, C224W, relative to structural features in the protein.



MAEKFDCHYCRDPLQGKKYVQKDGHHCCLKCFDKFCANTCVECRKPIGADSKEVHYKNRF
WHDTCFRCAKCLHPLANETFVAKDNKILCNKCTTREDSPKCKGCFKAIVAGDQNVEYKGT
VWHKDCFTCSNCKQVIGTGSFFPKGEDFYCVTCHETKFAKHCVKCNKAITSGGITYQDQP
WHADCFVCVTCSKKLAGQRFTAVEDQYYCVDCYKNFVAKKCAGCKNPITGFGKGSSVVAY
EGQSWHDYCFHCKKCSVNLANKRFVFHQEQVYCPDCAKKL

FIGURE 7

Human FHL1 isoform a and isoform b shared sequence

MAEKFDCHYCRDPLQGKKYVQKDGHHCCLKCFDKFCANTCVECRKPIGADSKEVHYKNRFWH
DTCFRCAKCLHPLANETFVAKDNKILCNKCTTREDSPKCKGCFKAIVAGDQNVEYKGTVWHKD
CFTCSNCKQVIGTGSFFPKGEDFYCVTCHETKFAKHCVKCNKAITSGGITYQDQPWHADCFVCV
TCSKKLAGQRFTAVEDQYYCVDCYKNFVAKKCAGXKNPITG (SEQ ID NO:1)

C224W mutation in human FHL1 isoform a (W is underlined)

MAEKFDCHYCRDPLQGKKYVQKDGHHCCLKCFDKFCANTCVECRKPIGADSKEVHYKNRFWH
DTCFRCAKCLHPLANETFVAKDNKILCNKCTTREDSPKCKGCFKAIVAGDQNVEYKGTVWHKD
CFTCSNCKQVIGTGSFFPKGEDFYCVTCHETKFAKHCVKCNKAITSGGITYQDQPWHADCFVCV
TCSKKLAGQRFTAVEDQYYCVDCYKNFVAKKCAG[redacted]KNPITGFGKGSSVVAYEGQSWHDYCFH
CKKCSVNLANKRFVFHQEQVYCPDCAKKL (SEQ ID NO:2)

C224W mutation in human FHL1 isoform b (W is underlined)

MAEKFDCHYCRDPLQGKKYVQKDGHHCCLKCFDKFCANTCVECRKPIGADSKEVHYKNRFWH
DTCFRCAKCLHPLANETFVAKDNKILCNKCTTREDSPKCKGCFKAIVAGDQNVEYKGTVWHKD
CFTCSNCKQVIGTGSFFPKGEDFYCVTCHETKFAKHCVKCNKAITSGGITYQDQPWHADCFVCV
TCSKKLAGQRFTAVEDQYYCVDCYKNFVAKKCAG[redacted]KNPITGKRTVSRVSRPVSKARKPPVCHG
KRLPLTLFPSANLRGRHPGGERTCPSWVVVLYRKNRSLAAPRGPGLVKAPVWWPMKDNPGTTT
ASTAKNAP (SEQ ID NO:3)

species/isoform conserved sequence

VAKKCX_1GX_2X_3NPIT (SEQ ID NO:4)

Representative mRNA encoding mutant human FHL1 isoform a; mutation of X (underlined) to nucleotide that results in C224W mutation associated with X-linked muscular myopathy

CGGAGGGGGCTCAGTCCGCAGCCGCCGCCGCCACCGCCGCGCCTCGGCCTCGGTGCAGGCA
GCGGCCGCCGCCGCCGAGACAGCTGCGCGGGCGAGCATCCCCACGCAGCACCTTGGAAGTT
GTTTTCAACCATATCCAGCCTTTGCCGAATACATCCTATCTGCCACACATCCAGCGTGAGGTC

