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A Gene for Late-Onset Fundus Flavimaculatus with Macular Dystrophy Maps to Chromosome 1p13

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Summary

Fundus flavimaculatus with macular dystrophy is an autosomal recessive disease responsible for a progressive loss of visual acuity in adulthood, with pigmentary changes of the macula, perimacular flecks, and atrophy of the retinal pigmentary epithelium. Since this condition shares several clinical features with Stargardt disease, which has been mapped to chromosome 1p21-p13, we tested the disease for linkage to chromosome 1p. We report here the mapping of the disease locus to chromosome 1p13-p21, in the genetic interval defined by loci D1S435 and D1S415, in four multiplex families (maximum lod score 4.79 at recombination fraction 0 for probe AFM217zb2 at locus D1S435). Thus, despite differences in the age at onset, clinical course, and severity, fundus flavimaculatus with macular dystrophy and Stargardt disease are probably allelic disorders. This result supports the view that allelic mutations produce a continuum of macular dystrophies, with onset in early childhood to late adulthood.

Introduction

Fundus flavimaculatus (FFM) with macular dystrophy is an autosomal recessive condition responsible for a gradual loss of visual acuity in adulthood. The disease starts at the end of the 2d decade or within the 3d decade and progresses slowly over many years. Finally, after >20 years of progression, visual acuity may be reduced to the level of counting fingers (final visual acuity 1/60–6/60). Color vision is initially normal, but an unclassifiable dyschromatopsia develops after several years of progression.

On the other hand, Stargardt disease is an autosomal recessive macular dystrophy of childhood, characterized by a bilateral loss of central vision over a period of several months (Stargardt 1909). It has an early onset (age 7–12 years), a rapidly progressive course, and a poor final outcome. Both conditions are associated with pigmentary changes of the central area of the retina (the macula), with perimacular yellowish spots of depigmentation and atrophy of the retinal pigmentary epithelium (RPE; Franceschetti and François 1965; Fishman 1976; Hadden and Gass 1976). We recently reported the mapping of Stargardt disease to chromosome 1p21-p13 in eight multiplex families (Kaplan et al. 1993) and suggested that this condition is genetically homogeneous in our series.

In fact, Stargardt macular dystrophy and FFM are now believed by most investigators to be different manifestations of the same autosomal recessive condition. Here we report on the mapping of the FFM gene to chromosome 1p13 and give genetic support to the view that Stargardt disease and FFM with macular dystrophy either result from allelic mutations or involve contiguous genes on chromosome 1p13.

Patients and Methods

Eleven affected individuals and 13 healthy relatives belonging to four unrelated families of French origin were recruited from genetic and ophthalmologic consultations (fig. 1). The time course of the disease was determined by interviewing at least one patient per family and, whenever possible, all affected siblings of the family. Minimal criteria for inclusion in the study were families with at least two affected children of either sex, born to healthy parents. For all pedigrees except family 4, both parents and all unaffected siblings were examined with the same degree of care and were excluded as manifesting carriers or as mildly affected individuals with dominant inherited disease (Stone et al. 1994; Zhang et al. 1994). The minimal criteria for diagnosis of FFM were (i) slowly progressive loss of visual acuity over a period of several years; (ii) onset by the end of the 2d decade onward; (iii) ophthalmoscopic evidence of alterations of the macula, with depigmentation and atrophy of the RPE, surrounded by yellowish flecks; (iv) fluorescein angiography showing a round or horizontal ovoid zone of hypofluorescence suggestive of atrophic pigment epithelium and surrounded by hyperfluorescent flecks (fig. 2); (v) visual field displaying a slightly diminished sensitivity

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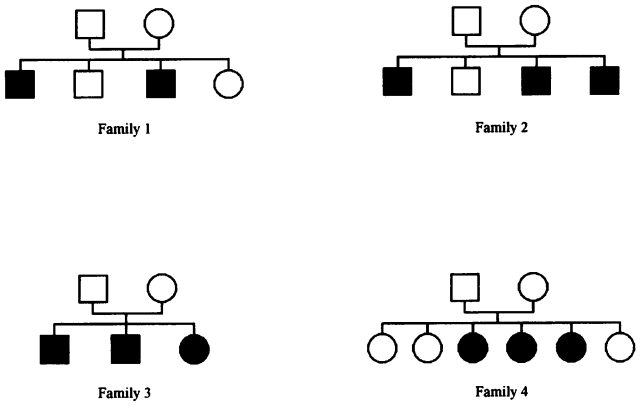

Figure 1 Late-onset FFM with macular dystrophy pedigrees

of central and midperipheral areas in the early stages and a central scotoma in the later stages; (vi) no (red-green) dyschromatopsia but mild errors of color vision (trita axis); and (vii) normal electroretinogram (ERG) in the early stages but, occasionally, photopic abnormal ERG in the advanced stage.

