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LETTER

Two novel mutations including a large deletion of the *SLC4A11* gene causing autosomal recessive hereditary endothelial dystrophy

INTRODUCTION

Congenital hereditary endothelial dystrophy (CHED) is an inherited disorder of the corneal endothelium characterised by bilateral non-inflammatory corneal clouding ranging from a diffuse haze to a ground-glass appearance. CHED can be inherited in an autosomal dominant (CHED1) or recessive (CHED2) manner. CHED2 usually presents at birth or early infancy. Bilateral corneal clouding can lead to visual impairment often accompanied by nystagmus in CHED2 patients requiring corneal transplantation.[1]

Mutations in the solute carrier family 4 member 11 (*SLC4A11*) gene have been identified in most patients with CHED2. With PCR sequencing of the entire coding and putative promoter regions of *SLC4A11*, there were, however, some clinically confirmed CHED2 patients with undetected *SLC4A11* mutations.[2]

CASE DESCRIPTION

Three affected siblings with CHED2 from a non-consanguineous Thai family were seen at the age of 7, 17 and 20 years, respectively. A diagnosis of CHED2 was made by clinical features, histopathological and confocal microscopic findings. All had corneal haze since birth. Nystagmus was present in the 20-year-old brother and the 7-year-old sister. None had sensorineural hearing loss. Both parents had clear corneas and denied a family history of corneal disorders.

To identify the genetic defects, we first performed PCR sequencing covering the entire coding region of *SLC4A11*. A novel c.778A>G mutation resulting in a lysine to glutamic acid substitution (p.K260E) at codon 260 was identified (figure 1A). No other variants were observed. PCR–RFLP (restriction fragment length polymorphisms) analysis showed that the mutation was present in all affected siblings and their mother but absent in their father (figure 1B). It is located at a highly conserved residue (figure 1C). As only one mutant allele was detected, PCR sequencing of the promoter region was performed and revealed no pathogenic