CCTCCAGCTACAAGGTGGGCACCATGGCGGAGAAGTTTGACTGCCACTACTGCAGGGATCCC

TTGCAGGGGAAGAAGTATGTGCAAAAGGATGGCCACCACTGCTGCCTGAAATGCTTTGACAA

GTTCTGTGCCAACACCTGTGTGGAATGCCGCAAGCCCATCGGTGCGGACTCCAAGGAGGTGC

ACTATAAGAACCGCTTCTGGCATGACACCTGCTTCCGCTGTGCCAAGTGCCTTCACCCCTTGG

CCAATGAGACCTTTGTGGCCAAGGACAACAAGATCCTGTGCAACAAGTGCACCACTCGGGAG

GACTCCCCCAAGTGCAAGGGGTGCTTCAAGGCCATTGTGGCAGGAGATCAAAACGTGGAGT

ACAAGGGGACCGTCTGGCACAAAGACTGCTTCACCTGTAGTAACTGCAAGCAAGTCATCGGG

ACTGGAAGCTTCTTCCCTAAAGGGGAGGACTTCTACTGCGTGACTTGCCATGAGACCAAGTT

TGCCAAGCATTGCGTGAAGTGCAACAAGGCCATCACATCTGGAGGAATCACTTACCAGGATC

AGCCCTGGCATGCCGATTGCTTTGTGTGTGTTACCTGCTCTAAGAAGCTGGCTGGGCAGCGT

TTCACCGCTGTGGAGGACCAGTATTACTGCGTGGATTGCTACAAGAACTTTGTGGCCAAGAA

GTGTGCTGGATG[redacted]AAGAACCCCATCACTGGGTTTGGTAAAGGCTCCAGTGTGGTGGCCTATG

AAGGACAATCCTGGCACGACTACTGCTTCCACTGCAAAAAATGCTCCGTGAATCTGGCCAAC

AAGCGCTTTGTTTTCCACCAGGAGCAAGTGTATTGTCCCGACTGTGCCAAAAAGCTGTAAAC

TGACAGGGGCTCCTGTCCTGTAAAATGGCATTTGAATCTCGTTCTTTGTGTCCTTACTTTCTG

CCCTATACCATCAATAGGGGAAGAGTGGTCCTTCCCTTCTTTAAAGTTCTCCTTCCGTCTTTT

CTCCCATTTTACAGTATTACTCAAATAAGGGCACACAGTGATCATATTAGCATTTAGCAAAA

AGCAACCCTGCAGCAAAGTGAATTTCTGTCCGGCTGCAATTTAAAAATGAAAACTTAGGTAG

ATTGACTCTTCTGCATGTTTCTCATAGAGCAGAAAAGTGCTAATCATTTAGCCACTTAGTGAT

GTAAGCAAGAAGCATAGGAGATAAAACCCCCACTGAGATGCCTCTCATGCCTCAGCTGGGAC

CCACCGTGTAGACACACGACATGCAAGAGTTGCAGCGGCTGCTCCAACTCACTGCTCACCCT

CTTCTGTGAGCAGGAAAAGAACCCTACTGACATGCATGGTTTAACTTCCTCATCAGAACTCT

GCCCTTCCTTCTGTTCTTTTGTGCTTTCAAATAACTAACACGAACTTCCAGAAAATTAACATT

TGAACTTAGCTGTAATTCTAAACTGACCTTTCCCCGTACTAACGTTTGGTTTCCCCGTGTGGC

ATGTTTTCTGAGCGTTCCTACTTTAAAGCATGGAACATGCAGGTGATTTGGGAAGTGTAGAA

AGACCTGAGAAAACGAGCCTGTTTCAGAGGAACATCGTCACAACGAATACTTCTGGAAGCTT

AACAAAACTAACCCTGCTGTCCTTTTTATTGTTTTTAATTAATATTTTTGTTTTAATTGATAGC

AAAATAGTTTATGGGTTTGGAAACTTGCATGAAAATATTTTAGCCCCCTCAGATGTTCCTGC

AGTGCTGAAATTCATCCTACGGAAGTAACCGCAAAACTCTAGAGGGGGAGTTGAGCAGGCG

CCAGGGCTGTCATCAACATGGATATGACATTTCACAACAGTGACTAGTTGAATCCCTTGTAA

CGTAGTAGTTGTCTGCTCTTTGTCCATGTGTTAATGAGGACTGCAAAGTCCCTTCTGTTGTGA
TTCCTAGGACTTTTCCTCAAGAGGAAATCTGGATTTCCACCTACCGCTTACCTGAAATGCAGG
ATCACCTACTTACTGTATTCTACATTATTATATGACATAGTATAATGAGACAATATCAAAAGT
AAACATGTAATGACAATACATACTAACATTCTTGTAGGAGTGGTTAGAGAAGCTGATGCCTC
ATTTCTACATTCTGTCATTAGCTATTATCATCTAACGTTTCAGTGTATCCTTACAGAAATAAA
GCAGCATATGAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 5);

Representative mRNA encoding mutant human FHL1 isoform b; mutation of X (underlined) to nucleotide that results in C224W mutation associated with X-linked muscular myopathy

