

A YAC Contig Encompassing the Recessive Stargardt Disease Gene (*STGD*) on Chromosome 1p

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Summary

Stargardt disease (STGD) and fundus flavimaculatus are infrequent autosomal recessive conditions characterized by a juvenile macular dystrophy and variable degrees of peripheral retinal changes. Linkage analysis performed in 47 STGD/fundus flavimaculatus families demonstrated significant linkage to 13 polymorphic DNA markers on chromosome 1p. The maximum combined two-point lod score was 32.7 (maximum recombination fraction [$\theta_{\max}$] = .006) with the polymorphic marker *D1S188*. Our data demonstrate that STGD and fundus flavimaculatus are the same disorder clinically and genetically and provide further evidence for genetic homogeneity of this phenotype. Analysis of recombination events on disease chromosomes placed the *STGD* gene within a 4-cM interval between markers *D1S435* and *D1S236*. A physical map was constructed of a YAC contig flanking *STGD*, from markers *D1S500* to *D1S495*, and includes the critical interval delineated by historical recombinants. This contig spans ~31 cM, with one gap (3–5 cM) that is outside the 4-cM critical region. Localization of *STGD* to a single YAC contig will facilitate its positional cloning.

Introduction

Stargardt disease (STGD) (MIM 248200; McKusick 1994) is an infrequent autosomal recessive retinal dystrophy that combines juvenile onset, central visual impairment, characteristic progressive “beaten metal” atrophy of the retinal pigment epithelium of the fovea, and various extents of orange-yellow flecks distributed around the macula and/or the midretinal periphery. Although apparently similar disorders of the macula had been identified earlier (Lang 1885; Batten 1897; Dujardin 1904; Jackson 1905; Oatman 1907), Stargardt in

1909 meticulously described with illustrations seven children in two families with onset of visual symptoms at ages 8–16 years, bilateral progressively atrophic macular dystrophy, and perimacular and peripheral “dirty grey-yellow spots” (Stargardt 1909). In subsequent publications, he added five more cases (Stargardt 1913, 1916, 1917, 1925). Reevaluation of one (family H.) of Stargardt’s original two families confirmed the progression of pigmentary atrophy and the appearance of flecks near the macula, nasal to the disk, and in the peripheral pigment epithelium (Rosehr 1954).

In 1962, Franceschetti introduced “fundus flavimaculatus” as a similar retinal disorder, typified by ill-defined yellow-white flecks in the deep layers of the posterior pole (Franceschetti 1963). With François in 1965, he reported 30 cases with the various manifestations of this condition (Franceschetti and François 1965) and later suggested that its discrimination from STGD may be difficult or impossible (Franceschetti 1965). Despite occasional nosologic separation (e.g., MIM 230100; McKusick 1994), most authors concur that fundus flavimaculatus is the same disorder defined initially by Stargardt (Irvine and Wergeland 1972; François et al. 1975; Fishman 1976; Hadden and Gass 1976; Anmarkrud 1979; Noble and Carr 1979).

Depending on biases of ascertainment, on geographic and ethnic origin, and on the era of identification, the disorder occurs equally in males and females, has been reported in twins, and is transmitted as an autosomal recessive (or isolated) trait in the offspring of unaffected parents (Stargardt 1913; Neame 1944; Franceschetti and François 1965; François et al. 1975; Hadden and Gass 1976). Occasional reports of a similar ophthalmoscopic picture transmitted as a dominant trait appear to represent dominant cone dystrophies or different macular disorders (Bonnet and Hugonnier 1943; Sorsby and Davey 1955; Turut et al. 1975; Krill 1977; Cibis et al. 1980; Fiori et al. 1980; Bither and Berns 1988; Lopez et al. 1990; Mansour 1992).

STGD begins typically at ages 6–15 years, although later onset of central visual impairment has been reported (Stargardt 1909; Franceschetti and François 1965; François et al. 1975; Fishman 1976; Hadden and Gass 1976; Anmarkrud 1979; Noble and Carr 1979).

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Early complaints of photodysphoria and altered clarity of vision yield to rapidly progressive losses of central acuity, often <20/100 at the initial ophthalmic examination (Moloney et al. 1983; Fishman et al. 1987). Diagnostic studies are typically normal or minimally abnormal. Assessment of visual fields shows central scotomas and normal to minimally constricted peripheries (Franceschetti and François 1965; Moloney et al. 1983). Responses to electroretinography, electrooculography, and dark adaptation are normal or nondiagnostic early in the disease and are mildly to moderately abnormal but unhelpful in advanced disease (Fishman 1976; Noble and Carr 1979). Color vision tested by classic pigment plates may be normal early but proceeds from nonspecific protan errors to severe dyschromatopsia (Birch et al. 1979).

Fluorescein angiography plays a crucial role in the secure diagnosis of STGD. Angiography enhances discrimination of the atrophic foveal lesion and atrophic pigment flecks and also demonstrates an unexpected "dark choroid" due to blockage of visualization of underlying choroidal fluorescence everywhere that the pigment epithelium is intact, typically including a small corona one-half-disk-diameter wide around the optic disk (Ernest and Krill 1966; Amalric et al. 1969; Bonnini et al. 1976; Anmarkrud 1979). This blockage appears to arise from the abnormal storage of a lipofuscin-like material throughout the retinal pigment epithelium, greater in localized patches yielding the flecks seen clinically (Klein and Krill 1967; Eagle et al. 1980; Green 1985; McDonnell et al. 1986; Steinmetz et al. 1991; Birnbach et al. 1994). Few other ocular or systemic disorders create a similar angiographic appearance (Cohen et al. 1993b). The dark or silent choroid is considered typical of STGD without or with pigment epithelial flecks (Gass 1987). No therapy alters the progression of this condition (Glenn et al. 1994).

Genetic linkage has been reported for autosomal dominant phenotypes similar to STGD, on chromosomes 6q11-q15 (Stone et al. 1994) and 13q34 (Zhang et al. 1994). Mutations causing an STGD phenocopy have also been reported for the peripherin/RDS gene on chromosome 6p (Weleber et al. 1993) and for a Leber hereditary optic neuropathy-associated mtDNA mutation (Heher and Johns 1993). However, in those affected individuals no dark choroid was observed (Weleber et al. 1993; Stone et al. 1994), the dark choroid was not reported (Heher and Johns 1993), or fluorescein angiography was not performed (Zhang et al. 1994).

