Short Report

European Journal of Human Genetics

Eur J Hum Genet 1996;4:237–241

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Assignment of Genes Coding for Brown Eye Colour *(BEY2)* and Brown Hair Colour *(HCL3)* on Chromosome 15q

Key Words
Eye colour
Hair colour
Genetic map
Linkage analysis
Chromosome 15
Pink-eye dilution

Abstract

Brown eye colour (BEY), or total brown iris pigmentation is one of the most common phenotypes of iris colour. Data of eye colour as well as hair colour were obtained for linkage analysis through an enquiry in our family material of 832 families from Copenhagen area. By exclusion mapping with 80 markers in 120 segregating families and 290 markers in 5 segregating families, we obtained some indication of a locus *BEY2* for brown eye colour on chromosome 15. For possible confirmation, we selected a total of 45 families from our DNA bank segregating for BEY. All these were tested for chromosome 15 markers in the area between *D15S11* and *CYP19*. We found a strong indication of tight linkage with the DNA polymorphism *D15S165* ($Z = 24.25$; $\theta_{M=F} = 0.010$) and with the flanking markers *D15S156* ($Z = 14.04$; $\theta_{M=F} = 0.0.029$) and *D15S144* ($Z = 12.99$; $\theta_{M=F} = 0.060$). *BEY2* is assigned to the region 15q11–15q21 by physically localized markers. A new locus for brown hair colour (HCL3) was localized by indication of linkage to *BEY2* in our 45 families segregating for BEY ($Z = 9.93$; $\theta_{M=F} = 0.10$). The gene *(DN10* or *P)* homologous to the pink-eye-dilution gene (p) in mice could be a candidate gene for *BEY2* or for *HCL3*.

Introduction

Brown eye colour (BEY), or total brown iris pigmentation (MIM# 227220) is in many populations the only phenotype of iris colour. Populations with other eye colours such as blue, grey, green and central brown are common among Caucasians [1]. Eye colour, as well as hair colour have been considered polygenic traits and stringent recognition of individual loci is accordingly difficult. However, if in some families segregation of one distinctive phenotype is apparent, and in other families another phenotype, pooling of each kind of family for linkage analysis may permit a recognition of individual major gene influences. Only few linkage studies of eye colour have been made. Gedde- Dahl [2] found positive lod scores between brown eye colour *BEY1* (later described as central brown eye colour) and the blood groups Colton (CO) and Kidd (JK). Another phenotype, 'green' eye colour *(GEY)*, has later been mapped to chromosome 19 by linkage to the blood types Secretor and Lutheran (LU). Also a gene for brown hair colour *(HCL1)* segregates with *GEY* ($Z = 5.6$; $\theta_{M=F} = 0.10$) [1]. No earlier linkage studies have been made of total brown eye (iris) colour.

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1018–4813/96/0044–0237$10.00/0

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Received:
September 5, 1995
Revision received:
February 2, 1996
Accepted: May 3, 1996

Table 1. Lod scores between total brown eye colour BEY and chromosome 15 loci in 45 informative families

Marker	Recombinations fractions ($\theta_{M=F}$)						
	0.01	0.05	0.10	0.20	0.30	Z_{max}	θ(M;F)
D15S11	−5.93	−0.19	1.67	2.39	1.74	2.84	0.500;0.094
D15S113	9.75	13.68	13.83	11.29	7.29	14.35	0.106;0.041
D15S156	13.70	13.87	12.89	9.93	6.22	14.67	0.001;0.065
D15S165	24.25	23.15	21.02	15.95	10.13	24.56	0.001;0.021
D15S144	10.80	12.95	12.62	10.02	6.39	13.31	0.028;0.090
D15S118	−4.52	1.54	3.28	3.55	2.43	4.35	0.500;0.096
D15S129	−8.92	−1.91	0.51	1.91	1.73	2.02	0.188;0.500
D15S1	−3.52	−1.42	−0.62	−0.05	0.07	0.08	0.500;0.300
CYP19	−7.85	−1.46	0.77	2.10	1.98	2.27	0.191;0.281
D15S2	−0.03	0.77	1.00	0.95	0.66	1.14	0.000;0.150

Materials and Methods

The family material consists of 832 families with at least 4 children living in the Copenhagen area. Serum, erythrocytes and lymphocytes were isolated from all blood samples and frozen for later typing [3]. DNA was extracted from Epstein-Barr transformed frozen lymphocytes. DNA amplification and RFLP analysis were made by standard methods [4].

