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Search for the best indicators for the presence of a *VPS13B* gene mutation and confirmation of diagnostic criteria in a series of 34 patients genotyped for suspected Cohen syndrome

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Key words: Cohen syndrome, *VPS13B* gene, neutropenia, chorioretinal dystrophy

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ABSTRACT

Background: Cohen syndrome is a rare autosomal recessive inherited disorder that results from mutations of the *VPS13B* gene. Clinical features consist of a combination of mental retardation, facial dysmorphism, post-natal microcephaly, truncal obesity, slender extremities, joint hyperextensibility, myopia, progressive chorioretinal dystrophy and intermittent neutropenia.

Patients and Methods: The aim of our study was to determine which of the above clinical features were the best indicators for the presence of *VPS13B* gene mutations in a series of 34 patients with suspected Cohen syndrome referred for molecular analysis of *VPS13B*.

Results: Fourteen *VPS13B* gene mutations were identified in 12 patients, and no mutation was found in 22 patients. The presence of chorioretinal dystrophy (92% versus 32%, $p=0.0023$), intermittent neutropenia (92% versus 5%, $p<0.001$) and postnatal microcephaly (100% versus 48%, $p=0.0045$) was significantly higher in the group of patients with a *VPS13B* gene mutation compared to the group of patients without a mutation. All patients with *VPS13B* mutations had chorioretinal dystrophy and/or intermittent neutropenia. The Kolehmainen diagnostic criteria provided 100% sensibility and 77% specificity when applied to this series.

Conclusion: From this study and a review of more than 160 genotyped cases from the literature, we conclude that, given the large size of the gene, *VPS13B* screening is not indicated in the absence of chorioretinal dystrophy or neutropenia in patients aged over 5 years. The follow-up of young patients could be a satisfactory alternative unless there are some reproductive issues.

INTRODUCTION

Cohen syndrome (CS) (OMIM 216550) is a rare autosomal recessive disorder first described in 1973, which involves a broad spectrum of clinical manifestations.¹ Based on the observations of 29 Finish patients with CS, Kivitie-Kallio and Norio² were the first to propose the essential features for CS diagnosis prior to the identification of the *COH1* gene: (1) non-progressive mental retardation, motor clumsiness, and microcephaly; (2) typical facial features including wave shaped eyelids, short philtrum, thick hair, and low hairline; (3) childhood hypotonia and joint hyperextensibility; (4) retinochoroidal dystrophy and myopia by 5 years of age; (5) periods of isolated neutropenia. These criteria were modified by Chandler *et al*³ to be more applicable to young patients, when there is not yet evidence of chorioretinal dystrophy (CRD) or patients with a more heterogeneous genetic background. These authors proposed that CS could be diagnosed in the presence of at least two of the following major criteria in a child with significant learning difficulties: (1) facial gestalt, characterised by thick hair, eyebrows and eyelashes, wave shaped, downward slanting palpebral fissures, prominent, beak-shaped nose, short, upturned philtrum with grimacing expression on smiling; (2) pigmentary retinopathy; (3) neutropenia (defined as $< 2000/\text{mm}^3$). The *VPS13B* gene was subsequently identified on chromosome 8q22-q23. This gene is composed of 62 exons that span a genomic region of around 864 kb and encodes a putative transmembrane protein of 4,022 amino acids with a complex domain structure (OMIM 607817).⁴ Although the exact function of *VPS13B* protein remains unknown, homology to the *Saccharomyces cerevisiae* VPS13 protein suggests a role in vesicle-mediated sorting and intracellular protein trafficking.⁴ Since the first identification of a *VPS13B* gene mutation,⁴ more than a hundred distinct *VPS13B* gene mutations have been identified⁴⁻¹⁴. Following identification of the *VPS13B* gene, the Chandler criteria were modified by the same team, since new clinical features were noted⁷. Patients were considered as having Cohen syndrome

