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SHORT COMMUNICATION

Is autosomal recessive deafness associated with oculocutaneous albinism a "coincidence syndrome"?

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Abstract Hearing impairment is frequently found associated with pigmentary disorders in many syndromes. However, total oculocutaneous albinism (OCA) associated with deafness has been described only once, by Ziprkowski and Adam (Arch Dermatol 89:151–155, 1964) in an inbred family. A syndrome associating deafness and OCA was suggested by the authors, but two separate recessive genes segregating in this inbred group were also proposed later by Fraser (OMIM # 220900). Combined deafness and total OCA were also observed by us in a family originally reported to be nonconsanguineous but in which haplotyping showed evidence of a common ancestry: the proband was affected by both diseases, one of his sisters had only OCA and another sister had only deafness. Both the proband and his deaf sister were found to be homozygotes for the 35delG mutation (*GJB2* gene), the most frequent cause of hereditary deafness. Linkage analysis with markers close to the four known OCA loci excluded linkage to OCA1, OCA2, and OCA3, and homozygosity in markers near OCA4 locus was observed. Sequencing of the corresponding gene (*MATP*) revealed a c.1121delT mutation, which leads to a stop codon at position 397 (L374*fs*X397). Clearly, the combined occurrence of deafness and albinism in this pedigree was due to mutations in two different genes, showing autosomal recessive inheritance. We speculate that the putative syndrome reported by Ziprkowski and Adam might have resulted from the co-occurrence of autosomal recessive deafness and albinism in the same pedigree, as suggested by Fraser.

Keywords Deafness · Pigmentary disorders · Oculocutaneous albinism · *GJB2* · *MATP*

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Introduction

Hereditary hearing impairment is frequently found associated with pigmentary disorders in genetic diseases such as Waardenburg syndrome, Tietz-Smith syndrome, and piebaldism. Nevertheless, in none of these syndromes is total oculocutaneous albinism (OCA) due to complete lack of melanin present, and only partial albinism, ocular or cutaneous, is reported. Oculocutaneous albinism is a group of autosomal recessive disorders characterized by total hypopigmentation of hair, skin, and eyes, and is usually associated with severe nystagmus, photophobia, and reduced visual acuity. OCA is not generally associated with deafness.

Ziprkowski and Adam (1964) described a Sephardic Jewish family from Morocco in which two children in each of two sibships born from consanguineous parents had congenital deafness with total albinism. The two

sibships shared a pair of great-great-grandparents. In one of the sibships, two sibs were doubly affected and three sibs had only congenital deafness. Fraser [1982, cited in Online Mendelian Inheritance in Man (OMIM) # 220900, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM] proposed that there might be two separate recessive genes segregating in this inbred group. Here we report on a Brazilian pedigree with individuals affected by deafness and/or OCA. Molecular analysis demonstrated that two independently segregating mutated genes, *GJB2* and *MATP*, were responsible for the phenotypes. The co-occurrence of two autosomal recessive phenotypes within the same family is most probably due to consanguinity.

Subjects and methods

Subjects

The proband (III-1, Fig. 1), born to parents who claimed nonconsanguinity, was examined by us at age 16 years. He presented with hypopigmentation of eyes, eyelashes, eyebrows and skin, and white-yellowish hair, contrasting with his normal pigmented sisters. The skin was milky-white, with some pinkish-white areas on sun-exposed skin regions. According to the parents' report, there was no increase in pigmentation in the skin and very little increase in pigmentation in the hair. His visual acuity was 20/150 in the right eye and 20/100 in the left eye (normal 20/20); he presented with strabismus, mild horizontal nystagmus, mild photophobia, and grayish iris; iris transillumination was present, pupillary red reflex was present; at ocular fundus exam, he presented with macular hypoplasia, visible choroidal vessels, and no pigment in the retinal pigment epithelium. He was also affected by moderate to severe sensorineural prelingual deafness (Fig. 2a).

A 12-year-old sister (III-4) also showed a similar hypopigmentation as her brother. Her visual acuity was 20/400p in both eyes; she presented with strabismus, severe nystagmus, mild photophobia, blue iris; iris transillumination was present; pupillary red reflex was present; at ocular fundus exam, she presented with macular hypoplasia, visible choroidal vessels, and no pigment in the retinal pigment epithelium. Her hearing was normal. Another of his sisters aged 7 years (III-6) was affected by profound sensorineural prelingual deafness (Fig. 2b), but had normal oculocutaneous pigmentation. His remaining four sisters had normal oculocutaneous pigmentation and no hearing impairment was reported (III-2, III-3, III-5, III-7).

