Polymorphism

RESEARCH

Identification of a c.286C>T mutation in the *SLURP1* gene in a patient with Mal de Meleda from India

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ABSTRACT

Mal de Meleda (MDM, OMIM#248300) is a very rare autosomal recessive skin disease caused by mutations in the *SLURP1* gene. It is characterized by classical progressive transgradiens hyperkeratosis of the palms and soles, hyperhidrosis and minor symptoms, such as perioral erythema, hyperkeratosis on elbows and knees, pseudo-ainhum, and nail abnormalities. Here, we report a mutation in the *SLURP1* gene (NM_020427.2: c.286C>T) in a 38 year old Indian male with Mal de Meleda that is compatible with severe clinical features of patients reported from Croatia and Korea with the same mutation.

KEYWORDS: Mal de Meleda, Hyperkeratosis, India.

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INTRODUCTION

Mal de Meleda (MDM) is an extremely rare form of palmoplantar keratoderma, first reported by Luca Stulli's (1826) [FatoviĆ-FerenčiĆ S, et.al., 2001]. Dramatic changes in the morphology of the epidermis in Mal de Meleda is contributed by secreted Ly6/uPAR-related protein-1 (Homo sapiens) in keratocytes and encoded by the *SLURP1* (MIM:606119) gene on chromosome 8q24.3. The mutations in the gene affect either the expression, integrity, or stability of the protein [Bertrand Favre, et.al., 2007]. The loss of function of *SLURP1* is controlled by the nAChR signaling pathway, leads to the uncontrolled growth of cells, resulting in thickening of the skin and abnormal keratinocyte apoptosis regulation; however, the clear mechanisms are still unexplored [Mal de Meleda - Genetics Home Reference NIH, 2020]. MDM is a rare autosomal recessive heritable genetic disorder reported in different parts of the world in 1 in 100,000 individuals [Perez C, et.al., 2016]. There are more than 100 cases reported worldwide to date, out of which the genetic cause is screened or explained in only a handful of cases [Meleda Disease - NORD (National Organization for Rare Disorders), 2020]. The spread of this heritable genetic condition to such geographically and environmentally diverse regions of the world is a matter of scientific exploration and concern. The MDM patients are always at the risk of developing frequent fungal/bacterial skin infections due to callused, thick skin along with a pathogen friendly environment (Pan, et.al., 2017). An unpleasant odour is another common characteristic of this condition, which requires regular use of moisturizers/keratolytics with antimicrobial properties. MDM is not life-threatening, but its chronic disfigured hyperkeratotic lesions impose great stress on the patient due to his/her condition in terms of living a normal social life, as a result of that it may lead to stress, anxiety, and depression in women predominantly as compared to men [Goodwin GM, 2006]. Due to an incurable chronic condition, the patient needs constant medical support and care throughout the lifetime. In this study, we investigated the genetic cause in a case with Mal de Meleda.

CASE STUDY

The onset of symptoms was from the first month of life, as revealed by the parents. In childhood, the patient complained of mild hyperkeratosis, followed by pain in the skin during winters; however, treatment by several allopathic and non-allopathic medications could not help; instead, the severity of symptoms increased. Now the patient, a 38-year-old male, was first diagnosed with Mal de Meleda (MDM) by a dermatologist (author-VAK) based on the symptoms: palmoplantar keratosis, diffuse, transgrediens, progrediens, erythromatous borders, inflammatory erythema, hyperkeratosis of elbows and knees, nail abnormalities, and conical tapering of fingers (Fig. 1).

Skin biopsy sections (H&E, scanning view) show the epidermis and dermis, where the epidermis indicates acanthosis with spongiosis and mild hyperkeratosis with focal parakeratosis. The superficial dermis shows thin-walled vessels with lymphomononuclear cells. Histopathology analysis suggests psoriasiform dermatitis (Fig. 2).

