Original Paper

Eur J Hum Genet 1994;2:132–138

H. Eiberg[a]
H.U. Møller[b]
I. Berendt[a]
J. Mohr[a]

[a] University Institute of Medical Biochemistry and Genetics, Department of Medical Genetics, Genome Group, Danish Centre for Genome Research, Copenhagen, and
[b] Department of Ophthalmology, University Hospital, Aarhus, Denmark

Assignment of Granular Corneal Dystrophy Groenouw Type I (CDGG1) to Chromosome 5q

Abstract

Granular corneal dystrophy Groenouw type I (CDGG1) is an autosomal dominant disease with complete penetrance. 124 blood samples were collected from a single Danish pedigree of seven generations. Linkage was discovered with markers on chromosome 5q, with IL9 ($Z = 15.96$; $\theta_M = 0.027$, $\theta_F = 0.00$) and D5S436 ($Z = 11.75$; $\theta_M = 0.00$, $\theta_F = 0.081$) flanking the disease locus most closely. The marker IL9 is located in the region 5q22-q32. By multilocus linkage analysis the most likely position of CDGG1 among 9 markers was: D5S396-IL9-CDGG1-D5S436-D5S210/D5S207-D5S434-D5S119-D5S211 and CDGG1-D5S402-D5S434. In each of two independent small pedigrees, in which a milder form of CDGG1 occurs, the disease gene was also linked to IL9 ($Z = 3.02$ at $\theta = 0.0$ in males and females); i.e. the severe and the milder forms may be allelic.

Key Words

Granular corneal dystrophy
Groenouw type I
Eye disease
Linkage analysis
Chromosome 5
Gene mapping

Introduction

Granular corneal dystrophy Groenouw type I (CDGG1) [ref. 1; MIM No. 121900] is an autosomal dominant disease of the cornea [2, 3]. There is complete penetrance, i.e. opacities in all carriers of the gene at 5 years of age and an increasing number of opacities through the following years [4, 5]. At around 50 years of age visual acuity is approximately 0.5, and at 70 years most patients have serious visual problems.

The opacities consist of greyish-white granules with sharp borders in the central area of the cornea. The distribution of granules varies between families [4], which may represent different mutant alleles or different loci. A Finnish family, also with autosomal dominant inheritance, but with mildly affected cases and later age at onset (between 15 and 20

Received: July 29, 1993
Revision received: February 2, 1994
Accepted: February 18, 1994

Hans Eiberg
Institute of Medical Biochemic and Genetics
Department of Genetics, Building 24.4
Blegdamsvej 3
DK-2200 Copenhagen N (Denmark)

1018–4813/94/
0022–0132$5.00/0

years) has been described [4, 6]. A recessive form has also been claimed [7], but these patients may have macular dystrophy of the cornea (Groenouw type II) [5]; Reis-Bücklers' corneal dystrophy has been proposed as a form of CDGG1 [8].

The granules have been shown to consist of a non-collagenous protein containing tyrosine, tryptophan, arginine and sulphur-containing amino acids, which may suggest a relationship to either amyloid or keratin [9]. Phospholipids [10] and immunoglobulins [11] have been found in the deposits.

In an earlier study, CDGG1 was scored for linkage against the blood group systems ABO, MNS and RH, but with negative results [12]. In a French family, association with an uncommon green eye colour in 6 patients out of 9 in two generations was observed [13]. Reanalysis of this pedigree with LIPED [14] gave a lod score below 1.0, while a study of 13 markers on chromosomes 1, 2, 4, 6, 8, 14 and 20 gave a positive lod score (z = 0.57) with GPT [15].

A linkage analysis with 35 markers in a large Danish family considered here [16] gave a positive lod score (z = 1.04) to the C1R serum protein (chromosome 12). The analysis of this family is here extended by inclusion of PCR markers [17–19]. Highly positive lod scores were found to the microsatellite markers IL9, D5S210, D5S119, D5S396, D5S402, D5S434 and D5S436, suggesting that the disease locus is on chromosome 5q, region 5q22-q33. Similar results were found in two subsequently examined smaller Danish families with a variant of CDGG1.

