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Localization of a Novel X-Linked Progressive Cone Dystrophy Gene to Xq27: Evidence for Genetic Heterogeneity

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Summary

Clinical reexamination and DNA linkage analysis were carried out in an X-linked progressive cone dystrophy (XLPCD) family, previously described by Pinckers and Timmerman in 1981. In a large pedigree segregating XLPCD, by use of $\geq$27 markers spanning the entire X chromosome, a novel locus for XLPCD was identified in Xq27. All other regions on the chromosome could be excluded. Since this novel locus is distinct from previously identified genes or regions involved in XLPCD, we further establish genetic heterogeneity underlying this disease entity.

Introduction

X-linked progressive cone dystrophy (XLPCD) is a hereditary eye disease characterized by photophobia, nystagmus, loss of visual acuity, abnormal color vision, and disturbed cone electroretinogram (ERG) (Pinckers et al. 1981; Verdoorn and Pinckers 1988; Pinckers and Deutman 1987). Eight families apparently segregating XLPCD have been reported elsewhere (Fleischman and O'Donnell 1981; Pinckers et al. 1981; Pinckers and Timmerman 1981; Heckenlively and Weleber 1986; Verdoorn and Pinckers 1988; Jacobson et al. 1989; Reichel et al. 1989; Keunen et al. 1990; Meire et al. 1994). According to these studies, subtle phenotypic differences exist between families segregating this disorder. Also, intrafamiliar differences have been described (Keunen et al. 1990; Hong et al. 1994). An overview of clinical characteristics of families for which the genetic locus is known is presented in table 1.

Electrophysiological examination is required for diagnosis. The ERG shows attenuated cone-mediated responses in the younger patients, whereas the older affected males may also demonstrate rod dysfunction (Pinckers et al. 1981; Heckenlively and Weleber 1986; Jacobson et al. 1989; Meire et al. 1994). Carriers may show decreased visual acuity and unilateral high myopia with amblyopia. Color-vision testing and foveal densitometry may be useful for carrier detection (Keunen et al. 1990; van Everdingen et al. 1992). Especially, a reduced ERG response elicited by a red-light stimulus appears to be indicative for carrier state (Meire et al. 1994).

XLPCD can be distinguished from another form of X-linked cone dysfunction, blue cone monochromatism (BCM). In contrast with XLPCD, BCM is a congenital, nonprogressive disorder in which the function of the cones that are sensitive for the blue part of the light spectrum remain intact.

Linkage analysis in three families assigned an XLPCD gene to the Xp21.1-p11.3 region, to the region between the loci DXS84 and ARAF1 (table 1) (Bartley et al. 1989; Hong et al. 1991, 1994; Bergen et al. 1993*a*). On the other hand, BCM and possibly another form of XLPCD were recognized as defects in the green and red cone pigment–gene cluster located in Xq28 (table 1) (Nathans et al. 1989; Reichel et al. 1989). Here we report a linkage analysis in an XLPCD family, previously described by Pinckers and Timmerman (1981), which suggests that the underlying molecular defect is uniquely located in Xq27.

Patients, Material, and Methods

Patients

The majority of patients were examined by A.J.L.G.P. at the Department of Ophthalmology of the University Hospital in Nijmegen. A number of individuals have been examined at the Netherlands Ophthalmic Research Institute and the Groot Ziekengasthuis in Den Bosch (table 2).

The pedigree of the family is presented in figure 1. All patients and potential carriers underwent full ophthalmological examination, including refraction and visual acuity testing, slit-lamp examination, and funduscopy. In addition, ERG and electrooculography were performed. Color vision was tested under standardized conditions, with the Tokyo Medical College test, The American Optical Hardy Rand Rittler test, the Ishihara test, Lanthony's New Color test, the desaturated Panel D-15 test of Lanthony, the anomaloscope Nagel type II, and the Farnsworth Munssell 100-Hue test.

