Am. J. Hum. Genet. 51:749–754, 1992

The Autosomal Dominant Familial Exudative Vitreoretinopathy Locus Maps on 11q and Is Closely Linked to D11S533

Yün Li,*,[1] Bertram Müller,‡ Christian Fuhrmann,† C. Erik van Nouhuys,§ Horst Laqua,† Peter Humphries,‖ Eberhard Schwinger,* and Andreas Gal*

*Institut für Humangenetik and †Augenklinik der Medizinische Universität, Lübeck; ‡Abteilung für Pädiatrische Genetik der Kinderpoliklinik, Ludwig-Maximilians-Universität, Munich; §Department of Ophthalmology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands; and ‖Department of Genetics, Trinity College, Dublin

Summary

Autosomal dominant familial exudative vitreoretinopathy (adFEVR) is a hereditary disorder characterized by the incomplete vascularization of the peripheral retina. The primary biochemical defect in adFEVR is unknown. The adFEVR locus has tentatively been assigned to 11q by linkage studies. We report the results of an extended multipoint linkage analysis of two families with adFEVR by using five markers (INT2, D11S533, D11S527, D11S35, and CD3D) from 11q13-q23. Pairwise linkage data obtained in the two families were rather similar and hence have not provided evidence for genetic heterogeneity. The highest compiled two-point lod score (3.67, at a recombination fraction of .07) was obtained for the disease locus versus D11S533. Multipoint analyses showed that the adFEVR locus maps most likely, with a maximum location score of over 20, between D11S533/D11S527 and D11S35, at recombination rates of .147 and .104, respectively. Close linkage without recombination (maximum lod score 11.26) has been found between D11S533 and D11S527.

Introduction

Familial exudative vitreoretinopathy (FEVR), a hereditary disorder of the human retinal vascular system, was first described by Criswick and Schepens (1969). It is characterized by a peripheral avascular zone of the retina, a zone that is bounded by atypical vessels developing exudates and retinal neovascularizations. The formation of peripheral fibrovascular masses causes both deformation of the posterior vascular bed and ectopic macula. The disease shows an extremely variable clinical expression. Minimally affected patients are generally free of any visual symptoms during life, while others may suffer from considerable visual impairment due to such late complications as retinal detachments, vitreous hemorrhage, and secondary glaucoma, any of which can lead to blindness at a young age. The incomplete vascularization of the peripheral retina seen in FEVR is believed to be the result of a premature arrest in retinal angiogenesis. However, the primary biochemical defect is unknown. The disease usually follows an autosomal dominant (ad) transmission (for review, see van Nouhuys 1982), although a few families with X-linked mode of inheritance have also been reported recently (Fullwood et al. 1991; Trese et al. 1991).

By applying a combination of molecular genetic methods, it has now become possible to isolate the gene and to identify the primary cause of any inherited monogenic disorder. The first step in this process (frequently called "positional cloning") is to determine the chromosomal location of the disease gene. In order to map the disease locus (EVR) in the human genome, we performed linkage studies in a large German family with adFEVR by analyzing the segregation of a great number of DNA polymorphisms, especially microsa-

Received April 10, 1992; revision received May 27, 1992.

Address for correspondence and reprints: Andreas Gal, M.D., Institut für Humangenetik, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, D-W2400 Lübeck 1, Germany.

1. On leave of absence from Department of Biology, Zhejiang Medical University, Hangzhou, Zhejiang, People's Republic of China.

