Identification of Recurrent Mutations in the ARS (Component B) Gene Encoding SLURP-1 in Two Families with Mal de Meleda

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Mal de Meleda is a rare, autosomal recessive form of palmoplantar keratoderma. The disease has been mapped to chromosome 8qter, and in a recent study mutations in the ARS gene have been identified in families with this disorder. Here, we report two unrelated families with mal de Meleda, in which two different homozygous mutations in the ARS gene were identified. These findings support the notion that mutations in the ARS gene are pathogenic in mal de Meleda. ***Key words: ARS (component B)/mal de Meleda/mutation/palmoplantar keratoderma/SLURP-1. J Invest Dermatol 120:96–98, 2003***

Mal de Meleda (MdM) (keratosis palmoplantaris transgrediens, OMIM# 248300) is a rare form of palmoplantar keratoderma (PPK). It is an autosomal recessive disorder characterized by erythema and hyperkeratosis of the palms and soles with sharp demarcation, extending to the dorsal aspects of the hands and feet (known as transgrediens) (Hovorka and Ehlers, 1897; Schnyder *et al*, 1969). The palmoplantar hyperkeratosis is usually accompanied by hyperhidrosis, maceration, fetid odor, and painful fissures. Other associated findings include nail abnormalities, keratotic plaques over the joints, perioral erythema, brachydactyly, and pseudoainhum (Bergman *et al*, 1993).

Recently, homozygous mutations in the ARS (component B) (ARS) gene were identified in families with MdM, implicating ARS as the susceptibility gene for this disease (Fischer *et al*, 2001). Subsequently, a patient with MdM lacking mutations in ARS was reported, suggesting genetic heterogeneity (van Steensel *et al*, 2002). In this report, we describe two unrelated families with MdM in which two different homozygous mutations in the ARS gene were identified. Of interest, these mutations are the same as reported previously by Fischer *et al.*

MATERIALS AND METHODS

Genomic DNA was extracted from whole blood using a QIAamp Blood Maxi Kit (Qiagen, Valencia, CA). 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.

Manuscript received May 31, 2002; revised September 6, 2002; accepted for publication September 9, 2002

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Abbreviations: ARS, ARS (component B); MdM, mal de Meleda; PPK, palmoplantar keratoderma.

RESULTS

Clinical data The clinical findings for both families have been described previously (Ayman *et al*, 2000). Family 1 was of Kurdish descent and family 2 of Turkish descent. Consanguinity was present in both families. All affected individuals showed classical findings of MdM, hyperkeratosis, and erythema of the palms and soles with transgrediens (**Figs 1*b*, 2*b***). In family 1, although both II-2 (36 y old) and II-7 (18 y old) had subungual hyperkeratosis of the toenails as well as hyperkeratotic plaques on the knees, these skin findings were absent in III-1 (2 y old). In II-1 (34 y old) of family 2, perioral erythema was observed with neither nail involvement nor hyperkeratotic plaques.

Mutation detection and confirmation In family 1, direct sequencing of the ARS gene flanking exon 2 showed a homozygous deletion of T at nucleotide 82 (82delT), resulting in a premature stop codon at residue 32. The affected individuals in this family, II-2, II-7, and III-1, all showed the homozygous 82delT mutation, whereas the carriers, II-3 and II-4, displayed a heterozygous sequence (**Fig 1*c***). 82delT abolishes a restriction endonuclease site for the enzyme *BspMI*, which was used to confirm the mutation (**Fig 1*d***).

In family 2, 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 C286T mutation was observed in the proband, her parents, I-1 and I-2, both displayed a heterozygous sequence (**Fig 2*c***). This mutation, designated C286T, creates a restriction endonuclease site for the enzyme *DdeI*, which was used to confirm the mutation (**Fig 2*d***). The sequence alterations found in these families were not observed in 50 unrelated, unaffected Turkish and Kurdish controls.

