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ABSTRACT

Background. Nicolaides-Baraitser and Coffin–Siris syndromes are emerging conditions with overlapping clinical features including intellectual disability and typical somatic characteristics, especially sparse hair, low frontal hairline, large mouth with thick and everted lips, and hands and feet anomalies. Since 2012, mutations in genes encoding six proteins of the BAF complex were identified in both conditions. **Methods and Results.** We have clinically evaluated a cohort of 1161 patients with intellectual disability from three different Italian centers. A strong clinical suspicion of either Nicolaides-Baraitser syndrome or Coffin-Siris syndrome was proposed in 11 cases who were then molecularly confirmed: 8 having *de novo* missense mutations in *SMARCA2*, two frame-shift mutations in *ARID1B* and one missense mutation in *SMARCB1*. Given the high frequency of the condition we set up a one-step deep sequencing test for all 6 genes of the BAF complex. **Conclusions.** These results prove that the frequency of these conditions may be as high as the most common syndromes with intellectual deficit (about 1%). Clinical geneticists should be well aware of this group of disorders in the clinical setting when ascertaining patients with intellectual deficit, the specific facial features being the major diagnostic handle. Finally, this work adds information on the clinical differences of the two conditions and presents a fast and sensitive test for the molecular diagnosis.

Key words: Nicolaides-Baraitser syndrome, Coffin–Siris syndrome, BAF-complex, *SMARCA2*, *ARID1B*, *SMARCB1*

INTRODUCTION

Nicolaides-Baraitser syndrome (NBS; OMIM#601358) and Coffin–Siris syndrome (CSS; OMIM#135900) are conditions with overlapping clinical characteristics. Both syndromes are characterized by intellectual disability (ID) with absent or limited speech and typical somatic morphology characterized by progression in the coarsening of the facial features with age, triangular face, sparse hair, low frontal hairline, hypertrichosis, thick anteverted alae nasi, long and broad philtrum, large mouth with thin upper lip and thick lower lip, and brachydactyly. Additionally there may be seizures, hypotonia, short stature and microcephaly while isolated or multiple congenital anomalies seem to be rare. [1,2]

The hallmark differences between the two conditions are hands and feet, as typically NBS patients present prominent finger joints and broad distal phalanges while CSS patients display hypoplasia or aplasia of the fifth finger nails with or without hypoplasia of the terminal phalanges. [3]

In 2012 Van Houdt et al performed exome analysis of 10 NBS patients and identified, in 8 of them, heterozygous variants in *SMARCA2*, which encodes the core catalytic unit of the switch/sucrose nonfermentable (SWI/SNF)-like chromatin remodeling complex (BAF complex) which plays an important role in several distinct processes, such as transcription, cell differentiation and DNA repair. [4,5] Since then, some tens of NBS patients with *SMARCA2* mutations have been reported. [6] One month later, mutations in *ARID1A, ARID1B, SMARCA4, SMARCB1* and *SMARCE1* genes, that are part of the BAF complex, have been identified in CSS patients. [7,8] Very recently, also mutations in *SOX11* have been associated with CSS syndrome. [9]

A clinical distinction between the two conditions is often challenging suggesting that these syndromes might represent a phenotypic spectrum rather than two distinct disorders. Tsurusaki et al. described a patient with a *SMARCA2* mutation who was previously diagnosed as CSS and then reclassified as NBS. [8]

On the other hand, Santen et al supports the clear phenotypic distinction between patients with mutation in *SMARCA2* and patients with mutations in the other components of BAF complex. The main points typical of NBS phenotype are the hair that becomes sparser with time, the progressive coarseness of the face, thinning of subcutaneous tissues in the face, broadened and thickened distal phalanges and normal nails. Furthermore, the emerging phenotype-genotype correlations are that *SMARCB1*-mutated patients have the most marked physical phenotype and severe cognitive and growth delay and patients mutated in *ARID1A* and *ARID1B* show considerable variability. [10]

In addition, *de novo* variants in *ARID1B* have been linked also to non-syndromic ID. Hoyer et al. identified nonsense or frameshift mutations in *ARID1B* in 0.9% of their cohort of patients with ID, showing that haploinsufficiency of *ARID1B* is a relatively frequent cause of moderate-to-severe ID and confirming that chromatin-remodeling defects are an important contributor to neurodevelopmental disorders. [11]

Here, we describe an Italian cohort of NBS-CSS patients diagnosed through an analysis of 1161 cases with ID.

