RAPID COMMUNICATION

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Genetic fine mapping of the gene for recessive Stargardt disease

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Abstract Stargardt disease (STGD) is one of the most frequent causes of macular degeneration in childhood. Linkage analysis in families with recessive STGD has recently shown genetic homogeneity and a location of the underlying gene at 1p22-p21 in a 4-cM interval. Haplotype analysis in seven Dutch STGD families with 11 highly polymorphic markers spanning the critical region has enabled us to refine the location of the underlying gene to a 2-cM region flanked by the loci DIS406 and DIS236. We have identified one 45-year-old nonpenetrant individual who carries two disease alleles. In another family, an affected individual inherited the paternal but not the maternal disease chromosome, suggesting genetic heterogeneity or a different mechanism leading to the disease in this family.

Introduction

Stargardt disease (STGD; MIM 248200; McKusick 1994) is the most common hereditary macular dystrophy and accounts for 7% of all retinal dystrophies (Stargardt 1909). Typically, patients suffer from decreased central vision, usually in the first or second decade of life. The fundus shows bilateral atrophic changes of the underlying retinal pigment epithelium (RPE) and the presence of orange-yellow flecks distributed around the macula and/or midretinal periphery. The existence of flecks has subsequently been described as fundus flavimaculatus (FFM) by Franceschetti (1963) and it is now generally accepted that STGD and FFM are different manifestations of the same disease. In FFM, there is generally a later age of on-

set (end of 2nd/3rd decade) and a progression that can last over more than 20 years (Franceschetti 1963; Fishman 1976; Noble and Carr 1979). Although Stargardt disease was originally described as an autosomal recessive trait, several large families have been reported with an autosomal dominant mode of inheritance (Bither and Berns 1988; Puech et al. 1989; Lopez et al. 1990; Mansour 1992).

Fluorescein angiography studies enable the visualization of the atrophic foveal lesion and atrophic pigment flecks and also demonstrate “dark choroid” because of blockage of the visualization of the underlying choroidal fluorescence where the RPE is intact. The blockage appears to arise from the abnormal storage of a lipofuscin-like material throughout the RPE; this material is concentrated in patches yielding the flecks seen clinically (Klien and Krill 1967; Green 1985).

Linkage studies in eight families have assigned a locus for STGD on the short arm of chromosome 1 (Kaplan et al. 1993). The same region has also been implicated in four multiplex French families with FFM, supporting the hypothesis that both disorders are allelic (Gerber et al. 1995). Recent linkage analysis performed in 47 STGD families suggests that recessive STGD is genetically homogeneous and has enabled genetic fine mapping of the underlying gene to a 4-cM region of 1p22-p21 that is demarcated by the loci DIS435 and DIS236 (Anderson et al. 1995). Here, we report linkage and haplotype analysis performed in seven Dutch STGD families.

Patients and methods

Patients

Seven Dutch STGD families containing at least two affected individuals were studied (Fig. 1). No genealogical data suggested consanguinity in any of these families. Detailed family histories and pedigrees were obtained through personal interviews (by C.B.H.) with appropriate family members. The anamnestic and ophthalmological information for each individual was reviewed and scored as affected or normal by a single observer (C.B.H.). For the purpose of the study, the essential features of STGD were: (1) a retinal disorder in multiplex families compatible with autosomal recessive

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inheritance, (2) onset of symptoms before the age of 20, (3) bilateral central vision loss with 'beaten-metal' foveal changes and/or yellow pigment epithelium deposits ('flecks') in the posterior pole of the eye, (4) normal caliber of the retinal vessels and no pigmented bone-spicules in the retinal periphery, (5) normal scotopic electroretinogram, and (6) typical 'dark choroid' in fluorescein angiography. All human studies have been reviewed by the Netherlands Medical Ethics Commission. All individuals gave their informed consent prior to inclusion in the study.

Linkage and haplotype analysis

DNA was extracted from leukocytes using the Promega Wizard genomic DNA purification kit, according to the manufacturer's instructions. Microsatellite DNA markers of the 1p22-p21 region, which physically map in two non-overlapping yeast artificial chromosome (YAC) contigs (Anderson et al. 1995), were used to genotype all individuals. DNA amplifications were performed in 96-well trays in a Techne thermocycler, with primer pairs as published for the markers D1S167 (Utsu 1346; Bowcock et al. 1992) and D1S311 (C81), available from the Genome Data Base; for the markers D1S188 (Mfd246), D1S406 (UT2069), D1S1170 (UT851; The Utah Marker Development Group, 1995; GDB, Johns Hopkins University, Baltimore), and the Génethon markers D1S206 (AFM113xf6), D1S236 (AFM205ta11), D1S420 (AFM199xb6), D1S424 (AFM203vd4), D1S435 (AFM217zb2), and D1S497 (AFM331vb1). Amplification was performed over 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min. In addition, an initial denaturation step of 4 min and a final extension step of 6 min were carried out. Genotypes were scored by at least two analyzers, without prior knowledge of the affected status. Two-point linkage analyses were performed with the program LINKAGE (Lathrop et al. 1985; version 5.03) and the subroutine Mlink. Allele frequencies determined in Centre d'Étude du Polymorphisme Humain families (Weissenbach et al. 1992; GDB) were used for lod score calculations. A penetrance of 95% was assumed because of the clinical and genetic data of the male ST7-12 and the findings reported by Anderson et al. (1995). A disease-allele frequency of 0.001 was used.