Extensive genetic counselling was conducted on the patient whose parents were consanguineous. The other family members were normal. Genetic testing included, the mother (55 years), unmarried brother (21 years) and spouse (22 years). The patient married late (at 37 years) and his wife was pregnant at the time of testing. The patient and family members recall no one else in the family and known distant relatives suffering from this disorder. Genetic testing by sequencing all 3 exons of the *SLURP1* gene revealed homozygous nonsense mutation c.286 C>T (p. R96X) in the patient, but the mother and unmarried brother were heterozygous, and the spouse was normal (Fig. 3 & 4).

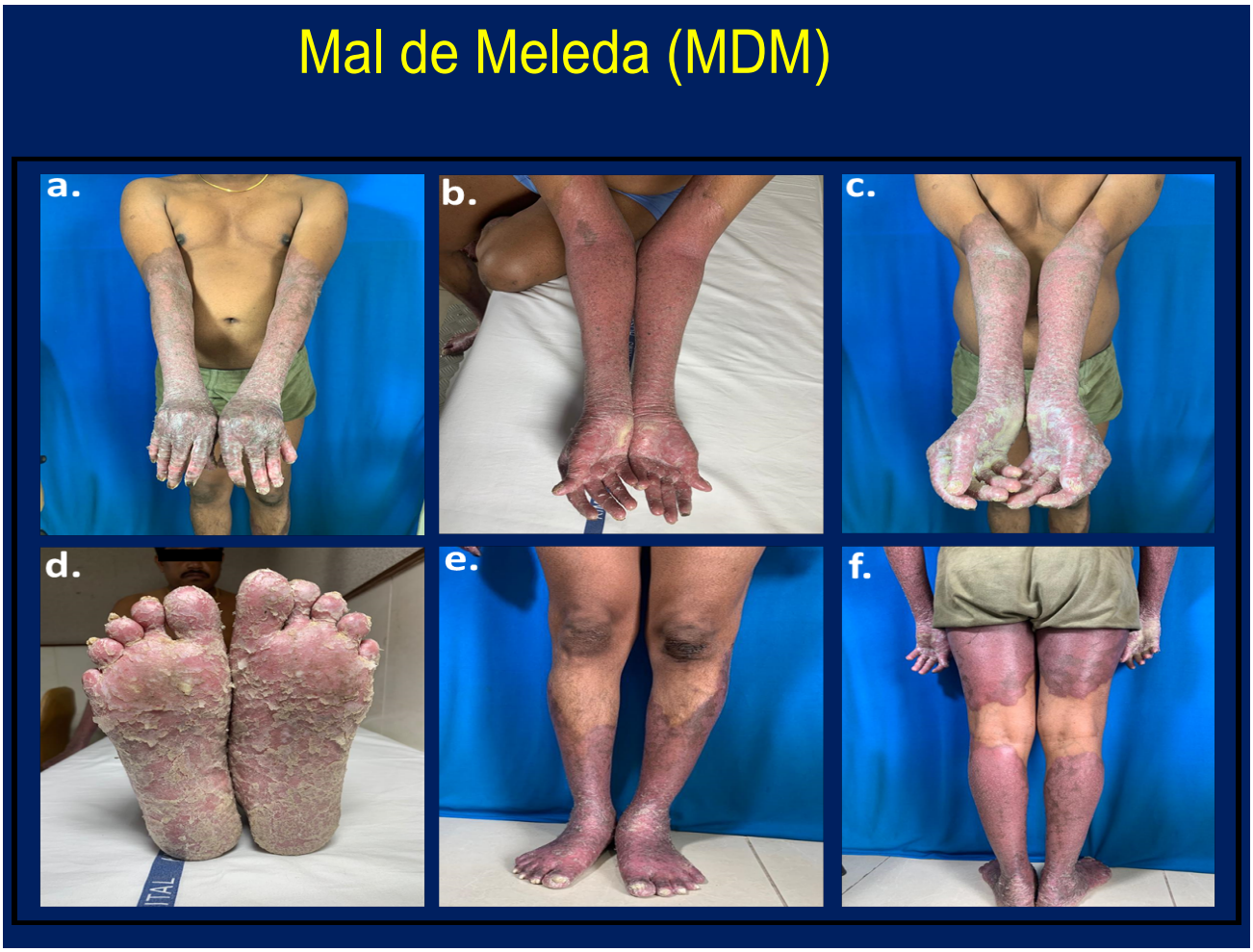

Fig. 1. 38-year-old male patient with rough, dry, thick, hard and callused skin of legs, feet, hands and palms.

