



ORIGINAL ARTICLE

Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing

Claire Redin,^{1,2} Bénédicte Gérard,³ Julia Lauer,³ Yvan Herenger,³ Jean Muller,^{1,3} Angélique Quartier,^{1,2} Alice Masurel-Paulet,⁴ Marjolaine Willems,⁵ Gaétan Lesca,⁶ Salima El-Chehadeh,⁴ Stéphanie Le Gras,⁷ Serge Vicaire,⁷ Muriel Philipps,⁷ Michaël Dumas,⁷ Véronique Geoffroy,⁸ Claire Feger,⁷ Nicolas Haumesser,^{1,2} Yves Alembik,⁹ Magalie Barth,¹⁰ Dominique Bonneau,¹⁰ Estelle Colin,¹⁰ Hélène Dollfus,¹¹ Bérénice Doray,⁹ Marie-Ange Delrue,¹² Valérie Drouin-Garraud,¹³ Elisabeth Flori,⁹ Mélanie Fradin,¹⁴ Christine Francannet,¹⁵ Alice Goldenberg,¹³ Serge Lumbroso,¹⁶ Michèle Mathieu-Dramard,¹⁷ Dominique Martin-Coignard,¹⁸ Didier Lacombe,¹² Gilles Morin,¹⁷ Anne Polge,¹⁶ Sylvie Sukno,¹⁹ Christel Thauvin-Robinet,⁴ Julien Thevenon,⁴ Martine Doco-Fenzy,²⁰ David Genevieve,⁵ Pierre Sarda,⁵ Patrick Edery,⁶ Bertrand Isidor,²¹ Bernard Jost,⁷ Laurence Olivier-Faivre,⁴ Jean-Louis Mandel,^{1,2,3} Amélie Piton^{1,2}

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/jmedgenet-2014-102554>).

For numbered affiliations see end of article.

Correspondence to

Amélie Piton, Translational Medicine & Neurogenetic, 67 400 Illkirch, France; amelie.piton@igbmc.fr
Jean-Louis Mandel, Translational Medecine & Neurogenetic, 67 400 Illkirch, France; jlmandel@igbmc.fr

Received 1 June 2014

Revised 31 July 2014

Accepted 3 August 2014

Published Online First

28 August 2014



CrossMark

To cite: Redin C, Gérard B, Lauer J, et al. *J Med Genet* 2014;51:724–736.

ABSTRACT

Background Intellectual disability (ID) is characterised by an extreme genetic heterogeneity. Several hundred genes have been associated to monogenic forms of ID, considerably complicating molecular diagnostics. Trio-exome sequencing was recently proposed as a diagnostic approach, yet remains costly for a general implementation.

Methods We report the alternative strategy of targeted high-throughput sequencing of 217 genes in which mutations had been reported in patients with ID or autism as the major clinical concern. We analysed 106 patients with ID of unknown aetiology following array-CGH analysis and other genetic investigations. Ninety per cent of these patients were males, and 75% sporadic cases.

Results We identified 26 causative mutations: 16 in X-linked genes (*ATRX*, *CUL4B*, *DMD*, *FMR1*, *HCFC1*, *IL1RAPL1*, *IQSEC2*, *KDM5C*, *MAOA*, *MECP2*, *SLC9A6*, *SLC16A2*, *PHF8*) and 10 de novo in autosomal-dominant genes (*DYRK1A*, *GRIN1*, *MED13L*, *TCF4*, *RAI1*, *SHANK3*, *SLC2A1*, *SYNGAP1*). We also detected four possibly causative mutations (eg, in *NLGN3*) requiring further investigations. We present detailed reasoning for assigning causality for each mutation, and associated patients' clinical information. Some genes were hit more than once in our cohort, suggesting they correspond to more frequent ID-associated conditions (*KDM5C*, *MECP2*, *DYRK1A*, *TCF4*). We highlight some unexpected genotype to phenotype correlations, with causative mutations being identified in genes associated to defined syndromes in patients deviating from the classic phenotype (*DMD*, *TCF4*, *MECP2*). We also bring additional supportive (*HCFC1*, *MED13L*) or unsupportive (*SHROOM4*, *SRPX2*) evidences for the implication of previous candidate genes or mutations in cognitive disorders.

Conclusions With a diagnostic yield of 25% targeted sequencing appears relevant as a first intention test for the diagnosis of ID, but importantly will also contribute to a better understanding regarding the specific contribution of the many genes implicated in ID and autism.

INTRODUCTION

Intellectual disability (ID) is a common neurodevelopmental disorder reported in 1.5–2% of children and adolescents.^{1,2} ID is defined by significant limitations in both intellectual functioning and adaptive behaviour with onset before the age of 18. Different classes of ID are conventionally defined upon IQ values (severe or profound, <35; moderate, 35–49 and mild, 50–70). However, in routine genetic practice, clinical assessment mainly based on records of developmental history, speech acquisition and patients' autonomy is used for classification in such subcategories.

Causes of ID can be environmental, genetic or multifactorial. Single genetic events are thought to account for a majority of cases, varying from large chromosomal anomalies or copy number variants (CNVs) affecting several genes to point mutations in single genes. These latter monogenic forms are characterised by an extreme genetic heterogeneity, with a hundred genes described as implicated in X-linked ID (XLID), and more associated to autosomal-recessive or autosomal-dominant forms. Altogether there are more than 500 genes proposed to cause ID with high penetrance when mutated^{3–9} underlying a phenotypic heterogeneity of the same extent in both severity and associated symptoms. This genetic heterogeneity has long limited the diagnostic offer for patients and families, which

was often restricted to fragile-X (MIM 300624) testing, array-CGH (comparative genomic hybridization) analysis and generic metabolic tests (see online supplementary figure S1). It may be complemented by sequencing a few genes associated to a specific syndrome evoked by patients' phenotype, yet the diagnostic yield remains low (1–2% for the recurrent fragile-X mutation; 10–15% for array-CGH and chromosomal analyses, higher in highly syndromic patients).^{10–12} A majority of patients remain therefore without molecular diagnosis, while it is of crucial importance for establishing recurrence risks and providing genetic counselling in the family. Moreover, such diagnosis often has direct consequences for the medical prognosis of patients or their optimised healthcare, and even (yet in still a minority of cases) can indicate specific therapeutic options.

To obviate this low diagnostic yield, we developed the simultaneous targeted sequencing of protein-coding exons of 217 genes associated with ID or autism spectrum disorders (ASDs) as primary clinically significant feature: 99 located on the X-chromosome, 118 on the autosomes. We report here the results of such strategy on a cohort of 106 ID patients with or without associated autistic-like features, negative for array-CGH, fragile-X and other specific genetic analyses. A causal mutation was detected in 25% of these patients, regardless the severity of their cognitive impairment. We illustrate cases in which the molecular diagnosis was immediately established, as well as other more complex situations. This highlights the challenge of interpreting variants generated by NGS technologies, already from targeted approaches restricted to a few hundred genes. This work demonstrates that a targeted sequencing approach is highly efficient for the diagnosis of ID, but also allows refining the clinical spectrum associated with mutations in certain genes, and confirming or questioning the involvement of other genes in cognitive disorders.

METHODS

Cohort of patients

DNA samples from 106 patients were addressed for testing through clinical geneticists from 16 public hospitals in France. Inclusion criteria for the patients were to be negative for the recurrent fragile-X mutation and for pathogenic CNVs via array-CGH testing, and availability of DNA samples from family members in order to conclude upon molecular findings. Additional specific genetic investigations had been performed on a majority of patients (on average: two genes tested per patient, **table 1**). Presence of multiple major congenital anomalies or suspected mitochondrial/peroxisomal disorders was an exclusion criterion. Clinical data were recorded following a standardised clinical record highlighting prenatal history, developmental history, neurological and behavioural disorders. ID severity was assessed by medical geneticists upon clinical evaluation and was not a discriminating inclusion criterion, although we encouraged inclusion of probands with moderate or severe ID. This study was approved by the local Ethics Committee of the Strasbourg University Hospital (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale (CCPPRB)). For all patients, a written informed consent for genetic testing was obtained from their legal representative.

Targeted genes and capture design

The 217 selected genes include 99 genes associated to XLID and 118 genes located on autosomes, implicated in dominant (45), recessive (66) or complex (7) forms of ID (see online supplementary table S8, further justification on gene selection is given in online supplementary methods). We targeted all

protein-coding exons of these genes, including 20 bp of intronic flanking sequences. The overall size of targeted regions is 1.034 Mbp. Corresponding 120 bp RNA baits were designed using SureDesign (<https://earray.chem.agilent.com/suredesign/>).

Library preparation and sequencing

DNA samples were extracted from peripheral blood or saliva. Sequencing libraries were prepared as described previously,¹³ performing individual in-solution SureSelect capture reaction for each DNA sample (Agilent, Santa Clara, California, USA). Paired-end sequencing (2×101-bp) was performed on an Illumina HiSeq 2000/2500, multiplexing up to 16 samples per sequencing lane.

Bioinformatic pipeline and variant ranking

Read mapping, variant calling and annotation was performed as described previously.¹³ Detected variants (short indels and single nucleotide variants (SNVs)) were ranked by VaRank (an in-house developed script), which incorporates the annotations retrieved by alamut-HT (putative effect on the protein, conservation scores, splice site predictions, allelic frequency in the 106 patients and in control cohorts such as Exome Variant Server (EVS) or 1000 genomes). Candidate variants were selected when harbouring a frequency compatible with the incidence of the disease: expectedly accounting for less than 0.1% of all ID cases, aka resulting in a disease frequency <0.002%.¹⁴ Using the EVS population as a subset of the general population, candidate variants were thus retrieved when reported in EVS: with a minor allele frequency lower than 0.45% for variants in autosomal-recessive genes (ie, with a frequency of homozygotes <0.002%), in no more than one carrier for variants in autosomal-dominant genes or in no more than one male for variants in X-linked genes (as we cannot exclude, although it is unlikely, that a particular carrier from the general population might have mild ID). We concomitantly excluded variants present more than twice in the cohort of 106 patients. Remaining variants predicted as potentially pathogenic and fitting with the mode of inheritance associated to the affected gene were further tested for validation (see online supplementary table S9).

CNVs detection pipeline

Putative heterozygous/homozygous/hemizygous structural variants or CNVs were highlighted using the previously described method based on a depth-of-coverage comparison between the index sample and eight other random samples from the same sequencing lane.¹³ For the X-chromosome, coverage was normalised according to the number of X-chromosomes of the patient.

Mutation validation

All candidate mutations were validated by Sanger sequencing and co-segregation analyses were performed as extensively as possible. Putative splicing mutations were confirmed either using a minigene *in vitro* assay with the SPL3B plasmid as described previously¹⁵ or using patients' fibroblasts or blood RNA when available. For apparent *de novo* variants, pedigree concordance was checked using polymorphic microsatellite markers (PowerPlex 16HS System, Promega). Mutations were considered as certainly causative when no doubt remained regarding their pathogenicity and an unambiguous diagnosis could thus be established. Such mutations co-segregated with the disease status in the family and were either truncating mutations or missense mutations that had been previously

Table 1 Description of the cohort of 106 patients with intellectual disability (ID) and global diagnostic results

	Cohort (n=106)	With conclusive genetic diagnosis (n=26)	Total	Yield (per category)
Gender				
Male	96 (91%)	14	8	22
Female	10 (9%)	2	2	4
<i>Total</i>	<i>106</i>	<i>16</i>	<i>10</i>	<i>26/106 (25%)</i>
Age				
[0–10]	57 (54%)	9	5	14
[10–20]	31 (29%)	5	4	9
>20	18 (17%)	2	1	3
Sporadic cases*				
Female	8 (7%)	2	2	4
Male	72 (68%)	7	7	14
<i>Total</i>	<i>80 (75%)</i>	<i>9</i>	<i>9</i>	<i>18/80 (23%)</i>
Familial history				
Male sib-pairs	12	5	0	5
Possible XLID†	8	2	1	3
Other (non-X-linked)	6	0	0	0
<i>Total</i>	<i>26 (25%)</i>	<i>7</i>	<i>1</i>	<i>8/26 (31%)</i>
Consanguinity	3 (3%)	0	0	0
ID severity				
Mild/borderline	12 (11%)	3	0	3
Moderate	49 (46%)	4	6	10
Severe	45 (42%)	9	4	13
Comorbidity				
Microcephaly (<−2 SD)	14 (13%)	3	2	5
Epilepsy	28 (26%)	5	2	6
Autistic traits	34 (32%)	7	2	9
Hypotonia	36 (34%)	4	4	8
Previous exploration				
CGH	106 (100%)			
Fragile-X	105 (99%)			
Karyotype	99 (96%)			
# Other genetic tests (mean per patient)	2			
MRI	53 (50%)			
Metabolism‡	82 (77%)			

*No familial first degree ID.

†Affected male relatives.

‡At least one biochemical test performed.

XLD, X-linked ID; in italic: total number per category.

convincingly published or that we confirmed with functional analyses. Mutations were considered as potentially causative when they appeared to co-segregate with ID in available members of the family and were predicted to be damaging, but further functional studies are needed to prove unambiguously their pathogenicity.

RESULTS

High-quality sequencing data ensure low rates of false-positive/negative calls for SNVs, indels and CNVs

Our strategy allowed generating a high-quality sequencing dataset, with a mean depth of coverage of 350× and an average per patient of 97.7% of targeted regions being well covered (>40×; see online supplementary table S1). Such coverage ensures a sensitivity of 99–99.9% of detecting SNVs and indels at any allelic state (Illumina Technical Note). We further assessed the sensitivity of SNV detection by comparing allelic states of SNPs detected by SNP-array (Affymetrix SNP Array 6.0) with the corresponding sites located in the targeted sequencing data of two patients and found no false-negatives after Sanger

sequencing validation of six SNPs showing discrepant allelic states between both methods (452 SNPs analysed). Interestingly, for these six SNPs, Sanger sequencing results were always in favour of targeted sequencing data suggesting a much higher accuracy (data not shown). No false positive was detected out of the 80 candidate variants located in well-covered regions that were tested for confirmation by Sanger sequencing.

This high-sequencing depth also ensured reliable CNVs calling. All CNVs detected by our pipeline were validated by Sanger sequencing, qPCR and/or confirmed retrospectively when looking at array-CGH data (see online supplementary table S2). Some were not initially mentioned in the array-CGH report because they were covered by only a few SNP probes (≤ 2 deleted probes, under the detection threshold).

Very few regions (a total of 3.9 kb, only 1.8 kb being protein coding) appear consistently poorly covered (coverage <40× in >90% of the samples; see online supplementary table S3). Those are mainly first exons or highly GC-rich regions that are a well-known burden in such capture strategy.

Cohort description and diagnostic yield

Patients harboured various degrees of cognitive impairment, although with a higher proportion of moderate or severe forms (46% and 42% respectively; **table 1**). The cohort was highly enriched in males. Among male probands, 68% were sporadic cases, the remaining had familial history of cognitive impairment mainly evocative of an X-linked mode of

transmission (**table 1**). We detected certainly causative mutations in 26/106 patients (**table 2**; see online supplementary figures S2–S19), leading to an overall diagnostic yield of 25% for the entire cohort (from 23% for sporadic cases to 31% for familial cases). Unexpectedly, the diagnostic yield appears unrelated to the severity of ID in patients (**table 1**).

Table 2 List of all causative/possibly causative mutations identified in our cohort

Patient ID	Sex	Gene	Mutation	Inheritance	Mode of inheritance	Degree of ID	Consistency with classic phenotype	See online supplementary figure #
Certainly-causative mutations								
APN-58	M	DYRK1A	chr21:g.38858865C>T; c.613C>T; p.Arg205*; htz	De novo	AD	++	Yes	S2
APN-87	M	DYRKA1	chr21:g.38858873del; c.621_624delinsGAA; p.Glu208Asnfs*3; htz	De novo	AD	++	Yes	S2
APN-63	M	GRIN1	chr9:g.140056661C>G; c.1733C>G; p.Pro578Arg; htz	De novo	AD	+++	Yes	S3
APN-14	M	MED13L	chr12:g.116406845_116406852del; c.6118_6125del; p.Gly2040Asnfs*32; htz	De novo	AD	++	Partially	S4
APN-46	M	RAI1	chr17:g.17698594_17698598del; c.2332_2336del; p.Gly778Glnfs*7; htz	De novo	AD	++	Partially	S5
APN-122	F	SHANK3	chr:g.51159168_51159183dup; c.2955_2970dup; p.Pro992Argfs*325; htz	De novo	AD	+++	Yes	S6
APN-38	M	SLC2A1	chr1:g.43395407G>A; c.724C>T; p.Gln242*; htz	De novo	AD	+++	Yes	S7
APN-139	M	SYNGAP1	chr6:g.33414346G>A; c.3583-6G>A; p.Val1195Alafs*27; htz; splice	De novo	AD	++	Yes	S8
APN-41	M	TCF4	chr18:g.53017622_53017625del; c.514_517del; p.Lys172Phefs*61; htz	De novo	AD	+++	Yes	S9
APN-117	F	TCF4	chr18:g.53017619G>A; c.520C>T; p.Arg174*; htz	De novo	AD	++	No	S9
APN-138	M	ATRX	chrX: g.76972632G>A; c.109C>T; p.Arg37* (rs122445108); hemz	Inherited (Ma)	XL	+++	Yes	S10
APN-137	M	CUL4B	chrX: g.119681009_119681010del; c.811_812del; p.Gln271Aspfs*11; hemz	Inherited (Ma)	XL	+++	Partially	S11
APN-42	M	DMD	chrX:g.31164440del; c.10889del; p.Arg3630Glnfs*27; hemz	Inherited (Ma)	XL	++	No	S12
APN-26	M	FMR1	Last exon deletion; hemz	Inherited (Ma)*	XL	+++	Partially	S13
APN-113	M	HCFC1; (ATRX)	chrX:g.153230153G>A; c.218C>T; p.Ala73Val; hemz; (chrX:g.76939735G>C, c.1013C>G, p.Ser338Cys; hemz)	Inherited (Ma) Inherited (Ma) Inherited (Ma)	XL; (XL)	+++	Yes (partially)	3
APN-82	M	IL1RAPL1	chrX:g.29935696_29935705del; c.894_903del; p.Trp299Thrfss*18; hemz	Inherited (Ma)	XL	++	Yes	S14
APN-68	M	IQSEC2	chrX:g.53268395G>A; c.3097C>T; p.Gln1033*; hemz	De novo	XL	+++	Yes	S15
APN-34	M	KDM5C	chrX:g.53228250C>G; c.2152G>C; p.Ala718Pro; hemz	De novo	XL	++	Partially	S16
APN-135	M	KDM5C	chrX:g.53240784dup; c.1296dup; p.Glu433*; hemz	Inherited (Ma)	XL	++	Partially	S16
APN-16	M	MAOA	chrX:g.43590942_43590943delinsTT; c.797_798delinsTT; p.Cys266Phe; hemz	Inherited (Ma)	XL	+/-	Yes	42
APN-130	F	MECP2	chrX: g.153296363G>A; c.916C>T; p.Arg306Cys (rs28935468); htz	De novo	XL	+++	Partially	S17
APN-142	F	MECP2	chrX:g.153296777G>A; c.502C>T; p.Arg168* (rs61748421); htz	De novo	XL	+++	Partially	S17
APN-3	M	MECP2	Complex rearrangement of exon 4; hemz	Inherited (Ma)	XL	+++	Yes	S17
APN-105	M	PHF8; (DOCK8)	chrX:g.54028583C>G; c.1249+5G>C; p.Tyr406Phefs*24; hemz; (chr9:g.407035G>T; c.3496G>T; p.Glu1166*; htz)	Inherited (Ma); (de novo)	XL; (AD)	+	Partially (No)	S18
APN-43	M	SLC9A6	chrX:g.135080258_135080262del; c.526_9_526_5del; p.??; splice disrupted; hemz	Inherited (Ma)	XL	+	Yes	Masurel-Paulet et al, in preparation
APN-110	M	SLC16A2	chrX:g.737490677>C; c.1412T>C ; p.Leu471Pro (rs122455132); hemz	Inherited (Ma)	XL	+++	Yes	S19

Continued

Table 2 Continued

Patient ID	Sex	Gene	Mutation	Inheritance	Mode of inheritance	Degree of ID	Consistency with classic phenotype	See online supplementary figure #
Possibly causative mutations								
APN-131	M	<i>SLC2A1</i> ; (<i>ANKRD11</i>)	chr1:g.43392779del; c.1412delG; p.Gly471Glufs*37; htz; (chr16:g.89348867G>T; c.4083C>A; p.His1361Gln; htz)	Inherited (Pa); Inherited (Ma)	AD; AD	+++	Partially	S7
APN-101	M	<i>TCF4</i>	chr18:g.52899907C>T; c.1487-5G>A; p.Arg495_Gly496insAla?; htz	De novo ? †	AD	++	No	S9
APN-99	M	<i>NLGN3</i>	chrX:g.70389249C>T; c.1849C>T; p.Arg617Trp; hemiz	Inherited (Ma)	XL	+++	Yes	S20
APN-70	M	<i>PQBP1</i>	chrX:g.48760294C>T; c.731C>T; p.Pro244Leu; hemiz	Inherited (Ma)	XL	++	No	S21

*Present in the three brothers. Mother untested, but most probably maternally inherited.