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Table 1

Main Clinical Characteristics of XLPCD and BCM for Which Genetic Defect Is Known

	Data for Disease (Gene Name)			
	XLPCD			
	Xp21.1-p11.2 (COD1)	Xq27 (COD2)	Xq28 (Not Assigned)	BCM, Xq28 (GCP and RCP)
Molecular defect:				
Status	Assigned by linkage analysis	Assigned by linkage analysis	Deletion in red cone–pigment gene	Deletion near 5′ of red cone–pigment gene
Reference(s)	Bartley et al. (1989); Bergen et al. (1993)	Present study	Reichel et al. (1989)	Nathans et al. (1989)
Main clinical characteristics:				
General	Reduced visual acuity; progressive macular atrophy	Early onset; reduced visual acuity; peripheral cone progresses to diffuse type	Reduced visual acuity; progressive macular atrophy	Reduced visual acuity; normal fundus; nystagmus
Color vision	Congenital normal, progressing to central acquired achromatopsia (type I Verriest)	Congenital normal, progressing to central acquired achromatopsia (type I Verriest)	Congenital protanopia, progressing to central acquired achromatopsia (type I Verriest)	Congenital achromatopsia, not progressive
ERG	Reduced cone ERG; in later stages reduced rod ERG	Reduced cone ERG; in later stages reduced rod ERG	Reduced cone ERG	Reduced cone ERG
References	Jacobson et al. (1989); Hong et al. (1994); Meire et al. (1994)	Pinckers and Timmerman (1981)	Reichel et al. (1989)	Nathans et al. (1989)

Molecular and Statistical Studies

Southern and PCR analyses were essentially performed as described elsewhere (Bergen et al. 1991, 1993*b*). Statistical analyses were performed by use of the computer program MLINK from the package LINKAGE 5.1. Markers are registered at the Human Genome Database. An XLPCD gene frequency of .00001 was used.

Results

Clinical Studies

The XLPCD segregating in the pedigree presented in figure 1 begins as a peripheral cone disease and progresses to a diffuse cone disease (Pinckers and Timmerman 1981). Affected males exhibit diminished visual acuity, myopia,

Table 2

Clinical Variability within XLPCD Family

Case	Visual Acuity	Myopia	Fundus	ERG[a]	Color Vision[a]
I-1/7	>.1	−8	Atrophy	Cone-rod	No color perception
I-3/4	>.1	−10	Atrophy	Cone-rod	No color perception
II-6/1	1.0	−5	Myopic	Cone	Normal
II-16/9	.1	−6	Myopic	EE	Type I, achromatopsia
II-18/15	.8	−9	Myopic	EE	EE
II-21/10	.3	−12	Myopic	Cone	EE
II-25/14	.3	−19	Myopic	Cone	Type I, early stage
III-5/2	.4	−4	Myopic	Cone	Type I, scotopization
III/6/3	1.0	+1	Normal	Normal	Normal
III-13/16	.4	−5	Myopic	EE	EE

Note.—I-4/13, II-5/5, and I-5/8 were examined elsewhere. Only directly comparable clinical on patients examined by A.J.L.G.P. according to specific standard procedures are given.

[a]EE = examined elsewhere (i.e., at the Netherlands Ophthalmic Research Institute or the Groot Ziekengasthuis in Den Bosch).

