Hum Genet (1989) 84:66–70

Human Genetics

Haplotype and multipoint linkage analysis in Finnish choroideremia families

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Summary. Multipoint linkage analysis of choroideremia (TCD) and seven X chromosomal restriction fragment length polymorphisms (RFLPs) was carried out in 18 Finnish TCD families. The data place TCD distal to PGK and DXS72, very close to DXYS1 and DXYS5 ($Zmax = 24$ at $\theta = 0$) and proximal to DXYS4 and DXYS12. This agrees with the data obtained from other linkage studies and from physical mapping. All the TCD males and carrier females studied have the same DXYS1 allele in coupling with TCD. In Northeastern Finland, 66/69 chromosomes carrying TCD had the same haplotype at loci DXS72, DXYS1, DXYS4, and DXYS12. The same haplotype is seen in only 15/99 chromosomes not carrying TCD. Moreover, in 71/104 non-TCD chromosomes, the haplotype at six marker loci is different from those seen in any of the 76 TCD chromosomes. This supports the previously described hypothesis that the large Northern Finnish choroideremia pedigrees, comprising a total of over 80 living patients representing more than a fifth of all TCD patients described worldwide, carry the same mutation. These linkage and haplotype data provide improved opportunities for prenatal diagnosis based on RFLP studies.

Introduction

Choroideremia (MIM No 30310; McKusick 1988), an X-linked progressive tapeto-choroidal dystrophy, causes night blindness and progressive constriction of visual fields finally leading to total blindness in affected males. Female carriers are usually symptomless, but their carriership can be unequivocally determined by ophthalmoscopy because of progressive degeneration of the pigment layer in the fundus of the eye (McCulloch and McCulloch 1948; Sorsby et al. 1952; Kärnä 1986).

Choroideremia is rare and one fourth of the reported cases come from Finland (Kärnä 1986). The majority of the approximately 100 presently living Finnish patients belong to three large Northern Finnish pedigrees and are thought to carry the same mutation (Kärnä 1986; Sankila et al. 1987). There is no clinical evidence of heterogeneity (Kärnä 1986).

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The choroideremia gene (TCD) has been localized to Xq21 by linkage studies (Nussbaum et al. 1985; Schwartz et al. 1986; Jay et al. 1986; Sankila et al. 1987; Lesko et al. 1987) and by physical mapping using patients with X chromosomal deletions associated with choroideremia and a variety of other clinical signs, such as mental retardation, deafness, and cleft lip and palate (Rosenberg et al. 1986; Hodgson et al. 1987; Nussbaum et al. 1987; Schwartz et al. 1988; Cremers et al. 1988). Locus DXS165 was found to be deleted in two unrelated choroideremia patients who had no other symptoms (Cremers et al. 1987). Physical mapping studies have shown that, of the presently available markers, DXS165 is closest to TCD, however, the closest polymorphic marker seems to be DXS95 (Cremers et al. 1989).

Although physical mapping may soon lead to the discovery of the gene itself, there is still a need for a precise genetic linkage map of the area. We present here results of linkage and haplotype analyses with seven marker loci from the proximal long arm of the X chromosome in 18 Finnish choroideremia families.

Materials and methods

Families

Seventeen families concentrated in three geographical locations in Northern Finland (Salla, Tornio river valley and Kainuu) and one family from Southern Finland (Kotka) with choroideremia were ascertained by ophthalmological examination. Our criteria for the diagnosis have been described earlier (Kärnä 1986; Sankila et al. 1987). A total of 43 patients, 53 carriers, 18 healthy brothers, 21 healthy sisters, and 26 unrelated spouses were included in the linkage and haplotype analysis.

DNA analysis and probes

DNA was extracted from leukocytes and digested with the appropriate restriction enzymes. The DNA probes were labeled either by nick-translation (Rigby et al. 1977) or random oligomer extension (Feinberg and Vogelstein 1983). The Southern

Table 1. Characteristics of the RFLPs used in this study

Locus	Probe	Enzyme	Alleles (kb)	Allele frequencies		References
				This study[a]	HGM9[b]	
PGK	pSPT-PGK	*Bgl*I	12 9	0.61 ($n = 31$) 0.39	0.75 ($n = 80$) 0.25	Singer-Sam et al. (1983)
DXS72	pX65H7	*Hin*dIII	0.7 7	0.39 ($n = 33$) 0.61	0.55 ($n = 44$) 0.45	Schmeckpeper et al. (1985)
DXYS1	pDP34	*Taq*I	12 11	0.40 ($n = 30$) 0.60	0.34 ($n = 100$) 0.66	Page et al. (1982)
DXYS5	p31	*Taq*I	4.8 2.8	0.45 ($n = 20$) 0.55	N.A.	J. Weissenbach and D. Page, unpublished results
DXYS4	p1	*Taq*I	7 10	0.03 ($n = 30$) 0.97	N.A.	Geldwerth et al. (1985) and D. Page, unpublished results
DXYS12	St25	*Taq*I	2.1 1.6	0.41 ($n = 30$) 0.59	0.23 ($n = 93$) 0.77	Koenig et al. (1985)
DXS3	19-2	*Msp*I	16 4.6	–	0.21 0.79	Aldridge et al. (1984)
		*Taq*I	2.95, 2.2 5.1	–	0.62 ($n = 98$) 0.38	Menlove et al. (1985)

