Novel compound heterozygous cadherin 3 mutations in hypotrichosis and juvenile macular dystrophy

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To the Editor: Hypotrichosis with juvenile macular dystrophy (HJMD, OMIM: 601553) is a rare autosomal recessive disorder characterized by short and sparse hair, progressive macular degeneration, decreased visual acuity, and even blindness in early life. Mutations responsible for HJMD have been identified in the cadherin 3 gene (*CDH3*, OMIM: 114021) on chromosome 16q22.1. This gene encodes P-cadherin, a calcium-dependent cell–cell adhesion protein that plays a significant role in the development of hair follicles[1] and the retinal pigmented epithelium.[2] To date, 30 HJMD pedigrees and 20 sporadic cases involving 36 mutations in the *CDH3* gene have been reported.

In this study, we present a sporadic Chinese HJMD case with novel compound heterozygous nonsense mutations and conduct a literature review of genotype-phenotype correlation in 116 HJMD individuals. This study was approved by the Ethics Committee of the Children's Hospital, Zhejiang University School of Medicine (No. 2022-IRB-046), and was conducted according to the *Declaration of Helsinki*. Informed consent was obtained from all participants. Blood samples were obtained from the proband and her parents, and genomic DNA was extracted using the TIANamp Blood DNA Kit (TIANGEN, Beijing, China). Whole exome sequencing (WES) was then performed on the proband. Exome capture was conducted using a Twist Comprehensive Exome kit (Twist Bioscience, South San Francisco, CA, USA) according to the manufacturer's protocol. The exome library was sequenced on the HiSeq 4000 platform (Illumina, San Diego, CA, USA) and aligned to the GRCh37/hg19 human reference sequence. Sanger sequencing was used to confirm the candidate mutations identified by WES. Total RNA was extracted from the blood samples for reverse transcription polymerase chain reaction (RT-PCR) analysis and Sanger sequencing of *CDH3* transcripts. Primers used are listed in Supplementary Table 1, http://links.lww.com/CM9/B93.

The proband was a 10-year-old girl, the first child of a non-consanguineous family. She was referred to our department because of hypotrichosis with short and slow-growing hair apparent from the age of 6 months. Her hair was almost never longer than its present presentation. Her medical history includes idiopathic thrombocytopenic purpura 9 years ago with a full recovery. There was no family history of abnormal hair growth or visual impairment. The proband presented scalp hypotrichosis with sparse, soft and curly hair, especially the peripheral hair [Figure 1A and 1B]. The hair pull test and scalp skin were normal. Her eyebrows and eyelashes were normal and she already had armpit hair. She had small papules on both cheeks and on the outer side of the upper arm. There were no associated abnormalities of her nails, teeth, or limbs. Dermoscopy revealed the hair diameter to be diverse and the percentage of pilosebaceous units with a single hair to be increased. Scanning electron microscopy showed the hair shaft had local deformation, peeling, and loss of cuticle but without complete 180° twists [Figure 1C and 1D]. Complete blood count and levels of zinc, iron, transferrin, ferritin, vitamin B12, antinuclear factor, and thyroid hormone levels were all within normal ranges. Bone age analysis (X-ray) found an early bone age.

Impairment of the proband's vision began at the age of 9 years. Her best-corrected visual acuity was 0.8 in left and right eyes. Fundus examination revealed a yellow lesion with a size of about 1 papillary diameter on the nasal side of the foveal center in the right macula [Figure 1E]. The left macula was normal. Optical coherence tomography showed partial continuity interruption in the outer retina [Figure 1F].

The WES analysis discovered compound heterozygous mutations, c.2102_2103delAT (NM_001793.5) and c.2158C > T (NM_001793.6), in the *CDH3* gene, which was confirmed by Sanger sequencing [Figure 1G and 1H].

