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ORIGINAL ARTICLE

Oculocutaneous albinism type 2 (OCA2) with homozygous 2.7-kb deletion of the P gene and sickle cell disease in a Cameroonian family. Identification of a common TAG haplotype in the mutated P gene

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Abstract In this study, we report on a Cameroonian family from the Ewondo ethnic group, presenting with three oculocutaneous albinism type 2 (OCA2) patients homozygous for the 2.7-kb deletion of the P gene. In one of these patients OCA2 was associated with sickle cell anaemia and in two with the sickle cell trait. We took this opportunity to determine single nucleotide polymorphism (SNP) haplotypes within the P gene in this family in comparison with a group of 53 OCA2 patients homozygous for the same mutation and with a matched unrelated full-coloured control group of 49 subjects, originating from seven different ethnic groups of Southern Cameroon including Ewondo. A combination of five exonic and intronic SNPs in the OCA2 gene was genotyped by sequencing PCR products. We found 3 different haplotypes (TAGCT, TAGTT and TAGCC with frequencies of 0.66, 0.28 and 0.06, respectively) associated with the mutation in the 53 OCA2 patients, while 11 different haplotypes were observed in the control group. These observations suggest that the mutation appeared on the relatively frequent haplotype TAGCT, and that the two other haplotypes are derived from two independent recombination events. These haplotypic data, associated with a value of 1/15,000 for the prevalence of the 2.7-kb mutation, a present effective population size of 10,000,000 for Cameroon and a recombination rate of 0.0031, allowed us to estimate that this mutation originated 4,100–5,645 years ago.

Keywords OCA2 · Sickle cell disease · P gene · βS-globin gene · SNPs · Cameroon

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Introduction

Type 2 oculocutaneous albinism (OCA2; MIM# 2032000)—an autosomal recessive disorder in which the biosynthesis of melanin pigment is reduced in the skin, hair and eyes—is associated with the ocular features of nystagmus, reduced visual acuity and misrouting of the optic fibres at the chiasma. OCA2 results from mutations in the P gene—the human homologue of the mouse *pink-eye dilution* (p) gene on chromosome segment 15q11.2–q12, which contains 25 exons, 23 of which are coding. This region encodes a 110-kDa protein integral to the melanosomal membrane with a predicted 12-transmembrane domain structure, resembling a channel or transporter (Rosemblat et al. 1994). Despite the critical role played by this 838-amino-acid P polypeptide in controlling tyrosinase processing and melanosome biogenesis, its precise biological function is still not defined. However, it is known to be required for normal pigmentation (Chen et al. 2002). OCA2

is the most common form of albinism, especially among Africans and African-Americans. There is a high incidence of OCA2 among specific African populations: 1 in 1,100 among the Ibo of Nigeria (Okoro 1975), 1 in 3,900 in South Africa (Kromberg and Jenkins 1982), 1 in 4,100 in Tanzania (Luande et al. 1985), 1 in 4,882 in Zimbabwe (Lund 1996), 1 in 5,000 in Nigeria (Barnicot, 1952), 1 in 7,900 among the Bamileke of Cameroon (Aquaron 1980, 1990) and 1 in 10,000 in African-Americans (Durham-Pierre et al. 1996). The most common mutation in the P gene among Africans and African-Americans is a 2.7-kb deletion that removes a single exon (exon 7; amino acids 216–270) and results in a frameshift mutation in the first luminal loop of the P polypeptide, producing a truncated and non-functional gene product (Durham-Pierre et al. 1994). This mutation was first detected in the homozygous state in a large family with seven albinos from an inbred population of triracial (Black, Caucasoid, and American-Indian) origin. This mutant P allele was also detected, in the heterozygous state, in four unrelated African-American OCA2 individuals, in a Zairean patient and in a Cameroonian patient, but not in any Caucasoids with OCA2, indicating an African origin for this allele (Durham-Pierre et al. 1994). This deletion allele is associated with a common haplotype, suggesting a founder effect (Stevens et al. 1995, 1997), and represents a high proportion of mutated P alleles in central, eastern and southern African countries: 33% (4/12 chromosomes) in the Central African Republic (Stevens et al. 1997), 65% (47/72 chromosomes) to 67% (233/346 chromosomes) in Cameroon (Puri et al. 1997; Aquaron and Berge-Lefranc 2002), 77% (20/26 chromosomes) in Tanzania (Spritz et al. 1995), 77% (131/170 chromosomes) in South Africa (Stevens et al. 1995), 79% (11/14 chromosomes) in Zambia (Stevens et al. 1995) and 94% (60/64 chromosomes) in Zimbabwe (Puri et al. 1997). In contrast, this mutant P allele has never been found in 30 OCA2 patients from west African countries: 1 albinism case in Nigeria (Spritz et al. 1995), and 29 cases in Niger, Mali, Togo, Burkina Faso (R. Aquaron and J.-L. Berge-Lefranc, unpublished results).

