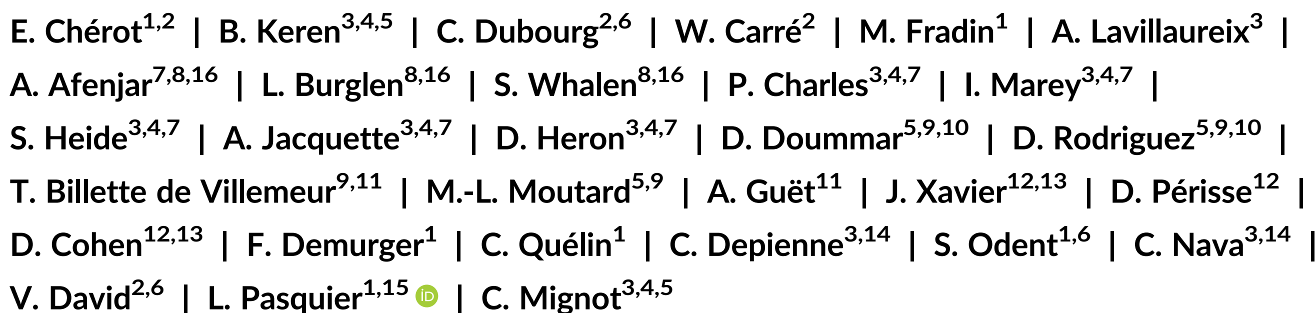
Received: 10 February 2017 | Revised: 7 July 2017 | Accepted: 10 July 2017

DOI: 10.1111/cge.13102

WILEY CLINICAL GENETICS

ORIGINAL ARTICLE

# Using medical exome sequencing to identify the causes of neurodevelopmental disorders: Experience of 2 clinical units and 216 patients



E. Chérot[1,2] | B. Keren[3,4,5] | C. Dubourg[2,6] | W. Carré[2] | M. Fradin[1] | A. Lavillaureix[3] | A. Afenjar[7,8,16] | L. Burglen[8,16] | S. Whalen[8,16] | P. Charles[3,4,7] | I. Marey[3,4,7] | S. Heide[3,4,7] | A. Jacquette[3,4,7] | D. Heron[3,4,7] | D. Doummar[5,9,10] | D. Rodriguez[5,9,10] | T. Billette de Villemeur[9,11] | M.-L. Moutard[5,9] | A. Guët[11] | J. Xavier[12,13] | D. Périsse[12] | D. Cohen[12,13] | F. Demurger[1] | C. Quélin[1] | C. Depienne[3,14] | S. Odent[1,6] | C. Nava[3,14] | V. David[2,6] | L. Pasquier[1,15] | C. Mignot[3,4,5]

[1]Service de Génétique Médicale, CLAD Ouest CHU Hôpital Sud, Rennes, France

[2]Laboratoire de Génétique moléculaire et Génomique, CHU Pontchaillou, Rennes, France

[3]Département de Génétique, AP-HP, Hôpital de la Pitié-Salpêtrière, Paris, France

[4]Groupe de Recherche Clinique (GRC) "déficience intellectuelle et autisme", UPMC, Paris, France

[5]Groupe de Recherche Clinique (GRC) "ConCer-LD", UPMC, Paris, France

[6]CNRS UMR 6290 (IGDR), Université de Rennes 1, Rennes, France

[7]Centre de Référence Déficiences Intellectuelles de Causes Rares Hôpital de la Pitié-Salpêtrière, Paris, France

[8]APHP, GHUEP, Hôpital Armand Trousseau, Centre de Référence 'Malformations et maladies congénitales du cervelet', Paris, France

[9]AP-HP, Service de Neuropédiatrie, Hôpital Armand Trousseau, Paris, France

[10]AP-HP, Hôpital Armand Trousseau, Centre de Référence Neurogénétique de l'Enfant à l'adulte, Paris, France

[11]AP-HP, Service de Pédiatrie, Hôpital Louis Mourier, Colombes, France

[12]AP-HP, Department of Child and Adolescent Psychiatry, Groupe Hospitalier Pitié-Salpêtrière et University Pierre and Marie Curie, Paris, France

[13]Sorbonne Universités, UPMC, CNRS UMR 7222, Institut des Systèmes Intelligents et Robotiques, Paris, France

[14]INSERM U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, Paris, France

[15]INSERM CIC pédiatrique 1414, CHU Pontchaillou, Rennes, France

[16]Département de Génétique, APHP, GHUEP, Hôpital Armand-Trousseau, Paris, France

**Correspondence**
Laurent Pasquier, Service de Génétique Médicale, CHU Hôpital Sud, Rennes, Bretagne, France.
Email: laurent.pasquier@chu-rennes.fr

**Funding information**
Assistance Publique des Hôpitaux de Paris (APHP); Rennes University Hospital Centre Hospitalier Universitaire de Rennes; la Fondation Groupama; Bio-Psy Labex

Although whole-exome sequencing (WES) is the gold standard for the diagnosis of neurodevelopmental disorders (NDDs), it remains expensive for some genetic centers. Commercialized panels comprising all OMIM-referenced genes called “medical exome” (ME) constitute an alternative strategy to WES, but its efficiency is poorly known. In this study, we report the experience of 2 clinical genetic centers using ME for diagnosis of NDDs. We recruited 216 consecutive index patients with NDDs in 2 French genetic centers, corresponded to the daily practice of the units and included non-syndromic intellectual disability (NSID, *n* = 33), syndromic ID (NSID = 122), pediatric neurodegenerative disorders (*n* = 7) and autism spectrum disorder (ASD, *n* = 54). We sequenced samples from probands and their parents (when available) with the Illumina TruSight One sequencing kit. We found pathogenic or likely pathogenic variants in 56 index patients, for a global diagnostic yield of 25.9%. The diagnosis yield was

Laurent Pasquier and Cyril Mignot contributed equally to this study.