TCCTATCTGCCACACATCCAGCGTGAGGTCCCTCCAGCTACAAGGTGGGCACCATGGCGGAG
AAGTTTGACTGCCACTACTGCAGGGATCCCTTGCAGGGGAAGAAGTATGTGCAAAAGGATG
GCCACCACTGCTGCCTGAAATGCTTTGACAAGTTCTGTGCCAACACCTGTGTGGAATGCCGC
AAGCCCATCGGTGCGGACTCCAAGGAGGTGCACTATAAGAACCGCTTCTGGCATGACACCTG
CTTCCGCTGTGCCAAGTGCCTTCACCCCTTGGCCAATGAGACCTTTGTGGCCAAGGACAACA
AGATCCTGTGCAACAAGTGCACCACTCGGGAGGACTCCCCCAAGTGCAAGGGGTGCTTCAAG
GCCATTGTGGCAGGAGATCAAAACGTGGAGTACAAGGGGACCGTCTGGCACAAAGACTGCT
TCACCTGTAGTAACTGCAAGCAAGTCATCGGGACTGGAAGCTTCTTCCCTAAAGGGGAGGAC
TTCTACTGCGTGACTTGCCATGAGACCAAGTTTGCCAAGCATTGCGTGAAGTGCAACAAGGC
CATCACATCTGGAGGAATCACTTACCAGGATCAGCCCTGGCATGCCGATTGCTTTGTGTGTG
TTACCTGCTCTAAGAAGCTGGCTGGGCAGCGTTTCACCGCTGTGGAGGACCAGTATTACTGC
GTGGATTGCTACAAGAACTTTGTGGCCAAGAAGTGTGCTGGATG[X]AAGAACCCCATCACTGG
GAAAAGGACTGTGTCAAGAGTGAGCCGCCCAGTCTCTAAAGCTAGGAAGCCCCCAGTGTGC
CACGGGAAACGCTTGCCTCTCACCCTGTTTCCCAGCGCCAACCTCCGGGGCAGGCATCCGGG
TGGAGAGAGGACTTGTCCCTCGTGGGTGGTGGTTCTTTATAGAAAAAATCGAAGCTTAGCAG
CTCCTCGTGGCCCGGGTTTGGTAAAGGCTCCAGTGTGGTGGCCTATGAAGGACAATCCTGGC
ACGACTACTGCTTCCACTGCAAAAAATGCTCCGTGAATCTGGCCAACAAGCGCTTTGTTTTCC
ACCAGGAGCAAGTGTATTGTCCCGACTGTGCCAAAAAGCTGTAA (SEQ ID NO:6)

Human FHL1 isoform a

NM_001449

>gi|34147646|ref|NM_001449.3| Homo sapiens four and a half LIM domains 1 (FHL1), mRNA