0002-9297/92/5104-0008$02.00

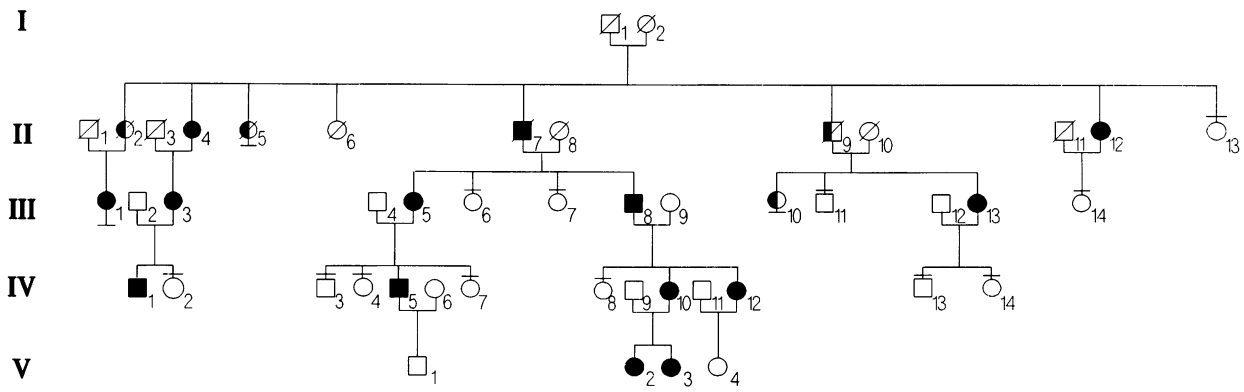

Figure 1 Pedigree of family 1. Completely blackened symbols denote individuals designated as affected, on the basis of ophthalmological examination; half-blackened symbols denote individuals designated as most likely affected, on the basis of history (no ophthalmological examination was performed); unblackened symbols with a bar above them denote individuals designated as not affected, on the basis of ophthalmological examination; and unblackened symbols without a bar above them denote individuals on whom no ophthalmological examination was performed.

tellite CA repeats. Positive lod scores (Z) suggestive of linkage have been obtained between EVR and two loci from 11q: INT2 (11q13) and D11S35 (11q22-q23) (Li et al. 1992). In an attempt to localize the disease locus more precisely, an extended multipoint linkage study has been initiated on the German family studied before and on a second family of Dutch origin by using five markers from 11q13-q23.

Families, Material, and Methods

Families

Pedigrees of the two families studied are shown in figures 1 and 2. Family 1 (fig. 1) is a large multigeneration German family described in detail elsewhere (Laqua 1980). In addition to the persons included in our preliminary linkage study (Li et al. 1992), DNA of four additional family members has been examined in the present analysis.

Family 2 (fig. 2) has also been reported earlier (as "family I," by van Nouhuys [1982]). All patients showed the typical signs of FEVR. II-4 and III-3 presented with minimal vascular alterations in the periphery of the retina, which could be considered as being within the range of normal variation. However, since adFEVR could not be excluded with certainty in these two family members, their phenotype was defined as "unknown" for linkage studies.

DNA Studies

DNA was isolated from peripheral blood samples of family members by standard methods. All DNA linkage markers used were of CA-repeat type. The most important information on the markers is summarized in table 1. PCR amplification conditions were according to the references given in table 1. PCR products were separated electrophoretically on polyacrylamide gels, stained with ethidium bromide, and visualized on a UV transilluminator.

Linkage Studies

Linkage analysis was performed by computer program LINKAGE v5.1 (Lathrop et al. 1984). Complete penetrance of the disease and a frequency of .0001 for the deleterious allele were assumed. For the multipoint analysis, equal recombination rates in males and females were used.

Results

Linkage analysis was performed in two families with adFEVR by using five markers from 11q13-q23. Pairwise Z values for the linkage relationships EVR

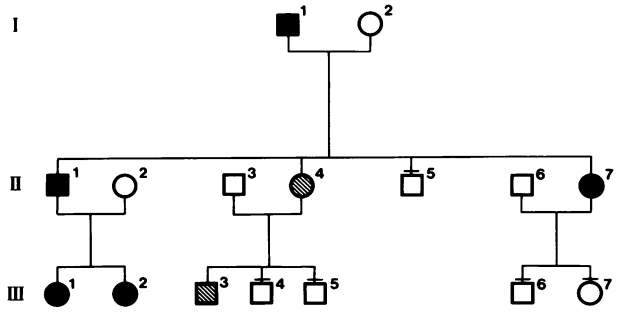

Figure 2 Pedigree of family 2. Hatched symbols denote that the disease phenotype unknown (see family description in text).

Table 1

Linkage Markers Used in Present Study

Locus	Chromosomal Localization	Repeat Type	Frequency		Reference
			Hetero-zygosity	PIC[a]	
INT2	11q13	$(CA)_n$	84.6		Polymeropoulos et al. 1990
D11S533 ...	11q13.5	Hexanucleotide		.80	Eubanks et al. 1991
D11S527 ...	11q13.5	$(CA)_n$		.88	Browne et al. 1990
D11S35	11q22-q23	$(CA)_n$		.86	Litt et al. 1990
CD3D	11q23	$(CA)_n$		.69	Weber et al. 1990

Note.—All polymorphisms were detected after PCR amplification.

versus marker loci in both families are shown in table 2. The highest Z (3.67, at a recombination fraction [θ] of .07) was obtained with D11S533. No recombination was detected between EVR and D11S533 in family 2, while in family 1 two recombinants were found: III-3, an affected female, and III-11, a nonaffected male. INT2, D11S527, and D11S35 all gave peak Z values of nearly 3 at θ = .11, .13, and .10, respectively. CD3D was informative only in family 1 and resulted in negative Z values throughout. Since the most proximal (INT2) and most distal (CD3D) of the loci studied here recombine with each other at a frequency of about 30%–40% (table 3 and authors' unpublished data), the positive Z values obtained with four of five loci from the 11q13-q23 region strongly suggest that EVR maps in this interval.

