Research letter

Novel compound heterozygous mutations in *KREMEN1* confirm it as a disease gene for ectodermal dysplasia

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Dear Editor, Ectodermal dysplasia (ED) is a heterogeneous group of disorders caused by mutations in at least 13 genes. Recently, a study reported Palestinian patients with ED from consanguineous families with a homozygous mutation in *KREMEN1* and proposed it to be a causative gene for the autosomal recessive ED 13, hair/tooth type (ECTD13; OMIM 617392).[1]

A Thai family, parents and two children affected with ED, was recruited. The study was exempted from review by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB584/60). Written informed consent from each participant was obtained according to the Declaration of Helsinki. Mutation analyses were performed as described previously.[2]

A 9-year-old Thai girl had sparse hair, thin eyebrows and eyelashes, dry skin and perioral hyperpigmentation (Fig. 1a–c). The proband, at age 6 years, showed only 13 primary teeth and five permanent teeth including two upper central incisors, two upper second molars and one second premolar (Fig. 1d–f). Her younger brother also manifested ED features similar to the proband (Fig. 1g–i). He had 16 primary teeth and 16 permanent teeth (Fig. 1j–l). Both siblings had agenesis of the permanent and primary upper lateral incisors and the permanent lower anterior teeth. The absent teeth were more pronounced in permanent than primary dentition, and more in the mandible than the maxilla. Their parents were nonconsanguineous and healthy. None of their relatives had any ED features.

Hair shaft analyses using light, polarized light and scanning electron microscopes of the siblings showed regular diameter, evenly distributed melanin granules and bright and polychromatic birefringence. The cuticle scales were flattened showing an imbricate pattern. Similar appearances were observed in the age- and sex-matched healthy Thai controls.

Exome and Sanger sequencing of the affected siblings and their parents revealed that the siblings harboured compound heterozygous mutations including a novel missense mutation, c.331T>A(p.C111S) in exon 3 (maternal allele) and novel frameshift deletion, c.1036_1046delGCCAACCTCAG(p.A346CfsTer27) in exon 7 (paternal allele) of *KREMEN1* (NM_032045.4).

p.C111S was not present in the Human Gene Mutation Database (HGMD; www.hgmd.cf.ac.uk/ac/index.php), the Exome Aggregation Consortium (ExAC; exac.broadinstitute.org) and an in-house database of 700 unrelated Thai exomes. It had a PolyPhen-2 score of 0·999;[3] SIFT 0·00;[4] and M-CAP 0·342[5] and is located in the highly conserved kringle domain. The alanine changed to cysteine at codon 346 introducing a premature stop codon in the extracellular domain of *KREMEN1*.

Issa *et al.* proposed, for the first time, that *KREMEN1* was another disease gene for ED.[1] However, as no functional studies have been performed for the p.F209S mutation, the pathogenicity and causative role of *KREMEN1* in ED are unconfirmed. More recently, homozygous p.T49R and p.F258_P259del were detected as likely to be pathogenic in two Turkish white probands with mild features of ED.[6] However, the two variants were not co-segregated with the phenotype in the two families. No functional studies were performed, leaving their pathogenicity debatable.

We identified two Thai siblings presenting with ED with compound heterozygous mutations in *KREMEN1*. One, p.C111S, is a missense. There is evidence that suggests that it is pathogenic. Firstly, it is absent in HGMD, ExAC and our in-house database. Secondly, it co-segregates with the phenotype in the family. Thirdly, *in silico* analyses identify it as pathogenic and an alignment of protein sequences has revealed that cysteine-111 is evolutionarily conserved. Fourthly, p.C111 is a part of disulfide bonds, which are essential in maintaining protein integrity.[7] The mutation is expected to cause unfavourable kringle domains, which play roles in various biological processes.

The other mutation is an out-of-frame deletion, p.A346CfsTer27, highly suggestive of its pathogenicity. It alters the conserved asparagine required for N-linked glycosylation producing a truncated protein. Each mutation is inherited from one parent. Taken together, the ED in the two patients is highly likely to be caused by mutations in *KREMEN1*.

Comparing our patients with the Palestinian patients, the absence of permanent upper lateral incisors and lower anterior teeth, and presence of upper central incisors, were found in all seven patients.[1] This aberrant dental pattern could be a pathognomonic feature of *KREMEN1* mutations. More individuals with *KREMEN1* mutations are needed to substantiate this observation.

