RESEARCH NOTE

Novel missense mutation (L1917P) involving sac-domain of *NSD1* gene in a patient with Sotos syndrome

FRANCESCO NICITA[1,*], LUIGI TARANI[2], ALBERTO SPALICE[1], MARINA GRASSO[3], LAURA PAPETTI[1], MASSIMILIANO CECCONI[3], CLAUDIO DI BIASI[4], FABIANA URSITTI[1] and PAOLA IANNETTI[1]

[1]*Department of Pediatrics, Child Neurology Division, Sapienza University of Rome, 00161 Rome, Italy*
[2]*Department of Pediatrics, Sapienza University of Rome, 00161 Rome, Italy*
[3]*Laboratory of Genetics, Galliera Hospital, 16128 Genoa, Italy*
[4]*Department of Radiology, Sapienza University of Rome, 00161 Rome, Italy*

[Nicita F., Tarani L., Spalice A., Grasso M., Papetti L., Cecconi M., Biasi C., Ursitti F. and Iannetti P. 2011 Novel missense mutation (L1917P) involving sac-domain of *NSD1* gene in a patient with Sotos syndrome. *J. Genet.* **90**, 147–150]

Introduction

Sotos syndrome (SS) (OMIM 117550) is an autosomal dominant disease belonging to the group of overgrowth conditions (Tatton-Brown and Rahman 2007). This syndrome was first described by Sotos in 1964 and diagnostic criteria were successively defined and reviewed (Sotos *et al.* 1964; Cole and Hughes 1994; Tatton-Brown *et al.* 2005). The diagnosis is based on the presence of three cardinal features that are characteristic facial appearance, learning disability and macrocephaly (occipito–frontal circumference, OFC > 97%) with frequent prenatal and postnatal overgrowth (Saugier-Veber *et al.* 2007). These cardinal features are reported in more than 90% of patients (Tatton-Brown and Rahman 2007). Typical facial characteristics include high, broad forehead, fronto–temporal sparse hairs, malar flushing, down-slanting palpebral fissures and a pointed chin. In addition, a broad series of major and/or minor anomalies may occur. Major features are present in more than 15% of patients, such as advanced bone age, neonatal jaundice, neonatal hypotonia, seizures, scoliosis, poor feeding in infancy, maternal pre-eclampsia, joint laxity, cardiac, renal and brain anomalies (Tatton-Brown and Rahman 2007). Minor anomalies are numerous (Tatton-Brown and Rahman 2007).

Recently, mutations of nuclear receptor set domain containing protein 1 gene (*NSD1*; 5q35.2-q35.3) have been identified as the genetic cause of SS (Kurotaki *et al.* 2002; Douglas *et al.* 2005). Since then more than 250 patients with SS have been reported; *NSD1* anomalies include mutations and 5q35 microdeletions (partial and encompassing *NSD1* gene); 5q35 microdeletions are more frequently found in Japan (Tatton-Brown and Rahman 2007).

Here we describe a patient with SS and *NSD1* missense mutation occurring in SAC (SET-associated cystein-rich) domain, still not described in the literature. This patient presents a wide clinical spectrum consisting of cardinal, major and minor features.

Material, methods and results

Our patient is a 5-year-old boy born from nonconsanguineous healthy Italian parents, after a cesarean delivery at 36th week, following an uneventful twin dichorionic pregnancy. Apgar scores were 9 and 10 at 1st and 5th min. Birth weight was 2,460 g (5th centile), birth length 52 cm (75th centile), OFC 38 cm (> 97th centile). Neonatal hypotonia was present. During the 2nd month the patient suffered from gastroesophageal reflux, needing for treatment with antacids till the 4th month. Developmental milestones were delayed: head control was achieved at 6 months and sitting without support at 10 months; he started to walk at 18 months while the first words were not still pronounced.

At the age of 2 the patient came for the first time to our attention for focal febrile status epilepticus, presenting with tonic–clonic seizures of the right side of the body and lasting 50 min, associated with acute respiratory insufficiency. Intravenous lorazepam, and successively, phenytoin were necessary to stop the seizures. Blood examinations were suggestive for infectious disease and chest X-ray showed an opacity of the upper right lobe, suggestive for lobar pneumonia, and was treated successfully with antibiotics. Post-ictal EEG revealed diffuse slow waves with rare

*For correspondence. E-mail: childneurology.sapienzaroma@live.it.

