Identification of novel locus for autosomal dominant butterfly shaped macular dystrophy on 5q21.2–q33.2

A I den Hollander, J J C van Lith-Verhoeven, F F J Kersten, J G A M Heister, C G F de Kovel, A F Deutman, C B Hoyng, F P M Cremers

**Participations and methods**

**Patients and ophthalmic examination**

We previously ascertained 13 members (eight affected and five unaffected) of a Dutch family with butterfly shaped macular dystrophy that was first described in 1970 by Deutman et al.1,2 Ophthalmic examination of the participating family members included best corrected Snellen visual acuity, slit lamp biomicroscopy, fundus examination, fluorescein angiography, electroretinography, electrooculography, colour vision, and dark adaptation testing.2

For this study, we ascertained and ophthalmologically examined eight additional family members. We obtained informed consent from all participating members. One member had a slightly diminished electroretinogram but a normal fundus appearance and therefore we excluded her from the study. For molecular analysis, we collected blood samples of all participating family members and isolated DNA by a standard extraction method.13

**Genotyping and linkage analysis**

We performed a genomewide linkage scan with 400 microsatellite markers distributed with an average spacing of 10 cM in the human genome (ABI Prism Linkage Mapping Set MD-10 version 2.5; Applied Biosystems, Foster City, CA, USA). We typed fluorescently labelled markers on an ABI Prism 3100 genetic analyser (Applied Biosystems) with GeneMapper software (Applied Biosystems). We binned genotyping data with the Linkage package; we assumed an autosomal dominant mode of inheritance, with a disease allele frequency of 0.001 and a penetrance of 0.95. We performed two point parametric linkage analysis of the genotyping data with the Linkage package; we assumed an autosomal dominant mode of inheritance, with a disease allele frequency of 0.001 and a penetrance of 0.95. We performed multipoint analysis for markers in the chromosome 5q21.2–q33.2 between markers D5S433 and D5S487, with a maximum multipoint logarithm of odds score of 4.05.

**The critical interval spans 46 cM (52 Mb) and contains the gene that encodes the α subunit of cyclic guanosine 5’ monophosphate phosphodiesterase 6A (PDE6A). Sequence analysis of the PDE6A gene did not show a pathologic mutation.**

**Key points**

- Butterfly shaped macular dystrophy is an autosomal dominant eye disease characterised by bilateral accumulation of pigment in the macular area, which resembles the wings of a butterfly. To date, butterfly shaped macular dystrophy has been associated only with mutations in the peripherin/RDS gene.

- It was described originally in a Dutch family in 1970. We previously excluded the involvement of the peripherin/RDS gene, the ROM-1 gene, and 44 known loci involved in retinal dystrophies in this family. A genomewide linkage scan identified a novel locus for butterfly shaped macular dystrophy on chromosome 5q21.2–q33.2 between markers D5S433 and D5S487, with a maximum multipoint logarithm of odds score of 4.05.

- The critical interval spans 46 cM (52 Mb) and contains the gene that encodes the α subunit of cyclic guanosine 5’ monophosphate phosphodiesterase 6A (PDE6A).

DNA sequence analysis

For mutation analysis of the PDE6A gene, we amplified 100 ng of genomic DNA by PCR under the following
conditions: initial denaturation for 5 minutes at 95 °C, denaturation for 1 minute at 94 °C, annealing for 1 minute at 55 °C, extension for 1 minute at 72 °C, and final extension for 10 minutes at 72 °C. Primers to amplify each exon and adjacent intron–exon boundaries of the PDE6A gene have been described.19 We performed sequencing of PCR products with BigDye Terminator chemistry (Applied Biosystems) on an ABI Prism 3730 or 3100 DNA analyser (Applied Biosystems).

RESULTS AND DISCUSSION

Previously, we described a Dutch family with butterfly shaped macular dystrophy and excluded involvement of the peripherin/RDS gene; the ROM-1 gene; four genes expressed in cone photoreceptors; all known non-syndromic macular, retinal pigment endothelium, and choroidal dystrophy loci; all known Leber congenital amaurosis loci; and all known non-syndromic congenital and stationary retinal disease loci in this family.12 Ophthalmological examination of 21 family members confirmed a definite diagnosis of butterfly shaped macular dystrophy in nine patients and an unaffected status in 11 members (fig 1). Affected patients presented with typical butterfly shaped pigmentations on ophthalmoscopy (fig 2). One member had a slightly diminished electrooculogram but a normal fundus appearance, and she therefore was excluded from the study.

We performed a genomewide scan to map the locus for butterfly shaped macular dystrophy in this family; we used a set of 400 microsatellite markers with an average spacing of 10 cM. Negative or non-significant two point logarithm of odds scores (\( \log_{10} \)) at \( \theta = 0.00 \) were obtained for all tested loci, except for 11 markers on chromosomes 2, 3, 5, and 12 (table 1). A maximum multipoint logarithm of odds score of
4.05 was seen for the region on chromosome 5 between markers D5S433 and D5S410.

We constructed haplotypes for the four chromosomal regions. We saw discordance of segregation of the chromosome 2 and 12 haplotypes with the butterfly shaped macular dystrophy phenotype (data not shown). The at risk chromosome 2 haplotype was present in all affected family members (II-7, III-1, III-3, III-4, and III-7). The regions that surrounded the loci on chromosomes 3 and 5 were saturated with more microsatellite markers, and we constructed haplotypes. The at risk chromosome 3 haplotype was present in five affected patients (II-8, III-2, III-9, III-11, and III-12) but it was present in two unaffected family members (II-7, III-4, and III-7). The interval between markers D5S433 and D5S487 (fig 1) shows that the responsible gene is located on 5q21.2–q33.2 in a 46 cM (52 Mb) interval between marker distance of 3 cM showed that the responsible gene is located on 5q21.2–q33.2 in a 46 cM (52 Mb) interval between markers D5S433 and D5S487 (fig 1).

The interval between markers D5S433 and D5S487 contains more than 450 genes and includes the PDE6A gene that encodes the α subunit of cyclic guanosine 5’ monophosphate phosphodiesterase 6A, a component of the phototransduction cascade. Mutations in the PDE6A gene have been found to cause autosomal recessive retinitis pigmentosa.19 20 Mutations in the ABCA4 and peripherin/RDS genes cause various retinal dystrophies, including retinitis pigmentosa and macular dystrophy.3 5 10 The PDE6A gene thus was considered to be a candidate gene for butterfly shaped macular dystrophy. We analysed the 22 exons of the PDE6A gene by sequencing in two affected patients (II-1 and II-8) but identified no sequence changes. This does not completely rule out the involvement of PDE6A, however, as larger deletions or insertions may have been missed by PCR based mutation analysis. Apart from PDE6A, no other obvious candidate genes were seen in the 52 Mb interval.

To date, butterfly shaped macular dystrophy has been associated only with mutations in the peripherin/RDS gene. In the family affected by butterfly shaped macular dystrophy described here, we excluded the peripherin/RDS gene and identified a novel locus on 5q21.2–q33.2. Linkage analysis in additional families affected by butterfly shaped macular dystrophy but without mutations in the peripherin/RDS gene may enable a refinement of the critical interval and facilitate the identification of the responsible gene.

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Conflicts of interest. None declared.

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REFERENCES

Table 1 Two point logarithm of odds scores of microsatellite markers on chromosomes 2, 3, 5, and 12 obtained in a genomewide linkage scan of a family affected by butterfly shaped macular dystrophy

<table>
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<th>Chromosome</th>
<th>Marker</th>
<th>Recombination fraction (ln Θ)</th>
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