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ARTICLE

Further delineation of neuropsychiatric findings in Tatton-Brown-Rahman syndrome due to disease-causing variants in *DNMT3A*: seven new patients

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Abstract

Tatton-Brown-Rahman (TBRS) syndrome is a recently described overgrowth syndrome caused by loss of function variants in the *DNMT3A* gene. This gene encodes for a DNA methyltransferase 3 alpha, which is involved in epigenetic regulation, especially during embryonic development. Somatic variants in *DNMT3A* have been widely studied in different types of tumors, including acute myeloid leukemia, hematopoietic, and lymphoid cancers. Germline gain-of-function variants in this gene have been recently implicated in microcephalic dwarfism. Common clinical features of patients with TBRS include tall stature, macrocephaly, intellectual disability (ID), and a distinctive facial appearance. Differential diagnosis of TBRS comprises Sotos, Weaver, and Malan Syndromes. The majority of these disorders present other clinical features with a high clinical overlap, making necessary a molecular confirmation of the clinical diagnosis. We here describe seven new patients with variants in *DNMT3A*, four of them with neuropsychiatric disorders, including schizophrenia and psychotic behavior. In addition, one of the patients has developed a brain tumor in adulthood. This patient has also cerebral atrophy, aggressive behavior, ID, and abnormal facial features. Clinical evaluation of this group of patients should include a complete neuropsychiatric assessment together with psychological support in order to detect and manage abnormal behaviors such as aggressiveness, impulsivity, and attention deficit-hyperactivity disorder. TBRS should be suspected in patients with overgrowth, ID, tall stature, and macrocephaly, who also have some neuropsychiatric disorders without any genetic defects in the commonest overgrowth disorders. Molecular confirmation in these patients is mandatory.

Introduction

Overgrowth syndromes (OGS) comprise a heterogeneous group of disorders whose main characteristic is that either the weight, height, or head circumference, (often also occurring together) are above the 97th centile or 2–3 standard deviations (SD) above the mean for age and gender [1]. Most of the OGS are associated with other clinical features that sometimes overlap between them, making the clinical diagnosis a challenge for pediatricians and geneticists.

Tatton-Brown-Rahman syndrome (TBRS) (MIM 615879) is a relatively recent described OGS disorder [2]. Cardinal features of this condition include overgrowth (mainly tall stature and macrocephaly), intellectual disability (ID), and distinctive facial appearance consisting of an oval face, horizontal eyebrows, and narrow palpebral fissures (Supplementary Table 1) [3]. TBRS is caused by heterozygous loss of function variants in the methyltransferase 3 alpha (*DNMT3A)* gene located on chromosome band 2p23.3. *DNMT3A* encodes an enzyme involved in epigenetic programming by de novo methylation,

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maintenance, and remodeling of DNA methylation patterns, and a more recently uncovered function in transcriptional activation [4, 5]. The DNA methyltransferase family of proteins comprises a group of enzymes that catalyze the transfer of a methyl group to DNA. Three active DNA methyltransferases have been described in mammals (*DNMT1*, *DNMT3A*, and *DNMT3B*). *DNMT1* is the gene responsible of ADCADN (Autosomal dominant cerebellar ataxia, deafness, and narcolepsy, MIM# 604121) [6]. Depression and psychosis have also been reported in patients with ADCADN [7], suggesting that variants in *DNMT1* may result in an aberrant gene expression or silencing of particular neuronal cells. *DNMT1* is also involved in sensory hereditary neuropathy type IE characterized by adult onset of progressive peripheral sensory loss associated with progressive hearing impairment and early onset dementia [8]. Immunodeficiency-centromeric instability-facial anomalies syndrome 1 (MIM# 242860) is caused by autosomal recessive variants in *DNMT3B*, being the most frequent clinical features the facial dysmorphism, ID, recurrent respiratory, skin, and gastrointestinal infections, and a variable immunodeficiency with constant decrease of IgA.

Both DNMT3A and DNMT3B proteins have a key role in epigenetic regulation by de novo methylation in the human genome [9]. DNMT3A contains three well established functional domains consisting in a tetrapeptide PWWP (Pro-Trp-Trp-Pro motif) domain, a polybromo homology domain (PHD) and a methyltransferase domain (MTase).

TBRS is a rare, possibly underdiagnosed condition with an unknown prevalence. Up to date, there are 79 patients formally reported or mentioned in the literature (55 summarized in [10]), most of them diagnosed by means of NGS DNA sequencing technologies, which has made possible the diagnosis of individuals with ID and/or overgrowth. Reported pathogenic variants in TBRS include missense, nonsense, and frameshift changes in *DNMT3A*, and at least one case had a microdeletion of 2p23 including this gene, supporting haploinsufficiency as the pathogenetic mechanism of this disorder [11]. Isolated cases and vertical transmission in some pedigrees demonstrated autosomal dominant inheritance with variable expressivity [3].

