Analysis of 21 Stargardt’s disease families confirms a major locus on chromosome 1p with evidence for non-allelic heterogeneity in a minority of cases


Abstract

Background—Autosomal recessive Stargardt’s disease is a macular degeneration characterised by a juvenile onset and a rapidly progressive course resulting in an atrophic macular area typically surrounded by yellowish retinal flecks.

Method—The disease locus has previously been assigned to markers from chromosome 1p21-p13 by genetic linkage analysis in eight multiplex Stargardt’s disease families.

Results—In an extended analysis, the assignment to chromosome 1p was confirmed in the majority of the 21 families with Stargardt’s disease who were studied. In addition, a series of recombinant chromosomes further narrowed the Stargardt’s disease region to an approximately 3 cM interval between markers at D1S424 and D1S497.

Conclusion—Multipoint linkage analysis most probably excludes this locus in three of these families suggesting non-allelic heterogeneity with at least one additional minor Stargardt’s disease locus.

In 1909, the German ophthalmologist Stargardt described a juvenile macular degeneration which was characterised by its familial occurrence, the manifestation of symptoms in the first or second decade of life, and a progressive course of the disease that eventually resulted in severe atrophic macular lesions typically surrounded by yellowish flecks in the retina. Even in advanced cases, loss of colour vision is generally mild and night blindness is not a common feature of the disease. Stargardt concluded that the clinical signs in this disorder should be the result of primary changes either in the choroid or the retina.

The symptoms in Stargardt’s disease usually begin at 6 to 15 years of age, although cases with later onset have been reported. It is a relatively frequent cause of macular degeneration in childhood and has been estimated to account for approximately 7% of all retinal conditions in this age group. Genetically, Stargardt’s disease is a heterogeneous disorder and can be inherited as an autosomal recessive and, less common, as an autosomal dominant trait generally with a later onset of clinical symptoms. By genetic linkage analysis two autosomal dominant Stargardt’s disease loci have recently been localised to chromosome 6q11-q15 and to chromosome 13q34, respectively.

As a first step in the effort to isolate the autosomal recessive Stargardt’s disease gene, the disease locus was assigned to chromosome 1p21-p13 between loci D1S424 and D1S236. The study was based on the analysis of eight families with Stargardt’s disease and suggested genetic homogeneity of the condition in this series. To define more precisely the genetic localisation of the gene, to identify additional recombination events in the critical interval between D1S424 and D1S236, and to test for possible genetic heterogeneity in this condition, we performed an extended analysis with a large number of autosomal recessive Stargardt’s disease families. Genetic heterogeneity is an important issue with regard to presymptomatic DNA testing, but is also critical for the isolation of the disease gene(s) itself.

Here, we report our results on the genetic linkage analysis in 21 Stargardt’s disease families. We confirm linkage of the disease locus to chromosome 1p21-p13 in the majority of our families. In addition, this study has identified a series of recombinant Stargardt’s disease chromosomes which together refine the previous localisation of the disease gene to a small interval on chromosome 1p between markers at D1S424 and D1S497. In three out of our 21 Stargardt’s disease families we found evidence for non-allelic heterogeneity of this condition suggesting the occurrence of at least one additional minor Stargardt’s disease locus.

Patients and methods

CLINICAL STUDIES

Forty-four affected and 28 non-affected at risk individuals belonging to 21 Stargardt’s disease families were recruited from the Eye Care Center Vancouver (Canada) and the Eye Clinic Tübingen (Germany) (Fig 1). At least one patient per family was seen by one of the authors (DW or AE), Dr W Macrae (Toronto) (family 15), or Dr CC Ewing (Saskatoon) (family 13). All families were of white origin except family 1 which originated from China. All affected family members were diagnosed with having typical features of Stargardt’s disease including the following criteria: (1) the
initial presence of fundus flavimaculatus with yellow ‘flecks’ in the macular and paramacular areas when patients were seen at younger age; (2) a normal appearance of the optic disc, blood vessels, and peripheral retina; (3) the presence of the typical dark choroid on fluorescein angiography; (4) normal to subnormal electro-oculogram (EOG), normal electroretinogram (ERG) for rods and cones in early stages, and abnormal dark adapted amplitudes of the ERG recordings in advanced cases; (5) mottled pigmentation of the macula; and (6) at later stages of the disease a central atrophy with RPE clumping. All patients, except those in family 2 (see below), developed loss of vision between the age of 8 and 30 years.