Sickle cell disease is an autosomal recessive disorder (MIM # 603903) characterised by chronic anemia with a haemoglobin concentration of around 80 g/l, painful swelling of the hands and/or feet at between 6 and 18 months of age. Survivors may also suffer recurrent and unpredictable severe painful crises as well as pneumonia or pulmonary infarction, bone or joint necrosis, cerebrovascular accidents or renal failure. Patients with sickle cell anemia are homozygous (SS) for a missense mutation (c.19 A > T) in the β-globin gene located on chromosome 11p15.5 that leads to replacement of the hydrophilic negatively charged amino acid (Glu) by the hydrophobic neutral amino acid (Val) (p.E6V) in the adult haemoglobin protein HbA, giving rise to the abnormal protein HbS. Heterozygous individuals (AS) are not symptomatic and have sickle-cell trait. The prevalence of sickle-cell trait ranges from 9.3 to 28.2% in Cameroon depending on ethnic group: 9.3% in albino Bamileke, 15.7% in black Bamileke, 17.1% in Pygmies, 20.7% in Ewondo and 28.2% in Eton (Gamet and Labes 1964; Nguematcha et al. 1973; Juhan and Kaptue 1974; Aquaron et al. 1984).

We know of only one case of albinism associated with sickle-cell anemia in an African-American family from Nashville, Tennessee (Massie and Hartmann 1957). Here, we report the study of a Cameronian family from the Ewondo ethnic group presenting with three OCA2 patients homozygous for the 2.7-kb deletion mutation in the P gene, one associated with sickle-cell anemia and two with sickle-cell trait. We took this opportunity to determine the single nucleotide polymorphism (SNP) haplotypes of the P gene in this family as well as in a group of 53 OCA2 patients homozygous for the same mutation and in a matched unrelated full-coloured control group of 48 subjects, all from seven different ethnic groups of South Cameroon including the Ewondo. We also identified haplotypes of the βS-globin gene in this family.

Subjects and methods

Study population

Propositus (IV-3), was a Cameroonian girl born from non-consanguineos parents in 1985 with clinical OCA2 (Fig. 1). Her skin was virtually white, her hair was pale golden yellow, and her irises were pale blue. Visual acuity was decreased and she had nystagmus. Her parents had normal pigmentation, originated from Yaounde and belong to the Ewondo ethnic group. She had one affected sister, four unaffected siblings, and an affected first cousin. Diagnosis of sickle cell anemia was made at the age of 6 years. She died at the age of 15 years from malaria. A total of 53 OCA2 patients homozygous for the 2.7-kb deletion allele and 48 matched unrelated full-coloured controls belonging to seven ethnic groups from South Cameroon was recruited: Douala, Bakundu, Bassa, Central-Bantus (Yambassa), Semi-Bantus (Bamileke), Beti-Pahouins (Boulou, Eton, Ewondo, Fang), and Maka (Dugast 1949). All albino subjects had been examined by one of us (R.A.) in main cities of Cameroon (Yaounde, the Cameroon capital, Douala, Bafoussam) and surroundings areas. The control subjects were recruited in Yaounde. Informed consent was obtained from all patients, or their legal guardian, and from control subjects enrolled in the study. Genomic DNA was isolated from peripheral blood leukocytes or from buccal cells using an automated device (Biorobot M48, Qiagen, Hilden, Germany).

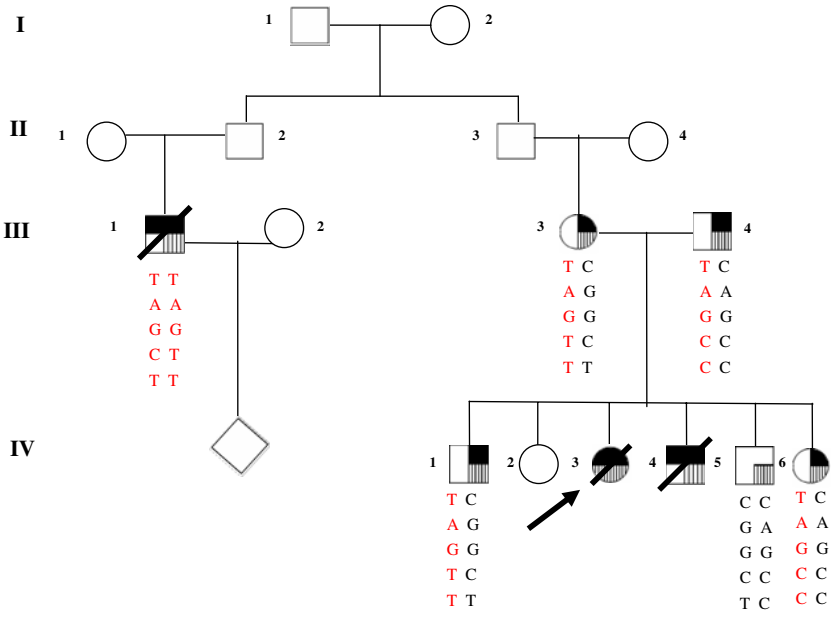

Fig. 1 Pedigree of the Cameroonian family with oculocutaneous albinism type 2 (OCA2) and sickle-cell anemia. The index case is indicated by an *arrow* (subject IV-3). *Black* and *hatched* symbols represent the two mutated alleles of the 2.7-kb deletion allele of the P gene and the βS-globin gene, respectively, in the heterozygous (1/4) or homozygous (2/4) state. Subject IV-2 has been tested only for HbS. The three different intragenic haplotypes, TAGCT, TAGCC and TAGTT, of the OCA2 gene are present in this family as indicated

