COMMENT

Methylation signatures in clinically variable syndromic disorders: a familial *DNMT3A* variant in two adults with Tatton-Brown–Rahman syndrome

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Tatton-Brown–Rahman syndrome (TBRS; OMIM #615879) is a rare overgrowth and intellectual disability (ID) syndrome caused by heterozygous loss-of-function variants in the *DNMT3A* gene. Other characteristic features include obesity and a distinctive facial appearance, including a round or long face, thick, horizontal eyebrows, narrow palpebral fissures and prominent upper incisors. Furthermore, psychiatric and behavioral problems, joint hypermobility, kyphoscoliosis, urogenital, cardiovascular issues and seizures have been reported [1–3]. Heterozygous pathogenic *DNMT3A* variants have been reported in about 100 TBRS cases, the vast majority being children. They include pathogenic missense, frameshift, splice site and nonsense variants as well as microdeletions. Most of the variants were found to be de novo, although rare cases of transmission from a less affected or mosaic parent were reported and showed autosomal dominant inheritance [1–5]. On the other hand, germline gain-of-function variants in this gene have been implicated in microcephalic dwarfism (OMIM #618724). The *DNMT3A* gene encodes for DNA methyltransferase 3 alpha. It is a key player in epigenetic programming by de novo methylation maintenance, essential for genome regulation and development. Pathogenic loss-of-function variants in *DNMT3A* have also been described as prevalent drivers in clonal hematopoiesis and adult hematological malignancies such as acute myeloid leukemia (AML) and are suggested to have a proliferative advantage in hematopoietic progenitor cells by epigenetic changes. Thus, aberrant methylation, amongst others caused by *DNMT3A* variants, appears to be an important contributing factor in cancer development [6, 7]. Overgrowth syndromes involve a heterogeneous group of disorders in which height, head circumference and/or weight are more than 2 standard deviations above the mean (>+2.0 SD). Often such syndromes, like other syndromic or neurodevelopmental disorders, present with overlapping clinical characteristics and sometimes molecular findings remain inconclusive, hampering a straightforward clinical diagnosis and management. Extensive clinical phenotyping and the use of additional diagnostic tools, including those for functional validation of genetic variants, are therefore crucial. An increasing number of genetic syndromes have unique peripheral blood methylation patterns, called episignatures, which can be used for diagnostic testing and for the interpretation of ambiguous genetic results [8, 9].

A 27-year-old man was referred to our hospital after tens of diagnostic investigations, presenting with postnatal tall stature and obesity, ID, brain atrophy, epilepsy and psychiatric issues. His father showed normal development without learning problems, autistic features, polyarthrosis and late-onset lymphocytosis. Corresponding symptoms among father and son were fetal macrosomia, kyphoscoliosis, macrocephaly and extensive cardiovascular problems, including cardiomyopathy, aortic root dilatation, mitral valve prolapse and arrhythmia. Detailed phenotypes of the proband and his father are shown in Table 1, Fig. 1, and Supplementary Document 1. Exome-based analysis revealed a missense variant c.2206C>T (p.(Arg736Cys)) in the *DNMT3A* gene (NM_022552.4) in 50% of the reads inherited from the father. In the latter, the variant was found in 40% of the reads in blood, buccal mucosa and eyebrow pluck. The variant is located in the methyltransferase domain, affects an evolutionary conserved amino acid and has been reported 6 times in gnomAD. Genes linked to clonal hematopoiesis, such as *DNMT3A*, can have increased allele frequencies in population databases due to somatic mosaicism. The majority of the prediction tools (10/14) predict the variant to be pathogenic. The variant has been reported in ClinVar with conflicting levels of pathogenicity (ClinVar_ID: 981259), although a different missense variant at the same position (p.(Arg736His)) has been reported to be pathogenic. In addition, other missense changes of neighboring amino acids have been reported as pathogenic in ClinVar and Decipher (ClinVar_ID:812892,391574; Decipher_patient:397855). EpiSign variant targeted analysis revealed a genome-wide DNA methylation profile consistent with TBRS for both proband and paternal blood samples (Fig. 2). EpiSign analysis was concordant with a methylation signature observed in patients with *DNMT3A* variants, as indicated by Euclidean clustering, multidimensional scaling and an elevated MVP score (proband = 1.0, father = 0.99), therefore confirming pathogenicity of this variant. Detailed protocols for whole exome sequencing and DNA methylation data analysis using EpiSign are described in Supplementary Document 2.