Somatic variants in *DNMT3A* have been reported in acute myeloid leukemia (AML, MIM# 601626) [12, 13] and other hematologic malignancies by epigenetic changes due to alterations in the methyltransferase activity of the enzyme. Thus, abnormal methylation pattern of DNMT3A seems to be a major factor for cancer development. Functional analysis of the variant p.Arg882His, which is located in the MTase domain of DNMT3A, showed strong changes in the flanking sequence preference of DNTM3A in different CpG sites, which in turn alters the DNA interaction of DNMT3A [14]. There is only one reported case of TBRS with development of AML [15], while there are others TBRS patients with the same *DNMT3A* variants (commonly associated with cancer) that have not presented neoplasia up to date [16]. Thus, the relationship between TBRS and neoplasia due to constitutive variants in *DNMT3A* is not clear yet. Besides, germline gain of function variants in *DNMT3A* have been recently implicated in microcephalic dwarfism, a group of conditions of profound size reduction in humans [29].

Schizophrenia (SZ) is a multifactorial neurological disorder with a heterogeneous complexity, making hard to establish the underlying genetic causative defect. Dysregulation in metabolic pathways represents an important and frequent mechanism in SZ. In fact, several reports have pointed out the importance of this mechanism in the physiopathogenesis of SZ [17–19]. Interestingly, DNMT3A is also involved in several metabolic pathways, including cysteine and methionine metabolism through the catalysis of the transfer of a methyl group from an S-adenosyl-L-methionine to S-adenosyl-L-homocysteine.

Herein, we report seven patients with novel disease-causing variants in *DNMT3A*, four of them with different degrees of neuropsychiatric disorders, encompassing psychotic behavior, schizophrenia, and hallucinations. We also briefly review all the TBRS cases reported so far.

Material and methods

Patients

Patients were selected from the Spanish Overgrowth Syndromes Registry (SOGRI), which includes about 2000 individuals and relatives with overgrowth disorders. This study has been approved by the ethical committee of Hospital Universitario La Paz and informed consent was obtained from all patients and/or parents. Neuropsychiatric findings were evaluated by an specialist for each patient.

In addition to the SOGRI patients, a revision of all previously reported individuals in the scientific literature has been made and the phenotypes of these cases have been compared with the SOGRI patients described in this report.

Genetic analysis

NGS libraries were constructed from peripheral blood leukocyte DNA with the Roche SeqCap EZ Kit with a custom NGS panel which contains 212 genes and seven genomic loci related to OGS (overgrowth panel v.2.3). NGS sequencing has been performed using a NextSeq500 equipment (Illumina, USA). Data analysis consisted first, in transforming the bcl files from the NextSeq500 sequencer to fastq files by means of the Illumina des-multiplexing tool bcl2fastq. Then, the

sequences were mapped to the UCSC human reference genome, hg19 (version February 2009) with Bowtie2 [20]. Duplicate reads were removed using Picard's MarkDuplicates function (http://picard.sourceforge.net/). Indel realignment and base quality score recalibration was performed afterward (RealignerTargetCreator, IndelRealigner and BaseRecalibrator functions from the suite GATK) following the best practices proposed by GATK [21]. Variant calling was performed over the realigned and recalibrated BAM files. The variants characterized were the result of an in-house consensus criterion between the outputs of the GATK variant callers UnifiedGenotyper and HaplotypeCaller. The consensus vcf files were filtered and annotated with Annovar [22]. In addition, the vcf file was enriched with prediction tools of pathogenicity provided by the proxy dbNSFP [23] (release 3.0) together with population data (Exac Non-Finnish European data) [24] and the CIBERER Spanish Variant Server (http://csvs.babelomics.org/), clinical, and genomic information. Candidate variants were validated by Sanger sequencing according to the standard procedures and electropherograms were analyzed with Sequencher v4.1.4 (Genecodes, USA). For patient four, whole exome sequencing was performed with the Ion AmpliSeq exome (ThermoFisher, USA), which captures >97% of the consensus coding sequences, >90% base on-target, and >90% coverage uniformity. Sequencing was performed by an Ion Proton instrument (ThermoFisher, USA) and reads were then aligned against the human reference sequence built 37/hg19. Interpretation of variants was made according to the American College of Medical Genetics and Genomics (ACMG) guidelines [25]. These guidelines classify variants in five groups: benign, likely benign, variant of unknown significance, likely pathogenic, and pathogenic.

Results

Clinical description of patients

Patient 1

A 25 years old male son of a non-consanguineous couple. He was born at term by Cesarean section due to cephalo-pelvic disproportion. Preeclampsia and macrosomia during pregnancy were observed. At birth: weight: 4.58 kg (+3.11 SD), height: 56 cm (+2.23 SD), occipitofrontal circumference: 38 cm (+1.3 SD), Apgar 9–10. He had generalized hypotonia, left hip subluxation, and macroglossia. Sitting at 7 month, crawling at 13 months, walking at 23 months. First syllables at 6 month, first words at 13 months, first phrases of two words at 28 months and three or more at 33 months.