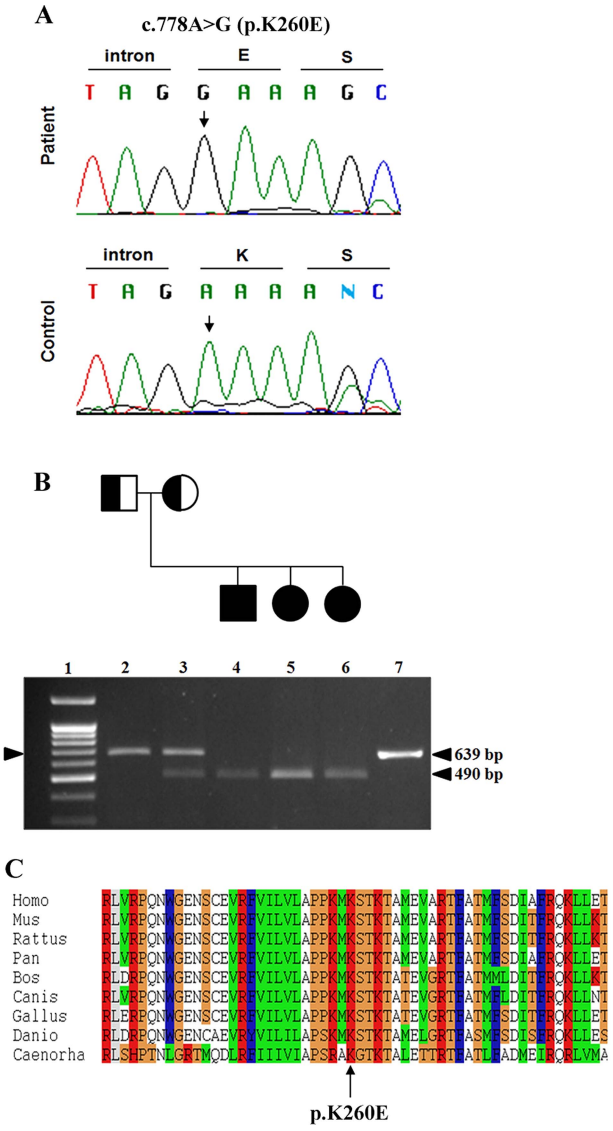

Figure 1 Analysis of the missense mutation. (A) Electropherograms of the patient and unaffected control. The c.778A>G mutation is indicated by an arrow. (B) PCR–RFLP (restriction fragment length polymorphisms) analysis for the c.778A>G mutation. *Avr*II digested the mutant allele into 490 and 149 bp products (an arrowhead). The wild-type allele does not harbour the recognition site, leaving the 639 bp PCR product intact. Note that the 149 bp band is not visualised. Lane 1, 100 bp marker; lane 2, father; lane 3, mother; lanes 4–6, affected family members; lane 7, uncut. The 500 bp band is indicated by an arrowhead. (C) Sequence alignment of SLC4A11 centring around residue 260 from various species. The lysine residue at codon 260 was conserved from *Homo sapiens* to *Caenorhabditis elegans*. The site of the p.K260E mutation is indicated by an arrow.

variants. These results suggested the possibility of a whole gene deletion occurring in the other allele. Further experiments using array comparative genomic hybridisation (CGH) covering chromosome 20 revealed that the patient had a deletion from position 3107501–3174468 which included the region where *SLC4A11* was located (3156063–3166373_NCBI36/hg18) (figure 2A). The presence of the heterozygous deletion was verified in all patients. The deletion was present in their father but absent in their mother (figure 2B, C). We also identified the precise deletion breakpoints by sequencing (figure 2D) and confirmed the deletion size of 67 733 base pairs.

DISCUSSION

We have described three affected siblings from a non-consanguineous family with CHED2. All were compound heterozygous for novel alterations, a single base pair transition (c.778A>G; p.K260E) and a 68 kb deletion encompassing *SLC4A11*.

Previous studies have failed to identify pathogenic mutations in some patients with clinically confirmed CHED2 using PCR