†Absent from the mother, deceased father (untested).

In bold: mutations previously reported in other patients.

-: no ID, +: mild ID, ++: moderate ID, +++: severe ID.

AD, autosomal dominant; F, female; hemiz, hemizygous; htz, heterozygous; ID, intellectual disability; M, male; Ma, maternally-inherited; MAOA, Monoamine Oxidase A enzyme; Pa, paternally inherited; XL, X-linked.

Sixteen mutations are located in genes of the X-chromosome: 14 point mutations or small indels (in *ATRX*, *CUL4B*, *DMD*, *HCFC1*, *IL1RAPL1*, *IQSEC2*, *KDM5Cx2*, *MECP2x2*, *MAOA*, *PHF8*, *SLC9A6*, *SLC16A2*), as well as two larger pathogenic events (one hemizygous complex rearrangement in *MECP2*, one hemizygous exon deletion in *FMR1*; see online supplementary figures S17 and S13). We identified 10 de novo point mutations or small indels in genes involved in autosomal-dominant/haploinsufficient forms of ID (in *DYRK1Ax2*, *GRIN1*, *MED13L*, *RAI1*, *SHANK3*, *SYNGAP1*, *SLC2A1*, *TCF4x2*). In four other patients, we identified potentially causative mutations (in *NLGN3*, *PQBP1*, *SLC2A1* and *TCF4*; see online supplementary figures S20, S21, S7 and S9, respectively), whose implication in cognitive impairment has to be further confirmed. Finally, missense variants that appeared at first likely to be pathogenic, notably based on the very high evolutionary conservation of the affected residues or on previous publications, appeared excluded as causal after further segregation analysis (in *FLNA*, *FMR1*, *HUWE1* or *MECP2*, see online supplementary figure S22).

Atypical type of mutations

Among the 26 certainly causative mutations identified, some were surprising by the nature of the mutation itself. In a boy with severe encephalopathy, epilepsy, hypotonia and microcephaly, we detected a highly complex rearrangement in exon #4 of *MECP2*, involving a 139 bp deletion flanked by the insertion of two sequences in inverted orientation derived from intron #2 still keeping the reading frame downstream of the rearrangement. Such event is inherited from the proband's mother who presents with speech delay and dyslexia (see online supplementary figure S17). This exon is known to be the one accumulating most mutations in patients, especially the 3' half, which is a recombination hotspot and the target of several deletions/duplications and less frequently inversions.^{16–18}

We report here for the first time an intragenic deletion affecting *FMR1* outside of the promoter/exon #1 region that is the target of the fragile-X syndrome CGG-expansion. Very few point mutations have been reported in coding regions. We identified a complete deletion of the last exon of *FMR1* in one patient and his two affected brothers unevenly presenting with

clinical features of fragile-X syndrome (see online supplementary figure S13).

We also identified a patient carrying a maternally inherited 10 bp deletion causing a frameshift in exon #7 of *IL1RAPL1*, while unexpectedly his affected brother bears a de novo deletion of the full exon highlighting that affected relatives may carry distinct mutations. We propose that small 10 bp deletion may have created a sequence conformation favouring further instability and leading to the larger deletion observed in the second brother (see online supplementary figure S14). Indeed, a large proportion of *IL1RAPL1* causative mutations are intragenic exon deletions or pericentric inversions,¹⁹ supporting that *IL1RAPL1* region is highly susceptible to recombination events.

Lastly, we identified a de novo Pro578Arg missense mutation in *GRIN1* in a male proband with severe ID, hypotonia, feeding disorders and very poor speech but no epilepsy, a phenotype similar to that associated to *GRIN1* missense mutations in some patients.^{20 21} A maternal uncle presented with similar features except for the poor speech and hypotonia (see online supplementary figure S3), initially suggesting an associated X-linked mode of inheritance. The finding of a de novo deleterious missense mutation excludes this latter hypothesis, further highlighting the prevalence of phenocopies in cognitive disorders.

Genotype–phenotype correlations: from expected to unexpected

The majority of certainly causative mutations were identified in patients whose clinical phenotype was retrospectively consistent with previous reports (table 2). For instance, the proband carrying a truncating mutation in *IQSEC2* presents with severe ID, no speech, motor developmental delay, severe epilepsy, strabismus and autistic features (see online supplementary figure S15), which matches the recently proposed clinical spectrum associated to mutations in this gene.^{22 23} Likewise, *DYRK1A* was originally found disrupted by translocations or deleted in several patients with ID and microcephaly.^{24–26} More recently, truncating mutations in this gene were shown to cause ID associated with primary microcephaly (sometimes borderline at -2 SD), growth retardation, developmental delay, facial dysmorphic traits, seizures and major feeding difficulties, with or without

associated autism.^{27–31} We report here two novel de novo truncating mutations in patients with similar clinical features but no epilepsy (see online supplementary figure S2). Nonetheless, in a few other cases (eg, mutations in *RAI1* or *MECP2*), the probands lacked some clinical features, thus the corresponding diagnosis of Smith-Magenis (MIM 182290) or Rett (MIM 312750) syndrome was not evoked by experienced clinical geneticists (see online supplementary figures S5 and S17). For instance, among the three patients with *MECP2* mutations, one female had the classic Rett phenotype, the other female proband presented with non-classic Rett phenotype (no regression episode, no hand-flapping), while the rarity of reports of *MECP2* encephalopathies in adolescent males precluded suspicion of the involvement of that gene in a patient (APN-3) who had undergone rather extensive prior genetic testing.

A few detected mutations were unexpected as they were detected in patients whose phenotype did not match previous descriptions. For instance, mutations in *TCF4* mainly cause Pitt-Hopkins syndrome (PHS, MIM 610954) characterised by severe motor retardation, absence of speech, characteristic dysmorphic traits, autistic features, intestinal problems and hyperventilation.³² We here describe truncating *TCF4* mutations in two patients, one with clinical manifestations highly suggestive of PHS, the other with less syndromic manifestations and no dysmorphic traits (see online supplementary figure S9). *TCF4* mutations were already reported in patients with non-syndromic ID, suggesting that such mutations were likely to be underdiagnosed.³³

Another patient and his affected brother both carry a distal frameshift mutation in *DMD* affecting the major muscle transcript encoding the dystrophin protein associated to Duchenne or Becker muscular dystrophy (DMD, MIM 310200; BMD, MIM 300376), and the brain-specific isoform Dp71. The index case presents with moderate ID, psychomotor retardation, no speech, behavioural disorders, dysmorphic traits but strikingly no muscular phenotype (see online supplementary figure S12). His brother harbours a milder phenotype with additional hypotonia and cerebellar dysplasia. Both harbour borderline-high CPK levels. The association of cognitive impairment with DMD/BMD has been extensively reported and correlated to truncating mutations affecting Dp71, yet never in the absence of a muscular phenotype.^{34–39} Our findings extend the recent report of a large family with affected males carrying an in-frame single amino acid deletion associated to mild ID and no muscular phenotype.⁴⁰

Confirmation of candidate genes for cognitive disorders

Some selected genes were only candidate ID or ASD genes at the time of the design, with single pieces of evidence in the literature. The identification of additional mutations in patients with similar phenotype definitively confirms their implication in cognitive disorders.

We reported a damaging missense affecting the function of the Monoamine Oxidase A enzyme (MAOA),^{41 42} which replicated for the first time in 20 years the implication of MAOA in autism/ID associated to significant behavioural disorders.^{41 42}

We also identified a probably pathogenic missense variant in *NLGN3* in a male and his cousin, both presenting with ID and autism (see online supplementary figure S20). In silico predictions, high conservation of the mutated residue across all neuroligin paralogs, and familial analysis are altogether in favour of a pathogenicity of this missense change. A definitive functional effect of this missense still needs to be demonstrated to clearly establish the diagnosis. The implication of this gene was never

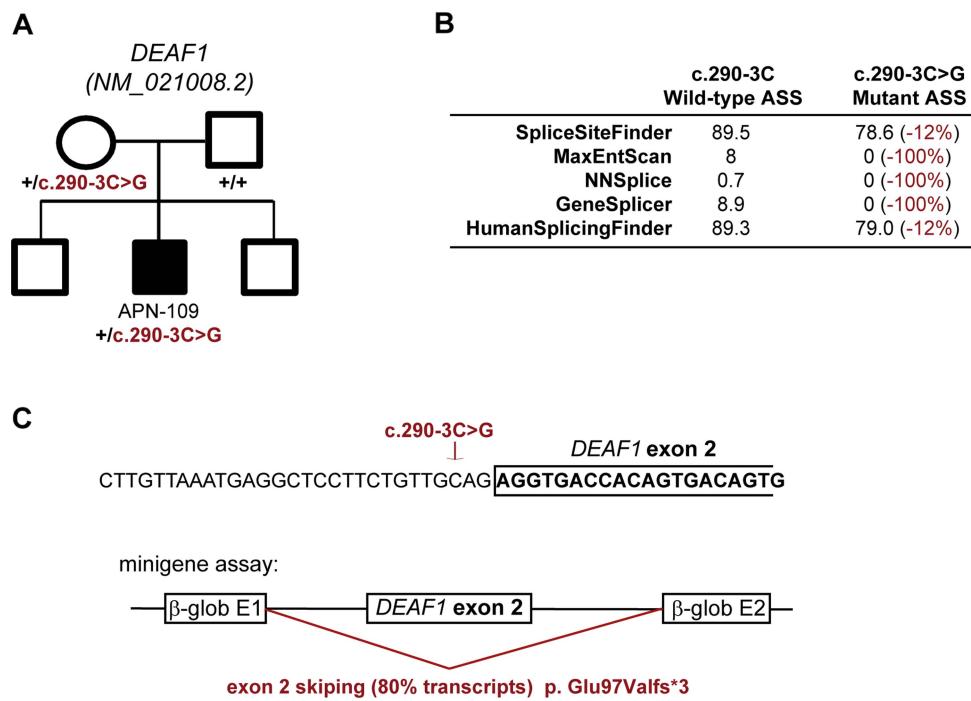
replicated since the initial publication,⁴³ although screened in several cohorts with comparable phenotypes.^{44–50}

Similarly, we identified a novel truncating point mutation in *MED13L* confirming the implication of this gene in ID (see online supplementary figure S4). Disruption of *MED13L* was initially associated with transposition of the great arteries (TGA), associated to ID in a single case with a chromosomal translocation.⁵¹ A homozygous missense mutation was then identified in two siblings from a consanguineous family presenting with non-syndromic ID, suggesting an implication of the gene in autosomal-recessive forms of ID justifying its selection in our panel.⁵ A total of five patients were more recently described with de novo intragenic CNVs or point mutations affecting *MED13L*, delineating a recognisable *MED13L*-haploinsufficiency syndrome characterised by hypotonia, moderate ID, variable cardiac defects, facial hypotonia and dysmorphic traits.^{52 53} Our patient with the *MED13L* mutation presents with concordant phenotype such as an open mouth appearance and muscular hypotonia but no cardiac defects. Due to the initially proposed autosomal-recessive mode of inheritance associated to ID, at first we did not consider this heterozygous truncating mutation as causative, as it may also have been the case for a heterozygous splicing mutation identified earlier in a large-scale exome sequencing study in one male with ASD.²⁸ Altogether, these findings suggest that ID associated to *MED13L*-haploinsufficiency syndrome is a relatively frequent condition.^{5 53}

Ambiguous mode of inheritance in ID-associated genes: the example of *DEAF1*

As for *MED13L*, the mode of inheritance associated to some genes is ambiguously described in literature. We identified an heterozygous variant affecting splicing in *DEAF1* inherited from the asymptomatic mother in a patient presenting with severe ID, developmental delay, poor speech, pain resistance, dysmorphic features and aggressive behaviour (figure 1), while the gene had been proposed as associated to autosomal-dominant forms of ID.^{7 8} The recent report of two additional individuals carrying de novo missense mutations narrowed the associated phenotype to moderate/severe ID, speech impairment, behavioural problems, high pain threshold, dysmorphic features and abnormal walking pattern, hence highly similar to the one of our proband.⁵⁴ Although in vitro validation studies suggest that the reported missense variants lead to an impaired function of *DEAF1*, the authors concluded that they presumably act as dominant-negatives incapacitating both normal and mutant proteins since truncating variants had been observed in asymptomatic individuals.⁵⁴ In parallel, a homozygous missense mutation clustering in the same SAND-domain with all three de novo missense mutations was reported in members of a consanguineous family presenting with ID, microcephaly and white matter abnormalities, therefore suggesting a possible autosomal-recessive mode of inheritance.⁵⁵ The pathogenic mechanism associated to *DEAF1* mutations is therefore unclear. Due to the highly similar clinical features of the herein reported proband and of probands carrying de novo missense mutations, the splice variant detected here may contribute to the phenotype of our patient, possibly through a recessive mode of inheritance (ie, acting in *trans* with another heterozygous variant) since haploinsufficiency appears tolerated in healthy individuals. Altogether those findings either suppose a similar phenotype for autosomal-dominant and autosomal-recessive mode of inheritance associated to *DEAF1* mutations, or a universal autosomal-recessive mode of inheritance with a second variant that has not

Figure 1 Inherited disrupting splice variant in *DEAF1*: what contribution to the phenotype? (A) Pedigree showing the maternally inherited splice variant in *DEAF1* (c.290-3C>G); (B) prediction scores for the effect of the herein described variant on splicing (prediction scores for acceptor splice sites (ASS) as computed by SpliceSite Finder, MaxEntScan, NNSplice, GeneSplicer and Human Splicing Finder for the consensus ASS with either the wild-type or the mutated allele); (C) localisation of the variant, and its resulting effect on splicing in vitro (minigene construct): 90% of abnormal transcripts: 80% with entire exon #2 skipped, and 10% using the alternative ASS c.290-16 both leading to a frameshift and a premature stop codon.



been yet identified, alike what was finally proven for Thrombocytopenia-absent radius (TAR) syndrome for instance.⁵⁶

Unsupportive evidences for proposed ID-associated mutations and genes

The identification in patients of a previously reported ID mutation should be considered with caution: indeed, as already discussed in previous publications, some ‘false-positive’ mutations are present among variants annotated as ‘pathogenic’ in dbSNP, OMIM or in published reports.^{13 14} We here question the causative effect of a few previously proposed mutations (see online supplementary table S4). For instance, one missense variant identified in *FLNA* (c.3872C>T, p.Pro1291Leu) was previously reported in a patient with FG syndrome (MIM 300321).⁵⁷ This variant was identified here in a patient with a different phenotype (see online supplementary figure S22) and is also reported in one male in EVS (presumably not presenting with cognitive disorders), raising doubt about its pathogenicity.

The identification of non-segregating truncating variants (ie, detected both in patients and healthy relatives) can also challenge the implication of genes in X-linked and autosomal-dominant forms of ID. In one family, we identified a frameshift variant in *SRPX2* in a male proband. It is most likely inherited from the deceased asymptomatic maternal grandfather since it was also detected in the mother and in three maternal aunts yet absent from the maternal grandmother. Despite recent functional evidences regarding the role of *SRPX2* in brain development,^{58 59} its definitive implication in cognitive disorders has already been questioned following the presence of the initially proposed mutations in EVS¹⁴ and in control individuals,⁶⁰ and subsequently to the identification of a missense mutation in *GRIN2A* co-segregating with the epileptic status in the initial *SRPX2* family.⁶¹ In another family, we identified a nonsense variant in *SHROOM4* in a male proband yet also in his unaffected brothers, a finding that further challenges the

implication of *SHROOM4* in X-linked cognitive disorders (see online supplementary table S5, figure 2).¹⁴

Patients carrying probably pathogenic variants in two ID-associated genes

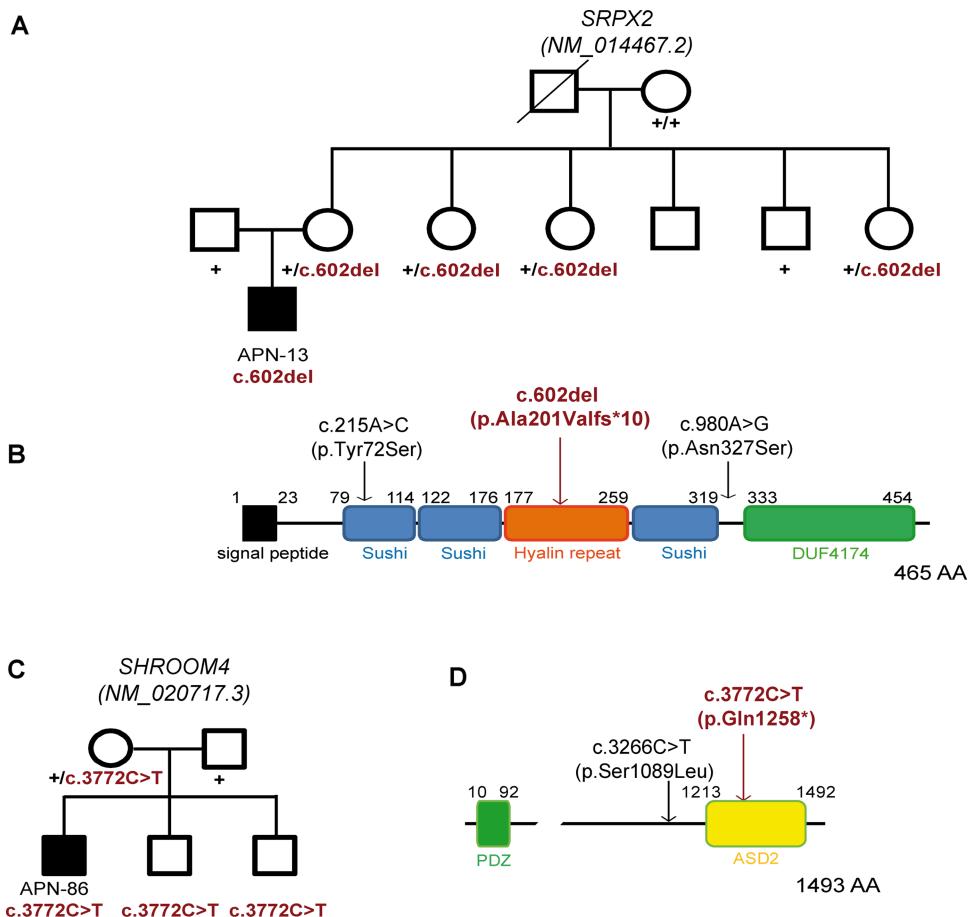
In three unrelated patients, we identified candidate variants in two separate genes, requiring the evaluation of different scenarios: (a) one single contributor while the second variant is innocuous, (b) one major contributor while the second variant acts as a modifier, and (c) both variants are implicated in the phenotype and have a synergistic effect.

In one male proband, we identified a maternally inherited splice variant leading to a frameshift in the X-linked gene *PHF8*, together with a de novo truncating variant in *DOCK8* (see online supplementary figure S18). Considering both the weak evidences implicating *DOCK8* in autosomal-dominant ID (two probands reported with translocations disrupting the gene), the clinical features consistent with a *PHF8* mutation (even in the absence of cleft lip/palate) and the X-inactivation bias identified in the mother, the *PHF8* variant alone is most likely to be responsible for the phenotype leaving the *DOCK8* variant as probably innocuous.^{62 63}

In another male proband, two possibly causative missense variants were identified in the XLID genes *ATRX* and *HCFC1* (figure 3). Patients’ phenotype was not evocative of an *ATRX* mutation (no dysmorphic traits, no urogenital abnormalities, absence of Heinz bodies), but perfectly matched the recent description of cobalamin-X metabolic disorder (*cbIX*, MIM 309541⁶⁴). We concluded to a causative effect of the *HCFC1* mutation (being one of the *cbIX* recurrent mutations), but cannot exclude a contributory effect of the *ATRX* variant based on current co-segregation data.

In the third family, the proband carries one distal truncating variant in *SLC2A1* and one missense variant in *ANKRD11*, each inherited from one asymptomatic parent. Some mutations in *SLC2A1* have already been associated to GLUT-1 deficiency (MIM 606777/612126) with incomplete penetrance.⁶⁵ The

Figure 2 Truncating variants not or ambiguously co-segregating with ID.
 (A) Pedigree of patient APN-13 carrying a frameshift variant in *SRPX2* (c.602del, p.Ala201Valfs*10) demonstrating the likely inheritance from the asymptomatic (deceased) maternal grandfather, yet a putative germinal mosaicism of a de novo mutation cannot be excluded.;
 (B) predicted functional domains of SRXP2 from Pfam indicating locations of the herein identified mutation (in red) and those previously described. DUF4174: domain of unknown function; (C) pedigree showing the non-segregating nonsense variant in *SHROOM4* (c.3772C>T; p.Gln1258*) in the family of patient APN-86; (D) location of the premature stop codon, which would disrupt the ASD2 domain of the protein.



proband presents with evocative symptoms of both *SLC2A1* and *ANKRD11* mutations (major hypotonia along with skeletal abnormalities) that might suggest a synergistic contribution of both variants to the phenotype (see online supplementary figure S7). No conclusion could be unambiguously drawn in this case, but such a di- or oligogenic mode of inheritance has already been proposed in neurodevelopmental disorders.^{66–68}

DISCUSSION

Targeted sequencing of 217 genes in a cohort of 106 patients with unknown genetic aetiology of ID led to a conclusive diagnostic yield of 25%. A majority of causative mutations identified are located in XLID genes, which can be partially explained by our male-enriched cohort. When excluding familial cases, X-linked mutations are found in 7/72 sporadic male cases, matching the proposed figure that 10% of males with sporadic ID carry mutations in X-linked genes.⁶⁹ Combining the results of recent trio-exome analyses leads to similar proportions (X-linked causative mutations in 7/66 males with sporadic ID)^{3–8}. The detection rate of X-linked mutations in our cohort is—as expected—higher in males with familial history of ID, but is also significant in females (2/10, both with *MECP2* mutations). Also unsurprisingly, mutations in autosomal-dominant/haploinsufficient ID genes are mostly found within sporadic cases.