It is not possible to determine on clinical grounds whether the manifestations of classic recessive STGD and fundus flavimaculatus are caused by variable expression of a single gene or by different genes acting independently or in some sequential manner. Because the nature of the biochemical defect(s) involved in the pathogenesis of the accumulated lipofuscin-like material

is unknown, gene-mapping studies provide one approach to define the mutation(s) responsible for the pleiotropic phenotype. Recently, genetic linkage in eight families assigned a locus, *STGD*, for classic recessive STGD, to the short arm of chromosome 1, with the best estimate at *D1S435* (Kaplan et al. 1993). Of these families, seven were French and one was Moroccan. Similarly, fundus flavimaculatus of later onset (2d and 3d decade) has been mapped to the same region (*D1S435-D1S415*) in four multiplex French families (Gerber et al. 1995). In this report we confirm the genetic mapping of this recessive *STGD* locus in other populations, provide further evidence for genetic homogeneity of STGD/fundus flavimaculatus, and localize the *STGD* gene to a single YAC contig.

Patients, Material, and Methods

Patients

Forty-seven families were informative for the design and objectives of this study (fig. 1). Of these, 45 were North American of northern and central European ancestry. Two consanguineous families (KKESH42 and KKESH71), in which the parents were first cousins, originated in Saudi Arabia but from different tribes. Families were ascertained (by R.A.L.) through the Medical Retina and Ocular Genetics Service of the Cullen Eye Institute, Baylor College of Medicine, Houston, and by regional, national, and international referral from ophthalmologists, geneticists, and support groups. Within each kindred, a detailed family history and pedigree were obtained through personal interviews (by R.A.L.) with appropriate family members. For individuals outside the Houston megalopolis, available ophthalmologic, photographic, fluorescein angiographic, electrophysiological, and psychophysiological records were recovered. The anamnestic and ophthalmological information for each individual was reviewed and scored as affected or normal by a single observer (R.A.L.), who obtained all blood samples and who had no prior knowledge of linkage data in any family. Each subject, or the responsible adult on behalf of minors or wards, signed a consent-for-participation form in these investigations that was approved by the Baylor Affiliates Review Board for Human Subject Research.

For the purposes of this study, the essential and defining features of STGD were (1) a retinal disorder in multiplex families with both parents living; (2) all pedigrees with at least two siblings affected with onset usually in the latter half of the 1st decade or in the 2d decade of life and compatible with autosomal recessive inheritance (family KKESH42 had a single affected child); (3) bilateral central visual loss with both "beaten-metal" foveal dystrophy and temporal pallor of the optic disk, documented by retinal color photography, without or with yellow pigment epithelial flecks in the macula and/or

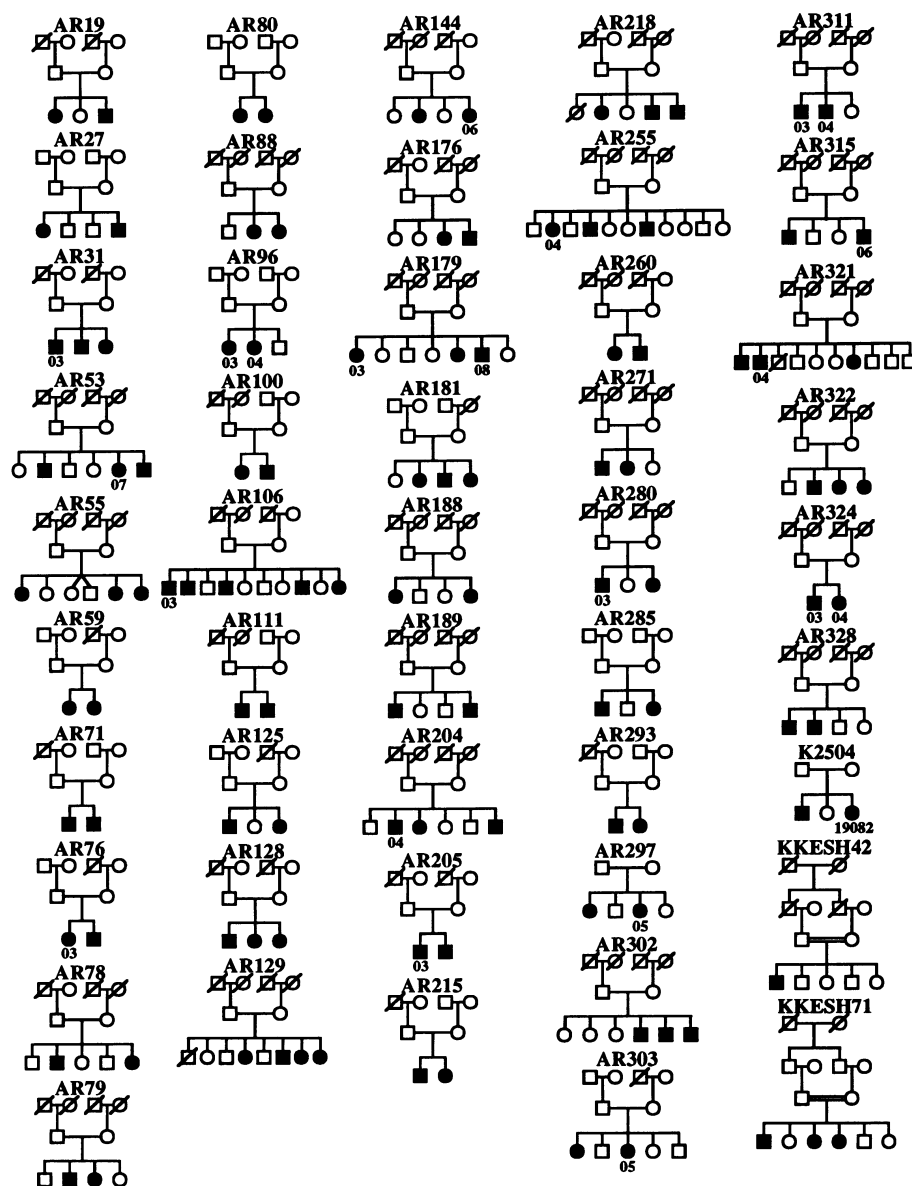


Figure 1 Pedigrees of 47 STGD families that show linkage to markers on chromosome 1p. Families labeled “AR” and “K” were ascertained in North America. Those labeled “KKESH” were enrolled from Saudi Arabia. Affected individuals are denoted by blackened symbols, and deceased family members are denoted by diagonal slashes. Affected individuals with disease chromosomes exhibiting informative crossover events are denoted by identification numbers below their respective pedigree symbols.

retinal periphery; and (4) the characteristic fluorescein angiographic feature of the dark choroid.