when 6 of the following 8 criteria were fulfilled: developmental delay, microcephaly, typical facial dysmorphism, obesity and slender extremities, sociable behaviour, joint laxity, myopia/retinal degeneration and intermittent neutropenia.⁷ In spite of considerable genotypic variability, positive patients fulfilled the diagnostic criteria with relative clinical homogeneity. Given the increasing number of requests for molecular testing in patients with suspected CS and the very large size of the gene, the aim of our study was to determine which of the above clinical features are the best indicators for the presence of *VPS13B* gene mutations by comparing patients carrying *VPS13B* mutations with negative patients in a series of 34 patients referred for molecular testing of *VPS13B* in suspected CS. The results should help clinicians to evaluate whether *VPS13B* mutations are likely to be responsible for a clinical phenotype combining a number of features. We also evaluated the current diagnostic criteria.

SUBJECTS AND METHODS

Subjects

A total of 34 patients from 29 families were ascertained for *VPS13B* testing and suspected CS at the molecular diagnostic laboratory of Dijon University Hospital in France. When a sample was received for *VPS13B* screening, a standardized comprehensive clinical form was sent to the referring physician. All blood samples received were screened for a *VPS13B* gene mutation, whether or not the patients presented the clinical criteria for the diagnosis of CS according to Chandler *et al*³. Written informed consent was obtained according to the French regulatory requirements for genetic testing.

Molecular genetic analysis

Each blood sample was processed for DNA extraction using the “salting-out” method.¹⁵ PCR analysis and sequencing of exons and exon-intron boundaries of the *VPS13B* gene as well as amplification of gene sequences encoding exons 1 to 62 were performed. All PCR products were directly sequenced using a BigDye terminator kit and an ABI Genetic Analyzer 3100 capillary sequencer according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). Primer sequences are available on request. Corresponding reference sequences of the genomic DNA sequences of the *VPS13B* gene were downloaded using Ensembl Genome Browser (Accession number ENSG00000132549). The SeqScape® software v2.5 package (Applied Biosystems, Foster City, CA) was used to visualize capillary trace electropherograms, for sequence assembly and alignment, and to search for comparisons with consensus and reference sequences and variations. Depending on the analyzed exon, only between twenty and thirty bases inside the intervening sequences donor site and acceptor site from the consensus splice junction sequences are clearly investigated by the sequencing analysis program and subjected to alignment with the reference sequence. Mutation nomenclature¹⁶ numbering was based on the current Ensembl transcript (Ensembl Transcript ID ENST00000358544), with +1 as the A of the ATG initiation codon. Mutations leading to premature truncations were considered pathogenic. When a missense mutation was found, its absence was verified in 220 control chromosomes.

Statistical analyses

The proportion of clinical features of the CS spectrum in the group of patients with *VPS13B* gene mutations was compared with that in the group of patients with negative *VPS13B* sequencing, using Fisher’s exact test. These analyses were possible because the age at molecular screening in the two groups was similar.

RESULTS

Clinical description

Patients with VPS13B mutations

VPS13B mutations were found in 12 patients (7 males and 5 females) originating from 8 families (Table 1), comprising one consanguineous Moroccan family and 7 non-consanguineous French families. The age range at CS suspicion was 2.5 to 43.0 years and that at genetic screening was 4.8 to 43.6 years. Except for two children aged 2.5 and 4.5 years, all patients were aged over 5 years at the time of diagnosis. The percentages of clinical features of the *VPS13B* spectrum are reported in Table 1. All patients had mental retardation, typical or evocative CS facial gestalt, microcephaly and slender extremities with narrow hands/feet. Truncal obesity was reported in all but one patient aged 4.5 years. CRD was reported in 11/12 patients (92%), diagnosed on fundus examination in 5 patients and on fundus and electroretinography (ERG) in 6. CRD was absent on fundus examination in a 19-year-old girl, but ERG was not performed. Early signs of CRD were observed at ERG as early as 16 and 24 months in 2 young siblings. Myopia was present in the 9 patients for whom the information was available. Neutropenia was reported in 11/12 patients. The clinical diagnosis of CS was fulfilled in all patients according to Kolehmainen *et al*⁷ criteria.