Audiological evaluation

The following hearing measurements were performed: acoustic immittance, including tympanometry and acoustic reflexes thresholds, tonal and vocal audiometry

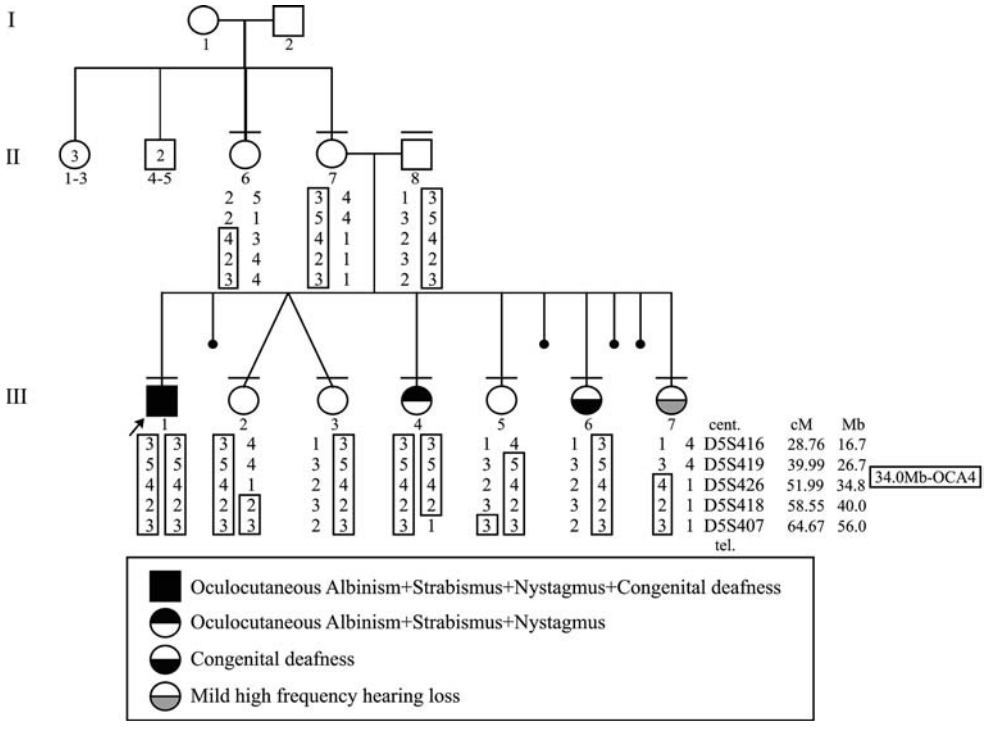

Fig. 1 Pedigree showing the haplotypes linked to *MATP* gene. The *boxed haplotype* segregates with the c.1121delT mutation. Individuals III-1, III-6, and III-7 are homozygotes for 35delG mutation and individuals II-7, II-8, and III-2 are heterozygotes for this mutation