Keywords. Sotos syndrome; *NSD1*; overgrowth; seizures; syncope; MRI.

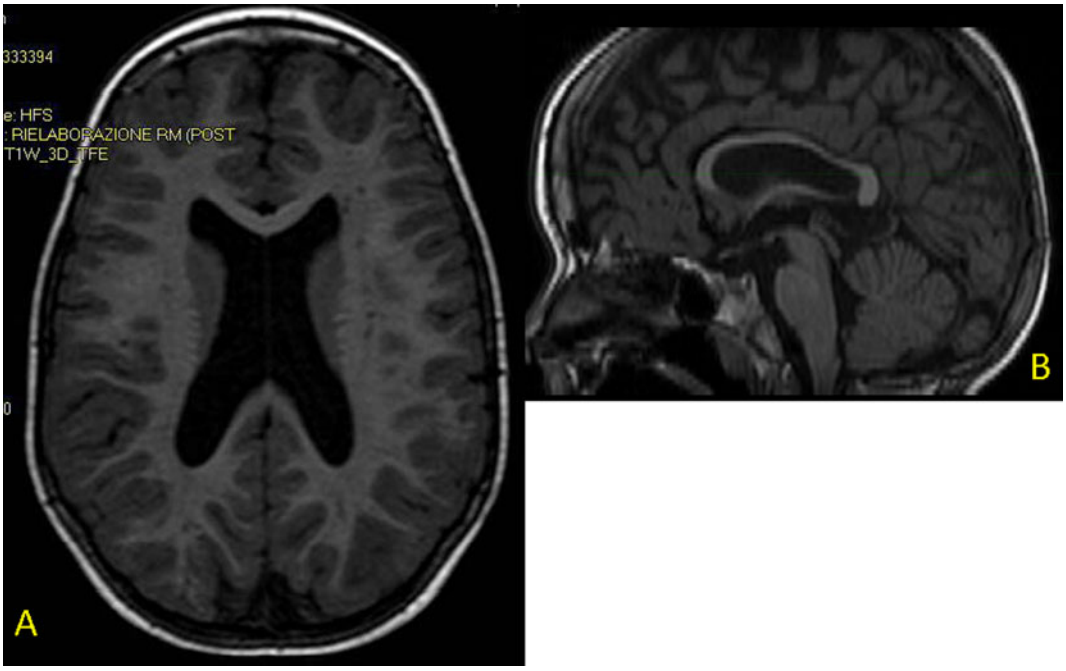

Figure 1. Cerebral MRI performed at 4 years of age. A, enlargement of lateral ventricles and prominent trigone, unilateral dilated Virchow–Robin spaces in the left deep white matter (corona radiata); dilation of frontal subarachnoid spaces. B, thinning of the posterior third of the corpus callosum body, pineal gland cyst.

spikes in left hemisphere. During the hospitalization, neurological examination revealed OFC = 52 cm (> 97th centile), diffuse muscular weakness and hypotonia, ligamentous laxity. Brain MRI, performed at 2 years and one month of age, discovered lateral ventricles enlargement with prominence of trigone and occipital horns, thinning of the posterior third of corpus callosum body, unilateral widening of Virchow–Robin spaces in the left deep white matter (corona radiata), dilatation of frontal subarachnoid spaces, pineal gland cyst (figure 1, a&b). Metabolic analysis gave normal results. Before discharge, antiepileptic treatment with phenobarbital 45 mg/day was started. Between the 2nd and the 3rd year of life, the patient experienced two brief febrile seizures and two episodes of bronchopneumonia. At the age of 4 years, the patient started to present frequent episodes characterized by pallor, perioral cyanosis, blurred vision, hypotonia with loss of consciousness and collapse lasting few seconds. Thus, a second hospitalization was required. Neurological examination confirmed the previous data. The child length was 110 cm (97th centile), weight was 17 kg (50th centile), OFC was 56 cm (> 97th); facial appearance of the patient is shown in figure 2. Results of blood examinations were all in the normal range, including glycemia and electrolytes. Cardiac echography revealed false tendons in left ventricle, left ventricular end diastolic dimension of 38 mm, slight mitral regurgitation. ECG showed a RSR pattern in precordial derivation V1–V2. Cerebral MRI confirmed the pathological findings of the previous scans. EEGs showed normal background activity without epileptic abnormalities. Head-up tilt test demonstrated the vasovagal origin of the syncope. Developmental evaluation discovered global delay of learning ability, prevailing on praxis, attention and communication abilities. Raven test, attention sustained,