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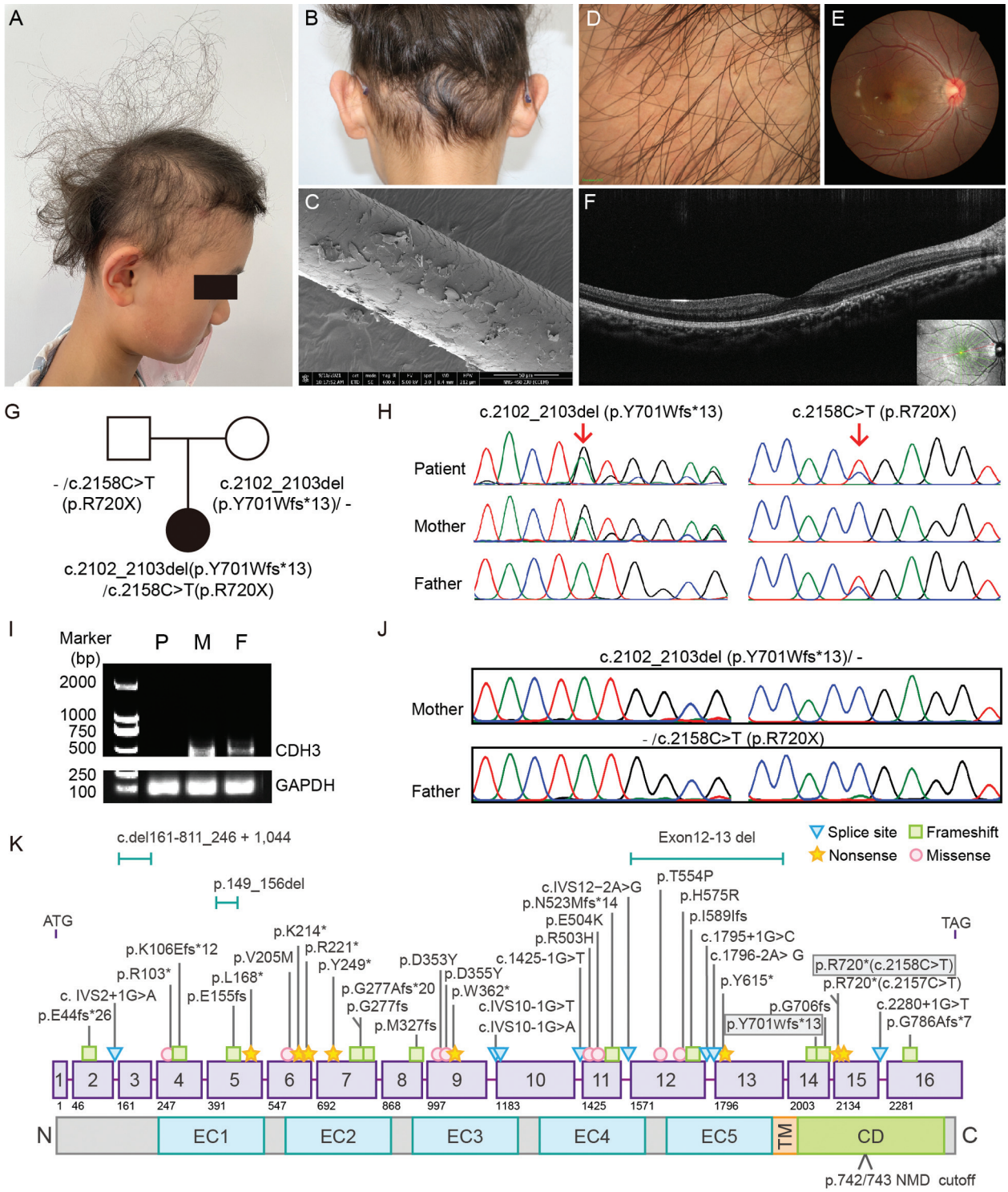

Figure 1: Genotypic and phenotypic characteristics of the HJMD patient. (A) and (B) Sparse, short, and slow-growing hair. (C) Scanning electron microscopy of the hair shaft. (D) Dermoscopy examination. (E) Color fundus photographs. (F) Optical coherence tomography. (G) The pedigree of the HJMD patient. (H) Sanger sequencing and WES-based analysis of two mutations. (I) RT-PCR analysis of the two mutations in the family. (J) cDNA sequencing analysis of the two mutations. (K) Review of mutations identified in the *CDH3* gene in patients with HJMD. The diagram includes protein domains, previously reported mutations, and mutations from this report (boxed). CD: Cytoplasmic domain; *CDH3*: Cadherin 3; EC: Extracellular; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HJMD: Hypotrichosis with juvenile macular dystrophy; NMD: Nonsense-mediated decay; RT-PCR: Reverse transcription polymerase chain reaction; TM: Transmembrane; WES: Whole exome sequencing.

