A Recurrent Mutation in the ARS (Component B) Gene Encoding SLURP-1 in Turkish Families with Mal de Meleda: Evidence of a Founder Effect

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Abstract

Mal de Meleda (MdM) (keratosis palmoplantaris transgrediens, MIM# 248300) is a rare form of palmoplantar keratoderma inherited in an autosomal recessive manner. It is characterized by erythema and hyperkeratosis of the palms and soles, extending to the dorsal aspects of the hands and feet (known as transgrediens palmoplantar keratoderma) (Hovorka and Ehlers, 1897; Schnyder et al, 1969). Some of the commonly associated findings include nail abnormalities, keratotic plaques over the joints, perioral erythema, brachydactyly, and pseudoainhum (Bergman et al, 1993). MdM was originally described and thus was thought to originate from the leper colonies living on the Adriatic island of Meleda, now Mljet in Croatia. MdM is not limited to Croatia and the island of Mljet, however. Indeed, families with MdM have been described in various European countries, as well as in the USA, northern Africa, the Middle East, and the Far East (Jee et al, 1985; Bergman et al, 1993; Chotzen et al, 1993; Urbina et al, 1995; Lestringant et al, 1997; Ayman et al, 2000).

The disease locus has been mapped to chromosome 8qter (Fischer et al, 1998), and in recent studies homozygous mutations in the ARS (component B) gene (ARS) have been identified in families with this disorder (Fischer et al, 2001; Ward et al, 2003). In this report, we describe three unrelated families of Turkish descent in which a homozygous nonsense mutation in the ARS gene was identified. Haplotype analysis with markers flanking the ARS gene on chromosome 8qter was performed in these three families and in one family we reported previously with the same mutation (Ward et al, 2003). The analysis revealed that the affected individuals in these families shared a common ancestral haplotype at the MdM locus, suggesting a founder effect. Of interest, this mutation, R96X, has previously been reported in three families from Croatia (Fischer et al, 2001). Three of our families live in the villages of Isparta and one in Antalya, both located in southern Turkey. Thus, it is possible that individuals with the R96X mutation relocated from Croatia, perhaps from the Meleda island, to southern Turkey carrying the mutation with them to this region.
Genomic DNA was extracted from whole blood using the PureGene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). The three exons of the ARS gene were amplified by polymerase chain reaction (PCR) using the primers described previously (Fischer et al, 2001). For mutation detection, the PCR products were sequenced using an Applied Biosystem 310 automated sequencing system. The PCR products were digested with BspMI or DdeI at 37°C for 3 h and analyzed on 1.5% agarose/TBE minigels.

Haplotype analysis was performed using the following markers from centromere to telomere: D8S1704, CNG002, CNG003, D8S1751, D8S1836, D8S2334. Microsatellite markers flanking the MdM locus at chromosome 8qter were chosen from the genetic map at the Center for Medical Genetics, Marshfield Medical Research Foundation (http://www.research.marshfieldclinic.org/genetics/), except for two markers CNG002 and CNG003 that were obtained from a previous report (Fischer et al, 2001). The primer sequences for PCR were obtained from the Genome Database (http://www.gdb.org). The PCR products were resolved on 6% nondenaturing polyacrylamide gels and visualized by ethidium bromide staining.

In all families MdM was inherited in an autosomal recessive manner (Figure 1). Family 1 is a large consanguineous family; however, a consanguinity loop was not found in families 2, 3, and 4. All affecteds exhibited transgrediens palmoplantar keratoderma with hyperhidrosis, typical of MdM (Figure 2a), and a variety of associated findings. Affecteds in family 1 had nail deformity, knuckle pads, hyperkeratotic plaques on the knees, and high arched palate. Individual V:14 also had retinitis pigmentosa. In family 2 perioral erythema was observed as the associated finding with neither nail involvement nor hyperkeratotic plaques. In family 3 nail dystrophy and in family 4 nail dystrophy and knuckle pads were observed. In none of the affecteds was brachydactyly or pseudoainhum noted.