Although we did not expect patients to harbour mutations in the same genes because of the small size of the cohort and the extensive genetic heterogeneity of ID, we had a few genes hit more than once: *MECP2* (despite being screened in 11% of patients prior to inclusion), *KDM5C*, *DYRK1A* and *TCF4*. Our results, intersected with the ones from other studies, highlight mutations within common genes (*MECP2*, *CUL4B*, *IL1RAPL1*,

IQSEC2, *KDM5C*, *SLC9A6*, *SLC16A2*, *DYRK1A*, *SLC2A1*, *SYNGAP1* or *TCF4*), suggesting that they are more frequently mutated in ID patients.^{3–9 20 28–30 70 71} If mutations in such ID genes are confirmed to account for a substantial number of patients, introducing as a diagnostic step massive multiplexed resequencing of such genes in large cohorts may be considered.²⁹

The extensive genetic and phenotypic heterogeneity of ID is the major hindrance for obtaining a precise molecular diagnosis. Direct sequencing strategies consisting in sequentially screening candidate genes are being replaced in diagnostic laboratories by more high-throughput NGS-based strategies: multiplex targeted sequencing of a few genes in large cohorts, selective targeted sequencing of up to several hundred genes, exome sequencing, while whole-genome sequencing (WGS), although powerful, notably for genome rearrangements and for CG-rich exons, remains in the research domain. Exome or full-genome strategies are more exhaustive alternatives and very attractive issues in the field of genetic diagnosis. Their universal approaches, whatever the clinical features, enhance the technical management of the workflow. However, the actual coverage proposed with the exome or full-genome strategies is still frequently insufficient (see online supplementary table S6), which may result in missing mutations.^{9 29} Also, the finding of a putative mutation in a gene never associated before to a given pathology marks the start of a research endeavour to validate the finding but is not per se a diagnostic result.

Targeted sequencing appears more appealing for well-defined pathologies in which most implicated genes have been uncovered or in clinically homogeneous entities (ie, Bardet-Biedl and related ciliopathies,¹³ retinal dystrophies,⁷² hearing loss,⁷³ etc).

Cognitive and behavioural genetics

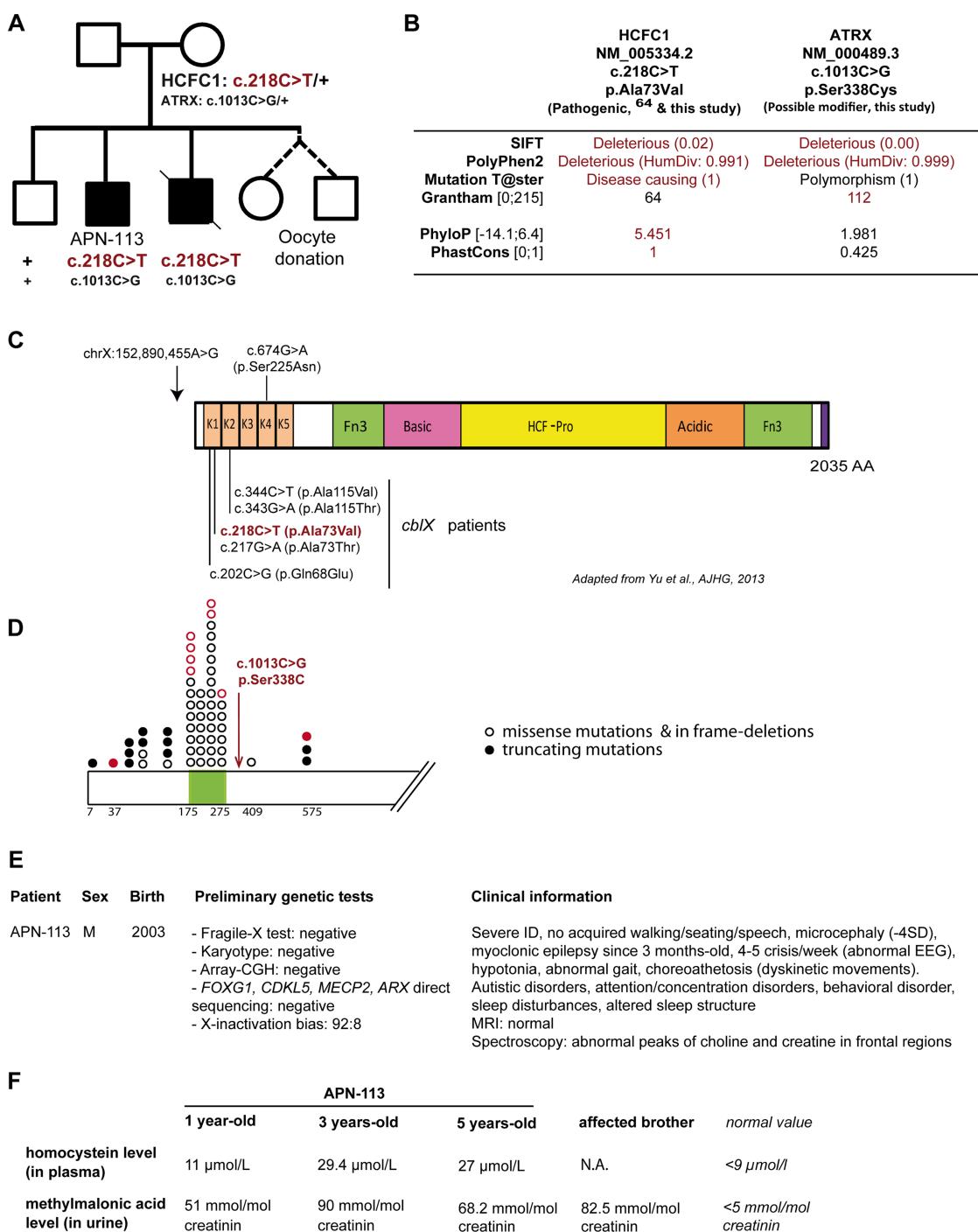


Figure 3 Patient carrying two probably damaging missense variants in *HCFC1* and *ATRX*: one causative mutation and one modifier variant? (A) Family tree of patient APN-113: proband carries a missense mutation in *HCFC1* (c.218C>T; p.Ala73Val) already reported in two patients with *cb/X* as well as another missense variant in *ATRX* (c.1013C>G, p.Ser338Cys). Both variants are maternally inherited, absent from the unaffected brother, but carried by the younger brother who died of sudden death at 2 months old; (B) associated predictions for the recurrent pathogenic missense mutation in *HCFC1*⁶⁴ and the possible modifier in *ATRX*, showing putative pathogenicity and moderate nucleotide conservation; (C) representation of *HCFC1* and its domains: kelch domains (K1–K5), Fn3 (fibronectin type 3), basic domain, HCF-proteolysis repeats (HCF-pro), acidic domain and nuclear localisation signal (NLS) domain. The initial mutations involved in milder non-syndromic ID are indicated above: a regulatory variant in the 5'UTR of *HCFC1* was identified by targeted massive parallel resequencing in a family with probable X-linked ID (XLID) (MRX3), which had for long remained unsolved. This variant was disrupting the functional binding site of the transcription factor YY1 within the *HCFC1* promoter region, leading to an upregulation of its expression in lymphoblastoid cells.⁷⁴ Subsequent screening of additional unsolved families identified one single co-segregating missense variant (c.674G>A; p. Ser225Asn) in *HCFC1*. The phenotype of both patients was rather mild: non-syndromic mild to moderate ID. In the bottom are indicated the mutations recently described in *cb/X* patients⁶⁴; (D) the *ATRX* missense variant is located close but outside of the hotspot for disease-causing missense mutations (in the zinc-finger binding domain, in green) reported in patients with *ATRX* mutations, in red: mutations reported independently in at least two patients. Mutations are indicated when affecting residues 1–700 (reported in OMIM, ClinVar or in⁷⁵), the rest of the protein is not represented; (E) Clinical information regarding APN-113 and previous genetic explorations, including X-inactivation status in the mother. *Cb/X* patients present with the same very severe phenotype: severe ID, early infantile epilepsy, choreoathetosis, microcephaly and more variable muscular hypotonia. Three of them are reported with early death in infancy⁶⁴; (F) biochemical abnormalities observed in the two affected brothers are similar to those previously observed.⁶⁴

We show here that it is also a powerful alternative for diagnostic purposes in ID (see online supplementary table S6). The positive diagnostic yield in our cohort of 106 patients is of 25% overall and 21% for sporadic cases, which is similar to the initial reports with the trio-exome strategy (32 highly likely causative mutations in a total of 151 patients, a 21% yield; see online supplementary table S7^{3 8}). Indeed, most of the mutations identified by exome sequencing affect genes included in our panel and would thus have been detected with our strategy. Our diagnostic yield is slightly lower than the 30% yield (on 211 patients) we now calculated from pooling the three published exome studies (including also²⁹ and using the updated data for² very recently reported in Gilissen *et al*⁹), a difference that can be accounted for by the recently recognised ID genes missing in our panel (see online supplementary table S7). Our relatively high diagnostic yield is not due to an overestimation regarding the pathogenicity of the identified variants as we were highly stringent regarding the classification of certainly causative mutations (eg, we did not include all probably damaging missense variants located in XLID genes that were detected in males). The high depth of coverage and the smaller portion of poorly covered regions achieved with our strategy ensure a high sensitivity and specificity of detecting pathogenic events in the regions of interest (SNVs, indels, but also CNVs, a topic that had not been addressed in previous exome studies). Also, the relative ease of variant analysis and smaller number of follow-up studies for candidate variants may contribute to this significant yield. As fewer candidate variants are identified per patient with this approach, they can also be analysed more thoroughly (putative effect on splicing for variants not affecting canonical splice sites, predictive impact on the protein through structural modelling, etc). With an even broader panel of genes (including, for instance, genes involved in ID associated to multiple congenital anomalies but found mutated in patients with a milder presentation), one can expect that the resulting diagnostic yield will be higher.

The limited number of sequenced genes with the targeted approach—restricted to those involved in cognitive disorders—also avoids the controversial issues raised by incidental findings. Nonetheless, the targeted sequencing approach will miss newly identified genes and theoretically prevents data reanalysis (ie, incorporating novel findings regarding genetics of ID). The major value of targeted sequencing for ID is that it should allow the application of such test to a much higher proportion of patients awaiting molecular diagnosis given the significantly lower cost of sequencing, but especially of data analysis, storage and interpretation. It should thus generate much more promptly large amounts of data regarding the spectrum of mutations and phenotypes associated to the many genes implicated in ID, and thus considerably increase our knowledge on the specific condition associated with each gene.

The substantial proportion of patients that remain without molecular diagnosis with either strategy raises several issues: whether a large fraction of ID-associated genes remain to be discovered, whether many mutations are missed because they are located in non-coding regions or lastly whether more complex genetic scenarios are implicated such as variants with reduced penetrance, oligogenic and/or multifactorial modes of inheritance.^{66–68} In particular, many disorders associated with autosomal-dominant inheritance are associated with incomplete penetrance and high intrafamilial phenotypic variability, although such scenario is mostly excluded from trio-studies that focus on de novo mutations. The recent results from Gilissen *et al* provide initial response on this issue: a substantial

proportion of such previously negative patients seem to carry pathogenic CNVs that could not be detected by array-CGH studies (and were not tested in the exome studies), other patients carry mutations located in newly identified genes or, mutations in known genes yet that had been missed by previous bioinformatic pipelines.⁹ With an estimated cumulative diagnostic yield of 62% (although derived from a small cohort of 50 patients, for WGS analysis), whole-genome analyses of larger cohorts of patients may shed some more light regarding the contribution of the abovementioned hypotheses to explain the remaining proportion of patients without molecular diagnosis.

Author affiliations

¹Département de Médecine translationnelle et Neurogénétique, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France

²Chaire de Génétique Humaine, Collège de France, Illkirch, France

³Laboratoire de diagnostic génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

⁴Centre de Génétique et Centre de Référence Anomalies du développement et Syndromes malformatifs, Hôpital d'Enfants, CHU de Dijon, Dijon, France

⁵Département de Génétique Médicale, Centre de Référence Maladies Rares Anomalies du Développement et Syndromes Malformatifs Sud-Languedoc Roussillon, Hôpital Arnaud de Villeneuve, Montpellier, France

⁶Département de Génétique Médicale, Hôpitaux Civils de Lyon, Lyon, France

⁷Plateforme de Biopuces et Séquençage, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France

⁸Plateforme de Bioinformatique de Strasbourg (BIPS), IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France

⁹Département de Génétique, CHU de Hautepierre, Strasbourg, France

¹⁰Département de Biochimie et de Génétique, CHU d'Angers, Angers, France

¹¹Laboratoire de Génétique Médicale, INSERM U1112, Faculté de Médecine de Strasbourg, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

¹²CHU de Bordeaux, Génétique Médicale, Université de Bordeaux, Laboratoire MRGM, Bordeaux, France

¹³Département de Génétique Médicale, CHU de Rouen, Rouen, France

¹⁴Service de Génétique Médicale, Centre De Référence Anomalies du Développement, CHU de Rennes, Rennes, France

¹⁵Service de Génétique Médicale, Hôtel Dieu, Clermont-Ferrand, France

¹⁶Laboratoire de Biochimie, CHU de Nîmes, Nîmes, France

¹⁷Unité de Génétique Clinique, CHU d'Amiens, Amiens, France

¹⁸Service de Génétique, Centre Hospitalier, Le Mans, France

¹⁹Service de Neuropédiatrie, Hôpital Saint Vincent de Paul, Groupe Hospitalier de l'Institut Catholique Lillois, Faculté Libre de Médecine, Lille, France

²⁰Service de Génétique, CHU de Reims, EA3801, Reims, France

²¹Service de Génétique Médicale, CHU de Nantes, Nantes, France

Acknowledgements This study was supported by grants and fellowships from Fondation pour la Recherche Médicale, Agence de Biomédecine and Fondation Jérôme Lejeune, APLM and CREGEMES. We thank the students who were involved in this project: Sébastien Kirsch, Audrey Creppy, Inès Bekkour and Grace Gan. We thank Nadège Calmels, Valérie Biancalana, Elsa Nourisson and all other members of the Genetic Diagnostic Laboratory of the Nouvel Hôpital Civil (Strasbourg) for their help with patients' DNA sample selection and preparation. We thank Cécile Pizot for the development of VaRank. We thank Damien Sanlaville, Christine Coubes, Delphine Héron, Sophie Naudion, James Lespinasse and Marie-Line Bichon for their contribution to the recruitment of patients, and all medical interns or genetic counsellors who participated in this project. Finally, we warmly thank all patients and their families for their implication in this study.

Contributors Study concept and design: CR, J-LM, AP. Clinical genetics investigations: AM-P, MW, GL, SE-C, YA, MB, DB, EC, HD, BD, M-AD, VD-G, EF, MF, CF, AG, SL, MM-D, DM-C, DL, GM, AP, SS, CT-R, JT, MD-F, DG, PS, PE, BI and LO-F. Acquisition, analysis and interpretation of data: CR, BG, JL, YH, JM, AQ, BJ, J-LM and AP. Drafting of the manuscript: CR, J-LM and AP. Critical revision of the manuscript for important intellectual content: BG and JM. Obtained funding: J-LM and AP. Administrative, technical or material support: NH, MD, CF, VG, SLG, MP and SV. Study supervision: BG, J-LM, AP.

Competing interests None.

Patient consent Obtained.

Ethics approval Comité de Protection des Personnes, France.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All the variants identified during the course of this study will be submitted to the variant database ClinVar: <http://www.ncbi.nlm.nih.gov/clinvar/>.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

