

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

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Butterfly shaped macular dystrophy was first described by Deutman et al. in 1970.¹ It is characterised by bilateral accumulation of pigmented or yellowish material at the level of the retinal pigment epithelium. Lesions consist of 3–5 “wings,” which resemble the wings of a butterfly. Affected patients present with a subnormal electrooculogram and normal or slightly diminished visual acuity. The disease is relatively benign, but it can progress with age to chorioretinal atrophy in the parafoveal and peripapillary regions.² Butterfly shaped macular dystrophy shares important similarities with age related macular degeneration—the most common cause of blindness in older patients.^{3–4} In both diseases, abnormal deposition of lipofuscin like material at the level of the retinal pigment epithelium is found, which results in loss of the overlying photoreceptors.²

Butterfly shaped macular dystrophy has an autosomal dominant inheritance pattern. To date, it has been associated only with mutations in the *peripherin/RDS* gene.^{5–12} We ascertained members of the family with butterfly shaped macular dystrophy that was described originally by Deutman et al. in 1970,¹ and we excluded *peripherin/RDS* as the causative gene in this family.² In addition, we excluded the *ROM-1* gene; four genes expressed in cone photoreceptors; all known non-syndromic macular, retinal pigment epithelium, and choroidal dystrophy loci; all known Leber congenital amaurosis loci; and all known non-syndromic congenital and stationary retinal disease loci.² This study aimed to identify the locus responsible for butterfly shaped macular dystrophy in this family with a genomewide linkage scan.

PARTICIPANTS 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 electrooculogram 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,

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*). Sequence analysis of the *PDE6A* gene did not show a pathologic mutation.

USA). We typed fluorescently labelled markers on an ABI Prism 3100 genetic analyser (Applied Biosystems) with GeneMapper software (Applied Biosystems). We binned alleles with the Excel macro linkage designer developed by van Camp and coworkers,¹⁴ and we checked Mendelian inheritance of alleles with PedCheck software.¹⁵ We performed two point parametric linkage analysis of the genotyping data with the Linkage package¹⁶; 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 5 interval (D5S644, D5S433, D5S2027, D5S471, D5S2115, D5S436, and D5S410) with Linkmap.¹⁶

We chose additional microsatellite markers for finemapping from the Marshfield and Génethon databases.^{17–18} We subjected samples to polymerase chain reaction (PCR) amplification with a standard cycling profile of 34 cycles at 94°C for 1 minute, 55°C for 2 minutes, and 72°C for 1 minute. We labelled PCR products by incorporating α [³²P]-2'-deoxycytidine 5'-triphosphate and separated them by electrophoresis on a 6.6% denaturing polyacrylamide gel. We performed haplotype analysis with Cyrillic 2.1 (Cherwell Scientific Publishing, Reading, UK).

DNA sequence analysis

For mutation analysis of the *PDE6A* gene, we amplified 100 ng of genomic DNA by PCR under the following

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

Chromosome	Marker	Recombination fraction (θ =)						Z_{\max}	θ_{\max}
		0.00	0.05	0.10	0.15	0.20	0.25		
2	D2S2216	1.44	1.29	1.13	0.96	0.78	0.60	1.44	0.00
3	D3S1304	1.76	1.63	1.47	1.29	1.10	0.89	1.76	0.00
5	D5S424	1.44	1.29	1.13	0.96	0.79	0.61	1.44	0.00
	D5S641	-2.61	0.37	0.62	0.67	0.64	0.54	0.67	0.15
	D5S428	-3.39	-0.46	-0.19	-0.07	-0.02	0.00	0.00	0.25
	D5S644	-0.21	1.45	1.60	1.56	1.42	1.22	1.60	0.10
	D5S433	-0.35	2.02	2.00	1.84	1.61	1.34	2.02	0.05
	D5S2027	3.38	3.08	2.76	2.42	2.07	1.69	3.38	0.00
	D5S471	1.45	1.37	1.27	1.15	1.01	0.85	1.45	0.00
	D5S2115	3.37	3.04	2.71	2.35	1.99	1.61	3.37	0.00
	D5S436	3.89	3.54	3.17	2.79	2.38	1.95	3.89	0.00
	D5S410	1.67	1.50	1.32	1.13	0.93	0.73	1.67	0.00
12	D12S351	1.46	1.74	1.76	1.67	1.50	1.27	1.76	0.10
	D12S346	1.46	1.74	1.76	1.67	1.50	1.27	1.76	0.10
	D12S78	0.22	0.69	0.87	0.91	0.87	0.76	0.91	0.15
	D12S79	1.52	1.68	1.66	1.56	1.39	1.19	1.68	0.05
	D12S86	-1.92	1.13	1.27	1.24	1.13	0.97	1.27	1.10

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 not seen in one affected patient (III-2), but it was present in two unaffected family members (III-3 and III-8). The at risk chromosome 12 haplotype was present in three unaffected family members (II-7, 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 not present in five affected patients (II-8, III-2, III-9, III-10, and III-11), but it was present in five unaffected family members (II-7, III-1, III-3, III-4, and III-7) (data not shown). Haplotypes of the chromosome 5 region showed complete segregation with the butterfly shaped macular dystrophy phenotype. Finemapping of the region with an average 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.^{9, 21–27} 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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Authors' affiliations

A I den Hollander, F F J Kersten, J G A M Heister, C G F de Kovel, F P M Cremers, Department of Human Genetics, University Medical Center Nijmegen, Nijmegen, Netherlands

J J C van Lith-Verhoeven, A F Deutman, C B Hoyng, Department of Ophthalmology, University Medical Center Nijmegen

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Correspondence to: A I den Hollander, Department of Human Genetics, University Medical Center Nijmegen, PO Box 9101, 6500 HB Nijmegen, Netherlands; a.denhollander@antrg.umcn.nl

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