European Journal of Human Genetics (2009) 17, 1378–1380
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www.nature.com/ejhg

LETTER

Mutation in the *SLC9A6* gene is not a frequent cause of sporadic Angelman-like syndrome

European Journal of Human Genetics (2009) **17,** 1378–1380; doi:10.1038/ejhg.2009.82; published online 27 May 2009

Angelman syndrome (AS) (MIM #105530) is a neurodevelopmental disorder characterized by a broad range of clinical features, such as profound mental retardation, impairment of verbal language, usually ataxia, subtle dysmorphic facial features, a peculiar behavioral profile, with a happy disposition and outbursts of laughter, and a seizure disorder associated with a characteristic electroencephalographic pattern.[1] It affects between 1:15 000 and 1:40 000 newborn children depending on ethnical and geographical areas.[2–5] Most cases of AS are sporadic, although several familial cases have been reported. A variety of different genetic mechanisms involving chromosome 15q11.2-13 (deletion, paternal uniparental disomy, imprinting center abnormality, and *UBE3A* (MIM #601623; 15q11.2) mutations) have been described in association with AS, all resulting in a deficiency of the ubiquitin-protein ligase E3A, which is involved in the process of ubiquitination.[6] In some cases, patients with AS share common features with patients affected by Rett syndrome (MIM #312750).[7] After extensive cytogenetic and molecular analysis of chromosome 15q11-13, there remains a small group of about 10–15% of AS patients, in whom no abnormality can be identified.

In a recent paper by Gilfillan *et al* (2008),[8] the authors investigated nine candidate genes in the Xq24-27.3 interval in patients with a phenotype mimicking AS (MIM #300243, Angelman-like syndrome) that did not indicate mutations in the *UBEA3* gene, as well as in the *MECP2* gene (MIM #300005), which is mutated in patients with Rett syndrome. They identified four mutations in the *SLC9A6* gene (MIM #300231; Xq26.3), which encodes the Na^+/H^+ exchanger 6 (NHE6) protein that is suspected to be involved in the regulation of the pH lumen in early recycling endosomes.[9,10] Three deletions and one nonsense mutations were shown to be deleterious for the function of the protein.

As we could not find mutations in the genes cited above (*UBE3A* and *MECP2*) and a characteristic molecular 15q abnormality (normal methylation and negative linkage to the critical 15q11-q13 region) in a cohort of patients with Angelman-like features, the aim of our study was to test these individuals for mutations in the *SLC9A6* gene. We analyzed 59 clinically well-documented boys from Caucasian or North-African origin by direct sequencing of the 16 encoding exons as well as exon–intron boundaries. All of them had the four clinical characteristics designated 'consistent' (present in 100%) in the diagnostic criteria for AS laid down by Williams *et al*[11]: severe developmental delay, no or minimal use of words, movement or balance disorder, and behavioral uniqueness. In addition, all had some of the other criteria described by Gilfillan *et al*[8] in their *SLC9A6* mutation patients, such as microcephaly and seizures. After informed consent, genomic DNA was extracted from peripheral blood lymphocytes according to the standard protocols and serves as a template for subsequent amplification of the regions of interest. Primer sequences and PCR conditions are available upon request to the corresponding author. Sequencing reactions were carried out with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Courtaboeuf, France) and loaded on the Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems). Output data were analyzed with Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA) and chromatograms were visually controlled by two independent operators.

In patient #50 (Supplementary information 1), we identified the c.25G>T variant in *SLC9A6* exon 1 substituting an alanine to a serine at position 9 of the protein (p.A9S) (Supplementary information 2, black arrow). As shown by sequence comparison (data not shown), alanine at codon 9 (NP_006350.1) is strictly conserved in several vertebrates (*Macaca mulatta*, *Bos taurus*, *Gallus gallus*, *Mus musculus* and *Rattus norvegicus*), suggesting its critical role for the function of the protein. This variant allele was transmitted by his unaffected mother (Supplementary information 2, white arrowhead). This male patient is the fifth child of related first-cousin parents from Egypt. He was born at term with normal weight (2940 g), height (46.5 cm), and head circumference (34 cm). From birth, his psychomotor development was delayed. His general development was delayed and he never established proper eye contact or obtained an expressive verbal language. He started walking independently at 18 months. Other features were hyperkinetic movements and deficit-attention disorder. At last examination, growth parameters were within normal range (−1 SD) but he showed microcephaly (−4 SD), no dysmorphic features except synophrys. Deep tendon reflexes were normal and there was no spasticity. Brain MRI, EEG, and metabolic explorations were normal as well as high-resolution karyotype and FISH with subtelomeric probes. Initially, he had been suspected of

