Using medical exome sequencing to identify the causes of neurodevelopmental disorders: experience of two clinical units and 216 patients

Running title: Medical exome and NDD

Elouan Chérot[1,2], Boris Keren[3,5,11], Christèle Dubourg[2,6], Wilfrid Carré[2], Mélanie Fradin[1], Alinoë Lavillaureix[3], Alexandra Afenjar[4,7], Lydie Burglen[7], Sandra Whalen[7], Perrine Charles[3,4,5], Isabelle Marey[3,4,5], Solveig Heide[3,4,5], Aurélia Jacquette[3,4,5], Delphine Heron[3,4,5], Diane Doummar[9,10,11], Diana Rodriguez[8,9,10,11], Thierry Billette de Villemeur[9,11], Marie-Laure Moutard[9,11], Agnès Guët[12], Jean Xavier[13,14], Didier Périsse[13], David Cohen[13,14], Florence Demurger[1], Chloé Quélin[1], Christel Depienne,[3,15] Sylvie Odent[1,6], Caroline Nava[3,15], Véronique David[2,6], Laurent Pasquier[1,16*], Cyril Mignot[3,4,5*]

1 Service de Génétique Médicale, CHU Hôpital Sud, CLAD Ouest, 35203 Rennes, France
2 Laboratoire de Génétique moléculaire et Génomique, CHU Pontchaillou, Rennes, France
3 AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, 75013, Paris, France
4 Centre de Référence Déficiences Intellectuelles de Causes Rares, Paris, France
5 Groupe de Recherche Clinique (GRC) "déficience intellectuelle et autisme" UPMC, 75013 Paris, France
6 CNRS UMR 6290 (IGDR), Université de Rennes 1, France.
7 APHP, GHUEP, Hôpital Armand Trousseau, Centre de Référence 'Malformations et maladies congénitales du cervelet', Paris, France
8 APHP, GHUEP, Hôpital Armand-Trousseau, Département de Génétique, 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 Groupe de Recherche Clinique (GRC) "ConCer-LD" UPMC, Paris, France
12 AP-HP, Service de Pédiatrie, Hôpital Louis Mourier, Colombes, France
13 AP-HP, Department of Child and Adolescent Psychiatry, Groupe Hospitalier Pitié-Salpêtrière et University Pierre and Marie Curie, Paris, France
14 Sorbonne Universités, UPMC, CNRS UMR 7222, Institut des Systèmes Intelligents et Robotiques, France
15 INSERM U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France
16 INSERM CIC pédiatrique 1414, CHU Pontchaillou, 35000 Rennes, France

* These two authors contributed equally to this work

Corresponding author:
Tel.: +33 2 99 26 67 44; fax: +33 2 99 26 67 45; E-mail address: laurent.pasquier@chu-rennes.fr

elouan.cherot@chu-rennes.fr; cyril.mignot@aphp.fr; melanie.fradin@chu-rennes.fr; boris.keren@aphp.fr; delphine.heron@aphp.fr; florence.demurger@chu-rennes.fr; christele.dubourg@chu-rennes.fr; caroline.nava@aphp.fr; wilfrid.carre@chu-rennes.fr; chloe.quelin@chu-rennes.fr; veronique.david@chu-rennes.fr; sylvie.odent@chu-rennes.fr; depiennc@igbmc.fr; alinoe.lavillaureix@aphp.fr; aurelia.jacquette@aphp.fr; alexandra.afenjar@aphp.fr; lydie.burglen@aphp.fr; sandra.whalen@aphp.fr; solveig.heide@aphp.fr; isabelle.marey@aphp.fr; perrine.charles@aphp.fr; jean.xavier@aphp.fr; didier.perisse@aphp.fr; david.cohen@aphp.fr; diane.doummar@aphp.fr; diana.rodriguez@aphp.fr; thierry.billette@aphp.fr; marielaure.moutard@aphp.fr; agnes.guet@aphp.fr; laurent.pasquier@chu-rennes.fr

Conflict of Interest: The authors declare no conflict of interest

ABSTRACT

Though whole exome sequencing is the gold standard for the diagnosis of neurodevelopmental disorders, it remains expensive for some genetic centers. Commercialized panels comprising all OMIM-referenced genes called “medical exome” constitute an alternative strategy to whole exome sequencing, but its efficiency is poorly known. In this study, we report the experience of two clinical genetic centers using medical exome for diagnosis of neurodevelopmental disorders.

We recruited 216 consecutive index patients with neurodevelopmental disorders in two French genetic centers, corresponded to the daily practice of the units and included non-syndromic intellectual disability (n=33), syndromic intellectual disability (n=122), pediatric neurodegenerative disorders (n=7) and autism spectrum disorder (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 higher in patients with intellectual disability as the main diagnosis (32%) than in patients with autism spectrum disorder (3.7%). Our results suggest that the use of medical exome is a valuable strategy for patients with intellectual disability when whole exome sequencing cannot be used as a routine diagnosis tool.

Keywords: medical exome, molecular strategy, intellectual disability, autism

INTRODUCTION

Intellectual disability (ID) is the most frequent neurodevelopmental disorder (NDD) affecting about 1-3% 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'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 two French genetic centers using the TruSight One sequencing panel, which targets genes associated with known phenotypes, for the diagnosis of NDD in 216 patients.