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Table 1. Characteristics of two adult patients with p.Arg736Cys variant in the *DNMT3A* gene.

Clinical features	Proband	Father
DNMT3A variant	c.2206C>T, p.(Arg736Cys)	c.2206C>T, p.(Arg736Cys)
Exon	6	6
Sex	M	M
Age at examination	25 years	60 years
Growth		
Birth parameters	W: 4.2 kg (>+2.5 SD); L: 54 cm (>+2.0 SD); HC: 39 cm (>+2.5 SD)	W: 4.85 kg (>+2.0 SD)
Fetal macrosomia	+	+
Height	210 cm (+4.5 SD) (at age 9: 180 cm (+7.2 SD)	192 cm (+1.7 SD)
Weight	160 kg (+2.8 SD) (at age 9: 74.5 kg (+3.1 SD)	
Head circumference	HC: 64 cm (+4.2 SD) (at age 9: 60.5 cm (+4.5 SD)	HC: 60 cm (+2.0 SD)
Postnatal overgrowth	+	+
Macrocephaly	+	+
Advanced bone age	+	NR
Epiphysiodesis	+	–
Overweight	+	–
Endocrinological abnormalities	–	NR
Development		
Developmental delay	+	–
Motor delay	+ (walking at 19 m)	–
Language delay	+ (two-word sentences at 2.5 years)	–
Mental retardation	+	–
Behavioral problems	–	+ (ASS)
Psychiatric problems	+ (depression, psychosis)	–
Epilepsy	+	–
Cardiovascular problems		
Cardiomyopathy	+ (age 19 years; decreased EF 34–40%)	+ (age 47; severe dilatation of right atrium and ventricle)
Aortic root dilatation	+ (age 19 years; 48 cm)	+ (age 47; 45 mm)
Mitral valve prolapse	+ (age 19 years)	+ (age 47)
Arrhythmia	+ (age 19 years; VES with ablation; NSVT with ICD)	+ (atrial fibrillation with chemical conversion)
Congenital abnormalities	–	–
Skeletal abnormalities		
Scoliosis	+	+ (operated at age 35)
Kyphosis	–	+
Artrosis	–	+ (poliarthrosis)
Hyperlaxity	–	–
Contractures	+ (finger)	–
Large hands and feet	+	–
Brain abnormalities		
Cerebral and cerebellar atrophy	+	NR
Pinealis cyst	+	NR
Additional findings	+ (T2 hyperintensity, suspicious for tumoral lesion, currently stable)	NR
Craniofacial features		
Long face	+	+
Synophrys	+	–
Thick eyebrows	+	+
Horizontal eyebrows	+	–
Downslanting palpebral fissures	+	–
Hypertelorism	–	+
Large upper central incisors	+	–

Table 1. continued

Clinical features	Proband	Father
Thin lips	–	+
High palate	+	–
Prominent chin	+	+
Other		
Hematological abnormalities	–	+ (monoclonal B-cell lymphocytosis, in follow-up)
Frequent infections	+ (pneumococcal meningitis, osteomyelitis and pneumonia)	–
Pneumothorax	+	–
Fractures	+ (pubic, elbow, toe, foot, vertebral)	–
Additional genetic testing		
ID and epilepsy gene panel	Maternally inherited hemizygote VUS in *BCORL1* gene (NM_001184772.2): g.130015195C>T (GRCh38/hg38), c.2423C>T, p.(Thr808Met), segregation showed the presence of the variant in two healthy brothers of our proband	NA
Cardiomyopathy, thoracic aneurysm gene panel	Negative	Negative
Connective tissue gene panel	NA	Negative
Other testing	Conventional and molecular karyotyping, fragile X syndrome, overgrowth panel	NA

ASS autism spectrum disorder, *EF* ejection fraction, *HC* head circumference, *ICD* implanted cardiac device, *L* length, *NA* not applicable, *NR* not reported, *NSVT* non-sustained ventricle tachycardia, *VES* ventricular extrasystoles, *VUS* variant of unknown significance, *W* weight.