CGGAGGGGGCTCAGTCCGCAGCCGCCGCCGCCACCGCCGCGCCTCGGCCTCGGTGCAGGCAGCGGCCGCC

GCCGCCGAGACAGCTGCGCGGGCGAGCATCCCCACGCAGCACCTTGGAAGTTGTTTTCAACCATATCCAG

CCTTTGCCGAATACATCCTATCTGCCACACATCCAGCGTGAGGTCCCTCCAGCTACAAGGTGGGCACCAT

GGCGGAGAAGTTTGACTGCCACTACTGCAGGGATCCCTTGCAGGGGAAGAAGTATGTGCAAAAGGATGGC

CACCACTGCTGCCTGAAATGCTTTGACAAGTTCTGTGCCAACACCTGTGTGGAATGCCGCAAGCCCATCG

GTGCGGACTCCAAGGAGGTGCACTATAAGAACCGCTTCTGGCATGACACCTGCTTCCGCTGTGCCAAGTG

CCTTCACCCCTTGGCCAATGAGACCTTTGTGGCCAAGGACAACAAGATCCTGTGCAACAAGTGCACCACT

CGGGAGGACTCCCCCAAGTGCAAGGGGTGCTTCAAGGCCATTGTGGCAGGAGATCAAAACGTGGAGTACA

AGGGGACCGTCTGGCACAAAGACTGCTTCACCTGTAGTAACTGCAAGCAAGTCATCGGGACTGGAAGCTT

CTTCCCTAAAGGGGAGGACTTCTACTGCGTGACTTGCCATGAGACCAAGTTTGCCAAGCATTGCGTGAAG

TGCAACAAGGCCATCACATCTGGAGGAATCACTTACCAGGATCAGCCCTGGCATGCCGATTGCTTTGTGT

GTGTTACCTGCTCTAAGAAGCTGGCTGGGCAGCGTTTCACCGCTGTGGAGGACCAGTATTACTGCGTGGA

TTGCTACAAGAACTTTGTGGCCAAGAAGTGTGCTGGATG[redacted]AAGAACCCCATCACTGGGTTTGGTAAAGGC

TCCAGTGTGGTGGCCTATGAAGGACAATCCTGGCACGACTACTGCTTCCACTGCAAAAAATGCTCCGTGA

ATCTGGCCAACAAGCGCTTTGTTTTCCACCAGGAGCAAGTGTATTGTCCCGACTGTGCCAAAAAGCTGTA

AACTGACAGGGGCTCCTGTCCTGTAAAATGGCATTTGAATCTCGTTCTTTGTGTCCTTACTTTCTGCCCT

ATACCATCAATAGGGGAAGAGTGGTCCTTCCCTTCTTTAAAGTTCTCCTTCCGTCTTTTCTCCCATTTTA

CAGTATTACTCAAATAAGGGCACACAGTGATCATATTAGCATTTAGCAAAAAGCAACCCTGCAGCAAAGT

GAATTTCTGTCCGGCTGCAATTTAAAAATGAAAACTTAGGTAGATTGACTCTTCTGCATGTTTCTCATAG

AGCAGAAAAGTGCTAATCATTTAGCCACTTAGTGATGTAAGCAAGAAGCATAGGAGATAAAACCCCCACT

GAGATGCCTCTCATGCCTCAGCTGGGACCCACCGTGTAGACACACGACATGCAAGAGTTGCAGCGGCTGC

TCCAACTCACTGCTCACCCTCTTCTGTGAGCAGGAAAAGAACCCTACTGACATGCATGGTTTAACTTCCT

CATCAGAACTCTGCCCTTCCTTCTGTTCTTTTGTGCTTTCAAATAACTAACACGAACTTCCAGAAAATTA

ACATTTGAACTTAGCTGTAATTCTAAACTGACCTTTCCCCGTACTAACGTTTGGTTTCCCCGTGTGGCAT

GTTTTCTGAGCGTTCCTACTTTAAAGCATGGAACATGCAGGTGATTTGGGAAGTGTAGAAAGACCTGAGA

AAACGAGCCTGTTTCAGAGGAACATCGTCACAACGAATACTTCTGGAAGCTTAACAAAACTAACCCTGCT

GTCCTTTTTATTGTTTTTAATTAATATTTTTGTTTTAATTGATAGCAAAATAGTTTATGGGTTTGGAAAC

TTGCATGAAAATATTTTAGCCCCCTCAGATGTTCCTGCAGTGCTGAAATTCATCCTACGGAAGTAACCGC

AAAACTCTAGAGGGGGAGTTGAGCAGGCGCCAGGGCTGTCATCAACATGGATATGACATTTCACAACAGT

GACTAGTTGAATCCCTTGTAACGTAGTAGTTGTCTGCTCTTTGTCCATGTGTTAATGAGGACTGCAAAGT

CCCTTCTGTTGTGATTCCTAGGACTTTTCCTCAAGAGGAAATCTGGATTTCCACCTACCGCTTACCTGAA

ATGCAGGATCACCTACTTACTGTATTCTACATTATTATATGACATAGTATAATGAGACAATATCAAAAGT

AAACATGTAATGACAATACATACTAACATTCTTGTAGGAGTGGTTAGAGAAGCTGATGCCTCATTTCTAC

ATTCTGTCATTAGCTATTATCATCTAACGTTTCAGTGTATCCTTACAGAAATAAAGCAGCATATGAAAAA

AAAAAAAAAAAAAAAAAA

NP_001440 (LIM Domains highlighted in Red;

>gi|21361122|ref|NP_001440.2| four and a half LIM domains 1 [Homo sapiens]

MAEKFDCHYCRDPLQGKKYVQKDGHHCCLKCFDKFCANTCVECRKPIGADSKEVHYKNRFWHDTCFRCAK

CLHPLANETFVAKDNKILCNKCTTREDSPKCKGCFKAIVAGDQNVEYKGTVWHKDCFTCSNCKQVIGTGS

FFPKGEDFYCVTCHETKFAKHCVKCNKAITSGGITYQDQPWHADCFVCVTCSKKLAGQRFTAVEDQYYCV

DCYKNFVAKKCAGCKNPITGFGKGSSVVAYEGQSWHDYCFHCKKCSVNLANKRFVFHQEQVYCPDCAKKL

Human FHL1 isoform b
AF098518

>gi|3851649|gb|AF098518.1|AF098518 Homo sapiens four and a half LIM domains 1 protein isoform B (FHL1) mRNA, complete cds