Material and Methods

Family Material

Blood samples from 124 individuals, all older than 5 years of age and comprising 52 affected and 72 non-affected persons, were collected from one large pedigree (family 1) [20]. Two additional small families independent of the large family and of each other (families 2 and 4) [20] were also collected for study of linkage and possible heterogeneity. Serum, erythrocytes and DNA were isolated from all blood samples of the three families and frozen for later typing [21].

Linkage Analysis

A total of 35 classical markers was tested and analysed for linkage [16]. Later 10 RFLP and 16 PCR systems were tested until a highly significant positive lod score was obtained using the computer program LIPED [14]. The scores L ($\theta_M = \theta_F$) of chromosome 5 markers, as shown in table 1, were calculated using the computer program LINKAGE [22]. The gene order was assessed both by manual haplotype drawing and ordering, and through multipoint analysis by the computer program LINKMAP.

DNA Amplification

The radioactive PCR was performed in a volume of 10 µl in microtitre plates (Hybaid). Reactants comprised 50 ng of template DNA, 1–3 µ*M* of non-label and ^{32}P-end-labelled primer, 0.1 m*M* of each dNTP, 1.5 m*M* $MgCl_2$, and 0.15 U of Taq DNA polymerase in buffer (Promega). The amplification was run in a programmable heating block (Omnigene, Hybaid) for 27 cycles comprising 94°C for 30 s, 55°C for 0.30 s and 72°C for 30 s. The amplified fragments were separated by electrophoresis for 1–3 h at 100 W in a 6% denaturing polyacrylamide sequencing gel (25 × 42 × 0.4 cm). Gels were fixed for 10 min (10% acetic acid), washed for 20 min, dried and exposed to X-ray film (XAR-5 Kodak) overnight.

Results and Discussion

By linkage analysis employing the computer program LINKAGE [22], lod scores of Z = 15.96 ($\theta_M = 0.027$, $\theta_F = 0.00$) to IL9, Z = 19.45 ($\theta_M = 0.00$, $\theta_F = 0.065$) to D5S210 and Z = 2.29 (θ_M 0.07, $\theta_F = 0.416$) to D5S119 were first obtained in the large pedigree (table 1). All markers are located on chromosome 5q, in the area 5q22-q32 for IL9 and 5q31.3-5q33.3 for D5S119 and D5S210 [23].

Only one crossing over was found between the disease locus (CDGG1) and IL9 and three crossing overs between D5S210 and CDGG1.

Table 1. Linkage relations of Groenouw I and chromosome 5 markers: lod (Z) scores at various recombination fractions of ($\theta_M = \theta_F$) in family 1