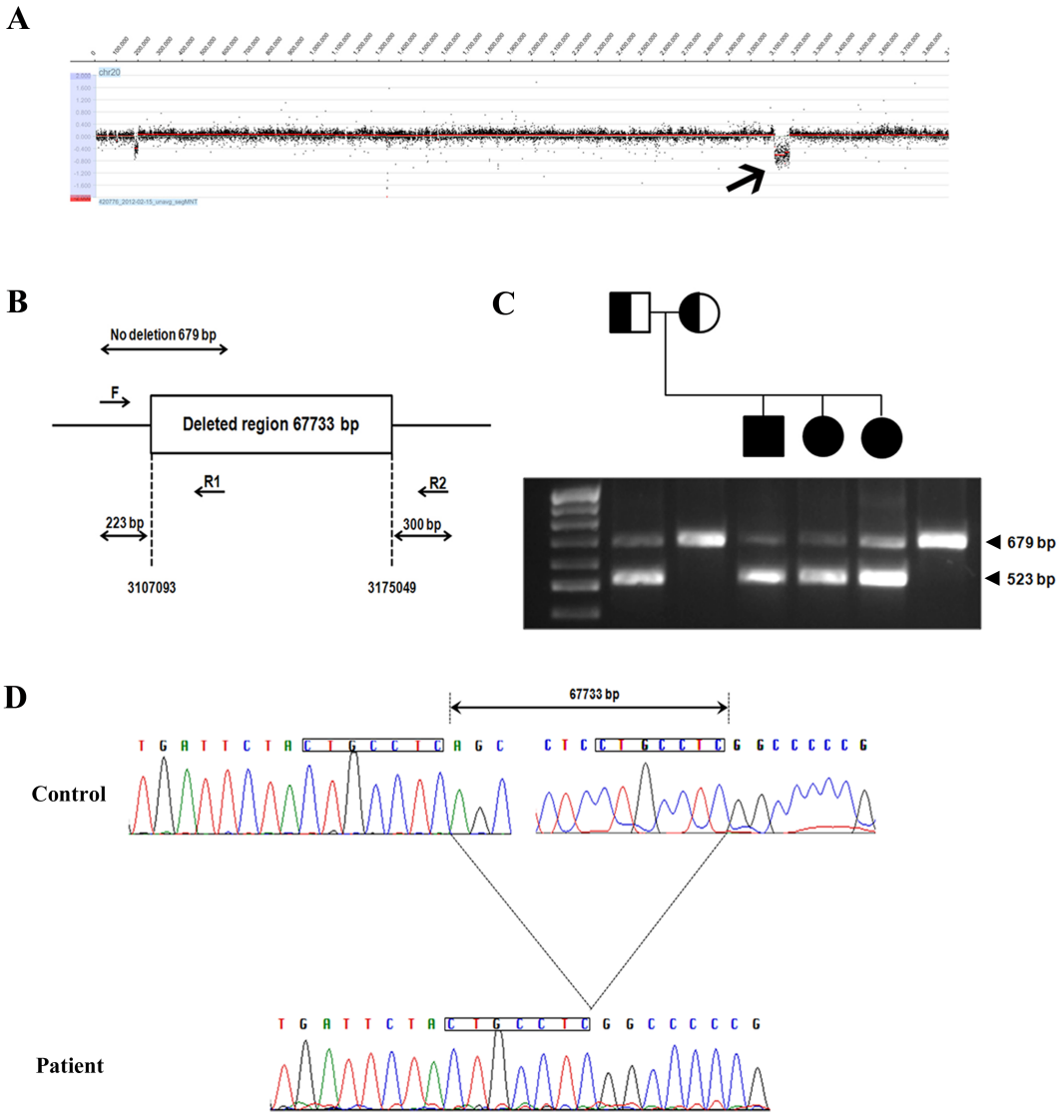

Figure 2 Analysis of the large deletion. (A) Array-comparative genomic hybridisation (CGH) analysis showed that the patient had a heterozygous deletion from position 3107501 to 3174468 (according to chromosome 20 of hg18) encompassing the *SLC4A11* gene. (B) Schematic representation of the deleted region identified in the patients showing the location of the primers and the expected size. (C) PCR analysis showing the deletion was present in all patients and their father but not present in their mother. The size of the PCR products was 523 bp and 679 bp for the deleted and non-deleted alleles, respectively (an arrowhead). (D) Electropherograms of the patient and unaffected control showing the precise breakpoints of the 67733 bp deletion and the flanking short direct repeats confirming the results from the array-CGH studies.

sequencing of the entire coding and promoter regions of *SLC4A11*.[2 3] Using array CGH, we were able to identify a novel 68 kb deletion encompassing *SLC4A11* as another disease-causing allele. This deletion resides in the structural variant (variation_5121, deletion type, 3076070–3238538_NCBI36/hg18), reported in the Database of Genomic Variants (DGV). In addition to the variation_5121, there are four deletion variants with different sizes encompassing *SLC4A11*. The deletion identified in our patient overlaps with these deletions. Copy number variations (CNVs) including microdeletions and microduplications have been demonstrated as a significant cause of structural variation in the genome and human diseases.[4–6] CNVs preferentially occur near or within the duplicated sequences. This newly identified 68 kb deletion is flanked by repeat sequences which can lead to aberrant recombination resulting in loss of the intervening sequence (figure 2D). The microdeletions at this region could be the disease-causing alleles responsible for CHED2 in the previously reported cases with unidentified mutations. It would be interesting to investigate what proportions of the chromosomal deletions contribute to CHED2. This information will have significant implications for developing an algorithm for genetic testing in CHED2 leading to more effective genetic counselling.

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Contributors VP collected the data, helped with interpretation of histological data and drafted the manuscript. PY performed molecular studies, analysed the data and drafted the manuscript. CC collected the data and helped with analysis of the images. KS and VS designed the study, undertook data analysis and interpretation and wrote the manuscript.

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