MATERIAL AND METHODS

Patients

We studied 216 index patients with a presumed genetic NDD but without molecular etiology recruited consecutively in the daily practice of two 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 four categories: 1) patients with non-syndromic ID (NSID), includes those with normal growth parameters and without dysmorphic features/malformations, neuromotor involvement (pyramidal, extrapyramidal, cerebellar syndrome) and sensory organ involvement, 2) patients with syndromic ID (SID), 3) patients with pediatric neurodegenerative disorders (NDEG), 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 12-18 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 two standard deviations (SD) 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], #51[16].

Sequencing technologies

All samples were prepared with the Illumina TruSight One preparation kit (which covers 4,813 genes associated with known phenotypes and 11,884,205 base pairs) and sequenced on either an Illumina MiSeq or NextSeq 500 sequencer using 2x150 bp sequencing kits. We performed ME sequencing in 196 parent-offspring trios (90.7%), 10 duos (proband + one parent; 4.6%) and 10 singletons (4.6%), including the three patients with a definite clinical diagnosis.

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 20x 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].

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 criteria[20–22], which classify variants according to 5 categories (class 1: benign, class 2: likely benign, class 3: uncertain significance, class 4: likely pathogenic, class 5: pathogenic). All variants of interest were discussed with clinicians and all molecular diagnoses were validated by referring clinicians.

RESULTS

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

Mode of inheritance and types of variants

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

We identified the molecular causes of NDD in 5/20 patients studied as index cases or in duos, including the three patients with a definite clinical diagnosis. Thus, we identified a pathogenic variant for 2/17 (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). Variant types include 33 variants leading to a premature termination codon (nonsense, frameshift, 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 two variants of patient #53), autosomal recessive disorders 7/7 (including two 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-Baraister 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.

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), 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).

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.

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, six 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 53/56 patients 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 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 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[23].

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[16,24]. 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[17]. 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: i) it affects only one of the two isoforms of the protein, while patients with *STXBP1*-related phenotypes usually have variants affecting both isoforms, ii) patient #55 had no epilepsy, ataxia or tremor, which are usually observed in patients with *STXBP1* mutations[24,25]. We finally classified this variant as likely pathogenic because i) it met biological criteria for class 4 variants, ii) an increasing number of *STXBP1* variants are identified in patients without seizures (7% in the largest series published so far[24]), iii) one reported patient with epileptic encephalopathy had a variant similarly affecting one isoform only[26].

Unexpected variants found in genes not associated with human diseases

The TruSight One gene panel contains 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[27]. 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 two other patients[28].

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[29] 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[30]. 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.

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 25-32% when index cases only are studied[4,5] and raises to 30-38.5% with trios[7,19], 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 4/7 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 *versus* 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[17] in which trio-based WES revealed a pathogenic variant in 2/64 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.

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 one 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 one patient) represents a 40% saving on sequencing reagents compared to WES in index cases at constant depth.

The diagnostic yield is markedly increased by the sequencing strategy of trios *versus* index cases with WES[19]. The aim of our study was not to compare trios *versus* 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. Though the cost of sequencing is three 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 analyzes.

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, i.e. those with ID or NDEG.

ACKNOWLEDGMENTS

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; Christel Depienne and Caroline Nava 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.

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. doi: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. doi: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. doi: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 J Neurol* 2017. doi:10.1093/brain/awx054.

17 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.

18 Grozeva D, Carss K, Spasic-Boskovic O *et al.* Targeted Next-Generation Sequencing Analysis of 1,000 Individuals with Intellectual Disability. *Hum Mutat* 2015; **36**: 1197–1204.

19 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 Off J Am Coll Med Genet* 2015; **17**: 578–586.

20 Richards CS, Bale S, Bellissimo DB *et al.* ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet Med Off J Am Coll Med Genet* 2008; **10**: 294–300.

21 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 Off J Am Coll Med Genet* 2015; **17**: 405–424.

22 Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *Am J Hum Genet* 2017; **100**: 267–280.

23 Hennekam RCM, Biesecker LG. Next-generation sequencing demands next-generation phenotyping. *Hum Mutat* 2012; **33**: 884–886.

24 Stamberger H, Nikanorova M, Willemsen MH *et al.* STXBP1 encephalopathy: A neurodevelopmental disorder including epilepsy. *Neurology* 2016; **86**: 954–962.

25 Mignot C, Moutard ML, Trouillard O *et al.*STXBP1-related encephalopathy presenting as infantile spasms and generelized tremor in three patients. *Epilepsia* 2011; **52**: 1820–1827.

26 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.

27 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.

28 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.

29 Cook DN, Kang HS, Jetten AM. Retinoic Acid-Related Orphan Receptors (RORs): Regulatory Functions in Immunity, Development, Circadian Rhythm, and Metabolism. *Nucl Recept Res* 2015; **2**. doi:10.11131/2015/101185.

30 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.

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_89346164insT, p.Pro2263Serfs*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):g.53227706G>A, p.Arg828*	maternally inherited	XL	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	NM_001172509.1:c.1627del,Chr2(GRCh37):g.200173596del, p.Arg543Alafs*3	*de novo*	AD	Glass syndrome
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

F : female, M : male, ht : heterozygous, hi : hemizygous, hm : homozygous, cht : compound heterozygous, AD : autosomal dominant, AR : autosomal recessive, XL : X-linked

Table 2. Likely pathogenic variants identified with ME in 17 patients with NDD and partial diagnoses made in two.

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, nonprogressive, 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	NM_006940.4:c.1895C>A,Chr12(GRCh37):g.23689480G>T, p.Thr632Asn	*de novo*	AD	Lamb-Shaffer syndrome
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

F : female, M : male, ht : heterozygous, hi : hemizygous, hm : homozygous, cht : compound heterozygous, AD : autosomal dominant, AR : autosomal recessive, XL : X-linked