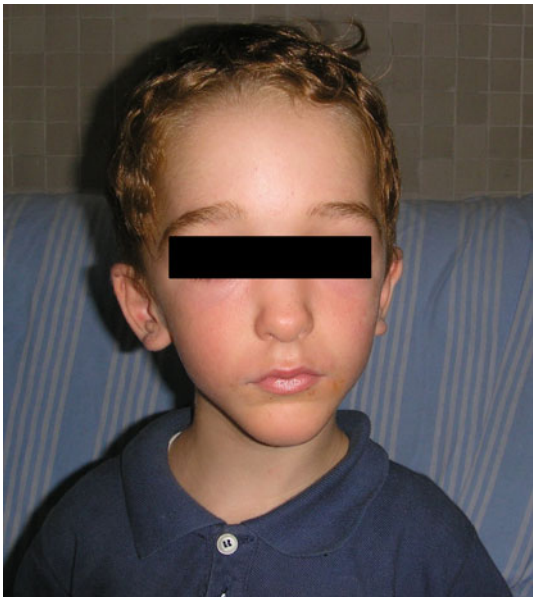

Figure 2. Facial appearance of patient. Round face with disproportionate prominence of forehead typical of macrodolichcephaly with receding hairline, apparent hypertelorism with down slanting palpebral fissures, prominent jaw and pointed chin, malar flushing, anteverted nostrils, mild micrognathia, high arched palate and large ears.

forward memory tests revealed a mild-moderate developmental delay. Hand X-ray identified a disharmonic advanced bone age, included between the 5th and the 6th years of life. Abdominal echography (liver, spleen, gall bladder, pancreas, bladder and kidneys) was normal.

Till the present time the patient has not experienced other episodes of febrile or afebrile seizures, and treatment with phenobarbital has been stopped during the second hospitalization. Episodes of syncope did not recur. Physiotherapy and psychomotor rehabilitation are performed weekly to improve developmental and physical outcome.

After informed consent was obtained, DNA was extracted from peripheral blood sample of the patient. Genetic analysis of exons 2–23 of *NSD1* gene was performed by means of denaturing high performance liquid chromatography, DHPLC (Transgenomics, Omaha, USA), and direct sequencing of the chromathographic variant on 3130xl sequencer (Applied Biosystems, Foster City, USA) and revealed a heterozygous missense mutation c.5750 T>C (p.Leu1917Pro; exon 18), localized in SAC domain. This missense mutation was not present in more than 750 patients and in 90 control individuals analysed. Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (sorting intolerant from tolerant, http://blocks.fhcrc.org/sift/SIFT.html) were used as tools to predict the effect of the mutation on the protein. Using polyphen-2 the variant L1917P is predicted to be 'possibly damaging' (score of 0.807), and using SIFT program is predicted to 'affect protein function' (score of 0.02).

Discussion

The differential diagnoses of SS include benign familial macrocephaly and the group of overgrowth syndromes, such as Beckwith–Wiedemann, Bannayan-Riley-Ruvalcaba and Weaver syndrome. These entities may share some clinical features, but positive molecular analysis of *NSD1* gene can confirm the diagnosis of SS (Baujat and Cormier-Daire 2007). The *NSD1* gene contains 23 exons and encodes a protein of 2696 amino acids, whose functions have not been fully elucidated, however, it is likely to play a role in transcriptional regulation (Tatton-Brown and Rahman 2007). *NSD1* anomalies are not found in 7%–35% of cases of SS. To date, the genotype–phenotype correlations are not completely defined; the main available studies suggest that patients with intragenic mutations are taller and with less severe developmental delay than patients with chromosomal microdeletions (Kurotaki *et al.* 2003; Nagai *et al.* 2003; Tatton-Brown *et al.* 2005).

