ORIGINAL ARTICLE

Exome sequencing identified a missense mutation of *EPS8L3* in Marie Unna hereditary hypotrichosis

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INTRODUCTION

Hereditary hypotrichosis is a kind of inherited hair disorder which can grossly be characterised into two major groups according to the absence (non-syndromic hereditary hypotrichosis) or presence (syndromic hereditary hypotrichosis) of extra-cutaneous features. Alopecia universalis congenita/alopecia areata congenita with papular lesions (AUC/APL), hereditary hypotrichosis simplex (HHS), localised autosomal recessive hypotrichosis and Marie Unna hereditary hypotrichosis (MUHH; OMIM 146550/612841) are different forms of non-syndromic hereditary hypotrichosis. AUC/APL is characterised by early-onset, complete hair loss including eyebrows, eyelashes, armpit hair, pubic hair and body hair, and/or small erythematous papules of head and neck. Individuals with HHS typically show normal hair at birth, but hair loss and thinning of the hair shaft start without characteristic hair shaft anomalies during early childhood and progress with age. The affected individuals of HHS are affected scalp hair and/or body hair. The individuals with localised autosomal recessive hypotrichosis have hypotrichosis simplex with shortened length of the hair shaft, skin fragility/ectodermal dysplasia and woolly hair with palmoplantar keratoderma and cardiomyopathy syndrome, and associated with absence or scarcity of eyebrows, eyelashes and pubic hair.

MUHH is a rare autosomal dominant congenital hair disorder which was first described by the German dermatologist Marie Unna.1 MUHH is distinguished from other forms of hypotrichosis by the presence of a twisting hair; affected individuals of this disease have normal, sparse or absent hair at birth, then develop to coarse, twisted and wiry hair during childhood and progress during puberty to an almost complete alopecia. Eyebrows, eyelashes and body hair are also markedly diminished or absent. No other ectodermal abnormalities are observed. So far, two linkage loci for MUHH have been mapped to chromosome 1p21.1–1q21.3.1,4 After that, an international collaboration discovered that MUHH is caused by heterozygous mutations in the second 5′-untranslated region of the HH gene (U2HR) recently.4 The MUHH pedigree in this study was reported previously by Yan et al and Yang et al.3 The former study found that affected individuals had little or no scalp hair at birth, wiry and irregular hair on the scalp that had been difficult to manage in childhood, and their forehead and parietal hair were bald or sparse in puberty. Eyebrows and eyelashes had always been thin. Axillary and pubic hair failed to develop. But the affected individuals have modest scarring alopecia and normal vertex hair. The sequel study had identified a locus for MUHH on chromosome 1p21.1–1q21.3 in this family.

For the past decades, the genes underlying Mendelian diseases have been identified through positional cloning, a process of meiotic mapping, physical mapping and candidate-gene sequencing. Recently, exome sequencing (also known as targeted exome capture) was demonstrated to be a cheaper but efficient strategy to selectively

ABSTRACT

Background Marie Unna hereditary hypotrichosis (MUHH) is an autosomal dominant disorder characterised by coarse, wiry, twisted hair developed in early childhood and subsequent progressive hair loss. MUHH is a genetically heterogeneous disorder. No gene in 1p21.1–1q21.3 region responsible for MUHH has been identified.

Methods Exome sequencing was performed on two affected subjects, who had normal vertex hair and modest alopecia, and one unaffected individual from a four-generation MUHH family of which our previous linkage study mapped the MUHH locus on chromosome 1p21.1–1q21.3.

Results We identified a missense mutation in *EPS8L3* (NM_024526.3: exon2: c.22G→A:p.Ala8Thr) within 1p21.1–1q21.3. Sanger sequencing confirmed the cosegregation of this mutation with the disease phenotype in the family by demonstrating the presence of the heterozygous mutation in all the eight affected and absence in all the seven unaffected individuals. This mutation was found to be absent in 676 unrelated healthy controls and 781 patients of other disease from another unpublished project of our group.

Conclusions Taken together, our results suggest that *EPS8L3* is a causative gene for MUHH, which was helpful for advancing us on understanding of the pathogenesis of MUHH. Our study also has further demonstrated the effectiveness of combining exome sequencing with linkage information for identifying Mendelian disease genes.
sequence the whole genome coding regions. It has been widely used to identify genes for rare monogenic diseases and can also provide molecular identification of Mendelian diseases when the clinical diagnosis is uncertain.6–9 Here, we identified a novel causative gene for MUHH by combining exome sequencing with the linkage information from our previous study.3

**MATERIALS AND METHODS**

**Clinical sample**

A four-generation MUHH family consisting of 21 individuals was first reported by Yan et al5 from Anhui province in China (figure 1A). There were nine affected individuals including four males and five female subjects. After obtaining written informed consent from all the participants, EDTA anticoagulated venous blood samples were collected from eight patients and seven controls. The clinical features, histological characteristics, light microscopic examination and disease history supported the diagnosis of MUHH.5 Sanger sequencing was performed to exclude U2HR mutation in this family. After that, two affected individuals (II5 and III10) and one unaffected individual (III2) were selected for exome sequencing. An additional 12 members (six cases and six controls) were evaluated in mutation validation analyses.