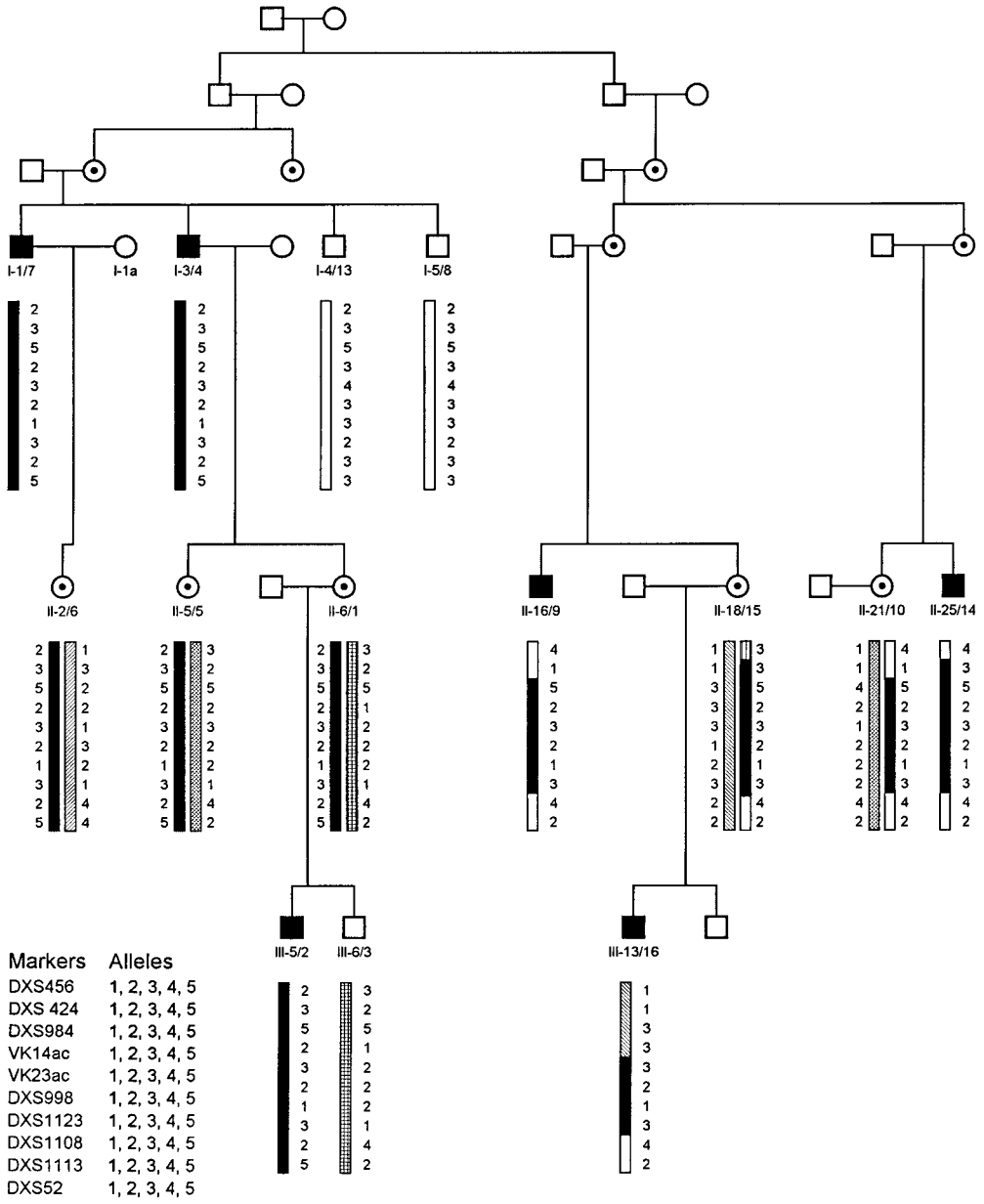

Figure 1 Segregation pattern of a number of X-chromosomal markers in the target region. DXS292 = VK14AC; and DXS297 = VK23AC.

disturbed cone ERG, and a type I color-vision defect. The type I acquired defect is observed in retinal disease primarily involving the photoreceptors of the posterior pole and is assumed to reflect a destruction of the macular cones. In the type I acquired defect the chromatic discrimination on the red and green axis progressively deteriorates. In an advanced stage, there is total colorblindness in the affected visual field, resembling congenital achromatopsia.

Heterozygous females in this XLPCD family have diminished visual acuity and myopia but, as a rule, a normal cone ERG and normal color vision. The only nonobligate carrier (II-21) was diagnosed as such because of a diminished visual acuity and high myopia, which could not be explained otherwise.

Reexamination of several family members over the past 15 years showed a slight progression in the males, whereas the findings in the heterozygote females remained constant. Clinical variability of the disease within the family is presented in table 2.