TCCTATCTGCCACACATCCAGCGTGAGGTCCCTCCAGCTACAAGGTGGGCACCATGGCGGAGAAGTTTGA

CTGCCACTACTGCAGGGATCCCTTGCAGGGGAAGAAGTATGTGCAAAAGGATGGCCACCACTGCTGCCTG

AAATGCTTTGACAAGTTCTGTGCCAACACCTGTGTGGAATGCCGCAAGCCCATCGGTGCGGACTCCAAGG

AGGTGCACTATAAGAACCGCTTCTGGCATGACACCTGCTTCCGCTGTGCCAAGTGCCTTCACCCCTTGGC

CAATGAGACCTTTGTGGCCAAGGACAACAAGATCCTGTGCAACAAGTGCACCACTCGGGAGGACTCCCCC

AAGTGCAAGGGGTGCTTCAAGGCCATTGTGGCAGGAGATCAAAACGTGGAGTACAAGGGGACCGTCTGGC

ACAAAGACTGCTTCACCTGTAGTAACTGCAAGCAAGTCATCGGGACTGGAAGCTTCTTCCCTAAAGGGGA

GGACTTCTACTGCGTGACTTGCCATGAGACCAAGTTTGCCAAGCATTGCGTGAAGTGCAACAAGGCCATC

ACATCTGGAGGAATCACTTACCAGGATCAGCCCTGGCATGCCGATTGCTTTGTGTGTGTTACCTGCTCTA

AGAAGCTGGCTGGGCAGCGTTTCACCGCTGTGGAGGACCAGTATTACTGCGTGGATTGCTACAAGAACTT

TGTGGCCAAGAAGTGTGCTGGATGCAAGAACCCCATCACTGGGAAAAGGACTGTGTCAAGAGTGAGCCGC

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AAC72390

>gi|3851650|gb|AAC72390.1| four and a half LIM domains 1 protein isoform B [Homo sapiens]

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CLHPLANETFVAKDNKILCNKCTTREDSPKCKGCFKAIVAGDQNVEYKGTVWHKDCFTCSNCKQVIGTGS

FFPKGEDFYCVTCHETKFAKHCVKCNKAITSGGITYQDQPWHADCFVCVTCSKKLAGQRFTAVEDQYYCV

DCYKNFVAKKCAGCKNPITGKRTVSRVSRPVSKARKPPVCHGKRLPLTLFPSANLRGRHPGGERTCPSWV

VVLYRKNRSLAAPRGPGLVKAPVWWPMKDNPGTTTASTAKNAP

Human FHL1 isoform c

AF220153

>gi|6942192|gb|AF220153.1|AF220153 Homo sapiens four and a half LIM domains 1 protein isoform C (FHL1) mRNA, complete cds, alternatively spliced

ATGGCGGAGAAGTTTGACTGCCACTACTGCAGGGATCCCTTGCAGGGGAAGAAGTATGTGCAAAAGGATG

GCCACCACTGCTGCCTGAAATGCTTTGACAAGTTCTGTGCCAACACCTGTGTGGAATGCCGCAAGCCCAT

CGGTGCGGACTCCAAGGAGGTGCACTATAAGAACCGCTTCTGGCATGACACCTGCTTCCGCTGTGCCAAG

TGCCTTCACCCCTTGGCCAATGAGACCTTTGTGGCCAAGGACAACAAGATCCTGTGCAACAAGTGCACCA

CTCGGGAGGACTCCCCCAAGTGCAAGGGGTGCTTCAAGGCCATTGTGGCAGGAGATCAAAACGTGGAGTA

CAAGGGGACCGTCTGGCACAAAGACTGCTTCACCTGTAGTAACTGCAAGCAAGTCATCGGGACTGGAAGC

TTCTTCCCTAAAGGGGAGGACTTCTACTGCGTGACTTGCCATGAGACCAAGTTTGCCAAGCATTGCGTGA

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AAF32351

>gi|6942193|gb|AAF32351.1|AF220153_1 four and a half LIM domains 1 protein isoform C [Homo sapiens]

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FFPKGEDFYCVTCHETKFAKHCVKCNKGLVKAPVWWPMKDNPGTTTASTAKNAP

Other isoforms:

AK09170

>gi|21750135|dbj|AK091702.1| Homo sapiens cDNA FLJ34383 fis, clone HCHON1000015, highly similar to SKELETAL MUSCLE LIM-PROTEIN 1

AGTCCGCAGCCGCCGCCGCCACCGCCGCGCCTCGGCCTCGGTGCAGGCAGCGGCTGCCGCCGCCGAGACA

GCTGCGCGGGCGAGCATCCCCACGCAGCACCTTGGAAGTTGTTTTCAACCATATCCAGCCTTTGCCGAAT

ACATCCTATCTGCCACACATCCAGCGTGAGGTCCCTCCAGCTACAAGGTGGGCACCATGGCGGAGAAGTT

TGACTGCCACTACTGCAGGGATCCCTTGCAGGGGAAGAAGTATGTGCAAAAGGATGGCCACCACTGCTGC

CTGAAATGCTTTGACAAGTTTGCCAAGCATTGCGTGAAGTGCAACAAGGCCATCACATCTGGAGGAATCA

CTTACCAGGATCAGCCCTGGCATGCCGATTGCTTTGTGTGTGTTACCTGCTCTAAGAAGCTGGCTGGGCA

GCGTTTCACCGCTGTGGAGGACCAGTATTACTGCGTGGATTGCTACAAGAACTTTGTGGCCAAGAAGTGT

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GGAGCAAGTGTATTGTCCCGACTGTGCCAAAAAGCTGTAAACTGACAGGGGCTCCTGTCCTGTAAAATGG

