

RESEARCH NOTE

Genetic analysis of a consanguineous Pakistani family with Leber congenital amaurosis identifies a novel mutation in *GUCY2D* gene

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Introduction

Leber congenital amaurosis (LCA) is an eye condition which ultimately leads to complete blindness. It is a rare hereditary disease with an early onset characterized by severe retinal degeneration, cataract, nystagmus, null pupillary response to light, photophobia and keratoconus (Chung and Traboulsi 2009). In the current study, we characterized a consanguineous Pakistani family affected by autosomal recessive LCA. By using genomewide homozygosity mapping we were able to link the disease to the LCA1 locus on the short arm of chromosome 17. The subsequent mutation analysis identified a novel 1-bp deletion mutation in exon 7 of the *GUCY2D* gene (c.1573delC), which presumably results in a frameshift leading to a premature truncated protein (p.Gln525Argfs*38).

Congenital LCA is genetically heterogeneous (Wang *et al.* 2011) with eighteen known causative genes; *AIPL1* (MIM: 604392), *CEP290* (MIM: 610142), *CRB1* (MIM: 604210), *CRX* (MIM: 602225), *GUCY2D* (MIM: 600179), *IMPDH1* (MIM: 146690), *KCNJ13* (MIM: 603208), *LCA5* (MIM: 611408), *LRAT* (MIM: 604863), *OTX2* (MIM: 600037), *RD3* (MIM: 180040), *RDH12* (MIM: 608830), *RPE65* (MIM: 180069), *RPGRIP1* (MIM: 605446), *SPATA7* (MIM: 609868) and *TULP1* (MIM: 602280) (RetNet (summaries of gene and loci causing retinal diseases) <https://sph.uth.edu/Retnet/>; updated in 2013). In addition to these genes, the allelic heterogeneous gene *IQCB1* has also been reported to be involved in the development of LCA. Physiologically these genes are associated with a variety of photoreception,

transduction and visual perception pathways (Wang *et al.* 2011).

Materials and methods

Sampling and phenotyping

In this study, the family analysed originates from Dera Ismail Khan, a city in the Khyber-Pakhtoonkhwa province of Pakistan. Patients were examined by an ophthalmologist for phenotypic diagnosis, where they underwent general eye examination, fundoscopy and photosensitivity examination. The study was carried out after having obtained informed written consent including consent for photograph presentation in the publication.

Genotyping and linkage analysis

Whole genome scan via SNP genotyping was performed using 250K NspI array (Affymetrix, Santa Clara, USA). Obtained data were analysed for homozygous stretches with dChip software (Lin *et al.* 2004). Segregation of the identified homozygous candidate region in the family was validated through STR based genotyping with markers D17S1828 (13.81 cM), D17S1584 (14.61 cM), D17S938 (19.66 cM), D17S578 (21.75 cM), D17S1353 (23.27 cM), D17S1796 (23.27 cM), D17S786 (25.53 cM) and D17S945 (30.48 cM) (Rutgers map B37 (Matise *et al.* 2007)). To prove the statistical significance of the identified homozygous locus, two point and multi point LOD score analyses were calculated with the online version of Superlink program (ver. 1.5) (Fishelson and Geiger 2002), assuming a disease allele frequency of 0.001 with full penetrance.

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Mutation screening

Primers for coding exons of *GUCY2D* gene were designed with Primer 3 software (ver. 0.4.0) (Rozen and Skaletsky 2000). DNA sequencing for mutation analysis and carrier screening was performed with a Genetic Analyzer 3130XL (Life Technologies,) using BigDye Terminator ver. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, USA). The obtained sequence data were further aligned to the human reference genome using the BLAT tool (<http://genome.ucsc.edu/cgi-ben/hgBlat?command=start>) of the UCSC Genome Browser (Kent 2002).

