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Report

In Southern Africa, Brown Oculocutaneous Albinism (BOCA) Maps to the *OCA2* Locus on Chromosome 15q: *P*-Gene Mutations Identified

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In southern Africa, brown oculocutaneous albinism (BOCA) is a distinct pigmentation phenotype. In at least two cases, it has occurred in the same families as tyrosinase-positive oculocutaneous albinism (OCA2), suggesting that it may be allelic, despite the fact that this phenotype was attributed to mutations in the *TYRP1* gene in an American individual of mixed ancestry. Linkage analysis in five families mapped the BOCA locus to the same region as the OCA2 locus (maximum LOD 3.07; $\theta = 0$ using a six-marker haplotype). Mutation analysis of the human homologue of the mouse pink-eyed dilution gene (*P*), in 10 unrelated individuals with BOCA revealed that 9 had one copy of the 2.7-kb deletion. No other mutations were identified. Additional haplotype studies, based on closely linked markers (telomere to centromere: D15S1048, D15S1019, D15S1533, *P*-gene 2.7-kb deletion, D15S219, and D15S156) revealed several BOCA-associated *P* haplotypes. These could be divided into two core haplotypes, suggesting that a limited number of *P*-gene mutations give rise to this phenotype.

Brown oculocutaneous albinism (BOCA; MIM 203290) or type IV oculocutaneous albinism (fig. 1) was first described in black individuals in Malawi (Stannus 1913) and later was named "brown albinism" by King et al. (1980), after their study in Nigeria. Affected individuals were noted to have cream to light tan skin, beige to light brown hair, and blue-green to brown irides with moderate transillumination defects, nystagmus, and reduced retinal pigment. They were found to be distinct from the rufous type of albinism (Kromberg et al. 1990). Ultrastructurally, their melanocytes are normal, but their melanosomes appear not to reach maturity, with only small amounts of melanin deposition (King and Rich 1986). Because of their lighter skin, affected individuals, particularly in Africa, are at greater risk of developing malignant skin lesions than are their normally pigmented counterparts (Kromberg 1985). The phenotype has not been described in white individuals, although King and Rich (1986) have suggested that, in this population, it may be analogous to autosomal recessive ocular albinism.

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BOCA is inherited as an autosomal recessive disorder. Two observations suggested the possible involvement of the human homologue of the mouse pink-eyed dilution gene (*P*) in the BOCA phenotype. The first was the occurrence of BOCA and OCA2 (caused by mutations in the *P* gene) in different members of the same family. Two families were found to fit this category. The second observation was that the BOCA phenotype is similar to that of OCA2, but represents a much milder form. The *P* gene and other candidate pigment loci were tested for linkage to BOCA. In this study, linkage analysis was performed in five families comprising 16 affected and 19 unaffected individuals. Diagnosis of the condition was based on a number of characteristic features: a light-brown skin that tans on exposure to sunlight; an accumulation of pigment, to a small degree, with age; brown hair; blue or brown irides; and presence of the visual anomalies associated with albinism: nystagmus, photophobia, reduced visual acuity, and sometimes strabismus (King et al. 1980).

The following candidate loci were identified, and polymorphic markers at or close to each locus were tested for linkage to the disorder in the 35 subjects: the *P* gene, which, when mutated, usually results in OCA2 (Rinchik et al. 1993); tyrosinase (*TYR*), a critical enzyme in the

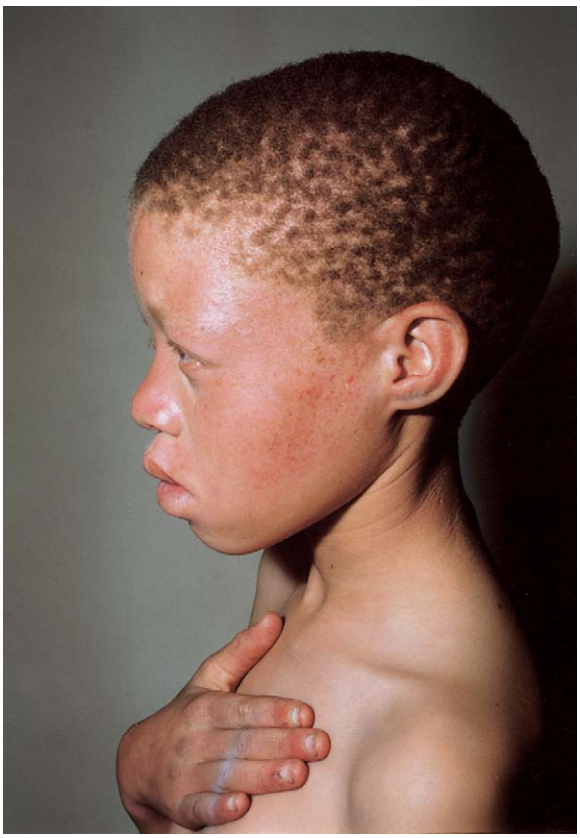

Figure 1 South African Bantu-speaking black subject with brown oculocutaneous albinism (BOCA).