At 30 months old he was hospitalized due to febrile seizures. Generalized developmental delay was detected with abnormal behavior. Physical examination at 12 years old: weight: 79.2 kg (+3.24 SD), height: 162.5 cm (+1.77 SD), head circumference: 57.8 cm (+2.79 SD). He has macrocephaly and his facial features include a long oval face with large forehead, thick eyebrows, hypertelorism, downslanting and narrow palpebral fissures, broad nasal base, deep philtrum, and high palate. The hands had thin fingers and there was an enlarged circumference of the leg with marked hypertrophy and mild bilateral hallux valgus. His karyotype was 46,XY, without pathogenic CNVs in the microarray analysis. MS-MLPA for Prader-Willi/Angelman syndromes did not show any abnormality. He was enrolled at SOGRI as a patient with non-syndromic OGS with ID.

A neuropsychiatric evaluation revealed crisis of aggressiveness to objects in response to auditory hallucinations, visual and kinesthetic hallucinatory symptomatology that disappeared when readjusting antipsychotics. At 20 years he was referred to the hospital due to a heteroaggressive episode with severe behavior alterations and psychotic symptoms due to persistent auditory hallucinations.

Patient 2

A male aged 26 years, son of a non-consanguineous couple. His parents were 29 and 32 years old at the moment of the child's birth, respectively. His maternal grandmother suffered from breast cancer. Pregnancy was controlled with maternal obesity. He was born at term; his mother suffered from hyperglycemia and eclampsia requiring Cesarean section. At birth: weight was 4.5 kg (+2.91 SD) and height 52 cm (+0.7 SD). Head circumference and Apgar information were not available. He was hospitalized at the neonatal period due to urinary infection with bilateral hydronephrosis demonstrated by dilatation of renal calyces. During his infancy he was operated of adenoid hypertrophy, strabismus, and ear tube drainage. He had mild ID. Physical examination at 14 years: weight: 73 kg (+1.6 SD), height: 184 cm (+2.59 SD), head circumference: 58 cm (+2.22 SD). Long oval face, large forehead, thick horizontal eyebrows, deep philtrum, scoliosis, enlarged breast button, and cutis laxa. A brain computed tomography evidenced discrete frontotemporal atrophy.

He was initially enrolled at SOGRI with the diagnosis of Sotos syndrome (SoS) (MIM 117550) (Fig. 1). His karyotype was 46,XY. Previous MLPA and Sanger sequencing of the 23 exons of *NSD1* gene did not find deletions/duplications or single nucleotide pathogenic variants.

Patient 3

A 48-year-old male, referred to Neurology Department because of paranoid schizophrenia, aggressiveness, and