Results

Clinical outcomes

Nine family members (five nonaffected and four affected) participated in the present study. General eye examinations showed that all patients were completely blind since birth. The common phenotypes among all patients included congenital cataract, keratoconus, nystagmus, characteristic poking of eye and no pupillary response to light (figure 1, a&b). In addition to the common LCA phenotype two of the patients (IV-1 and IV-16) also had mydriasis. Patients were able to distinguish between day and night in their childhood, but this ability was lost completely while reaching adulthood. The slit-lamp examination showed a moderate form of retinal cells degeneration and the overall clinical analysis of the patients confirmed a LCA phenotype.

Molecular analysis

Homozygosity mapping and linkage analysis: Complete genome screening for homozygosity mapping in individual IV-12 revealed a 6.012 Mb large homozygous stretch at the LCA1 locus (MIM: 204000) on chromosome 17p13.1 (7 906 Mb) which was flanked by the two SNP markers rs4790161 (3889843 bp, hg19) and rs3786085 (9902533 bp, hg19) (Genome Reference Consortium human genome build GRCh 37/hg19, Feb. 2009). Subsequent STS marker analysis in the whole family confirmed the segregation of the LCA1 locus with the disease. While all affected patients displayed the homozygous disease associated haplotype (indicated in

green, see figure 2a) from markers D17S938 to D17S786, the nonaffected family members were either heterozygote or did not carry the disease-associated haplotype at all, as for example individual IV-14. Linkage analysis of genotype data generated a maximum two-point LOD score of 2.85 for markers D17S938 and D17S1353, and a significant multipoint LOD score of 3.25 for markers D17S938, D17S578, D17S1353, D17S1796 and D17S786 at a recombination fraction $\theta = 0$ (table 1).

Mutation analysis

GUCY2D, the gene for LCA1, was first reported by Perrault et al. (1996). Sequence analysis of all coding exons of the *GUCY2D* gene in all affected individuals showed a novel single base pair deletion (c.1573delC) in exon 7. This deletion distorted the reading-frame and resulted in a premature stop codon 37 amino acids after deletion (p.Gln525Argfs*38). Segregation analysis of this deletion among all unaffected family members revealed a heterozygous carrier status for all except individual IV-14, who was homozygous for the wild-type sequence (figure 2c).

Discussion

GUCY2D encodes for the retinal-specific guanylate cyclase 2D protein, which has three domains: transmembrane domain, protein kinase domain and guanylate cyclase domain. Subcellular localization showed that GUCY2D is present in disc membranes of outer photoreceptor segments (Liu et al. 1994) and it has the function to restore the level of cGMP in the retina after its depletion which is induced by a photo-mediated phosphodiesterase reaction (Burns and Baylor 2001).

In the current study, the molecular analysis of a Pakistani family with five generations affected from autosomal recessive LCA was conducted (see figure 1a). The molecular diagnostics of this family revealed a single nucleotide deletion (c.1573delC) in exon 7 of the *GUCY2D* gene, resulting in a premature truncation of the protein 37 amino acids after the deletion (p.Gln525Argfs*38) (figure 2b).

Due to the premature stop codon, the c-terminal region which includes the protein tyrosine kinase and guanylate

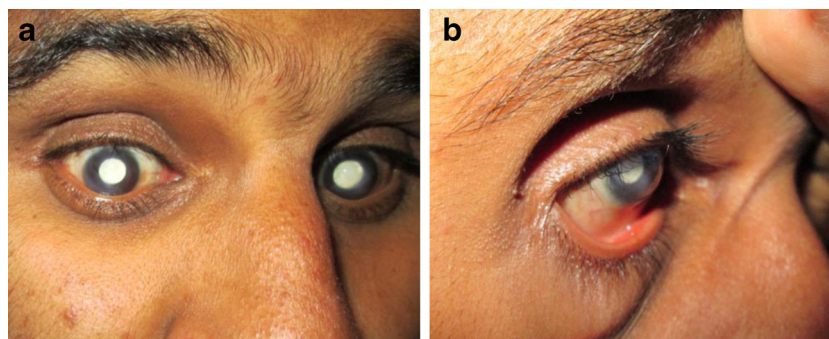


Figure 1. Patient IV:16 shows (a) bilateral cataract and (b) keratoconus.