[a] *n*, Number of X chromosomes studied
[b] Pearson et al. (1987); N.A., not available

blots and hybridizations were performed according to published methods (Southern 1975; Page et al. 1982). The restriction fragment length polymorphisms (RFLPs) used are listed in Table 1.

Linkage analysis

Two-point linkage analysis was performed using the LINKAGE program (Lathrop et al. 1984). The allele frequencies in the population under study were calculated from spouses of affected males or carrier females (Table 1). The disease gene frequency was set to 10^{-3}. The disease gene was assumed to be fully penetrant both in affected males and heterozygous female carriers. Multipoint linkage analysis was performed using LINKMAP (Lathrop et al. 1984).

Results

Two-point linkage

The two-point maximum-likelihood estimates of recombination frequencies between TCD and each of seven marker loci are shown in Table 2. Two marker loci, DXYS1 and DXYS5, showed very close linkage to TCD. The lod scores for the maximum likelihood recombination distance of O centiMorgans were 13.63 for DXYS1 and 6.88 for DXYS5. The other marker loci used, PGK, DXS72, DXYS4, DXYS12, and DXS3, were found to reside within 5 to 7 centiMorgans from TCD with lod scores varying from 3.14 to 8.90.

Table 2. Two-point linkage results between TCD and marker loci

	θ_{max}	Lod	95% confidence interval
TCD vs PGK (pSPT-PGK)	0.07	4.04	0 – 0.21
TCD vs DSX72 (pX65H7)	0.05	6.09	0 – 0.16
TCD vs DXYS1 (pDP34)	0.00	13.63	0 – 0.05
TCD vs DXYS5 (p31)	0.00	6.88	0 – 0.08
TCD vs DXYS4 (p1)	0.07	3.62	0 – 0.24
TCD vs DXYS12 (St25)	0.06	3.14	0 – 0.25
TCD vs DXS3 (p19-2)	0.05	8.90	0.01 – 0.15

Multipoint linkage

For the multipoint analysis, a fixed map of marker loci with the following order was used: PGK-DXS72-(DXYS1,DXYS5)-(DXYS4,DXYS12)-DXS3 (Davies et al. 1987; D. Page, unpublished). The following estimates of the genetic distances between the markers were used: PGK-DXYS1 $\theta = 0.05$ (Kwan et al. 1988), DXYS1-DXYS12 $\theta = 0.02$ (Arveiler et al. 1987), DXYS1-DXS3 $\theta = 0.044$ (Drayna and White 1985). DXYS12-DXS3 was estimated as $\theta = 0.02$, and PGK-DXS72

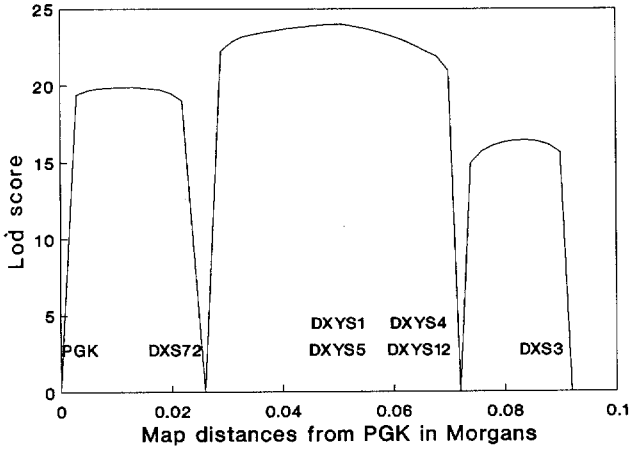

Fig. 1. Results of LINKMAP analysis when TCD is moved along the fixed map PGK-DXS72-(DXYS1,DXYS5)-(DXYS4,DXYS12)-DXS3. The confidence interval for the location of TCD is between DXS72 and (DXYS4,DXYS12). *Zmax* is 24.00 at (DXYS1,DXYS5). The map distances from PGK in Morgans are indicated on the *abscissa* and the lod score values on the *ordinate*