While not essential for diagnosis, clinical ophthalmological examination for an STGD diagnosis often reveals a diminution of the foveal light reflex and the umbo, an elliptical zone of fine granularity of the subfoveal retinal pigment epithelium evolving to a “beaten metal” sheen

(dependent on the angle of the incident illumination), and mild temporal pallor of the optic disk. Among European Americans, the fundus has a distinctive orange-vermilion hue, which may be quite difficult to discriminate from normal in heavily pigmented races. Depending on the age of the affected individual, soft yellow flecks appear in the retinal pigment epithelium, occasionally

wreathing the fovea but typically beginning in the infero-nasal quadrant and belting the midperiphery of the retina. Initially small, round, to oblong, these become irregular, linear, stellate, to pisciform; they tend to orient with their long axis circumferential with the macula and may become contiguous to confluent, forming irregular reticular patterns in the periphery. As they atrophy, either marginal or central hyperpigmentation may yield to irregular zones of atrophic scars with dense pigment-epithelial metaplasia (Stargardt 1909; Franceschetti and François 1965; Fishman 1976; Hadden and Gass 1976; Noble and Carr 1979).

Among 131 families ascertained with STGD/fundus flavimaculatus, 51 families with only a single affected individual were excluded from consideration. Thirty-three other families were excluded from analysis if affected individuals had (1) evidence of autosomal dominant inheritance; (2) any history of night blindness, loss of peripheral vision, or "retinitis pigmentosa"; (3) cataracta complicata or cells in the vitreous; (4) substantially abnormal electroretinographic or electrooculographic responses; (5) no fluorescein angiography performed or no dark choroid documented; (6) neurological disease (including loss of cognition or seizures); (7) drug exposures (especially to antimalarial and agents known to cause crystalline retinopathies); or (8) any "atypical" maculopathies in which a unique diagnosis of STGD could not be established.

Linkage Analysis

DNA was extracted from leukocytes by standard procedures (Lewis et al. 1990). Microsatellite DNA markers of known chromosomal location were used to genotype all available individuals. DNA amplifications were performed in 96-well trays in a Techne[®] thermocycler for 30 cycles, with primer pairs and reaction conditions as published for marker *D1S167* (Utsu1346) (Bowcock et al. 1992) and the Génethon markers *D1S206* (AFM113xf6), *D1S207* (AFM116xb2), *D1S236* (AFM205ta11), *D1S424* (AFM203vd4), *D1S435* (AFM217zb2), and b337xe1 (AFMb337xe1) (Gyapay et al. 1994; J. Weissenbach, personal communication). All primer sequences and reaction conditions are available from the Genome Data Base, for marker *D1S188* (Mfd246) and the Utah markers *D1S375* (UT494), *D1S401* (UT1344), *D1S406* (UT2069), *D1S1163* (UT5782), *D1S1170* (UT851), *D1S1172* (UT5019), *D1S1174* (UT5210), and *D1S1541* (UT967) (Utah Marker Development Group 1995) (GDB, John Hopkins University, Baltimore; URL: gopher://gopher.gdb.org/11/Database-local/GDB). Genotyping was performed as published (Leppert et al. 1994), and genotypes were scored by at least two observers, in a fashion masked with respect to affected status. Two-point linkage analyses were performed with the MLINK subroutine of the program LINKAGE (Lathrop et al. 1985)

with an autosomal recessive model of disease inheritance, a penetrance of .95, and a disease-allele frequency of .001; siblings were scored as unaffected if they had reached age 10 years with no feature or sign of disease.

Construction of YAC Contig

YACs were positive for the 20 Génethon/CHLC markers mapped to chromosome 1 (Cohen et al. 1993a) (available from the QUICKMAP database, version 1.1, with the Infocloner program [URL: <http://www.cephb.fr/bio/infocloner.html>]). These include *D1S206* (AFM113-xf6), *D1S207* (AFM116xb2), *D1S208* (AFM120xa5), *D1S223* (AFM168yb2), *D1S226* (AFM184xe11), *D1S236* (AFM205ta11), *D1S415* (AFM183yg7), *D1S420* (AFM199xb6), *D1S424* (AFM203vd4), *D1S430* (AFM210vc9), *D1S435* (AFM217zb2), *D1S454* (AFM080xd3), *D1S465* (AFM270zf5), *D1S486* (AFM297zg1), *D1S488* (AFM299ze9), *D1S495* (AFM323ya5), *D1S497* (AFM331vb1), *D1S500* (AFM343vf9), *D1S540* (CHLC.GATA4H05.129), and *D1S551* (CHLC.GATA6A05.942).

A sequence-tagged site (STS)-based screening approach (Green and Olson 1990; Kwiatkowski et al. 1990) identified additional YAC clones from the CEPH "mega-YAC" genomic library (Bellanné-Chantelot et al. 1992), with primers to 20 markers: *D1S167* (Utsu1346), *D1S188* (Mfd246), *D1S311* (C81), *D1S375* (UT494), *D1S401* (UT1344), *D1S406* (UT2069), *D1S551* (CHLC.GATA6A05.942), *D1S1163* (UT5782), *D1S1170* (UT851), *D1S1172* (UT5019), *D1S1174* (UT5210), *D1S1541* (UT967), *D1S1556* (MH161), *D1S1692* (WI-1235), *D1S2154* (WI-3099), *D1S2172* (WI-5166), *F3* (human tissue factor), AFMb337xe1 (b337xe1), WI-2994, and WI-5213. An STS landmark-mapping approach based on PCR (Chumakov et al. 1992a, 1992b) overlapped YAC clones to assemble the YAC contig. A total of 73 STSs were used in the landmark map of these YAC clones. In addition to those listed, these include *D1S1547* (AFM283wc1), *D1S1587* (CHLC.ATA1D01.31740), *D1S1607* (CHLC.GATA48C11.31731), *D1S1658* (CHLC.GATA45B07), *D1S1671* (CHLC.GATA67E04), *D1S1672* (CHLC.GATA68F04), *D1S1691* (WI-1224), *D1S1698* (WI-1974), *D1S1712* (WI-2540), *D1S1713* (WI-2628), *D1S1714* (WI-2658), *D1S2102* (WI-5391), *D1S2166* (WI-3294), *D1S2176* (WI-5518), *D1S2185* (EST11511), AFM354td9, AFMa109wh5, AFMa125xc9, GATA-P6158 (CHLC.GATA4H05.P6158), GATA-P17293 (CHLC.GATA32F02.P17293), UTR-9717, UTR-9740, WI-1423, WI-2443, WI-2864, WI-3235, WI-4684, WI-6896 (HSCOSH092), WI-7126 (UTR-01663), WI-7282 (UTR-03968), WI-7559 (UTR-00346), WI-7967 (UTR-05488), WI-8243 (EST110331), WI-9122 (UTR-01919), WI-9124 (UTR-01937), WI-9681 (HSC16B052), and WI-9944. Primer sequences and reaction conditions are available from GDB or the Whitehead Institute/MIT