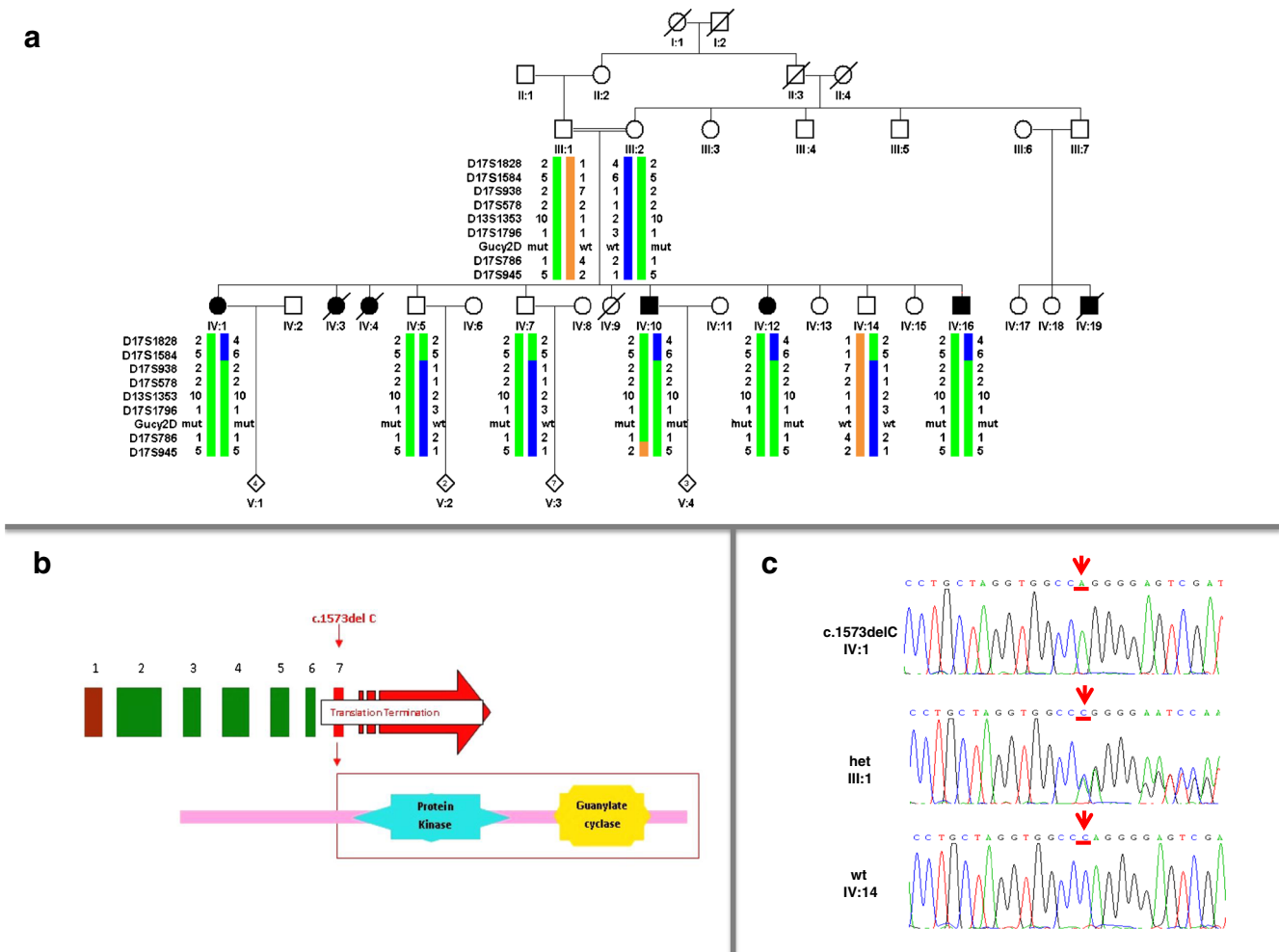


Figure 2. (a) Five generation pedigree with the haplotypes for dinucleotide repeat markers D17S1828 (13.81 cM), D17S1584 (14.61 cM), D17S938 (19.66 cM), D17S578 (21.75 cM), D17S1353 (23.27 cM), D17S1796 (23.27 cM), D17S786 (25.53 cM) and D17S945 (30.48 cM) with GUCY2D located between D17S1796 and D17S786. Absolute allele sizes are down-coded. The smallest allele size for a given marker in this family is indicated by 1; all larger alleles are given relative to the smallest allele corresponding to the additional number of dinucleotide repeats. Bold symbols represent affected family members (IV-1, IV-3, IV-4, IV-10, IV-12, IV-16 and IV-19). (b) Graphical representation of the deleterious mutation at genomic and protein level. (c) Sequence chromatogram of patient IV:1, heterozygous carrier III:1 and healthy individual IV:14 who carry two wild-type alleles. Lines and arrows indicate deletion.

Table 1. Two-point and multipoint LOD score between markers (D17S1828, D17S1584, D17S938, D17S578, D17S1353, D17S1796, D17S786 and D17S945) and LCA disease.

Marker	Genetic position in cM (Rutgers map, build 37)	Physical position in bp (Feb. 2009 (GRCh37/hg19))	Two-point LOD score (at $\theta = 0$)	Multipoint LOD score
D17S1828	13.81	3810359	0.1	-1.81
D17S1584	14.61	4345021	0.25	0.23
D17S938	19.66	6249268	2.85	3.25
D17S578	21.75	6823880	1.835	3.25
D17S1353	23.27	7617413	2.849	3.25
D17S1796	23.27	7787163	1.835	3.25
D17S786	25.53	8811709	2.849	3.25
D17S945	30.48	9823223	2.849	3.25

cyclase domains, is lost. Hence, it can be speculated that the cGMP level cannot be restored, which is essential in the phototransduction pathway in cone/rod cells where cGMP acts as a second messenger. With this assumption, we suggest that this loss of function mutation impairs the vision mechanism and is thus responsible for the LCA and thereby observed early onset of blindness. Although autosomal dominant mutations in *GUCY2D* have been reported for cone-rod dystrophy 6 (CORD6), mutation carriers in our family had normal visual perception and did not display any LCA or CORD6 associated symptoms (Kitiratschky *et al.* 2008; Ugur Iseri *et al.* 2010).