Analysis of the 2.7-kb deletion allele of the P gene

PCR-based screening for the 2.7-kb deletion allele of the P gene was performed as described by Durham-Pierre et al. (1994). The method used primers located on both side of the deletion (MHB107 and MHB71) and an internal primer (MHB72) to discriminate between normal and mutated alleles. Two PCR products are generated by the assay and separated by gel electrophoresis; the larger (420 bp) fragment is indicative of the deletion, and the smaller (240 bp) fragment is amplified from the normal non-deleted allele. DNA from obligate carriers gave both fragments after PCR amplification.

OCA2 SNP genotyping and estimation of the age of mutation

A combination of five exonic and intronic common SNPs of the OCA2 gene (NCBI SNP ID: rs1800401, rs1800410, rs1900758, rs1800419, rs8025804) were genotyped by sequencing PCR products from exons 9, 13 and 22 and intronic flanking sequences (Jannot et al. 2006) in 53 unrelated OCA2 patients with the 2.7-kb deletion in the homozygous state and 48 ethnically matched full-coloured unrelated controls (Table 1). Haplotype identification was inferred from unphased genotypes using Arlequin ver3.0 software (Excoffier et al. 2005). Family members were genotyped for the same SNPs and the phase was inferred from segregation analysis assuming the absence of intragenic recombination.

These haplotypic data allowed us to estimate the age of the mutation (i.e. the time since its appearance in the population) as well as its growth rate, using the method developed by Austerlitz et al. (2003). This method uses as inputs the current number of carriers of the mutation in the population and the level of linkage disequilibrium with the surrounding haplotype. The number of carriers can be deduced from the prevalence of the disease and the total effective size of the present population. According to data published in the literature concerning the prevalence of OCA2 in African and African-American patients (1 in 3,900–1 in 10,000) with the 2.7-kb deletion mutation allele (33–94%), the prevalence of the 2.7-kb deletion mutation can be estimated at between 1/3,900 and 1/33,000. We thus tried four possible values: 1/500, 1/5,000, 1/15,000 and 1/30,000. For the effective population size, we assumed four possible values: 100,000, 1,000000, 10,000,000 and 100,000,000.

The method also requires an independent knowledge of the recombination rate of the whole haplotype. Two recombination rates were used: 0.00477 and 0.0031. The first was deduced from the physical length of the haplotype (167.09 kb) and the ratio between the physical and genetic length in the region (1 cM for 350 kb). The second was estimated from the fine scale genetic map published by Myers et al. (2005) between the two recombining markers rs1900758 (intron 13) and rs1800419 (exon 22) (http://www.stats.ox.ac.uk/mathgen/Recombination.html). The age estimated in generations was converted into years by assuming an average generation length of 25 years.

βS haplotypes analysis

Haplotype analysis was performed as described by Elion et al. (1992). The following polymorphic sites were

studied: *Xmn*I –158 bp to Gγ, *Hin*dIII within Gγ and Aγ, *Hinc*II within $\psi\beta$ and the sequence from position –555 to –392 upstream of the β-globin gene cap site.

Results

The 2.7-kb deletion allele and HbS analysis

Figure 1 shows the family pedigree of the six analysed subjects and two deduced genotypes for the 2.7-kb deletion allele: three homozygous patients, two sisters (IV-3 and IV-4) who died at 15 and 2 years of age, respectively, and their uncle (III-1) deceased at 39 years, one normal subject (IV-5) and four heterozygous black subjects: father (III-3), mother (III-4), son (IV-1) and daughter (IV-6). The patients' haemoglobin status was ascertained by cellulose acetate electrophoresis. One was SS (IV-3), one was AA (IV-2) and the other seven were heterozygous AS.

