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RESEARCH

Dominantly and Recessively Inherited Cornea Plana Congenita Map to the Same Small Region of Chromosome 12

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Cornea plana congenita occurs in a mild autosomal dominant (CNA1) and a more severe autosomal recessive (CNA2) form. We recently assigned a CNA2 locus to a region on chromosome 12 by linkage analysis and excluded linkage to that locus in two Finnish CNA1 families. Here we describe a Cuban pedigree in which 14 members are affected with dominantly inherited cornea plana. By linkage analysis this phenotype was mapped to the immediate vicinity of markers *D12S82* and *D12S351* on 12q, that is, precisely the same small region (3 cM or less) to which CNA2 previously had been assigned. Our results support the existence of at least three genetically distinct forms of cornea plana. It remains to be determined whether recessive and dominant cornea plana are caused by different mutations of a single gene or whether the region in 12q harbors two or more genes whose mutations cause corneal maldevelopment.

The classification of cornea plana congenita has been based on inheritance patterns and clinical signs. McKusick's catalog (1994) lists an autosomal dominant form (CNA1, MIM 121400) and an autosomal recessive form (CNA2, 217300). With the recent assignment of a recessive form to chromosome 12q (Tahvanainen et al. 1995a) and the exclusion of that locus as a cause of dominantly inherited cornea plana in two Finnish families (Tahvanainen et al. 1996), the two forms could be proven to be genetically distinct.

Clinical signs shared by the dominant and recessive forms of cornea plana include reduced corneal curvature, indistinct border of corneal limbus, and arcus lipoides at an early age. The two forms are best distinguished from each other by a round and opaque thickening, ~5 mm in width located centrally. This thickening occurs in most cases of the recessive form but never in the dominant form. Additional anomalies such as malformations of the iris, a slit-like pupil, and adhesions between the iris and cornea are more prevalent in the recessive form (Eriksson et al. 1973).

In this study we describe a newly discovered pedigree from Cuba showing dominant inheritance of cornea plana. By linkage analysis its locus was assigned to the same small chromosome region as CNA2. The result was confirmed by observing an extended conserved haplotype in all known affected individuals in three generations.

RESULTS

Pairwise lod scores between the disease phenotype (CNA1) and 12 marker loci are shown in Table 1. No obligate recombinations were observed between CNA1 and markers *D12S88*, *D12S365*, *D12S316*, *D12S82*, *D12S351*, *D12S322*, *D12S311*, and *D12S95*. The closest markers with which recombination was observed were *D12S319* on the centromeric and *D12S327* on the telomeric side, defining an interval of ~4 cM. The highest lod score of 6.98 was observed with marker *D12S322*, and the second highest lod score of 6.20 with marker *D12S351*. These two markers also displayed the highest lod scores in our previous study of CNA2.

The results of eight-point linkage analysis are shown in Figure 1. With marker *D12S81* as an

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Table 1. Pairwise lod Scores between Affection Status and 12 Marker Loci

Locus	0.00	0.001	0.01	0.05	0.10	0.20	0.30	0.40	Z_{\max}	θ_{\max}	90% confidence limits
<i>D12S81</i>	$-\infty$	3.71	4.61	4.87	4.60	3.66	2.48	1.12	0.04	4.88	$0.001 < \theta < 0.18$
<i>D12S319</i>	$-\infty$	1.30	2.26	2.75	2.71	2.19	1.44	0.62	0.07	2.77	$0.003 < \theta < 0.26$
<i>D12S88</i>	0.64	0.81	1.40	1.91	1.97	1.67	1.14	0.54	0.09	1.98	$0.002 < \theta < 0.33$
<i>D12S365</i>	5.36	5.36	5.31	5.05	4.65	3.68	2.53	1.24	0.00	5.36	$0.00 < \theta < 0.14$
<i>D12S316</i>	1.44	1.70	2.39	2.89	2.89	2.40	1.58	0.60	0.07	2.93	$0.002 < \theta < 0.27$
<i>D12S82</i>	6.15	6.14	6.05	5.63	5.08	3.90	2.61	1.24	0.00	6.15	$0.00 < \theta < 0.10$
<i>D12S351</i>	6.20	6.19	6.10	5.69	5.16	4.00	2.72	1.35	0.00	6.20	$0.00 < \theta < 0.10$
<i>D12S322</i>	6.98	6.97	6.86	6.40	5.81	4.57	3.20	1.64	0.00	6.98	$0.00 < \theta < 0.09$
<i>D12S311</i>	4.95	4.94	4.88	4.60	4.18	3.22	2.10	0.95	0.00	4.95	$0.00 < \theta < 0.13$
<i>D12S95</i>	2.89	2.89	2.88	2.79	2.60	2.09	1.47	0.76	0.00	2.89	$0.00 < \theta < 0.24$
<i>D12S327</i>	$-\infty$	-5.69	-2.17	0.23	0.98	1.22	0.88	0.37	0.18	1.23	$0.05 < \theta < 0.44$
<i>D12S309</i>	$-\infty$	-3.17	-0.25	1.48	1.90	1.81	1.30	0.66	0.13	1.95	$0.02 < \theta < 0.36$