In our patient, the presence of the three cardinal features and several other major/minor conditions associated with SS allowed us to suppose a clinical diagnosis of 'typical Sotos syndrome'. Genetic analysis of *NSD1* gene revealed a novel missense mutation and confirmed the diagnosis, since the mutation is placed within functional domains of the protein product (Tatton-Brown *et al.* 2005), and the involved amino-acid (leucine at position 1917) is highly conserved up to chicken considering 11 species (Alamut Mutation Interpretation Software v. 1.5; www.interactive-biosoftware.com).

Unfortunately, we were not able to perform parental analysis, however, the absence of this mutation in more than 750 patients and 90 control individuals indicates that it is not a polymorphism and therefore strongly supports its pathogenic role. The *NSD1* mutation carried by our patient has not been reported in the literature yet; it involved the SAC domain, one of the *NSD1* gene functional domains. In patients with SS, missense mutations are typically placed in functional domains at 3′ end of the gene, however missense mutations of SAC domain can be considered quite rare: in the largest genotype–phenotype study reporting *NSD1* mutations in patient with SS, only 7/266 mutations involved the SAC domain (Tatton-Brown *et al.* 2005).

The neurological involvement seems to be predominant in our patient, and it is characterized by diffuse hypotonia, mild-moderate mental retardation, episodes of febrile seizures with or without status epilepticus, and episodes of syncope. All these findings have been previously associated with SS, with the exception of syncope. Seizures may appear in about 50% of patients but they are febrile in half the cases (Baujat and Cormiere-Daire 2007). Status epilepticus has been rarely reported and associated with poor outcome (Korematsu *et al.* 1995). There are no data on whether those patients with febrile seizures later developed idiopathic seizures (Leventopoulos *et al.* 2009). In our patient the ictal manifestations started at the 2nd year of life with febrile focal status epilepticus lasting 50 min and requiring treatment with intravenous lorazepam and phenytoin. Subsequently, the patient experienced other few and brief episodes of febrile seizures, and afebrile seizures have not been observed till now. Since treatment with phenobarbital has been stopped, seizures did not recur. The vasovagal origin of the syncope has been demonstrated by the tilt test and by the absence of epileptic activity at EEG. At present episodes of syncope have been considered as benign and selflimiting; in fact, although initially numerous (about 10 episodes), they became less frequent and then disappeared without needing further treatment. It is know that neurally-mediated syncope is quite frequent among the population, however episodes of syncope are not included in the numerous anomalies affecting patients with SS.

The available neuroradiological studies demonstrate that some cerebral anomalies recur in patients with SS. Ventricles enlargement, prominence of trigone and/or occipital horn, increased extra-cerebral fluid, midline anomalies such as cavum vergae and/or septum pellucidum, thinning or hypoplasia of corpus callosum are frequently reported (Schaefer *et al.* 1997; Aoki *et al.* 1998; Melo *et al.* 2000, 2002; Horikoshi *et al.* 2006). Some of these anomalies have been discovered in our patient together with dilatation of Virchow–Robin spaces, whose clinical and pathogenic significance remains unclear, although they are considered

rare in healthy subjects (Barkhof 2004; Groeschel *et al.* 2006). However, dilatation of Virchow–Robin spaces has never been reported in cases of SS and it may be a casual association that should be confirmed in larger case studies.

Conclusions

The pathogenic role of *NSD1* gene in SS has been recently discovered and since then a large number of mutations has been reported. Therefore, the identification of a novel mutation, falling in rarely involved domain such as SAC domain, adds a useful contribution to the current knowledge of the molecular basis of SS.

References

Aoki N., Oikawa A. and Sakai T. 1998 Serial neuroimaging studies in Sotos syndrome (cerebral gigantism syndrome). *Neurol. Res.* **20**, 149–152.

Barkhof F. 2004 Enlarged Virchow–Robin spaces: do they matter? *J. Neurol. Neurosurg. Psychiatry* **75**, 1516–1517.