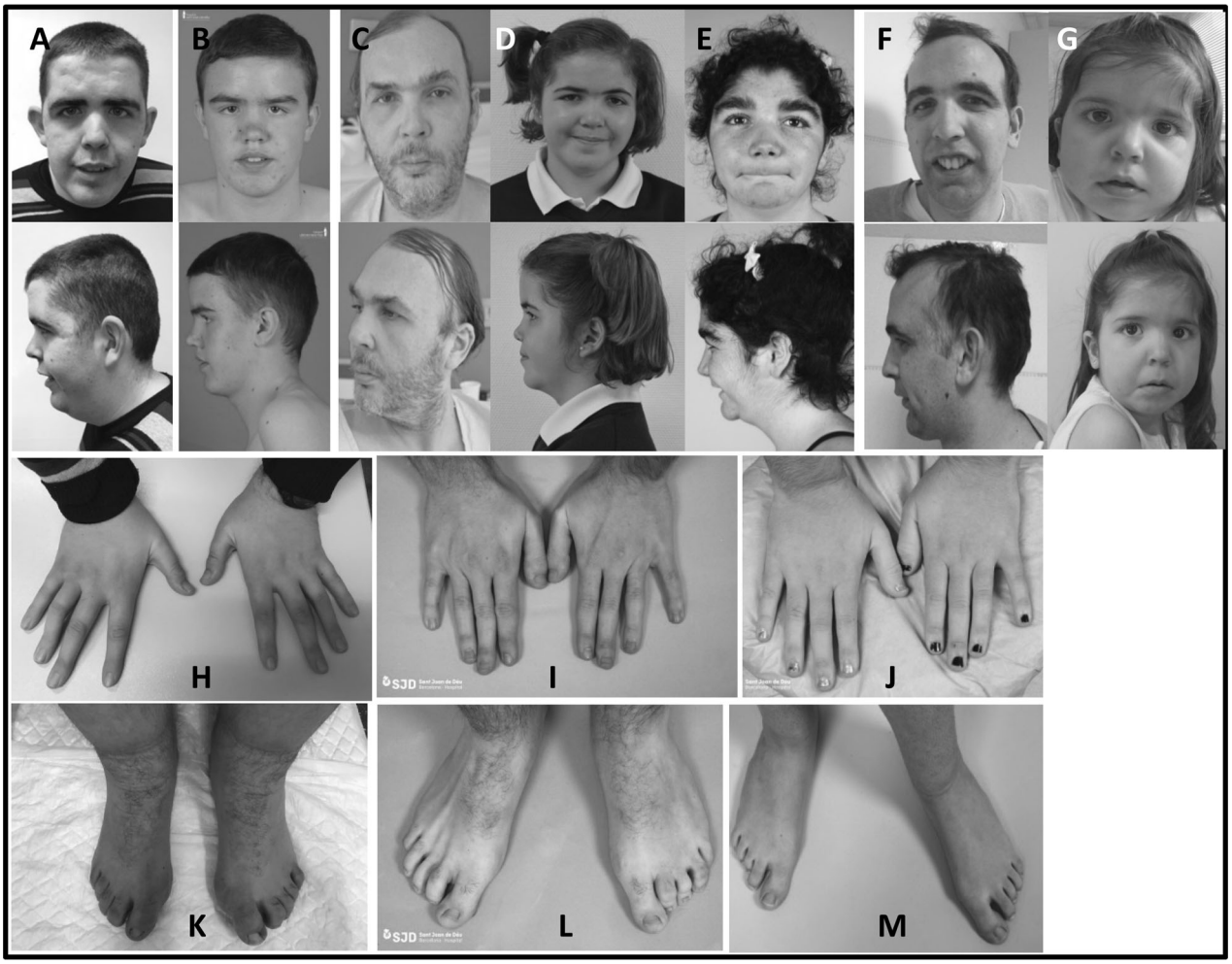

Fig. 1 Phenotypic features of seven patients with variants in *DNMT3A*. **a–g** (Patients 1–7, respectively): Note facial dysmorphic features with macrocephaly, horizontal and thick eyebrows. Also, large hands with large fingers and abnormal feet. **h** and **k**: patient 1; **i** and **l**: patient 2; **j** and **m**: patient 5

cerebral atrophy. He had been followed-up due to a diagnosis of paranoid behavior. He also has moderate ID with tall stature, joint hypermobility, multiple nevus, mitral regurgitation, abnormal facial shape with long oval face, thick eyebrows, and narrow palpebral fissures (Fig. 1c).

The patient was admitted at the hospital several times since 2010, because of lack of pharmacopsychiatric adherence. One year ago, a MRI screening showed abnormalities compatible with a cerebral tumor, which was later confirmed with a biopsy. The pathology report demonstrated that he had a benign glioma without histological signs of aggressiveness. He also presented impossibility to control the sphincters and difficult to sleep with prolonged drowsiness.

Patient 4

A 10-year-old female, third child of a non-consanguineous parents. Normal pregnancy, preterm birth at 36 weeks with 3300 g of weight (90th centile). Developmental delay was detected during first months of life. Autonomous ambulation was at 26 months of life. At 2 years she presented an episode of febrile seizure. At 6 years old, she suffered for speech delay without behavior abnormalities, needing a speech therapist. Anthropometric parameters were in the medium-high centile: 105.5 cm height (75–90 centile), 21.5 kg (97th centile). She has an abnormal facial shape including broad forehead, midface hypoplasia with short nasal bridge and short neck (Fig. 1d).

Patient 5

A 17-year-old female, the daughter of a non-consanguineous couple with neither history of cancer nor ID. The couple has had three previous spontaneous abortions. Delivery was at term. Birth weight, height, and head circumference were 2.9 kg (50th centile), 51 cm (75th centile), and 35 cm (75th centile), respectively.

Since birth, overgrowth was evident with tall stature and macrocephaly. She also presented facial dysmorphic features such as anteverted nares, downslanting palpebral

fissures, ocular hypertelorism, long philtrum, low anterior hairline, thick eyebrow, thin upper lip vermilion, large earlobes, and hypertrichosis. Abnormalities of the skeletal system included genu valgum and recurvatum, broad hallux, joint hypermobility with laxity and pes planus (Fig. 1i, l). She presented moderate ID with cognitive impairment, motor delay, delayed gross motor development with delayed speech and language development, seizures, and stereotypic behavior (Fig. 1e).