Markers	θ =	0.05	0.10	0.20	0.30	0.40	Z_{max} at	θ(M;F)
CDGG1-D5S396		8.95	8.40	6.49	4.15	1.73	9.63	8.3;0.0
CDGG1-IL9		15.13	13.85	10.81	7.40	3.40	15.96	2.7;0.0
CDGG1-D5S436		10.93	10.26	8.19	5.72	2.98	11.75	0.0;8.1
CDGG1-D5S402		2.81	2.77	2.11	1.28	0.55	3.20	0.0;12.0
CDGG1-D5S210		18.64	17.55	14.13	9.90	5.11	19.45	0.0;6.5
CDGG1-D5S207		9.46	9.50	7.91	5.49	2.65	9.73	4.3;9.4
CDGG1-D5S434		1.40	3.46	4.10	3.18	1.59	4.55	10.4;23.6
CDGG1-D5S119		−3.76	−0.83	1.00	1.12	0.57	2.29	7.0;41.6
CDGG1-D5S211		−15.42	−8.73	−3.07	−0.81	−0.03	0.25	33.6;49.1
D5S396-IL9		7.81	7.27	5.26	3.01	1.06	7.81	4.5;4.6
D5S396-D5S436		4.41	4.49	3.46	2.08	0.82	4.69	5.6;12.0
D5S396-D5S402		1.66	1.68	1.21	0.65	0.21	7.81	4.5;4.6
D5S396-D5S210		7.08	7.08	5.48	3.27	1.56	7.60	3.0;11.0
D5S396-D5S207		6.47	6.29	4.91	3.10	1.25	6.54	4.5;7.6
D5S396-D5S434		3.62	4.47	3.90	2.40	0.84	4.55	9.5;13.9
D5S396-D5S119		−3.55	−0.92	0.74	0.82	0.35	0.90	23.9;27.0
D5S396-D5S211		−9.59	−5.19	−1.78	−0.52	−0.10	0.93	19.9;54.3
IL9-D5S436		12.87	12.17	9.43	6.11	2.72	13.14	2.3;8.3
IL9-D5S402		9.49	8.24	5.67	3.18	1.17	10.70	0.0;0.0
IL9-D5S210		15.19	14.27	11.08	7.30	3.43	15.22	4.6;3.6
IL9-D5S207		12.26	11.02	8.16	5.15	2.24	13.16	0.0;3.3
IL9-D5S434		4.48	5.73	5.14	3.28	1.25	6.85	6.8;20.2
IL9-D5S119		−1.83	0.55	1.76	1.43	0.58	2.25	13.6;39.1
IL9-D5S211		−10.88	−5.84	−1.85	−0.44	−0.04	0.23	34.8;53.9
D5S436-D5S402		8.63	7.59	5.38	3.13	1.16	9.60	0.0;0.0
D5S436-D5S210		16.56	14.88	11.10	7.13	3.27	17.83	0.0;3.0
D5S436-D5S207		11.52	10.48	7.94	5.12	2.26	12.41	0.0;5.4
D5S436-D5S434		6.72	7.00	5.66	3.58	1.47	7.48	4.7;15.4
D5S436-D5S119		−0.84	0.78	1.41	1.04	0.43	2.80	8.2;45.2
D5S436-D5S211		−11.13	−5.99	−1.88	−0.42	−0.01	0.11	36.8;48.9
D5S402-D5S210		8.78	7.56	5.14	2.88	1.05	9.99	0.0;0.0
D5S402-D5S207		7.62	6.65	4.66	2.68	1.00	8.54	0.0;0.0
D5S402-D5S434		3.38	3.42	2.61	1.51	0.52	4.61	0.0;22.2
D5S402-D5S119		−1.35	−0.23	0.39	0.33	0.10	0.69	16.8;47.3
D5S402-D5S211		−6.83	−3.91	−1.48	−0.50	−0.11	0.00	50.4;51.8
D5S210-D5S207		16.24	14.46	10.75	6.92	3.11	17.97	0.0;0.0
D5S210-D5S434		10.10	10.09	7.94	5.01	2.11	10.55	4.2;10.6
D5S210–D5S119		−0.45	1.62	2.37	1.77	0.74	4.43	5.1;36.3
D5S210-D5S211		−14.23	−7.65	−2.34	−0.40	0.02	0.15	39.1;43.3
D5S207-D5S434		6.42	6.69	5.50	3.56	1.42	7.02	4.0;13.5
D5S207-D5S119		−0.95	0.89	1.73	1.38	0.61	2.64	7.3;34.1
D5S207-D5S211		−9.51	−4.96	−1.42	−0.25	0.01	0.31	25.8;47.5
D5S434-D5S119		3.14	3.86	3.39	2.17	0.88	3.90	11.2;12.3
D5S434-D5S211		−9.43	−4.76	−1.26	−0.13	0.11	0.22	32.5;44.2
D5S119-D5S211		−9.64	−4.94	−1.26	−0.06	0.18	0.50	23.1;48.3

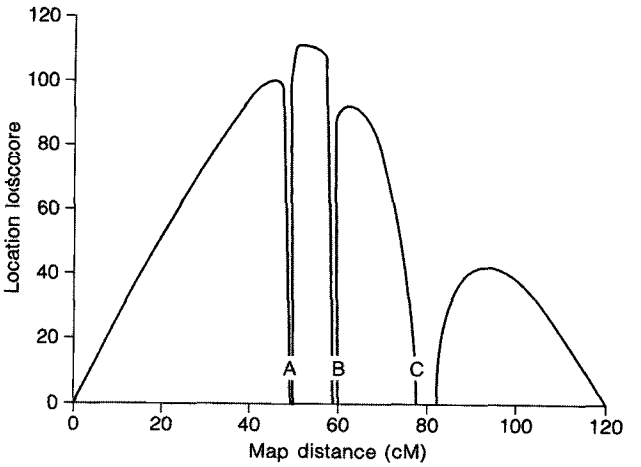

Fig. 1. LINKMAP scores of CDGG1 against the markers IL9 (A), D5S210 (B) and D5S119 (C). The most likely position of the disease was found to be between A and B, i.e. the order IL9-CDGG1-D5S210-D5S119, with a maximum location lod score difference of Z = 12 against the most likely other possibility.