Table 1
Z Values for STGD and 13 Chromosome 1p Markers in 47 STGD Families

Locus	Z AT $\theta =$							$Z_{\max}$	$\theta_{\max}$
	.001	.01	.05	.10	.20	.30	.40		
D1S207	-21.60	-4.12	6.61	9.10	7.94	4.58	1.37	9.26	.121
D1S375	-8.73	1.87	8.06	9.10	7.33	4.16	1.25	9.10	.099
D1S167	11.86	18.16	20.29	18.70	13.12	7.03	2.04	20.33	.043
D1S435	23.37	23.81	22.09	19.14	12.77	6.67	1.91	23.83	.008
b337xe1	23.30	23.66	21.58	18.41	11.94	6.07	1.70	23.72	.006
D1S188	32.36	32.63	30.00	25.89	17.14	8.84	2.49	32.73	.006
D1S1170	20.00	19.56	17.52	14.88	9.62	4.89	1.38	20.04	.000
D1S424	25.78	26.16	24.07	20.76	13.76	7.14	2.03	26.21	.006
D1S406	30.93	31.23	28.70	24.74	16.33	8.40	2.36	31.31	.006
D1S236	24.95	26.33	24.92	21.79	14.68	7.70	2.21	26.35	.013
D1S1163	17.70	20.14	19.82	17.49	11.78	6.16	1.76	20.47	.022
D1S206	-7.26	4.09	10.23	10.84	8.25	4.50	1.30	10.91	.086
D1S1174	-7.58	3.76	9.92	10.55	8.01	4.33	1.24	10.62	.086

Center for Genome Research (WICGR-URL: <http://www-genome.wi.mit.edu/>). Novel primers for *D1S1170* (UT851) were selected from sequence data available in the GenBank database for STS screening: forward (5'-GCCTTGGAA-GGAAAACTTCTTTC-3') and reverse (5'-GACGAG-GCCTTGCTATCCTGCCTA-3'). Five contigs assembling 59 STSs are available as part of the WICGR, Human Genetic Mapping Project, Data Release 7 (May 1995). Sequences of the primers for the genes *F3*, *GNAT2*, and *RPE65* were selected from sequence data available from GenBank (URL: http://ncbi.nlm.nih.gov/genbank/query_form.html). These include *F3* (A, 5'-ACTAAGTCAGGA-GATTGGAAGCA-3'; and B, 5'-CTCCAGGTAAGG-TGTGAAGTCTGGG-3'), *GNAT2* (A, 5'-ATGATGATG-CGGGAGGAATTAC-3'; and B, 5'-ACTCCATCTCCA-AAGAATGG-3'), and *RPE65* (A, 5'-GAGCAGACAAA-AGCCTGCT-3'; and B, 5'-GCTCAACTCAGTGCT-TTCTG-3').

Individual YAC clones were grown in AHC media (Brownstein et al. 1989), and DNA was isolated (Green and Olson 1990). Amplified PCR reaction products were loaded in a 2% agarose gel and electrophoresed in 90 mM Tris-borate/2.0 mM EDTA at 50–70 V for 3–10 h. Gels were stained with ethidium bromide and were photographed with transillumination in UV light.

Results

Linkage Analysis

Three hundred seventeen individuals from 47 STGD families (fig. 1) including 106 affected individuals were sampled and genotyped with 13 highly polymorphic DNA markers on chromosome 1p. Significant linkage was obtained in a region that flanks *D1S435* and spans an approximate genetic distance of 22 cM from *D1S207* to *D1S1174* (table 1). Two-point linkage analysis dem-

onstrated that the maximum combined two-point lod score ($Z_{\max}$) for all families was 32.7 ($\theta_{\max} = .006$) for the marker *D1S188* and the disease locus. The $Z_{\max}$ ranged from 32.7 ($\theta_{\max} = .006$) to 9.1 ($\theta_{\max} = .099$), for all 13 markers examined. These data provide evidence for genetic homogeneity of this disorder.

Three additional markers in the general region also demonstrated significant evidence for linkage in a subset of these families. The Z values are for *D1S401* in 29 families ($Z = 7.1$; $\theta = .05$); *D1S1172* in 33 families ($Z = 7.7$; $\theta = .05$); and *D1S1541* in 8 families ($Z = 4.1$; $\theta = .05$).