DISCUSSION

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 and a gene structure of four exons, the

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first of which is untranslated (Adermann *et al*, 1999). Although members of the Ly-6/uPAR family have been implicated in transmembrane signal transduction, cell activation, and cell adhesion, the function of SLURP-1 remains unknown. The SLURP-1 mRNA expression has been demonstrated by reverse transcription PCR in keratinocytes from plantar and truncal skin, the foreskin, the scalp skin, and fetal bladder. Its mRNA expression was not observed in most tissues, however, including adult liver, lung, kidney, colon, uterus, and fibroblasts (Fischer *et al*, 2001).

In 2001, 12 families of Algerian descent and seven families from Croatia with MdM were shown to exhibit mutations in the ARS gene. Specifically, three different homozygous mutations, 82delT, 178 + 1G-A, and C286T, were identified in these families (Fischer *et al*, 2001). Of interest, the two families we examined also exhibited homozygous 82delT and C286T mutations in the ARS gene. These mutations are observed in amino acids that are conserved among species in which ARS has been cloned. Both mutations result in premature stop codons, and thus are predicted to lead to the synthesis of truncated protein or to the degradation of transcripts via nonsense mediated mRNA decay. C286T 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. Although C286T mutation is towards the end of the protein, the truncated protein lacks a cysteine implicated in one of the highly conserved disulfide bridges.

MdM is characterized by transgressive PPK and a variety of associated findings, and shows clinical variability among the patients. Recently some patients with classical MdM phenotype have failed to show linkage to the MdM interval (Lestringant *et al*, 2001) or lacked mutations in the ARS gene (van Steensel *et al*, 2002), suggesting genetic heterogeneity. The two families described here both exhibit classical transgressive PPK. In addition, patients in family 1 with the 82delT mutation demonstrate hyperkeratotic plaques (lichenoid plaques) on the knees and subungual hyperkeratosis of the toenails. The patient in family 2 with the C286T mutation only has perioral erythema as the associated finding. Our patients lacked brachydactyly, pseudoainhum, and knuckle pads. The identification of recurrent mutations suggests that these may be hot-spot mutations within the ARS gene, or may implicate a common ancestral allele. Further work in the mutational spectrum of ARS in other MdM families will help to clarify these issues. Our data confirm the findings of Fischer