Another marker, D5S211, which maps to the area 5q33.3-5qter gave negative lod scores. For more precise localisation, the additional marker D5S207 [19] and later the markers D5S396, D5S434, D5S436 and D5S402 [18] were considered.

Reference linkage maps of 5q including PCR markers show the following order: IL9-GRL-D5S210/D5S207-CSF1R/D5S119-D5S211 [23] and -D5S396-D5S436/D5S402-D5S434- [18]. Through four-point analysis using ILINK, LINKMAP and haplotype analysis (fig. 1) with IL9 (A), D5S210 (B) and D5S119 (C) the most likely position of CDGG1 was found to be: IL9-CDGG1-D5S210-D5S119. For the purpose of positioning the disease locus, the Weissenbach marker D5S396 was analysed against the two flanking markers IL9 (A) and D5S210 (B), and D5S396 was found to be in the order: D5S396-IL9-D5S210 (fig. 2). We could then position the disease locus into the map of Weissenbach et al. [18] as follows: D5S396-CDGG1-D5S436-D5S434 (fig. 3).

In an attempt to connect the two established linked groups, the following order was suggested: D5S396-IL9-CDGG1-D5S436-D5S210/D5S207-D5S434-D5S119-D5S211. This order was derived from the following four-point analysis by the program ILINK: D5S396-IL9-CDGG1-D5S436; CDGG1-D5S436-D5S210-D5S434; D5S210-D5S434-D5S119-D5S211 and D5S436-D5S207-D5S434-D5S119. The order of markers in each group was supported over the next most likely order with odds > 1,000:1, regardless of whether likelihoods were calculated assuming equal female:male recombination fractions or allowing for differences in the female:male recombination frequences. We found no recombination between the marker D5S402 against the markers D5S207, D5S210 and D5S436. ILINK analysis of D5S402 gave the order CDGG1-D5S402-D5S434. No recombinations between D5S207 and D5S210 or between D5S402 and D5S436 were found in this or the CEPH material [18].

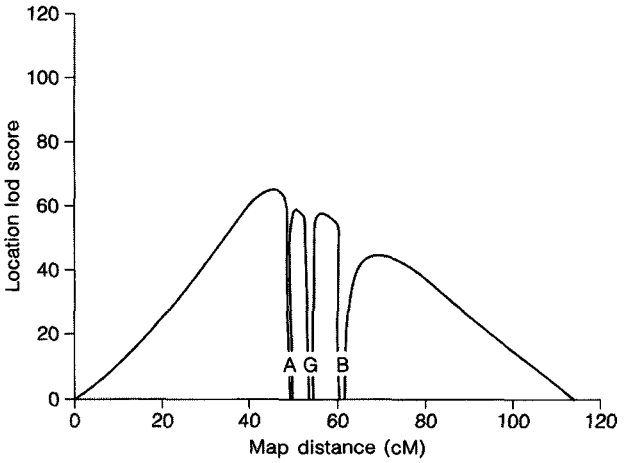

Fig. 2. Scores of D5S396 in the fixed marker position IL9-CDGG1-D5S210 (A-G-B). The most likely position of D5S396 was found to be proximal to A, i.e. the order: D5S396-IL9-CDGG1-D5S210.