REFERENCES

- Leonard H, Wen X. The epidemiology of mental retardation: challenges and opportunities in the new millennium. *Ment Retard Dev Disabil Res Rev* 2002;8:117–34.
- Maulik PK, Mascarenhas MN, Mathers CD, Dua T, Saxena S. Prevalence of intellectual disability: a meta-analysis of population-based studies. *Res Dev Disabil* 2011;32:419–36.
- de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA, Vissers LE. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med* 2012;367:1921–9.
- Lubs HA, Stevenson RE, Schwartz CE. Fragile X and X-linked intellectual disability: four decades of discovery. *Am J Hum Genet* 2012;90:579–90.
- Najmabadi H, Hu H, Garshasbi M, Zemotjal T, Abedini SS, Chen W, Hosseini M, Behjati F, Haas S, Jamali P, Zecha A, Mohseni M, Puttmann L, Vahid LN, Jensen C, Moheb LA, Bieneck M, Larti F, Mueller I, Weissmann R, Darvish H, Wrogemann K, Hadavi V, Lipkowitz B, Esmaeeli-Nieh S, Wieczorek D, Kariminejad R, Firouzabadi SG, Cohen M, Fattahizadeh Z, Rost I, Mojahedi F, Hertzberg C, Dehghan A, Rajab A, Banavandi MJ, Hoffer J, Falah M, Musante L, Kalscheuer V, Ullmann R, Kuss AW, Tzschach A, Kahrizi K, Ropers HH. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 2011;478:57–63.
- Ropers HH. Genetics of early onset cognitive impairment. *Annu Rev Genomics Hum Genet* 2010;11:161–87.
- Vissers LE, de Ligt J, Gilissen C, Janssen I, Steehouwer M, de Vries P, van Lier B, Arts P, Wieskamp N, del Rosario M, van Bon BW, Hoischen A, de Vries BB, Brunner HG, Veltman JA. A de novo paradigm for mental retardation. *Nat Genet* 2010;42:1109–12.
- Rauch A, Wieczorek D, Graf E, Wieland T, Endele S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donato N, Dufke A, Cremer K, Hempel M, Horn D, Hoyer J, Joset P, Ropke A, Moog U, Riess A, Thiel CT, Tzschach A, Wiesener A, Wohlleber E, Zweier C, Ekici AB, Zink AM, Rump A, Meisinger C, Grallert H, Sticht H, Schenck A, Engels H, Rappold G, Schrock E, Wieacker P, Riess O, Meitinger T, Reis A, Strom TM. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 2012;380:1674–82.
- Gilissen CH-KJ, Thung DT, van de Vorst M, van Bon BW, Willemsen MH, Kwint M, Janssen IM, Hoischen A, Schenck A, Leach R, Klein R, Tearle R, Bo T, Pfundt R, Yntema HG, de Vries BB, Kleefstra T, Brunner HG, Vissers LE, Veltman JA. Genome sequencing identifies major causes of severe intellectual disability. *Nature* 2014;511:344–7.
- Biancalana V, Beldjord C, Taillandier A, Szapiro-Tapia S, Cusin V, Gerson F, Philippe C, Mandel JL. Five years of molecular diagnosis of Fragile X syndrome (1997–2001): a collaborative study reporting 95% of the activity in France. *Am J Med Genet A* 2004;129A:218–24.
- Rauch A, Hoyer J, Guth S, Zweier C, Kraus C, Becker C, Zenker M, Hüffmeier U, Thiel C, Rüschendorf F, Nürnberg P, Reis A, Trautmann U. Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. *Am J Med Genet A* 2006;140:2063–74.
- van Karnebeek CD, Jansweijer MC, Leenders AG, Offringa M, Hennekam RC. Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness. *Eur J Hum Genet* 2005;13:6–25.
- Redin C, Le Gras S, Mhamdi O, Geoffroy P, Stoezel C, Vincent MC, Chiarazzi P, Lacombe D, Ouertani I, Petit F, Till M, Verloes A, Jost B, Chaabouni HB, Dollfus H, Mandel JL, Muller J. Targeted high-throughput sequencing for diagnosis of genetically heterogeneous diseases: efficient mutation detection in Bardet-Biedl and Alstrom syndromes. *J Med Genet* 2012;49:502–12.
- Piton A, Redin C, Mandel JL. XLID-causing mutations and associated genes challenged in light of data from large-scale human exome sequencing. *Am J Hum Genet* 2013;93:368–83.
- Piton A, Jouan L, Rochefort D, Dobrzeniecka S, Lachapelle K, Dion PA, Gauthier J, Rouleau GA. Analysis of the effects of rare variants on splicing identifies alterations in GABA_A receptor genes in autism spectrum disorder individuals. *Eur J Hum Genet* 2013;21:749–56.
- Archer HL, Whatley SD, Evans JC, Ravine D, Huppke P, Kerr A, Bunyan D, Kerr B, Sweeney E, Davies SJ, Reardon W, Horn J, MacDermot KD, Smith RA, Magee A, Donaldson A, Crow Y, Hermon G, Miedzybrodzka Z, Cooper DN, Lazarus L, Butler R, Sampson J, Pilz DT, Laccone F, Clarke AJ. Gross rearrangements of the MECP2 gene are found in both classical and atypical Rett syndrome patients. *J Med Genet* 2006;43:451–6.
- Lebo RV, Ikuta T, Milunsky JM, Milunsky A. Rett syndrome from quintuple and triple deletions within the MECP2 deletion hotspot region. *Clin Genet* 2001;59:406–17.
- Ravn K, Nielsen JB, Skjeldal OH, Kerr A, Hulten M, Schwartz M. Large genomic rearrangements in MECP2. *Hum Mutat* 2005;25:324.
- Behncke A, Hinderhofer K, Bartsch O, Numann A, Ipach ML, Damatova N, Haaf T, Dufke A, Riess O, Moog U. Intragenic deletions of IL1RAPL1: report of two cases and review of the literature. *Am J Med Genet A* 2011;155A:372–9.
- Epi KC, Epilepsy Phenome/Genome P, Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, Epstein MP, Glauzer T, Goldstein DB, Han Y, Heinzen EL, Hitomi Y, Howell KB, Johnson MR, Kuzniecky R, Lowenstein DH, Lu YF, Madou MR, Marson AG, Mefford HC, Esmaeeli Nieh S, O'Brien TJ, Ottman R, Petrovski S, Poduri A, Ruzzo EK, Scheffer IE, Sherr EH, Yuskaits CJ, Abou-Khalil B, Alldredge BK, Bautista JF, Berkovic SF, Boro A, Cascino GD, Consalvo D, Crumrine P, Devinsky O, Dlugos D, Epstein MP, Fiol M, Fountain NB, French J, Friedman D, Geller EB, Glauzer T, Glynn S, Haut SR, Hayward J, Helmers SL, Joshi S, Kanner A, Kirsch HE, Knowlton RC, Kossoff EH, Kuperman R, Kuzniecky R, Lowenstein DH, McGuire SM, Motika PV, Novotny EJ, Ottman R, Paolicchi JM, Parent JM, Park K, Poduri A, Scheffer IE, Shellhaas RA, Sherr EH, Shih JJ, Singh R, Sirven J, Smith MC, Sullivan J, Lin Thio L, Venkat A, Vining EP, Von Allmen GK, Weisenberg JL, Widness-Walsh P, Winawer MR. De novo mutations in epileptic encephalopathies. *Nature* 2013;501:217–21.
- Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, Park AR, Spiegelman D, Dobrzeniecka S, Piton A, Tomitori H, Daoud H, Mascicotte C, Henrion E, Diallo O, Group S, Shekarabi M, Marineau C, Shevell M, Maranda B, Mitchell G, Nadeau A, D'Anjou G, Vanasse M, Srour M, Lafreniere RG, Drapeau P, Lacaille JC, Kim E, Lee JR, Igarashi K, Huganir RL, Rouleau GA, Michaud JL. Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. *Am J Hum Genet* 2011;88:306–16.
- Shoubridge C, Tarpey PS, Abidi F, Ramsden SL, Rujirabanjerd S, Murphy JA, Boyle J, Shaw M, Gardner A, Proos A, Puusepp H, Raymond FL, Schwartz CE, Stevenson RE, Turner G, Field M, Walikonis RS, Harvey RJ, Hackett A, Futreal PA, Stratton MR, Gecz J. Mutations in the guanine nucleotide exchange factor gene IQSEC2 cause nonsyndromic intellectual disability. *Nat Genet* 2010;42:486–8.
- Tran Mau-Them F, Willems M, Albrecht B, Sanchez E, Puechberty J, Ende S, Schneider A, Ruiz Pallares N, Missrian C, Rivier F, Girard M, Holder M, Manouvrier S, Touitou I, Lefort G, Sarda P, Moncla A, Drunat S, Wieczorek D, Geneviève D. Expanding the phenotype of IQSEC2 mutations: truncating mutations in severe intellectual disability. *Eur J Hum Genet* 2014;22:289–92.
- Möller KS, Kubart S, Hoeltzenbein M, Heye B, Vogel I, Hansen CP, Menzel C, Ullmann R, Tommerup N, Ropers HH, Turner Z, Kalscheuer VM. Truncation of the Down syndrome candidate gene DYRK1A in two unrelated patients with microcephaly. *Am J Hum Genet* 2008;82:1165–70.
- van Bon BW, Hoischen A, Hehir-Kwa J, de Brouwer AP, Ruivenkamp C, Gijsbers AC, Marcelis CL, de Leeuw N, Veltman JA, Brunner HG, de Vries BB. Intragenic deletion in DYRK1A leads to mental retardation and primary microcephaly. *Clin Genet* 2011;79:296–9.
- Yamamoto T, Shimojima K, Nishizawa T, Matsuo M, Ito M, Imai K. Clinical manifestations of the deletion of Down syndrome critical region including DYRK1A and KCNJ6. *Am J Med Genet A* 2011;155A:113–19.
- Courcet JB, Faivre L, Malzac P, Masrel-Paulet A, Lopez E, Callier P, Lambert L, Lemesle M, Thevenon J, Gigot N, Duplomb L, Ragon C, Marle N, Mosca-Boidron AL, Huet F, Philippe C, Moncla A, Thauvin-Robinet C. The DYRK1A gene is a cause of syndromic intellectual disability with severe microcephaly and epilepsy. *J Med Genet* 2012;49:731–6.
- Iossifov I, Ronenius M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee YH, Narzisi G, Leotta A, Kendall J, Grabowska E, Ma B, Marks S, Rodgers L, Stepansky A, Troge J, Andrews P, Bekritsky M, Pradhan K, Ghiban E, Kramer M, Parla J, Demeter R, Fulton LL, Fulton RS, Magrini VJ, Ye K, Darnell JC, Darnell RB, Mardis ER, Wilson RK, Schatz MC, McCombie WR, Wigler M. De novo gene disruptions in children on the autistic spectrum. *Neuron* 2012;74:285–99.
- O'Rourke BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, Carville G, Kumar A, Lee C, Ankenman K, Munson J, Hiatt JB, Turner EH, Levy R, O'Day DR, Krumm N, Coe BP, Martin BK, Borenstein E, Nickerson DA, Mefford HC, Doherty D, Akey JM, Bernier R, Eichler EE, Shendure J. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* 2012;338:1619–22.
- O'Rourke BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, Turner EH, Stanaway IB, Vernot B, Malig M, Baker C, Reilly B, Akey JM, Borenstein E, Rieder MJ, Nickerson DA, Bernier R, Shendure J, Eichler EE. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* 2012;485:246–50.
- Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, Hardison M, Person R, Bekheirnia MR, Leduc MS, Kirby A, Pham P, Scull J, Wang M, Ding Y, Plon SE, Lupski JR, Beaudet AL, Gibbs RA,

- Eng CM. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med* 2013;369:1502–11.
- 32 Amiel J, Rio M, de Pontual L, Redon R, Malan V, Boddaert N, Plouin P, Carter NP, Lyonnet S, Munnich A, Colleaux L. Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *Am J Hum Genet* 2007;80:988–93.
- 33 Hamdan FF, Daoud H, Patry L, Dionne-Laporte A, Spiegelman D, Dobrzeniecka S, Rouleau GA, Michaud JL. Parent-child exome sequencing identifies a de novo truncating mutation in TCF4 in non-syndromic intellectual disability. *Clin Genet* 2013;83:198–200.
- 34 Bushby KM, Appleton R, Anderson LV, Welch JL, Kelly P, Gardner-Medwin D. Deletion status and intellectual impairment in Duchenne muscular dystrophy. *Dev Med Child Neurol* 1995;37:260–9.
- 35 Daoud F, Angeard N, Demerle B, Martie I, Benyaou R, Leturcq F, Cosseme M, Deburgrave N, Saillour Y, Tuffery S, Urtizberea A, Toutain A, Echenne B, Frischman M, Mayer M, Desguerre I, Estournet B, Revelliere C, Penisson B, Cuisset JM, Kaplan JC, Heron D, Rivier F, Chelly J. Analysis of Dp71 contribution in the severity of mental retardation through comparison of Duchenne and Becker patients differing by mutation consequences on Dp71 expression. *Hum Mol Genet* 2009;18:3779–94.
- 36 Emery AE, Skinner R, Holloway S. A study of possible heterogeneity in Duchenne muscular dystrophy. *Clin Genet* 1979;15:444–9.
- 37 Kerr TP, Sewry CA, Robb SA, Roberts RG. Long mutant dystrophins and variable phenotypes: evasion of nonsense-mediated decay? *Hum Genet* 2001;109:402–7.
- 38 Taylor PJ, Betts GA, Maroulis S, Gilissen C, Pedersen RL, Mowat DR, Johnston HM, Buckley MF. Dystrophin gene mutation location and the risk of cognitive impairment in Duchenne muscular dystrophy. *PLoS ONE*;5:e8803.
- 39 Zellweger H, Niedermeyer E. Central nervous system manifestations in childhood muscular dystrophy (CMD). I. Psychometric and electroencephalographic findings. *Ann Paediatr* 1965;205:24–42.
- 40 de Brouwer AP, Nabuurs SB, Verhaart IE, Oudakker AR, Hordijk R, Yntema HG, Hordijk-Hos JM, Voesenek K, de Vries BB, van Essen T, Chen W, Hu H, Chelly J, den Dunnen JT, Kalscheuer VM, Aartsma-Rus AM, Hamel BC, van Bokhoven H, Kleefstra T. A 3-base pair deletion, c.9711_9713del, in DMD results in intellectual disability without muscular dystrophy. *Eur J Hum Genet* 2014;22:480–5.
- 41 Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* 1993;262:578–80.
- 42 Piton A, Poquet H, Redin C, Masurel A, Lauer J, Muller J, Thevenon J, Herengen Y, Chancenotte S, Bonnet M, Pinoit JM, Huet F, Thauvin-Robinet C, Jaeger AS, Le Gras S, Jost B, Gerard B, Peoc'h K, Launay JM, Faivre L, Mandel JL. 20 ans apres: a second mutation in MAOA identified by targeted high-throughput sequencing in a family with altered behavior and cognition. *Eur J Hum Genet* 2014;22:776–83.
- 43 Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet* 2003;34:27–9.
- 44 Blasi F, Bacchelli E, Pesaresi G, Carone S, Bailey AJ, Maestrini E. Absence of coding mutations in the X-linked genes neuroligin 3 and neuroligin 4 in individuals with autism from the IMGSAC collection. *Am J Med Genet B Neuropsychiatr Genet* 2006;141B:220–1.
- 45 Gauthier J, Bonnel A, St-Onge J, Karemra L, Laurent S, Mottron L, Fombonne E, Joobter R, Rouleau GA. NLGN3/NLGN4 gene mutations are not responsible for autism in the Quebec population. *Am J Med Genet B Neuropsychiatr Genet* 2005;132B:74–5.
- 46 Liu Y, Du Y, Liu W, Yang C, Wang H, Gong X. Lack of association between NLGN3, NLGN4, SHANK2 and SHANK3 gene variants and autism spectrum disorder in a Chinese population. *PLoS One* 2013;8:e56639.
- 47 Vincent JB, Kolozsvari D, Roberts WS, Bolton PF, Gurling HM, Scherer SW. Mutation screening of X-chromosomal neuroligin genes: no mutations in 196 autism probands. *Am J Med Genet B Neuropsychiatr Genet* 2004;129B:82–4.
- 48 Wermter AK, Kamp-Becker I, Strauch K, Schulte-Korne G, Remschmidt H. No evidence for involvement of genetic variants in the X-linked neuroligin genes NLGN3 and NLGN4X in probands with autism spectrum disorder on high functioning level. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:535–7.
- 49 Yan J, Oliveira G, Coutinho A, Yang C, Feng J, Katz C, Sram J, Bockholt A, Jones IR, Craddock N, Cook EH Jr, Vicente A, Sommer SS. Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients. *Mol Psychiatry* 2005;10:329–32.
- 50 Ylisaukko-oja T, Rehnstrom K, Auranen M, Vanhala R, Alen R, Kempas E, Ellonen P, Turunen JA, Makkonen I, Riikonen R, Nieminen-von Wendt T, von Wendt L, Peltonen L, Jarvela I. Analysis of four neuroligin genes as candidates for autism. *Eur J Hum Genet* 2005;13:1285–92.
- 51 Muncke N, Jung C, Rudiger H, Ulmer H, Roeth R, Hubert A, Goldmuntz E, Driscoll D, Goodship J, Schon K, Rappold G. Missense mutations and gene interruption in PROSIT240, a novel TRAP240-like gene, in patients with congenital heart defect (transposition of the great arteries). *Circulation* 2003;108:2843–50.
- 52 Asadollahi R, Oneda B, Sheth F, Azzarello-Burri S, Baldinger R, Joset P, Latal B, Knirsch W, Desai S, Baumer A, Houge G, Andrieux J, Rauch A. Dosage changes of MED13L further delineate its role in congenital heart defects and intellectual disability. *Eur J Hum Genet* 2013;21:1100–4.
- 53 van Haelst MM, Monroe GR, Duran K, van Binsbergen E, Breur JM, Giltay JC, van Haften G. Further confirmation of the MED13L haploinsufficiency syndrome. *Eur J Hum Genet* 2014 Apr 30. Published Online First. doi: 10.1038/ejhg.2014.69
- 54 Vulto-van Silfhout AT, Rajamanickam S, Jensik PJ, Vergult S, de Rocker N, Newhall KJ, Raghavan R, Reardon SN, Jarrett K, McIntyre T, Bulinski J, Ownby SL, Huggenvik JI, McKnight GS, Rose GM, Cai X, Willaert A, Zweier C, Endeide S, de Ligt J, van Bon BW, Lugtenberg D, de Vries PF, Veltman JA, van Bokhoven H, Brunner HG, Rauch A, de Brouwer AP, Carvill GL, Hoischen A, Mefford HC, Eichler EE, Vissers LE, Menten B, Collard MW, de Vries BB. Mutations Affecting the SAND Domain of DEAF1 Cause Intellectual Disability with Severe Speech Impairment and Behavioral Problems. *Am J Hum Genet* 2014;94:649–61.
- 55 Faqeih EA, Al-Owain M, Colak D, Kenana R, Al-Yafeey Y, Al-Dosary M, Al-Saman A, Albalawi F, Al-Sarar D, Domiaty D, Daghestani M, Kaya N. Novel homozygous DEAF1 variant suspected in causing white matter disease, intellectual disability, and microcephaly. *Am J Med Genet A* 2014;164A:1565–70.
- 56 Albers CA, Paul DS, Schulze H, Freson K, Stephens JC, Smethurst PA, Jolley JD, Cvejic A, Kostadima M, Bertone P, Breuning MH, Debili N, Deloukas P, Favier R, Fiedler J, Hobbs CM, Huang N, Hurles ME, Kiddie G, Krapels I, Nurden P, Ruivenkamp CA, Sambrook JG, Smith K, Stemple DL, Strauss G, Thys C, van Geet C, Newbury-Ecob R, Ouwehand WH, Ghevaert C. Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome. *Nat Genet* 2012;44:435–9, S1–2.
- 57 Unger S, Mainberger A, Spitz C, Bahr A, Zeschnick C, Zabel B, Superti-Furga A, Morris-Rosendahl DJ. Filamin A mutation is one cause of FG syndrome. *Am J Med Genet A* 2007;143A:1876–9.
- 58 Salmi M, Bruneau N, Cillario J, Lozovaya N, Massacrier A, Buhler E, Cloarec R, Tsintsadze T, Watrin F, Tsintsadze V, Zimmer C, Villard C, Lafitte D, Cardoso C, Bao L, Lesca G, Rudolf G, Muscatelli F, Pauly V, Khalilov I, Durbec P, Ben-Ari Y, Burnashev N, Represa A, Szepetowski P. Tubacin prevents neuronal migration defects and epileptic activity caused by rat Srxp2 silencing in utero. *Brain* 2013;136(Pt 8):2457–73.
- 59 Sia GM, Clem RL, Huganir RL. The human language-associated gene SRPX2 regulates synapse formation and vocalization in mice. *Science* 2013;342:987–91.
- 60 Reinthal EM, Lal D, Jurkowski W, Feucht M, Steinbock H, Gruber-Sedlmayr U, Ronen GM, Geldner J, Haberlandt E, Neophytou B, Hahn A, Altmuller J, Thiele H, Toliat MR, Lerche H, Nurnberg P, Sander T, Neubauer BA, Zimprich F. Analysis of ELP4, SRPX2, and interacting genes in typical and atypical rolandic epilepsy. *Epilepsia* 2014 Aug. Published Online First. doi: 10.1111/epi.12712.
- 61 Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, Salmi M, Tsintsadze T, Addis L, Motte J, Wright S, Tsintsadze V, Michel A, Doummar D, Lascelles K, Strug L, Waters P, de Bellescize J, Vrielynck P, de Saint Martin A, Ville D, Ryvlin P, Arzimanoglou A, Hirsch E, Vincent A, Pal D, Burnashev N, Sanlaville D, Szepetowski P. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. *Nat Genet* 2013;45:1061–6.
- 62 Griggs BL, Ladd S, Saul RA, DuPont BR, Srivastava AK. Dicator of cytokinesis 8 is disrupted in two patients with mental retardation and developmental disabilities. *Genomics* 2008;91:195–202.
- 63 Laumonnier F, Holbert S, Ronce N, Faravelli F, Lenzner S, Schwartz CE, Lespinasse J, Van Esch H, Lacombe D, Gozlet C, Phan-Dinh Tuy F, van Bokhoven H, Fryns JP, Chelly J, Ropers HH, Moraine C, Hamel BC, Brault S. Mutations in PHF8 are associated with X linked mental retardation and cleft lip/cleft palate. *J Med Genet* 2005;42:780–6.
- 64 Yu HC, Sloan JL, Scherer G, Brebner A, Quintana AM, Achilly NP, Manoli I, Coughlin CR II, Geiger EA, Schneck U, Watkins D, Suormala T, Van Hove JL, Fowler B, Baumgartner MR, Rosenblatt DS, Venditti CP, Shaikh TH. An X-linked cobalamin disorder caused by mutations in transcriptional coregulator HCFC1. *Am J Hum Genet* 2013;93:506–14.
- 65 Leen WG, Klepper J, Verbeek MM, Leferink M, Hofste T, van Engelen BG, Wevers RA, Arthur T, Bahi-Buisson N, Ballhausen D, Bekhof J, van Bogaert P, Carrilho I, Chabrol B, Champion MP, Coldwell J, Clayton P, Donner E, Evangelista A, Ebinger F, Farrell K, Forsyth RJ, de Goede CG, Gross S, Grunewald S, Holthausen H, Jayawant S, Lachlan K, Laugel V, Leppig K, Lim MJ, Mancini G, Marina AD, Martorell L, McMenamin J, Meuwissen ME, Mundy H, Nilsson NO, Panzer A, Poll-The BT, Rauscher C, Rouselle CM, Sandvig I, Scheffner T, Sheridan E, Simpson N, Sykora P, Tomlinson R, Trounce J, Webb D, Weschke B, Scheffer H, Willemsen MA. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. *Brain* 2010;133(Pt 3):655–70.
- 66 Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, Itsara A, Vives L, Walsh T, McCarthy SE, Baker C, Mefford HC, Kidd JM, Browning SR, Browning BL, Dickel DE, Levy DL, Ballif BC, Platky K, Farber DM, Gowans GC, Wetherbee JJ, Asamoah A, Weaver DD, Mark PR, Dickerson J, Garg BP, Ellingwood SA, Smith R, Banks VC, Smith W, McDonald MT, Hoo JJ, French BN, Hudson C, Johnson JP, Ozmore JR, Moeschler JB, Surti U, Escobar LF, El-Khechen D, Gorski JL, Kussmann J,