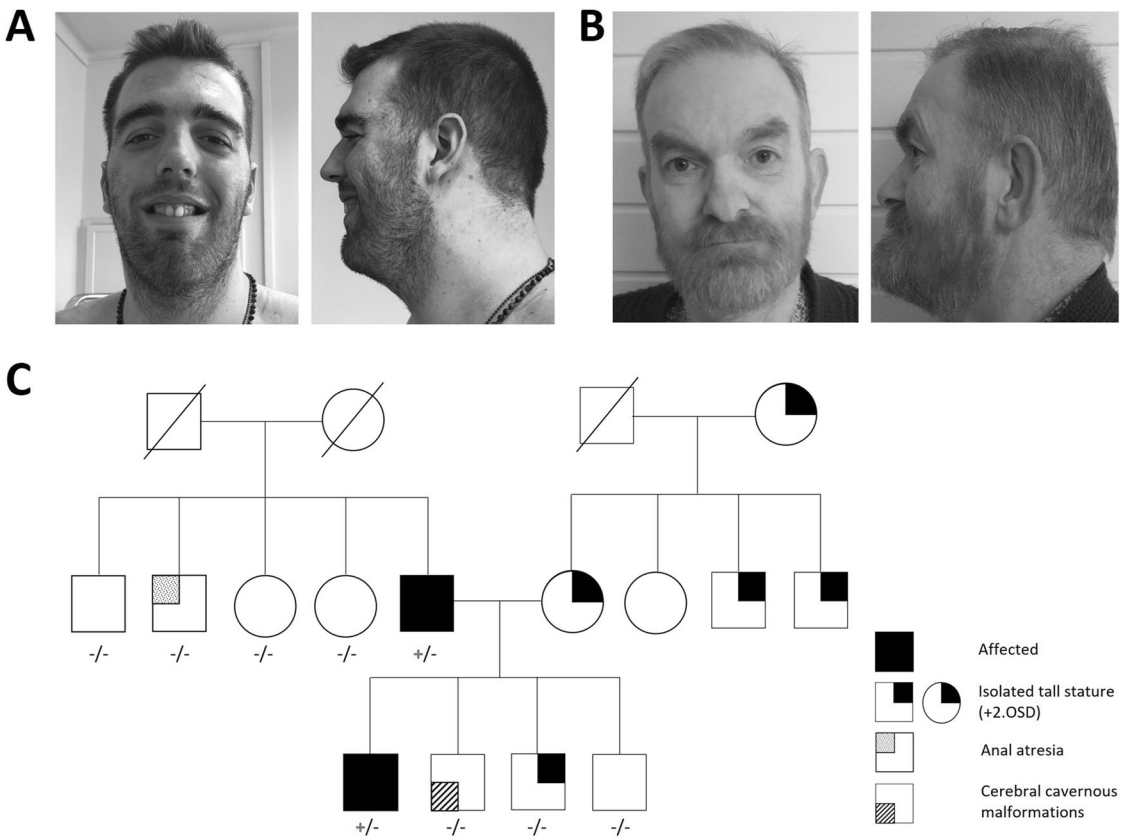

Fig. 1 Clinical pictures and pedigree of the two TBRS patients. A Clinical pictures of the proband showing a dolichocephalic skull and elongated face, full eyebrows with synophris, downslanting of the palpebral fissures, prominent upper central incisors, pronounced chin. **B** Clinical pictures of the father showing large forehead, full eyebrows, hypertelorism, low-hanging columella, thin lips and prominent chin. **C** Pedigree of the family. The *DNMT3A* p.(Arg736Cys) variant was not present in the sibs of the proband, neither in the paternal family members, the latter all presenting with normal stature (160–180 cm).