SUBJECTS AND METHODS

Patients

A cohort of 1161 patients with ID was included in the medical study. All patients were clinically evaluated by at least two expert clinical geneticists (FM, AR, LG, AS).

Sanger sequencing

Direct sequencing of the purified PCR products, obtained with the same primers and PCR conditions described for amplicon library preparation, was performed in both directions (PE Big

Dye Terminator Cycle Sequencing Kit) on an ABI Prism 310 genetic analyser (PE Applied Biosystems, Forest City, CA, USA) and analyzed with the Sequencer software.

Mutation analysis by Next Generation Sequencing

Mutation detection in *SMARCA2*, *SMARCA4*, *SMARCE1*, *SMARCB1*, *ARID1A* e *ARID1B* genes was performed by locus-specific amplification followed by 454 Junior sequencing (Roche, Mannheim, Germany) as already described. [12] Fusion primers were designed to generate tiled amplicons ranging in size between 200-300 bp segments (http://454.com/downloads/my454/documentation/gs-junior/method-manuals/454SeqSys_AmpliconLibraryPrepMethodManual_Jun2013.pdf) (Supplementary table 1). Processed and quality-filtered reads generated by Genome Sequencer software were analyzed using the latest version (2.9) of NGS Amplicon Variant Analyzer (AVA).

In silico variant analysis

All variants were checked in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/)

Pathogenicity of non-synonymous variations was assessed by in silico analysis using 3 prediction programs: SIFT (http://sift.jcvi.org/www/SIFT_BLink_submit.html), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), and PhyloP (http://genome.ucsc.edu/cgi-bin/hgGateway). The tools SIFT and PolyPhen-2 can predict the potential impact of an amino acid substitution on the structure and function of a human protein. The analysis produces categorical output that is tolerated/not tolerated for SIFT and benign/possibly damaging/probably damaging for PolyPhen-2. Furthermore, PolyPhen-2 gives a score between 0 and 1 (a higher score suggests a more damaging variation) and it indicates a value for sensitivity and specificity of the result. Only variants not tolerated for SIFT and possibly/probably damaging by PolyPhen-2 were considered as functionally relevant. PhyloP measures the phylogenetic conservation of a nucleotide at a specific

position and provides a numerical value, ranging from -3.69 to +6.94. Positive scores are assigned to sites predicted to be conserved and negative scores to sites predicted to be fast evolving.

RESULTS

Patients. Among 1161 patients, a strong suspicion of NBS/CSS was raised on clinical genetic grounds in 11 subjects: 7 males and 4 females, with age at diagnosis ranging between 11 months and 35 years (median age of 11 years-3 months). Clinical and genetic data of these patients are summarized in Table 1. Figures 1-3 show the facial gestalt, hands and feet anomalies and X-rays of the cohort with pictures at different ages in several patients. None of these clinical pictures has been reported elsewhere while a summary of the clinical data of 5 patients (cases 1-3, 9, 10, table 1) was already published. [3,4] The clinical suspicion was mainly based on the facial gestalt, including sparse hair, low frontal hairline, high nasal bridge with thick and prominent columella especially in patients in childhood, big mouth with thin upper lip, everted lower lip, and prominent chin prevalently in adolescence and adulthood (Fig. 1). Secondly, other characteristics were searched such as hand and foot anomalies mainly represented by prominent interfalangeal joints, asymmetry in finger length with shortness of the first phalanx of the first finger and broadening of the first phalanx especially of the toe (Fig. 2 and 3). The great majority of patients did not show major internal organ malformations (Tab. 1). All patients carried a mutation in one of the 6 genes of the BAF complex: 8 in *SMARCA2*, two in *ARID1B* and one in *SMARCB1* (Table 1).

