Clinical Report

Angelman Syndrome Associated With Oculocutaneous Albinism Due to an Intragenic Deletion of the P Gene

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Angelman syndrome (AS) is a neurodevelopmental disorder characterized by mental retardation, speech impairment, ataxia, and happy disposition with frequent smiling. AS results from the loss of expression of a maternal imprinted gene, UBE3A, mapped within 15q11-q13 region, due to different mechanisms: maternal deletion, paternal UPD, imprinting center mutation, and UBE3A mutation. Deletion AS patients may exhibit hypopigmentation of skin, eye, and hair correlating with deletion of P gene localized in the distal part of Prader-Willi (PWS)/AS region. Our patient presented developmental delay, severe mental retardation, absence of speech, outbursts of laughter, microcephaly, ataxia, hyperactivity, seizures, white skin, no retinal pigmentation, and gold yellow hair. His parents were of African ancestry. The SNURF-SNRPN methylation analysis confirmed AS diagnosis and microsatellite studies disclosed deletion with breakpoints in BP2 and BP3. All of the 25 exons and flanking introns of the P gene of the patient, his father, and mother were investigated. The patient is hemizygous for the deleted exon 7 of the P gene derived from his father who is a carrier of the deleted allele. Our patient manifests OCA2 associated with AS due to the loss of the maternal chromosome 15 with the normal P allele, and the paternal deletion in the P gene. As various degrees of hypopigmentation are associated with PWS and AS patients, the study of the P gene in a hemizygous state could contribute to the understanding of its effect on human pigmentation during development and to disclose the presence of modifier pigmentation gene(s) in the PWS/AS region. © 2003 Wiley-Liss, Inc.

KEY WORDS: hypopigmentation; albinism; P gene; Angelman syndrome

INTRODUCTION

Angelman syndrome (AS) is characterized by neurodevelopmental delay, severe mental retardation, speech impairment, ataxia, happy disposition with frequent laughter, macrostomia, wide-spaced teeth, and protruding tongue [Williams et al., 1995]. AS results from the loss of expression of a maternal expressed imprinted gene, UBE3A, mapped within the 15q11-q13 chromosome region [Kishino et al., 1997; Matsuura et al., 1997; Nicholls and Knepper, 2001]. Different mechanisms can lead to the AS phenotype and although the most common genetic mechanism is a maternal deletion of the PWS/AS region which is responsible for about 70% of the cases, ~5% of the patients present paternal uniparental disomy (UPD), 1–2% show mutation in the imprinted center (IC), ~10% of the patients present mutation in the UBE3A gene, and in the remaining cases the genetic mechanism is still unknown [Nicholls and Knepper, 2001].

Both PWS and AS deletion patients show a great consistency in deletion size and identify three hotspots for chromosome breakage in the 15q11-q13 region, referred to as BP1, BP2 (proximal breaks), and BP3 (distal break) [Christian et al., 1999].

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The human P gene consists of 25 exons spanning between 250 and 600 kb of the chromosome region 15q11-q13. The P protein appears to be an integral membrane protein of melanosomes [Lee et al., 1995]. Many studies have demonstrated that mutations in the P gene are associated with the tyrosinase-positive oculocutaneous albinism (OCA, type II), the most prevalent type of albinism worldwide [Ramsay et al., 1992; Rinchik et al., 1993; Durham-Pierre et al., 1994, 1996; Lee et al., 1994; Stevens et al., 1997].

PWS and AS patients with paternal or a maternal deletion, respectively, exhibit hypopigmentation of the skin, eyes, and hair when compared to their parents that is correlated with the deletion of one copy of the P gene localized in the distal part of the 15q11q-13 region [King et al., 1993; Spritz et al., 1997].

AS patients may occasionally present the clinical features of OCA2 due to a mutated P gene in the remaining paternal chromosome 15. Indeed, Saitoh et al. [2000] described a patient with AS and OCA2 for whom they found a paternal P gene missense mutation (1441G→A, A481T) and deletion of the maternal chromosome 15q11-q13. Durham-Pierre et al. [1994] found that the 2.7 kb deletion spanning the whole exon 7 of the P gene is a frequent mutation among people of African origin, and they briefly described an AS patient who carries a single paternally derived P allele with the 2.7 kb deletion. Here we report a patient with Angelman syndrome and OCA2 due to a 2.7 kb deletion present in the remaining paternally inherited chromosome 15.

**CLINICAL REPORT**

The propositus is a Brazilian boy who was born to healthy non-consanguineous parents. At birth, the mother was 24 and the father 32 years of age. He was born in the 31st week of gestation, with birth weight of 1750 g, and apgar score of 3 (1 min) and 6 (5 min). Developmental milestones were delayed: he sat with support at 4 years of age and unsupported after the age of 5 years, walked alone between 6 and 7 years of age. He was referred for genetic studies at age 7½ to disclose the diagnosis of AS as he presented neuro-psychomotor developmental delay, severe mental retardation, absence of speech, happy disposition with outbursts of laughter, microcephaly, brachycephaly, ataxia, hyperactivity, seizures with onset at the age of 1½ years, and albinism with gold yellow hair (Figure 1A,B). His father and mother are of African ancestry and the father has darker skin than the mother. The mother indicated that a maternal grandmother's sister had albinism.