- Salbert B, Lacassie Y, Biser A, McDonald-McGinn DM, Zackai EH, Deardorff MA, Shaikh TH, Haan E, Friend KL, Fichera M, Romano C, Gecz J, DeLisi LE, Sebat J, King MC, Shaffer LG, Eichler EE. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet* 2010;42:203–9.
- Leblond CS, Heinrich J, Delorme R, Proepper C, Betancur C, Huguet G, Konyukh M, Chaste P, Ey E, Rastam M, Anckarsater H, Nygren G, Gillberg IC, Melke J, Toro R, Regnault B, Fauchereau F, Mercati O, Lemiere N, Skuse D, Poot M, Holt R, Monaco AP, Jarvela I, Kantojarvi K, Vanhala R, Curran S, Collier DA, Bolton P, Chiocchetti A, Klauck SM, Poustka F, Freitag CM, Waltes R, Kopp M, Duketis E, Bacchelli E, Minopoli F, Ruta L, Battaglia A, Mazzone L, Maestrini E, Sequeira AF, Oliveira B, Vicente A, Oliveira G, Pinto D, Scherer SW, Zelenika D, Delepine M, Lathrop M, Bonneau D, Guinchat V, Devillard F, Assouline B, Mouren MC, Leboyer M, Gillberg C, Boeckers TM, Bourgeron T. Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. *PLoS Genet* 2012;8:e1002521.
- Schaaf CP, Sabo A, Sakai Y, Crosby J, Muzny D, Hawes A, Lewis L, Akbar H, Varghese R, Boerwinkle E, Gibbs RA, Zoghbi HY. Oligogenic heterozygosity in individuals with high-functioning autism spectrum disorders. *Hum Mol Genet* 2011;20:3366–75.
- Mandel JL, Chelly J. Monogenic X-linked mental retardation: is it as frequent as currently estimated? The paradox of the ARX (Aristless X) mutations. *Eur J Hum Genet* 2004;12:689–93.
- Nava C, Lamari F, Heron D, Mignot C, Rastetter A, Keren B, Cohen D, Faudet A, Bouteller D, Gilleron M, Jacquette A, Whalen S, Afenjar A, Perisse D, Laurent C, Dupuits C, Gautier C, Gerard M, Huguet G, Caillet S, Leheup B, Leboyer M, Gillberg C, Delorme R, Bourgeron T, Brice A, Depienne C. Analysis of the chromosome X exome in patients with autism spectrum disorders identified novel candidate genes, including TMLHE. *Transl Psychiatry* 2012;2:e179.
- Tarpey PS, Smith R, Pleasance E, Whibley A, Edkins S, Hardy C, O'Meara S, Latimer C, Dicks E, Menzies A, Stephens P, Blow M, Greenman C, Xue Y, Tyler-Smith C, Thompson D, Gray K, Andrews J, Barthorpe S, Buck G, Cole J, Dummore R, Jones D, Maddison M, Mironenko T, Turner R, Turrell K, Varian J, West S, Widaa S, Wray P, Teague J, Butler A, Jenkinson A, Jia M, Richardson D, Shepherd R, Wooster R, Tejada ML, Martinez F, Carvill G, Goliath R, de Brouwer AP, van Bokhoven H, Van Esch H, Chelly J, Raynaud M, Ropers HH, Abidi FE, Srivastava AK, Cox J, Luo Y, Mallya U, Moon J, Parnau J, Mohammed S, Tolmie JL, Shoubridge C, Corbett M, Gardner A, Haan E, Rujirabanjerd S, Shaw M, Vandeleur L, Fullston T, Easton DF, Boyle J, Partington M, Hackett A, Field M, Skinner C, Stevenson RE, Bobrow M, Turner G, Schwartz CE, Gecz J, Raymond FL, Futreal PA, Stratton MR. A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. *Nat Genet* 2009;41:535–43.
- Simpson DA, Clark GR, Alexander S, Silvestri G, Willoughby CE. Molecular diagnosis for heterogeneous genetic diseases with targeted high-throughput DNA sequencing applied to retinitis pigmentosa. *J Med Genet* 2011;48:145–51.
- Brownstein Z, Friedman LM, Shahin H, Oron-Karni V, Kol N, Abu Rayyan A, Parzefall T, Lev D, Shalev S, Frydman M, Davidov B, Shohat M, Rahile M, Lieberman S, Levy-Lahad E, Lee MK, Shomron N, King MC, Walsh T, Kanaan M, Avraham KB. Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families. *Genome Biol* 2011;12:R89.
- Huang L, Jolly LA, Willis-Owen S, Gardner A, Kumar R, Douglas E, Shoubridge C, Wieczorek D, Tschach A, Cohen M, Hackett A, Field M, Froyen G, Hu H, Haas SA, Ropers HH, Kalscheuer VM, Corbett MA, Gecz J. A noncoding, regulatory mutation implicates HCFC1 in nonsyndromic intellectual disability. *Am J Hum Genet* 2012;91:694–702.
- Gibbons RJ, Wada T, Fisher CA, Malik N, Mitson MJ, Steensma DP, Fryer A, Goudie DR, Krantz ID, Traeger-Synodinos J. Mutations in the chromatin-associated protein ATRX. *Hum Mutat* 2008;29:796–802.

SUPPORTING INFORMATION

SUPPORTING METHODS.....	2
SUPPORTING FIGURES.....	3
Figure S1. Decisional trees for the molecular diagnosis of intellectual disability.	3
Figure S2. <i>DYRK1A</i> , two de novo truncating causative mutations	4
Figure S3. <i>GRIN1</i> , one causative <i>de novo</i> missense mutation	5
Figure S4. <i>MED13L</i> , one causative <i>de novo</i> truncating mutation	7
Figure S5. <i>RAII</i> , one causative <i>de novo</i> truncating mutation.....	9
Figure S6. <i>SHANK3</i> , one causative <i>de novo</i> truncating mutation	10
Figure S7. <i>SLC2A1</i> , one causative <i>de novo</i> truncating mutation and one potentially-causative inherited truncating mutation	11
Figure S8. <i>SYNGAP1</i> , one causative <i>de novo</i> mutation affecting splicing.....	13
Figure S9. <i>TCF4</i> , two <i>de novo</i> truncating causative mutations and one heterozygous potentially-causative splice site mutation	15
Figure S10. <i>ATRX</i> , one maternally-inherited causative nonsense mutation.....	17
Figure S11. <i>CUL4B</i> , one causative frameshift mutation	18
Figure S12. <i>DMD</i> , one causative frameshift mutation in two affected brothers without muscular phenotype	20
Figure S13. <i>FMRI</i> : one causative exon deletion in 3 affected brothers.....	22
Figure S14. <i>IL1RAPL1</i> , one causative frameshift mutation and exon 7 deletion in two affected brothers.....	24
Figure S15. <i>IQSEC2</i> , one causative <i>de novo</i> nonsense mutation	25
Figure S16. <i>KDM5C</i> , two causative mutations	26
Figure S17. <i>MECP2</i> , three causative mutations	28
Figure S18. <i>PHF8</i> , one causative mutation affecting splicing	30
Figure S19. <i>SLC16A2/MCT8</i> , one causative missense mutation.....	31
Figure S20. <i>NLGN3</i> , one potentially-causative missense mutation	33
Figure S21. <i>PQBP1</i> , one potentially-causative missense mutation in two affected brothers.....	34
Figure S22. Other candidate variants not or ambiguously co-segregating with ID in probands' families	36
REFERENCES	39

SUPPORTING METHODS

Gene selection justification and design strategy

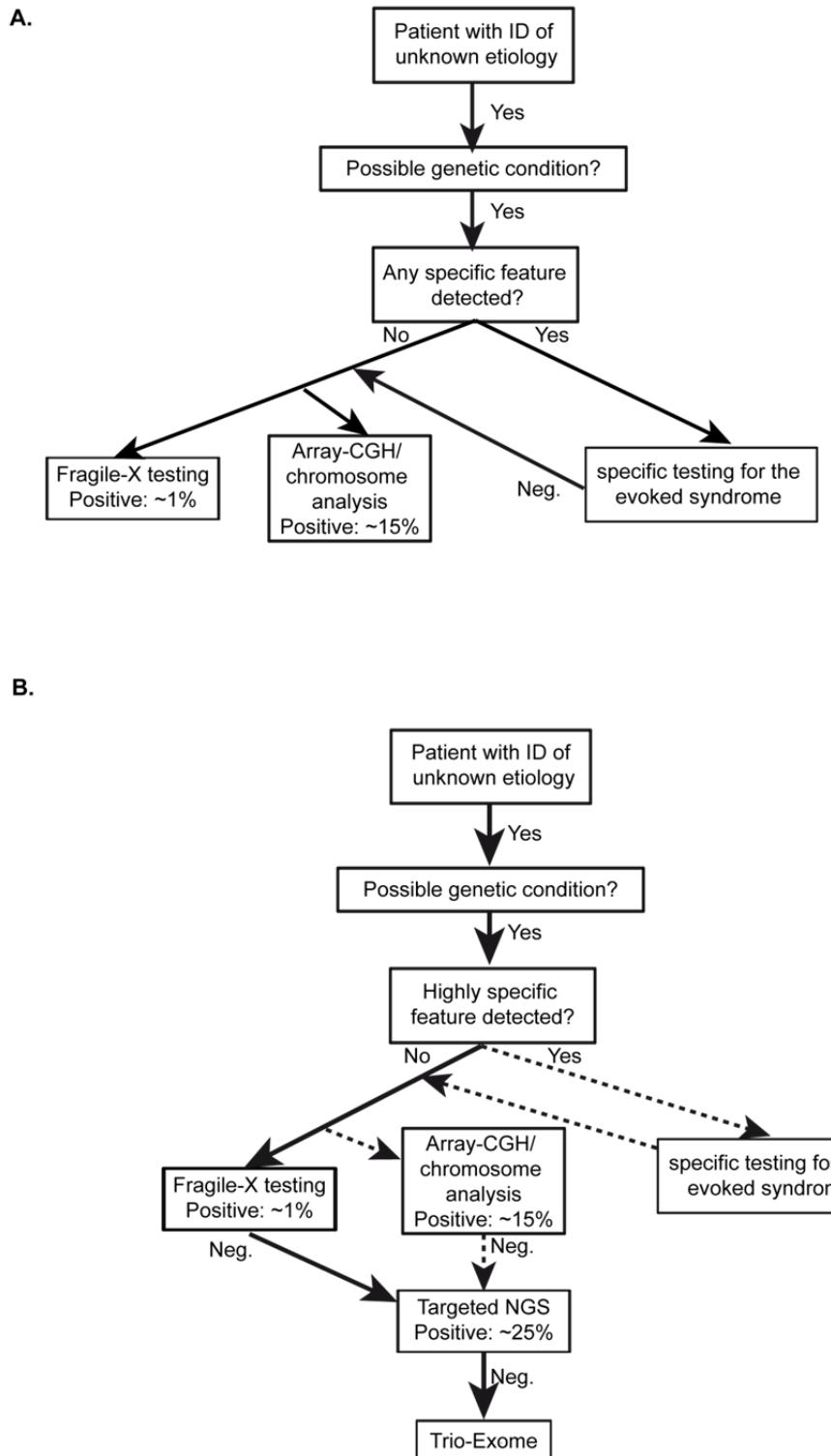
The design of the capture baits in terms of target genes was performed in November 2011 and therefore omits genes for which implication in ID was identified subsequently. Our strategy for including genes was based on the following scientific and economic considerations. First, we were limited to a total of 1-Mb of sequences as this size corresponded to a major price threshold for the manufacture of the array. Next, as we wanted to estimate the contribution of X-linked mutations in affected males and since we assumed that the vast majority of XLID genes had already been identified [1-3], we included almost all X-linked ID or ASD genes known at that time (99 in total, seven being excluded subsequently to our critical analysis of XLID genes, namely *ABCD1*, *AGTR2*, *FANCB*, *IKBKG*, *MTM1*, *PGK1* and *RAB40AL*; [4]). For autosomal genes, we favored those involved in non-syndromic ID/ASD but also included some implicated in syndromic forms. We included most of the genes described as involved in autosomal dominant/haploinsufficient forms of ID or ASD. Considering autosomal recessive genes, we only included those for which convincing mutations had been reported in at least two families or in single families but with additional evidences supporting their implication in ID (functional validations, CNV encompassing the gene in patients with ID, etc; [5-7]). Selected genes had to be associated to conditions in which intellectual disability (or ASD) is the major clinical concern, hence excluding diseases with major involvement of other organs (ex: genes encoding subunits of mitochondrial electron transfer complex since their involvement can be suspected on the basis of metabolic investigations, most genes responsible for severe brain anomalies detectable by MRI such as Joubert syndrome, leukodystrophies...). Conversely, we did include the distal part of *DMD* encoding the brain expressed DP71 transcript, as a few reported patients carrying mutations affecting DP71 presented with mild muscle involvement while had been initially ascertained on the basis of ID/autism [8]. In total, we selected 45 genes associated to an autosomal dominant/haploinsufficiency type of ID, 66 to an autosomal recessive one, and 7 to other complex or unclear mode of transmission (**Table S1**).

Capture baits tiling

RNA capture baits were designed via eArray to achieve a tiling frequency of 5x (i.e. number of independent probes covering each nucleotide of a targeted region; a probe being considered as an orphan if it is >100-bp away from any neighboring probe). Some segments were left without bait due to multi-mapping thus requiring manual design. The number of probe replicates was then customized according to their GC-content or associated region complexity in order to maximize the pull-down efficiency (60<%GC<65: x4, 65<%GC<70: x5, 70<%GC: x7, orphan baits: x10, custom baits for uncovered regions: x5, total: 53,000 baits).

SUPPORTING FIGURES

Figure S1. Decisional trees for the molecular diagnosis of intellectual disability.



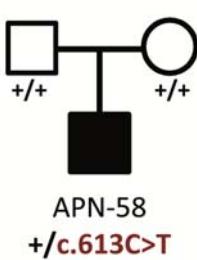
A: Current tree used in routine in most diagnostic centers; **B:** Proposed new decisional tree integrating NGS-approaches. Dashed-lines: steps that might become obsolete upon analysis of cost effectiveness and reliability (targeted sequencing may replace array-CGH for reliable detection of CNVs).

Figure S2. *DYRK1A*, two de novo truncating causative mutations

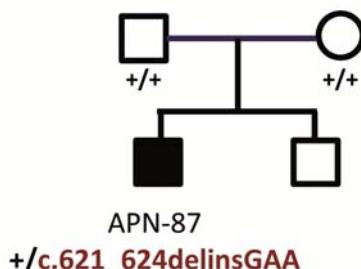
APN-58: c.613C>T (NM_001396.3), p.Arg205*, heterozygous *de novo*

APN-87: c.621_624delinsGAA (NM_001396.3), p.Glu208Asnfs*3, heterozygous *de novo*

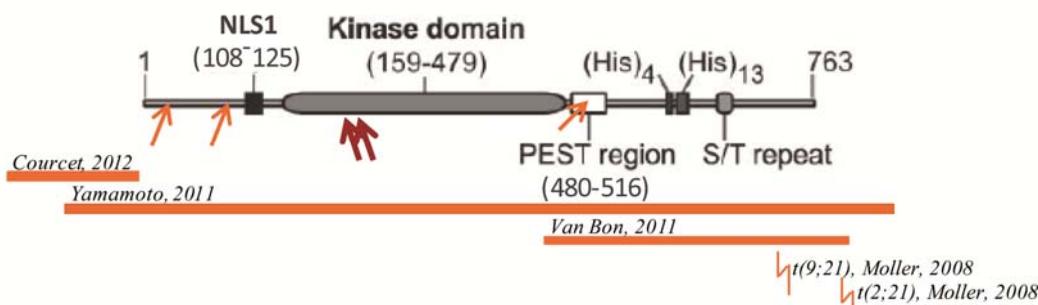
A.



B.



C.



A. Pedigree of patient APN-58; **B.** Pedigree of patient APN-87; **C.** Representation of the protein DYRK1A with its putative domains, localization of causative mutations identified in this study (red arrows) and of previously reported mutations (orange arrows), deletions (orange bars), translocations (orange lightning) in patients [9-13].

Previous implication of *DYRK1A* in cognitive disorders:

Trisomy of *DYRK1A* was for long proposed having a major role in the cognitive impairment observed in Down syndrome patients. Recently, several mutations were identified in patients with ID or ASD, and very similar clinical phenotype: microcephaly, growth retardation, developmental delay, major speech impairments, feeding difficulties, stereotypic movements, large low-set ears, abnormal hair growth and specific facial features (micrognathia, hypotelorism, thin lips and large nose with long philtrum; [9 10 12-16]).

Patient APN-58 (male, born in 1998)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative; Karyotype: normal

Telomeres MLPA: normal; *MED12*, *ZDHHC9*, *FBN1*, *TGFBR1* and *TGFBR2* direct sequencing: negative

Clinical information:

Moderate ID, borderline microcephaly (-2 SD), developmental delay (delayed sitting/walking/speech acquisition), hyperactivity, feeding disorders (sucking difficulties). Pectus excavatum, arachnodactyly, dysmorphic traits.

Patient APN-87 (male, born in 2009)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative

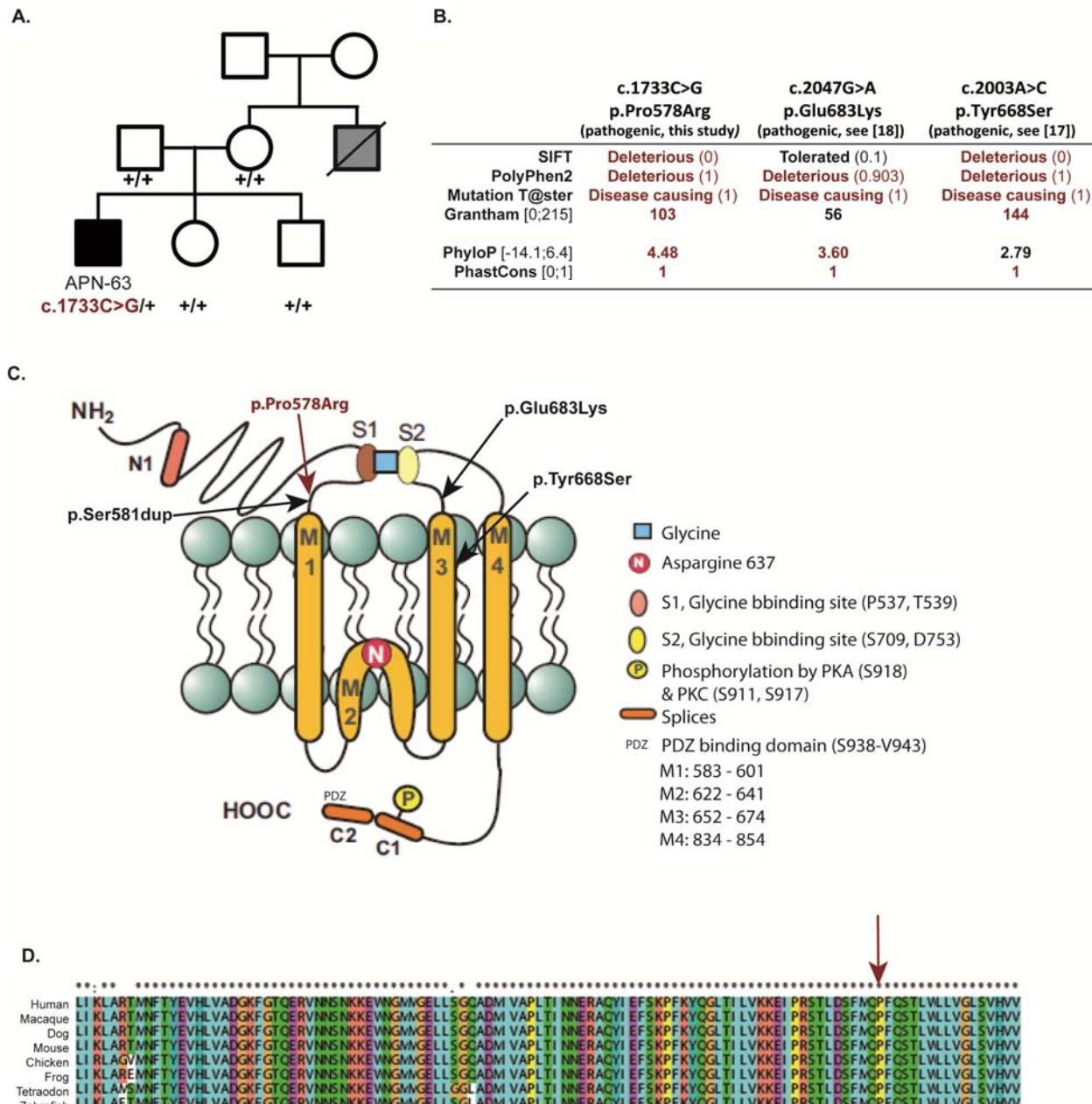
Clinical information:

Moderate ID, developmental delay (late sitting/walking/speech acquisition). Microcephaly (-4 SD) Dysmorphic traits. Hyperopia, astigmatism. Sleep disturbances, stereotypic movements. Hypertonia, opisthotonus episodes, feeding disorders, constipation.

MRI: discrete hydrocephaly, dilatation of lateral ventricles.

Figure S3. GRIN1, one causative *de novo* missense mutation

APN-63: c.1733C>G (NM_001185090.1), p.Pro578Arg, heterozygous *de novo*



A. Pedigree of patient APN-63; **B.** Associated prediction scores comparing the pathogenic missense mutation identified in this study with two pathogenic previously described missense mutations [17 18], showing a predicted deleterious status and a high conservation at the nucleotide level for the missense p.Pro578Arg; **C.** Representation of GRIN1 protein with the different domains. Mutations that were reported in ID patients are also indicated (black arrows), with the herein detected mutation in red (adapted from Parsons et al., 2007; [19]); **D.** Alignment of GRIN1 protein orthologs using Clustalw showing the high conservation of the affected residue Pro578.

Previous implications of *GRIN1* in cognitive disorders

Following the evidence for the implication of NMDA (N-methyl-D-aspartate) type ionotropic glutamate receptors in schizophrenic-like behavior in mouse models, various studies have screened cohorts of schizophrenic patients but failed to identify any *GRIN1* mutations [20-22]. Later on, *de novo* mutations (one missense, p.Glu683Lys and one in-frame duplication, p.Ser581dup) were reported in patients with moderate to severe non-syndromic ID [18]. Both mutations resulted in decreased efficiency of the NMDAR channel. More recently, one patient was lately identified as carrying a *de novo* missense (p.Tyr668Ser) and presenting with early infantile epilepsy, no regression, severe ID, acquired relative microcephaly and no speech at 18 [17].

Patient APN-63 (male, born in 1972)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative; Karyotype: negative

MED12, ZDHCC19, UPF3B, FBNI, TGFBR2 direct sequencing: negative

FraxA/FraxE: negative

Clinical information:

Proband: Severe ID, poor speech. Severe hypotonia and feeding disorders in early infancy, developmental delay (walking/speech), behavioral and mood disorders. Arachnodactyly, mild *pectus excavatum*.