© 2017 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

higher in patients with ID as the main diagnosis (32%) than in patients with ASD (3.7%). Our results suggest that the use of ME is a valuable strategy for patients with ID when WES cannot be used as a routine diagnosis tool.

**KEYWORDS**

autism, intellectual disability, medical exome, molecular strategy

## 1 | INTRODUCTION

Intellectual disability (ID) is the most frequent neurodevelopmental disorder (NDD) affecting about 1% to 3% of the population worldwide. More than 700 genes are known to cause ID[1] and this large genetic heterogeneity is challenging for diagnosis because the phenotype in many patients is either non-syndromic or corresponds to syndromes that are either not recognized or for which the molecular cause is still unknown. To decipher the genetic etiologies of NDD, chromosomal microarray is a first tier diagnosis tool,[2] together with the search for a *FMR1* gene 5′ untranslated region (5′UTR) triplet amplification. When these investigations are negative and if no targeted genetic testing is considered, clinical geneticists have to choose between gene panels[3] and whole-exome sequencing (WES). Because of the extreme genetic heterogeneity of NDD and because each genetic cause is very rare, the probability to find molecular causes of NDD tends to increase with the number of analyzed genes, which implies that WES is a more appropriate tool than gene panels.[4–7] However, the cost of WES is still high and unaffordable in daily practice for many genetic centers that have to choose between locally designed gene panels and commercialized gene panels comprising all known disease-related genes, called "medical exome" (ME). A few articles reported the use of ME for the diagnosis of genetic disorders.[8,9] We report here the experience of 2 French genetic centers using the TruSight One sequencing panel, which targets genes associated with known phenotypes, for the diagnosis of NDD in 216 patients.

## 2 | MATERIAL AND METHODS

### 2.1 | Patients

We studied 216 index patients with a presumed genetic NDD but without molecular etiology recruited consecutively in the daily practice of 2 clinical genetic centers (Groupe Hospitalier Pitié-Salpêtrière and Centre Hospitalier Universitaire of Rennes). Inclusion criteria were: (1) the family asks for the establishment of a definite risk for a first-degree relative of having a child with the disease of the index case, (2) negative previous genetic testing including chromosomal microarray analysis, fragile X testing, as well as normal metabolic screening and/or targeted genetic studies varying from one patient to another.

The overall series included 133 males and 83 females. Ages at disease onset ranged from 1 to 56 years. Consanguinity was reported in 17 families (7.8%).

Three patients only had a definite clinical diagnosis (Nicolaides-Baraitser syndrome [MIM 601358], Coffin-Siris syndrome [MIM 135900] and microcephaly, lymphoedema, retinal dysplasia syndrome [MIM 152950]) with unavailable or expensive molecular testing.

We classified the neurodevelopmental phenotypes of the patients into 4 categories: (1) patients with non-syndromic ID (NSID), includes those with normal growth parameters and without dysmorphic features/malformations, neuromotor involvement (pyramidal, extrapyramidal, and cerebellar syndrome) and sensory organ involvement, (2) patients with syndromic ID (SID), (3) patients with pediatric neurodegenerative disorders (NDEG), and (4) patients with autism spectrum disorder (ASD). Patients with early developmental delay and ID meeting the criteria of the Autism Diagnosis Interview (ADI) for ASD were classified as having ID. Patients meeting the ADI criteria for ASD with normal early development during the first 12 to 18 months of life followed by autistic regression and those with ASD and preserved intelligence were classified in the ASD category. We considered macrocephaly and microcephaly in patients with a head circumference above or below 2 SDs to the mean, respectively. We considered epilepsy as a non-specific feature.

Informed consent was obtained from all individual participants, parents or legal representatives, included in the study. Samples from each center were sent to local laboratories.

The following patients have been previously reported in articles: #3 and #4,[10] #8,[11] #13,[12] #14,[13] #23,[14] #26,[15] and #51.[16]

### 2.2 | Sequencing technologies

All samples were prepared with the Illumina (Illumina Inc, San Diego, CA) TruSight One preparation kit (which covers 4813 genes associated with known phenotypes and 11 884 205 bp) and sequenced on either an Illumina MiSeq or NextSeq 500 sequencer using 2 × 150 bp sequencing kits. We performed ME sequencing in 196 -parent-offspring trios (90.7%), 10 duos (proband + 1 parent; 4.6%) and 10 singletons (4.6%), including the 3 patients with a definite clinical diagnosis.

### 2.3 | Bioinformatics pipeline

Alignment on the reference genome was made with BWA-mem and variant calling algorithms were Freebayes and the GATK Unified Genotyper and Haplotype Caller in Rennes. Annotations from ANNOVAR were added. The Basespace cloud computing platform (with BWA 2.1 and GATK Unified Genotyper 1.6) and the Variant Studio software

provided by Illumina were used in La Pitié-Salpêtrière. More than 95% of targets were covered with a 20× depth of sequencing in both centers. Only point variants and small indels were investigated with this pipeline, as usually done in WES series.[4,7–9]

### 2.4 | Variant validation and interpretation

All variants with a potential deleterious effect were confirmed by Sanger sequencing and were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/ with submissions ID: SUB2218315 and SUB2313153; Organizations ID: 505806 and 506058). Pathogenicity of variants was ascertained according to the ACMG (American College of Medical Genetics) criteria,[17–19] which classify variants according to 5 categories (class 1: benign, class 2: likely benign, class 3: uncertain significance, class 4: likely pathogenic, and class 5: pathogenic). All variants of interest were discussed with clinicians and all molecular diagnoses were validated by referring clinicians.