Facial Gestalt

Figure 1 shows faces of NBS patients on the left and CSS on the right at different ages from infancy to adulthood (Fig. 1). All patients have similar facial features especially at a very young age. Palpebral fissures are narrow and down slanting; eyebrows are thick (5 NBS and 2 CSS) and synophrys is frequent (4 NBS and 1 CSS), eyelashes are long and prominent (4 NBS and 2 CSS). The nose has large base, high bridge, thick and anteverted nares and thick low-hanging columella (NBS). Philtrum is broad and long; the upper vermillion tends to be thin, while the lower vermilion is thick and everted. The mouth is wide.

Intellectual disability, speech impairment and behavioral disorders

All patients showed ID of variable degree ranging from mild (CSS due to *ARID1B*) to moderate (NBS). All probands presented speech impairment, being able to utter only few single words. Expressive language was more impaired than motor abilities. Hearing loss was excluded in all cases. Four patients (3 NBS and CSS due to *ARID1B*) showed behavioural anomalies such as hyperactivity, aggressiveness, and psychosis, while three patients were described as friendly and happy (2 NBS and 1 CSS). Five patients (NBS) showed seizures.

Growth Parameters

All patients were born at term. Only in 3 patients growth parameters at birth were in the lower range. At the time of the evaluation six of them were underweight, three patients showed short stature (1 NBS and 2 CSS), of prenatal onset in two of them, and four out of 8 patients showed postnatal microcephaly (3 NBS and 1 CSS). Among the six underweight patients a history of feeding problems in infancy was recorded. Evident body length disproportion was not ascertained in any patient.

Ectodermal anomalies

Low anterior hairline with sparse scalp hair has been a constant feature present since the neonatal period. In case 4, cut hair was examined under light microscopy at x4 and x10 magnification and demonstrated hair shaft abnormalities including trichoschisis and trichorrhexis nodosa-like defects. Body hypertrichosis was reported in four patients (3 NBS and 1 CSS).

Wrinkling skin in the face, distal limbs and neck is present in two NBS patients. Eczema is present in 4 patients (3 NBS and 1 CSS mutated in *ARID1B*). CSS patients with either *SMARCB1* or *ARID1B* mutations showed dystrophic nails (Fig. 2). Fifth toe nail hypoplasia was present in four patients (1 NBS and all CSS) and in the NBS patient 5th finger nail hypoplasia was also reported. Teeth do not show unusual features in our series of patients, however, in two NBS cases a delayed dentition is reported.

Limbs and Joints

Gradually distal phalanges become broad (3 NBS and 1 CSS mutated in *ARID1B*) and inter-phalangeal joint swelling develops (7 NBS) (Fig. 2). The feet show bilateral sandal gap (six NBS cases), broad hallux and thickening of the distal phalanges of toes (four cases) (Fig. 2). Short distal phalanges, metacarpal or metatarsal are frequent (Fig. 2). In two NBS cases delayed bone age and scoliosis are reported. In one CSS patient joint hyperlaxity was ascertained while three patients (2 NBS and 1 CSS) showed inguinal/umbilical hernia.

X-ray anomalies

For 6 patients X-rays of hands and/or feet were available, were performed at different ages and showed some characteristic signs in feet and hands (Fig. 3). In particular, for the two patients with X-rays performed at 11.4 years and 13 years, ivory epiphyses in distal phalanges of hands and feet were clearly noted. In most patients regardless of age, broad first metatarsals and metacarpals were noted.

Other features

The whole cohort of patients did not show a high prevalence of congenital anomalies. In three patients brain MRI showed corpus callosum hypoplasia (1 NBS and 2 CSS). Two NBS patients showed heart anomalies: echogenic intracardiac foci and septal aneurysm; one NBS patient had right duplex kidney.

Assessment of the NGS-based diagnostic test.