Haplotype analysis of the P gene and age of the 2.7-kb deletion mutation

Haplotype analysis of the P gene in five members of the family showed that three different haplotypes, TAGCC, TAGCT and TAGTT, differing in the 3′ portion of the gene were associated with the 2.7-kb deletion mutation (Fig. 1). This observation indicated that the mutant allele was not inherited from a close common ancestor, and prompted us to determine the haplotype frequencies both among 53 OCA2 patients and 48 matched unrelated full-coloured control Cameroonian subjects. Haplotype frequencies were deduced from genotypic data in both groups (Table 1). Haplotypic diversity appears to be reduced to 3 in OCA2 patients compared to the 11 variants found in controls. A common haplotype TAG determined by the three most 5′ SNPs (rs1800401, rs1800410 and rs1900758) is found in all 53 OCA2 patients (100%) studied although this haplotype was found in only 21% of the controls. No 2.7-kb deletion mutation was found in any subject of the control group. This is in keeping with the hypothesis that the 2.7-kb deletion is a founder mutation that occurred only once in Africa. However, some haplotypic diversity in the 3′ region of the gene (rs1880419 and rs8025804) was found among the 53 OCA2 patients, determining three different haplotypes, TAGCT, TAGTT and TAGCC with frequencies of 0.66, 0.28 and 0.06, respectively, with corresponding frequencies in the control group of 0.13, 0.02 and 0.06 (Table 1). These observations suggest that the mutation appeared on the relatively frequent haplotype TAGCT, and that the other two haplotypes now found in patients are

Table 1 Intragenic haplotype frequencies from 53 Cameroonian oculocutaneous albinism type 2 (OCA2) patients and from 48 unrelated matched full-coloured controls, deduced from a combination of five exonic and intronic common single nucleotide polymorphisms (SNPs) of the OCA2 gene (NCBI SNP ID: rs1800401, rs1800410, rs1900758, rs1800419, rs8025804) genotyped by sequencing PCR products from exons 9, 13 and 22 and intronic flanking sequences (Jannot et al. 2006). Haplotype frequencies were inferred from unphase genotypic data using the ELB algorithm (Excoffier and Slatkin 1995) implemented using Arlequin software ver 3.0 (Excoffier et al. 2005)

rs1800401	rs1800410	rs1900758	rs1800419	rs8025804	Haplotype frequency
C/T[a] Exon 9 R305W	A/G intron 13 pos +26	A/G intron 13 pos +113	T/C Exon 22 A776A	T/C intron 22 pos+75	
OCA2 patients (n = 53)					
T	A	G	C	T	0.66
T	A	G	T	T	0.28
T	A	G	C	C	0.06
Controls (n = 48)					
C	G	G	C	T	0.20
C	A	G	T	T	0.19
C	A	G	C	C	0.14
T	A	G	C	T	0.13
C	G	G	T	T	0.10
C	A	G	C	T	0.08
T	A	G	C	C	0.06
C	G	G	C	C	0.04
C	A	A	C	C	0.03
T	A	G	T	T	0.02
C	A	A	C	T	0.01

[a] Major allele/minor allele

derived from two independent recombination events between markers rs1900758 and rs1800419. Interestingly, these two markers are separated from each other by hot spots of recombination (http://www.stats.ox.ac.uk/mathgen/Recombination.html).

The growth rates and ages of the 2.7-kb deletion mutation as estimated from the haplotypic data for the various hypotheses on prevalence of the mutation and population size are given in Table 2 . If we take a value of 1/15,000 for the prevalence of the 2.7 kb mutation (this mutation represents 67% of mutated alleles for a frequency of OCA2 of 1/7,900–1/10,000 in Cameroon) and a present effective population size of 10,000,000 (the most recent population evaluation at http://www.statistics-cameroon.org), this yields an age between 4,100 and 5,645 years assuming a recombination rate of 0.0031 or between 2,650 and 3,610 years with a recombination rate of 0.00477 (Table 2).

Haplotype analysis of the βS-globin gene

Polymorphic sites for the enzymes *Xmn*I, *Hin*dIII, *Hin*cII and the sequence from position –555 to –392 upstream of the β-globin gene cap site define four specific patterns, named Senegal, Bantu, Benin and Cameroon types, linked to the βS-globin gene from Africa. Single nucleotide variations were observed at four positions: –551, –553, –521 and –491 with a repeated purine/pyrimidine structure (AT) xTy, which exhibits variations in length and configuration, just downstream at position at –543. Sequence polymorphism in this region is also characteristic for the Senegal, Bantu, Benin and Cameroon types. Each type differs from the reference sequence: TC(AT)7T7CA. All the AS subjects (mother, father, children, uncle) carry a Benin-type βS chromosome: TT(AT)8T4CC. In addition, on the βA chromosome, the mother carries an atypical, previously undescribed, haplotype that was also transmitted to her two heterozygous girls: +AT TT(AT)6T8CC.