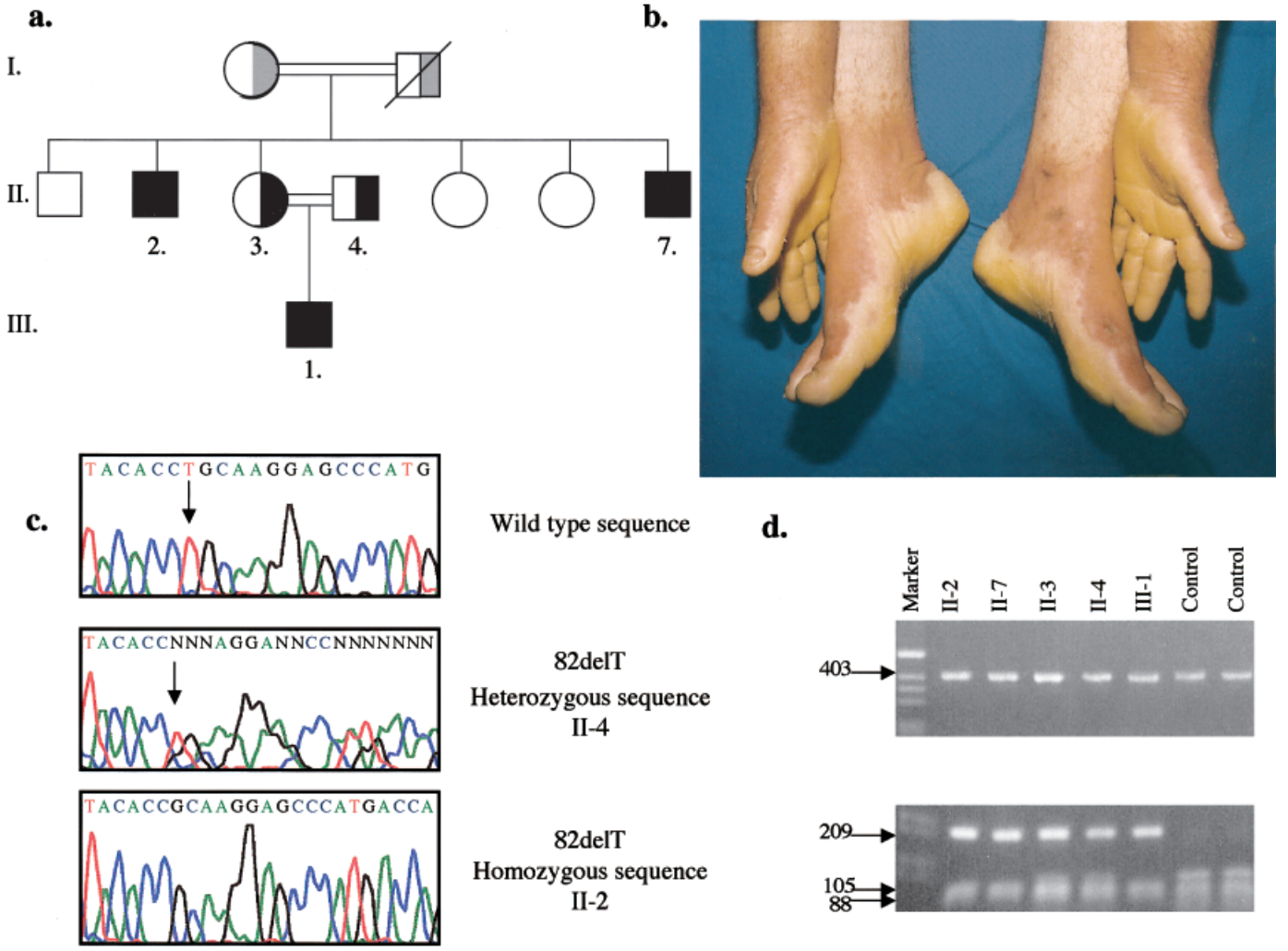


Figure 1. Clinical data and genetic analysis of family 1 with MdM. (*a*) Pedigree of the family. *Gray symbols* indicate family members that are predicted to be carriers, whose DNA have not been analyzed. (*b*) Palmoplantar erythema and keratoderma with transgrediens. (*c*) DNA sequence of exon 2 from control, heterozygous carrier, and homozygous affected individuals. (*d*) The top figure shows the 403 bp PCR amplification of exon 2 of the ARS gene, in II-2, II-3, II-4, II-7, III-1, and two control samples. The bottom figure shows *BspMI* digestion of the PCR samples, which cuts the wild-type allele in 120, 105, 90, and 88 bp fragments. The 82delT mutation abolishes the *BspMI* site at nucleotide 120 of the PCR product, and therefore digestion of the mutant allele results in three fragments: 209, 105, and 88 bp.