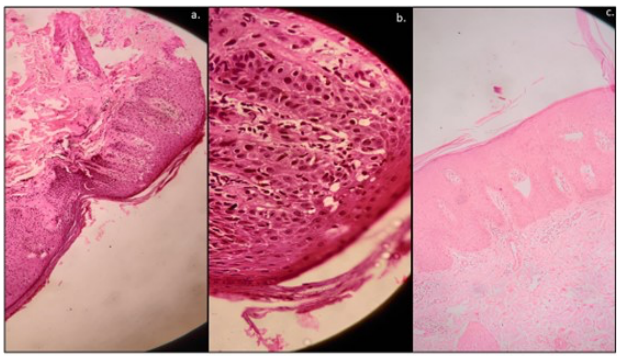

Fig. 2. Skin H & E- Sections showing epidermis and dermis. Epidermis shows acanthosis with spongiosis and mild hyperkeratosis with focal parakeratosis present. The superficial dermis shows thin-walled vessels with lymphomononuclear cells.

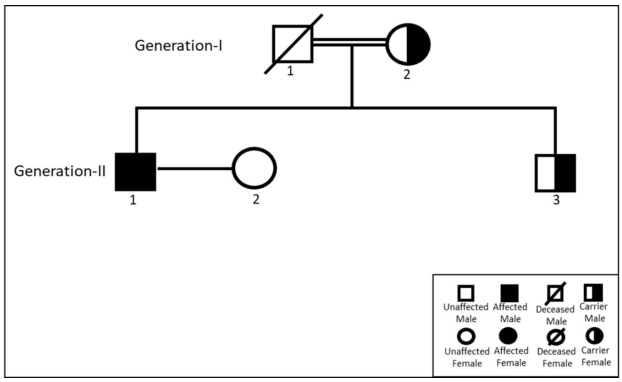

Fig. 3. Pedigree chart of the family showing distribution of the mutation (rs121908317) p. Arg96Ter at position 286 (c. C286T). Generation-I: 1. Deceased father, 2. Mother (55 years, carrier). Generation-II: 1. Patient (38 years, homozygous), 2.

Patient's spouse (22 years, normal), 3. Brother (21 years, carrier). Family samples were collected to confirm the autosomal recessive mode of inheritance. Sample from spouse was collected to predict the risk in future offspring(s) of the family.

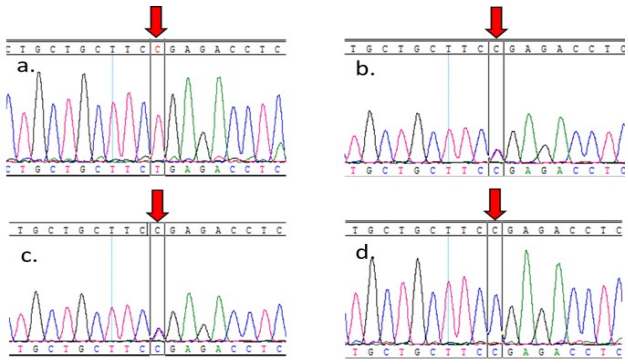

Fig. 4. Electrograms of the mutation c.C286T), p. Arg96Ter with a homozygous condition (TT) in the patient (a), heterozygous(C/T) in brother (b) and mother (c) of the subject, and homozygous normal (CC) in wife (d).