arbitrary starting point, the maximum multi-point lod score of 8.3 was reached at a map distance of 2.0 cM. The location corresponds to marker *D12S82*. Marker *D12S351* is located 1 cM telomeric to *D12S82* and has an lod score almost

as high, 7.8. As shown in Figure 1 the only remaining marker within a ± 3 lod score interval is *D12S311*. This suggests the immediate vicinity of *D12S82* and *D12S351* as the best supported region for this dominant cornea plana gene. The

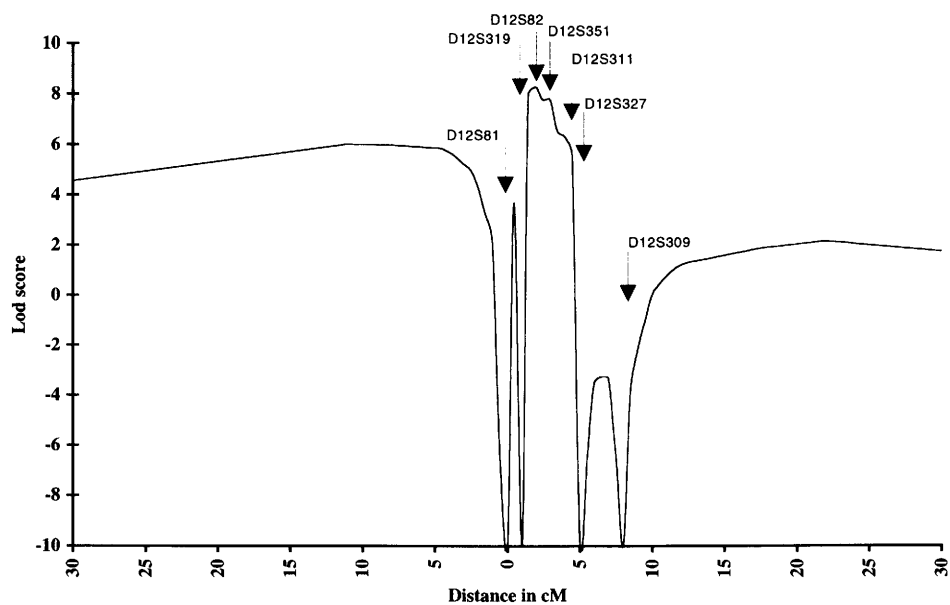


Figure 1 Eight-point linkage analysis between affection status and seven marker loci on chromosome 12 computed using the LINKMAP program. Marker *D12S81* was chosen as the starting point (zero). Sex-average distances based on Haldane map function are shown. The centromere is to the left.

CNA1 AND CNA2 MAP TO THE SAME REGION OF CHROMOSOME 12

recessive form of cornea plana was mapped previously to exactly the same location by linkage analysis; in addition, the highest value of linkage disequilibrium occurred with marker *D12S351* (Tahvanainen et al. 1995b).

The result of haplotype analysis (Table 2) gives evidence of a single haplotype shared by all patients. In its best preserved core composed of markers *D12S365*, *D12S316*, *D12S82*, *D12S351*, *D12S322*, *D12S311*, and *D12S95*, the alleles are identical in all patients. Deviations from the most common haplotype occur at the more outlying loci. These delineate inferred historical recombinations reinforcing the most probable location of the disease gene between markers *D12S88* and *D12S327*.

DISCUSSION

Our results show that mutation in a gene located in the long arm of chromosome 12 causes dominantly inherited cornea plana congenita in a Cuban pedigree (Fig. 2). To our knowledge this is the largest cornea plana congenita pedigree reported thus far that shows unambiguous dominant transmission of the trait. The assignment was surprising as we recently had excluded this location

by linkage studies in two Finnish CNA families also displaying dominant inheritance (Tahvanainen et al. 1996). Thus, a planned genome-wide search for linkage in the Cuban family did not become necessary.