## 3 | RESULTS

Detailed results are available in Tables 1 and 2. ME sequencing revealed 1 or 2 pathogenic (class 5) variants in 39 of 216 patients (17.9%), 1 or 2 likely pathogenic (class 4) variants in 17 of 216 (7.8%) and 1 or 2 variants partially explaining the phenotype in 2 of 216 others (0.9%). Excluding the latter category, the overall diagnostic yield was 25.9%.

### 3.1 | Mode of inheritance and types of variants

Of the 58 full or partial diagnoses, 40 (69%) were dominant disorders due to (1) heterozygous de novo variants ($n$ = 35 including 2 in the same gene for patient #53), (2) variants inherited from an unaffected parent with proven or suspected somatic mosaicism ($n$ = 5) and (3) variant inherited from an affected father ($n$ = 1). Nine patients (15.5%) had recessively inherited disorders related to (1) compound heterozygous variants ($n$ = 6), (2) homozygous variants ($n$ = 2) or (3) paternally inherited variant combined with a maternally inherited gene deletion ($n$ = 1). Nine patients (15.5%) had an X-linked disorder due to (1) de novo variants ($n$ = 2), (2) maternally inherited variants ($n$ = 5), (3) 1 variant inherited from a mosaic father.

We identified the molecular causes of NDD in 5 of 20 patients studied as index cases or in duos, including the 3 patients with a definite clinical diagnosis. Thus, we identified a pathogenic variant for 2 of 17 (11%) patients without definite clinical diagnosis studied as index cases or in duos, and 27% in parent-offspring trios (all without definite clinical diagnosis).

Altogether, we identified 64 variants including 47 novel mutations (Table S1, Supporting Information). Variant types include 33 variants leading to a premature termination codon (nonsense, frameshift, and canonic splice site variants) and 31 missense variants. Truncating/missense variants were distributed as follows according to the mode of inheritance: autosomal dominant disorders 22/19 (including the 2 variants of patient #53), autosomal recessive disorders 7/7 (including 2 homozygous variants), X-linked disorders 4/5.

Intriguingly, for patient #53, we identified 2 heterozygous de novo variants in the same gene *SMARCA2* responsible for Nicolaides-Baraitser syndrome. We were not able to answer about the cis/trans position. Indeed, we confirmed that both variations were not present in parent's DNA within Sanger sequencing method and were not located on the same read, looking at our NGS data.

### 3.2 | Rate of diagnoses and phenotypes

Diagnostic yields according to the phenotypic categories defined above (Table S1) were as follows: NSID 30% (10/33), SID 32% (40/122), NDEG 57% (4/7), and ASD 3.7% (2/54). The number of diagnoses relative to the number of tested patients with SID was not significantly different from the number of diagnoses made in those with NSID. On the contrary, the number of diagnoses made in all patients with ID (50/155, 32%) was significantly higher than the number of diagnoses made in those with ASD ($P$ = 0.0002, Fisher's exact test).

Pathogenic variants identified in *KAL1* in patient #57 and *PRODH* in patient #58 partly explained their SID phenotypes (hypogonadotrophic hypogonadism and ID with schizophrenia, respectively).

## 4 | DISCUSSION

Many previous studies have shown that WES is an excellent option for genetic testing in patients with NDD when fragile X syndrome, chromosomal imbalances and other hypotheses with available targeted genetic studies have been ruled out.[4–7] The huge genetic heterogeneity of NDD and the rarity of each cause imply that the likelihood of finding pathogenic variants increases with the number of the studied genes. ME, i.e. panels including all disease-associated genes, is an alternative strategy when WES is not available or too expensive. In the only article reporting of the use of the TruSight One gene panel in the daily practice, this panel was applied to the diagnosis of all kinds of genetic diseases.[8] Our study on patients with NDD only provides more insight into the use of ME in a context of extreme genetic heterogeneity.

### 4.1 | Mutated genes and associated phenotypes

As in series of patients with NDD studied by WES,[4–7] genetic heterogeneity was the rule in our series since we found pathogenic variants in 48 different genes involved in NDD (excluding *KAL1*). Eight genes were found mutated twice (*ARID1B*, *STXBP1*, *SCN2A*, *SYNGAP1*, *TCF4*, *ANKRD11*, *ADNP* and *ATP1A3*). As expected, 6 of these genes are among the most frequently mutated in the DDD study reporting pathogenic variants found by WES in individuals with developmental disorders.[7]

Most gene panels are targeted to the molecular investigation of patients with particular phenotypes. Because of the large clinical and molecular heterogeneity of NDD, the search for its etiology requires testing with panels comprising hundreds of genes or an informative clinical examination allowing the targeting of specific panels. Using ME sequencing, we identified the etiology of NDD in 53 of 56 patients