Patients without a VPS13B mutation

The 22 patients (7 males, 15 females) in whom no *VPS13B* gene mutation was found originated from 21 families, of which 1 was consanguineous. The mean age at molecular screening was 15.8 ± 7.7 years. The percentages of clinical features of the CS spectrum are reported in Table 1. In particular, CRD was observed in 6/19 ascertained patients. One patient had neutropenia. Kolehmainen's criteria for CS were fulfilled in 5/22.

***VPS13B* gene mutations**

Table 2 summarizes the mutations found in this series as well as the predicted consequence of each mutation at the protein level. A total of 14 different mutations, of which 11 have never been published, were identified in the 8 families. All mutations except 3 resulted in premature truncation (Table 2). One patient had, on the same allele on exon 56, a missense mutation c.10880C>T followed by an 18-base-pair (bp) deletion (c.10883_10900delCGAGGCAGCTTGTGCACG) leading to the deletion of 6 amino acids. A homozygous missense mutation (c.4907T>A, p.I1636N) was identified in the 2 Moroccan siblings born to consanguineous parents. The pathogenic nature of the mutation was suspected for several reasons: i) isoleucine at position 1636 is a non-polar amino acid (whereas the mutant asparagine residue is polar), ii) the hydrophobic nature of the amino acid side chain at this position was 100% preserved following the alignment of proteins from different animal species (supplementary figure 1), iii) the cosegregation of the mutation in the family (supplementary figure 2), iv) the absence of this variant in 100 healthy controls. Only one truncating heterozygous *VPS13B* mutation was found in two patients, but these patients were considered as having CS in the presence of the typical clinical picture of the condition.

Statistical analyses

Significant differences were found between the group of patients with *VPS13B* mutations and the group of patients without *VPS13B* mutations. Indeed, the probability of finding CRD, neutropenia, microcephaly and myopia was higher in the group of patients with *VPS13B* mutations (Table 1). Conversely, there was no significant difference for the presence of facial gestalt, narrow extremities and truncal obesity. In this series, the sensitivity of CDR and neutropenia was 92% for both parameters whilst specificity was 68% and 95%, respectively.

DISCUSSION

The hallmarks of CS include mental retardation, facial dysmorphism, chorioretinal dystrophy and neutropenia, and patients exhibit high clinical homogeneity. Following identification of the *VPS13B* gene, it was discovered that patients with *VPS13B* mutations, who did not fulfil CS clinical criteria were exceptional.⁴⁻¹² Contrary to other examples in the literature¹⁷, the identification of the *VPS13B* gene has not made it possible to enlarge the clinical spectrum of CS. On the contrary, patients with suspected CS based on the presence of evocative facial gestalt but in the absence of ophthalmologic manifestations were reclassified as having Cohen-like syndrome, since no *VPS13B* mutations were found on either allele.⁸ Only Kolehmainen *et al*⁷ has given a brief description of patients negative for *VPS13B* mutations. The aim of this study was to compare the clinical features of patients with *VPS13B* mutations with those in patients without *VPS13B* mutations in order to give clues to the clinician on the indication for *VPS13B* screening according to the clinical phenotype, especially since molecular screening of *VPS13B* is a time-consuming task. We showed that all patients with *VPS13B* mutations had either CRD or neutropenia. The presence of microcephaly, found in all patients with *VPS13B* mutations in this series, was another clinical feature that can help to distinguish between CS and differential diagnoses. Conversely, there was no significant difference for the presence of evocative dysmorphism, obesity and slender extremities. Therefore, except in young children, these features, in the absence of neutropenia or CRD, are not sufficient to suspect CS. Of note, the assessment of facial gestalt is subjective whereas CRD and neutropenia may be assessed objectively. According to the London Dysmorphology DataBase, around 80 syndromes associate mental retardation and retinopathy,¹⁸⁻¹⁹ while only a few syndromes associate neutropenia and mental retardation. This explains why the probability of finding a *VPS13B* mutation in patients with neutropenia (specificity of 95% in

our series) in a context of suspected CS is even higher than it is in patients with associated CRD (specificity of 68% in our series)