Genetic analysis was performed only for those patients with a clinical suspicion of either NBS or CSS. The DNA of 5 patients carrying mutations already reported was used as positive control for the set-up of the NGS test (Cases 1-3, 9, 10 of table 1). [3,4] To analyse sequence variations genes of the BAF complex we used a strategy based on the locus-specific amplification of genomic DNA, amplifying each amplicon separately, followed by Roche 454 resequencing. Fusion primers for a total of 150 amplicons were designed in order to cover all coding sequence and intronic boundaries of the following genes: *ARID1A, ARID1B, SMARCA2, SMARCA4, SMARCB1* and *SMARCE1* (Supplementary table 1). The amplicons of one patient fill a sixth of a Junior Roche slide space. The obtained mean Reads sequence depth is of about 100X. In the same slide up to 6 patients can be placed. Obtained results were initially confirmed by Sanger sequencing, during the assessment phase. Subsequently, the confirmation of the mutation was carried out by a second NGS experiment in which the sample fills 1/900 of a Picotiter device Junior Roche space. In this case the confirmation of the mutation is carried out at a depth 10 times greater (1000X). Using 454 GS Junior sequencing, the analysis for point mutations of all genes of the BAF complex can be completed in 5 working days.

The newly identified mutations include five mutations in the *SMARCA2* gene (Cases 4-8, table 1) and one mutation in *ARID1B* (Case 11, Table 1). All *SMARCA2* mutations are missense, involving different aminoacids, and occurred in the region of the gene encoding the ATPase domain

(exons 15-25; from codon 748 to codon 1213). All these mutations are predicted to be functionally important by the computer-based algorithms. In particular two of them (case 4-5, table 1) were classified as “most likely pathogenic” variants and the other three were classified as “likely pathogenic” variants. The mutation identified in *ARID1B* is a frame-shift mutation in exon 9. All these mutations were private and in each of the 4 out of 6 individuals for whom DNA from both parents was available, the variant was confirmed to be *de novo*.

DISCUSSION

The clinical diagnosis of this group of conditions is mainly based on the facial gestalt and physical features. The clinical distinction between NBS and CSS is often challenging especially at younger age suggesting that these syndromes might represent a phenotypic spectrum rather than two distinct disorders. A patient with a *SMARCA2* mutation who was previously diagnosed as CSS was then reclassified as NBS. [8]

The identification of our 11 cases was mainly guided by the facial gestalt (Fig. 1). The facies is easily recognizable from the first months owing to the presence of the characteristic triangular shape, low frontal hairline, sparse hair, bushy eyebrows with/without synophrys, broad and long philtrum, big mouth with thin upper lip and thick and everted lower lip. With the progression from childhood to adolescence, facial gestalt becomes progressively coarse, especially in CSS (Fig. 1). In NBS, especially in the transition from childhood to adulthood, prominence of the columella becomes prevalent and the overall impression of the face is triangular (Fig. 1). Therefore, the differential diagnosis between the two conditions is a dysmorphological subtlety and it is more easily achieved with increasing age.

Developmental delay/ID is a cardinal feature in patients with a disorder of the BAF complex (severe/profound in *SMARCB1*, *SMARCE1*, and *ARID1A* mutations; variable in *SMARCA4*, *SMARCA2*, and *ARID1B* mutations). [13] Moreover, speech impairment was also observed in all subjects and was usually more severe than the intellectual status. [13] The behavioral disorders such

as hyperactivity, aggressiveness, psychosis and autistic trait have been reported in the literature. [13] Our results confirm the presence of ID in all patients and the variability of ID severity that in our cohort is milder in *ARID1B* mutated patients and prevalently moderate in *SMARCA2* mutated cases. Also the behavioural features have great variability ranging from aggressiveness and hyperactivity to sociable without a distinction between NBS and CSS patients.

Our cohort, although small, indicates also the tendency to short stature and the presence of eczema in a fraction of cases. Internal organ anomalies, which usually represent a parameter for the differential diagnosis between NBS and CSS, are almost absent in our series.