For DNA analyses, EDTA blood samples (20 ml) were collected, and DNA was prepared from lymphocyte pellets by SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension. For genotyping using PCR, hypervariable microsatellites were used (Weissenbach et al. 1992; Gyapay et al. 1994). Genomic DNA (200 ng) was amplified using 0.5 U of *Taq* polymerase (Boehringer Mannheim) in a buffer containing 1.5 mM $MgCl_2$, 20 μM each deoxynucleotide, and 20 μM primers, in a final volume of 20 μl. Amplification conditions were 95°C for 10 min, followed by 30 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension of 10 min at 72°C. Amplified DNA (1 μl) was mixed with 2 μl of formamide and 0.4 μl of loading buffer (xylene cyanol/bromophenol blue/glycerol), electrophoresed at 1,400 V for 4 h on a 6% denaturing polyacrylamide gel, and was transferred onto charged nylon membranes (Quiabran; Quiagen). Membranes were hybridized with a (^{32}P) dCTP $(CA)_{10}$ oligonucleotide labeled by terminal deoxynucleotide transferase (Boehringer Mannheim). Hybridization was carried out for 90 min at 42°C in 0.13 M sodium phosphate buffer pH 7.2, 0.25 M NaCl, 7% SDS, and 10% polyethylene glycol. Blots were washed for 10 min at room temperature in 2 × SSC and 0.1% SDS and were autoradiographed. Markers containing short tracts of (CA)n repeats were chosen from the Généthon linkage map (Gyapay et al. 1994), and all nucleotide sequences are available in the Genome Data Base. Linkage analysis was performed using the MLINK and LINKMAP options of the 5.1 version of the LINKAGE program (Lathrop et al. 1985).

Results

Pairwise linkage data using the polymorphic markers of the 1p13 region gave positive lod score (Z) values in all four FFM families (table 1). The maximum Z (Z_{max}) value was obtained for probe AFM217zb2 at the D1S435 locus (Z_{max} = 4.79 at recombination fraction [θ] 0). All families displayed positive lod score values (not shown; available on request), and the cumulative Z_{max} values were consistently >3.0 (table 1).

The location-score method (Ott 1985, pp. 112–115) was used to estimate the position of the disease gene. The order pter–D1S435–(.02)–D1S424–(.02)–D1S236–(.00)–D1S415–cen has been established elsewhere by analysis of CEPH reference families (recombination estimates are in parentheses [Weissenbach et al. 1992]). The maximum-likelihood estimate of the disease gene was over the locus D1S424 (Z = 5.20), but the odds against alternative orders were not significantly different, since no recombination events were observed in our families (fig. 3).

Considering that a dominant mutation of the RDS gene has been reported in several families with macular degeneration, we also tested FFM for possible linkage to the RDS locus on chromosome 6p (Kumar-Singh 1994). No linkage of the disease gene to the RDS locus was found in our series (Z_{max} = 0.014 at θ = .20; not shown).

Discussion

Stargardt disease and FFM with macular atrophy are autosomal recessive macular dystrophies that share several clinical features—namely, a decrease of visual acuity and pigmentary changes of the central area of the retina (the macula), with depigmentation and atrophy of the RPE and with perimacular yellowish spots termed "fundus flavimaculatus." For this reason, Stargardt disease and FFM

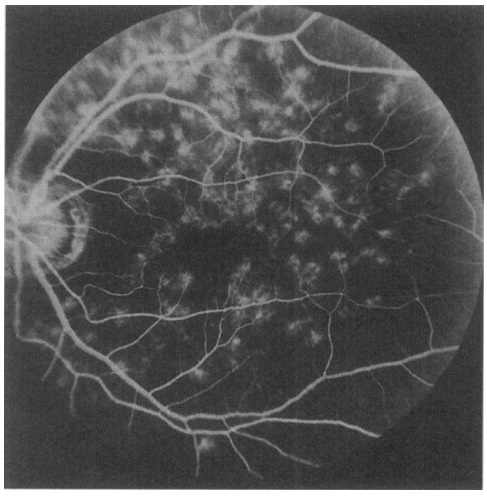

Figure 2 FFM with macular dystrophy phenotype: typical fluorescein angiograph showing FFM (see text).