**TABLE 1** Pathogenic variants identified with ME in 39 patients with NDD

| Patient # | Sex | Mutated gene | Status | Variants | Inheritance | Variant type | OMIM/ClinVar phenotype |
|---|---|---|---|---|---|---|---|
| 1 | M | *ADNP* | ht | NM_015339.2:c.517C>T, Chr20(GRCh37): g.49510734G>A, p. Arg173* | De novo | AD | Helsmoortel-van der Aa syndrome |
| 2 | M | *ADNP* | ht | NM_001282531.1: c.2156dup,Chr20 (GRCh37):g.49509095dup, p.Tyr719* | De novo | AD | Helsmoortel-van der Aa syndrome |
| 3 | F | *ANKRD11* | ht | NM_001256182.1: c.2647G>T,Chr16 (GRCh37):g.89350303C>A, p.Glu883* | De novo | AD | KBG syndrome |
| 4 | F | *ANKRD11* | ht | NM_001256182.1: c.6786_6787insA, Chr16(GRCh37):g. 89346163_893461 64insT, p.Pro2263 Serfs*10 | Paternally inherited (father affected) | AD | KBG syndrome |
| 5 | F | *ARHGEF9* | ht | NM_015185.2:c.865C>T, ChrX(GRCh37): g.62893977G>A, p. Arg289* | Paternally inherited (mosaicism 24% of reads) | XL | Epileptic encephalopathy, early infantile, 8 |
| 6 | F | *ARID1B* | ht | NM_020732.3:c.5830C>T, Chr6(GRCh37): g.157528105C>T, p. Arg1944* | Paternally inherited (mosaicism 7% of reads in blood) | AD | Coffin-Siris syndrome 1 |
| 7 | M | *ATRX* | hi | NM_000489.3:c.4865C>T, ChrX(GRCh37): g.76889145G>A, p. Ala1622Val | Maternally inherited (a brother is affected) | XL | Mental retardation-hypotonic facies syndrome, X-linked-1 |
| 8 | M | *DYRK1A* | ht | NM_001396.4:c.932C>T, Chr21(GRCh37): g.38862744C>T, p. Ser311Phe | De novo | AD | Mental retardation, autosomal dominant 7 |
| 9 | M | *EFTUD2* | ht | NM_004247.3: c.1775_1779del,Chr17 (GRCh37): g.42937354_42937358del, p.Val592Alafs*12 | Maternally inherited (mosaicism 2/300 reads in blood) | AD | Mandibulofacial dysostosis, Guion-Almeida type |
| 10 | M | *FOXP1* | ht | NM_032682.5:c.1349-5_1350del,Chr3(GRCh37): g.71026872_71026878del, p.? | De novo | AD | Mental retardation with language impairment and with or without autistic features |
| 11 | M | *GNAS* | ht | NM_000516.4:c.772C>T, Chr20(GRCh37): g.57484792C>T, p. Arg258Trp | De novo | AD | Albright hereditary osteodystrophy |
| 12 | M | *GRIA3* | hi | NM_007325.4:c.1964T>C, ChrX(GRCh37): g.122561878T>C, p. Phe655Ser | Maternally inherited (mosaicism 10% reads in blood) | XL | Mental retardation, X-linked, syndromic, Wu type |
| 13 | F | *GRIN2B* | ht | NM_000834.3:c.1966C>T, Chr12(GRCh37): g.13761581G>A, p. Gln656* | De novo | AD | Epileptic encephalopathy, early infantile, 27 |
| 14 | F | *HNRNPU* | ht | NM_031844.2(HNRNPU): c.16delinsATT,Chr1 (GRCh37): g.245027594delinsAAT, p. Val6Ilefs*4 | De novo | AD | registered as Phenotype in ClinVar |
| 15 | M | *HPRT* | hi | NM_000194.2:c.47G>T,ChrX (GRCh37): g.133607408G>T, p. Gly16Val | Maternally inherited | XL | Lesch-Nyhan syndrome |
| 16 | M | *KDM5C* | hi | NM_004187.3:c.2482C>T, ChrX(GRCh37): | Maternally inherited | XL | |

(*Continues*)

**TABLE 1** (Continued)

| Patient # | Sex | Mutated gene | Status | Variants | Inheritance | Variant type | OMIM/ClinVar phenotype |
|---|---|---|---|---|---|---|---|
| | | | | g.53227706G>A, p.Arg828* | | | Mental retardation, X-linked, syndromic, Claes-Jensen type |
| 17 | F | *KDM6A* | ht | NM_001291415.1:c.2988+1G>C,ChrX(GRCh37):g.44936072G>C, p.? | De novo | XL | Kabuki syndrome 2 |
| 18 | F | *KIF1A* | ht | NM_001244008.1:c.920G>A, Chr2(GRCh37):g.241715306C>T, p.Arg307Gln | De novo | AD | Mental retardation, autosomal dominant 9 |
| 19 | M | *LARS2* | cht | NM_015340.3:c.1987C>T, Chr3(GRCh37):g.45557711C>T, p.Arg663Trp and NM_015340.3:c.371A>T,Chr3(GRCh37):g.45458981A>T, p.Asn124Ile | Biparental transmission | AR | Perrault syndrome |
| 20 | F | *MED13L* | ht | NM_015335.4:c.5588+1G>A, Chr12(GRCh37):g.116413319C>T, p.? | Apparently de novo germline mosaicism (sister and brother affected) | AD | Mental retardation and distinctive facial features with or without cardiac defects |
| 21 | M | *MFSD8* | cht | NM_152778.2:c.1444C>T, Chr4(GRCh37):g.128841898G>A, p.Arg482*, maternally inherited and NM_152778.2:c.416G>A,Chr4(GRCh37):g.128864930C>T, p.Arg139His, paternally inherited | Biparental transmission | AR | Ceroid lipofuscinosis, neuronal, 7 |
| 22 | M | *MICU1* | cht | NM_006077.3:c.1048C>T, Chr10(GRCh37):g.74183021G>A, p.Gln350*, maternal inherited NM_006077.3:c.40del,Chr10(GRCh37):g.74326512del, p.Ala14Leufs*20 , paternal inherited | Biparental | AR | Myopathy with extrapyramidal signs |
| 23 | F | *NAA10* | ht | NM_003491.3:c.384T>G, ChrX(GRCh37):g.153197526A>C, p.Phe128Leu | De novo | XL | Ogden syndrome |
| 24 | M | *NFIX* | ht | NM_001271043.2:c.97del, Chr19(GRCh37):g.13135880del, p.Ala33Leufs*32. | De novo | AD | Sotos syndrome 2 |
| 25 | M | *PANK2* | cht | NM_153638.2:c.1235+1G>T, Chr20(GRCh37):g.3891478G>T, p.?, paternally inherited and NM_153638.2:c.1561G>A,Chr20(GRCh37):g.3899342G>A, p.Gly521Arg | Biparental transmission | AR | Neurodegeneration with brain iron accumulation 1 |
| 26 | M | *POGZ* | ht | NM_015100.3:c.1810G>T, Chr1(GRCh37):g.151384217C>A,p.Glu604* | De novo | AD | White-Sutton syndrome |
| 27 | M | *RAI1* | ht | NM_030665.3:c.2966_2969del,Chr17(GRCh37):g.17699228_17699231del, p.Lys989Serfs*74 | De novo | AD | Smith-Magenis syndrome |
| 28 | M | *SATB2* | ht | | De novo | AD | Glass syndrome |