In order to determine the position of EVR relative to the marker loci examined, multipoint analyses were performed in the two families by using the four loci showing linkage to EVR. The order of marker loci was fixed as 11cen–INT2–D11S533/D11S527–D11S35–11qter according to their physical locations (table 1). Intermarker distances were deduced mainly from our own results (table 3) and were in line with those obtained by linkage studies in large reference pedigrees (Julier et al. 1990). D11S533 and D11S527 showed

Table 2

Results of Pairwise Linkage Analyses between the Locus for adFEVR and Five Loci from 11q

	Z at θ of								
	0	.001	.05	.10	.20	.30	.40	$\hat{\theta}$	Z_{max}
INT2:									
Family 1 ...	$-\infty$	−1.16	1.86	2.06	1.80[a]	1.21	.50		
Family 2 ...	$-\infty$	−1.05	.47	.59	.51	.30	.09	.11	2.65
Total	$-\infty$	−2.22[a]	2.33	2.65	2.31[a]	1.51	.58[a]		
D11S533:									
Family 1 ...	$-\infty$	−.86	2.12	2.28	1.94	1.29	.53		
Family 2 ...	1.71	1.71	1.51	1.31	.89	.48	.14	.07	3.67
Total	$-\infty$	.85	3.63	3.59	2.84[a]	1.77	.67		
D11S527:									
Family 1 ...	$-\infty$	−4.72	1.55	2.20	2.22	1.61	.71		
Family 2 ...	.81	.81	.77	.70	.52	.30	.10	.13	2.96
Total	$-\infty$	−3.91	2.32	2.90	2.74	1.91	.80[a]		
D11S35:									
Family 1 ...	$-\infty$	.81	2.29	2.30	1.87	1.17	.37		
Family 2 ...	$-\infty$	−1.33	.22	.37	.36	.23	.07	.10	2.66
Total	$-\infty$	−.52	2.51	2.66[a]	2.23	1.39[a]	.44		
CD3D:									
Family 1 ...	$-\infty$	−2.51	−.78	−.46	−.19	−.07	−.02	.50	0

[a] Slight discrepancy from the sum of the two numbers is due to rounding error.

Table 3

Results of Marker-to-Marker Linkage Analyses

	INT	D11S533	D11S527	D11S35	CD3D
INT		.05	.12	.21	.23
D11S533	6.59		.00	.18	[.06]
D11S527	3.74	11.26		.15	.32
D11S35	.88	1.12	2.86		.09
CD3D	.24	[−2.16]	.09	1.29	

NOTE.—Z_{max} values are given below the diagonal; and the θ value of the Z_{max} is given in the corresponding cell above the diagonal. If Z values were negative throughout, θ values at $Z \leq -2.00$ are given in square brackets.

no recombination (maximum Z [Z_{max}] = 11.26 at θ = 0), and therefore, for the sake of simplicity, we used only one of them for each multipoint analysis.

Four-point analyses showed that EVR maps most likely between D11S527/D11S533 and D11S35 (table 4). Although a location of EVR outside the region defined by the four marker loci used was less probable, data were not strong enough in each case to consider this latter possibility as highly unlikely. Using the most likely order of loci that was obtained in the multipoint analyses, we calculated a maximum location score of 21.4 (equivalent to Z_{max} = 4.652) for localizing the disease locus between D11S527 and D11S35, at recombination rates of .147 and .104, respectively (fig. 3). As expected, similar data were obtained when D11S527 was replaced by D11S533 (location score of 20.4 for a position similar to that in the first analysis; not shown).