The primary classification of eye and hair colour was made on the basis of an enquiry on 832 families [1]. We asked the families to classify their own eye colour by the following main categories: purely blue, blue, grey, brown, and 'don't know'; further we asked them to say whether there were brown spots or a brown ring around the pupil. In the case of hair colour (scored both as child and as adult), the categories were the following: white, blond, brown, black, red or grey. We had DNA from 48 families in which brown eye colour was segregating, and succeeded in contacting all these families for further enquiry. Three families turned out to be distinctive, since all their 'brown eyed' persons turned out to have green spots (or a green area) around the pupil i.e. the phenotype for central brown eye colour *(BEY1)*.

In the 45 families with fully brown eye colour, 25 fathers and 21 mothers had brown eye colour. The intensity of brown eye colour in supposed heterozygote carriers (i.e. brown-eyed parents with at least one non-brown-eyed child) varied between persons from dark brown to yellow-brown. Spouses and children with green-brown eye colour were assumed to have the gene 'green' on the locus *BEY1/GEY*, and for the purpose of linkage analysis as non-brown. An excess above a Mendelian expectation of non-brown eye colour was found among the children: 123 children with non-brown eyes (100 with blue and 23 with green) against 104 children with brown eye colour (123:104). This deviation is non-significant statistically, and the familial distribution of total brown eye colour *BEY2* is compatible with an autosomal dominant model.

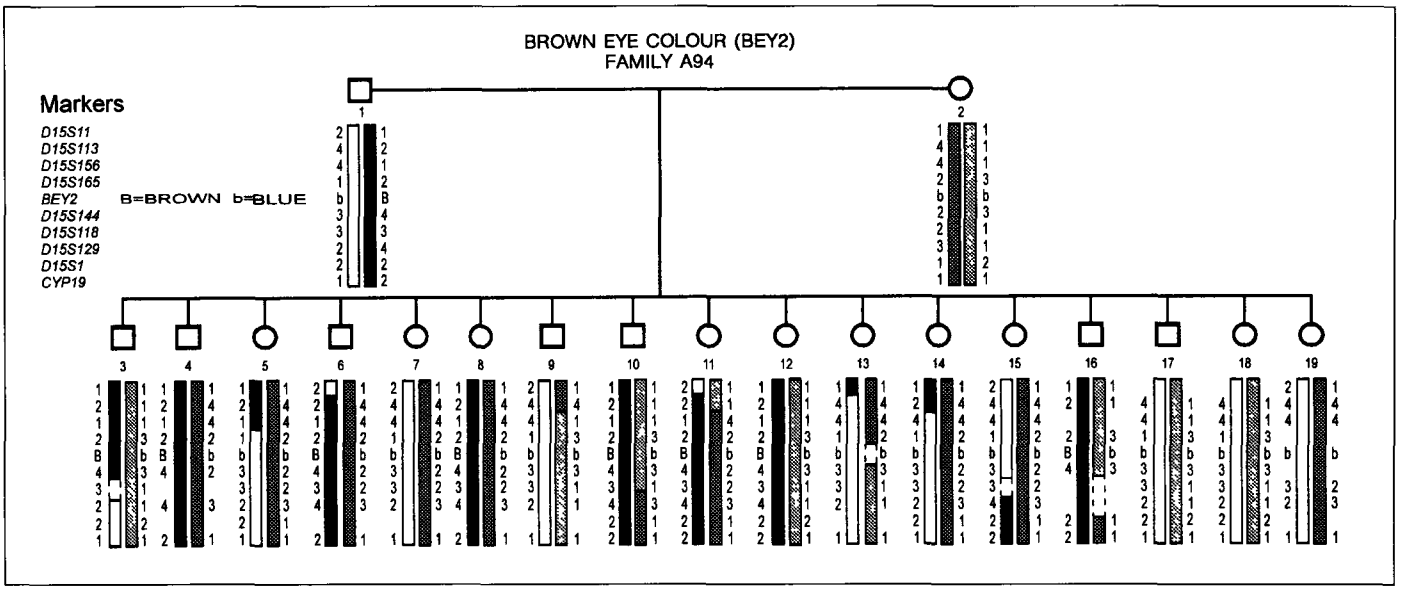
After reexamination of one apparently aberrant family, we have no case of deviation from Mendelian inheritance in the 832 families tested, such as two blue-eyed parents with no brown spots or central brown ring having children with total brown eyes. On the other hand there were cases of blue-eyed parents, one with brown spots, having children with 'brownish' eyes with green spots (GEY phenotype) in our material.

Brown and black hair colour were considered as dominant to blond and 'white' (flaxen). Only persons with blond hair both in childhood and as adults were considered as 'blond'. Persons with brown and black hair colour were considered to have at least one major gene component for brown hair colour. In this preliminary study, brown hair colour was considered as a simple dominant Mendelian trait.