To determine the most likely position of STGD relative to the 13 linked markers ordered on a YAC contig, recombinants segregating with STGD in these 47 families were analyzed for the region between markers *D1S207* and *D1S1174*. Twenty-two disease chromosomes exhibited informative crossover events between the disease locus and flanking polymorphic loci. These recombination events are summarized as table 2 and substantively refine the STGD locus to a 4-cM interval flanked by the markers *D1S435* and *D1S236*. Importantly, haplotypes of the recombinant individuals were completely compatible with the marker order derived from the physical map shown as figure 2. The maternal haplotype for family AR324 demonstrated a recombination event between *D1S375* and *D1S167*; however, it was not possible to designate unambiguously which child received the recombinant haplotype. Haplotype analysis in all 45 STGD families with unrelated parents showed no significant evidence for linkage disequilibrium for five markers in the 4-cM STGD region between *D1S435* and *D1S236* (data not shown). Haplotype analysis identified four cases of nonpenetrant offspring each of whom carries two STGD alleles (data not shown). In every such instance,

Table 2**Summary of 22 Informative Recombinants Localizing the STGD Locus on Chromosome 1p**

Locus	AR31-03 (paternal)	AR31-03 (maternal)	AR53-07 (maternal)	AR76-03 (paternal)	AR96-03 (maternal)	AR96-04 (paternal)	AR106-03 (maternal)	AR144-06 (paternal)	AR179-03 (maternal)	AR179-08 (maternal)	AR204-04 (paternal)
<i>D1S207</i>	...	...	...	R	R	R	N	...	...	R	R
<i>D1S375</i>	...	...	...	R	...	R	N	...	...	R	R
<i>D1S167</i>	...	...	...	R	...	R	R	...	...	...	R
<i>D1S435</i>	...	...	...	...	...	...	R	...	...	...	N
<i>b337xe1</i>	...	...	...	...	...	...	N	...	...	...	N
<i>D1S188</i>	...	...	...	...	...	...	...	...	...	...	...
<i>D1S1170</i>	...	...	...	...	...	...	...	...	...	...	...
<i>D1S424</i>	...	...	...	...	...	...	...	...	...	...	...
<i>D1S406</i>	...	...	...	...	...	...	...	...	...	...	...
<i>D1S236</i>	...	...	...	...	...	...	...	R	...	...	...
<i>D1S1163</i>	R	N	...	...	...	...	...	R	R	...	...
<i>D1S206</i>	R	R	R	...	...	...	...	R	R	...	...
<i>D1S1174</i>	R	R	R	...	...	...	...	R	R	...	...

NOTE.—“paternal” and “maternal” denote parental origin of chromosome. R = recombinant; N = not informative; and an ellipsis (...) denotes a nonrecombinant.

affected siblings at the onset of symptomatic disease were older than their unaffected sibling carrying two *STGD* alleles. During the preparation of the manuscript of this article, one of these individuals subsequently demonstrated the clinical diagnosis of STGD. In family AR88 (fig. 1), one of the affected siblings shows the inheritance of only one disease chromosome (paternal) for all markers in the 4-cM critical region, suggesting that STGD may be genetically heterogeneous or that other mechanisms may be operative in this family. The Z value for family AR88 with the marker *D1S188* is negative ($Z = -1.97$; $\theta = .001$). We cannot exclude this family for phenotypic reasons, since both affected siblings met our clinical criteria for inclusion in this study. Furthermore, this sibling inherits correctly for all 13 highly polymorphic markers.

The four affected offspring of the two Saudi consanguineous families (KKESH42 and KKESH71) demonstrated homozygosity for alleles at 10 loci in a contiguous region (*D1S435*–*D1S1174*) that includes the 4-cM *STGD* interval (data not shown). Heterozygosity for alleles at loci telomeric to this region was observed in these four affected offspring. As expected, the affected offspring are likely homozygous by descent for both the disease gene and the flanking chromosomal interval, since STGD is infrequent and the affecteds are products of consanguineous marriages. Incidentally, family AR293 showed evidence of homozygosity for this same *D1S435*–*D1S1174* interval; however, despite intensive inquiry, historical evidence for consanguinity in this family does not exist.

Physical Map

To characterize physically the *STGD* gene region, a YAC contig spanning ~31 cM from *D1S500* to *D1S495* was constructed from 256 mega-YAC clones. For the 20 Génethon/CHLC DNA markers mapped to this chromosome 1p region, 111 specific YAC addresses (“YAC

hits”) identified 86 YAC clones (Cohen et al. 1993a). Only 16 of these YACs are level 1 resolution, i.e., connect two or more STSs. No level 1 YACs were present in the 4-cM *STGD* region. We identified 103 YAC clones (72 novel) with 124 specific YAC addresses for 17 markers (*D1S167*, *D1S188*, *D1S311*, *D1S375*, *D1S406*, *D1S551*, *D1S1163*, *D1S1170*, *D1S1174*, *D1S1556*, *D1S1692*, *D1S2154*, *D1S2172*, AFMb337xe1, *F3*, WI-2994, and WI-5213), by an STS screening approach (Green and Olson 1990; Kwiatkowski et al. 1990). Of these YACs, 31 previously had been identified as positive for Génethon/CHLC markers within this chromosomal 1 region: thus, *D1S311*, *D1S375*, and *D1S551* localize with *D1S207*, *D1S208*, *D1S226*, *D1S454*, and *D1S488*; AFMb337xe1 with *D1S435*; *D1S406* and WI-5213 with *D1S424*; *D1S1556*, *D1S2154*, *D1S2172*, and *F3* with *D1S236*; *D1S1163* with *D1S415*; WI-2994 with *D1S415*, *D1S420*, *D1S497*, and *D1S540*; and *D1S1174* and *D1S1692* with *D1S206*, *D1S223*, and *D1S486*. Five WICGR contigs within this region identified 37 additional STSs and 98 YACs. These contigs span a genetic distance of ~15 cM; however, less than one 1-cM coverage was assembled within the 4-cM *STGD* region.

We ordered these 256 YAC clones with 73 STSs and assembled the extended YAC contig (fig. 2A–C) by an STS landmark-mapping approach (Chumakov et al. 1992a, 1992b). As high as 19-fold level 1 YAC coverage was obtained for flanking markers. Approximately 40% of the YACs from this library are chimeric (Bellanné-Chantelot et al. 1992); the degree of chimerism for these YAC clones has not been assayed. One gap (3–5 cM) currently remains in this contig, between *D1S167* and WI-6896; however, no gaps are present within the 4-cM *STGD* region. Experiments are in progress to cover this remaining gap and to identify additional YACs within this open region.

We identified 14 additional YAC clones (694e6,

AR205-03 (maternal)	AR255-04 (maternal)	AR280-03 (maternal)	AR297-05 (maternal)	AR303-05 (maternal)	AR311-03 (maternal)	AR311-04 (paternal)	AR315-06 (paternal)	AR321-04 (paternal)	AR324-(03/04) (maternal)	K2504-19082 (maternal)
...	R	R	R	...	...	R	...	R	R	R
...	R	R	R	...	...	R	...	...	R	R
...	...	...	R	...	...	...	...	...	...	R
...	...	...	...	...	...	...	...	...	...	N
...	...	...	...	...	...	...	...	...	...	N
...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...
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...	...	...	...	...	...	...	...	...	...	...
R	...	...	...	...	...	...	...	...	...	...
N	...	...	...	...	...	...	...	...	...	...
N	...	...	...	N	R	...	R	...	...	...
R	...	...	...	R	R	...	N	...	...	...