melanin biosynthesis pathway that displays activity in BOCA above the mean value for brown-haired white individuals (King and Rich 1986); tyrosinase-related protein 1 (*TYRP1*), which has been implicated in an OCA phenotype classified as BOCA by Boissy et al. (1996), but which the present authors have shown to be rufous oculocutaneous albinism (ROCA) in southern African populations (Manga et al. 1997); and tyrosinase-related protein 2 (*TYRP2*), which codes for another enzyme in the melanin pathway (Sturm et al. 1994).

Polymorphic markers at the selected loci were characterized in each family, and two-point linkage analysis was carried out. *TYR, TYRP1,* and *TYRP2* were excluded from linkage to BOCA (table 1). Linkage to the *P* locus could not be excluded but remained equivocal with individual *P* gene–associated markers.

P gene–associated haplotypes based on the markers D15S10-D15S11-D15S97-GABRB3-GABRA5-IR10-CMW were therefore constructed for each family and were used as a single "marker" to test for linkage. The markers were ordered according to a consensus map based on data from Kuwano et al. (1992), Mutirangura et al. (1993), and Kedda et al. (1994). A maximum LOD of 3.07 ($\theta = 0$) was obtained for two-point linkage analysis, suggesting the involvement of the *P* gene in the etiology of BOCA. Once linkage had been established to markers mapping to chromosome 15q11-q13 and the *P* gene had been identified as the most likely candidate, mutation analysis was carried out for the *P* gene in affected individuals. A further five unrelated subjects with BOCA were included in the mutation screen. The strategy was to look first for structural alterations, using Southern blotting. Secondly, an exon-by-exon screen using SSCP and sequence analysis was carried out for each of the 25 exons (Lee et al. 1995). The 2.7-kb deletion, which removes exon 7 of the *P* gene (Durham-Pierre et al. 1994) and is known to account for the majority (77%) of OCA2 cases in southern Africa (Stevens et al. 1997), was also screened for in affected individuals.

The 2.7-kb deletion was found in the heterozygous state in 9 of 10 unrelated individuals with BOCA, giving it a frequency of .45 in this group. The mutation has a high carrier rate, 1 in 78, in the normally pigmented population (Stevens et al. 1997). Thus, the frequency is significantly higher in BOCA subjects ($\chi^2 = 38.2$; $P = 6.39 \times 10^{-10}$), confirming that *P* is involved in the BOCA phenotype. No definitive pathogenic mutations, other than the 2.7-kb deletion, were identified in the nine subjects tested, although many polymorphic variants were observed (Kerr et al. 2000).

Additional haplotype analysis was performed with closely linked microsatellite markers to determine whether there was a single or multiple origins for BOCA *P* alleles. The following markers were used (telomere→centromere): D15S1048, D15S1019, D15S1533, *P*-gene 2.7-kb deletion, D15S219, and D15S156. The distance between the outer markers is ~1.03 Mb, according to the Draft Genome Browser. The order of all these markers and their primer sequences, excluding D15S1533, were obtained from the Genome Database. D15S1533 represents a new polymorphic microsatellite marker that was detected on a partially sequenced BAC (RP11-322N14, AC017046). This BAC contains some of the *P*-gene exons. An algorithm designed by A. Christoffels was used to detect AC repeats of >10 units. This BAC contains 14 unordered segments, of which segment 11 contained an AC repeat of interest. The primers for D15S1533 are: (F) 5′-CTTGGCAACATCCCTGTATCA-3′ and (R) 5′-TGAATGCCATTATTTCATTCCTT-3′. The exact position of D15S1533 in relation to the 2.7-kb deletion of *P* is not known.

Haplotype analysis using the closely linked markers is shown together with the BOCA pedigrees in figure 2 and is detailed in table 2. Five different (nondeletion) haplotypes probably derived from two ancestral haplotypes were observed to be segregating with the brown allele, suggesting that there is a small number of mutations (possibly one common mutation) associated with