FAMILY 2
In family 2 of Canadian descent the older of the two patients was last seen by an ophthalmologist in 1987 and showed patchy retinal pigment epithelium (RPE) clumping in both maculae and mid-peripheral flecks. She developed loss of vision in her early 40s. Fluorescein angiography revealed the presence of a dark choroid and the red-free fundus photographs demonstrated peripheral and central pisciform (fishtail-like) flecks typical for Stargardt’s disease. ERG data were not available. Her younger sister was diagnosed independently by a retinal surgeon in Sydney (Australia) and appeared to be less severely affected. At the time of examination her vision was 20/20 in each eye. She showed a symmetrical appearance of patchy RPE clumping at both maculae. In the mid-peripheral retina a number of white flecks were present. On fluorescein angiogram the RPE changes were much more widespread than was visible ophthamoscopically and the background appearance was extremely dark quite consistent with a diagnosis of Stargardt’s disease.

FAMILY 12
At age 29 the vision of the patient in the Canadian family 12 was 20/20 in both eyes. Three years later her vision dropped to 20/200 in both eyes. Fluorescein angiography revealed a bull’s eye pattern of RPE atrophy around the fovea in both eyes. The RPE did not have much pigment (blond fundus) so the choroidal vasculature was seen quite well in the early stages of the angiogram. A ring of hyperfluorescence was seen around the fovea with late leakage of dye in both eyes. No flecks were noted on colours or angiogram. A dark choroid sign was absent. When her vision was 20/20 ERG recordings in both the light and dark adapted state and 30 Hz flicker and oscillatory potentials were normal. The light/dark ratio on EOG was 2.5 in both eyes.

FAMILY 17
All four affected siblings of the German family 17 were diagnosed with symptoms typical of Stargardt’s disease. Glare sensitivity and visual loss began at about 16 years of age. Visual acuity decreased rapidly to 20/200 within 4–6 years after onset of symptoms. All have been examined first between 16 and 25 years of age. Central fundus reflexes were absent, fine RPE atrophy could be seen in the macular region, and all but one had a variable amount of fishtail-like yellowish deposits in the deeper retinal layers at the posterior pole. A dark choroid sign was present. All had a 10° central scotoma. The ERG was normal for rods and cones.

DNA and statistical analysis
DNA was extracted by standard procedures. Genotyping of family members was done using the polymerase chain reaction (PCR) and microsatellite markers at loci D1S207, D1S435, D1S424, D1S497, D1S206, D1S167, D1S188 (GDB-ID G00-036-738), and AMY2B. PCR conditions for each marker set were taken from the literature. Amplified products were mixed with denaturing loading buffer and electrophoresed on a 6% denaturing polyacrylamide gel. The gel was dried and exposed to Kodak XOMAT-AR film.

Pairwise and multipoint linkage analyses were performed using the MLINK and LINKMAP routines of the LINKAGE program package Version 5.2. Stargardt’s disease was analysed as an autosomal recessive trait with complete penetrance, no occurrence of phenocopies, and a gene frequency of 0.004. For five-point calculations the allele systems were reduced to three alleles. The allele frequencies were adjusted accordingly. Recombination fractions were calculated according to the mapping function of Kosambi and were assumed to be equal in both sexes. The order and genetic distances of the chromosome 1 markers were derived from established framework maps (Figs 2 and 3). Homogeneity of

Figure 1  Pedigrees of the 21 Stargardt’s disease families. The closed symbols indicate an ascertained affection status, while open symbols represent individuals who were examined and clinically normal. DNA samples from all living individuals shown in the pedigrees were obtained and included in this study.
Table 1  Pairwise linkage analysis between Stargardt’s disease and eight markers from chromosome 1p21-p13

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Linkage was analysed with the program HOMOQ.17 The data used to compute the conditional probabilities of belonging to the linked type of families were taken from multipoint linkage calculations—that is, five point analyses for each family with marker alleles at loci D1S167-D1S435-D1S188-D1S497-Stargardt’s disease.