**MATERIALS AND METHODS**

**Southern Blotting**

The methylation status of the PWS/AS region was assessed by Southern blotting. The genomic DNA of the patient was extracted from peripheral blood leukocytes by standard procedures, digested with XbaI + NotI, run on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized as described [Nicholls et al., 1989]. The probe used was a 0.6 kb EcoRI-NotI fragment, which contains exon 1 of SNURF-SNRPN [Glenn et al., 1996; Gray et al., 1999].

**Analysis of Microsatellite Markers**

Microsatellite analyses were also performed on DNA from the patient and his parents in order to identify the genetic mechanism envolved. Three microsatellite
markers within the critical region 15q11-q13, 4-3RCA (D15S11), LG6-1CA (D15S113), and GABRB3CA (GABRB3) were typed in a multiplex PCR reaction as previously described [Mutirangura et al., 1993], and one marker outside the critical region was also tested (D15S131). To detect the extent of the deletion, we used markers D15S542, D15S543, D15S1002, and D15S1048 as described by Amos-Landgraf et al. [1999].

**Mutation Study of the P Gene**

All of the 25 exons and flanking introns of the P gene were PCR-amplified by the primer pairs described previously [Lee et al., 1995]. The products were agarose gel-purified by GFX PCR and Gel Band Purification Kit (Amersham Pharmacia Biotech, Uppsala, Sweden), and direct-sequenced with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Sequence reactions were run on Perkin-Elmer 310 Automatic sequencer and analyzed by use of LASERGENE software (DNASTAR, Madison, WI). The three primer assay system developed by Durham-Pierre et al. [1994] was employed to detect the 2.7 kb intragenic deletion of the P gene. The sequences of the primers were 5’-TTTGCCTAGGTTTCAAGGGGAG-3’ (MHB51), 5’-GCGTGCTGCTATGGC-3’ (MHB72), and 5’-GGAG-GTGTCATTCTGACTGTGA-3’ (MHB71). The primer pair MHB51 and MHB71 gives an 820 bp PCR product for the 2.7 kb intragenic deleted allele, whereas the primer pair MHB72 and MHB71 gives a 240 bp PCR product for the normal allele.

**RESULTS**

The methylation analysis of exon 1 of the SNURF-SNRPN bicistronic gene showed a typical methylation pattern for AS, thus confirming the diagnosis. Microsatellite analysis of loci mapped within and outside the PWS/AS region revealed that the genetic mechanism involved is a maternally derived deletion and the breakpoints are in BP2 (between the markers D15S542 and D15S543) and BP3 (between the markers D15S1002 and D15S1048) [Amos-Landgraf et al., 1999; Christian et al., 1999] (data not shown).

To analyse the molecular basis for the albinism in this AS patient, all of the exons and flanking introns of the P gene were direct sequenced. All of the exons and flanking introns were normal except exon 7 that was not amplified. Therefore the patient could have an intragenic deletion of the P gene. PCR analysis for detecting the deletion using the primer pairs described by Durham-Pierre et al. [1994] were used. The patient is hemizygous for the deleted exon 7 of the P gene that is derived from the father who is a carrier of this allele; the mother has a normal allele (Fig. 2).

**DISCUSSION**

Our patient manifests recessively inherited OCA2 associated with AS because he has lost the maternal chromosome 15 carrying one P allele and has a mutated P allele in the paternal 15. The distal breakpoint of the chromosome deletion is in BP3, which is distal of the P gene, and is thus included in the deletion. The prevalence of the OCA2 resulting from the 2.7 kb deletion of exon 7 of the P gene is 1 out of 1,800 in individuals of African origin [Durham-Pierre et al., 1994]. Theoretically, about 1 out of 20 persons of African origin carries this mutation.

The intragenic deletion of the P gene results in a frameshift mutation in the first luminal loop, predicting a truncation of the polypeptide and hence a non-functional gene product [Durham-Pierre et al., 1994]. Because the maternal P gene is lost and the paternal P gene has intragenic deletion, the patient has no functional P gene product. This is reflected in his white skin, gold hair, and no retinal pigmentation. The color of the skin and hair of the patient has not changed so far at least by the age of 12 years old. This is different from the patient described by Saitoh et al. [2000] whose hair color changed from gold to black after some years due to the hemizygous A481T missense mutation that appears to result in a partially functional protein. These cases can help us to understand the full spectrum of the AS condition associated with P mutations.

AS patients can present hypopigmentation resulting from reduced expression of the P gene, due to mutations in promoter region or some polymorphic changes that influence the P gene expression. Other unknown gene(s) in PWS/AS region can also be deleted and also be responsible for pigmentation. Besides, AS patients present the gene in hemizygous state while normal carriers...
of OCAII mutation are not hypopigmented because they still have a normal allele.

As various degrees of hypopigmentation are associated with PWS and AS deletion patients [Spritz et al., 1997], the study of the P gene present in a hemizygous state could contribute to the understanding of its effect on human pigmentation during development, and to disclose the presence of additional or modifier pigmentation gene(s) in the PWS/AS region.

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