Table 3. Distribution of 29 different haplotypes observed in the phase-known X chromosomes of 43 patients, 33 carriers, 18 healthy brothers and 17 healthy sisters, and 26 unrelated spouses belonging to four different pedigrees. The complete haplotype of both X chromosomes could not be determined in all females. The alleles are marked as follows: PGK, Aa; DXS72, Bb; DXYS1, Dd; DXYS5, Ee; DXYS4, Ff; DXYS12, Gg. The capital letter refers to the larger fragment size, and the small letter to the smaller fragment size. The haplotype shared by the majority of the Salla patients and all Kainuu patients is underlined. *, The X chromosomal allele of DXYS5 could not be distinguished from the Y chromosomal allele

Pedigree	Haplotype	Haplotype in coupling with TCD (no. of X chromosomes)		Haplotype in repulsion with TCD (no. of X chromosomes)			
		Patients	Carriers	Healthy brothers	Healthy sisters	Carriers	Spouses
Salla	A-B-d-E-f-g	27	31	2	1	1	–
	A-B-d-*-f-g	4	–	–	–	1	–
	a-b-d-E-f-g	1	–	–	–	–	–
	A-b-d-E-f-g	1	–	1	2	6	–
	A-B-d-E-F-g	1	–	–	–	1	–
	a-B-d-E-f-g	–	–	–	1	1	1
	Other (25)	–	–	10	21	20	26
Kainuu	a-B-d-E-f-g	1	–	–	–	–	–
	a-B-d-*-f-g	3	–	–	–	–	–
	A-B-d-E-f-g	–	–	1	1	–	–
	A-B-d-E-F-g	–	–	–	1	–	–
	A-B-d-*-F-g	–	–	1	–	–	–
Tornio river valley	A-b-d-e-f-g	2	2	–	–	–	–
	A-B-D-E-f-g	–	–	2	–	–	–
	A-B-d-e-f-g	–	–	–	–	2	–
Kotka	A-B-d-e-f-g	2	–	–	–	–	–
	A-B-d-e-f-G	1	–	–	–	–	–
	A-B-D-E-f-g	–	–	1	–	–	–
Total		43	33	18	27	32	27

as well as DXS72-DXYS1 were estimated to be $\theta = 0.025$ judging from the above map distances and order. For the purposes of these calculations, DXYS1-DXYS5, and DXYS4-DXYS12 were estimated as $\theta = 0$ based on physical proximity (D. Page, unpublished results).

In the LINKMAP analysis, the position of the TCD gene was moved along the fixed genetic map described above. Figure 1 shows that TCD lies very close to (DXYS1, DXYS5) ($Zmax = 24$ at $\theta = 0$) but its exact location in relation to the marker loci cannot be determined; the confidence interval is between DXS72 and (DXYS4, DXYS12).

Haplotype analysis

The haplotype analysis was performed at six loci flanking the TCD gene: PGK, DXS72, DXYS1, DXYS5, DXYS4, and DXYS12. There were a total of 180 completely or nearly completely typed chromosomes (the allele at DXYS5 could not be typed) of which 76 carried the TCD mutation and 104 did not. Among these chromosomes, 29 different haplotypes were observed (Table 3). All 43 patients and 33 phase-known carriers studied had the same haplotype for TCD and DXYS1 (TCD/pDP34, 11 kb). The majority of Salla patients (31/34), all phase-known Salla carriers (31/31) and all Kainuu patients (4/4) had the same haplotype at DXS72, DXYS1, DXYS5, DXYS4, and DXYS12 in coupling with TCD. Four Salla patients and three Kainuu patients could not be scored for DXYS5. Assuming that these patients have the same haplotype for the entire region, then 66/69 Salla and Kainuu haplotypes in coupling with TCD are identical. Salla and Kainuu are in Northeastern Finland. The haplotypes of the patients and phase-known carriers from the Tornio river valley and of two patients from Kotka differed from the Salla-Kainuu haplotype regarding DXS72 and DXYS5 in the Tornio river valley, and DXYS5 in Kotka. A different haplotype in the third Kotka patient was the result of a crossover between TCD and DXYS12. The Tornio river is in Northwestern Finland whereas Kotka is in the South of the country.

The haplotypes in repulsion with TCD differed markedly from the haplotypes in coupling with TCD; 71/104 chromosomes not carrying TCD had haplotypes different from any of the 76 chromosomes carrying TCD.

Discussion

The finding of several patients with large overlapping X-chromosomal deletions of different sizes associated with choroideremia has facilitated the physical mapping of choroideremia in relation to various DNA markers (Rosenberg et al. 1986; Hodgson et al. 1987; Nussbaum et al. 1987; Schwartz et al. 1988; Cremers et al. 1988, 1989). These patients had various clinical abnormalities in addition to choroideremia; hence the deletion of other functionally important genes is likely. In contrast, two unrelated choroideremia patients carrying an interstitial deletion comprising DXS165 reported by Cremers et al. (1987) had no other abnormal phenotypic features. This suggests that the deletions in the latter patients are small. The closest physically mapped polymorphic marker near TCD known at present is DXS95 (Cremers et al. 1989), but it has a

relatively poor polymorphism information content (0.18) (Davatelis et al. 1985).

