# 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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**Mal de Meleda is a rare form of palmoplantar keratoderma, and recently mutations in the ARS (component) B gene have been identified in families with this disease. We identified a recurrent nonsense mutation, R96X, in four families of Turkish descent. In this report, we demonstrate that these families share a common ancestral haplotype at the mal de Meleda locus, suggesting a founder effect.** ***Key words: ARS (component B)/ founder/mal de Meleda/mutation/SLURP-1. J Invest Dermatol 120:967–969, 2003***

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 (**Fig 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 (**Fig 2*a***), 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 brachydactly 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

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Abbreviations: *ARS*, ARS (component B); MdM, mal de Meleda.

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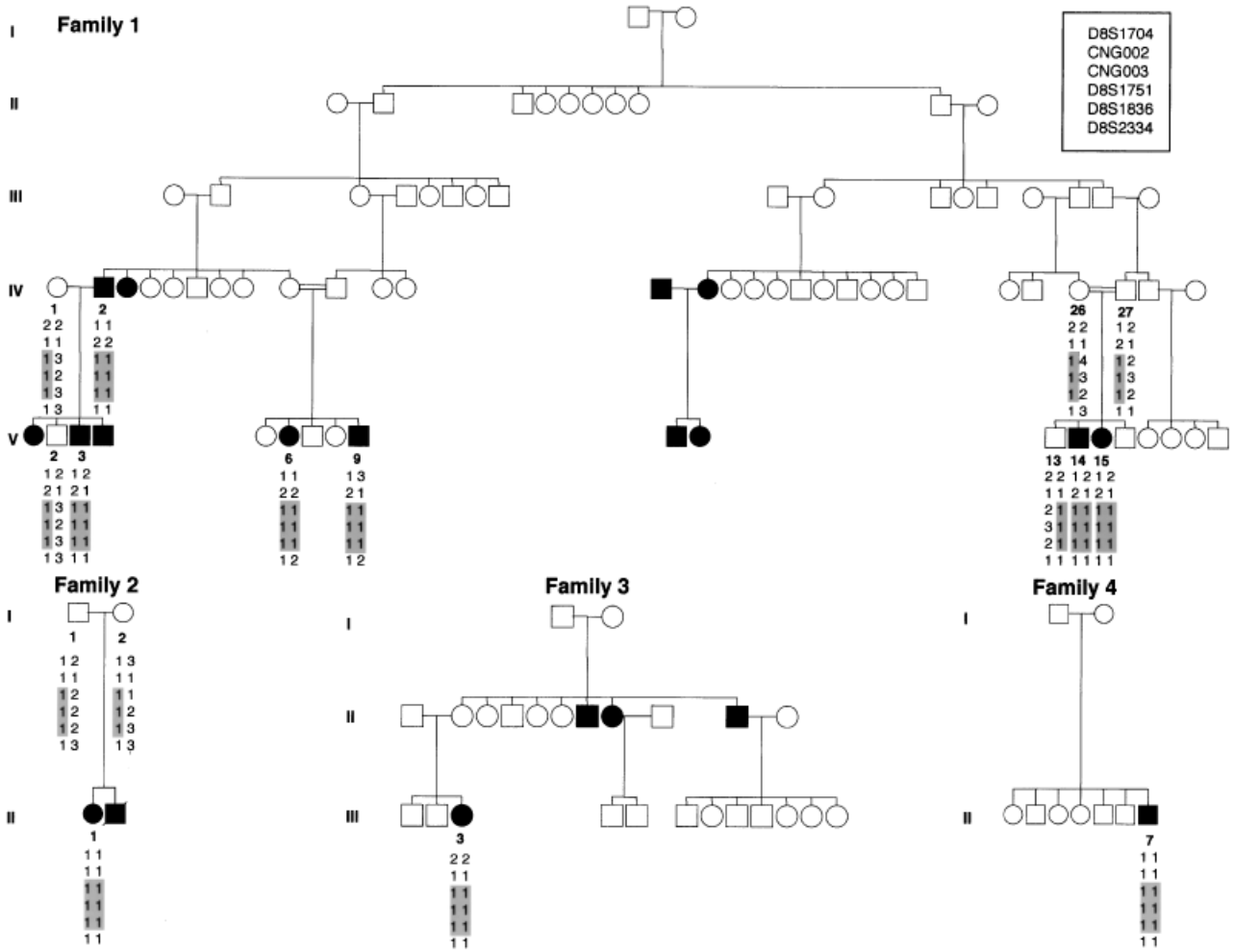