Results

TWO POINT LINKAGE ANALYSIS

The genotypes of the 105 members of the 21 Stargardt’s disease families (Fig 1) were determined for loci D1S207, D1S167, D1S435, D1S188, D1S424, D1S497, D1S206, and AMY2B spanning a genetic distance of approximately 29.5 cM (Kosambi) on chromosome 1p21-p13. Two point lod scores at standard recombination fractions for Stargardt’s disease versus the microsatellite markers derived from these loci are listed in Table 1.

The combined lod scores from 18 Stargardt’s disease families, excluding families 2, 12, and 17, demonstrated significant linkage of the disease phenotype to six out of the eight markers tested (Table 1). D1S424 has not been analysed in some families and, in addition, has been uninformative in others, thus resulting in a maximum lod score below 3 (1.58 at Θ of 0.07). However, two critical recombination events in families 11 and 19 demonstrate that D1S424 is the closest proximal marker flanking the disease locus (Fig 2). D1S207 reached a combined Zmax of 1.85 at a genetic distance Θ of 0.17. The highest combined lod score was obtained for microsatellite marker at D1S497 with a Zmax of 8.92 at Θ of 0.01 (Table 1).

In order to assess a possible significant association of marker alleles with the disease...
phenotype in our families we determined the genotypes for each marker on a single polyacrylamide gel. However, comparison of the absolute allele sizes at all loci tested has not provided any evidence for a common disease allele in our Stargardt’s disease cohort (data not shown).

The individual two point linkage data for each family demonstrated that the disease phenotype in families 2 and 17 recombined with most of the markers tested within the 29.5 cM interval (Fig 2). In family 12 most markers were not informative. However, the remaining markers suggested no linkage to the disease in this family. The three families had conditional probabilities of < 5% (p=0.04) of being linked to the Stargardt’s disease locus ($\chi^2 = 3.054$ at df =1) (Fig 2). Thus, families 2, 12, and 17 were analysed separately in all further calculations. Absence of linkage was demonstrated for markers at loci D1S207, D1S424, D1S206, and AMY2B (Table 1). Slightly positive $Z_{max}$ values (ranging from $Z_{max} = 0.22$ to 0.47) were obtained for markers at D1S167, D1S435, D1S188, and D1S497, although at genetic distances clearly outside the most likely region of the Stargardt’s disease locus (ranging from $\Theta=0.18$ to 0.27).

**Mapping the Stargardt’s Disease Locus by Multipoint Linkage Analysis**

To determine the most likely location of the Stargardt’s disease locus relative to the eight markers tested, we performed a series of substantial fine scale analyses using data from 18 Stargardt’s disease families (excluding families 2, 12, and 17) (solid lines) or in a separate calculation data from families 2, 12, and 17 (broken lines) (Fig 3).

Multipoint lod score calculations without the genotyping data from families 2, 12, and 17 resulted in a maximum lod score of 12.98 within the interval between loci D1S424 and D1S497 (at recombination distances of 0.018 and 0.012, respectively) (Fig 3). An alternative location of Stargardt’s disease between D1S497 and D1S206 (lod score 12.05) was significantly excluded (p< 0.05).

**Analysis of Recombinant Stargardt’s Disease Chromosomes**

Based on the individual two point linkage data for each of the 21 families, the Stargardt’s disease chromosomes were grouped in three classes (Fig 2).

The first and second group included those chromosomes that were likely to carry the disease gene (conditional probabilities of ≥ 95% of being linked to chromosome 1p21-1p13).

The first class revealed no recombination events between the markers analysed and the disease locus while the second group contained chromosomes with crossing overs between Stargardt’s disease and one or several markers utilised in the study. Recombinant chromosomes in families 11, 19, and 20 define interval D1S424-D1S497 as the minimum candidate region most likely harbouring the Stargardt’s disease gene (Fig 2). The remaining recombinant chromosomes are consistent with a location of the disease gene in the D1S424/ D1S497 interval. The third group consists of chromosomes most likely not linked to Stargardt’s disease (conditional probabilities of ≤5%). Most markers tested reveal a recombination event with the disease phenotype.