Table 1

Pairwise Lod Scores between FFM with Macular Dystrophy and Four Microsatellites of Chromosome 1p

		Z AT θ =							
LOCUS	MARKER	0	.01	.05	.10	.20	.30	Z_{max}	θ
D1S435	AFM217zb2	4.79	4.68	4.28	3.72	2.57	1.40	4.79	.00
D1S424	AFM203vd4	4.64	4.53	4.10	3.55	2.42	1.31	4.64	.00
D1S236	AFM205ta11	3.07	3.00	2.74	2.38	1.61	.85	3.07	.00
D1S415	AFM183yg7	3.16	3.09	2.82	2.47	1.72	.95	3.16	.00

are often regarded as closely related conditions (Fishman 1976; Hadden and Gass 1976). Yet, the two diseases differ in the age at onset, clinical course, severity, and electrophysiological changes. Indeed, Stargardt disease has an onset in childhood; the macular changes are typical, and flavimaculatus flecks are present in most patients. The main symptom—i.e., loss of visual acuity—is concomitant with macular changes and may occasionally precede them (Hadden and Gass 1976). The deterioration in visual acuity is rapid, and the final visual outcome is poor. Color vision is altered early and shows a predominant deuteranopia. The ERG is normal in the early stages and can show a selective alteration of the photopic function in the later stages.

By contrast, FFM with macular dystrophy (Moloney et al. 1983), also called "Stargardt disease type II" or "Stargardt disease of late-onset" (Merin et al. 1978), has a much later onset. The decrease of vision is gradual and slowly progressive over many years, with a final visual acuity of 1/60–6/60. The age at onset has a significant effect on the severity of the disease: the earlier the onset, the poorer the visual outcome. The rod system appears to be affected slightly, a feature that is never observed in Stargardt disease (Rosehr 1954; Weleber 1994). Color vision is initially subnormal and displays an unclassifiable dyschromatopsia after several years of evolution. Finally, the ERG is normal in the early stages and shows photopic and, occasionally, scotopic alterations in the later stages.

In fact, Stargardt macular dystrophy and FFM are now believed to be various manifestations of the same autosomal recessive condition (Weleber 1994). If visual acuity loss begins in the first 2 decades, the term "Stargardt disease" is preferred. If the disease begins later in life and has a more progressive course, the term "FFM" is favored (Weleber 1994). Here we report on the mapping of FFM to chromosome 1p13, in the genetic interval where the gene for Stargardt disease has recently been mapped (D1S435-D1S415; Kaplan et al. 1993). These results give genetic support to the view that the two conditions either are allelic disorders or result from mutations of distinct genes on chromosome 1p13. Yet, the reported intrafamilial variability, with early- and late-onset cases present within the same pedigrees, suggests that allelic mutations at a single locus might account for the diversity of clinical profiles in macular dystrophies. Moreover, considering the diversity of the age at onset (17–60 years) in our adult patients with FFM, the present study addresses the intriguing questions of the relevance of this locus in age-related macular dystrophies and, more generally, a possible continuum of macular degeneration that is associated with different mutations at this locus and that has onset in early childhood to late adulthood.

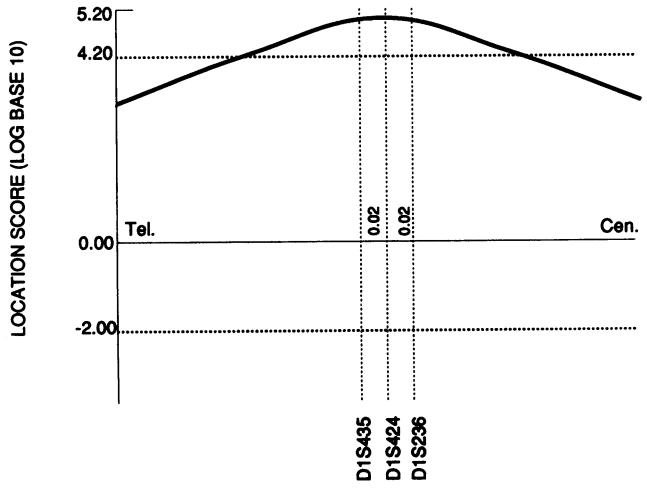

Figure 3 Support for location of the FFM with macular dystrophy gene, with respect to chromosome 1p markers. Likelihood estimates are given in log base 10. Distances between marker loci are shown (in cM) along the abscissa. The maximum score for the disease-causing gene is over the D1S424 locus.

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