In this paper, we present linkage data that place TCD very close to DXYS1 and DXYS5. Indeed, the absence of recombination between TCD and these two markers prevented us from determining the order and orientation of these three loci with respect to each other by LINKMAP analysis. However, by physical mapping methods, TCD has been placed between DXS72 and DXYS1 (Cremers et al. 1989). Our results are compatible with this finding.

Genealogical studies reported previously have provided tentative evidence that all known choroideremia families in Northern Finland (Salla, Kainuu, and Tornio river valley pedigrees) descend from one founder couple born around 1640 (Forsius et al. 1980; Kärnä 1986; Sankila et al. 1987). Considering the close linkage of TCD to DXYS1 and the apparent worldwide rarity of new mutations leading to choroideremia, it is unlikely to be a coincidence that TCD is in coupling with the same allele of DXYS1 in all Finnish TCD patients so far studied (Table 3). Instead, it reinforces the idea that TCD and DXYS1 are very closely linked, and suggests that all Northern Finnish choroideremia patients carry the same mutation. Once it becomes possible directly to characterize TCD and its mutations, it should be possible to determine the number of mutations accounting for the disease worldwide. If the strength of linkage disequilibrium is inversely proportional to the distance between loci, then the absence of clear-cut allelic association between TCD and DXYS5 predicts that DXYS5 is further away from TCD than DXYS1 (Table 3). We note that, unlike the results of our previous study (Sankila et al. 1987), we now have found one recombination between TCD and DXYS12. Based on physical mapping results, the order is indeed cen-TCD-DXYS1-DXYS5-DXYS12 (Cremers et al. 1989). Given this order of loci, the absence of total linkage disequilibrium between TCD and DXYS5, or TCD and DXYS12, is not surprising.

We now present extensive haplotype data that support the hypothesis that a single TCD mutation was recently introduced into Northeastern Finland. Considering the chromosomal region within which TCD is located, and which comprises the loci DXS72, DXYS1, DXYS5, DXYS4, and DXYS12, 66 out of 69 haplotypes in coupling with TCD from Salla and Kainuu are identical (Table 3). (In 7 of the 35 patients, the allele at DXYS5 could not be determined with certainty because of the presence of a similarly sized fragment emanating from the Y chromosome). Haplotype differences in the remaining 3 patients from Salla resulted from a crossover proximal of DXYS1 in two patients, and distal of DXYS5 in one patient. Interestingly, in the Tornio river valley, which is in Northwestern Finland but only some 300 km from Salla and Kainuu, the haplotype differed from that dominant in Salla and Kainuu with respect to two loci, namely DXS72 and DXYS5. It should therefore be considered whether the Tornio river valley TCD patients might be genealogically distant from, in spite of being geographically relatively close to, Salla and Kainuu. The three patients from Kotka (in distant Southern Finland) also have haplotypes that differ from that prevalent in Salla and Kainuu. As expected, this suggests genealogical distance. However, as mentioned above, all patients and phase-known carriers share the TCD-DXYS1 haplotype; this is compatible with a single choroideremia mutation.

The significance of these haplotype data in showing TCD to be recently introduced by one or only very few founders is reinforced by the findings in non-TCD chromosomes. The common TCD haplotype for four marker loci was found in only 17 out of 104 non-TCD chromosomes. Moreover, 71 out of 104 such chromosomes had haplotypes not seen in any of the 76 chromosomes carrying the TCD mutation.

Many Finnish families with choroideremia have indicated an interest in prenatal diagnosis of male fetuses provided the accuracy of the determination is high. As long as the mutation itself cannot be identified in all families, the importance of segregation analysis is evident. Using flanking markers, the accuracy of diagnosis is now close to 100% and most women are heterozygous for at least one closely linked marker on each side of TCD. Moreover, in Northeastern Finland, haplotype analysis can be used as an adjunct to segregation analysis.

Acknowledgements. We thank Dr. J. Weissenbach for probes p1 and p31 and L. Brown for her help in characterizing the polymorphisms detected by p1 and p31. We thank Dr. J. Singer-Sam for probe pSPT-PGK, Dr. B. J. Schmeckpeper for probe pX65H7, Dr. J.-L. Mandel for probe St25, and Dr. G. Bruns for probe 19-2. This investigation was supported by grants from the Sigrid Juselius Foundation, the Academy of Finland, the Paulo Foundation, and the National Institutes of Health.

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Received April 18, 1989 / Revised June 30, 1989