**Discussion**

Stargardt’s disease has previously been mapped to markers from chromosome 1p21-1p13 by genetic linkage analysis. This study was based on the genotyping data from eight Stargardt’s disease families and suggested genetic homogeneity of the condition in this group. The question of genetic homogeneity in Stargardt’s disease is a crucial one and has important implications for both the isolation of the disease gene itself and also for the accuracy and reliability of presymptomatic DNA testing. In this study we have addressed this question by analysing a large number of clinically well characterised Stargardt’s disease pedigrees.

Our findings show that the majority of Stargardt’s disease families analysed are linked to the known disease locus on chromosome 1p21-1p13. However, at least three families in our series have a high probability of being unlinked to the chromosome 1 locus. This may even be an under-representation since multiplex families with only two sibs have some probability of belonging to the linked type group by coincidence. Thus, presymptomatic DNA testing should be performed with caution and should only be offered in families where significant linkage to the known Stargardt’s disease locus on chromosome 1p21-1p13 can be demonstrated.

Although the affected members of the three unlinked families have been seen by at least...
one of the authors and have unambiguously been diagnosed with Stargardt’s disease, at present, the possibility of a misdiagnosis cannot entirely be ruled out. It is known that, particularly at early stages of the disease, the infantile and juvenile forms of neural ceroid lipofuscinoses, CNL1 and CNL3, may present with signs somewhat similar to Stargardt’s disease. However, one of the main characteristics of CNL is the slow but progressive deterioration of intellectual ability. There are no signs of dementia in any of our patients unlinked to chromosome 1p21-p13. Nevertheless, only recently the CNL1 gene on chromosome 1p32 encoding the palmitoyl protein thioesterase has been cloned and disease causing mutations have been identified in CNL1 and CNL3 patients, respectively. It will now be possible to assess these two genes in the patients of our three unlinked Stargardt’s disease families.

In the original report on the mapping of the Stargardt’s disease locus to chromosome 1p some confusion existed about the exact order of markers used. In particular, locus D1S435 was localised between D1S424 and D1S236 at a distance of 4.2 and 2.0 cM, respectively. Given this order, D1S435 did not reveal recombination with the disease. Since then, high resolution linkage maps have been constructed placing D1S435 more distal to D1S424 and suggesting the following order: 1pter - D1S207 - D1S167 - D1S435 - D1S188 - D1S424 - D1S236 - D1S497 - D1S206 -AMY2B-1cen. Based on this locus order re-evaluation of the original genotyping data demonstrated a location of the Stargardt’s disease locus between D1S424 and D1S236 with crossing over between the disease and all markers tested.

In our series we have identified 11 recombinant Stargardt’s disease chromosomes most likely linked to the chromosome 1p locus that all are consistent with the more recent mapping of the disease gene. In addition, two recombinant chromosomes (families 11 and 19, Fig 2) may be helpful in further refining the disease locus by analysing additional markers from within the D1S424-D1S497 interval. Thus far, all known markers within the critical region still recombine with the disease. Therefore, additional tightly linked markers will be needed to further narrow the Stargardt’s disease locus and to finally facilitate the cloning of the disease gene.

In summary, we have confirmed the location of a gene causing Stargardt’s disease on chromosome 1p and have shown that most of our families have a high probability of being linked to this disease locus. In addition, our data strongly suggest genetic heterogeneity for autosomal recessive Stargardt’s disease which may account for as many as 14% (3/21) of cases not linked to 1p21-p13. Finally, the identification of additional recombinant chromosomes in families with a high probability of being linked to chromosome 1p will likely narrow the disease locus and, thus, facilitate the timely cloning of the major Stargardt’s disease gene.

The authors wish to thank the families for their kind cooperation, Dr W Maarse (Toronto), G Harris (Vancouver), C C Ewing (Saskatoon), T Pierce (Edmonton), R Pruett (Boston) for providing history and clinical information on some of the disease’s disease families, W Woll (Würzburg) for helping with the statistical analysis, and H Stöhr for critically reading the manuscript.