Patient 6

A 30-year-old male patient who was referred to the Department of Clinical Genetics due to Marfanoid-like features (tall stature and aortic root dilatation), ID, and schizophrenic behavior. Pregnancy was normal and delivery at term with a weight of 4000 g (90th centile), height of 55 cm (>97th centile); head circumference was not reported. Family history revealed that the father suffered for aortic root dilatation and lung cancer and the mother presented lupus erythematosus without any cardiac defect. During the early childhood, mild to moderate global developmental delay was detected with speech and language delay. Therefore, he attended to a special school. He was operated of both umbilical hernia and micrognathia. Tall stature and macrocephaly were evident from infancy and facial dysmorphic features included high forehead with high anterior hairline, deeply set eyes, downslanted palpebral fissures, ocular hypertelorism, low-set ears, micrognathia, and central incisor macrodontia (Fig. 1). He also suffered for myopia. He manifested psychotic episodes with schizophrenia symptoms since 18 years old. MRI did not show any abnormality, and echocardiogram confirmed the artery root dilatation of 57 mm, requiring replacement surgery. Initial molecular tests discarded Marfan syndrome (*FBN1* variants negative), *TGFRB1* and *TGFRB2* negative (both Sanger sequencing and MLPA were normal) (data not shown), *SKY* negative (only exon 1). CGH microarrays did not show any rearrangement. Due to the suspicious of an OGS, whole exome sequencing was requested.

Patient 7

This girl is the second child of a non-consanguineous Caucasian couple. Her father and her older sister presented macrocephaly without ID. Pregnancy without complications. She was born at term by Cesarean section because of macrosomia. An abdominal mass was detected at 2 months of age; final diagnosis was Neuroblastoma. She undertook surgery at 6 months of age. Developmental assessment at 8 month-old showed remarkable hypotonia and global developmental delay. She also has marked joint laxity and pes planus. She walked independently at 24 months of age. Language delay was also noted with dysarthria and hypophonia. At 5-year-old she attends ordinary school with support.

The patient was referred to the clinical genetics department when she was 2 years and 6 months of age. At that moment anthropometric parameters showed weight 13.9 kg (0 SD), height 94.7 cm (+2 SD), and OFC 52.5 cm (>+2 SD). She presented macrocephaly with turricephaly and broad forehead, downslanted palpebral fissures, and midface hypoplasia.

Comprehensive clinical examination of the patient suggested the possibility of an OGS. To uncover the underlying molecular cause of the phenotype displayed by the patient, whole exome sequencing was performed. A variant in *DNMT3A* (NM_175629.2:c.919 C>T):p.(Pro307Ser) was detected. The variant has not been previously reported in public databases (dbSNP, 1000 Genomes Project, Exome Variant Server, Genome Aggregation Database). Segregation analysis showed that this variant was a de novo event in the proband.

Molecular results

Seven variants in seven unrelated patients were detected by NGS analysis (Table 1 and Fig. 2) (one frameshift, one splice site, one nonsense, and four missense). Three of them are localized within the S-adenosyl-L-methionine (SAM)-dependent MTase c5-type protein domain, two are located within the DNMT1 and DNMT3b interaction motif (Zinc Finger, FYVE/PHD-type) (Table 1) and the last one is located within the PWWD domain. Segregation analysis in available families showed that all of the variants were de novo. None of the variants except the p.(Arg736His) were previously described in the control population databases (gnomAD exomes, gnomAD genomes, Kaviar, 1000 G, ESP). Therefore, this variant was classified as VUS ("variant of unknown significance"). The rest of variants were classified as pathogenic or likely pathogenic. For variant p.(Leu737Phe), the same change has been previously detected in somatic cells in an individual with ovarian serous carcinoma [26].

Three out of the seven variants produce aberrant proteins; two would lead to truncated proteins and one a splice site variant predicting an exon skipping. These three changes may lead to mRNA degradation through the nonsense mediated decay (NMD) machinery. The rest of the changes are missense variants, three classified as likely pathogenic and one as VUS.

Discussion

Since the first publication of variants in *DNMT3A* related to an overgrowth disorder in 2014 [2], 55 patients have been compiled so far in a recent review [24]. There are other five individuals included by genetic laboratories in