Baujat G. and Cormier-Daire V. 2007 Sotos syndrome. *Orphanet J. Rare Dis.* **2**, 36–42.

Cole T. R. and Hughes H. 1994 Sotos syndrome: a study of the diagnostic criteria and natural history. *J. Med. Genet.* **31**, 20–32.

Douglas J., Tatton-Brown K., Coleman K., Guerrero S., Berg J., Cole T. R. *et al.* 2005 Partial NSD1 deletions cause 5% of Sotos syndrome and are readily identifiable by multiplex ligation dependent probe amplification. *J. Med. Genet.* **42**, e56.

Groeschel S., Chong W. K., Surtees R. and Hanefeld F. 2006 Virchow–Robin spaces on magnetic resonance images: normative data, their dilatation, and a review of the literature. *Neuroradiology* **48**, 745–754.

Horikoshi H., Kato Z., Masuno M., Asano T., Nagase T., Yamagishi Y. *et al.* 2006 Neuroradiologic findings in Sotos syndrome. *J. Child. Neurol.* **21**, 614–618.

Korematsu S., Goto K., Ishihara T., Ishiwa S., Izumi T. and Ogawa T. 1995 Status epilepticus in two patients with Sotos syndrome. *No To Hattatsu* **27**, 29–34.

Kurotaki N., Imaizumi K., Harada N., Masuno M., Kondoh T., Nagai T. *et al.* 2002 Haploinsufficiency of NSD1 causes Sotos syndrome. *Nat. Genet.* **30**, 365–366.

Kurotaki N., Harada N., Shimokawa O., Miyake N., Kawame H., Uetake K. *et al.* 2003 Fifty microdeletions among 112 cases of Sotos syndrome: low copy repeats possibly mediate the common deletion. *Hum. Mutat.* **22**, 378–387.

Leventopoulos G., Kitsiou-Tzeli S., Kritikos K., Psoni S., Mavrou A., Kanavakis E. *et al.* 2009 A clinical study of Sotos syndrome patients with review of the literature. *Pediatr. Neurol.* **40**, 357–364.

Melo D. G., Pina-Neto J. M., Acosta A. X., Daniel J., de Castro V. and Santos A. C. 2000 Neuroimaging and echocardiographic findings in Sotos syndrome. *Am. J. Med. Genet.* **90**, 432–434.

Melo D. G., Acosta A. X., Salles M. A., Pina-Neto J. M., Castro J. D. and Santos A. C. 2002 Sotos syndrome (cerebral gigantism): analysis of 8 cases. *Arq. Neuropsiquiatr.* **60**, 234–238.

Nagai T., Matsumoto N., Kurotaki N., Harada N., Niikawa N., Ogata T. *et al.* 2003 Sotos syndrome and haploinsufficiency of NSD1: clinical features of intragenic mutations and submicroscopic deletions. *J. Med. Genet.* **40**, 285–289.

Saugier-Veber P., Bonnet C., Afenjar A., Drouin-Garraud V., Coubes C., Fehrenbach S. *et al.* 2007 Heterogeneity of NSD1 alterations in 116 patients with Sotos syndrome. *Hum. Mutat.* **28**, 1098–1107.

Schaefer G. B., Bodensteiner J. B., Buehler B. A., Lin A. and Cole T. R. 1997 The neuroimaging findings in Sotos syndrome. *Am. J. Med. Genet.* **68**, 462–465.

Sotos J. F., Dodge P. R., Muirhead D. D., Crawford J. D. and Talbot N. B. 1964 A syndrome of excessively rapid growth and acromegalic features and a nonprogressive neurologic disorder. *N. Engl. J. Med.* **271**, 109–116.

Tatton-Brown K., Douglas J., Coleman K., Baujat G., Cole T. R., Das S. *et al.* 2005 Genotype–phenotype associations in Sotos syndrome: an analysis of 266 individuals with NSD1 aberrations. *Am. J. Hum. Genet.* **77**, 193–204.

Tatton-Brown K. and Rahman N. 2007 Sotos Syndrome. *Eur. J. Hum. Gen.* **15**, 264–271.

Received 7 June 2010; in revised form 8 September 2010; accepted 27 September 2010
Published on the Web: 19 May 2011