(*Continues*)

**TABLE 1** (Continued)

| Patient # | Sex | Mutated gene | Status | Variants | Inheritance | Variant type | OMIM/ClinVar phenotype |
|---|---|---|---|---|---|---|---|
| | | | | NM_001172509.1:c.1627del, Chr2(GRCh37): g.200173596del, p. Arg543Alafs*3 | | | |
| 29 | M | *SCN1A* | ht | NM_001165963.1: c.5726C>T,Chr2(GRCh37): g.166848059G>A, p. Thr1909Ile | De novo | AD | Dravet syndrome |
| 30 | F | *SCN2A* | ht | NM_021007.2:c.2558G>A, Chr2(GRCh37): g.166198975G>A, p. Arg853Gln | De novo | AD | Epileptic encephalopathy, early infantile, 11 |
| 31 | M | *SCN8A* | ht | NM_014191.3:c.4394A>T, Chr12(GRCh37): g.52183177A>T, p. Asp1465Val | De novo, possible paternal mosaicism (3/251 reads in blood) | AD | Epileptic encephalopathy, early infantile, 13 |
| 32 | M | *SYNGAP1* | ht | NM_006772.2:c.490C>T, Chr6(GRCh37): g.33400564C>T, p. Arg164* | De novo | AD | Mental retardation, autosomal dominant 5 |
| 33 | M | *SYNGAP1* | ht | NM_006772.2:c.3190C>T, Chr6(GRCh37): g.33411519C>T, p. Gln1064* | De novo | AD | Mental retardation, autosomal dominant 5 |
| 34 | M | *TCF4* | ht | NM_001243226.2: c.2039G>A,Chr18 (GRCh37):g.52896224C>T, p.Arg680His | De novo | AD | Pitt-Hopkins syndrome |
| 35 | M | *TCF4* | ht | NM_001243226.2: c.2263_2264del,Chr18 (GRCh37): g.52895514_52895515del, p.Ser755Leufs*57 | De novo | AD | Pitt-Hopkins syndrome |
| 36 | M | *UNC80* | cht | NM_032504.1:c.2399del, Chr2(GRCh37): g.210690698del, p. Leu800Trpfs*19, paternal inherited and NM_032504.1: c.4150G>T,Chr2(GRCh37): g.210752852G>T, p. Glu1384*, maternal inherited | Biparental | AR | Hypotonia, infantile, with psychomotor retardation and characteristic facies 2 |
| 37 | M | *UPF3B* | hi | NM_080632.2:c.846+1G>A, ChrX(GRCh37): g.118974608C>T, p.? | Maternally inherited | XL | Mental retardation, X-linked, syndromic 14 |
| 38 | M | *ZEB2* | ht | NM_014795.3:c.3170G>A, Chr2(GRCh37): g.145147493C>T, p. Cys1057Tyr | De novo | AD | Mowat-Wilson syndrome |
| 39 | M | *ZMYND11* | ht | NM_006624.5:c.76C>T, Chr10(GRCh37): g.226028C>T, p.Arg26Trp | De novo | AD | Mental retardation, autosomal dominant 30 |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; cht, compound heterozygous; F, female; hi, hemizygous; hm, homozygous; ht, heterozygous; M, male; ME, medical exome; NDD, neurodevelopmental disorder; XL, X-linked.

without definite clinical diagnoses. The corresponding disorders had not been suggested by physicians because characteristic features of the disease were absent or too mild to be noted, or because the syndrome was not recognized. As examples, patient #3 with *ANKRD11* variant had a mild KBG phenotype, patient #7 had a variant in *ATRX* but a non-syndromic phenotype, even after a reverse phenotyping, patient #10 had a variant in *FOXP1* but the corresponding syndrome was poorly known, patient #15 had a phenotype suggestive of Lesch-Nyhan disease but with a mildly (and overlooked) elevated uricemia, patient #25 had *PANK2* variants but a brain magnetic resonance imaging (MRI) that did not show the characteristic "eye-of-the-tiger" sign before the analysis. These clinical pitfalls were overcome using ME. The >30% of diagnostic yield in patients with SID and in those with NSID suggests that ME could be used as a second-line genetic test in patients without clinical diagnoses instead of sequential studies on smaller panels chosen on the basis of clinical signs. It is of note that