Angelman-like syndrome because of the association of profound mental retardation, secondary microcephaly, hyperkinetic behavior and absent speech, but relevant genetic examinations regarding this diagnosis had been negative. Interestingly, his 25-year-old sister, who also presented with profound mental retardation, does not carry the c.25G>T allele (Supplementary information 2, black arrowhead). Acute analysis of the phenotype of this patient shows several discrepancies with her brother. In the neonatal period, she presented with severe intrauterine growth retardation (birthweight 1000 g). From early infancy, she had delayed developmental milestones, started walking at 3 years old and never developed expressive language. At last evaluation, growth parameters were below −2 SD and head circumference was measured at 47.5 cm (below −2 SD). She presented dysmorphic features that consist of ptosis, brachymetacarpia, and clinodactyly of the fifth finger. Neurological examination showed a combination of spasticity and dystonic posture, but no ataxia. Seizures and sleep disorders have never been reported. Hence, she presents profound mental retardation without Angelman-like features as observed in her brother, but with additional signs that consist of dysmorphic features, growth retardation, spasticity, and dystonia. Moreover, AS features such as walking with the arms flexed at the elbows and open mouth with drooling were not observed. Karyotype was normal. Although consanguinity may suggest an autosomal recessive disorder, all tested parameters (blood and urine) exclude both amino acid metabolic and organic acid disorders, as well as other metabolic diseases, such as Congenital Disorders of Glycosylation (CDG) syndrome and lysosomal diseases. EEG as well as cardiac and renal ultrasound were normal. Cerebral MRI showed moderate cerebellar atrophy. To investigate whether this variant can be found in the general population, we screened 400 unaffected male individuals. It was found in one subject from the Egyptian origin, thus suggesting that the variant is a rare polymorphism, with an estimated ~0.4% frequency (2/459).

Two additional intronic single nucleotide polymorphisms (SNPs) were found in other patients: the c.1538+8G>A transition in intron 11 (patient #15), which has been referred to as rs6654310 in the HapMap database; and the c.1725−4G>A transition in intron 14 (patient #22) that has not been reported yet, but unlikely alters splicing as it is located one nucleotide upstream of the three base pair C/T-A-G consensus 3′-splice site in humans.

In our cohort of 59 patients showing Angelman-like features, no unambiguous disease-causing mutation was found. An earlier report found *SLC9A6* mutations in 4 out of 73 families with patients having an AS-like phenotype, and suggest that disruption of *SLC9A6* should be considered in male patients with a non-15q11-13-related AS phenotype, particularly when X-linked inheritance in the family is suspected.[8] Two recent studies (Schwartz *et al*, Raymond *et al*, personal communications) reported different frequencies of mutations within the *SLC9A6* gene in AS-like patient cohorts (0–10%). These data as well as ours highlight that in most of cases, the genetic basis of Angelman-like phenotype remains unknown and other unidentified genes and/or genetic abnormalities are undoubtly involved in conditions with phenotypical overlap. As suggested in the earlier report, weight (that appears low in patients with *SLC9A6* mutations) and EEG pattern (rapid background frequency of 10–14 Hz) should be taken into account in the choice of patients to be considered for *SLC9A6* gene screening.

Acknowledgements

This work was supported by Institut National de la Santé et de Recherche Médicale (ANR-Maladies Rares ANR-06-MRAR-003-01, and ANR E-Rare EuroRETT Network).

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Supplementary information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)