Molecular Studies

The XLPCD pedigree was studied with $\geq$27 markers spanning the entire X chromosome (table 3). Except for markers from the Xq27 region, one or multiple recombination events were found between each of these markers and the disease locus. The segregation of alleles of markers in the Xq27 region in the family is shown in figure 1.

Close linkage without recombination was found be-

Table 3

Two-Point Linkage for Markers along Human X Chromosome and XLPCD

Locus	Maximum Recombination Fraction	Maximum LOD Score	LOD Score at Recombination Fraction of					
			.00	.01	.05	.10	.20	.30
KAL			−∞	−4.82	−2.18	−1.16	−.36	−.07
DXS207			−∞	−6.76	−3.39	−2.04	−.86	−.32
DXS999			−∞	−5.44	−2.74	−1.66	−.72	−.28
DXS451			−∞	−2.07	−.80	−.35	−.06	−.04
DMD49			−∞	−2.92	−.99	−.33	.08	.13
DYS-I			−∞	−4.31	−2.25	−1.42	−.66	−.29
DXS1058			−∞	−2.65	−1.30	−.76	−.29	−.09
MAOB			−∞	−5.31	−2.61	−1.53	−.59	−.19
DXS255			−∞	−1.22	.00	.38	.54	.43
DXS14			−∞	−2.81	−1.47	−.93	−.45	−.22
DXS453			−∞	−6.13	−2.80	−1.49	−.45	−.08
DXS441			−∞	−6.37	−3.02	−1.71	−.06	−.16
DXS1002			−∞	−6.67	−3.32	−2.01	−.89	−.42
DXS3			−∞	−7.47	−4.03	−2.61	−1.30	−.63
DXS456			−∞	−2.34	−.99	−.46	−.04	.10
DXS1106			−∞	−.59	.00	.17	.22	.18
DXS424			−∞	−2.14	−.81	−.32	.06	.15
DXS1114			−∞	.05	.61	.73	.67	.49
DXS984			−∞	−.8	−.18	−.00	.06	.02
DXS292			−∞	.73	1.22	1.26	1.03	.68
DXS297	.00	2.54	2.54	2.50	2.29	2.03	1.48	.93
DXS998	.00	2.06	2.06	2.02	1.84	1.61	1.15	.70
DXS1123	.00	2.60	2.60	2.55	2.33	2.07	1.52	.97
DXS1113			−3.49	.66	1.15	1.18	.94	.58
DXS52			−3.67	.48	.98	1.02	.811	.48

tween the XLPCD locus (COD2) and the loci DXS297, DXS998, and DXS1123, with maximum LOD scores of 2.54, 2.06, and 2.60, respectively. For all other markers, at least one recombination event between the XPLCD locus and these markers was observed, resulting in negative LOD scores in two-point linkage analysis. Analysis of the Xq27 haplotypes suggests that the XLPCD locus in this family is located in the chromosomal region between the loci DXS292 and DXS1113 (fig. 1).

Two-point linkage analysis with different combinations of several distinct markers along the entire X chromosome resulted in negative or low LOD scores along the chromosome, except for the Xq27 region. Significant linkage without recombination was found between the COD2 locus and the markers DXS297, DXS998, and DXS1123, with LOD scores of 2.54, 2.06, and 2.60, respectively.

Multipoint linkage analysis with different combinations of markers yielded negative multipoint LOD scores along the entire chromosome, except for marker combinations in the Xq27 region, and thus resulted in exclusion of the position of the disease locus elsewhere. Multipoint linkage analysis suggests that COD2 is located between DXS292 and DXS1113, with a maximum multipoint LOD score of 10.8 at the DXS1123 locus (fig. 2).