Discussion

In this study, we report a very rare case of an OCA2 Cameroonian girl from the Ewondo ethnic group with a homozygous 2.7-kb deletion of the P gene associated with sickle cell anemia, i.e. homozygous for the βS-globin gene. Cameroon is of considerable anthropological interest for two historical reasons (Spedini et al. 1999). First, the peopling of Cameroon involved a process of sedentarisation into three nuclei that are ancient and well documented. The oldest nucleus of sedentarisation was identified in northern Cameroon in the lake Chad region around 6,000 years before present (BP). A second nucleus of sedentarisation was identified in western Cameroon, along the mountain slopes of the Nigerian–Cameroon plateau by a proto-Bantu group around 4,000 BP. A third nucleus of sedentarisation has been identified in the south–central region of Yaounde around 3,000 BP. Second, Cameroon has been invaded by numerous migratory waves of people with different origins and languages, hence this country may be regarded as the first meeting point between the Bantu and Sudanese cultures and peoples. Cameroon is an equatorial country located between latitudes 2° and 13°N, and has three distinct habitats: equatorial forest in the south and central region, savanna and the Sahel in the northern regions. Equatorial forest, located between latitudes 2° and 6°N, encompasses approximately five administrative provinces: Centre, East, Littoral, West and South. Population inhabitants in this area are quite homogenous according to their ethnic languages, cultural attitudes, and their use of French. They belong to the Benue-Congo subfamily 3c, also referred to as the Bantu group (Spedini et al. 1999). On the other hand, this area encompasses numerous ethnic groups: Douala, Bakundu, Bassa, Central-Bantus (Banen, Yambassa), Semi-Bantus (Bamileke, Bamoun), Beti-Pahouins (Boulou, Eton, Ewondo, Fang) and Maka, who are affected by two frequent monogenic diseases: OCA2 and sickle cell anemia.

OCA2 results from mutations in the P gene; the most frequent mutation in Cameroon is the 2.7-kb deletion, which accounts for 67% (233/346 chromosomes) of mutant P alleles. The 2.7-kb deletion allele is less common among the Bamileke ethnic group (94/170 alleles, i.e. 55%) than in all the other ethnic groups combined, namely Banem, Bamoun, Bassa, Ewondo and Douala (103/128 alleles i.e. 80%) (Aquaron and Berge-Lefranc 2002). According to the second population census (1987) in Cameroon, there were 10,490,655 inhabitants, but even if this census does not provide information on the total size of each ethnic group, we could estimate the Ewondo people to 460,000 individuals (Imbert 1973). Based on recent unpublished data, we were able to evaluate the incidence of OCA2 in the Ewondo ethnic group to 1/11,500 (40/460,000 individuals) and the prevalence of the 2.7-kb deletion mutation to 80% (45/56 chromosomes). The Ewondo migrated to central–southern Cameroon from their northern Cameroon homeland, southern Adamawa, which was invaded in the late eighteenth century by some Baboute groups (Alexandre and Binet 1958).

Using Southern blot analysis, Stevens et al. (1995) have shown that the 2.7-kb deletion allele, which accounts for 77% (131/170 chromosomes) of the mutated alleles in southern Africa, is associated predominantly with one common haplotype (A; 0.78), although it is also associated with other less frequent haplotypes (B, E, F and G)

Table 2 Estimates from the haplotypic data of the growth rate and the age of the mutation, using the method developed by Austerlitz et al. (2003), for the various hypotheses regarding the prevalence of the 2.7-kb deletion of the P gene, the present effective population size of the population and two recombination rates, $\theta = 0.0031$ and 0.00477