We also evaluated the clinical criteria used to diagnose CS in our series. All patients with *COH1* mutations fulfilled the Kolehmainen criteria, and among patients with no *COH1* mutations, 5/22 fulfilled Kolehmainen's criteria. These criteria, therefore, provided 100% sensitivity and 77% specificity. These results are totally consistent with those reported by Kolehmainen *et al*⁷. All patients with *VPSI3B* mutations fulfilled their clinical diagnostic criteria. Out of 24 patients with no *VPSI3B* mutation and full clinical data, only 2 fulfilled their clinical criteria. Among the 22 Cohen-like patients, none had neutropenia, and only one out of the seven patients with myopia and/or retinal dystrophy had the typical facial appearance of CS – although the patient did not sufficiently fulfil the other criteria for a true diagnosis of CS.

Table 3 shows the prevalence of neutropenia in previous series reporting *VPSI3B* mutations. This prevalence was high except in the series reported by Hennies *et al*⁶. He found only 10 patients with neutropenia in a series of 18 patients in spite of repeated haematological examinations. These results could not be assigned to any specific ethnic group or genotype-phenotype correlation. Table 3 also shows the prevalence of CRD in previous series. When considering all of the 160 patients with *VPSI3B* mutations reported in the literature and assessed for CRD and neutropenia, CRD was found in 92% of patients aged over 5 years (86% if the age of patients was not taken into account). The difficulties in diagnosing CRD in young patients (below 5 years of age) have been discussed by others.² When CS is suspected, CRD may be diagnosed at the early stage of the disease using an electroretinogram (ERG) instead of, or in addition to a fundus examination. Indeed the amplitude of ERG waves may be reduced occur as soon as the photoreceptor cells are impaired while in examinations of the fundus, bull eye maculopathy or optic disc pallor with narrow vessels may be the only

symptoms. Typical pigmentary lesions are observed later and only after cell death. This was clearly demonstrated by Chandler *et al.*: the early onset of CRD was evidenced using ERG in 80% of children under 5 years with suspected CS.^{3,20} The absence of CRD in a 19-year-old patient and its presence in 2 siblings aged 16 and 24 months in our series show the large variability in age at diagnosis of CRD in CS. These are other examples showing that this feature cannot be mandatory for the diagnosis of CS. Only 6% of patients had neither neutropenia nor CRD; this fell to 4% if children aged ≤ 5 years were excluded.

We cannot exclude the possibility of undetected *VPS13B* mutations and in particular genomic deletions of the *VPS13B* gene as recently described.¹³ Although this hypothesis is very likely in our 2 families in which only one pathogenic mutation was identified, it is unlikely when no pathogenic mutations are identified after genomic sequencing of the entire gene in non-consanguineous families.

In conclusion, our study showed that CRD and neutropenia appear to be the best predictors for the presence of *VPS13B* mutations. Given the large size of the gene, and since the probability of finding a *VPS13B* mutation in the absence of these key features is very low, screening for *VPS13B* mutations is not recommended unless they are present. Because of the age-dependent onset of CRD, caution should be exercised in young children, in whom follow-up could be a reasonable alternative. However, *VPS13B* screening could be offered in such cases when the reproductive context is indicative since there is a 25% risk of recurrence.

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COMPETING INTEREST

None declared

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Legends to supplementary figures

Supplementary figure 1: Sequence alignment of conserved region including the position 1636 where the amino acid isoleucine (I) is mutated to asparagine (N) in VPS13-like proteins in different species. This position is indicated by an (*) and always occupied by a non-polar amino acid residue.

Supplementary figure 2: Sequence analysis of *COH1* gene exon 31 in probands from the family with Cohen syndrome. The homozygous nature of the missense mutation c.4907T>A in the probands and the heterozygous nature of the same mutation in the consanguineous parents are shown.