**TABLE 2** Likely pathogenic variants identified with ME in 17 patients with NDD and partial diagnoses made in 2

| Patient # | Sex | Mutated gene | Status | Variants | Inheritance | Variant type | OMIM/ClinVar phenotype |
|---|---|---|---|---|---|---|---|
| 40 | F | *ARID1B* | ht | NM_020732.3:c.5025+1G>A, Chr6(GRCh37): g.157525131G>A, p.? | De novo | AD | Coffin-Siris syndrome 1 |
| 41 | F | *ATP1A3* | ht | NM_152296.4:c.2224G>T, Chr19(GRCh37): g.42479820C>A, p. Asp742Tyr | De novo | AD | Cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensoryneural hearing loss |
| 42 | M | *ATP1A3* | ht | NM_001256214.1:c.499A>G, Chr19(GRCh37): g.42490279T>C, p. Met167Val | De novo | AD | CAPOS syndrome |
| 43 | F | *AP4S1* | hm | NM_007077.4:c.289C>T, Chr14(GRCh37): g.31542174C>T, p.Arg97* | Biparental transmission | AR | Spastic paraplegia 52, autosomal recessive |
| 44 | M | *CAMTA1* | ht | NM_015215.3:c.2863C>T, Chr1(GRCh37): g.7737742C>T, p. Arg955Trp | De novo | AD | Cerebellar ataxia, non-progressive, with mental retardation |
| 45 | F | *COG5* | cht | NM_006348.3:c.2324C>T, Chr7(GRCh37): g.106851608G>A, p. Pro775Leu, paternally inherited (class 4) and NM_006348.3: c.1508dup,Chr7(GRCh37): g.106924076dup, p. Gly505Trpfs*3 maternally inherited (class 5) | Biparental transmission | AR | Congenital disorder of glycosylation, type IIi |
| 46 | F | *FOXG1* | ht | NM_005249.4:c.545C>T, Chr14(GRCh37): g.29237030C>T, p. Pro182Leu | De novo | AD | Rett syndrome (congenital variant) |
| 47 | M | *PEX16* | hm | NM_057174.2:c.104T>G, Chr11(GRCh37): g.45939259A>C, p. Leu35Arg | Biparental inheritance | AR | Peroxysome biogenesis disorder 8B |
| 48 | F | *KIF11* | ht | NM_004523.3:c.862_871del, Chr10(GRCh37): g.94373206_94373215del, p.Ile288Profs*3 | De novo | AD | Microcephaly, lymphoedema, retinal dysplasia |
| 49 | M | *PHIP* | ht | NM_017934.5:c.3892C>T, Chr6(GRCh37): g.79664960G>A, p. Arg1298* | De novo | AD | Registered as a phenotype in ClinVar (syndromic mild ID) |
| 50 | F | *RORA* | ht | NM_134260.2:c.1118del, Chr15(GRCh37): g.60795790del, p. Arg373Profs*17 | De novo | AD | None |
| 51 | M | *SCN2A* | ht | NM_021007.2:c.4160_4161del,Chr2(GRCh37):g.166231382_166231383del, p.Lys1387Serfs*4 | De novo | AD | Early-infantile epileptic encephalopathy 11 |
| 52 | M | *SLC6A1* | ht | NM_003042.3:c.223G>A, Chr3(GRCh37): g.11059120G>A, p. Gly75Arg | De novo | AD | Myoclonic-atonic epilepsy |
| 53 | M | *SMARCA2* | Both ht | NM_001289396.1: c.3495G>C,Chr9(GRCh37): g.2115860G>C, p. Gln1165His and NM_001289396.1: c.3917G>A,Chr9(GRCh37): g.2123873G>A, p. Arg1306Lys | Both de novo | AD | Nicolaides-Baraitser syndrome |
| 54 | M | *SOX5* | ht | | De novo | AD | Lamb-Shaffer syndrome |

(*Continues*)

**TABLE 2** (Continued)

| Patient # | Sex | Mutated gene | Status | Variants | Inheritance | Variant type | OMIM/ClinVar phenotype |
|---|---|---|---|---|---|---|---|
| | | | | NM_006940.4:c.1895C>A, Chr12(GRCh37): g.23689480G>T, p. Thr632Asn | | | |
| 55 | F | *STXBP1* | ht | NM_001032221.3: c.1706C>T,Chr9(GRCh37): g.130453057C>T, p. Ser569Phe | De novo | AD | Early-infantile epileptic encephalopathy 4 |
| 56 | F | *STXBP1* | ht | NM_003165.3:c.1082C>T, Chr9(GRCh37): g.130435512C>T, p. Thr361Ile | De novo | AD | Early-infantile epileptic encephalopathy 4 |
| Partial diagnoses | | | | | | | |
| 57 | M | *KAL1* | hi | NM_000216.2:c.422G>A, ChrX(GRCh37): g.8565194C>T, p. Ser141Asn | Maternally inherited | XL | Hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1) |
| 58 | M | *PRODH* | cht | NM_016335.4:c.1397C>T, Chr22(GRCh37): g.18905859G>A, p. Thr466Met,rs2870984 | *PRODH* gene variant paternally inherited with *PRODH* deletion maternally inherited | AR | Hyperprolinemia, type I |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; cht, compound heterozygous; F, female; hi, hemizygous; hm, homozygous; ht, heterozygous; M, male; ME, medical exome; NDD, neurodevelopmental disorder; XL, X-linked.

the secondary evaluation of many patients of our series allowed a retrospective clinical (or radiological) validation of the suspected variant based on subtle, most of the time overlooked, clinical signs. This pleads for a close dialogue between molecular and clinical geneticists, as anticipated by Hennekam and Biesecker.[20]