Three mL intravenous blood from each participant was collected in EDTA tubes after obtaining informed consent. DNA was extracted using the phenol-chloroform method (Aggarwal RK, et.al., 1992), followed by PCR amplification of the *SLURP1* exons. Sequencing was carried out on a 3500 genetic analyzer from Applied Biosystems using protocols recommended by the supplier. All three exons of the *SLURP1* gene were sequenced to screen for any known/novel variation(s) and or mutation(s) in the amplified exons. Direct sequencing revealed a known pathogenic mutation in the homozygous condition in the patient at position NM_020427.3(SLURP1): c.286C>T (rs121908317), changing arginine at position 96 to a stop codon (Figure 4). This mutation altered the protein conformation, resulting in a truncated version of the protein, which lacks cysteine in the highly conserved disulphide bridges. The mutation was reported as pathogenic in ClinVar and was previously reported in three Croatian families [Fischer J, et.al., 2001] and one Korean case (Pan, et.al., 2017). The presence of the homozygous mutation (NM_020427.3, c.286C>T) in the patient and heterozygous condition in the mother and brother further confirmed the pathogenic nature of this mutation (Figure 3). However, the spouse of the subject was homozygous normal. In silco analysis for the pathogenicity of this mutation through Combined Annotation Dependent Depletion (CADD) revealed a score of 38 (top 1% most deleterious). The mutation taster predicted this mutation as disease-causing with a probable value of 0.964, which leads to a truncated version of the protein, probably causing nonsense-mediated mRNA decay (NMD) and thereby causing MDM.

DISCUSSION

The *SLURP1* gene is well known for its implication in MDM [Mal de Meleda Disease - NORD (National Organization for Rare Disorders), 2020; Nellen RGL, et.al., 2015; Radiono S, et.al., 2017; Bchetnia M, et.al., 2015; Taylor JA, et.al., 2016]. Our study from India also confirms the involvement of this gene in the pathogenesis of MDM. This is the first case reported in India with mutation in the *SLURP1* gene (NM_020427.3:c.286C>T), which was earlier reported by Fischer J, et.al., (2001) in three Croatian and by Oh, et.al., (2011) in one Korean families. The minor allele frequency of this mutation in 1000G was 0.0002 (1/5008) and 0.00103 in the Sage South Asian Genomes & Exomes database, which is extremely rare, but comparatively higher incidence in South Asian populations. A case pertaining to MDM had been reported from the Indian subcontinent in 2015 (first case), but harbouring a different frameshift mutation, c.58+5G>T, in the first intron (Nellen, et.al., 2015). This case reported classical phenotype traits like palmoplantar keratoderma, diffuse, transgrediens, nail abnormality, conical tapering fingers and histopathological findings of acanthosis and mild epidermolytic keratosis, but differs from the present case with more severe erythematous borders and inflammatory erythema. Whereas the Korean case aged 15 years involved exon 3 mutations in compound heterozygous state, p.R96X and p.G86R, presented with severe traits similar to the

present case (p.R96X/p. R96X). Mutations in the third and last exon prevent the translation of the last Cys residue of the protein, part of the LY6/PLAUR CCX4CN motif, and thereby the formation of the highly conserved fifth disulphide bridge. This shortening probably destabilizes the whole structure and particularly the third loop of the three-finger fold. The comparison of phenotypic traits infers the type of mutations that determine severity. The frameshift mutation (c. 58+5G) reported earlier in the Indian population is novel, whereas the p.R96X was earlier reported in Croatian and Korea populations. We are unable to comment on the origin of this mutation, though ancient genomic footprints in India cannot be ruled out until further haplotype studies are conducted. Genetic counselling may help the family in terms of the heterozygous younger brother opting for pre-marital or prenatal testing. Besides, the patient's wife (wild homozygous) delivered their first child (now 6 months old), and no symptoms were detected. The Genome Foundation, India, has the provision to maintain a rare genetic disorders registry and follow-up on the counselling outcomes.

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Author's contribution

Prof V.R. Rao conceived the concept. Dr. Vaggu Anand Kumar performed the initial diagnosis and referred the patient for genetic screening. Dr. Gaurav Gupta and Ms. Gargi Deshmukh have performed the experiments and prepared the manuscript. Prof V.R. Rao finalized the manuscript for submission. All authors have read and approved the final version of the manuscript for submission.

Conflict of interest

Authors have no conflict of interest.