MRI, scanner, echocardiography: normal

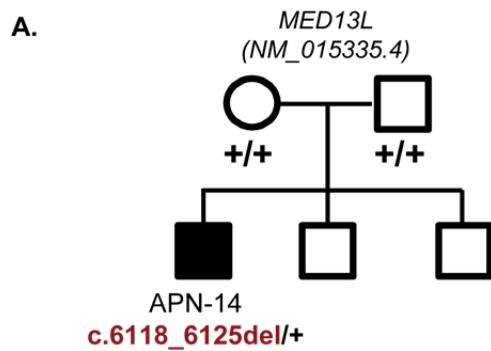
Skin biopsy: normal fibrilin

Plasma homocysteine levels: normal

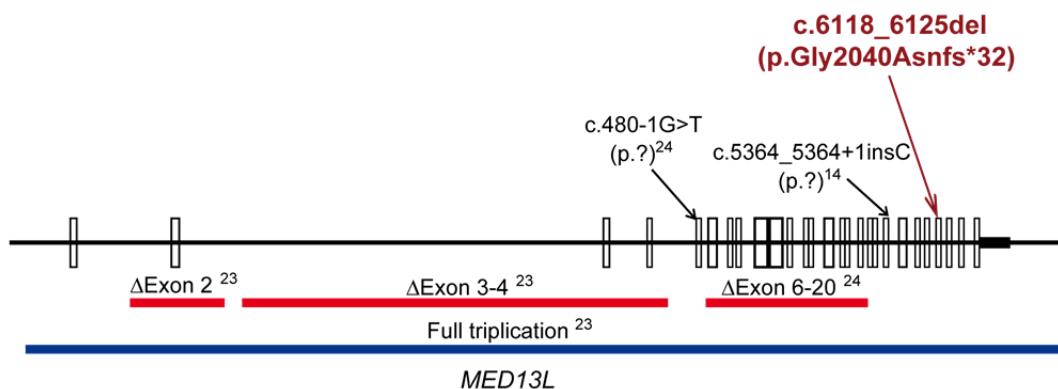
Maternal Uncle: Developmental delay, behavioral and mood disorders (aggression), but normal speech and no hypotonia. Within the last 10 years of life: cognitive decline, anxiety, significant swallowing disorders. Deceased in 2010 from a heart attack.

Figure S4. *MED13L*, one causative *de novo* truncating mutation

APN-14: c.6118_6125del (NM_015335.4), p.Gly2040Asnfs*32, heterozygous *de novo*



B.



A. Pedigree of patient APN-14; **B.** Visualization of the previously reported intragenic deletions and point mutations associated or probably-associated to *MED13L*-haploinsufficiency syndrome [14 23 24], and the point mutation identified in our patient.

Previous implication of *MED13L* in ID

A chromosomal balanced translocation disrupting *MED13L* was described in a patient with transposition of the great arteries (TGA) and intellectual disability. Three heterozygous missense mutations were then identified in patients with isolated TGA and a homozygous missense mutation in two siblings with non-syndromic ID from a consanguineous family [6 25]. More recently, Asadollahi et al. described three patients with copy number changes affecting *MED13L* and delineated a recognizable *MED13L*-haploinsufficiency syndrome characterized by hypotonia, moderate ID, variable degrees of conotruncal heart defect, facial hypotonia and dysmorphic traits (upslanting palpebral fissures, flat nasal root with bulbous tip, deep philtrum, micrognathia, large low-set ears, and broad forehead; [23]). This haploinsufficiency syndrome delineated by ID, uneven cardiac defects, developmental and speech delays, and hypotonic open mouth appearance was further confirmed by the report of two additional patients, carrying a *de novo* splice site mutation and a *de novo* exon deletion respectively[24].

Patient APN-14 (male, born in 1996)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: duplication in 15q11.2 of 2.1-Mb (encompassing *CYFIP1*), considered as non pathogenic

Myotonic dystrophy: negative for DM1/*DMPK* repeat expansions

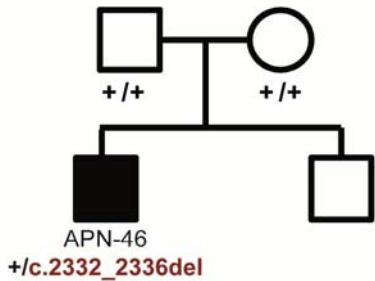
PAX6 direct sequencing: negative

Clinical information: Moderate ID, motor development delay, muscular hypotonia, dysarthria, facial dysmorphic traits (round face, hypertelorism, everted lower lip, low set ears), buccal dysprasia, coloboma. Echocardiography (2010): normal.

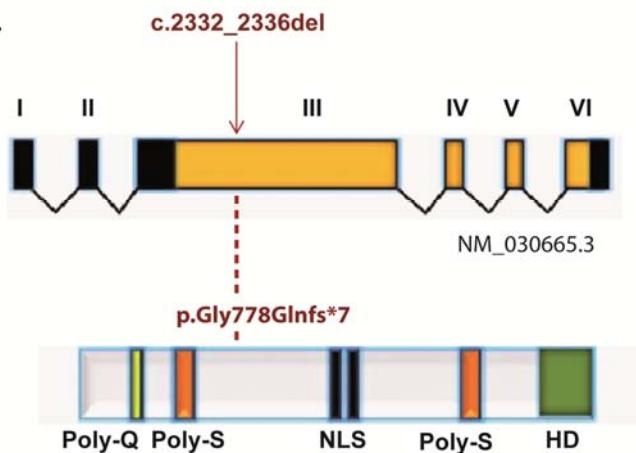
Figure S5. *RAII*, one causative *de novo* truncating mutation

APN-46: c.2332_2336del (NM_030665.3), p.Gly778Glnfs*7, heterozygous *de novo*

A.



B.



A. Pedigree of patient APN-46; **B.** Representation of RAI1 at both gene and protein levels: polyglutamine (Poly-Q) and polyserine (Poly-S) tracts, bipartite nuclear localization signals (NLS), and C-terminal plant homeodomain (PHD), (adapted from [26]).

Previous implication of *RAII* in cognitive disorders

Smith-Magenis syndrome (SMS; OMIM #182290, *607642) is a complex disorder characterized by variable degree of intellectual disability, sleep disturbances, craniofacial and skeletal anomalies, self-injurious and attention-seeking behaviors, and speech and motor delay. SMS is caused by either a heterozygous ~4-Mb deletion in 17p11.2 or heterozygous mutations in *RAII*[26-28].

Patient APN-46 (male, born in 2009)

Preliminary genetic tests:

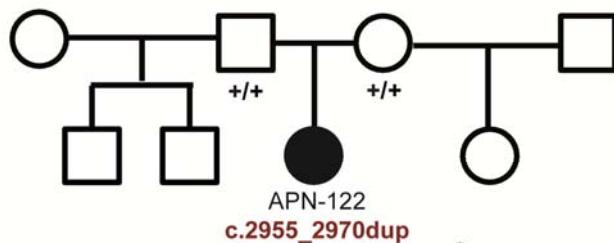
Fragile-X test: negative; Array-CGH array: negative
ARX, Prader-Willi, *GAMT* direct sequencing: negative

Clinical information: Moderate ID, **developmental delay**, **sleep disorders**, **severe behavioral disorders** (auto and hetero aggressivity), **no speech**. **Facial hypotonia**, **frontal bossing**, **upslanting palpebral fissures**, **midface hypoplasia**, a broad square-shaped face with depressed nasal bridge. Normal stature and OFC but overweight. Sleep disturbances after reevaluation. Hypermetropia, **strabismus** and astigmatism.
 (in bold: classical features of Smith Magenis syndrome, from [26])

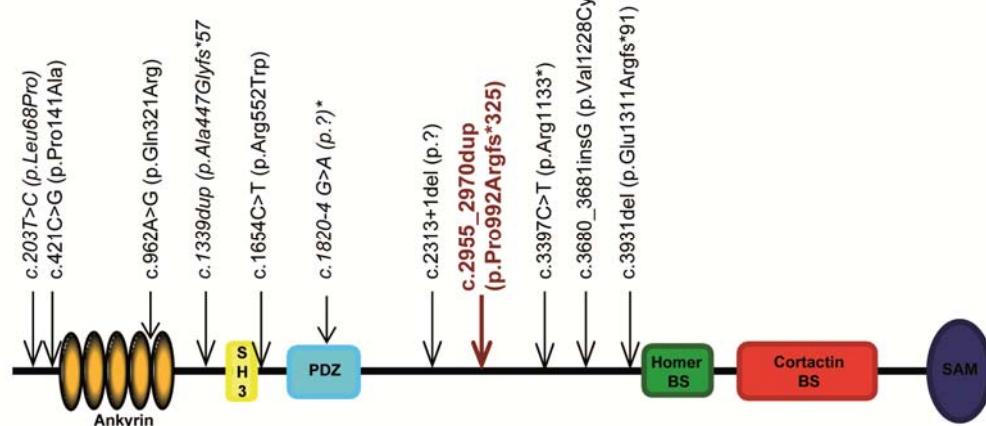
Figure S6. SHANK3, one causative *de novo* truncating mutation

APN-122: c.2955_2970dup (NM_001080420.1), p.Pro992Argfs*325, heterozygous *de novo*

A.



B.



A. Pedigree of patient APN-122; **B.** Representation of protein SHANK3 and its putative functional domains, and distribution of all reported point mutations in ASDs, ID and SCZ patients (deletions, translocations and duplications are not represented; adapted from [29-30]). Italic: mutations inherited from an unaffected parent. *: variant also detected in EVS (MAF=0.02%, 2/6237 individuals). SH3: Src homology-3 domain; PDZ: PDZ domain; SAM: sterile α-motif domain.

Previous implication of SHANK3 in cognitive disorders

SHANK3 is the major haploinsufficient gene implicated in Phelan-McDermid syndrome (PMS, OMIM #606232) that is caused by a deletion in 22q13 encompassing several other genes and whose characteristic phenotype is: moderate to severe ID, absent or severe speech delay, neonatal hypotonia, and minor facial dysmorphic traits [31]. Heterozygous *de novo* point mutations in SHANK3 have also been described in patients with autism spectrum disorders and schizophrenia associated to moderate to severe ID and poor language [29-30, 32-35]. Shank3 knock-out mice harbor concordant phenotype, with self-injurious repetitive grooming, and deficits in social interactions and communication [36-39].

Patient APN122 (female, born in 2003)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative; Karyotype: negative

RAIL and *MECP2* direct sequencing: one variant paternally inherited, considered as non-pathogenic

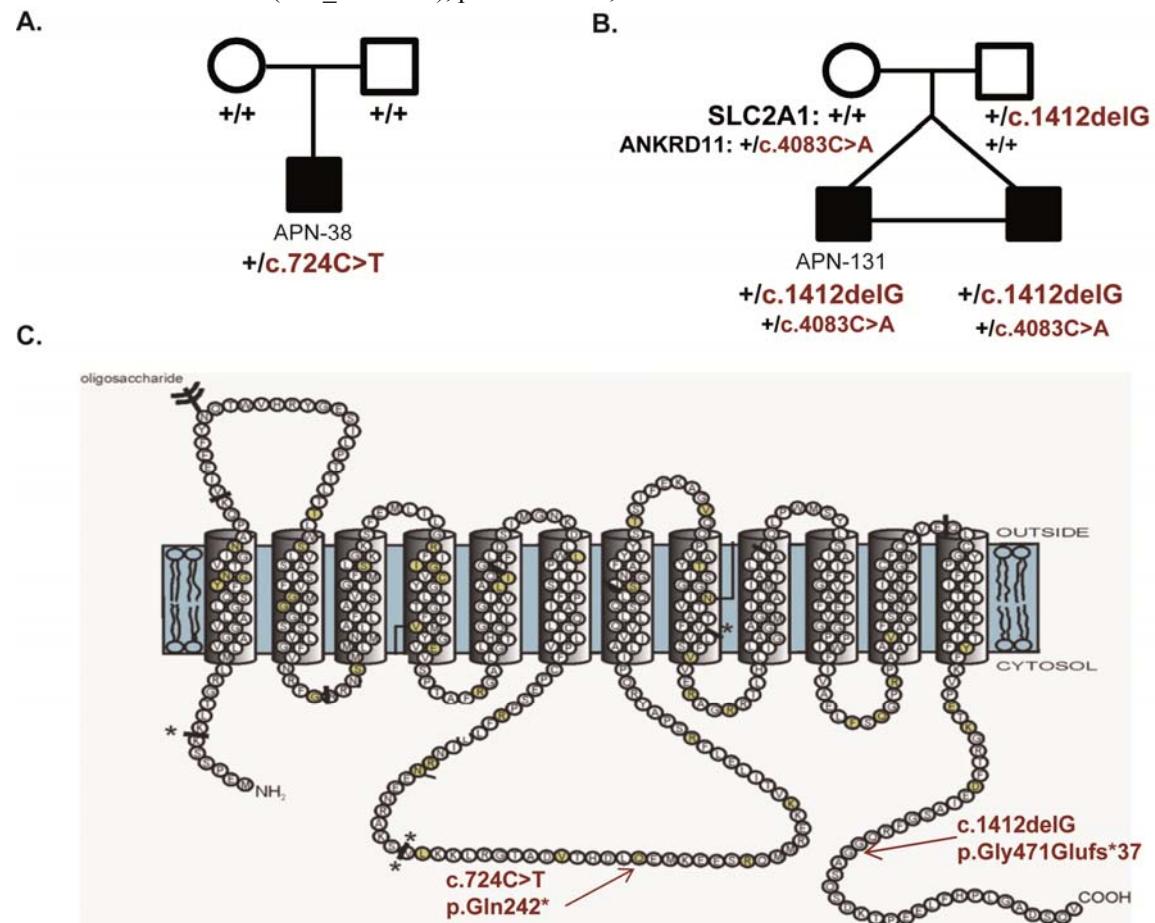
Clinical information:

Severe ID, developmental delay (acquired walking at 18 months-old, first words at 3 years-old followed by speech regression), no acquired reading/writing. Abnormal EEG without epilepsy, autistic traits, attention deficit/concentration disorder, behavioral disorders, sleep disturbances, distal spasticity, facial dysmorphic traits (upward philtrum, laterally-inserted eyebrows, bulbous nose).

Figure S7. *SLC2A1*, one causative *de novo* truncating mutation and one potentially-causative inherited truncating mutation

APN-38 : c.724C>T (NM_006516.2), p.Gln242*, heterozygous *de novo*

APN-131: c.1412delG (NM_006516.2), p.Gly471Glufs*37, heterozygous, inherited from unaffected father and *ANKRD11*: c.4083C>A (NM_013275.5), p.His1361Gln, inherited from unaffected mother



A. Pedigree of patient APN-38; **B.** Pedigree of patient APN-131; **C.** Structure of the SLC2A1 transporter and localization of the two herein described mutations reported in patients (in red; adapted from [40]).

Previous implication of *SLC2A1* in ID

Heterozygous mutations in GLUT1 transporter (*SLC2A1*) are responsible for GLUT1 deficiency syndrome-1 (GLUT1DS1, MIM #606777), a neurologic disorder characterized by infantile-onset epileptic encephalopathy associated with cognitive impairment (from learning disabilities to severe intellectual disability), delayed development, acquired microcephaly, hypotonia, motor incoordination (ataxia and dystonia), and spasticity [41 42]. Other forms of GLUT1DS can include ID, dysarthria, intermittent ataxia but no clinical seizures. Some clinical features such as sleep disturbances and headache can unevenly be described [43]. Low CSF glucose (less than 40 mg/dl) and low CSF lactate are detected in patients with GLUT1DS1. A ketogenic or modified Atkins diet often result in marked clinical improvement of the motor and seizure symptoms [41].

Previous implication of *ANKRD11* in ID

Mutations in *ANKRD11* are responsible for KBG syndrome (MIM #148050), characterized by macrodontia, distinctive craniofacial features (triangular face, ptosis, hypertelorism, prominent nasal bridge, anteverted nostrils, long philtrum, large and prominent ears, ...), short stature, costovertebral anomalies, clinodactyly, cryptorchidism and neurologic involvement that includes global developmental delay, seizures, and mild to moderate intellectual disability [44]. A mouse model carrying a missense mutation in *ankrd11* presents with important craniofacial anomalies [45].

Patient APN-38 (male, born in 2005)Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: amplification 18q21.31 and 18q21.32 inherited from the mother and concluded as non-pathogenic

ARX direct sequencing: negative; Prader-Willi/Angelman test: negative

Clinical information: Severe ID, no speech. Hypotonia with ataxia, epilepsy starting from 14-months of age.

Patient APN-131 (male, born in 2003)Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative

ARX, DMPK, CREBBP, EP300, ZEB2, PYCR1 direct sequencing: negative

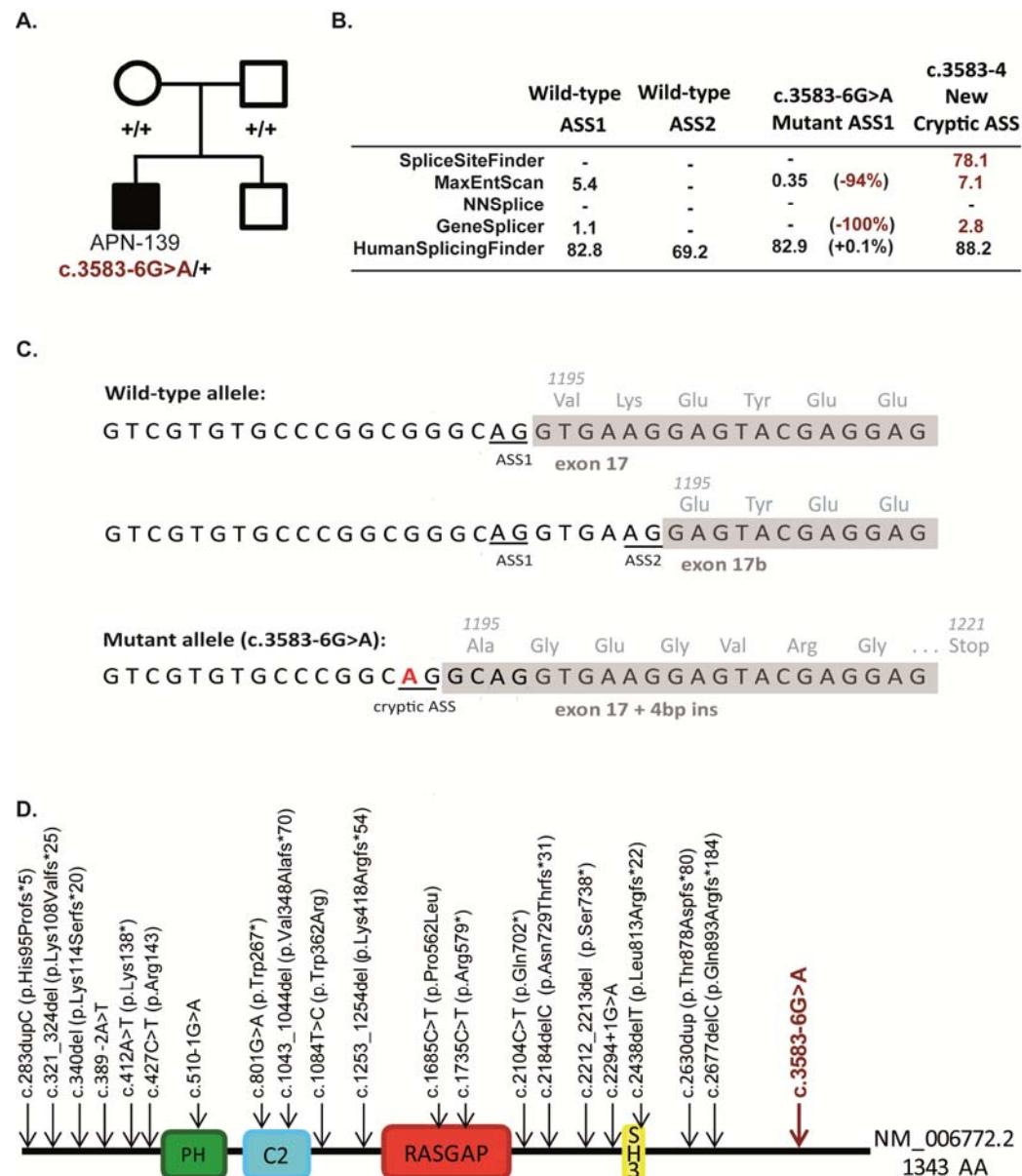
Prader-Willi/Angelman test: negative

Clinical information: Severe ID, hypotonia, hyperlaxity, developmental delay. Dorsal hemivertebra, cryptorchidism, neonatal hypoglycemia, dysmorphic features (hypertelorism, asymmetrical eyelids, thin upper lip). OFC: -2SD. No epilepsy.

Clinical phenotype of the monozygotic twin brother: highly similar.