A total of 80 classical markers was used for analysis of linkage [3] as well as 50 RFLP and 160 PCR systems. Two-point lod score for an initial exclusion mapping was obtained with the computer program LIPED [5]. The lod scores $L(\theta_M = \theta_F)$ and multipoint lod scores as shown in table 1 and figure 2 were calculated using the program Fastlink [6]. Exclusion mapping was done with the programme exclude [7]. *BEY2**Brown was considered as a dominant allele and gene frequency and penetrance were set to 0.10 and 1.00, respectively. For hair colour, gene frequencies and penetrance were postulated to be: p = 0.5 for the supposed recessive gene 'blond', p = 0.5 for the dominant gene 'brown', penetrance 1.00. The DNA markers *D15S2* [8] *D15S11, D15S113, D15S156, D15S165, D15S144, D15S118, CYP19, D15S129* [8–11] were used for the linkage studies.

Results

Our first effort to map *BEY2* was by exclusion mapping with 80 markers in 120 segregating families and with 290 markers in 5 segregating families in the Copenhagen family material (fam. 604–1505). By this approach, only the chromosome region 15q stood out as a possibility. The computer program LIPED gave a lod score of Z = 1.00. ($\theta_{M=F}$ = 0.10) to D15S2 [8]. We then typed five large families for the DNA microsatellite markers *D15S11, D15S113, D15S156, D15S165, D15S144, D15S118, CYP19, D15S129* [8–11] to test for linkage. Two of these



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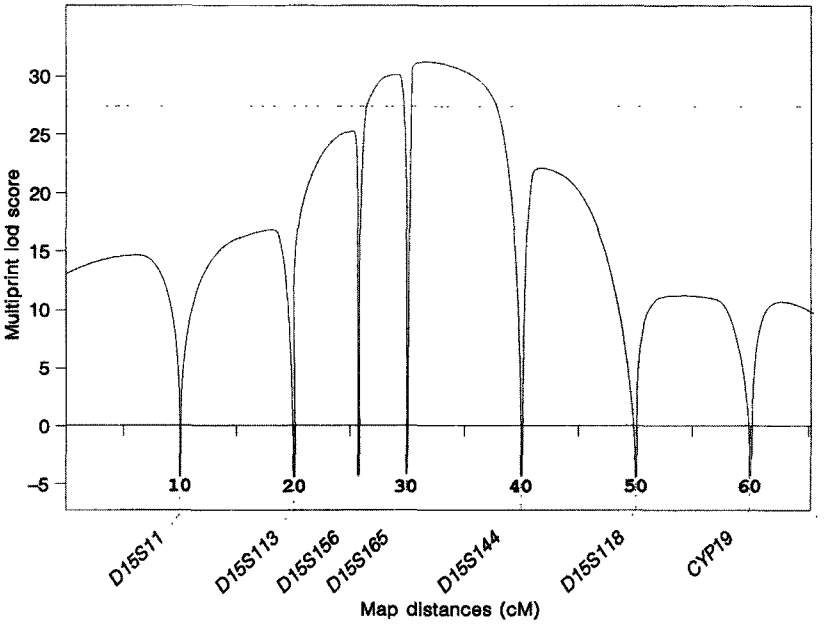

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Fig. 1. A family with 17 children segregating for BEY. By use of this family we found the order of chromosome 15 markers to be: *D15S11 - D15S113 - D15S156 - (D15S165, BEY2) - (D15S144, D15S118) - (D15S129, D15S1, CYP19).* The marker *D15S2* is not shown, but it segregates together with *D15S129, D15S1* and *CYP19.*

Fig. 2. Multipoint analysis with 7 markers from chromosome 15q using 45 families segregating for total brown eye colour *(BEY2).* The horizontal dashed line indicates the significance level for exclusion (odds 1:1,000). *BEY2* is mapped between the markers *D15S156* and *D15S144* close to *D15S165.*

very large families (A04 and A94) with respectively 10 and 17 children, were each sufficient to show a genetic linkage (lod score ≥ 3) to the marker *D15S165.* Family A94 favours the following order: *D15S11-D15S113-D15S156-(D15S165, BEY2)-(D15S144, D15S118)-(D15S129, D15S1, CYP19)* (fig. 1). *D15S2* segregated with the markers *D15S129, D15S1* and *CYP19.* Later a total of 45 families were used to narrow down the region for a possible *BEY2* locus. The multipoint lod score was calculated with markers positioned in accordance with the Généthon map [9, 11] and the genomic database (GDB). The markers and distances used for the multipoint analysis were: *D15S11*-(0.1)-*D15S113*-(0.06) - *D15S156* - (0.04) - *D15S165* - (0.1) - *D15S144* - (0.1) - *D15S118*-(0.1)-CYP19. The most likely order for all families was calculated to be *D15S156-D15S165-*