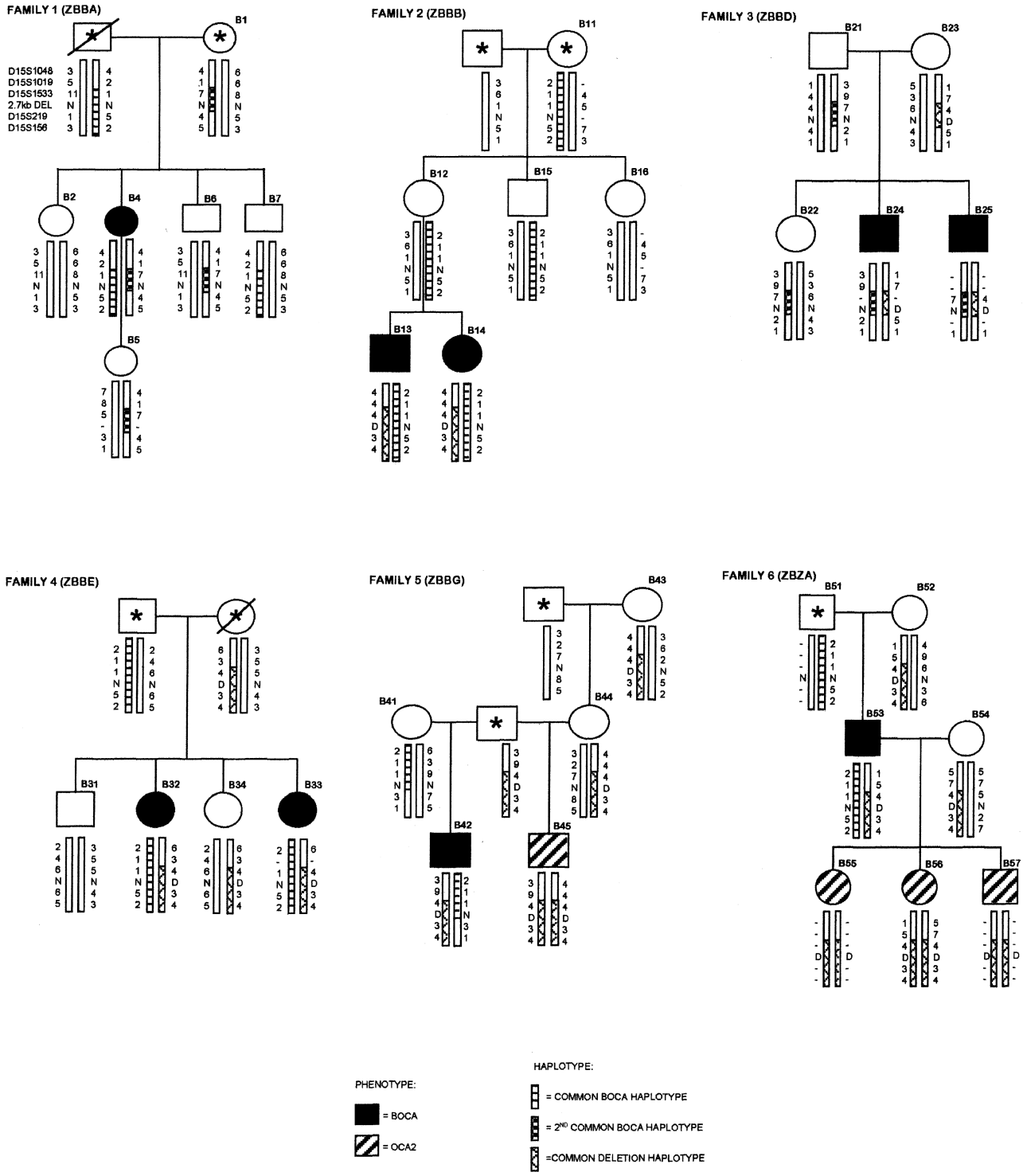

Figure 2 Pedigrees and haplotype data of markers flanking the *P* gene. Marker order is shown in family 1 (telomere→centromere): D15S1048, D15S1019, D15S1533, 2.7-kb deletion, D15S219, and D15S156. The haplotypes in individuals marked with an asterisk (*) have been inferred from data obtained from other family members. Dashes within haplotypes indicate that a result was not obtained for that marker in that individual, but, in most cases, it can be inferred from other family members. Families 5 and 6 contain individuals affected with BOCA and OCA2.

Table 1

Two-Point LOD-Score Analysis between Candidate Pigment Loci and BOCA

Candidate Locus[a]	Chromosomal Position	θ 0	.10	.20	.30	.40	.50	Reference for Markers
TYR	11q14-q21	$-\infty$	−.54	−.06	.04	.02	.00	Morris et al. (1991)
TYRP1	9p23	$-\infty$	−.78	−.31	−.11	−.02	.00	Box and Sturm (1994)
TYRP2	13q13-q32	$-\infty$	−.63	−.27	−.11	−.03	.00	Sturm et al. (1994)
P haplotype[b]	15q11-12	3.07	2.26	1.46	.73	.20	.00	

[a] The markers used for *TYR, TYRP1,* and *TYRP2* were intragenic and recombinations were observed, excluding each locus.