In addition, the unpublished exome sequencing data of ethically and geographically matched 1457 subjects including 676 unrelated controls and 781 unrelated patients of other disease were used to filter variants. All the 1457 subjects were excluded MUHH by at least two dermatologist examinations.

Figure 1  (A) The genealogical tree. ‘+’ in pedigree indicates those who are subjected to exome sequencing, and ‘−’ in pedigree indicates those who had undergone Sanger sequencing. (B) Chromatogram of the heterozygous c.22G>A variant resulting in the EPS8L3 Ala8Thr substitution. This figure is only reproduced in colour in the online version.
RESULTS
We conducted exome sequencing in two affected individuals (II3 and III10) and one unaffected individual (II2) from one Chinese MUHH family where locus was mapped to 1p21.1–1q21.3.5 A 5.85 Gb of average sequence was generated per individual as paired-end, 90-bp reads. After discarding the reads that had duplicated start sites and then mapping to the human reference genome (NCBI Build 37, hg19), 2.44 Gb mapped, targeted exome sequences with a mean depth of 64.83-fold were achieved on average, 88.41% of the exome was covered at least 10-fold.

As mentioned in previous studies, we focused our analyses primarily on NS variants, SS and coding insertions/deletions (indels) that are more likely to be pathogenic mutations10 11 (table 1). In addition, we predicted that variants underlying MUHH are rare and thus unlikely to be identified previously. We therefore selected the variants that were absent from the most updated dbsNP database (V135) for further analysis. Assuming a dominant model, we found 91 novel NS/SS/indels mutations in untranslated regions (UTRs) with 200 bp 5’ of initiation codon or 3’ of termination codon.

DISCUSSION
In this study, we identified a missense mutation in EPOS8L3 (NM_024526.3: exon2: c.22G->A:p.Ala8Thr) which located within the linkage region 1p21.1–1q21.3 in this family.12 This mutation was predicted to be damaging by the ANNOVAR program15 16 and affect a conserved amino-acid residue by PhastCons software.17 Subsequently, we confirmed that the mutation showed a complete cosegregation with the disease phenotype in the family, carried by all the eight affected and absent in all the seven unaffected individuals. We further confirmed the absence of the mutation in additional 676 controls and 781 subjects of other disease. Taken together, our findings have strongly implicated EPOS8L3 as a new gene for MUHH.

We then analysed the mutation in all the available affected and unaffected individuals (including three samples performed with exome sequencing) of this family by Sanger sequencing. All the eight affected individuals carried this heterozygous mutation in EPOS8L3 (NM_024526.3: exon2: c.22G->A:p.Ala8Thr) which was absent in the seven unaffected family members, suggesting complete cosegregation between the mutation and MUHH phenotype (figure 1B). Furthermore, this mutation was not detected in additional exome sequencing data of 676 unrelated, ethnically and geographically matched controls, as well as within the exome sequencing data of 781 unrelated, ethnically and geographically matched patients of other disease. All these results suggested that this mutation is a causal variant for MUHH, instead of a rare polymorphism.
in mouse. In addition, defective and excessive transforming growth factor-β/EGFR signalling leads to abnormal hair morphogenesis and hairless phenotype, respectively. Taken together, these data suggested that EPS8L3 mutation might cause MUHH by resulting in hair follicle reduction through hairspheres. As the bulge region of the hair follicle serves as a role in maintaining stem cells. Of course, further study is warranted to determine whether this relationship between EPS8 family and stem cells could contribute to MUHH or not.

In conclusion, our study identified EPS8L3 as a disease gene for MUHH by combining exome sequencing with previously established linkage information in a large multi-generation MUHH family of Chinese population. Further biological studies of EPS8L3 would shed new insights into the genetic aetiology and pathophysiology of MUHH. Our study has also demonstrated the effectiveness of exome sequencing, combined with linkage analysis, in discovering disease genes for Mendelian disorders.

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Contributors XZ, BRG, LGQ, TJ, L-D, JS-JL and YC wrote the manuscript and revised it; J-CH, G-QS, YX, ML and XJ performed other data analysis; GC, HY and JL performed blood samples collection and selection; PL extracted Genomic DNA; X-JZ and SY performed other DNA samples; XZ, B-RG, L-QC, TJ, L-DS, J-JL and YC performed other data analysis; HC, P-GW and MG performed other DNA samples. All authors contributed to this study and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was approved by the Ethical Committee of Anhui Medical University and conducted according to the Declaration of Helsinki Principles.

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