Hands and feet are characteristic with an overall shortening of bone segments, broadening of the interphalangeal joints, especially in *SMARCA2*-mutated patients, broadening of the distal phalanges, more typical of CSS patients and tendency to have broad hallux. We also found that the hypoplasia/aplasia of the fifth finger distal phalanges, a major criterion for the clinical diagnosis of CSS, is interestingly clinically present, in mild form, in 3 *SMARCA2* mutated patient (patient 1, 10 and 11). One important diagnostic handle, if X-rays of hands/feet are available, is the presence of ivory epiphyses, as illustrated in Figure 2.

We have provided the clinical information of 11 patients with mutations in the components of the SWI/SNF complex. Our patients were recruited from a total of 1161 patients with syndromic intellectual disability afferent to three different Italian centres. It indicates that the frequency of NBS/CSS conditions may be as common as the best known intellectual disability associated syndromes such as fragile X syndrome, as already proposed. [14] Therefore, clinical geneticists should consider NBS/CSS in the clinical setting during the ascertainment of patients with syndromic ID.

One-step deep sequencing test for all 6 genes of the complex (*SMARCA2*, *SMARCA4*, *SMARCE1*, *SMARCB1*, *ARID1A* e *ARID1B)* was developed. In this study we applied GS Junior sequencing (Roche 454) to simultaneously analyze 6 genes implicated in NBS/CSS: *SMARCA2*, *SMARCA4*, *SMARCE1*, *SMARCB1*, *ARID1A* and *ARID1B.* The use of the 454 technology can

reduce time of analysis and it is the most efficient in order to rapidly test patient belonging to "SWI/SNF-complex syndromes", also in terms of flexibility since probably in the near future additional genes belonging to the same pathway will be discovered as causing ID syndromes.

Competing interests disclosure

Authors disclose no competing interest.

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FIGURE LEGENDS

Figure 1. Facial gestalt of reported patients. Note progression of facial features in NBS (part A) and CSS (part B) patients at different ages from top (younger ages) to bottom (older ages).

Figure 2. Clinical pictures at different ages of hands and feet of NBS (part A) and CSS (part B) patients. Notice the gradual broadening of distal phalanges and the inter-phalangeal joint swelling of fingers, thickening of the distal phalanges of toes and short distal phalanges of fingers and toes.

Figure 3. Hands and feet X-rays of NBS (part A) and CSS (part B) patients. Part A: 1) Left Hand X-rays of patient 6 show short distal phalanges and cone-shaped epiphyses at medial phalanges. 2) Left Hand X-rays of patient 4 show short distal phalanges and slightly cone-shaped epiphyses at medial phalanges. 3) Left Hand X-rays of patient 1 show ivory epiphysis of the 2^{nd} finger distal phalanx. Right Hand X-rays show Ivory epiphysis of the 2^{nd}, 3^{rd} and 5^{th} finger distal phalanges. 4) Hands X-rays of patient 8 show ivory epiphysis of the 3^{rd} and 5^{th} finger distal phalanges of the left hand and ivory epiphysis of the 2^{nd} and the 5^{th} finger distal phalanges. 5) Right foot x-rays of patient 1 demonstrate ivory epiphyses of the 2^{nd} and the 4^{th} toe distal phalanges, tarsal fusions and broad first metatarsal. Left foot X-rays demonstrate ivory epiphyses of the 2^{nd} toe distal phalanx, tarsal fusions, broad first metatarsal and cone-shaped epiphysis of the proximal phalanx of the first toe. 6) Right foot X-rays of patient 8 show ivory epiphyses of the proximal phalanx of the first toe, ivory epiphyses of the distal phalanx of the 2^{nd} toe and broad first ray. Part B: 1) Patient 1 left hand X-rays showing short fifth finger phalanges. 2) Patient 9 hands X-rays showing enlarged metacarpals of the first finger and cone shaped epiphyses. 3) Feet X-rays of patient 9 showing broad first ray.