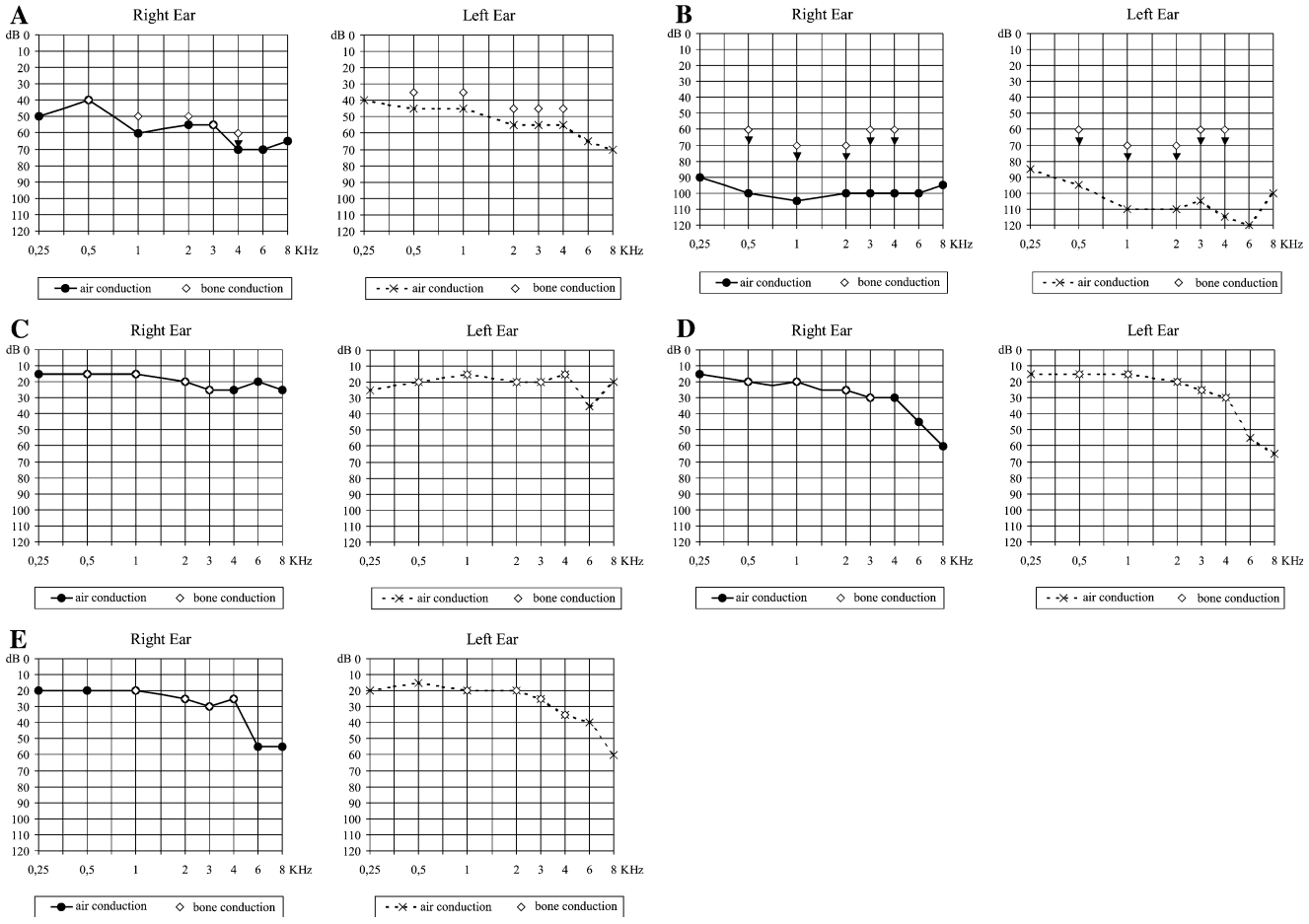

Fig. 2a–e Tonal audiometries of the patients. **a** Patient III-1 at 15 years. **b** Patient III-6 at 6 years. **c** Patient III-7 at 3 years and 6 months, **d** 4 years and 7 months, and **e** 5 years

with conditioning methods according to patient's age; transiently evoked oto-acoustic emissions and distortion product were also registered. We considered a response to the oto-acoustic emissions test to occur when the values obtained with the relationship sign/noise were greater than 6 dBNPS (Hall and Mueller 1997).

Molecular genetic analysis

This study was approved by CONEP, the Brazilian National Committee on Ethics in Research. DNA samples were obtained after written informed consent of all family members enrolled in the study. DNA was extracted from 300 µl of whole blood using a GFX Genomic Blood DNA Purification Kit (Amersham Biosciences).

35delG mutation screening

The 35delG mutation in the *GJB2* gene was tested by allele-specific PCR using previously described primers (Scott et al. 1998). The presence of the 35delG mutation was confirmed by sequencing with primers F-5′ GTG TTG TGT GCA TTC GTC TTT TC-3′ and R-5′ ACC TTC TGG GTT TTG ATC TCC TC 3′, using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Buckinghamshire, UK) and MegaBACE 1000 DNA Analysis System (Amersham Biosciences).

Linkage analysis

Microsatellite markers (ABI PRISM Linkage Mapping Set v. 2.5-MD10 kit, Applied Biosystems) close to loci DFNB1 (D13S175 and D13S1275), OCA1 (D11S901, D11S4175), OCA2 (D15S128, D15S1002, D15S165), OCA3 (D9S286, D9S285, D9S157), and OCA4 (D5S416, D5S419, D5S407, D5S426, D5S418) were amplified. Fluorescent-labeled PCR products were analyzed in MegaBACE 1000 DNA Analysis System with the software Genetic Profiler version 1.5 (Amersham Biosciences).