Prevalence of the 2.7-kb mutation	Present effective population size	Estimated growth rate	Estimated age (generations)	Estimated age (years)
$\theta = 0.0031$				
1/500	100,000	1.0316 (1.0226, 1.0482)	211.4 (177.7, 254.5)	5285.0 (4442.5, 6362.5)
	1,000,000	1.0488 (1.0392, 1.0673)	193.0 (165.9, 230.5)	4825.0 (4147.5, 5762.5)
	10,000,000	1.0666 (1.0566, 1.0863)	182.1 (160.4, 214.7)	4552.5 (4010.0, 5367.5)
	100,000,000	1.0848 (1.0745, 1.1053)	175.0 (157.2, 203.4)	4375.0 (3930.0, 5085.0)
1/5,000	100,000	1.0235 (1.0153, 1.0383)	226.3 (189.6, 272.1)	5657.5 (4740.0, 6802.5)
	1,000,000	1.0401 (1.0307, 1.0578)	200.8 (170.4, 241.1)	5020.0 (4260.0, 6027.5)
	10,000,000	1.0576 (1.0478, 1.0768)	186.9 (162.7, 221.8)	4672.5 (4067.5, 5545.0)
	100,000,000	1.0756 (1.0655, 1.0958)	178.2 (158.6, 208.6)	4455.0 (3965.0, 5215.0)
1/15,000	100,000	1.0199 (1.0124, 1.0336)	235.5 (197.8, 282.4)	5887.5 (4945.0, 7060.0)
	1,000,000	1.0360 (1.0268, 1.0532)	205.4 (173.4, 247.0)	5135.0 (4335.0, 6175.0)
	10,000,000	1.0534 (1.0437, 1.0723)	189.6 (164.1, 225.8)	4740.0 (4102.5, 5645.0)
	10,000,000	1.0713 (1.0613, 1.0912)	180.0 (159.4, 211.4)	4500.0 (3985.0, 5285.0)
1/30,000	100,000	1.0203 (1.0128, 1.0342)	242.2 (204.0, 289.8)	6055.0 (5100.0, 7245.0)
	1,000,000	1.0366 (1.0273, 1.0538)	208.8 (175.8, 251.2)	5220.0 (4395.0, 6280.0)
	10,000,000	1.0539 (1.0442, 1.0729)	191.5 (165.1, 228.4)	4787.5 (4127.5, 5710.0)
	100,000,000	1.0719 (1.0618, 1.0918)	181.2 (160.0, 213.2)	4530.0 (4000.0, 5330.0)
$\theta = 0.00477$				
1/500	100,000	1.0539 (1.0396, 1.0807)	134.4 (113.3, 161.7)	3360.0 (2832.5, 4042.5)
	1,000,000	1.0813 (1.0660, 1.1111)	123.6 (106.7, 147.3)	3090.0 (2667.5, 3682.5)
	10,000,000	1.1097 (1.0937, 1.1415)	117.1 (103.6, 137.7)	2927.5 (2590, 3442.5)
	100,000,000	1.1390 (1.1223, 1.1724)	112.8 (101.6, 130.8)	2820.0 (2540, 3270)
1/5,000	100,000	1.0410 (1.0277, 1.0654)	142.8 (119.7, 171.9)	3570.0 (2992.5, 4297.5)
	1,000,000	1.0675 (1.0526, 1.0960)	128.2 (109.3, 153.7)	3205.0 (2732.5, 3842.5)
	10,000,000	1.0954 (1.0797, 1.1262)	120.0 (104.9, 142.1)	3000.0 (2622.5, 3552.5)
	100,000,000	1.1242 (1.1079, 1.1568)	114.8 (102.5, 134.0)	2870.0 (2562.5, 3350.0)
1/15,000	100,000	1.0351 (1.0227, 1.0579)	148.0 (124.1, 177.9)	3700.0 (3102.5, 4447.5)
	1,000,000	1.0610 (1.0463, 1.0887)	130.9 (110.9, 157.2)	3272.5 (2772.5, 3930.0)
	10,000,000	1.0886 (1.0731, 1.1190)	121.6 (105.7, 144.5)	3040.0 (2642.5, 3612.5)
	100,000,000	1.1173 (1.1011, 1.1495)	115.9 (103.0, 135.7)	2897.5 (2575, 3392.5)
1/30,000	100,000	1.0316 (1.0198, 1.0532)	152.0 (127.6, 182.4)	3800.0 (3190, 4560.0)
	1,000,000	1.0569 (1.0425, 1.0842)	132.9 (112.3, 159.8)	3322.5 (2807.5, 3995.0)
	10,000,000	1.0844 (1.0690, 1.1144)	122.8 (106.4, 146.2)	3070. (2660, 3655.0)
	100,000,000	1.1129 (1.0968, 1.1449)	116.7 (103.4, 137.0)	2917.5 (2585, 3425)

differing from the ancestral haplotype A by at least a single site. Haplotype A was not found on non-OCA chromosomes (78/78). Stevens et al. (1997) have also shown that this mutation deletion has a similar frequency among OCA2 patients from Zambia (11/14 chromosomes; 79%) but was associated with three haplotypes, the most common being the same haplotype as in southern Africa but occurring at a lower frequency (3/6; 0.50). Because the polymorphisms studied by these authors were defined only by a combination of probe and enzyme, any relationship to our present data is difficult to establish. However, in agreement with the latter report, we also show that the 2.7-kb deletion allele is associated with different intragenic haplotypes. The finding that the 2.7-kb deletion is found on three different haplotypes: TAGCT (0.68), TAGCC (0.26) and TAGTT (0.06) sharing the same 5′ core TAG is likely to reflect two ancestral intragenic recombinations that resulted in the present haplotypic diversity associated with this mutation. It is noteworthy that hot spots of recombination are located between the 5′ and the 3′ parts of the haplotype between markers rs1900758 (intron 13) and rs1800419 (exon 22) (Myers et al. 2005 and

http://www.stats.ox.ac.uk/mathgen/Recombination.html). These observations suggest that, within African populations in Cameroon, the mutation appeared on the relatively frequent haplotype TAGCT and that the two other haplotypes are derived from two independent recombination events. This may, at first, appear surprising, because albinism, a detrimental autosomic trait, is expected to affect the Darwinian fitness of affected individuals. Darwinian fitness (w), which reflects the ability of albinos to survive and reproduce, may have a lower value relative to non-albinos because of death at an earlier age, resulting mainly from solar radiation-induced skin cancer, or because of environmental accidents like malaria for our two albino girls. As skin cancer, which is the most frequent reason for death in equatorial regions, occurs mainly during the second to fourth decade of life, after reproductive age, we expect that the Darwinian fitness of albinos will not be severely decreased. Thus, natural selection has little or no influence on the frequency of the OCA2 deletion allele. For example, with a selection coefficient "s" of 0.5 measuring the degree of selection against albinos, the Darwinian fitness will be $w = 1 - 0.5 = 0.5$, and with an assumed mutation rate (μ) at the P locus of 2.5×10^{-5}, the frequency of albinos at equilibrium in a large population would be $q^2 = \mu/s = 5 \times 10^{-5}$ or 1/20,000, which is the usual value found in the world (Hedrick 2003). If $s = 0.3$ or 0.2, then the equilibrium incidence of albinism is only 1/12,000 and 1/8,000, the incidence values found in Cameroon. However, as the mutation rate at the P locus and the selection coefficient are unknown, the high frequency of the OCA2 deletion allele requires other explanations such as heterozygote advantage and/or cultural selection and/or chance processes: founder effect, bottleneck effect or genetic drift (Stern 1960).