Discussion

adFEVR is a trait with manifestations only in the eye. In spite of extensive studies (see van Nouhuys 1991), the basic biochemical defect in adFEVR remains unknown. Ophthalmological features of affected persons are very similar to those seen in retinopathy of prematurity (ROP), a nongenetic condition often observed after neonatal oxygen administration to prematurely born infants. If it is assumed that both ROP and adFEVR result from a disturbance in the late development of the retinal vascular system, one can speculate that the two disorders have a similar etiology—i.e., inhibition of vascularization of the fetal retina by elevated local oxygen tension. In this context it is interesting that the gene (COX8) for subunit VIII of cytochrome C oxidase, the terminal enzyme of the mitochondrial respiratory chain, maps in 11q12-q13 (Rizzuto et al. 1989). However, physical mapping data suggest that COX8 is situated proximal to INT2 (see Junien and van Heyningen 1991), while the most likely position of EVR is, according to our linkage data, distal to INT2, in 11q13.5-q22. Thus, the data presented in this communication do not provide support for COX8 being a candidate gene for adFEVR.

In the absence of any candidate gene, cloning and subsequent identification of the gene can be achieved by genetic and physical mapping of the disease locus ("positional cloning"). A detailed genetic map of the long arm of chromosome 11 has recently been constructed (Julier et al. 1990). Of the 31 loci assigned in that map, three (INT2, D11S35, and CD3D) were included in our study. Marker-to-marker linkage data from the present study and those of Julier et al. (1990) are rather similar and have been used to deliver the primary map for multipoint analyses in our study. While the physical locations of D11S533 and D11S527 are rather well established, their positions on the linkage map are defined less precisely. Our data suggest that both D11S533 and D11S527 map between INT2 and D11S35 and that they are closely linked to each other. Since no recombination was de-

Table 4

Multipoint Analysis for Various Positions of the adFEVR Locus and Four Marker Loci

Order	Odds[a]
FEVR–INT2–D11S527–D11S35	6.18×10
INT2–D11S527–FEVR–D11S35	1
INT2–D11S527–D11S35–FEVR	1.06×10
FEVR–INT2–D11S533–D11S35	3.35
INT2–D11S533–FEVR–D11S35	1
INT2–D11S533–D11S35–FEVR	8.38

[a] Only odds less than 1×10^2 are given. In each case, the odds against the order are presented relative to the most likely order.

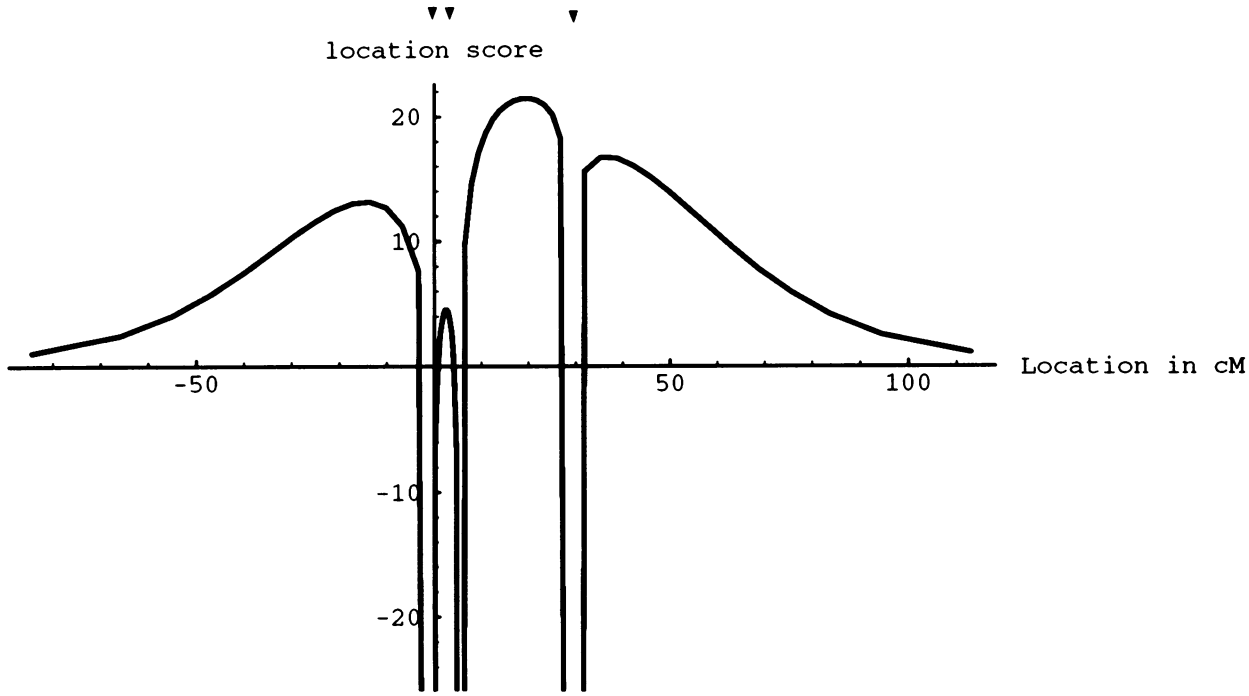

Figure 3 Mapping the locus for adFEVR by multipoint linkage analysis. Genetic distance was calculated by using Kosambi's mapping function. Marker loci, marked by arrowheads, were placed at 0 cM (INT2), 5 cM (D11S527), and 29 cM (D11S35).

tected between D11S533 and D11S527 in the two families studied by us, the order of the two loci cannot be determined at present.