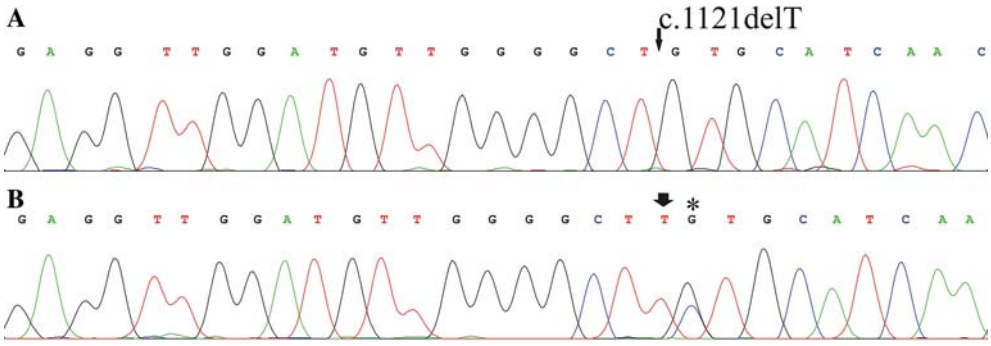

Fig. 3 **a** Partial sequence of exon 5 in the proband (III-1) bearing the c.1121delT mutation. **b** Sequence of a control sample, heterozygous for the L374F polymorphism (*)

MITF gene analysis

SSCP analysis of the eight exons of *MITF* gene was performed. PCR primers were designed using the ''Primer3'' program (Rozen and Skaletsky 2000). Products were electrophoresed on 5% MDE gel (3% glycerol) (BioWhittaker Molecular Applications, Rockland, ME, USA, formerly FMC Bioproducts) at 6 W for 10–14 h at 20°C, and visualized after silver staining (Bassam et al. 1991).

MATP gene analysis

The seven exons of the *MATP* gene were sequenced. PCR primers were also designed using the ''Primer3'' program (Rozen and Skaletsky 2000). PCR products were purified and sequenced using a DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences) and analyzed with the MegaBACE 1000 DNA Analysis System (Amersham Biosciences).

Results and discussion

Considering the hypothesis of a syndrome involving both hearing loss and pigmentation disorder to explain our findings, *MITF* seemed a good candidate gene, since it is found to be mutated in Waardenburg syndrome type 2 and in Tietz-Smith syndrome, both characterized by a combination of pigmentation anomalies and deafness. However, no mutation in the *MITF* gene was detected in the proband (III-1).

To test the hypothesis of two independent mutations causing the phenotypes, we screened the family for the 35delG mutation in *GJB2*, the most frequent cause of autosomal recessive nonsyndromic deafness. Individuals III-1, III-6, and III-7 were homozygotes for 35delG mutation and individuals II-7, II-8, and III-2 heterozygotes for this mutation (Fig. 1). The homozygotes for this mutation were also homozygous for two microsatellites mapped close to the *GJB2* gene. We infer that this was the probable genetic cause of deafness in the proband and his sister.

However, it was surprising that III-7, who was also a homozygote for the 35delG mutation, demonstrated normal hearing thresholds until almost 4 years of age(Fig. 2c). The hearing measurements had first been conducted with III-7 because of her family history of hearing loss and not because of parental concerns (Fig. 2c). Later examination showed that oto-acoustic emissions were partially absent, and audiometric thresholds indicated mild high-frequency hearing loss (Fig. 2d). Based on the different hearing thresholds observed at 3 years (Fig. 2c), 4 years (Fig. 2d), and 5 years (Fig. 2e), we hypothesize that she is manifesting a later-onset, progressive hearing loss. This contrasts strongly with the majority of 35delG homozygotes, who exhibit a prelingual congenital hearing loss or a fast progression of hearing loss shortly after birth. Further, it is unusual for a 35delG homozygote to be observed speaking and hearing at the age of four. Bolz et al. (2004) also reported a 35delG homozygote who did not exhibit any hearing impairment before the age of 12 years, although no audiometry testing had been performed to confirm this. Pagarkar et al. (2006) recently described two siblings homozygous for the 35delG mutation who passed hearing tests in early infancy and later developed progressive hearing loss. Our results, together with those of Pagarkar et al., illustrate that a surprising phenotypic variability associated with 35delG homozygosity can exist. It seems likely that such phenotypic variability is caused by as-yet-unknown environmental factors and/or modifier genes.

We went on to investigate the genetic cause of albinism in the pedigree and performed linkage analysis with microsatellites close to the four known OCA loci. Our results allowed exclusion of linkage to loci OCA1, OCA2, and OCA3. Both individuals affected by OCA exhibited homozygosity in a region spanning ~30 cM on chromosome 5, which includes the OCA4 locus (Fig. 1). None of the normally pig-

mented sisters of the proband were homozygotes for this region.