The frameshift deletion (c.2102-2103_delAT) in exon 14 results in the replacement of tyrosine (Y) by tryptophan (W) at position 701 of the amino acid sequence and thus a stop codon (TGA) occurred at position 713 caused by frameshift (p.Y701Wfs*13). The nonsense mutation (c.2158C > T) in exon 15 results in an arginine codon (CAG) being substituted by a stop codon (TAG) at position 720 of the amino acid sequence (p.R720*). Further Sanger sequencing showed that the proband inherited the deletion mutation from her mother and the

nonsense mutation from her father [Figure 1H]. Neither mutation was previously reported for Human Gene Mutation Database (HGMD) and both were absent in the ClinVar, HGMD, gnomAD, and dbSNP databases.

Gel electrophoresis of RT-PCR products showed that a *CDH3* band was detected in the parents, but not in the proband who carried the compound heterozygous mutations [Figure 1I]. cDNA sequencing analysis further confirmed that transcripts containing either of the two mutations were not expressed [Figure 1J]. Both loss-of-function mutations arose before the predicted cutoff (p.742/743) for nonsense-mediated decay (NMD). These results indicate that these mutations trigger NMD or cause truncation of the P-cadherin protein, thereby preventing cytoplasmic domain binding with β-catenin, which will inhibit β-catenin/Wnt signaling in the hair follicle and expression of P-cadherin in the retinal pigment epithelium.[1,2]

We have reviewed 95 familial HJMD cases from 30 pedigrees and 21 sporadic HJMD cases [Supplementary Table 2, http://links.lww.com/CM9/B93]. The patients with HJMD have been reported throughout the world but are mainly prevalent in the Middle East. Only one case with hypotrichosis but no visual symptoms had been reported in China. Of 113 patients whose detailed clinical data were available, the male/female ratio was 48:65, the average onset of scalp hypotrichosis was approximately 1 month of age, while decreased visual acuity developed from approximately 9 years of age. No significant gender or ethnic differences in dermatological characteristics or ophthalmological features were observed among patients, but they did show strong phenotypic heterogeneity. Some phenotypic differences were also observed between our case and previous patients. Unlike most previously reported patients who had generalized sparse hair all over scalp, the hair loss in our patient was mainly focused on the peripheral hair while the central scalp hair was relatively longer and denser. More interestingly, the armpit hair growth in our patient was not consistent with the appearance of hypotrichosis in HJMD. However, armpit hair growth and early bone age could be associated with precocious puberty. Our findings expand the manifestation spectrum of HJMD.

Thirty-eight distinct *CDH3* mutations, including eight nonsense, eight splicing, eight missense, eleven frameshift, and three long fragment deletion mutations have been reported in HJMD patients [Figure 1K]. These mutations are scattered throughout the *CDH3* gene, with 26% located in exons 11 and 12, which may represent a slight hotspot of mutations. In addition, despite the difference in length, the number of mutations in the extracellular domain (84%) is much more than that in the intracellular domain (16%). The five calcium-binding extracellular domains are critical for protein function by mediating dimerization. All reported missense mutations are located within the extracellular domains, which may indicate the most significant impact on function.[3] Interestingly, we also found that most patients with mutations in the intracellular cytoplasmic domain had later onset of visual symptoms.

To date, no apparent relationship between genotype and phenotype has been identified. Saeidian *et al*[4] compared 28 families of various ethnic origins and found no correlation between clinical characteristics and the type or location of *CDH3* mutations. Leibu *et al*[5] analyzed 16 Israeli patients for two mutations and found no genotype–phenotype correlation with regard to fundus appearance. However, they observed that the visual acuity of patients with the p.M327fs mutation was better than that of patients with p.R503H for the same age and the same level of cone-mediated retinal function. We reviewed a total of 116 HJMD patients and also found it difficult to draw conclusions for phenotype–genotype correlation regarding *CDH3* mutations based on phenotypic heterogeneity due to a limited sample size.

In summary, we report a Chinese case with two novel *CDH3* pathogenic mutations, which were in the intracellular cytoplasmic domain and presumably lead to nonsense-mediated mRNA decay. Our report provides more genetic evidence for the importance of the intracellular domain and also more phenotypic evidence for the complexity of genotype–phenotype correlations in HJMD.

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Conflicts of interest

None.

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