695f11, 737d2, 739d12, 799g10, 799g11, 842g3, 852g5, 854a9, 914b12, 937c11, 956g9, 975c5, and 983b5) for the markers *D1S401*, *D1S1172*, and *D1S1541* by an STS screening approach (Green and Olson 1990; Kwiatkowski et al. 1990). These markers identified no YACs already placed on the contig, and these YACs were not identified by any STS already ordered on the contig. Landmark mapping could not order these markers physically with respect to the STSs that assembled the YAC contig, suggesting that the markers may be contained within the 3–5-cM gap. This hypothesis is supported by the inspection of these markers in the recombination events and by the fact that each marker yielded a significant *Z* value that peaked at a recombination fraction (θ) consistent with placement in this gap. Moreover, *D1S401* and *D1S1172* identified four YACs in common; subsequent analysis revealed that primers for these two polymorphic markers flank the same tetranucleotide repeat and should be designated as only one STS.

This physical mapping approach allowed the alignment of five genetic linkage maps (fig. 2D). STS screening with primers for the *F3* locus identified the same YACs as with primers for *D1S236*, thus aligning the NIH/CEPH comprehensive genetic linkage map (NIH/CEPH Collaborative Mapping Group 1992) with the 1993–94 Généthon genetic linkage map (Gyapay et al. 1994). A YAC contig assembling 15 STSs with 17 level 1 YACs covering the 4-cM *STGD* region with a minimum of twofold YAC coverage is shown as figure 3. STS screening with primers for the cone transducin alpha subunit (*GNAT2*) (Wilkie et al. 1992; Morris and Fong 1993) mapped to chromosome 1p13, and that with primers for retinal pigment epithelium-specific protein (*RPE65*) localized to 1p31 (Hamel et al. 1994) was negative for all YACs in this region. Thus these genes are

not contained within the YAC contig for this region and are likely excluded as possible candidates for *STGD* (fig. 3), as suggested by others who reported no disease-specific mutations in the coding region of *GNAT2* among 66 unrelated *STGD* patients (Magovcevic et al. 1995).

Discussion

Linkage analysis in an independent set of 47 *STGD*-recessive families (45 North American and 2 Saudi Arabian) demonstrated statistically significant evidence for localization of a major *STGD* gene to markers on chromosome 1p and provided further evidence for genetic homogeneity of this disorder. Each affected individual among these families had characteristic features of either *STGD* or fundus flavimaculatus, supporting the hypothesis that these are the same genetic disorder. The best estimate for the *STGD* locus is the 4-cM interval between *D1S435* and *D1S236*.

Physical mapping was performed by an STS landmark-mapping approach to establish a YAC contig across the region flanking the *STGD* locus. This YAC contig contains the 4-cM region between *D1S435* and *D1S236*, including the respective *STGD*-flanking recombinants, thus suggesting that *STGD* is present on this YAC contig. Our mapping strategy has permitted the alignment of five genetic linkage maps and physically placed additional markers with respect to the 1993–94 Généthon human genetic linkage map (Gyapay et al. 1994).

Genetic linkage has been reported elsewhere in a set of eight autosomal recessive *STGD* families to markers on chromosome 1p21-p13, with no evidence for genetic heterogeneity (Kaplan et al. 1993). The $Z_{\max}$ for *STGD* was reported at the locus *D1S435* in an interval delineated by key recombination events with markers *D1S424*