In the last couple of years, numerous mutations in *GUCY2D* have been reported, highlighting it as one of the most important genes in LCA disease screening. In the last decade, intensive studies on treatment strategies have been successfully conducted. Mihelec *et al.* (2011) demonstrated a strong rescue of the LCA phenotype in *GUCY2E*^{-/-} (mouse homologue of the human *GUCY2D*) knockout mice models by delivering the human *GUCY2D* transgene via a rAAV2/8 viral vector into subretinal tissue. Moreover, several research groups have recently revealed the great potential of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) for treating retinal degenerative diseases in humans and mice (Carr *et al.* 2009; Meyer *et al.* 2011; Schwartz *et al.* 2012).

Conclusion

Even in the area of next generation sequencing, homozygosity mapping in combination with subsequent candidate gene sequencing is a cost and time-effective method to detect disease causing mutations. This study highlights the usefulness of homozygosity mapping as the first step in the diagnosis of autosomal recessive diseases with great genetic heterogeneity in families with a consanguineous background.

Revealing the disease causing mutation allows genetic counselling and a straightforward mutation screening of consanguineous couples in the family to calculate the individual risk for LCA in their offspring. Although, a therapeutic approach is currently unavailable, the above mentioned advances in retinal degenerative research raise the hope of a targeted gene or stem cell therapy in the near future.

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