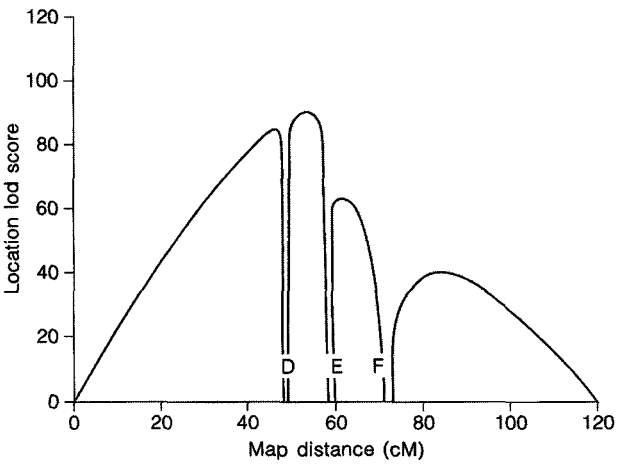

Fig. 3. Location of CDGG1 in the fixed marker sequence of D5S396-D5S436-D5S434 (D-E-F) [18]. The most likely position of the disease gene seems to be between D5S396 (D) and D5S436 (E).

Heterogeneity

Two small pedigrees (families 2 and 4) in which the mode of inheritance is also autosomal dominant, but with a later age at onset and milder expression, both gave a positive lod score to IL9 [Z = 2.11 in family 2 and Z = 0.903 (θ_M, θ_F) = (0,0) in family 4. The severe and the milder forms might represent different alleles at the same locus. The disease in these three Danish families is associated with entirely different haplotypes, which suggest that they are all of different mutational origin. Reis-Bücklers' disease and granular dystrophy have some common clinical signs and the

same locus may be involved [8]. Linkage analysis in Reis-Bückler families might clarify this question.

Disease Gene

About 24 genes (ADRA1B, ADRB2, ARH9, CAMK4, CCA, CD14, CD49B, CSF2, DHLAG, DTD, DTS, EGR1, FBN2, FGFA, GRL, IL3, IL4, IL5, IFR1, LGMD1, PDEA, SPARC, TCOF1, TCF7) have been mapped in the region of IL9 (5q22-q32). Many of these are endothelial cell growth factors and receptors. The marker GRL (glucocorticoid receptor gene) which maps to 5q31-32, between IL9 and D5S210, although being close to CDGG1, is not an obvious candidate gene, but is important for more precise localization of CDGG1.

The locus for fibrillin 2 is involved in a Marfan-like disease [24]. The eye is also involved in this syndrome, i.e. dislocation of the lens, but the pathology is very different from that of CDGG1, and the fibrillin 2 locus is not therefore a serious candidate. SPARC (osteonectin) is a calcium-binding acid secreted glycoprotein [25], rich in cysteine. SPARC may be considered a candidate gene for CDGG1 because it is rich in sulphur-containing amino acids, and because, through inhibition of calcification or assembly or modification of glycoprotein compounds, it is involved in the production and assembly of extracellular matrix material (in bone and other tissue). So far, no human genetic disorder has been associated with mutations in the SPARC gene. To identify the gene for CDGG1 we plan to screen the region between IL9 and D5S243/D5S210 for cDNAs from a cDNA library using YAC and cosmid contigs from this region.

Acknowledgements

The skilful assistance of Ms. Joanna Amenuvor, Ms. Annemette Olsen, Ms. Lillian Rasmussen, Mr. Jens Klausen, and Ms. Mona Kristensen is gratefully acknowledged. We thank Dr. Claes Wadelius, Akademiske Sjukhuset, Department of Clinical Genetics, Uppsala, for supplying PCR primers. This work was supported by grants from the Danish Medical Research Council, The Danish Eye Research Foundation (Øjenfonden) and The Danish Society for Eye Disease and Blindness (Værn om synet).