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CATATTAGCATTTAGCAAAAAGCAACCCTGCAGCAAAGTGAATTTCTGTCCGGCTGCAATTTAAAAATGA

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GTGATGTAAGCAAGAAGCATAGGAGATAAAACCCCCACTGAGATGCCTCTCATGCCTCAGCTGGGACCCA

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CAAGAGGAAATCTGGATTTCCACCTACCGCTTACCTGAAATGCAGGATCACCTACTTACTGTATTCTACA

TTATTATATGACATAGTATAATGAGACAATATCAAAAGTAAACATGTAATGACAATACATACTAACATTC

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>lcl|Sequence 1 ORF:197..670 Frame +2
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FVFHQEQVYCPDCAKKL

AX747139

>gi|32131527|emb|AX747139.1| Sequence 664 from Patent EP1308459

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ACATCCTATCTGCCACACATCCAGCGTGAGGTCCCTCCAGCTACAAGGTGGGCACCATGGCGGAGAAGTT

TGACTGCCACTACTGCAGGGATCCCTTGCAGGGGAAGAAGTATGTGCAAAAGGATGGCCACCACTGCTGC

CTGAAATGCTTTGACAAGTTTGCCAAGCATTGCGTGAAGTGCAACAAGGCCATCACATCTGGAGGAATCA

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AAACTTAGGTAGATTGACTCTTCTGCATGTTTCTCATAGAGCAGAAAAGTGCTAATCATTTAGCCACTTA

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TTCCTGCAGTGCTGAAATTCATCCTACAGAAGTAACCGCAAAACTCTAGAGGGGGAGTTGAGCAGGCGCC

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GTCTGCTCTTTGTCCATGTGTTAATGAGGACTGCAAAGTCCCTTCTGTTGTGATTCCTAGGACTTTTCCT

CAAGAGGAAATCTGGATTTCCACCTACCGCTTACCTGAAATGCAGGATCACCTACTTACTGTATTCTACA

TTATTATATGACATAGTATAATGAGACAATATCAAAAGTAAACATGTAATGACAATACATACTAACATTC

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TCAGTGTATCCTTACAGAAATAAAGCAGCATATGAAT

>lcl|Sequence 1 ORF:197..670 Frame +2
MAEKFDCHYCRDPLQGKKYVQKDGHHCCLKCFDKFAKHCVKCNKAITSGGITYQDQPWHADCFVCVTCSK
KLAGQRFTAVEDQYYCVDCYKNFVAKKCAGCKNPITGFGKGSSVVAYEGQSWHDYCFHCKKCSVNLANKR
FVFHQEQVYCPDCAKKL*

Mouse FHL1:

Related mRNA sequences from GenBank: Mouse: AK128904; U77039; AK158966; U41739; BC029024; BC031120; AF114380; BC059009; AF294825; BC055725

NM_010211

>gi|116517333|ref|NM_010211.2| Mus musculus four and a half LIM domains 1 (Fhl1), transcript variant 3, mRNA

AGTCCTGTGCTGCCGCTGTCGCCGCTGCGCTTTGGTCTCGGAGCTGGCAGCGGCCGCCGGTGCCGCCTAG

ACAGCTGCGCGGGCAACTGGTAGCTGTTCTTAGCTGTGCCCAGTCCTTCTGGAACACATCCTGTGTGAGG

TCCCTCCAGCTATAAGGTGGGCACCATGTCGGAGAAGTTCGACTGTCACTACTGCAGGGACCCCTTGCAG

GGGAAGAAGTACGTGCAGAAGGATGGCCGTCACTGCTGCCTGAAGTGCTTTGACAAGTTCTGCGCCAACA

CCTGCGTGGACTGCCGCAAGCCCATAAGCGCTGATGCCAAGGAGGTGCATTATAAGAATCGCTACTGGCA

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AAGATCCTGTGCAACAAGTGCGCTACTCGGGAGGACTCCCCCAGGTGCAAAGGGTGCTTCAAGGCCATTG

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CAAGCAAGTCATTGGGACCGGAAGCTTCTTCCCGAAAGGGGAGGACTTCTACTGTGTGACTTGCCATGAG