[b] The markers screened for in the *P* haplotype (D15S10, D15S11, GABRB3, D15S97, GABRA5, IR10, and CMW, of which IR10 [Nicholls et al. 1989] and CMW [Rich et al. 1988] were screened by Southern blot analysis and the remainder by PCR and polyacrylamide gel electrophoresis) all showed Z_{max} values at $\theta = 0$, except D15S11 ($\theta = .10$); and the Z_{max} values for the markers were 0.77, 1.19, 2.41, 2.47, 0.40, and 0.84, respectively. When combined into a *P* haplotype, they gave an overall Z_{max} of 3.07 at $\theta = 0$.

this phenotype. In three of the six families (families 2, 4, and 6), the OCA2 2.7-kb–deletion core haplotype appeared with the same BOCA haplotype. This common BOCA haplotype was not seen in 18 chromosomes of control individuals from the same population, suggesting that it is not common in the general population (data not shown).

Linkage analysis was not performed on these markers, because there were no observed recombination events within families, which confirms tight linkage between these markers and the *P* gene. Two ancestral hot spots for recombination are suggested, as depicted in table 2 (between D15S1019 and D15S1533 and between the 2.7-kb deletion of *P* and D15S219). The region most often conserved in BOCA patients is between D15S1533 and D15S156 indicating that the pathogenic mutations giving rise to this phenotype are within this region. The BOCA-associated haplotypes are conserved over a larger region (D15S1048–D15S156) than are the 2.7-kb deletion haplotypes (conserved from D15S1533 to

Table 2

***P*-Gene–Associated Haplotypes in BOCA Subjects**

Family	D15S1048	D15S1019	D15S1533	2.7-kb Deletion[a]	D15S219	D15S156
2(ZBBB)	2	1	1	N	5	2
4(ZBBE)	2	1	1	N	5	2
6(ZBZA)	2	1	1	N	5	2
1(ZBBA)	4	2	1	N	5	2
5(ZBBG)	2	1	1	N	3	1
1(ZBBA)	4	1	7	N	4	5
3(ZBBD)	3	9	7	N	2	1
4(ZBBE)	6	3	4	D	3	4
5(ZBBG)	3	9	4	D	3	4
6(ZBZA)	1	5	4	D	3	4
2(ZBBB)	4	4	4	D	3	4
3(ZBBD)	1	7	4	D	5	1

Note.—Boxed areas indicate conserved regions of the haplotype. With respect to the OCA2 individuals within these families, the OCA2 individual in family 5 shares the same deletion haplotype with the BOCA individual in family 2, whereas the OCA2 individuals in family 6, in addition to the deletion haplotype obtained from their BOCA parent, have a different deletion haplotype (5-7-4-D-3-4) that shows the core haplotype (boxed region) but, again, no conservation telomeric to the core haplotype. Microsatellite analyses were performed by amplification of products with fluorescently labeled primers and electrophoresis on a 4.2% polyacrylamide gel using an ABI Prism 377 DNA sequencer. GeneScan software was used to size the amplimers. An allele-numbering system for the various product sizes, at each locus, was used to simplify the data. The numbering was used consistently throughout the study (details not shown).

[a] N = no deletion; D = 2.7-kb *P*-gene deletion.

D15S156), indicating that at least one BOCA mutation is likely to be evolutionarily more recent than the 2.7-kb deletion.

On the basis of the data presented here, there is good support for the hypothesis that BOCA in southern African individuals is caused by mutations in the *P* gene, despite the fact that no additional pathogenic mutations were identified. This is contrary to findings in one BOCA subject presented by Boissy et al. (1996), in whom mutations were found in the *TYRP1* gene. The phenotype in that case may have been modified by the genetic background and mixed ancestry of the individual. BOCA is a mild phenotype, and, in the affected southern African individuals, the second mutation may well be a milder mutation, possibly in the promoter region (down-regulating expression) or in other unscreened regions of the *P* gene.

We propose that BOCA mutations are rare, and that the phenotype is only expressed when present together with another mutation at the OCA2 locus. Individuals who do not carry a severe OCA2 mutation but are homozygous for the milder BOCA mutations may be expected to be phenotypically normal, but perhaps tend towards the lighter part of the spectrum of normal skin-color variation.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Draft Genome Browser, http://genome-test.cse.ucsc.edu/goldenPath/hgTracks.html (for marker distances)

Genbank, http://www.ncbi.nlm.nih.gov/Genbank/ (for RP11-322N14 BAC [accession number AC017046])

Genome Database, http://www.gdb.org/ (for primer sequences and marker order)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for BOCA [MIM 203290])

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