We identified variants in genes associated with phenotypes different from those of our patients. Variants in *SCN2A* (MIM 182390) and *STXBP1* (MIM 602926) are responsible for epileptic encephalopathies.[16,21] Patient #30 with a de novo *SCN2A* variant had an unusual phenotype associating ASD and mild ID without seizures. We considered this variant as the cause of the NDD because rare patients with ASD had been previously reported.[22] The largest series of patients with *SCN2A* variants published thereafter (including ours) demonstrated that 15% of them had no epilepsy but ID with or without ASD. The de novo missense variant in *STXBP1* found in patient #55 is of interest because: (1) it affects only 1 of the 2 isoforms of the protein, while patients with *STXBP1*-related phenotypes usually have variants affecting both isoforms, (2) patient #55 had no epilepsy, ataxia or tremor, which are usually observed in patients with *STXBP1* mutations.[21,23] We finally classified this variant as likely pathogenic because (1) it met biological criteria for class 4 variants, (2) an increasing number of *STXBP1* variants are identified in patients without seizures (7% in the largest series published so far[21]), (3) 1 reported patient with epileptic encephalopathy had a variant similarly affecting 1 isoform only.[24]

## 4.2 | Unexpected variants found in genes not associated with human diseases

The TruSight One gene panel contains not only genes known for human diseases but also some genes considered as good candidates for human diseases (at the time of its design), including *UNC80*, *PHIP* and *RORA*.

We identified biallelic truncating mutations in *UNC80* in patient #36 with global developmental delay, microcephaly, marked hypotonia and chorea/dystonia. The clinical significance of this result remained briefly uncertain until the publication of an article reporting biallelic variants in *UNC80* in patients with a similar phenotype.[25] Likewise, the de novo heterozygous truncating variant identified in *PHIP* in patient #49 with syndromic ID became the likely cause of his NDD after the publication of 2 other patients.[26]

We found a de novo heterozygous truncating variant in *RORA* in patient #50 with ataxia, epilepsy and severe ID. *RORA* encodes the retinoic-acid orphan receptor alpha expressed in the brain[27] and is intolerant to loss-of-function variants (pLi 0.95 in ExAC http://exac.broadinstitute.org). Most patients with heterozygous deletions of this gene have ID and epilepsy.[28] However, no deleterious point variant in *RORA* has been reported to date. Available data suggest that the heterozygous truncating variant found in patient #50 may be the cause of her NDD. Thus, the TruSight One panel may provide excellent candidate variants even for a few "candidate" genes.

## 4.3 | Diagnosis rate

We obtained a global diagnostic yield of 25.9%. The diagnostic yield of WES for all types of genetic diseases, most of which are developmental disorders, is 25% to 32% when index cases only are studied[4,5] and raises to 30% to 38.5% with trios,[7,29] mainly because variants are discovered in "new" genes. Thus, our results are lower but close to those obtained with WES performed with index cases only. However, the diagnosis rate is obviously limited with ME because new genes involved in NDD are regularly identified.

We identified a pathogenic mutation in 4 of 7 patients with NDEG but this number is too small to discuss the efficiency of ME in this clinical context. The 32% of diagnoses in patients with ID vs 3.7% in those with ASD is related to our classification of NDD. Some patients meeting the ADI criteria for ASD were classified in the SID and NSID groups because of early developmental delay suggesting that ASD was a manifestation of their NDD rather than the NDD itself. As examples, this was the case for patient #2 with a variant in *ADNP* and for patient #32 with a variant in *SYNGAP1*. Patients with ASD in our study roughly corresponds to the "essential" ASD group defined in a previous article[22] in which trio-based WES revealed a pathogenic variant in 2 of 64 patients (3.1%). This result is close to ours and suggests that careful selection of patients with NDD may help defining subgroups of patients with a higher probability of achieving a molecular diagnosis.

### 4.4 | Proposition of a rational use of ME in a context of economic constraints

Despite the diagnostic efficiency of WES for patients with NDD, some genetic centers cannot use it in their daily practice because of its high cost. The TruSight One gene panel covers 12 Mb of the genome while WES kits cover about 60 Mb. When studying trios with the ME, 36 Mb are sequenced, which is 60% of the coverage for 1 WES. Given that the cost per base is set with a given sequencing kit and that the cost of library preparations is comparable between kits, ME in trios (for 1 patient) represents a 40% saving on sequencing reagents compared with WES in index cases at constant depth.

The diagnostic yield is markedly increased by the sequencing strategy of trios vs index cases with WES.[29] The aim of our study was not to compare trios vs index cases (or duos) with ME. As demonstrated by the previous series of ME,[8] the "index case" strategy may reveal pathogenic variants when specific genes are suspected. Because of the low rate of diagnoses obtained with index cases (or duos) in our first sequencing series of patients without clinical diagnosis, we decided to use trio-based sequencing when possible. Although the cost of sequencing is 3 times higher than with index cases, this strategy greatly facilitates the downstream analyses and variant interpretation by allowing to detect de novo and compound heterozygous variants and reduces the cost of Sanger cosegregation analysis.

We conclude that for centers that do not use WES for routine diagnoses, trio-based ME may be considered as a useful alternative strategy to investigate NDD. The rate of diagnoses can be further improved by selecting patients with the highest likelihood of achieving a molecular diagnosis, that is, those with ID or NDEG.

## ACKNOWLEDGEMENTS

This work was funded by Assistance Publique des Hôpitaux de Paris (APHP), by a grant from the Rennes University Hospital (AAP innovations médicales, 2014) and la Fondation Groupama. C.D. and C.N. are supported as members of the Bio-Psy Labex. We would like to thank the families for their participation in the study. We are grateful to Artem Kim for carefully reading this manuscript. We also thank our technicians in La Pitié-Salpêtrière (Corinne Mach, Valérie Olin, Claude Estrade, Aurélie Lafitte, Sabina Karagic, Elodie Lejeune) and in Rennes (Sophie Marzelière, Valérie Perrault) for their contribution to this work. The authors declare they have no competing financial interests in relation to the work described.