Results

Forty-three individuals from seven STGD families including 20 affected individuals were sampled and genotyped for highly polymorphic DNA markers from chromosome 1p. Significant linkage was obtained for the markers D1S188, D1S236, D1S406, D1S420, and D1S424 (Table 1). Two-point linkage analysis demonstrated that the maximum two-point lod score (Z_{max}) for all families combined was 5.35 ($\Theta_{max} = 0.04$) for the marker D1S188 and the disease locus. We observed at least one recombinant for each marker.

To determine the most likely position of the STGD gene relative to the chromosome 1p markers, haplotypes were constructed in these seven families. Four disease chromosomes exhibited crossover events between the disease locus and flanking polymorphic loci. In the affected female ST2-4, the maternal haplotype shows a recombination between D1S435 and D1S188, the latter of which cosegregates with the STGD phenotype (Fig. 1a). Alternatively, the recombinant maternal haplotype is present in her brother ST2-3. This is less likely, since it would require an additional recombination between D1S435 and D1S188 in the unaffected male ST2-5. In family ST3, the

Table 1 Pairwise lod scores between STGD and markers on chromosome 1p

Locus	Lod scores (Z) at recombination fraction (Θ)						Z_{max}	Θ_{max}
	0.00	0.01	0.05	0.10	0.20	0.30		
D1S311	-8.87	-1.44	0.34	0.84	0.89	0.57	0.95	0.15
D1S167	-5.25	0.40	1.48	1.65	1.34	0.78	1.65	0.10
D1S435	-7.93	0.77	2.59	2.92	2.39	1.39	2.92	0.10
D1S188	1.85	5.07	5.30	4.83	3.41	1.85	5.35	0.04
D1S1170	-0.77	1.94	2.25	2.07	1.45	0.79	2.25	0.05
D1S424	-3.81	2.65	3.48	3.36	2.50	1.40	3.50	0.06
D1S406	-3.60	2.19	3.10	3.05	2.26	1.23	3.14	0.07
D1S236	0.91	3.39	3.60	3.27	2.29	1.23	3.63	0.04
D1S497	-13.68	-2.44	0.41	1.29	1.44	0.91	1.51	0.16
D1S420	3.81	3.84	3.72	3.35	2.38	1.30	3.84	0.01
D1S206	-12.25	-2.11	0.54	1.34	1.48	0.99	1.55	0.16

paternal haplotype demonstrated a cross-over event between D1S406 and D1S236, the former of which cosegregated with the disease phenotype (Fig. 1b). In this family, it was not possible to determine which of the paternal haplotypes in the two affected children most likely corresponded to the recombinant haplotype. Together, these two recombinants confirm the location of the STGD gene in the D1S435-D1S236 interval, as described by Anderson et al. (1995).

In ST1-15, the maternal chromosome demonstrated a recombination event between D1S406 and D1S497 (Fig. 1c). Surprisingly, the unaffected sister of ST1-15, ST1-17, apparently carries the same haplotypes. Unfortunately, the marker D1S236 was not informative to reveal a possible difference between ST1-15 and ST1-17 with respect to the position of the crossover(s) in the maternal haplotype. Based on the occurrence of three crossover events, alternative haplotypes can be constructed for ST1-8, ST1-14, ST1-15, ST1-16, and ST1-17. In this situation, the maternal chromosomes in ST1-15 and ST1-17 are not recombined. Instead, the maternal chromosomes in ST1-14 and ST1-16 show a recombination between D1S406 and D1S497. In addition, a recombination event between the latter two markers results in different (deduced) STGD haplotypes in ST1-6 and ST1-8. This solution seems less attractive, because the healthy individual ST1-17 would carry two disease chromosomes and thus show non-penetrance. Regardless of which of the two possibilities is correct, there is a crossover event in an affected individual between D1S406 and D1S497. Other individuals in family ST1 showed perfect concordance between the presence or absence of two disease alleles and their phenotype. Together, these results refine the location of the STGD locus to a 2-cM interval flanked by the markers D1S406 and D1S236.