Special attention was given to the segregation patterns of the markers surrounding the COD1 region in Xp21.1-p11.3, as well as to the markers closely linked to the red and green cone–pigment genes (RCP and GCP, respectively) in Xq28. The COD1 gene region was analyzed by multipoint linkage analysis including the mark-

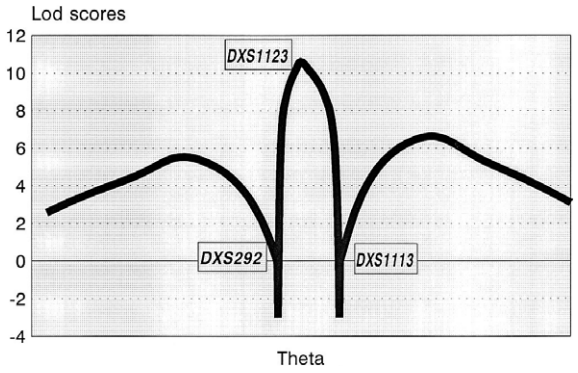

Figure 2 Multipoint analysis of the XLPCD locus. Map distances (*horizontal axis*) between the markers used were 3 cM for DXS292–DXS1123 and 5 cM for DXS1123–DXS1113, as summarized from the genetic maps from the Genome Database and CEPH and from the physical map of the region as presented on the Internet by the Whitehead Institute. DXS292 is present on the consensus map of the X chromosome.

ers DMD49, DYS-I, DXS1058, MAOB, and DXS255. In the entire interval, multipoint LOD scores were <0 (results not shown).

The possible involvement of the RCP and GCP genes in the XLPCD in this family was checked by analysis of the segregation patterns of two markers, one distally and one proximally very closely linked to this gene cluster. Both markers, DXS8103 and DXS8069, reveal recombination events with the XLPCD locus. Consequently, multipoint linkage analysis including these markers resulted in negative LOD scores as well (not shown). In addition, Southern blot analysis with an RCP/GCP cDNA probe (kindly provided by J. Nathans) did not reveal any structural abnormalities in the DNA of the patients, compared with healthy individuals.

Discussion

The disease segregating in our pedigree exhibits subtle clinical differences compared with what is seen in other published pedigrees (table 1). The disease onset in our pedigree is early childhood, which is later than in the family described by Fleischman and O'Donnell (1981) but much earlier than in other pedigrees. The disease described here is progressive but reveals a comparatively normal fundus on funduscopy: only little pigment clumping is seen, and no tapetal reflex can be observed. In contrast with the families described by Jacobson et al. (1989) and Meire et al. (1994), who described families with a central cone disease, early stages of the disorder in our family are characterized by peripheral cone degeneration. Similar to the family described by Reichel et al. (1989), the patients in the pedigree described here seem to have lost primarily red cone function. In all XLPCD pedigrees described so far, including ours, heterozygotes show functional abnormalities in response to a red-light stimulus. A summary of clinical findings in those families for which the genetic defect is known is presented in table 1.

Reichel et al. (1989) described a progressive-cone-dystrophy family in which the clinical features apparently were associated with a deletion in the red-pigment gene in Xq28. Yet another form of X-linked cone dysfunction, BCM, apparently due to a deletion near the 5′ end of the red cone–pigment gene, has been described by Nathans et al. (1989). Although BCM and the XLPCD described by Reichel et al. (1989) are congenital disorders, clinically it may be very difficult to distinguish these disease entities from later-onset XLPCD, when early medical records of the patients involved are not available. Even the Berson test for BCM cannot differentiate BCM from X-linked progressive cone dystrophy (Pinckers 1992).

The linkage results for the family described here indicate that a second gene for XLPCD, COD2, resides in an 8-cM genomic interval in the Xq27 region. Consequently, the results presented here provide evidence for genetic heterogeneity in XLPCD. This confirms the statement of Verdoorn and Pinckers (1988), who put forward the possible existence of more than one type of X-linked cone dystrophy. The clinical differences observed in XLPCD may reflect an equally heterogeneous situation at the molecular level. Similarly to other genetic eye diseases, such as retinitis pigmentosa, "progressive cone dystrophy" may be a term to describe a group of diseases caused by distinct defects in one of multiple genes scattered along the human genome.

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