Table 1: Clinical features of the CS spectrum in patients with or without *VPS13B* gene mutations

Clinical features	Patients with <i>VPS13B</i> mutations*	Patients without <i>VPS13B</i> mutations*	p
Mean age at screening	18±12 years	16±7 years	
Mental retardation	12/12 (100%)	22/22 (100%)	NS
Compatible facial gestalt	12/12 (100%)	17/22 (77%)	NS
Microcephaly	11/11 (100%)	10/21 (48%)	0.0045
Joint hyperextensibility	9/11 (82%)	7/14 (50%)	NS
Slender extremities/ Tapering fingers	12/12 (100%)	19/22 (86%)	NS
Truncal obesity	11/12 (92%)	18/22 (82%)	NS
Myopia	9/10 (90%)	11/22 (50%)	0.049
CRD	11/12 (92%)	6/19 (32%)	0.0023
Neutropenia	11/12 (92%)	1/21 (5%)	<0.001
Fulfillment of Kolehmainen's criteria [2004]	12/12 (100%)	5/22 (23%)	<0.001

NS: not statistically significant

* N positive/N assessed patients

Table 2: Mutations identified in *VPSI3B* gene from 12 patients (8 families) with Cohen Syndrome

Patients (Family)	Nucleotide change (amino-acid change)	Exon	Source
P1 F1	c.436C>T (p.R146X) Second mutation not found	5	Novel
P2, P3 F2	c.10139_10143dupCGCCA (p.A3380fsX3396) Second mutation not found	56	Novel
P7 F6	c.1220delA (p.Q407fsX418) c.7286delT (p.V2429fsX2430)	9 40	Novel Novel
P8, P9 F7	c.4907T>A (p.I1636N) c.4907T>A (p.I1636N)	31 31	Novel Novel
P10, P11 F8	c.2074C>T (p.R692X) c.5426_5427dupAG (p.Q1810fsX1830)	15 34	^{4,10} ^{4,10}
P15 F11	c.3427C>T (p.R1143X) [c.10880C>T; c.10883_10900delCGAGGCAGCTTGTGCACG]* ([p.T3627I;p.A3628_H3633del])	23 56	Novel Novel
P18, P19 F14	c.916_917delGA (p.D306fsX9) c.1006C>T (p.Q336X)	7 8	Novel Novel
P21 F16	c.477_480delACTA (p.I159fsX21) c.11859_11860insAA (p.N3954fsX60))	5 62	Novel Novel

*These two sequence variations were found on the same allele

Table 3: Study of the occurrence of CRD and/or neutropenia in patients with *VPS13B* mutations reported in the literature and in the present series (N positive/N assessed patients)

	CRD	Neutropenia	CRD or neutropenia
Kolehmainen et al., 2003	31/31	31/31	31/31
Kolehmainen et al., 2004	28/29	25/26	29/29
Falk et al., 2004	8/8*	NR	8/8
Hennies et al., 2004	13/20**	10/18	17/20
Kondo et al., 2004	2/2	2/2	2/2
Mochida et al., 2006	4/7*	3/7	5/7
Seifert et al., 2006	13/21***	14/16	16/21
Katzaki et al., 2007	9/10*	9/10	10/10
Seifert et al., 2009	11/12*****	6/11	12/12
Balikova et al., 2009	8/8	7/8	8/8
Present series	11/12*****	11/12	12/12
Total	138/160 (86%)		150/160 (94%)
(% of positive/assessed patients)	128/139 (92%) if children aged ≤ 5 years are excluded	118/141 (84%)	133/139 (96%) if children aged ≤ 5 years are excluded

NR: not reported;

* including 1 patient aged ≤ 5 years; ** including 7 patients aged ≤ 5 years; *** including 6 patients aged ≤ 5 years; **** including 3 patients aged ≤ 5 years; ***** including 2 patients aged ≤ 5 years.