In this communication we have presented the results of two-point and multipoint analyses strongly suggesting that the locus for adFEVR maps in 11q13-q23. Nevertheless, additional markers from this chromosomal region should be tested to confirm our initial observation and to determine more accurately the relative order of loci. Linkage data obtained in the two families are rather similar and hence do not provide evidence for genetic heterogeneity. However, the analysis of other families with adFEVR should be carried out in the future to investigate this issue.

Hitherto, in case of positive family history, diagnosis of adFEVR has been made exclusively by ophthalmoscopy and fluorescein angiography. However, ophthalmological diagnosis is complicated by the extremely variable expression of the disease, especially in cases with vitreous "organizations," in persons with minimal vascular anomalies, and in children. The availability of genetic markers closely linked to the disease locus now allows an indirect genotype analysis in families seeking advice. Secondary complications of adFEVR, such as neovascularization and retinal detachment, are best treatable at early stages, and therefore it is important to diagnose adFEVR at a young age.

Acknowledgments

This study has been financially supported by Deutsche Forschungsgemeinschaft grant Ga 210/5-2 and by Commission of the European Community grant ERB4002PL-910247.

References

Browne DL, Gault J, Thompson MB, Hauge XY, Evans GA, Litt M (1990) Dinucleotide repeat polymorphism at the D11S527 locus. Nucleic Acids Res 19:4790

Criswick VG, Schepens CL (1969) Familial exudative vitreoretinopathy. Am J Ophthalmol 68:578–594

Eubanks JH, Selleri L, Hart R, Rosette C, Evans GA (1991) Isolation, localization, and physical mapping of a highly polymorphic locus on human chromosome 11q13. Genomics 11:720–729

Fullwood P, Jones J, Bundey S, Fielder AR, Kilpatrick MW (1991) Genetic linkage analysis in X-linked vitreoretinopathy. Invest Ophthalmol Vis Sci 32 [Suppl]: 1138

Julier C, Nakamura Y, Lathrop M, Connell P, Leppert M,

Litt M, Mohandas T, et al (1990) A detailed genetic map of the long arm of chromosome 11. Genomics 7:335–345

Junien C, van Heyningen V (1991) Report of the Committee on the Genetic Constitution of Chromosome 11. 11th International Workshop on Human Gene Mapping (HGM11). Cytogenet Cell Genet 58:459–554

Laqua H (1980) Familial exudative vitreoretinopathy. Graefes Arch Clin Exp Ophthalmol 213:121–133

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Li Y, Fuhrmann C, Schwinger E, Gal A, Laqua H (1992) The gene for autosomal dominant exudative vitreoretinopathy (Criswick-Schepens) is on the long arm of chromosome 11. Am J Ophthalmol 113:712–713

Litt M, Sharma V, Luty JA (1990) Dinucleotide repeat polymorphism at the D11S35 locus. Nucleic Acids Res 18:590

Polymeropoulos MH, Xiao H, Rath DS, Merril CR (1990) Dinucleotide repeat polymorphism at the int-2 protooncogene locus (INT2). Nucleic Acids Res 18:7468

Rizzuto R, Nakase H, Darras B, Francke U, Fabrizi GM, Mengel T, Walsh F, et al (1989) A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and nonmuscle tissues. J Biol Chem 264:10595–10600

Trese MT, Hartzer MK, Shastry BS (1991) X-chromosome-linked forms of familial exudative vitreoretinopathy. Invest Ophthalmol Vis Sci 32 [Suppl]: 692

van Nouhuys CE (1982) Dominant exudative vitreoretinopathy and other vascular developmental disorders of the peripheral retina. Doc Ophthalmol 54:1–414

——— (1991) Signs, complications, and platelet aggregation in familial exudative vitreoretinopathy. Am J Ophthalmol 111:34–41

Weber JL, Kwitek AE, May PE (1990) Dinucleotide repeat polymorphisms at the D11S419 and CD3D loci. Nucleic Acids Res 18:4036