**Figure 1. Pedigrees with MdM, showing haplotypes for markers at 8qter.** The order of the markers is indicated in the upper right corner. The disease-associated haplotype is highlighted.

the proband, the parents displayed a heterozygous sequence (**Fig 2*b***). This mutation, designated R96X, creates a restriction endonuclease site for the enzyme *DdeI*, which was used to confirm the mutation (**Fig 2*c***). 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 (**Fig 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

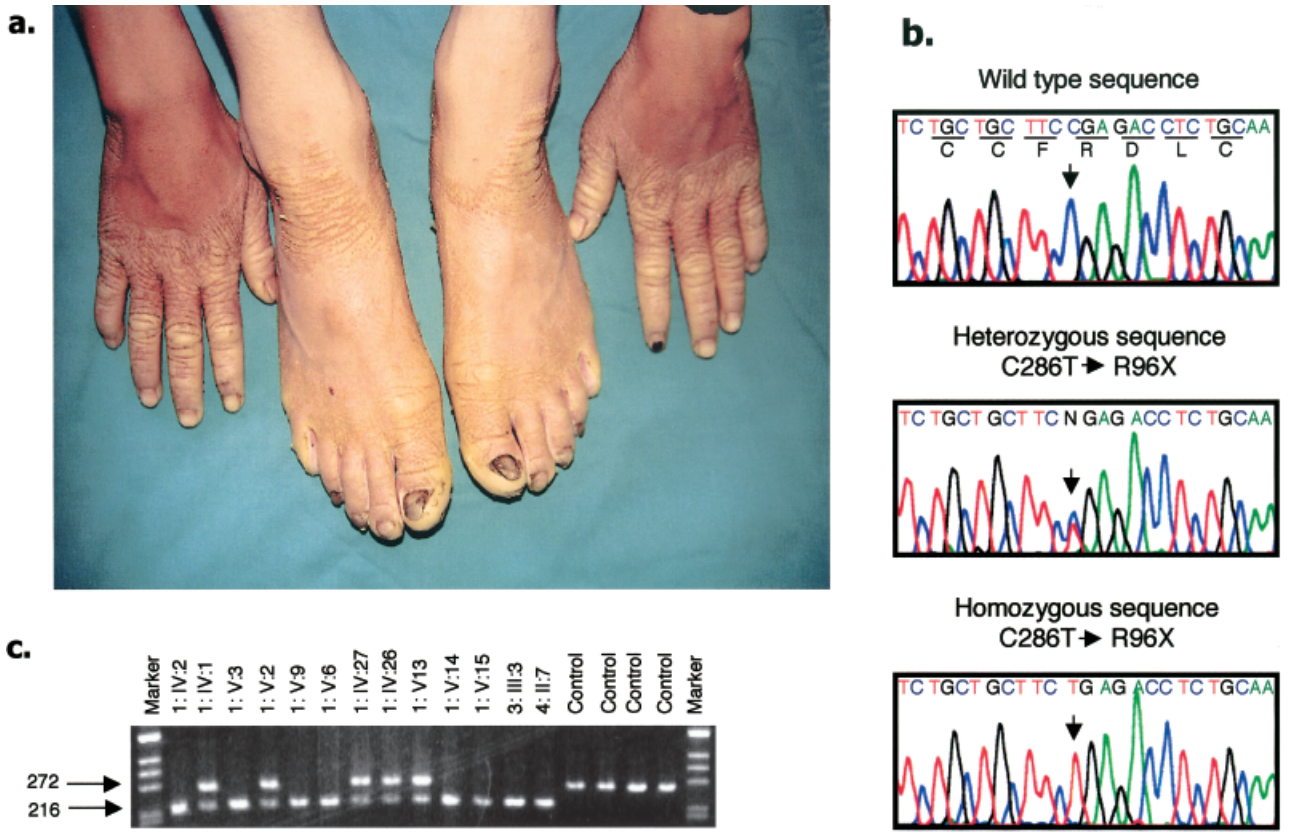



**Figure 2. Clinical data and genetic analysis of families 1, 3, and 4 with MdM.** (*a*) Keratoderma with transgrediens. (*b*) DNA sequence of exon 3 from controls, heterozygous carriers, and homozygous affected individuals. Encoding amino acid sequence is indicated on the wild-type sequence. (*c*) Restriction endonuclease analysis with the enzyme *DdeI*. *DdeI* 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 *DdeI* 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.

estimation of the frequency of the founder mutation within this population.

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