Our siblings' hair patterns were comparable with controls. These suggest that hair structure is unlikely to be affected by

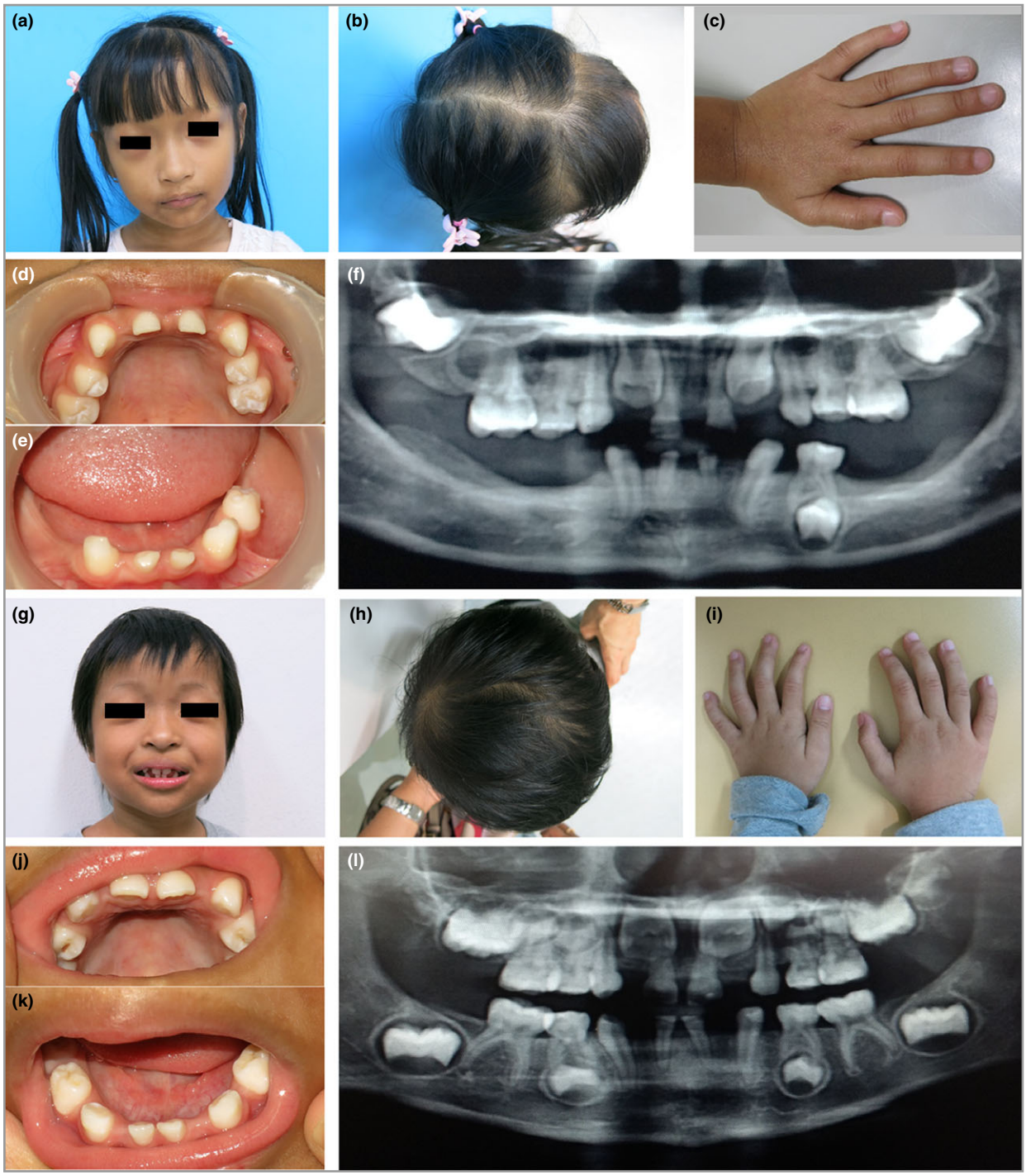

Fig 1. Clinical and radiographic manifestations of the siblings. (a–e) The photographs of the face, head, hands, maxilla and mandible of the proband at age 6 years showed sparse hair, thin eyebrows and eyelashes, dry skin, perioral hyperpigmentation, normal nails, severe dysplastic edentulous arch, high-arched palate and agenesis of multiple primary teeth. (f) Panoramic radiograph of the proband at age 9 years revealed only five permanent teeth including the upper central incisors and second molars, and lower left second premolar. (g–k) Clinical photographs of affected brother at 3 years of age showed sparse hair, eyebrows, eyelashes and teeth. (l) Panoramic radiograph of the proband's brother, at age 5 years, revealed 12 missing permanent teeth including upper lateral incisors, lower anterior teeth, lower second premolars and lower second molars.

KREMEN1 mutations. The sparse hair phenotype observed in participants with *KREMEN1* could be a result of multiple factors such as reduced follicular units, decreased number of hairs per unit, slow growth rate or early exfoliation.

In conclusion, our study that has identified the second and third mutations found in *KREMEN1* confirms that *KREMEN1* is a human disease gene for ECTD13. Agenesis of permanent maxillary lateral incisors and mandibular anterior

teeth could be a unique manifestation of *KREMEN1* mutations.

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