In family ST6, two affected brothers (ST6-3 and ST6-4) show identical haplotypes for the STGD region (Fig. 1d). Their affected sister (ST6-5) only carries one disease chromosome, the paternal chromosome, which is recom-

Fig. 1 Segregation of simple tandem repeat alleles in families ST2 (a), ST3 (b), ST1 (c), and ST6 (d). Chromosomal segments presumed to carry an STGD defect are boxed by solid lines (maternal disease allele) or by interrupted lines (paternal disease allele). In cases where markers were uninformative, they are boxed by stipples in the offspring. The parental alleles in family ST1 and the haplotypes in individuals ST2-2 and ST6-1 were deduced. Haplotypes were constructed assuming a minimal number of recombinants

combined between the markers D1S236 and D1S497. The maternal chromosome does not carry a STGD defect. ST6-5 is a 45-year-old patient who has suffered from a central scotoma and visual loss since the age of 18. Visual acuity at the age of 19 was 10/20 in both eyes and this gradually declined to the level of fingercounting at the age of 30.

Funduscopy showed 'fish-tail' yellowish flecks at the posterior pole; the photopic and scotopic electroretinogram was normal. Color vision tests revealed a red-green color defect and a diminished red-sensitivity.

Haplotype analysis identified one individual (ST7-12) showing non-penetrance, i.e., he inherited two STGD alleles but did not show the phenotype at the age of 42 years. His visual acuity is 20/20 in both eyes and no abnormalities have been seen in funduscopy.

Relevant recombination events found in our families and in two other studies (Kaplan et al. 1993; Anderson et al. 1995) are schematically depicted in Fig. 2. Haplotypes of all recombinant individuals are completely compatible with the marker order derived from the physical map as recently published by Anderson et al. (1995).

Fig. 2 Schematic representation of informative recombinants in the STGD critical region as deduced from Kaplan et al. (1993; *single asterisk*), Anderson et al. (1995; *two asterisks*), and our own studies. *Top* The physical order of polymorphic markers as published by Anderson et al. (1995). The genetic distances indicated below the loci were derived from Gyapay et al. (1994) and Matisse et al. (1994). *Filled circles* connected with a *line* represent segments of chromosomes that do not carry the STGD defect(s); *open circles* indicate alleles of markers that cosegregate with the STGD phenotype (*N* = not informative)

Discussion

Linkage analysis in seven families with recessive STGD has confirmed the location of a major recessive STGD gene at chromosome 1p22-p21. Haplotype analysis has enabled us to reduce the critical region for STGD from a 4-cM interval, as described recently by Anderson et al. (1995), to a 2-cM region, flanked by D1S406 and D1S236. Family 3 of Kaplan et al. (1993) was not included in Fig. 2. Based on the observed recombinations in this family, the proposed marker order D1S207-D1S424-D1S435 is incompatible with the physical map of Anderson et al. (1995) and with the order of these loci as predicted from crossover events in the study of Anderson et al. (1995) and our study (Fig. 2).

In one family (ST6), we encountered an affected female, who, based on the haplotype analysis, only carried one disease allele. A similar finding was made by Anderson et al. (1995), who, as in our case, could not exclude the relevant family (AR88) since affected siblings met the clinical criteria for STGD. A few explanations may account for this observation. First, recessive STGD in some families might result from a defect located elsewhere on an autosome. Genetic heterogeneity has been shown for autosomal dominant STGD, loci of which have been mapped to chromosomes 6q11-q15 (Stone et al. 1994) and 13q34 (Zhang et al. 1994). Second, the STGD phenotype in family ST6 might be dominantly inherited through one of the parents who carries a novel germline mutation. If the dominant mutation is present in the STGD gene at 1p22-p21, it would be predicted to have arisen on the paternal D1S406-D1S236 segment of chromosome 1, which is present in all three affected cases, but not in the unaffected individual. For diseases of the retina, the occurrence of dominant and recessive mutations in the same gene is not unprecedented, as different types of mutations in the human rhodopsin gene have been shown to result in

autosomal dominant and recessive forms of retinitis pigmentosa (Dryja et al. 1990; Rosenfeld et al. 1992; Kumaramanickavel et al. 1994). Others have found mutations in some patients with an FFM-like phenotype or with other macular dystrophies in the retinal degeneration slow (RDS)/peripherin gene (Weleber et al. 1993; Wells et al. 1993). To study the putative involvement of the RDS gene and the two dominant STGD loci mentioned above, mutation and haplotype analysis respectively, will be performed in the future.

In one individual, ST7-12, we found evidence of non-penetrance. Thus, in our set of families, penetrance of the STGD phenotype was observed in 20 of 21 individuals (95%). This is in good agreement with the findings of Anderson et al. (1995), who have reported three cases showing nonpenetrance among a total of 109 individuals (penetrance: 97%).

Future research will concentrate on the cloning of novel highly polymorphic markers that lie in the D1S406-D1S236 region and that can be used to analyze the recombinant haplotypes of ST1-15, ST1-17, and ST3-3/4, and the three proximal crossovers published by Kaplan et al. (1993) and Anderson et al. (1995). The YAC cloning study by Anderson et al. (1995) indicates that the refined critical region for STGD, which is flanked by D1S406 and D1S236, corresponds to approximately 3 Mb of DNA. Since, on average, such a region will contain 100 genes, and no 'obvious' candidate genes have been mapped in this region, further genetic or physical fine mapping of the STGD gene will be required for the successful application of the positional cloning approach.

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