The genetic length of the critical interval harboring the *CNA1* gene is somewhat smaller when derived from haplotype analysis than when deduced from obligatory recombinations detected by linkage analysis. This difference is attributable to the effects of deficient information provided by individuals in the pedigree with unconfirmed phenotype who were not available for genotyping. This result does not reflect a discrepancy between the two methods but raises the possibility that a disease chromosome may have been introduced into the pedigree more than once. Nonetheless, the results of the haplotype analysis are consistent with the hypothesis that one ancestral disease chromosome is shared by all affected individuals in this pedigree. This is a reasonable assumption in this rare disorder.

Somewhat surprisingly, these results document the existence of at least one recessively and two dominantly inherited forms of cornea plana. Earlier clinical descriptions are not necessarily at variance with this notion, in that they show

Table 2. Disease Haplotypes for Each Affected Individual Constructed Assuming the Smallest Number of Recombinations

Affected Individual	Alleles at marker locus <i>D12S#</i>												
	81	319	88	365	316	82	351	322	311	95	327	309	
1	8	8	6	ND	3	4	4	6	3	10	14	4	
2	8	8	6	1	3	4	4	6	3	10	5	3	
3	8	8	6	1	3	4	4	6	3	10	14	4	
4	8	8	6	1	3	4	4	6	3	10	5	3	
5	8	8	6	1	3	4	4	6	3	10	5	3	
6	8	8	6	1	3	4	4	6	3	10	5	3	
7	8	8	8	1	3	4	4	6	3	10	14	4	
8	8	8	6	1	ND	4	4	6	3	10	14	4	
9	8	8	8	1	3	4	4	6	3	10	14	4	
10	8	8	8	1	3	4	4	6	3	10	14	4	
11	8	8	6	1	3	4	4	6	3	10	5	3	
12	8	8	6	1	3	4	4	6	3	10	5	3	
13	8	8	6	1	3	4	4	6	3	10	5	3	
14	8	8	6	1	3	4	4	6	3	10	5	3	

(ND) Not determined.

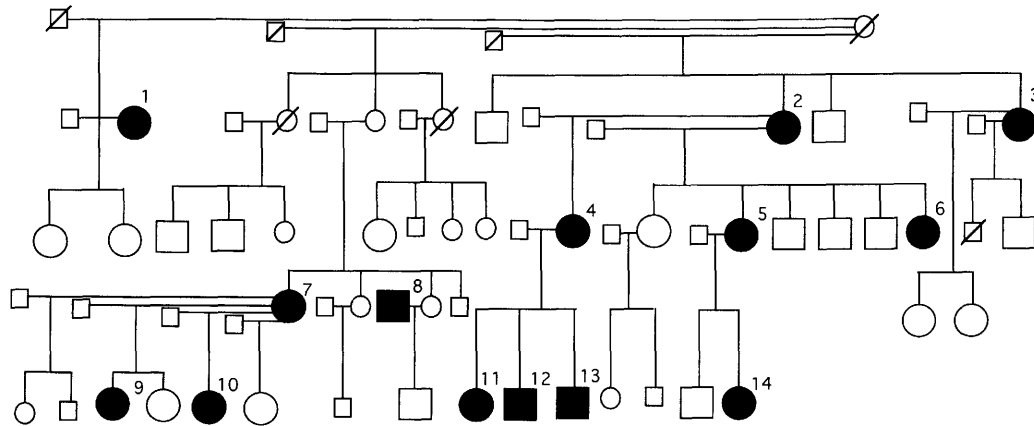


Figure 2 Pedigree of a four-generation family from Cuba displaying dominant cornea plana. To trim the size and display the study strategy, those individuals who were included in the linkage study are shown with larger symbols than the others. The absence of the cornea plana trait among those individuals who are marked with smaller symbols is not necessarily based on ophthalmologic examination in all cases. Those individuals were assigned disease status unknown in the linkage study. Numbers to the *right* of the symbol for each affected individual correspond to those shown in Table 2.

marked variation in phenotype (Friede 1921; Kokkott 1937; Larsen and Eriksen 1949; Eriksson et al. 1973; Ruprecht and Nauman 1974; Elliot et al. 1985). Two clinical types of cornea plana were suggested previously (Larsen and Eriksen 1949). Therefore, it is not entirely surprising that we found genetic heterogeneity in dominant cornea plana.

The most interesting of our findings is the colocalization of the loci for a dominantly and recessively inherited form of the "same" disease. Clearly this could be attributable to one of two alternative situations. Either both diseases are caused by mutations in the same gene or there are two or more genes adjacent to each other whose mutations can lead to a disturbance of corneal development.