Figure S8. *SYNGAP1*, one causative *de novo* mutation affecting splicing

APN-139: c.3583-6G>A (NM_006772.2), p.Val1195Alafs*27, heterozygous *de novo*



A. Family pedigree of patients APN-139; **B.** Prediction scores for acceptor splice sites (ASS) determined by MaxEnt, NNsplice, GeneSplicer or Human splicing Finder (HSF): prediction scores for the normal acceptor sites ASS1 (exon17) and ASS2 (exon17b) with the wild-type allele are given. The variations of scores for ASS1 with the c.3583G>A mutation are also indicated, as well as the prediction score for the new alternative cryptic ASS created by the mutation. **C.** Patient blood mRNA was analyzed and confirmed the use of this alternative cryptic ASS at c.3583-4 when the mutation is present instead of the usual ASS1 or ASS2 sites. Four nucleotides are inserted, causing a frameshift that leads to a premature truncated protein (1210 aminoacids instead of 1343); **D.** Schematic representation of protein SYNGAP1 with its different domains (PH: Pleckstrin homology-like, C2, RASGAP: Ras-GTPase activating and SH3 domains) and distributions of all SYNGAP1 mutations identified in patients since 2009.

Previous implication of *SYNGAP1* in cognitive disorders

To date, about 20 or more heterozygous truncating or missense mutations have been identified in *SYNGAP1* in patients with moderate to severe ID, with or without associated severe epilepsy or autism [46-50].

Patient APN-139 (male, born in 2008)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative

Clinical information: Moderate ID, no speech, psychomotor delay, stereotypic movements, behavioral disorders with hetero and auto-aggressivity, hypotonia, cerebellar syndrome.

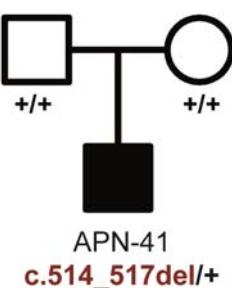
Figure S9. TCF4, two *de novo* truncating causative mutations and one heterozygous potentially-causative splice site mutation

APN-41: c.514_517del (NM_001083962.1), p.Lys172Phefs*61, heterozygous *de novo*

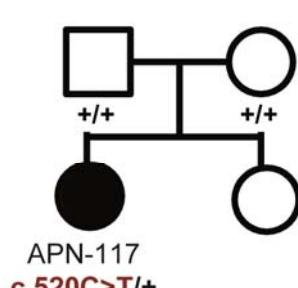
APN-117: c.520C>T (NM_001083962.1), p.Arg174*, heterozygous *de novo*

APN-101: c.1487-5G>A (NM_001083962.1), p.Arg495_Gly496insAla?, heterozygous putative splice site mutation, which may create a new cryptic splice site that would result in the in-frame addition of a glutamine codon

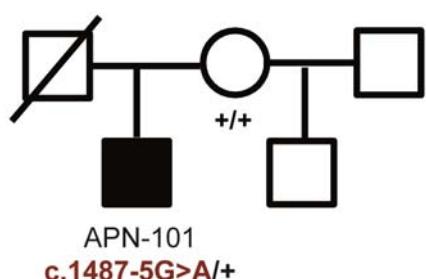
A.



B.



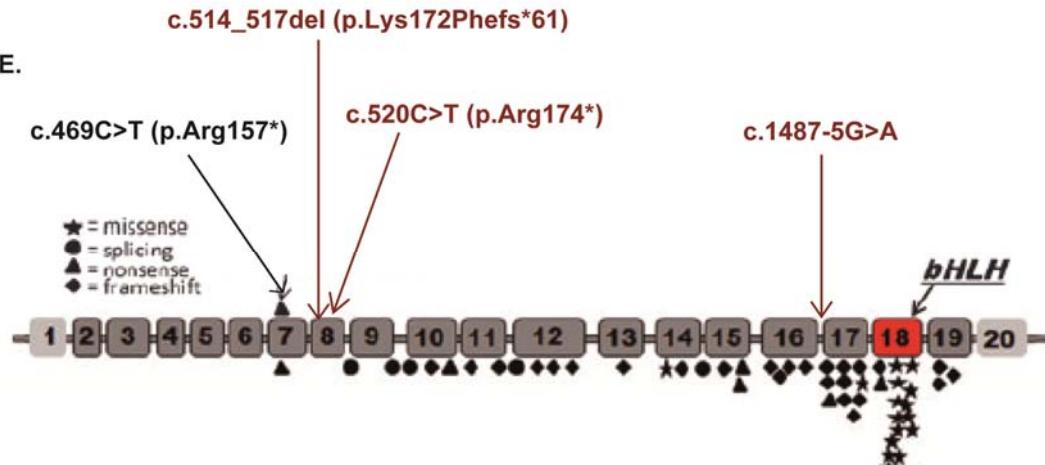
C.



D.

	Wild-type ASS	c.1487-5G>A Mutant ASS (Possibly pathogenic)	c.1487-3 New Cryptic ASS
SpliceSiteFinder	92.87	0 (-100%)	92.43
MaxEntScan	8.54	7.55 (-12%)	9.59
NNSplice	0.98	0.98	0.98
GeneSplicer	12.39	9.28 (-25%)	10.86
HumanSplicingFinder	92.62	92.69 (+0.1%)	89.69

E.



A-C. Pedigrees of patients carrying mutations in *TCF4*; **D.** Prediction scores for acceptor splice sites (ASS) as computed by SpliceSite Finder, MaxEntScan, NNSplice, GeneSplicer and Human Splicing Finder for the consensus ASS with either the wild-type or the mutated allele, and for the newly created cryptic ASS (*-3*) that adds one in frame alanine residue (tested by minigene assay); **E.** Representation of *TCF4* with location of the previously-described Pitt-Hopkins mutations (diamonds, triangles, stars and circles) and the one associated with non-syndromic ID (black arrow; adapted from [51]).

Previous implication of *TCF4* in cognitive disorders

Missense and truncating mutations in *TCF4* cause Pitt-Hopkins syndrome (MIM #610954) whose classical phenotype is: severe motor delay and intellectual disability, no speech, characteristic dysmorphic features (wide mouth), stereotypic hand movements, constipation and hyperventilation (later onset: after 6-7 years old; [52]). Recently, a truncating mutation was identified in *TCF4* in a patient with non-syndromic ID [51].

Patient APN-41 (male, born in 2008)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: 8p23.2 deletion inherited from the mother

Myotonic dystrophy: negative for DM1/*DMPK* repeat expansions

Prader-Willi test: negative

Clinical information: Severe ID with no speech. Constipation, stereotypic movements, frequent ear infections, motor development delay, hypotonia, no epilepsy, no breathing abnormality. No familial history.

Patient APN-117 (female, born in 2002)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative; Telomeric rearrangement: negative

17p11.23 (SMS), 22q11.2: negative

UBE3A, *MECP2* direct sequencing: negative

Myotonic dystrophy: negative for DM1/*DMPK* and DM2/*ZN9* repeat expansions

Clinical information: Moderate ID, motor development delay, poor speech, partial autonomy. Attention and sleep disorder, hypotonia, cerebellar syndrome. No epilepsy. Left epicanthus, hemangioma. Echocardiography, electromyography and EEG: normal.

Patient APN-101 (male, born in 1986)

Preliminary genetic tests:

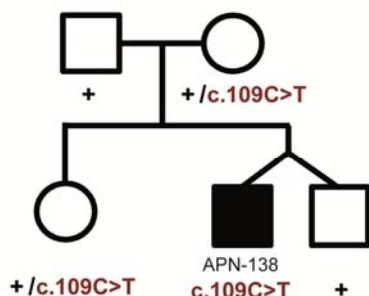
Fragile-X test: negative; Array-CGH: negative; Karyotype: negative

Clinical information: Moderate ID with normal speech, partial autonomy. Autistic features, attention deficit disorders. Facial dysmorphic traits: facial asymmetry, malar hypoplasia and earlobe hypoplasia.

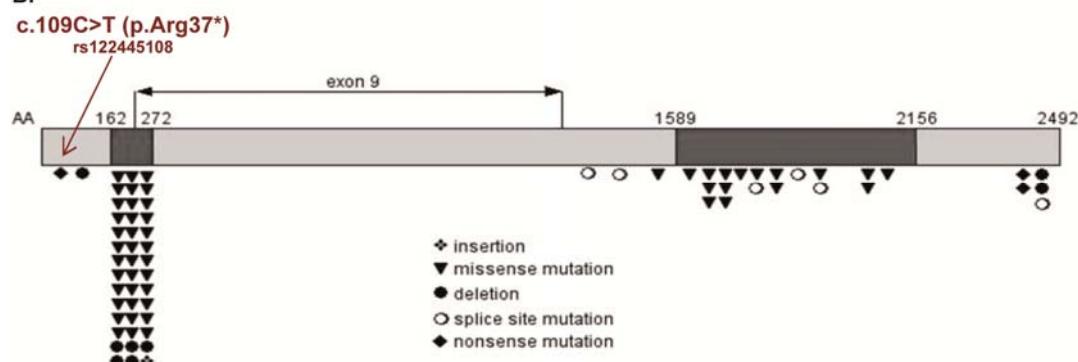
Figure S10. ATRX, one maternally-inherited causative nonsense mutation

APN-138: c.109C>T (NM_000489.3), p.Arg37* (rs122445108), hemizygous, maternally inherited

A.



B.



A. Pedigree of patient APN-138; **B.** Representation of the ATRX protein with distribution of mutations identified in patients with ID (adapted from [53]).

Previous implication of ATRX in cognitive disorders

Mutations in this gene are reported in patients with Alpha-thalassemia/mental retardation syndrome (MIM #301040) or Mental retardation-hypotonic facies syndrome (MIM #309580) [53 54]. These mutations are mainly missense mutations but truncating mutations have also been identified (81 missense mutations, 18 mutations affecting splicing, 8 nonsense mutations and 12 small indels; [55]). Clinical features of ATR-X syndrome include: ID, moderate to severe motor delay, characteristic dysmorphic facies (microcephaly, hypertelorism, epicanthus, anteverted nostrils, carp-shaped mouth), hypotonia, mild-to-moderate anemia secondary to alpha-thalassemia, and genital anomalies. Most patients carrying the c.109C>T, p.Arg37* mutation present with classical ATR-X syndrome, including characteristic facies [56 57].

Patient APN-138 (male, born in 2001)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: 15q15.1 duplication paternally inherited; Karyotype: negative

RSK2, EHMT1 direct sequencing: negative

Clinical information: Moderate ID, mild motor delay (acquired walking: 20 months-old), microcephaly (-4SD) facial dysmorphic traits (epicanthus, short palpebral fissures, short nose with anteverted nostrils, carp-shaped mouth, triangular nose), protruding tongue, attention/concentration disorders, stereotypic movements, anxiety. Cryptorchidism, birthmark (on the leg) and minor hand anomalies.

Detection of Heinz bodies (2003): negative

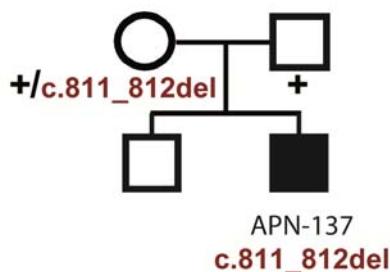
New test performed subsequently to NGS results (2013): 1.2% Heinz bodies detected.

X-inactivation bias detected in the mother (99:1) and the sister (98:2), subsequently to NGS results (2013).

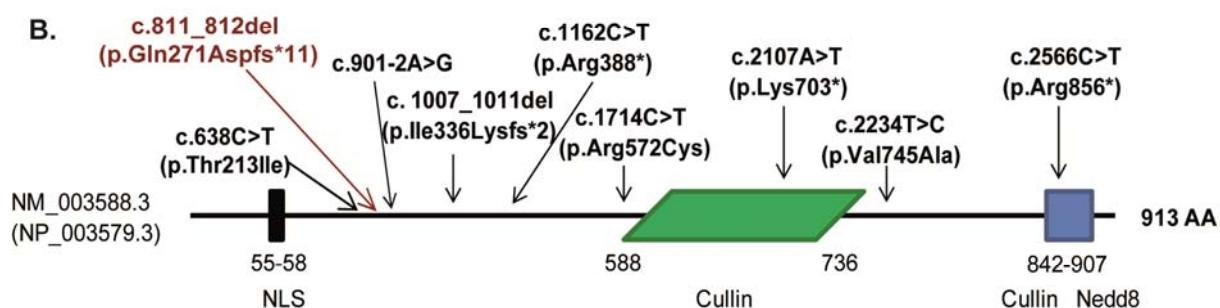
Figure S11. *CUL4B*, one causative frameshift mutation

APN-137: c.811_812del (NM_003588.3), p.Gln271Aspfs*11, hemizygous, maternally-inherited

A.



B.



C.

Clinical findings	Patients	
	Number	%
Intellectual disability	29/29	100%
Motor delay	12/12	100%
Speech delay	25/25	100%
Short stature	14/18	78%
Seizures	12/17	71%
Tremors	13/19	68%
Gait ataxia	13/19	68%
Aggressive outbursts	13/22	59%
Macrocephaly	8/18	44%
Obesity	15/25	60%
Pes cavus	8/14	57%
Small testes	12/20	60%
Prominent lower lip	12/23	52%
Kyphosis	4/19	21%
Abnormal toes	11/14	79%

A. Family tree of patient APN-137; B. Representation of protein CUL4B with its different domains. Patient's mutation (in red) and mutations previously identified in ID (in black) are also indicated C. Table of recurrent clinical findings and their incidence in patients carrying *CUL4B* mutations (adapted from [58]).

Previous implication of *CUL4B* in ID

Missense and truncating mutations in *CUL4B* were reported in several families with ID, short stature, hypogonadism, abnormal gait, and more variable features such as speech delay, prominent lower lip and tremor (Cabezas type) [58-60].

Patient APN-137 (male, born in 2009)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative; Karyotype: negative

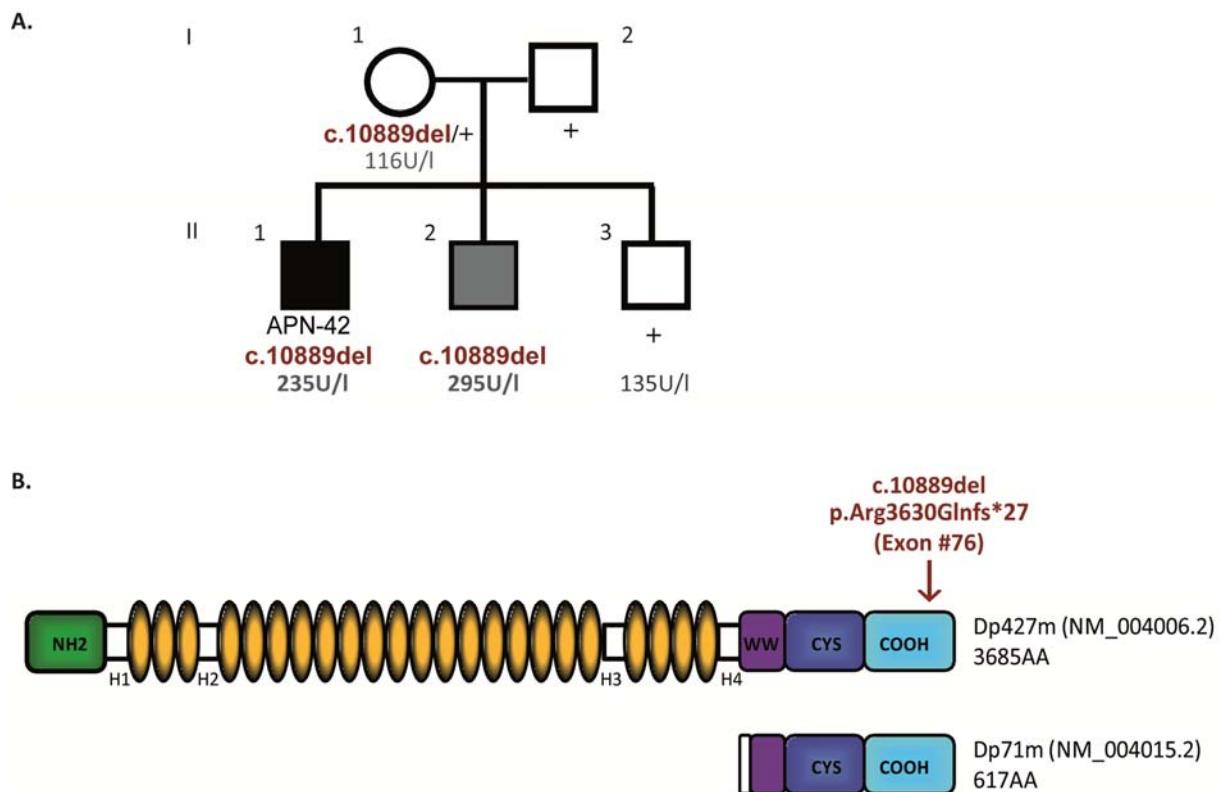
Angelman/Prader Willi test: negative

RSK2, ZEB2 (Mowat-Wilson syndrome) direct sequencing: negative

Clinical information: Severe ID, motor delay (no acquired walking at 4 years-old), absence of speech, autistic traits, sleep disturbances, behavioral disorders, constipation. Facial dysmorphic traits (wide mouth with prominent lower lip, depressed nasal bridge).

Figure S12. DMD, one causative frameshift mutation in two affected brothers without muscular phenotype

APN-42: c.10889del (NM_004006.2), p.Arg3630Glnfs*27, hemizygous, maternally-inherited



A. Pedigree of patient APN-42: his brother II-2 presents with important hypotonia, cerebellar dysplasia and developmental delay, while the youngest brother II-3 is unaffected. Serum Creatin PhosphoKinase (CPK) levels are indicated for all three brothers and the mother, showing slightly elevated levels for affected probands (normal values: <300 U/l); **B.** The protein encoded by the longest DMD transcript, DP427m, is represented with the different domains: N-terminal domain involved in actin binding (green), spectrin repeats (yellow), WW and EF/ZZ (CYS) domains (purple) involved in β -dystroglycan binding and the C-terminal domain (blue). The protein encoded by the shortest DMD transcript Dp71, which is the most abundant in brain is also represented. Localization of the mutation is indicated in red, and is one of the most distal truncating variant ever described in the gene (premature stop codon lies at the beginning of exon #77). The absence of muscle phenotype in our patient may be explained by an inefficient nonsense-mediated mRNA decay, allowing the translation of C-terminal truncated dystrophin and Dp71 that still contain the domain associated to the sarcoglycan/dystroglycan complex in the muscle, but lack the distal part of Dp71 that may be necessary for brain function.

Previous implication of DMD in ID

Mutations in *DMD* can cause Duchenne muscular dystrophy (DMD, MIM #310200) or the milder form Becker muscular dystrophy (BMD, MIM #300376). Muscular dystrophy is often accompanied by intellectual disability, especially when the mutation affects also the DP71 isoform and is located within the C-ter of the protein [8 61 62]. Depending on the specific mutation in the C-ter region, the muscular phenotype varies from mild BMD to DMD. The variability of phenotypes reported in patients with distal frameshift mutations (affecting exon #70 and higher) most probably implicates variable efficiency of nonsense mediated mRNA decay (NMD) [63]. More recently, a single amino-acid deletion in the EF domain was identified in DMD within the linkage region of one large family with non-specific mild to moderate XLID [64]. There was no muscular phenotype in the affected members of the family and CPK levels in the proband were just above the normal threshold (279U/l vs. normal range in Netherland: 30-200U/l).

Patient APN-42 (male, born in 2003)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: heterozygous 8p23.2 deletion (exons 2-5 of *MCPH1*) inherited from the mother; Karyotype: negative

ARX direct sequencing: negative

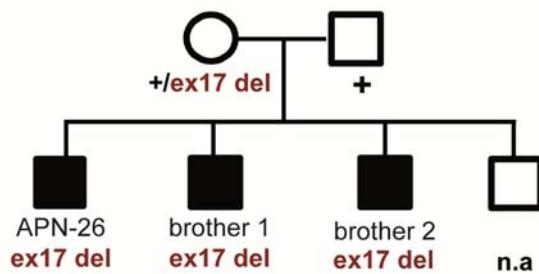
Clinical information: Moderate ID, absence of speech, developmental delay, important behavioral disorders, facial dysmorphic traits (narrow forehead, pointed nose, hanging columella, low-set ears with posterior rotation). Slightly elevated CPK-levels 235U/L but within normal range (20-300U/l)

Affected brother II-2 (male,born in 2009): Important hypotonia, maybe due to fetal distress (APGAR Score: 4 at 1 minute and 10 at 5 minutes). Cerebellar dysplasia and developmental delay. Slightly elevated CPK-levels 295U/L but within normal range (20-300U/l).