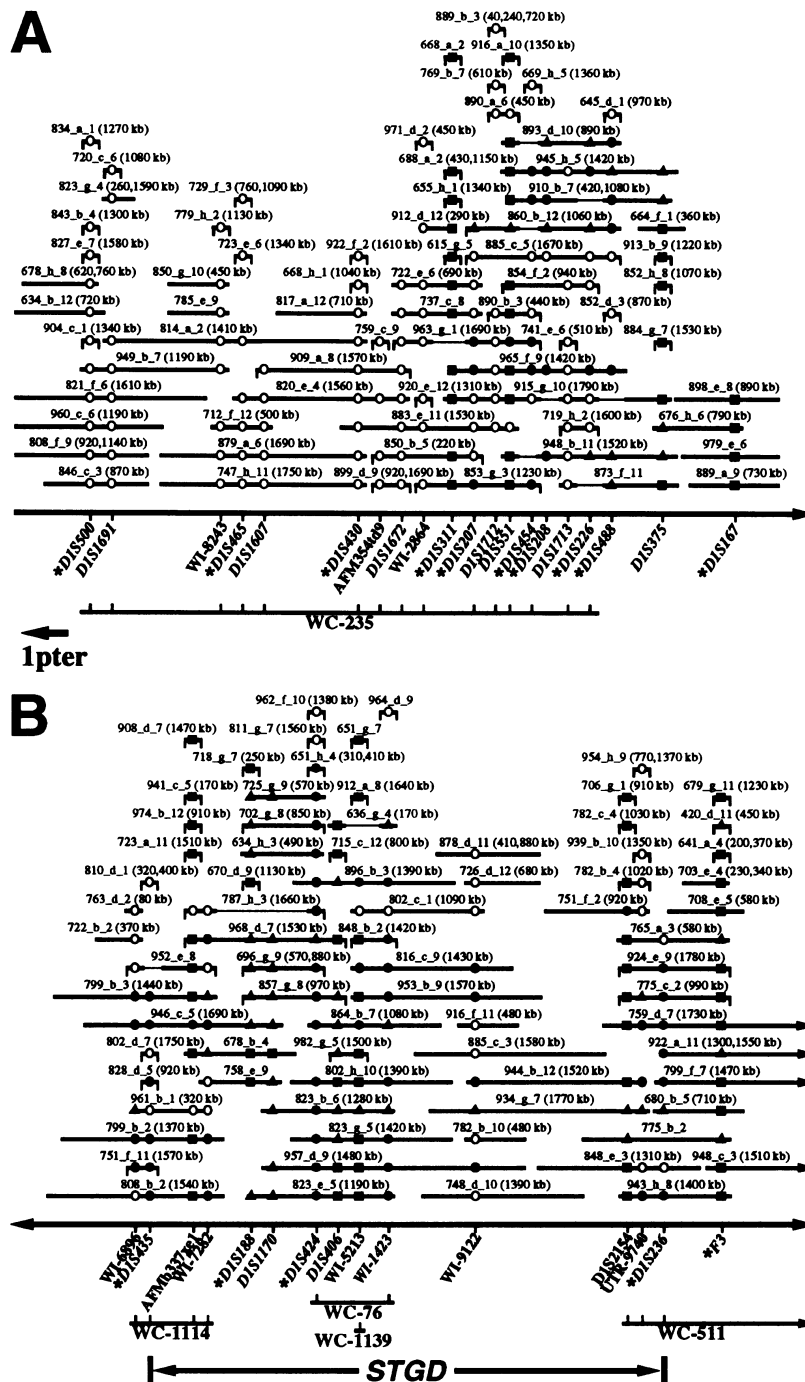
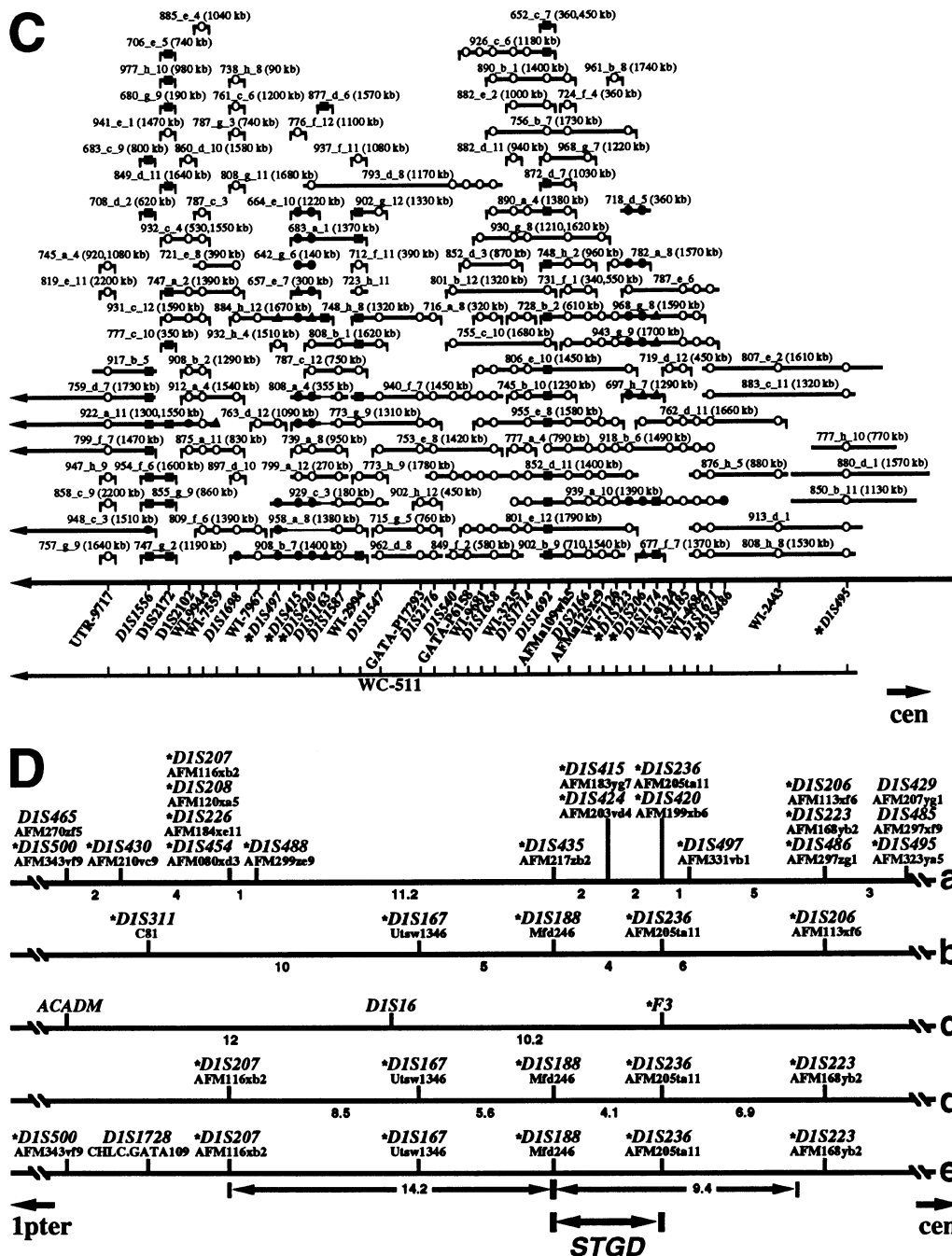


Figure 2 YAC contig of the flanking telomeric region (A), 4-cM STGD interval (B), and flanking centromeric region (C). Horizontal bars represent 256 mega-YAC clones from the CEPH mega-YAC genomic library. Squares denote YACs identified by STS screening at Baylor College of Medicine; triangles denote YACs positive for STSs by landmark mapping; and circles denote YACs identified by STS screening at other institutions (WICGR, G  n  thon, and CEPH) (blackened circles denote YACs that were verified at Baylor). This figure represents 689 specific YAC addresses for 73 STSs. The thicker horizontal line beneath the YACs represents the best physical ordering of STS markers, as determined by STS landmark mapping. YAC clones are drawn approximately to scale and have a size range of 40–2,200 kb, with an average size of 1,121 kb. Some YACs could not be drawn to scale, since they were not positive for a flanking STS, possibly because these YACs are



chimeric; these YACs are depicted by a horizontal bar flanked by two short vertical bars. Some YACs that are positive for flanking STSs and shown to be deleted for an internal STS are depicted by a thin line in the region deleting an STS. Arrows indicate connections to the other sections of the YAC contig, in panels A–C. Horizontal lines below the markers represent five contigs assembled by the WICGR. An asterisk (*) denotes a polymorphic marker represented in the YAC contig and the genetic maps. D, Genetic maps for the 31-cM region encompassing the STGD interval. The horizontal lines represent five published genetic linkage maps aligned for the characterization of this region: a, 1993–94 Génethon human genetic linkage map (Gyapay et al. 1994); b, Multimap (Matise et al. 1994); c, NIH/CEPH Map (NIH/CEPH Collaborative Mapping Group 1992); d, CHLC integrated map (Buetow et al. 1994); and e, comprehensive human linkage map (Murray et al. 1994). Map distances reported as θ values were converted to centimorgans, by the Kosambi map function (Ott 1991).