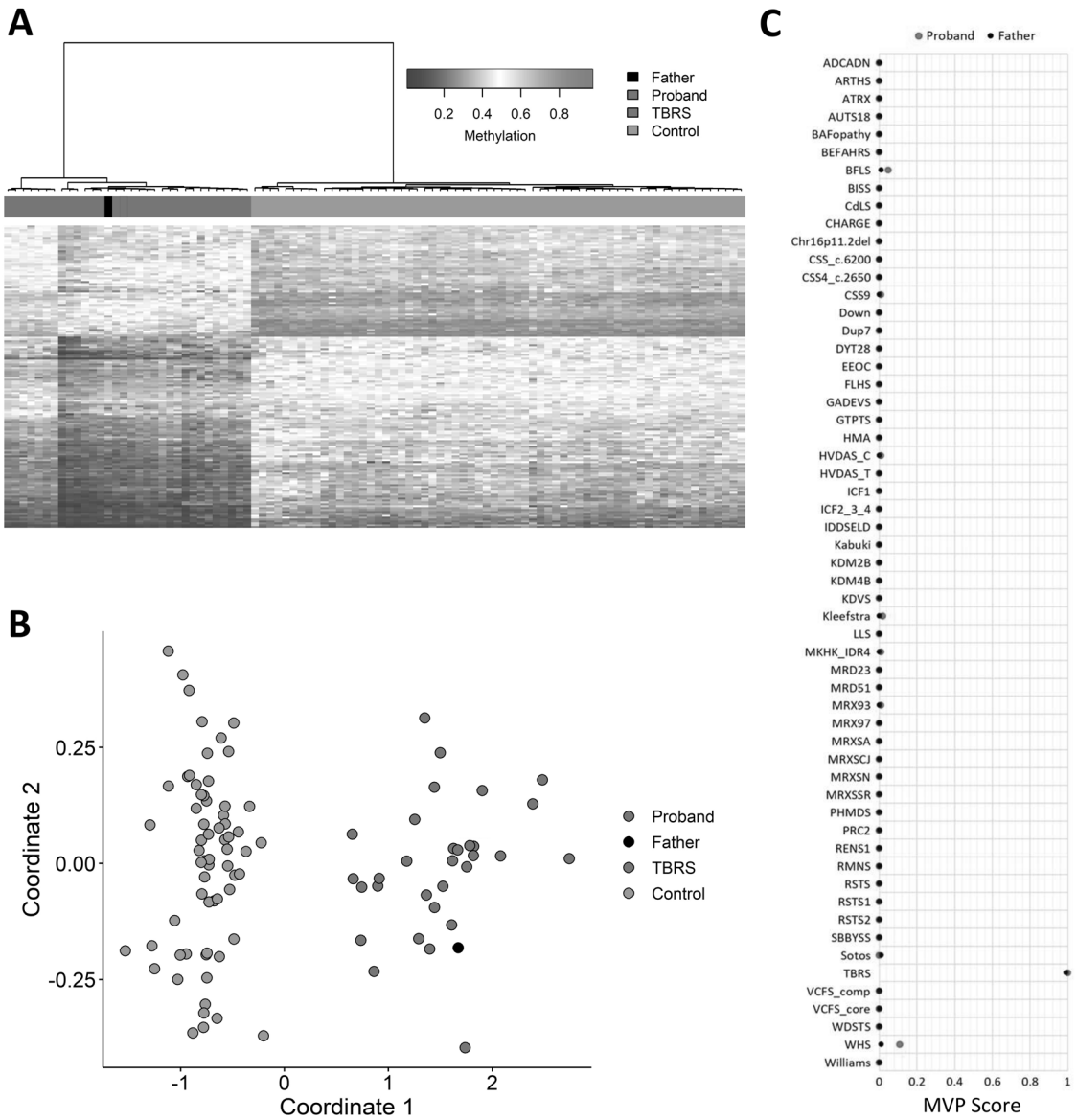

Fig. 2 EpiSign (DNA methylation) analysis of peripheral blood from the proband and his father with the p.(Arg736Cys) *DNTM3A* variant. **A** Hierarchical clustering and **B** multidimensional scaling plots indicate the proband (red) and father (black) both have a DNA methylation signature similar to subjects with a confirmed TBRS episignature (blue) and distinct from controls (green). **C** MVP score, a multi-class supervised classification system capable of discerning between multiple episignatures by generating a probability score for each episignature. The elevated scores for TBRS show an episignature similar to the TBRS reference.

To date, about 100 TBRS patients with pathogenic loss-of-function variants in the *DNMT3A* gene have been reported. The syndrome is characterized by postnatal overgrowth (83%) and ID (18% mild, 65% moderate and 16% severe; few cases with borderline low intelligence) and clear developmental delay. In addition, the most commonly reported features are joint hypermobility (74%), obesity (67%) and hypotonia (54%). Furthermore, behavioral and psychiatric issues have been reported in about half of cases, kyphoscoliosis in 33% and seizures in 22%. Several publications report on individuals presenting with urogenital abnormalities including cryptorchidism (11%), vesicoureteral reflux and hypospadias, brain abnormalities such as ventriculomegaly (7%) and Chiari malformation (5%) and hematological (4%) and other malignancies (5%). Finally, cardiovascular problems (10%) were reported including congenital heart defects (ASD, VSD), aortic root enlargement (3%), mitral valve prolapse and cardiomyopathy (3%). These latter were initially thought to be incidental co-occurring findings [1–6]; however, Cecchi et al. suggest an association of TBRS with aortic disease and cardiomyopathy [5].