The first hypothesis of one gene causing two disorders with different modes of inheritance is not without precedents. Examples of different mutations in one gene causing either dominant or recessive mode of inheritance include von Willebrand disease (Zhang et al. 1992), myotonia congenita (George et al. 1993; Lorenz et al. 1994), and epidermolysis bullosa (Christiano et al. 1993, 1994). Although the majority of mutations are recessive to wild type, the molecular mechanisms leading to a dominant mode of inheritance have been reviewed recently (Wilkie 1994). In short, the categories of dominant inheritance include alterations in gene dosage or protein activity, ectopic or temporally altered expression, domi-

nant-negative effects, altered structural proteins, and toxic or new protein functions.

The second hypothesis of adjacent genes causing similar disorders may be even more likely, as there is abundant documentation of gene families or groups of genes that cluster in a chromosomal region and whose protein products have similar functions or participate in similar metabolic pathways. One example that might be relevant to cornea plana is the keratin gene cluster at 12q13 (Yoon et al. 1994). Keratin genes *KRT3* and *KRT12* encode the major keratins expressed in adult corneal epithelium in a number of species (Schermer et al. 1986; Kasper et al. 1988; Chaloin-Dufau et al. 1990). In the developing mouse eye *KRT12* is first expressed at embryonic day 15 in corneal epithelium, later in all layers of the cornea, and at no stage in any other tissue than the cornea (Kurpacus et al. 1994). Neither the cytogenetic localization nor the nucleotide sequence of *KRT12* (type I keratin) is known. Most of the genes for type I keratins are located on chromosome 17, but keratin 18 (*KRT18*) is located next to its partner keratin 8 (*KRT8*) in the chromosome 12 cluster. We are attempting to determine whether any keratin genes, especially *KRT12*, might be located in the CNA region.

Determination of the molecular mechanisms leading to cornea plana requires the cloning of candidate genes in the disease region and the discovery of disease-causing mutations. Such work is in progress in our laboratory.

MATERIALS AND METHODS

Patients

The patients were discovered and diagnosed in and around Moron City, Cuba. All patients belong to a four-generation pedigree (Fig. 2). Typical symptoms of dominant cornea plana observable in patients include reduced corneal curvature, hazy corneal limbus, and arcus lipoides. The corneal refraction of affected persons varies between 34.5 and 42.75 diopters (D), (mean in males 39.8 D, and in females 39.6 D) and the corneal diameter varies between 8.75 and 11.25 mm (mean in males 10.3 mm, and in females 9.9 mm). For comparison, in unaffected family members the mean corneal refraction is 43.6 D in males and 44.2 D in females and the mean horizontal diameter is 11.4 mm in males and 11.3 mm in females.

Linkage and haplotype analysis

Blood samples were collected from 14 affected persons and 16 unaffected relatives who were most relevant for linkage studies and who consented to take part in the study. DNA was isolated from blood leukocytes. The analyses were based on polymorphic markers from the Généthon collection (Gyapay et al. 1994) and published information on their order and distances. Allele numbering was based on the current on-line Genome Data Base version (Pearson 1991; Pearson et al. 1992; Cuticchia et al. 1993). The PCRs were done using published protocols (Weber et al. 1989). For linkage analyses the programs of the LINKAGE program package (Lathrop et al. 1984) were used. The 90% confidence limits shown in Table 1 are based on the ± 1 lod unit method. For multipoint analysis marker genotypes were reduced to three-allele loci with equal allele frequencies. When several markers were reported without known intermarker distance and order, only the most informative marker from such a group was chosen for the multipoint analysis. As no significant sex difference was observed by CILINK analysis, sex-average recombination fractions were used in the construction of the multipoint map. All computations assumed dominant inheritance with complete penetrance. Haldane map function was used for the multipoint map. The disease allele frequency was arbitrarily set to 0.001. A LINKMAP analysis was carried out against a fixed map of four marker loci at the time in the order centromere–*D12S81*–*D12S319*–*D12S82*–*D12S351*–*D12S311*–*D12S327*–*D12S309*–telomere with distances of 1, 1, 1, 1, and 3 cM, respectively, between the markers.

Likely haplotypes were constructed based on a minimum number of recombinations for those loci in which the haplotypes could not be determined by studying parents. The relative order of the four markers *D12S319*, *D12S88*, *D12S365* and *D12S316*, and the three markers *D12S311*, *D12S322*, and *D12S95* has not yet been determined by recombinational analysis in pedigrees from the Centre d'Etude du Polymorphisme Humain (Gyapay et al. 1994). This ambiguity was taken into account in the interpretation of the results.

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