BEY2-D15S144 using the LINKMAP program of the FASTLINK package, with a multipoint lod score of Z = 32.7. The next likely order *D15S156-BEY2-D15S165-D15S144* gave a multipoint lod score of Z = 32.2 (odds = 1:3.78). Families with central brown eye colour *BEY1/GEY* gave negative lod scores for these chromosome 15 markers (D15S165: Z = −20 at $\theta_{M=F} = 0.0$ and D15S144: Z = −10 at $\theta_{M=F} = 0.0$).

Further in our 45 families segregating for BEY we got a significant lod score between BEY and brown hair colour Z = 9.93 at $\theta_{M=F} = 0.10$ and also to the closely linked markers *D15S165* (Z = 3.66 at $\theta_{M=F} = 0.20$ *D15S144* (Z = 2.91 at $\theta_{M=F} = 0.20$). LINKMAP analysis could not place *HCL3* among the chromosome 15 markers like BEY2; hair colour is presumably heterogeneous with common dominant alleles on at least two loci.

Discussion

We have earlier reported a significant indication of linkage between the phenotypes 'green' eye colour *(GEY)* and 'brown' hair colour *(HCL1)* Z = 5.06 at $\theta_{M=F} = 0.00$ [1]. Here, we report a linkage between *BEY2* and brown hair colour as well, with a positive lod score Z = 9.93 at $\theta_{M=F} = 0.10$. The two assayed distances within each of the two eye-hair colour syntenies are presumably unrealistically high, because of the genetic heterogeneity represented by the two brown hair colour loci referred to here. It is not possible to distinguish phenotypically between the two postulated brown hair genotypes. There is an association between eye colour and hair colour in Denmark. This may be due to linkage disequilibrium (recent immigration) and quite likely also to physiological association (pleiotropy of certain pigment genes). In our material we found 56 informative matings concerning BEY and hair colour, and in 51 of these families the two traits are inherited together (phase cis), while in only five families the two traits are separated when transmitted to the offspring (phase trans). We have analysed three of these phase trans families and BEY and brown hair colour do segregate with chromosome 15 markers. This supports the assumption of linkage disequilibrium between *BEY2* and *HCL3*, as an explanation for the excess of the apparent phase cis. The disequilibrium may be due to recent immigration of people with brown hair and brown eye colour. There was an association between brown eye colour and brown hair colour in our 45 selected families: among 46 parents with brown eye colour 44 had brown hair colour while among 44 spouses with blue eye colour only 26 had brown hair colour.

In conclusion, we assign a gene *BEY2* to chromosome 15q, lod score (Z = 24.25; $\theta_{M=F} = 0.010$) with the DNA microsatellite marker *D15S165*. The most likely order of *BEY2* was *D15S156-D15S165-BEY2-D15S144*. This region is flanked by the regionally assigned markers *CYP19* (15q21) and *D15S11* (15q11-q12) [8].

In the region on chromosome 15q11-q21, a gene for human skin colour, the albinism II gene (OCA2), and the human homoloque (P or DN10) to pink-eye dilution (p) in the mouse may be of interest as candidates for *BEY2* or *HCL3* [12]. However, mutations in the *OCA2* and *P* gene in humans give skin colour deviations [13–15]. Duplication of the *DN10* locus may be present in 6% of patients with Prater-Willi syndrome *(PWS)* and give dark skin [16]. This makes it less likely, that these genes are responsible for *BEY2* or *HCL3*. The loss of function of the *p* locus in mice results in pink-eyed dilution. This suggests that another gene or perhaps a cluster of genes for pigmentation in different tissues is located in this area.

Eventually, once the supposed *BEY2* and *HCL3* genes are found and sequenced, this may provide an interesting new possibility in forensic genetics: the use of a DNA marker to recognize an easily identifiable common physiological trait.

Acknowledgements

The skilful assistance of Ms. Joanna Amenuvor, Ms. Annemette Olsen, Ms. Lillian Rasmussen, Mr. Jens Klausen, Ms. Mona Kristensen is gratefully acknowledged. We thank Dr. Claes Wadelius, Akademiska Sjukhuset, Dept. Clin. Genet., Uppsala, Sweden, for delivering of PCR primers. This work was supported by grants from the Danish Medical Research Council.

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