ACCAAGTTCGCCAAACATTGCGTGAAGTGCAACAAGGCCATCACATCTGGAGGAATCACTTACCAGGATC

AGCCCTGGCATGCCGAGTGCTTTGTGTGTGTTACCTGCTCTAAGAAGCTGGCTGGGCAGCGTTTCACCGC

TGTGGAGGACCAGTATTACTGCGTGGATTGCTACAAGAACTTTGTGGCCAAGAAGTGTGCTGGATGCAAG

AACCCCATCACTGGGTTTGGTAAAGGCTCCAGTGTGGTGGCCTATGAAGGACAATCCTGGCACGACTACT

GCTTCCACTGCAAAAAATGCTCCGTGAATCTGGCCAACAAGCGCTTTGTATTTCATAATGAGCAGGTGTA

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>CCDS30148.1_prot length=280
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CKGCFKAIVAGDQNVEYKGTVWHKDCFTCSNCKQVIGTGSFFPKGEDFYC
VTCHETKFAKHCVKCNKAITSGGITYQDQPWHAECFVCVTCSKKLAGQRF
TAVEDQYYCVDCYKNFVAKKCAGCKNPITGFGKGSSVVAYEGQSWHDYCF
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AK158966

>gi|74186514|dbj|AK158966.1| Mus musculus visual cortex cDNA, RIKEN full-length enriched library, clone:K530020N06 product:four and a half LIM domains 1, full insert sequence

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GCGGCCTCCGGCCTCCGCTGCCTCGCCCACGTTGGGGGCTGAGGAACCTGGGGCTCCAAGGTCCCTTAGG

GCAACTGGTAGCTGTTCTTAGCTGTGCCCAGTCCTTCTGGAACACATCCTGTGTGAGGTCCCTCCAGCTA

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GCCGCAAGCCCATAAGCGCTGATGCCAAGGAGGTGCATTATAAGAATCGCTACTGGCACGACAACTGCTT

CCGCTGTGCCAAGTGCCTTCACCCCTTGGCCAGTGAGACCTTTGTGTCCAAGGATGGCAAGATCCTGTGC

AACAAGTGCGCTACTCGGGAGGACTCCCCCAGGTGCAAAGGGTGCTTCAAGGCCATTGTGGCAGGAGACC

AGAACGTGGAGTACAAGGGCACCGTCTGGCATAAAGACTGCTTCACCTGCAGCAACTGCAAGCAAGTCAT

TGGGACCGGAAGCTTCTTCCCGAAAGGGGAGGACTTCTACTGTGTGACTTGCCATGAGACCAAGTTCGCC

AAACATTGCGTGAAGTGCAACAAGGCCATCACATCTGGAGGAATCACTTACCAGGATCAGCCCTGGCATG

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TCCCTCGTGGGTGGTGGTTCTTTATAGAAAAAATCGAAGCTTAGCAGCTCCTCGAGGCCCGGGTTTGGTA

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CTGTAACTTGACAGGGGCTCCTGTCCTGTAAAATGGCATTGGAACCATTCTTTGTGTCCTTTGCTCCCTC

CCTCCCTCTGTACCATCCATAGGGCAAGAGTGGGCTTTCACCTCTTTAAAGTTGCTCTTTCCGTCTTTTC

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TCATAGAGCAGAAAAGTGCTAACCATGTAGCCACTTCACGATGTAAACGAGAAGCATAGGCGATAAAGCT

CCCACTGAGACACCTTTGGGGCTCAGTCTGGATGCGCTGTGCGGTCACGTGACTGCGGTGTAAGAGTTGC

AGCGGCTGCTCCAACTCCCTTCTCGCCTTCTCTGGGCAGTTAAGAACTTGCCAGAATGCATGGTTTAACT

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TGGCGTGTTCTCTGAGCGTTCCTACTTTAAAGCATGGAACACACAGGTGATTTGAAGCATCTAAGCAGAT

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CTCCTGTCCTTTTTAATTTTTTTTTTAAATTTTGTTTTAATGAGTAGTAAAATAGTTTATGGGTTTGGAA