The classic example of heterozygote advantage is resistance to infection by the malaria parasite (*Plasmodium falciparum*) in heterozygotes for sickle-cell anemia (Allison 1954), and more recently for G6PD deficiency, α+ thalassemia, and haemoglobin C (Kwiatkowski 2005). The application of novel haplotype-based techniques has demonstrated that malaria-protective genes have been subject to recent positive selection (Kwiatkowski 2005). Oettle (1963) speculated that the lighter skin colour of the OCA2 heterozygote, as demonstrated by skin reflectance (Roberts et al. 1986), may confer a social advantage, as individuals with lighter skins may be preferable as marriage partners. This hypothesis seems not to be true from the longstanding experience of one of us (R.A.) in Cameroon. If, as very often, children of albinos who are obligate heterozygotes have lighter skin than the full-coloured parent, we are not aware of any such advantage for OCA2 heterozygotes. The same conclusion about the absence of heterozygote advantage at the P locus was drawn in Amerindian populations with OCA2 albinism (Woolf 2005), particularly among the Navajo, which present a 122.5-kb deletion of the P gene (Yi et al. 2003).

The additional factor of cultural selection for albinos was suggested in Amerindians with OCA2. An intense negative cultural selection against San Blas Cuna albinos, culminating in infanticide, and almost complete marriage discrimination against albino males, has been documented. On the contrary, albinos were not ostracised or looked upon as being inferior among the Hopi and Zuni Indians, and they married full-coloured individuals and had healthy offspring (Woolf 2005). Hopi males with albinism were traditionally allowed to remain in the villages, thereby avoiding bright sunlight and its detrimental effects on them, and "had ample opportunity to engage in sexual activity" (Hedrick 2003). In Cameroon, we know that the average number of children, obligate heterozygotes, in albino families (full-coloured male/albino female mating or reverse, even though many were not married) is about the same as in their black counterparts. We also heard that females with albinism were preferred by full-coloured individuals to engage in sexual activity because they are white. In Cameroon traditional permissive sexual mores was observed. On the other hand, mating between albinos is not prohibited in Africa but is a very rare event. We had the opportunity in Mali to observe such a couple with four albino children (Aquaron 2000). In conclusion, it is difficult to establish if a mating advantage "m" of males and/or females exists.

An interesting example of a founder effect is described in the Bamileke society. The Bamileke society is organised as small kingdoms each headed by a king. In the West province, 131 kingdoms have been individualised and 88, i.e. 67%, have less than 5,000 inhabitants (Barbier and Nkwi 1977). The founder effect is illustrated in the Balengou kingdom where the eighth and ninth kings, at the beginning of the twentieth century had albinism. These kings were polygamists, with 80–100 wives and 200–300 children, who were obligate heterozygotes; on the other hand, in the Bamileke tribe, marriages tended to occur within the same clan or town (Puri et al. 1997) with a high endogamic rate of around 95% (Spedini et al. 1999). A hypothetical scenario would be that the founder effect was the cause of the initial high proportion of albinos in this small endogamous population, which was then spread by migrating groups to other geographic regions followed by the rapid increase in size of that population (Woolf 2005). This scenario could be possible because (1) Balengou had less than 5,000 inhabitants around 1900, (2) albinism occurs most frequently in the Bamileke group [70% (190/273 registered albinos) even if the 2.7-kb deletion was less common: 55% (94/170 alleles) than in other ethnic groups (Aquaron 1990; Aquaron and Berge-Lefranc 2002)], (3) the Bamileke population are the predominant ethnic group

in Cameroon with approximately 2 million in a population of 10 million with a high fecundity rate (Puri et al. 1997), (4) they are located mainly in the west province but for economic reasons also in the two main cities of Cameroon: Yaounde, the administrative capital, and Douala, the economic port where they represent, respectively, 20 and 40% of the population (Marguerat 1975).