## Ethical statement

The study was carried out in accordance with the policies of La Pitié-Salpêtrière and Rennes University Hospital.

## Conflict of interest

The authors declare no conflict of interest.

## REFERENCES

1. Vissers LELM, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet*. 2016;17:9-18.
2. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*. 2010;86:749-764.
3. Redin C, Gérard B, Lauer J, et al. Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing. *J Med Genet*. 2014;51:724-736.
4. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of Mendelian disorders. *N Engl J Med*. 2013;369:1502-1511.
5. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*. 2014;312:1870-1879.
6. Thevenon J, Duffourd Y, Masurel-Paulet A, et al. Diagnostic odyssey in severe neurodevelopmental disorders: towards clinical whole-exome sequencing as a first-line diagnostic test. *Clin Genet*. 2016;89(6):700-707. https://doi.org/10.1111/cge.12732.
7. Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. *Nature*. 2015;519:223-228.
8. Pajusalu S, Kahre T, Roomere H, et al. Large gene panel sequencing in clinical diagnostics – results from 501 consecutive cases. *Clin Genet*. 2017;[Epub ahead of print]. https://doi.org/10.1111/cge.13031.
9. Okazaki T, Murata M, Kai M, et al. Clinical diagnosis of Mendelian disorders using a comprehensive gene-targeted panel test for next-generation sequencing. *Yonago Acta Med*. 2016;59:118-125.
10. Goldenberg A, Riccardi F, Tessier A, et al. Clinical and molecular findings in 39 patients with KBG syndrome caused by deletion or mutation of ANKRD11. *Am J Med Genet A*. 2016;170:2847-2859.
11. Ruaud L, Mignot C, Guët A, et al. DYRK1A mutations in two unrelated patients. *Eur J Med Genet*. 2015;58:168-174.
12. Platzer K, Yuan H, Schütz H, et al. GRIN2B encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects. *J Med Genet*. 2017;54(7):460-470. https://doi.org/10.1136/jmedgenet-2016-104509.
13. Depienne C, Nava C, Keren B, et al. Genetic and phenotypic dissection of 1q43q44 microdeletion syndrome and neurodevelopmental phenotypes associated with mutations in ZBTB18 and HNRNPU. *Hum Genet*. 2017;136:463-479.
14. Saunier C, Støve SI, Popp B, et al. Expanding the phenotype associated with NAA10-related N-terminal acetylation deficiency. *Hum Mutat*. 2016;37:755-764.
15. Stessman HAF, Willemsen MH, Fenckova M, et al. Disruption of POGZ is associated with intellectual disability and autism spectrum disorders. *Am J Hum Genet*. 2016;98:541-552.
16. Wolff M, Johannesen KM, Hedrich UBS, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain*. 2017;[Epub ahead of print]. https://doi.org/10.1093/brain/awx054.

17. Richards CS, Bale S, Bellissimo DB, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. *Genet Med*. 2008;10:294-300.
18. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405-424.
19. Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *Am J Hum Genet*. 2017;100:267-280.
20. Hennekam RCM, Biesecker LG. Next-generation sequencing demands next-generation phenotyping. *Hum Mutat*. 2012;33:884-886.
21. Stamberger H, Nikanorova M, Willemsen MH, et al. STXBP1 encephalopathy: a neurodevelopmental disorder including epilepsy. *Neurology*. 2016;86:954-962.
22. Tammimies K, Marshall CR, Walker S, et al. Molecular diagnostic yield of chromosomal microarray analysis and whole-exome sequencing in children with autism spectrum disorder. *JAMA*. 2015;314:895-903.
23. Mignot C, Moutard ML, Trouillard O, et al. STXBP1-related encephalopathy presenting as infantile spasms and generalized tremor in three patients. *Epilepsia*. 2011;52:1820-1827.
24. Milh M, Villeneuve N, Chouchane M, et al. Epileptic and non-epileptic features in patients with early onset epileptic encephalopathy and STXBP1 mutations. *Epilepsia*. 2011;52:1828-1834.
25. Shamseldin HE, Faqeih E, Alasmari A, Zaki MS, Gleeson JG, Alkuraya FS. Mutations in UNC80, encoding part of the UNC79-UNC80-NALCN channel complex, cause autosomal-recessive severe infantile encephalopathy. *Am J Hum Genet*. 2016;98:210-215.
26. Webster E, Cho MT, Alexander N, et al. De novo PHIP-predicted deleterious variants are associated with developmental delay, intellectual disability, obesity, and dysmorphic features. *Cold Spring Harb Mol Case Stud*. 2016;2:a001172.
27. Cook DN, Kang HS, Jetten AM. Retinoic acid-related orphan receptors (RORs): regulatory functions in immunity, development, circadian rhythm, and metabolism. *Nucl Receptor Res*. 2015;2:101185. https://doi.org/10.11131/2015/101185.
28. Yamamoto T, Mencarelli MA, Di Marco C, et al. Overlapping microdeletions involving 15q22.2 narrow the critical region for intellectual disability to NARG2 and RORA. *Eur J Med Genet*. 2014;57:163-168.
29. Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. *Genet Med*. 2015;17:578-586.


## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Chérot E, Keren B, Dubourg C, et al. Using medical exome sequencing to identify the causes of neurodevelopmental disorders: Experience of 2 clinical units and 216 patients. *Clin Genet*. 2017;1–10. https://doi.org/10.1111/cge.13102