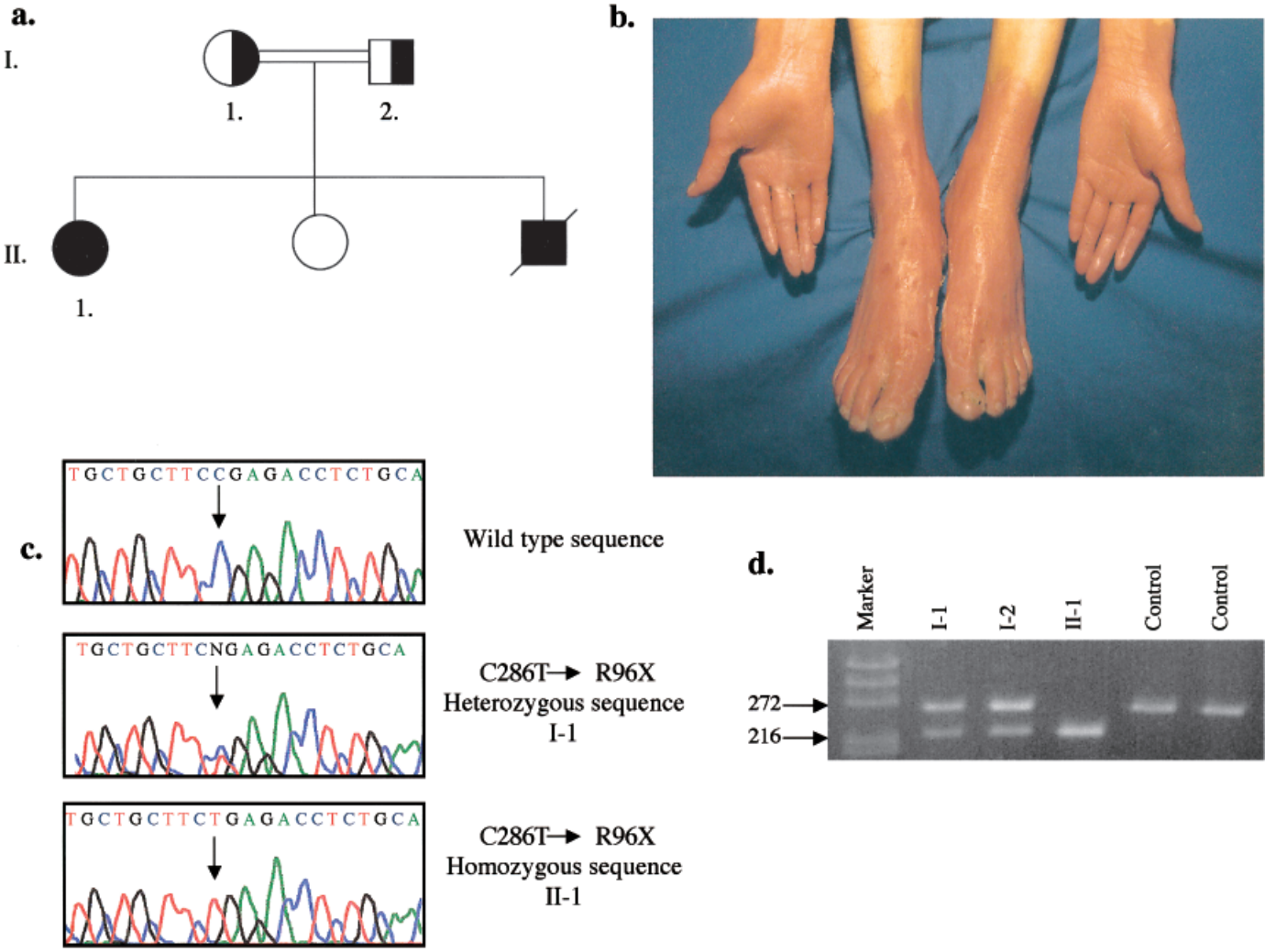


Figure 2. Clinical data and genetic analysis of family 2 with MdM. (*a*) Pedigree of the family. (*b*) Clinical data. (*c*) DNA sequence of exon 3 from control, heterozygous carrier, and homozygous affected individuals. (*d*) Restriction endonuclease analysis with the enzyme *DdeI*. *DdeI* digestion cuts the 448 bp PCR product into 272, 90, 65, and 21 bp fragments on the wild-type allele. The C286T 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.

et al and support the notion that mutations in the ARS gene are pathogenic in MdM.

We appreciate the participation of the patient and family members in the study. We are grateful to those who participated in the unrelated, unaffected, Turkish and Kurdish control group. This work was supported in part by the National Institute of Arthritis and Musculoskeletal and Skin Diseases Training Grant (K.M.W), the Dermatology Foundation (J.T.C.), the Waterbor Burn and Cancer Foundation (J.T.C.), and the Irving Center for Clinical Research at Columbia University (J.T.C.).

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