Finally, it would seem that the 2.7-kb deletion allele is a predominantly Bantu-specific marker that has attained a high frequency by the combination of higher viability, some mating advantage of males and females with albinism, founder effect and by genetic drift or some other unknown selective force. The high endogamic rate (97–99%) in the Bamileke, Bassa, and Ewondo ethnic groups favours genetic drift (Spedini et al. 1999), but these values seem to us overestimated because, for example, of the 40 Ewondo albinos actually recorded, 10 are related to another ethnic group, giving an endogamic rate of 75% (unpublished results).

A number of other Bantu-specific markers have been described, including βS-associated haplotypes (Panier et al. 1984) and a 9-bp deletion in the mitochondrial DNA control region of sub-Saharan African populations (Soodyall et al. 1996).

It is generally accepted that the Bantu languages originated in the Middle-Benue valley between Nigeria and Cameroon approximately 3,000–4,000 years ago and then spread to other regions of sub-Saharan Africa (Spedini et al. 1999).The presence of the 2.7-kb deletion mutation in Negroids of central African origin (Cameroon, Gabon, Congo, Democratic republic of Congo, Central African Republic) and in the Bantu-speaking Negroids of southern Africa (Zambia, Zimbabwe, Southern-Africa) suggests that the mutation likely arose before the divergence of these groups, estimated to have occurred 2,000–3,000 years ago (Stevens et al. 1995, 1997). We were able to estimate the age of the mutation using intragenic markers located on either side of a recombination hot spot, thus allowing us to use recombination rates from a fine-scale genetic map (Myers et al. 2005), the estimated frequencies of different haplotypes associated with the mutation, the prevalence of the 2.7 kb mutation and the present effective population size (Table 2). With a value of 1/15,000 for the prevalence of the 2.7 kb mutation and an effective population size of 10,000,000, the age of the mutation was estimated at between 4,100 and 5,645 years for a recombination rate of 0.0031. These estimations are sensitive to several factors, including the recombination rate (the higher the recombination rate the lower the estimate) and possible selective positive and negative forces that are not taken into account here since their nature and direction remain highly speculative. Nevertheless, our data are consistent with estimations of the time of divergence of different African populations and the times of the second and third nucleii of sedentarisation in western and south-central regions of Cameroon 3,000–4,000 BP.

The βS mutation responsible for sickle-cell disease has appeared independently in different parts of the world in different genetic backgrounds. βS genes are found in linkage disequilibrium with five different restriction haplotypes in the β-globin gene cluster. Four haplotypes in Africa correspond to the Senegal, Bantu, Benin, and Cameroon types, respectively. Additional DNA sequence polymorphisms have been reported to be associated with these restriction haplotypes. They included single nucleotide variations at –551, –543, –521 and –419 upstream of the β-globin gene cap site, and a tandem repeated motif of a (AT) xTy structure located downstream of position –543, which defines four specific African patterns: Senegal, Bantu, Benin: TT(AT)8T4CC and Cameroon: TC(AT)8T5CA) types. Each of these types is different from the reference sequence TC(AT)7T7CA. In Cameroon, the Benin haplotype was found in 84% of cases in different ethnic groups: Bamileke, Bassa, Boulou, Ewondo, Yambassa. The remaining groups carry the Cameroon haplotype, restricted to the nearby Eton ethnic group (Lapoumeroulie et al. 1992; Elion et al. 1992). The Bantu haplotype is absent in Central and Southern Cameroon and is found only in Central and Southern Africa, in agreement with the Bantu expansion hypothesis, i.e. a journey that probably began in the Congo and ended in South Africa (Nagel 2004). All HbS carriers in our family carry the Benin haplotype. It is interesting to note that the βS-globin gene has been linked to the 5′ Benin haplotype for only 2,000–3,000 years BP, i.e. the approximate time of origin of the HbS gene (Steinberg et al. 1998). However, it has been found that 5–10% of chromosomes have less common haplotypes, usually referred to as "atypical" haplotypes frequently resulting from crossovers between a typical βS haplotype and a different βA-associated haplotype (Zago et al. 2000). These atypical haplotypes seem to be at higher risk for cerebrovascular accidents in Cameroon (Njamnshi et al. 2006). The normal atypical haplotype +AT TT(AT)6T8CC found in the mother and two children could be due to repetitive polymorphism.

Association of two distinct recessive disorders such as OCA2 and sickle-cell anemia, located on different chromosomes, is very rare. In a literature search we found two such observations: the first linked MacArdle disease and fatal hepatopathy due, respectively, to a homozygous nonsense mutation (p.V456M) in the PYGM gene, on chromosome 11q13 associated with a homozygous 4-bp GATT duplication in the deoxyguanosine kinase gene on chromosome 2p13 (Mancuso et al. 2003); the second was oculocutaneous albinism type 4 and complement component six deficiency due, respectively, to a homozygous deletion in the MATP (SLC45A2) gene (c.264delC) and to

a homozygous nonsense mutation (p.S91X) in the C6 gene, located in close proximity (approximately 7 Mb), on the short arm of chromosome 5 (Ikinciogullari et al. 2005). The manifestation of two rare conditions results from the respective high frequency of the two mutated alleles in a given population.

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