References

1 McKusick VA: Mendelian Inheritance in Man, ed 10. Baltimore, The Johns Hopkins University Press, 1992, p 271.
2 Groenouw A: Knötchenförmige Hornhauttrübungen (Noduli corneae). Arch Augenheilkd 1890;21: 281–289.
3 Groenouw A: Knötchenförmige Hornhauttrübungen vererbt durch vier Generationen. Klin Monatsbl Augenheilkd 1933;90:577–580.
4 Møller HU: Inter-familial variability and inter-familial similarities of granular corneal dystrophy, Groenouw type I. Acta Ophthalmol (Copenh) 1989;67:669–677.
5 Møller HU: Granular corneal dystrophy Groenouw type I: Clinical and genetic aspects. Acta Ophthalmol (Copenh) 1991;69 (suppl 198): 1–40.
6 Forsius H, Eriksson AW, Kärna J, Tarkkanen A, Aurekoski H, Frants RR, Damsten M: Granular corneal dystrophy with late manifestation. Acta Ophthalmol (Copenh) 1983; 61:514–528.
7 Padma T, Murty JS, Reddy PR: A rare family of recessive granular corneal dystrophy with the incidence of haemoglobulin and haptoglobin. Afro-Asia J Ophthalmol 1982;1:38–42.
8 Møller HU: Granular corneal dystrophy Groenouw type I (GrI) and Reis-Bücklers' corneal dystrophy (R-B): One entity? Acta Ophthalmol (Copenh) 1989;67:678–684.
9 Garner A: Histochemistry of corneal granular dystrophy. Br J Ophthalmol 1969;53:799–807.
10 Rodrigues MM, Streeten BW, Kracher JH, Laibson PR, Salem N, Passonneau J, Chock S: Microfibrillar protein and phospholipid in granular corneal dystrophy. Arch Ophthalmol 1983;101:802–810.

11 Møller HU, Bojsen-Møller M, Schrøder HD, Nelson ME, Vegge T: Immunoglobulins in granular corneal dystrophy Groenouw type I. Acta Ophthalmol (Copenh), in press.
12 Bourquin JB, Babel J, Klein D: Nouvel arbre généalogique de dystrophie cornénne granuleuse (Groenouw I). J Génét Hum 1954;3:137–146.
13 Nizetic B, Sakic D: Dégénération nodulaire de la cornée (Groenouw) liée à la couleur de l'iris. Acta Genet Stat Med 1957;7:274–276.
14 Ott J: A computer programme for linkage analysis of general human pedigree. Am J Hum Genet 1973; 28:528–529.
15 Kömpf J, Ritter H, Lisch W, Weidle EG, Baur MP: Linkage analysis in granular corneal dystrophy (Groenouw I), Schnyder's crystalline corneal dystrophy, and Reis-Bücklers' corneal dystrophy. Graefes Arch Clin Exp Ophthalmol 1989;227: 538–540.
16 Møller HU, Eiberg H, Kruse TA: Linkage relations of the locus for granular corneal dystrophy Groenouw type I with 35 polymorphic systems. Acta Ophthalmol (Copenh) 1989;67:721–723.
17 Eiberg H, Møller J, Berendt I, Mohr J: Assignment of granular corneal dystrophy Groenouw type I (CDGG1) to chromosome 5q: Close linkage to IL9, D5S210 and D5S119 (abstract). ESHG meeting, Barcelona, May 1993.
18 Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M: A second-generation linkage map of the human genome. Nature 1992;359: 794–801.
19 Weber JL, Polymeropoulos MH, May PE, Kwitek AE, Xiao H, McPherson JD, Wasmuth JJ: Mapping of human chromosome 5 microsatellite DNA polymorphisms. Genomics 1991;11:695–700.
20 Møller HU: Granular corneal dystrophy Groenouw type I – 115 Danish patients. Acta Ophthalmol (Copenh) 1990;68:297–303.
21 Eiberg H, Nielsen LS, Klausen J, Dahlén M, Kristensen M, Bisgaard ML, Møller N, Mohr J: Linkage between serum cholinesterase 2 (CHE2) and γ-crystalline gene cluster (CRYG): Assignment to chromosome 2. Clin Genet 1989;35:313–321.
22 Lathrop GM, Lalouel JM: Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 1984;36:460–465.
23 Wasmuth JJ, Bishop DT, Westbrook CA: Report of the committee on the genetic constitution of chromosome 5. Cytogenet Cell Genet 1991;58:261–294.
24 Lee B, Godfrey M, Vitale E, Hori H, Mattei MG, Sarfarazi M, Tsipouras P, Ramirez F, Hollister DW: Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 1991;352:330–334.
25 Swaroop A, Hogan BLM, Francke U: Molecular Analysis of the cDNA for human SPARC/osteonectin/ BM-40: Sequence, expression, and localization of the gene to chromosome 5q31-q33. Genomics 1988;2: 37–47.