Table 1 List of variants detected in *DNMT3A* in patients

Family	Variant ID		Exon/ Intron	mutation type	Protein domain	Pathogenic prediction			ACMG prediction	Inheritance	LOVD ID
	Genomic coordinate (hg19)	cDNA and protein location				Population frequency[a]	COSMIC	Pathogenic predictors[b]			
Family I	chr2: g.25463246_25463246del	NC_000002.12 (NM_022552.4): c.2246_2247del:p. (Arg749Profs*7)	19	Frameshift	SAM dependent MTase c5-type	0	N/A	(2/2)+35	P	de novo	#00231357
Family II	chr2:g.25467407T>G	NC_000002.12 (NM_022552.4): c.1667+2T>G	IVS10	Splicing	Zinc Finger, FYVE/PHD-type	0	N/A	(2/2)+29.6	LP	de novo	#00231367
Family III	chr2:g.25463284C>T	NC_000002.12 (NM_022552.4): c.2209C>T:p. (Leu737Phe).	19	Missense	SAM dependent MTase c5-type	0	COSM74415[c]	(8/9)+27.9	LP	ND	#00231370
Family IV	chr2:g.25463286C>T	NC_000002.12 (NM_022552.4): c.2207G>A:p. (Arg736His)	19	Missense	SAM dependent MTase c5-type	4,81E–02	N/A	(6/9)+24.7	VUS	de novo	#00231373
Family V	chr2:g.25467449C>T	NC_000002.12 (NM_022552.4): c.1627G>A:p. (Gly543Ser)	14	Missense	Zinc Finger, FYVE/PHD-type	0	N/A	(8/10)+25.1	LP	de novo	#00231374
Family VI	chr2:g.25467194C>A	NC_000002.12 (NM_022552.4): c.1681G>T:p. (Glu561*)	15	Nonsense	Zinc Finger, FYVE/PHD-type	0	N/A	(4/4)+41	P	de novo	#00231375
Family VII	chr2:g.25470555G>A	NC_000002.12 (NM_022552.4): c.919C>T p. (Pro307Ser)	8	Missense	PWWD	0	N/A	(9/9)+27.3	LP	de novo	#00245882

Details of SNVs detected in six patients with TBRS. Variants were classified according to the ACGM guidelines. Reference sequence for variants nomenclature is NC_000002.12 (NM_022552.4). Database for *DNMT3A* uploaded variants is http://www.lovd.nl/DNMT3A

DC Disease causing, *M* medium, *T* tolerated, *D* damaging/deleterious, *P* pathogenic, *LP* likely pathogenic, *N/A* not applicable, *ND* not determine

[a]gnomAD exomes, gnomAD genomes, Kaviar, 1000 G phase III, ESP

[b]dbNSFP (MutationTaster, MutationAssessor, FATHMM, FATHMM-MKL, MetaSVM, MetalR, Provean, LRT, SIFT)+CADD phred score

[c]Ovarian serous carcinoma [26]

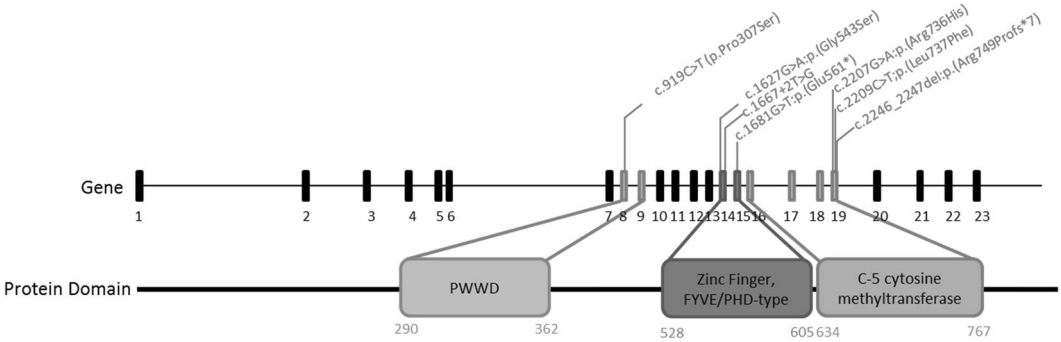

Fig. 2 *DNMT3A* gene NC_000002.12(NM_022552.4) and DNMT3A protein structure. Details of the seven disease-causing variants described in this work (one at PWWD, three at the Zinc Finger and three at the C-5 cytosine methyltransferase domains)

the ClinVar database with disease-causing variants and TBRS but without additional clinical information of them. Here we describe seven novel patients with *DNMT3A* variants emphasizing on the neuropsychiatric findings of this entity.

Clinical features of the published patients with TBRS are listed in Supplementary Table 1. The most common clinical features (frequency above 75%) of these patients include macrocephaly, tall stature, and ID. Other less common features (between 25% and 75% of frequency) are horizontal and thick eyebrows, weight above+2 SD/ obesity, narrow palpebral fissures, broad forehead, hypotonia, and scoliosis. Clinical findings such as the facial gestalt and large hands and feet in present patients are shown in Fig. 1. Facial dysmorphic features allowing distinction with SoS are triangular face with pointed chin and macrodolichocephaly [27]. Also, advanced bone age is very common in SoS (≈80–85% of the cases) but not in TBRS. Skeletal abnormalities are not usually seen in TBRS, helping to distinguish from Weaver syndrome, in which patients suffered for clinodactyly, scoliosis, talipes equinovarus, and pectus excavatum/carinatum in a highest proportion [28].