Thus, the seven exons of the *MATP* gene, already defined as the OCA4 gene (Newton et al. 2001), were sequenced in III-1 and III-4. Exon 5 showed a homozygous deletion of a T at position 1121 of the cDNA (genBank accession number AF172849) in both individuals (Fig. 3). Position c.1121 corresponds to the transmembrane domain 8 of the MATP protein (genBank accession number NP_057264). This frameshift mutation leads to a stop codon in exon 5 at position 397 of the protein (L374*fs*X397). All other family members (II-6, II-7, II-8, III-2, III-3, III-5, III-6, and III-7) tested were found to be heterozygous for the c.1121delT mutation. Interestingly, this deletion is in the same codon where the L374F polymorphism (c.1122G>C) is located, and was recently associated with normal variation in skin color (Inagaki et al. 2004): the 374Phe allele is significantly associated with fair hair, skin and eye color in different Caucasian populations, while the 374Leu allele presents a significantly higher frequency in African and Asian populations (Graf et al. 2005; Nakayama et al. 2002; Yuasa et al. 2004). All our subjects were homozygotes for the 374Leu allele. This finding is not surprising given the admixture of Brazilian populations, with the contribution of Europeans, native Americans, and Africans.

In conclusion, the combination of deafness and albinism within the same individual presented in this report occurred as two independent autosomal recessive diseases due to mutations in two different genes, *GJB2* and *MATP*, respectively. This is the first report of OCA resulting from mutations in the *MATP* gene in Brazil. Although the parents did not acknowledge consanguinity, the microsatellite data for both the deafness and OCA loci indicate that they shared a common ancestor. Given the high prevalence of *GJB2* mutations in all populations, we speculate that the association of complete albinism and deafness reported by Ziprkowski and Adam resulted by chance from consanguineous matings as proposed by Fraser (1982, cited in OMIM #220900).

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References

Bassam BS, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Bolz H, Schade G, Ehmer S, Kothe C, Hess M, Gal A (2004) Phenotypic variability of non-syndromic hearing loss in patient heterozygous for both c.35delG of *GJB2* and the 342-kb deletion involving *GJB6*. Hear Res 188:42–46

Graf J, Hodgson R, van Daal A (2005) Single nucleotide polymorphisms in the *MATP* gene are associated with normal human pigmentation variation. Hum Mutat 25:278–84

Hall JW, Mueller HG (1997) Otoacoustic emissions. In: Hall JW, Mueller HG (eds) Audiologists desk reference. Singular, San Diego, pp 235–288

Inagaki K, Suzuki T, Shimizu H, Ishii N, Umezawa Y, Tada J, Kikuchi N, Takata M, Takamori K, Kishibe M, Tanaka M, Miyamura Y, Ito S, Tomita Y (2004) Oculocutaneous albinism type 4 is one of the most common types of albinism in Japan. Am J Hum Genet 74:466–471

Nakayama K, Fukamachi S, Kimura H, Koda Y, Soemantri A, Ishida T (2002) Distinctive distribution of AIM1 polymorphism among major human populations with different skin color. J Hum Genet 47:92–94

Newton JM, Cohen-Barak O, Hagiwara N, Gardner JM, Davisson MT, King RA, Brilliant MH (2001) Mutations in the human orthologue of the mouse underwhite gene (uw) underlie a new form of oculocutaneous albinism, OCA4. Am J Hum Genet 69:981–988

Pagarkar W, Bitner-Glindzicz M, Knight J, Sirimanna T (2006) Late postnatal onset of hearing loss due to GJB2 mutations. Int J Pediatr Otorhinolaryngol 70(6):1119–1124

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, NJ, pp 365–386

Scott DA, Kraft ML, Carmi R, Ramesh A, Elbedour K, Yairi Y, Srisailapathy CR, Rosengren SS, Markham AF, Mueller RF, Lench NJ, Van Camp G, Smith RJ, Sheffield VC (1998) Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. Hum Mutat 11:387–394

Yuasa I, Umetsu K, Watanabe G, Nakamura H, Endoh M, Irizawa Y (2004) *MATP* polymorphisms in Germans and Japanese: the L374F mutation as a population marker for Caucasoids. Int J Legal Med 118:364–366

Ziprkowski L, Adam A (1964) Recessive total albinism and congenital deaf-mutism. Arch Dermatol 89:151–155