In all three families, a homozygous C-to-T transition at nucleotide 286 in exon 3, leading to a conversion of an arginine residue (CGA) to a stop codon (TGA) at amino acid 96 was identified. Whereas the homozygous R96X mutation was observed in the proband, the parents displayed a heterozygous sequence (Figure 2b). This mutation, designated R96X, creates a restriction endonuclease site for the enzyme DdeI, which was used to confirm the mutation (Figure 2c). The sequence alterations found in these families were not observed in 100 unrelated, unaffected Turkish controls.

To test whether these families shared the same haplotype at the MdM locus, we performed haplotype analysis in three families reported here and in one family we previously reported carrying the same mutation, R96X, in the ARS gene (family 2) (Ward et al, 2003). Ten affected and seven unaffected individuals were genotyped using six microsatellite markers spanning approximately
3.65 cM in the region. The disease gene is located between (or telomeric to) the markers CNG003, D8S1751, and D8S1836. Recombination events in individuals V:6, V:9, V:14, and V:15 (family 1) identified the same boundary as the one established previously (Fischer et al, 2001). Affecteds in all four families showed homozygosity at the MdM locus, whereas none of the unaffecteds were homozygous for both markers (Figure 1).

The ARS gene is predicted to consist of three translated exons, and encodes a 103 amino acid protein, SLURP-1 (secreted Ly-6/uPAR related protein 1). SLURP-1 belongs to the Ly-6/uPAR family of proteins, which typically contain a highly conserved pattern of disulfide bridges (Adermann et al, 1999). R96X mutation occurs in a CpG-containing arginine codon found in exon 3 of the ARS gene. Deamination of CpG dinucleotides is the most common mechanism underlying point mutations in humans. This mutation results in a premature stop codon, and thus the mutant allele is predicted to encode a truncated protein that is missing the last eight amino acids. Although R96X is towards the end of the protein, the truncated protein lacks a cysteine implicated in one of the highly conserved disulfide bridges.

To date, the nonsense mutation R96X has been identified in three Croatian families and four Turkish families (including the ones reported here) (Fischer et al, 2001; Ward et al, 2003). The identification of recurrent mutations suggests that these may be hot-spot mutations within the ARS gene, or alternatively a common ancestral allele. To determine whether the mutation in the Turkish population originated from a common ancestor, we analyzed the R96X allele signatures in four Turkish families. Our results showed that all four families shared a common haplotype at the MdM locus, suggesting a founder effect. Although the unambiguous identical-by-descent sharing of this haplotype cannot be demonstrated without the genotyping of certain married-in individuals who were unavailable for this study, the coincidental identical-by-state sharing of alleles in perfect concordance with the segregation of the same point mutation in four unrelated families seems highly improbable. Furthermore, the markers used for haplotype analysis were highly polymorphic. Of interest, three Croatian families with the mutation R96X had been shown to share the same haplotypes at the MdM locus (Fischer et al, 2001). It would be interesting to compare the haplotypes of Croatian and Turkish families with R96X. Future mutational analysis of additional families with MdM from Turkey and neighboring countries will provide a better estimation of the frequency of the founder mutation within this population.

References

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Pedigrees with MdM, showing haplotypes for markers at 8qter. The order of the markers is indicated in the upper right corner. The disease-associated haplotype is highlighted.
Clinical data and genetic analysis of families 1, 3, and 4 with MdM. (a) Keratoderma with transgrediens. (b) DNA sequence of exon 3 from controls, heterozygous carriers, and homozygous affected individuals. Encoding amino acid sequence is indicated on the wild-type sequence. (c) Restriction endonuclease analysis with the enzyme Ddel. Ddel digestion cuts the 448 bp PCR product into 272, 90, 65, and 21 bp fragments on the wild-type allele. The R96X mutation creates a new Ddel site at nucleotide 216 of the PCR product, and therefore digestion of the mutant allele results in fragments of 216, 90, 65, 56, and 21 bp.