All the cases presented in this report were macrosomic newborns and have macrocephaly and ID in the follow-up. Anthropometry (when available) at birth showed that five had weight >2 SD. Macrocephaly is a common clinical sign but not present in all cases (50%). Almost all reported cases have an impaired neurocognitive development, mainly mild, or moderate ID (~85% [10]) like the patients describe herein and only ~15% were reported with severe ID. Additional neuropsychiatric abnormalities described in our series include profound global developmental delay, schizophrenia, aggressiveness, attention deficit-hyperactivity disorder, impulsivity, stereotypic behavior, cognitive impairment, and sleep disturbances. Abnormalities of the vision were not reported in TBRS cases, but this is well-known to be present in ~75% of individuals with Weaver syndrome [29], therefore this can give a clue for differential diagnosis between these two conditions.

Facial *gestalt* in TBRS is different from others overgrowth disorders. It includes a long oval face, thick horizontal eyebrows, narrow palpebral fissures, and marked philtrum. This would be useful to distinguish from others well-known disorders such as SoS, Beckwith-Wiedemann (BWS) (MIM 130650), Simpson-Golabi-Behmel (SGBS) (MIM 312870), Weaver (277590), and Fragile X (MIM 300624) syndromes, among others. Nevertheless, TBRS is still difficult to be distinguished from some recently described overgrowth disorders such as the Thauvin-Robinet-Faivre (TROFAS) (MIM 617107), Tenorio (MIM 616260), Rahman (MIM 617537), Kosaki (MIM 616592), and Luscan-Lumish (LLS; MIM 616831), syndromes because only a few patients have been reported so far. Thus, more individuals and a more detailed clinical description are necessary to have a better understanding of their clinical characteristics. Clinical comparison between TBRS and Weaver, Sotos, and Malan syndrome is detailed in Supplementary Table 3.

For example, patients reported with TROFAS by Akawi et al. [29], have thick eyebrows similar to those with TBRS, but it is not clear whether this feature is typical of the syndrome or corresponds to a family trait. LLS patients [30] have high forehead but their facial findings are clearly different from those observed in SoS and TBRS syndrome.

DNMT3A encodes for a DNA methyltransferase (MTase) alpha 3, a member of the MTases involved in several human development processes by establishing DNA methylation patterns during development, specifically in histones proteins and CpG sites within the genome, playing an important role in paternal and maternal imprinting [31]. Specifically, DNMT3A has three main functional domains. First, the PWWD domain, which is basically a core of Pro-Trp-Trp-Pro and that is present in proteins functioning as transcriptional factors regulating developmental process. Seven variants were detected in TBRS in this domain in previous reports [10] (Supplementary Table 2). We have detected one individual with a missense variant within this domain. Missense gain-of-function variants within the

PWWD domain were recently reported in patients with microcephalic dwarfism and the authors suggested that the substitutions impairs the binding of the H3K36me2 and H3K36me3 histone residues, leading to an abnormal DNA methylation in patient cells [32]. Secondly, the zinc finger FYVE/PHD-type domain that is involved in protein-protein and protein-DNA binding interactions. We identified three variants at this domain, one stop gain, one splicing and one missense variant. Thirdly, the SAM dependent MTase C-5 cytosine methyltransferase domain is essential in these proteins to carry out the methyltransferase activity that predominantly regulates the methylation patterns at CpG sites in many imprinted regions. It is recruited to tri-methylated Lys-36 of histone H3 (H3K36me3). Thus, the majority of disease-causing variants associated with TBRS are located within exons that encode for this domain. In our series, we detected three additional variants in this domain, two missense variants located in contiguous codons (p.(Arg736His and p.(Leu737Phe)) and one frameshift variant, which was predicted to be degraded by the NMD mRNA processing machinery. Remarkable, the three described patients with variants in the SAM dependent MTase c5-type –all in exon 19- had neuropsychiatric disorders, including schizophrenia with paranoid behavior, which could be related with defects in this domain of DNMT3A and the phenotypic spectrum of the disease. In fact, genetic analysis of *DNMT1, DNMT3A, DNMT3B*, and *DNMT3L* in a cohort of patients with schizophrenia, revealed several SNPs in *DNMT1* and *DNMT3B* that are significantly associated with this neuropsychiatric disorder [18]. Specifically, for *DNMT3B* it has been found that the presence of the polymorphism rs2424932 increases the risk of developing schizophrenia in males but not in females, and the SNP rs1569686 correlates with early onset schizophrenia and also with a family history of schizophrenia [18]. Schizophrenia is a multifactorial disease with a heterogeneous etiology, including genetic predisposition, environmental aspects, drug abuse, and a comorbidity of other diseases. Hundreds of differentially expressed genes have been found to be altered after microarray expression of brain tissue of patients with schizophrenia compared with control samples, but the specific mechanisms and dysregulation that produce this neuropsychiatric disorder remain unclear. Several biological pathways have been found to be involved in schizophrenia: synaptic [33], mitochondrial [17], immune system [34], GABA-ergic [35], and oligodendrocytic [36].