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>lcl|Sequence 1 ORF:224..1195 Frame +2
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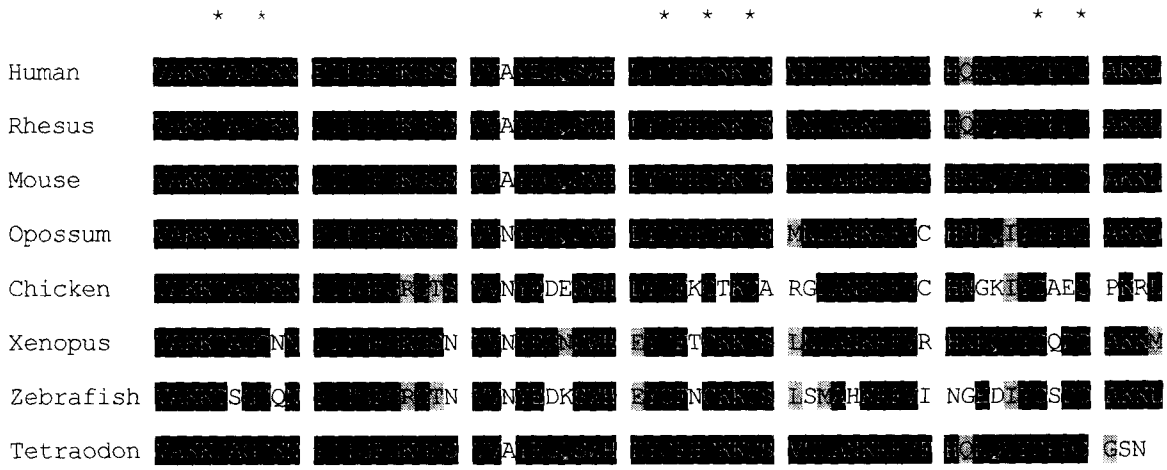
FFPKGEDFYCVTCHETKFAKHCVKCNKAITSGGITYQDQPWHAECFVCVTCSKKLAGQRFTAVEDQYYCV
DCYKNFVAKKCAGCKNPITGKRTVSRVSHPVSKARKSPVCHGKRLPLTLFPSANLRGRHPGGERTCPSWV
VVLYRKNRSLAAPRGPGLVKAPVWWPMKDNPGTTTASTAKNAP*

FIGURE 8.

Comparative analysis of 4th LIM domain of FHL1

BOXSHADE analysis, indicating Zn-binding cysteine residues (*), and position of mutation Cys224Trp (C224W) (*). The domain, and cysteine residues is highly conserved across vertebrates, from primates through to amphibians and primitive fish.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/001062

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 14/47*** (2006.01) , ***C07K 16/18*** (2006.01) , ***C07K 7/08*** (2006.01) , ***C12N 15/12*** (2006.01) , ***C12Q 1/68*** (2006.01) , ***G01N 33/53*** (2006.01) (more IPCs on the last page)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 14/47 (2006.01) , ***C07K 16/18*** (2006.01) , ***C07K 7/08*** (2006.01) , ***C12N 15/12*** (2006.01) , ***C12Q 1/68*** (2006.01) , ***G01N 33/53*** (2006.01) and ***C07K 14/705*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, PubMed, Delphion, GenomeQuest and Scopus. Keywords: Four and a Half LIM domains protein 1, FHL-1, Skeletal muscle LIM protein 1, SLIM-1, muscular myopathy and X-linked.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HAUSER K. ET AL. Identification of isoforms of the exocytosis-sensitive phosphoprotein PP63/parafusin in *paramecium tetraurelia* and demonstration of phosphoglucomutase activity THE BIOCHEMICAL JOURNAL 1997 323: 289 - 296 ISSN: 0264-6021 see the abstract and pages 294 - 295	1 - 7
Y	LEE S. M. Y. ET AL. (1) Chromosomal mapping, tissue distribution and cDNA sequence of Four and a Half LIM domain protein 1 (FHL1) GENE 1998 216: 163 - 170 ISSN: 0378-1119 see whole document	1 - 7

[ ] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 2008 (14-07-2008)	29 August 2008 (29-08-2008)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Ken Steinberg 819- 934-7929

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/001062

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 8 - 13

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 8 - 13 and 21 is directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claim.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEE S. M. Y. ET AL. (2) Characterization of a brain specific nuclear LIM domain protein (FHL1B) which is an alternatively spliced variant of FHL1 GENE 1999 237: 253 - 263 ISSN: 0378-1119 see whole document	1 - 7
A	SCHESSL J. ET AL. Proteomic identification of FHL1 as the protein mutated in human reducing body myopathy. THE JOURNAL OF CLINICAL INVESTIGATION Mar 2008 118: 904 - 912 ISSN: 0021-9738	
A	HOLASKA J. M. ET AL. Lmo7 is an emerin-binding protein that regulates the transcription of emerin and many other muscle-relevant genes. HUMAN MOLECULAR GENETICS 2006 15(23): 3459 - 3472 ISSN: 0964-6906	
A	MCGRATH M. J. ET AL. (1) Skeletal Muscle LIM protein 1 (SLIM1/FHL1) induces {alpha}5{beta}1-integrin-depndent myocyte elongation. AMERICAN JOURNAL OF PHYSIOLOGY. CELL PHYSIOLOGY Aug 2003 285: 1513 - 1526 ISSN: 0363-6143	
A	MCGRATH M. J. ET AL. (2) Four and a Half LIM protein 1 binds myosin-binding protein C and regulates myosin filament formation and sarcomere assembly. MCGRATH M. J. ET AL. THE JOURNAL OF BIOLOGICAL CHEMISTRY Mar 17, 2006 281(11): 766 - 7683 ISSN: 0021-9258	

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