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REFERENCES

FatoviĆ-FerenčiĆ S, Holubar K. The portrait and paper of a forgotten hero-Luca Stulli (1772-1828) and the Mal de Meleda of yesteryear: A 175-year anniversary. J Invest Dermatol [Internet]. Elsevier; 2001 [cited 2021 Feb 15]; 116:198–9.

Bertrand Favre, Laure Plantard, Lorène Aeschbach and Noureddine Brakch. SLURP1 Is a Late Marker of Epidermal Differentiation and Is Absent in Mal de Meleda. Journal of Investigative Dermatology. 2007 127(2):301-8.

Mal de Meleda - Genetics Home Reference - NIH [Internet]. [cited 2020 Aug 11].

Perez C, Khachemoune A. Mal de Meleda: A Focused Review [Internet]. Am. J. Clin. Dermatol. Springer International Publishing; 2016 [cited 2020 Jul 31]. p. 63–70.

Meleda Disease - NORD (National Organization for Rare Disorders) [Internet]. [cited 2020 Aug 11].

Pan Y, Zhao H, Chen A, Huang X. A Mal De Meleda patient with severe flexion contractures of hands and feet. Medicine

(Baltimore) [Internet]. Lippincott Williams and Wilkins; 2017 [cited 2020 Aug 11];96: e7972.

Oh YJ, Lee HE, Ko JY, Ro YS, Yu HJ. A sporadic case of Mal de Meleda caused by gene mutation in SLURP1 in Korea. Ann Dermatol [Internet]. Korean Dermatological Association; 2011 [cited 2020 Aug 3]; 23:396–9.

Shah K, Nasir A, Irfan Ullah, Shahzad S, Khan S, Ahmad W. A novel homozygous mutation disrupting the initiation codon in the SLURP1 gene underlies mal de Meleda in a consanguineous family. Clin Exp Dermatol [Internet]. Blackwell Publishing Ltd; 2016 [cited 2020 Aug 11]; 41:675–

Goodwin GM. Depression and associated physical diseases and symptoms [Internet]. Dialogues Clin. Neurosci. Les Laboratoires Servier; 2006 [cited 2020 Aug 11]. p. 259–65. Available from: www.dialogues-cns.org

Aggarwal RK, Lang JW, Singh L. Isolation of high-molecular-weight DNA from small samples of blood having nucleated erythrocytes, collected, transported, and stored at room temperature. Genet Anal Biomol Eng. 1992;9:54–7

Fischer J, Bouadjar B, Heilig R, Huber M, Lefèvre C, Jobard F, et al. Mutations in the gene encoding SLURP1 in Mal de Meleda [Internet]. Hum. Mol. Genet. 2001. Available from: http://genome.ucsc.edu/

Nellen RGL, Claessens T, Subramaniam R, Betkerur J, Prashanth A, Steijlen PM, et al. A novel mutation in SLURP1 in patients with mal de Meleda from the Indian subcontinent [Internet]. J. Dermatol. Sci. Elsevier Ireland Ltd; 2015 [cited 2020 Jul 31]. p. 76–8.

Radiono S, Pramono ZAD, Oh GGK, Surana U, Widiyani S, Danarti R. Identification of novel homozygous SLURP1 mutation in a Javanese family with Mal de Meleda. Int J Dermatol [Internet]. Blackwell Publishing Ltd; 2017 [cited 2020 Jul 31]; 56:1161–8.

Bchetnia M, Bozgia M, Laroussi N, Ben Brick AS, Charfeddine C, Ben Halim N, et al. The first Mal de Meleda case in Libya: Identification of a SLURP1 mutation. Int J Dermatol [Internet]. Blackwell Publishing Ltd; 2015 [cited 2020 Jul 31]; 54:1426–8.

Taylor JA, Bondavalli D, Monif M, Yap LM, Winship I. Mal de Meleda in Indonesia: Mutations in the SLURP1 gene appear to be ubiquitous. Australas J Dermatol [Internet]. Blackwell Publishing; 2016 [cited 2020 Jul 31];57: e11–3.