The two above-described related adult patients with TBRS due to a previously unreported pathogenic missense variant in *DNMT3A* further contribute to refine the clinical phenotype of this syndrome. They present with a spectrum of overgrowth, ID, obesity, behavioral and neuropsychiatric problems, epilepsy, cardiovascular disease and hematological issues including lymphocytosis. So far, most described individuals with TBRS are children. Interestingly, these two adult patients present with extensive cardiovascular issues including aortic dilatation, mitral valve prolapse, cardiomyopathy and arrhythmia. Especially given the rare reports on adults, cardiovascular problems might appear to be a paramount feature of the TBRS spectrum. Furthermore, we describe the first TBRS patient without a developmental phenotype, although we cannot exclude mosaicism with the absence of the variant in the central nervous system. Appropriate surveillance for patients with TBRS is crucial and includes growth monitoring,

extensive neurological, neuropsychiatric, musculoskeletal and genitourinary follow-up, as well as regular cardiological evaluation including echocardiography with ECG and CBC testing.

Clinical variability and reduced penetrance for some features in TBRS, like in many genetic disorders, was already known, but clinical diagnostics become challenging when the main features of rare syndromes are less evident. Therefore, in-depth phenotyping is important while emphasizing the need for attention for inherited variants. Differential diagnoses of TBRS encompass Sotos, Weaver and Malan syndromes and should also include Marfan syndrome because of the emerging cardiovascular phenotype and incomplete penetrance of the ID phenotype. On the other hand, for patients with (familial) thoracic aneurysm and cardiomyopathy, TBRS should be considered in the differential diagnosis and gene panels should be adapted accordingly. Facial gestalt can play a role as well in differentiating overgrowth syndromes. Some of the typical characteristics such as low set (horizontal) thick eyebrows, course face, long or round face and prominent upper incisors were recognized in our patients. Of note, the recognizability of TBRS was illustrated by the use of Face2gene, which suggested TBRS in our proband as the second hit after Sotos syndrome using a frontal picture.

Aberrant epigenetic regulation affecting DNA methylation and histone modification drives the pathogenesis in several overgrowth syndromes. The heterozygous p.(Arg736Cys) *DNMT3A* variant is located in the methyltransferase domain similar to many other reported pathogenic variants [1–6]. It is suggested that TBRS-associated *DNMT3A* loss-of-function variants have reduced methyltransferase activity, displaying methylation loss in genes involved in development and growth, resulting in increased cell numbers during organ formation, overgrowth and mirroring the observed neurocognitive disfunctions [7]. DNA methylation signatures are new means to improve diagnostic yield in patients with suspected genetic disorders. They can guide towards a specific syndrome and targeted testing of relevant genes. Moreover, for a variant of unknown significance in a gene of interest, this can be an important argument to assume causality. Based on analysis of over 40 syndromes, disorder-specific peripheral blood episignatures have been identified and used for functional validation of variants. For some, methylation changes might be subtle, but for defects in genes involved in direct regulation of methylation marks, such as *DNMT3A*, extensive changes are observed [8, 9]. More precisely, it was shown that patients with inactive DNMT3A show strong hypomethylation, which was confirmed in our patients. Moreover, they fit the profile observed in patients with TBRS, providing an additional argument for the pathogenicity of the variant. In a recent report, a patient with tall stature, ID and congenital myopathy similarly showed an aberrant methylation signature matching with TBRS [10]. It is of high importance that tools for functional assessment of genetic variants become implemented in daily diagnostics and an increasing number of publications illustrate the promising perspectives of these technologies as they can have important implications for genetic diagnostics, management and counseling as well as determination of recurrence risks.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

CK saw the patients, contributed to variant interpretation, and wrote the manuscript. ED contributed to data analysis and exome-based analysis. OMV saw the patients, contributed to variant interpretation, and assisted in manuscript preparation. ED was involved in exome data analysis. JK, HM, MA, and BS performed methylation studies and the EpiSign analysis.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

Informed consent for trio-based whole exome sequencing and for the publication of clinical data and photographs were obtained from the patients. Ethical approval was not required by our ethics committee, given the fact that this research concerns a case report.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41431-023-01459-w.

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