

Ophthalmology 2018: Mutation in the PYK2-binding domain of PITPNM3 causes autosomal dominant cone dystrophy (CORD5) in two Swedish families

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Abstract

Autosomal dominant cone dystrophy is a rare disease predominantly affecting cone photoreceptors. Here we refine the CORD5 locus previously mapped to 17p13 from 27 to 14.3 cM and identified a missense mutation, Q626H in the phosphatidylinositol transfer membrane-associated protein in 2 Swedish families. In our study, the mutation causing CORD5 is located in the C-terminal region interacting with a member of non-receptor protein tyrosine kinases, PYK2.

Introduction

Progressive cone or cone-rod dystrophies are characterized by a defective cone function and demonstrated by the abnormalities in cone-mediated electro-retinogram components. The presenting symptoms are defective colour vision, impaired central visual acuity and sensitivity to light. A variant of autosomal-dominant cone dystrophy has been mapped to 17p12–p13 in the Swedish family. Two other reports, one from United Kingdom and one from the United States and also showed linkage of dominant cone dystrophy to chromosome 17p. The disorder in the British family, designated as the CORD6 was found to be caused by the mutations in GUCY2D gene. Mutations with in GUCY2D gene also cause Leber congenital amaurosis and autosomal recessive retinitis pigmentosa. In the USA family, the disease mapped to 17p12–p13 was later reported to be caused by same mutation in British CORD6 family, and therefore it was concluded by the CORD5 and CORD6 having the same disease.

Materials and methods

Patients and clinical examination

To identify the gene causing CORD5, we analysed two multi-generation in a Swedish families originating from the same geographical area in Northern Sweden. Family 151, reported previously, is five-generation family with total of 48 individuals of which 18 will be affected. Family 152 is a seven-generation family of 32 individuals with 15 affected and two individual's with unknown disease status. Informed the consent was obtained from all individual's participating in the study and the research following to the tenets of Declaration of Helsinki was approved by the Ethics Committee of University of Umea. All individuals were classified as unaffected were symptom less, and had normal visual acuity and normal colour vision. In family 152 the corresponding clinical examinations in progress, however have not yet been completed. A full ophthalmologic examination included assessment of visual acuity and

evaluation of the anterior segment and the fundus. Visual fields were tested in a Goldmann perimeter by using standard objects. In selected cases, a more detailed investigation was performed. The course of dark adaptation was determined by using a Goldmann-Weekers adaptometer. Colour vision was tested with the pseudoisochromatic plates. Electrooculography, and full-field, single flash and flicker ERGs were recorded by using the UTAS-E 2000 and UTAS-E 3000-mf LKC technologies Inc.,

Genotyping, linkage analysis, and mutational screening

DNA from 80 individuals was extracted from peripheral blood lymphocytes according to standard protocols reported by Balciuniene et al. Twelve microsatellite markers included in the Rutgers Combined Linkage-Physical Map were used for genotyping. The markers mapped to the 17p13 in proximity to D17S938 were situated approximately 2 cM apart. The GDB Human Genome Database was used for the information about microsatellite markers and primer sequences. PCR mixes were prepared in 7.5 ml reactions using 0.3U of AmpliTaq Gold, 0.75 ml of GeneAmpPCRbuffer II, 0.075 mM of dNTP, 0.75 mM of MgCl₂, and 0.1 mM of primers. Polymerase chain reaction was performed with a temperature profile of 95°C for 10 min, 94°C for 15 s, 55°C for 15 s, 72°C for 30 s for 10 cycles, 89°C for 15s, 55°C for 15s and 72°C for 30s for 20 cycles, and the final extension at 72°C for 10 min. Fragment analysis of the samples was performed on a 3730xl DNA analyser, and the collected raw data were analysed with ABI Prism GeneMapper Software v3.0.

For bidirectional sequencing of phosphatidylinositol transfer membrane-associated protein, coding exons and adjacent intrinsic sequences were amplified from genomic DNA of two affected patients from each family and one unaffected individual. Primer pairs designed with Primer software is listed. PCR amplification of the 19 exons was performed in 25 ml reactions with 0.5 U of AmpliTaq Gold, 2.5 ml of PCR buffer II 10, 1.5 mM of MgCl₂, 0.1 mM of dNTP, 0.4 mM of primers, and 50 ng of genomic DNA. PCR cycling consisted of an initial denaturing step at 94°C for 5 min; then 30 cycles of denaturing at 94°C for 1 min, annealing at temperature dependent on primer sequence for 1 min, and extension at 72°C for 1 min; and final elongation step at 72°C for 7 min. PCR products were purified using MicroSpin columns. The sequencing reactions were performed using Big Dyes Terminator v3.1. The products of sequencing reactions were run on 3730xl DNA analyzer.

Results and discussion

Two Swedish families, are originating from same geographical area in the Northern Sweden were included in the study. The majority of the patients in family 151 had subnormal visual acuity and light sensitivity from childhood. Signs of macular degeneration were observed to early as well. There was a progressive decrease of VA leading to legal blindness in the early adulthood. Electro-physiological tests showed a progressive loss of photoreceptor function restricted to the cones. The macular regions were presented a normal appearance at early stages of the disease. In the young patient a bull's-eye maculopathy could be seen. In more advanced cases, findings varied from pigment mottling to pronounced central chorioidal atrophy. There was no peripheral restriction of the visual field and patients did not have any history of nyctalopia. Photopic ERGs representing cone function were greatly diminished or absent, whereas scotopic ERGs showed normal amplitudes. The EOG was normal. Dark adaptation curves showed absence of a normal cone segment, but the final rod dark adaptation threshold was not elevated.

In summary, this study adds one more gene on 17p, which causes the retinal degeneration. We can provide the evidence of CORD5 in Swedish patients was distinct clinical entity and describe the first disease causing mutation within the PYK2-binding domain in the PITP family.

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