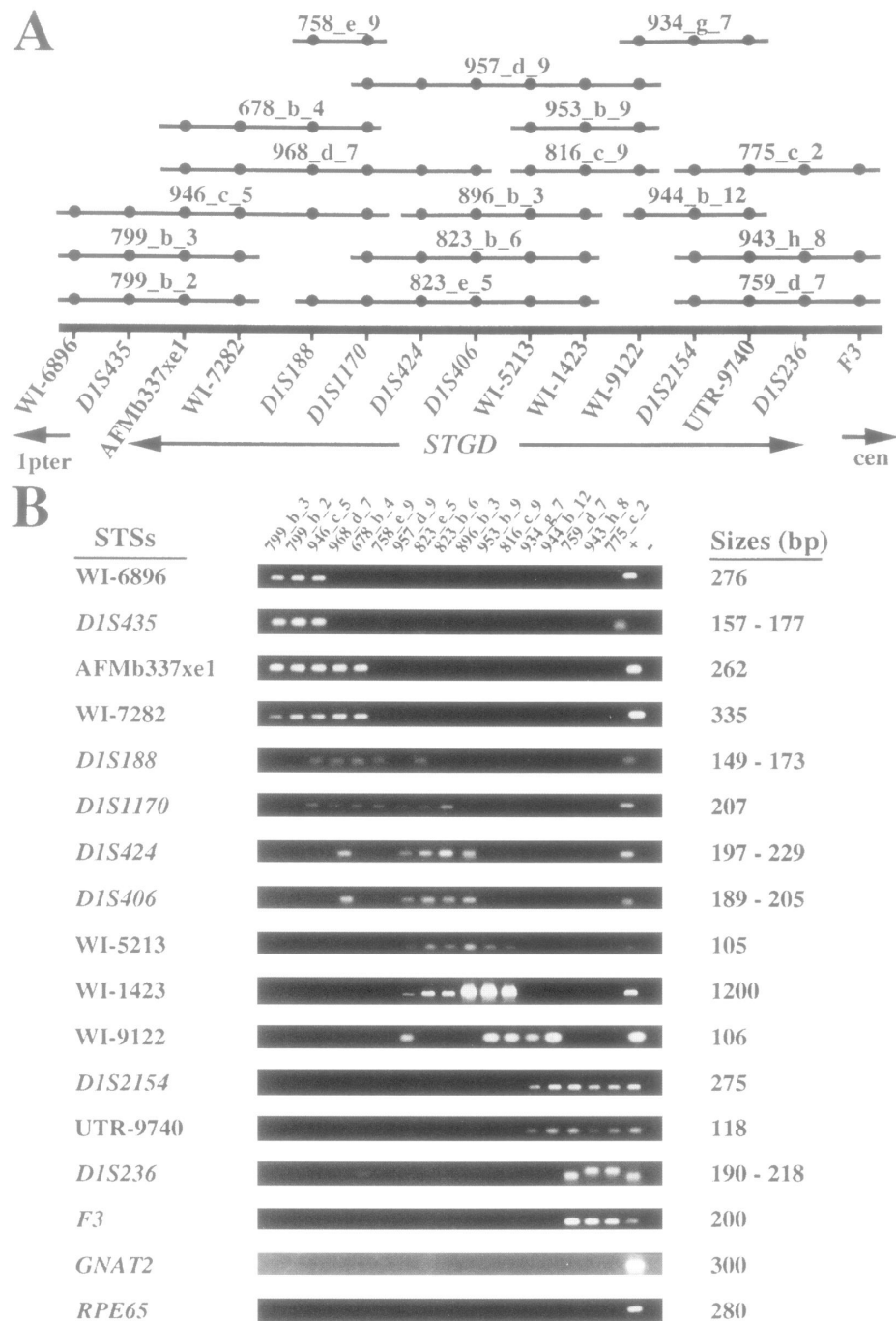


Figure 3 Minimal YAC contig of the 4-cM *STGD* region. In panel A, YACs are represented by horizontal bars, with black dots indicating verified YAC hits. The thicker line below the YACs represents the physical ordering of 15 STSs assembled in this region. In panel B, gel-electrophoresis results of PCR analyses for 17 YACs are arranged horizontally in order from the telomeric side (*left*) to the centromeric side (*right*). Positive and negative controls represent human genomic DNA as template and no DNA, respectively. The 15 STSs are ordered vertically in the left-hand margin, from the telomeric side (*top*) to the centromeric side (*bottom*). Analyses for *GNAT2* and *RPE65* screening are included below the 15 STSs. Sizes of the STSs are arranged vertically in the right-hand margin.

and *D1S236*, in family 3 and family 5, respectively. Interestingly, a recombination event in family 3 was reported between *STGD* and two telomeric markers (*D1S207* and *D1S424*), which could refine substantively the 4-cM *STGD* region determined here; however, that study observed no recombination event with the marker *D1S435*. Although consistent with the marker order that that study reports (*D1S207–D1S424–D1S435*), that order is not compatible with what we demonstrate here (*D1S207–D1S435–D1S424*) on the basis of our YAC contig (figs. 2 and 3), a recombination event that we observed in individual AR106-03 (table 2), and the 1993–94 Généthon human genetic linkage map (Gyapay et al. 1994). Similarly, fundus flavimaculatus of later onset has been mapped to the same region in four multiplex families (Gerber et al. 1995). The position of the disease gene was reported at the locus *D1S424* within the 4-cM interval (*D1S435–D1S236*) consistent with the marker order that we show here. No key recombination events were reported in these families.

Refined localization by both genetic and physical mapping provides the next step toward identifying the *STGD* gene on chromosome 1p. Delineating a 4-cM region defines a realistic target for subsequent isolation of *STGD* by positional cloning techniques. Eventual identification of the genetic defect responsible for *STGD* will provide insight into the pathogenic processes in this hereditary macular dystrophy and may illuminate another step in normal retinal function.

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