Metabolic pathways have also been seen to have a major role in schizophrenia due to the reduction in expression involved in the regulation of ornithine and polyamine metabolism, mitochondrial malate shuttle system, the transcarboxylic acid cycle, aspartate and alanine metabolism, and ubiquitin metabolism [17]. Also, DNMT3A participates in several metabolic pathways, including the regulation of the cysteine and methionine metabolism by creating a protein complex with DNTM1 and DNMT3B, which catalyzes the conversion of S-adenosyl-L-methionine to S-adenosyl-L-homocysteine+DNA containing 5-methylcytosine. SAM is an enzyme that catalyzes transmethylation reactions. The function is similar to the C5-cytosine MTase domain of *DNMT3A*. This enzyme was found to be increased in post-mortem brains of patients with schizophrenia [37]. Thus, it is possible that defects in the catalytic function of DNMT3A caused by constitutive variants in the SAM dependent MTase c5-type domain found in families 1, 3, and 4 might produce the alterations of metabolic pathways that could finally lead to neuronal defects and the schizophrenic or schizophrenic-like phenotype. Therefore, metabolite accumulations in the brain prefrontal cortex can be observed and lead to changes in both gene expression and protein function that finally lead to the dysfunction in neurons' communication in response to stimulus.

Recent studies have suggested a possible relationship between methylation hallmarks in dnmt3a and social behavior in adolescent and adult rats, showing that in rats, which suffered for stressful social experience, dnmt3a levels were elevated compared with the control group. Specifically, methylation of several positions in the histone (H_3K4, H_3K9, and H3K14ac) was different between these two groups [38].

Previous descriptions of patients with variants in exon 19 of *DNMT3A* have not reported schizophrenia, although neuropsychiatric symptoms such as depression, anxiety, attention deficit- hyperactivity disorder, and behavior problems have been clearly described [39]. Furthermore, one of the patients described in the present report (patient 3; Fig. 1c) developed a brain tumor with right intraventricular lesions caused by glial proliferation without histological signs of aggressiveness and another patient developed a neuroblastoma requiring surgery at the age of 6 months. A potential link among germline variants in *DNMT3A* and tumors has to be further explored in order to investigate whether individuals with *DNMT3A* abnormalities have an increased risk of tumor development.

Patient 4 was clinically diagnosed with Simpson-Golabi-Behmel syndrome, but *GPC3* analysis was negative. After that, Beckwith–Wiedemann was also suspected but 11p15.5 MS-MLPA was also negative. Therefore, she was included in whole exome sequencing, which reveals a de novo missense variant in *DNMT3A:*p.(Arg736Hys). This variant is located within the C-5 cytosine methyltransferase domain, in which most of the disease-causing variants in TBRS were described. In addition to the de novo *DNMT3A* variant, a second variant was also found in *COL11A1* (NM_001854.3:p.(Ile708Thr)), in this patient who was inherited from her father. Variants in *COL11A1* are related with Stickler syndrome type II in an autosomal dominant

inheritance mode. Thus, the variant in *COL11A1* in this patient may have contributed with the phenotypic characteristics. In fact, ophthalmological and hearing defects are commonly related to Stickler syndrome, such as myopia and auditory impairment, and clinical screening of these features have to be considered in the follow-up of the patient and her father. This observation reinforces the notion that about 0.5–1.5% of patients that are screened by WES have more than one disease-causing variant associated with more than one different monogenic disease [40–43].

In patient 6 and his father, aortic root dilatation was detected, which in combination with the tall stature suggested a Marfan syndrome as the first diagnosis. *FBN1* variants were discarded, and then other genes were screened, including *TGFBR1*, being all negative. There is no previous evidence for cardiac anomalies in TBRS, suggesting that this finding is probably not caused by the variant in *DNMT3A* and maybe other genetic/disruptive defects can be involved.

In summary, we report seven new patients with TBRS with novel variants. Among the clinical features of these patients, schizophrenia, hallucinations, seizures, delayed speech, and language development and an abnormal psychiatric behavior seem to be important findings to be added and to follow-up in adult individuals with this disorder. Therefore, after molecular confirmation of TBRS, neuropsychiatric evaluation is strongly recommended in these patients in order to prevent stigmata during childhood and adolescence. In our cohort, overweight and obesity are less frequent than previous reports and none of the patients suffered for severe ID. None of our patients present pes planus compared with the 20% previously reported. Although one of the patients developed a brain tumor, it is unclear its relationship with constitutive genetic defects in *DNMT3A*. Due to the high overlap between several OGS, massive paralleled techniques such as customized targeted panels, whole exome sequencing or even whole genome sequencing should be the first molecular approach for these patients.

It is also necessary to carry out further studies focusing on methylation signatures, based on the effect of these genes in chromatin regulation, and therefore, to inquire how the changes in these genes are affecting the methylation signature.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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