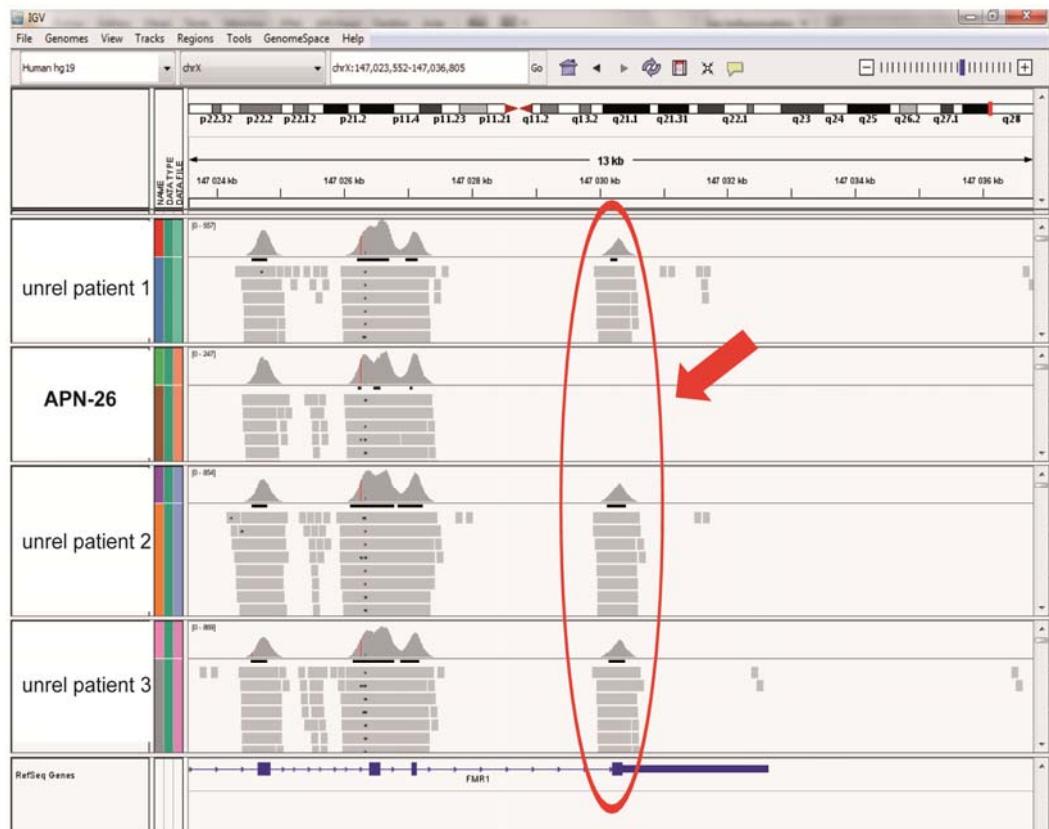
Figure S13. *FMR1*: one causative exon deletion in 3 affected brothers

APN-26: exon 17 hemizygous deletion, maternally inherited

A.



B.



A. Pedigree of patient APN-26; **B.** Visualization of the sequencing data in Integrative Genome Viewer (IGV) showing deletion of the last coding exon in proband,

Previous implication of *FMR1* in ID

Mutations in *FMR1* are responsible for fragile-X syndrome (MIM #300624). The vast majority of reported patients carry the trinucleotide (CGG)_n repeat expansion of greater than 200 repeats. Fragile-X syndrome is characterized by moderate to severe ID, macroorchidism and characteristic facial features (long face, large ears, and prominent jaw). Additional clinical features may include hyperlaxity, skeletal manifestations (scoliosis, pectus excavatum), strabismus and epilepsy.

Other reported mutations (besides the CGG expansion) in patients are: 1) a *de novo* missense mutation (p.Ile304Asn reported in a single patient with a severe form of fragile-X syndrome [65]; 2) a *de novo* frameshift (c.373delA; p.Thr125Leufs*35) in a patient with developmental delay and typical facies, 3) an inherited splice mutation in an adult male with classical fragile-X syndrome [66]; 4) an inherited stop mutation (p.Ser27*) in a man with classic features of fragile-X syndrome [67]; and 5) a potentially-pathogenic missense variant (c.413G>A; p.Arg138Gln) in a patient with intellectual disability [68].

Family of patient APN-26 (male, born in February, 1954)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative

Direct sequencing of *MEDI2*, *ZDHHC9*, *UPF3B*, *FBN1*, *TGFBR2*, *MECP2*, *ARX*: negative

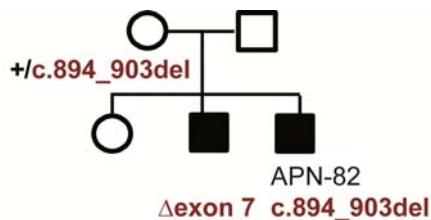
Clinical information:

- APN26: Moderate to severe ID with epilepsy, scoliosis, pectus excavatum, strabismus
- Brother 1: Moderate ID without epilepsy, facial features evocative of Fragile-X syndrome (long face and large ears), pectus excavatum, arachnodactyly.
- Brother 2: Mild ID without epilepsy.

No other familial history of ID reported in families of the three maternal aunts or of the two maternal uncles.

Figure S14. *ILIRAPL1*, one causative frameshift mutation and exon 7 deletion in two affected brothers

APN-82: c.894_903del (NM_014271.3), p.Trp299Thrfs*18, hemizygous mutation maternally-inherited
 APN-82's brother: exon 7 deletion, hemizygous *de novo*



Pedigree of patient APN-82, showing the two different hemizygous mutations in *ILIRAPL1* carried by both affected brothers: one deletion of 10-bp in exon 7 identified in the proband and maternally inherited, and a *de novo* deletion of the entire exon 7 in the affected brother. PCR confirmed that exons 6 and 8 are not deleted in *ILIRAPL1* in APN-82's brother. Variants in genes located on each side of *ILIRAPL1* confirmed that the deletion of exon 7 appeared on the chromosome originally carrying the small 10-bp deletion, suggesting that this small deletion might have provoked a genomic instability leading to the larger deletion observed in the second brother.

Previous implication of *ILIRAPL1* in ID

Mainly pericentric inversions and numerous intragenic deletions have been reported in patients with ID or ASD, supporting that *ILIRAPL1* is highly susceptible to abnormal recombination events [69]. Two small deletions and two truncating point mutations have also been described.

Nature of reported mutations	References
Pericentric inversions	
Pericentric inversion (inv(X)(p21.3q27.1)	[70]
Pericentric inversion (inv(X)(p21.3q27.1)	[71]
Pericentric inversion (inv(X)(p22.1q13)	[72]
Intragenic deletions	
del exons 1-5	[73]
del exon 2	[74]
del exons 2-5	[75]
del exons 2-5	[73]
del exons 3-5	[74]
del exons 3-5	[76]
del exons 3-7	[77]
del exon 7	<i>present study</i>
del exons 3-11	[78]
Small deletions	
del 7nts in exon 9	[77] (ASD patient)
del 10 nts in exon 7	<i>present study</i>
Point mutations	
c.1377C>A p.Y459*	[76]
c.1460G>A p.W487*	[79]

Patient APN-82 (male, born in 2009)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative

Clinical information: Moderate ID, speech delay, hypotonia. At 29 months: weight: 12kg, height: 88.cm (-1SD), OFC: 48.5cm (-1SD).

MRI: some anomalies detected.

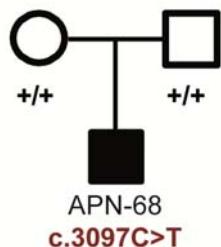
Affected brother (male, born in 2006)

Clinical information: Moderate ID with pervasive developmental disorders, delayed motor development, poor speech, anxiety, hypotonia.

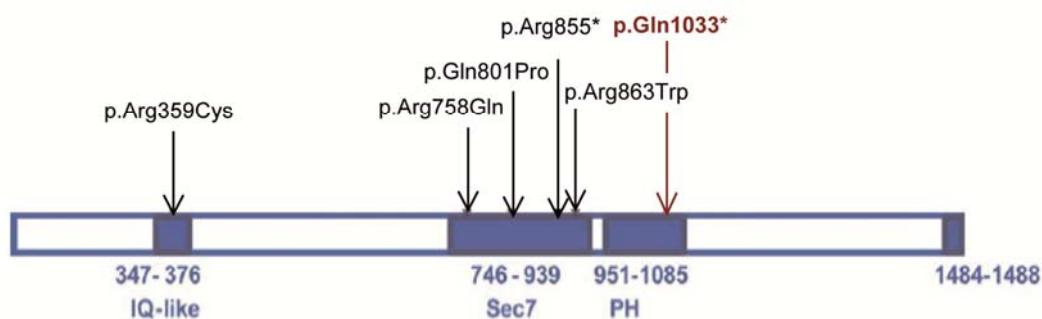
Figure S15. *IQSEC2*, one causative *de novo* nonsense mutation

APN-68: c.3097C>T (NM_00111125.1), p.Gln1033*, hemizygous *de novo*

A.



B.



A. Pedigree of patient APN-68; **B.** Representation of *IQSEC2* and distribution of the mutations previously reported in ID patients (adapted from [80]).

Previous implication of *IQSEC2* in ID

Missense mutations in *IQSEC2* (p.Asn801Pro; p.Arg758Asn; p.Arg359Cys, p.Arg863Trp) were identified in four large families with NS-ID (some individuals had ASD or epilepsy) [80]. All four mutations affect the GEF (guanine nucleotide exchange factor) activity of *IQSEC2*. Recently, a *de novo* nonsense mutation (p.Arg855*) was identified in a boy with ID and unspecific facial features with convergent strabismus [50]. Tran Mau-Them F et al., reported two additional male patients with duplications disrupting *IQSEC2* [81]. They compared the phenotype of all patients with *IQSEC2* truncating mutations, describing a severe syndromic XLID phenotype characterized by severe ID, progressive microcephaly with postnatal onset, severe delayed motor and speech development, midline hand stereotypic traits, additional autistic traits and seizures.

Patient APN-68 (male, born in 2005)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: dupXp22.3 inherited from the mother, concluded as non-pathogenic *MECP2*, *ARX* and *CDKL5* direct sequencing: negative.

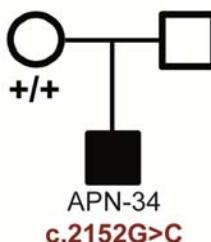
Clinical information: Severe ID, no acquired language, reduced autonomy, motor developmental delay, severe epilepsy, strabismus, stereotypic features and behavioral disorder. The phenotype overlaps that was reported by Tran Mau-Them et al. for patients carrying truncating *IQSEC2* mutation but without microcephaly [81].

Figure S16. *KDM5C*, two causative mutations

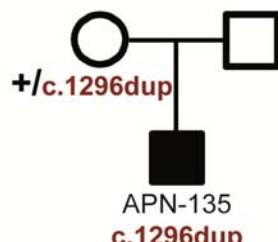
APN-34: c.2152G>C (NM_004187.3), p.Ala718Pro, hemizygous *de novo*

APN-135: c.1296dup (NM_004187.3), p.Glu433*, hemizygous, maternally inherited

A.



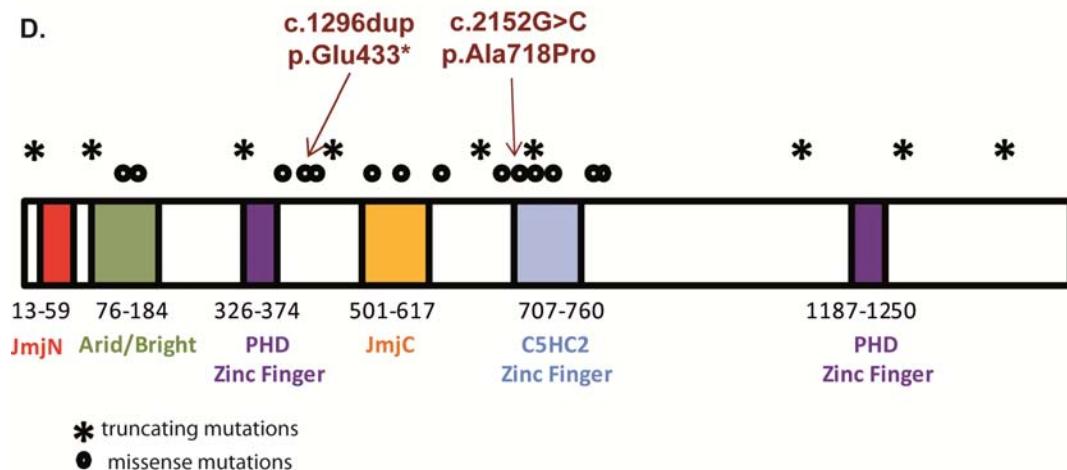
B.



C.

c.2152G>C p.Ala718Pro Pathogenic, <i>this study</i>	
SIFT	Deleterious (0)
PolyPhen2	Deleterious (HumDiv: 1)
Mutation T@ster	Disease causing (1)
Grantham [0;215]	27
PhyloP [-14.1;6.4]	4.97
PhastCons [0;1]	1

D.



A-B. Family trees of both patients with *KDM5C/JARID1C* mutations detected; **C.** Associated prediction scores of the pathogenic missense mutation identified in this study showing a predicted deleterious status and a high conservation at the nucleotide level; **D.** Representation of *KDM5C* and its domains with localization of these detected mutations and those previously reported in ID patients.

Previous implications of *KDM5C* in ID

The resequencing of 47 genes in the Xq28 region in MRX families led to the identification of 7 mutations in *KDM5C* [82]. Many other mutations have been described afterwards,[83-86] in other patients with intellectual disability. Recurrent clinical symptoms include hyperreflexia, short stature, aggressive behavior and seizures. Microcephaly is reported unevenly [87]. Mutations in *JARID1C/KDM5C* appear to be a frequent cause of intellectual disability.

Patient APN-34 (male, born in 2004)Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative; Karyotype: negative

Subtelomeric rearrangement: negative

FOXP2 direct sequencing: negative

Clinical information: Moderate ID with hyperactivity and friendly behavior despite some aggressive bursts, poor language with speech apraxia, pyramidal syndrome, strabismus and hypermetropia. At 8 years-old: OFC=53 cm (mean), height=125 cm (mean), weight = 29 kg (90th centile).

Patient APN-135 (male, born in 2004)Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative; Karyotype: negative

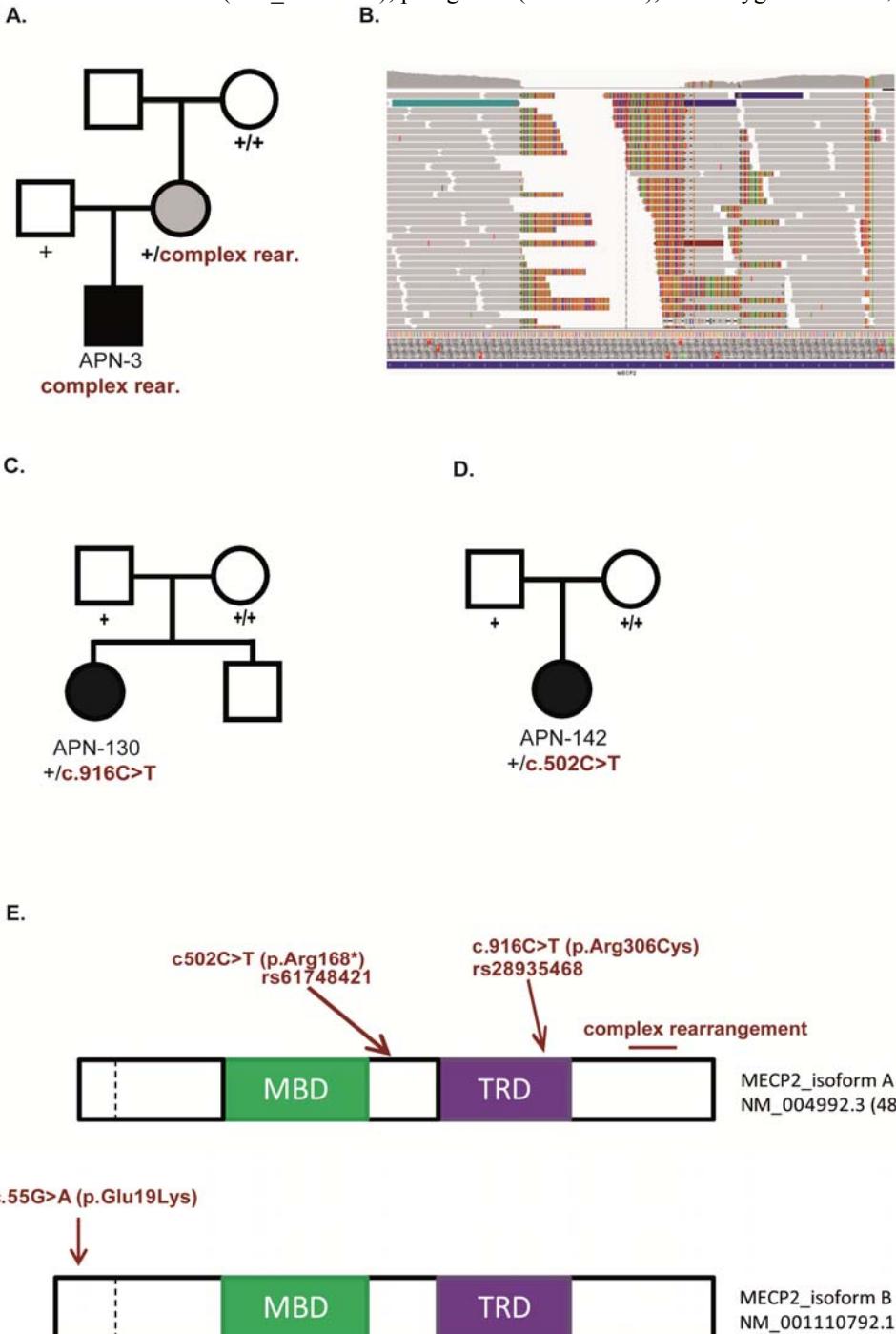
ARX direct sequencing: negative

Clinical information: Moderate ID, poor language (only a few words), sleep disturbances, PDD diagnosed. Behavioral disturbances (aggression). Minor facial features, clinodactyly, astigmatism.

At birth, weight: 2600 g, height: 45 cm, OFC: 34 cm. At 9 years-old, weight: 25 kg, height: 119 cm (-2SD), OFC: 51 cm. Treatment with growth hormone since November, 2009.

Figure S17. MECP2, three causative mutations

- APN-3: c.[954A>T; 957_960delins4bp;1060delCins 6bp; 1097_1235delins50bp](NM_004992.3); (p.[Glu318Asp; Val320His; Arg354_Val412delins41]) hemizygous complex rearrangement
- APN-130: c.916C>T (NM_004992.3); p.Arg306Cys (rs28935468), heterozygous *de novo*, known mutation
- APN-142 : c502C>T (NM_004992.3); p.Arg168* (rs61748421), heterozygous *de novo*, known mutation



A. Pedigree of patient APN-3 carrying the complex rearrangement; B. IGV (Integrative Genome Viewer) view of the sequencing data showing complex rearrangement in proband; C., D. Pedigrees of APN-130 and APN-142 carrying *de novo* mutations, previously reported in Rett patients; E. Representation of both *MECP2* isoforms (A and B) and localization of the four variants/mutations identified in patients.

Previous implication of *MECP2* in cognitive disorders

MECP2 is mutated in Rett syndrome (MIM #312750), a severe neurodevelopmental disorder that affects mostly females, and characterized by an early-stage arrested development and regression of skills, speech loss, specific stereotypic features (“hand-flapping”), microcephaly, seizures and intellectual disability. Since the discovery of *MECP2* as the cause of Rett syndrome in 1999 [88], *MECP2* mutations have also been reported in males [89-95]. These males phenotypically have classical Rett syndrome when the mutation arises as somatic mosaicism or when they have an extra X-chromosome [89-96]. Otherwise, males with *MECP2* mutations show diverse phenotypes: from severe early-onset congenital encephalopathy associated with microcephaly, hypotonia, seizures, respiratory irregularities, constipation and intellectual disability with various neurological symptoms to mild cognitive impairment [95]. The last exon of *MECP2* is known to be the one accumulating most mutations in patients with the first 5' half mainly subject to point mutations, while the 3' half is a hotspot for rearrangements [97-99].

Patient APN-3 (male, born in 2001)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative

FISH 22q13, 1qter, 1p36, 15q11-q13, Coffin-lowry, CDG, Angelman/Prader-Willi, Telomeric rearrangement screening: negative

X-inactivation bias: negative

Clinical information: Severe ID, epilepsy, myotonia, ataxia, hypotonia. Progressive myoclonic encephalopathy with cerebellar and pyramidal syndrome. Overweight. Microcephaly (-3SD), dysmorphic traits, photophobia.

His mother presents with speech delay, dyslexia, sleep and attention disorders, and overweight.

Proband has a young maternal half-brother born in September 2009 who presents with similar clinical features (severe encephalopathy, ataxia, hypotonia, sleep and behavioral disorder) and also carries the mutation.

Patient APN-130 (female, born in 2003)

Preliminary genetic tests:

Fragile-X test: negative; Karyotype: negative; Array-CGH: negative;

FISH 17p11.2: negative

Angelman/Prader-Willi, *GNAS1* direct sequencing: negative

Telomeric rearrangements screening by MLPA: negative

Clinical information: Normal development during the first year. Walking at 12 months. Stagnation of development starting at 14 months-old, with walking denial during 20 months subsequently to a fall. Severe ID, absence of speech. Autistic features, which appeared between 1-2 years-old, but no typical Rett phenotype. At 10 years, presence of some median hand movements. Behavioral disorders including hyperactivity and sleep disorders. Hyperactivity and aggressive behaviors progressively improved starting from 8 years-old.

Overweight (+5SD) in a context of familial obesity. Facial dysmorphic traits (narrow forehead, prominent cheeks, small and deep-set eyes, turned-up nose). Birth measurements: weight: 4100g, height: 53cm, OFC: 33 cm. At 9 year-old: weight: 66 kg (+5SD), height: 141cm (+1.5SD), OFC: 53.5cm (+1SD).

Patient APN-142 (female, born in 2006)

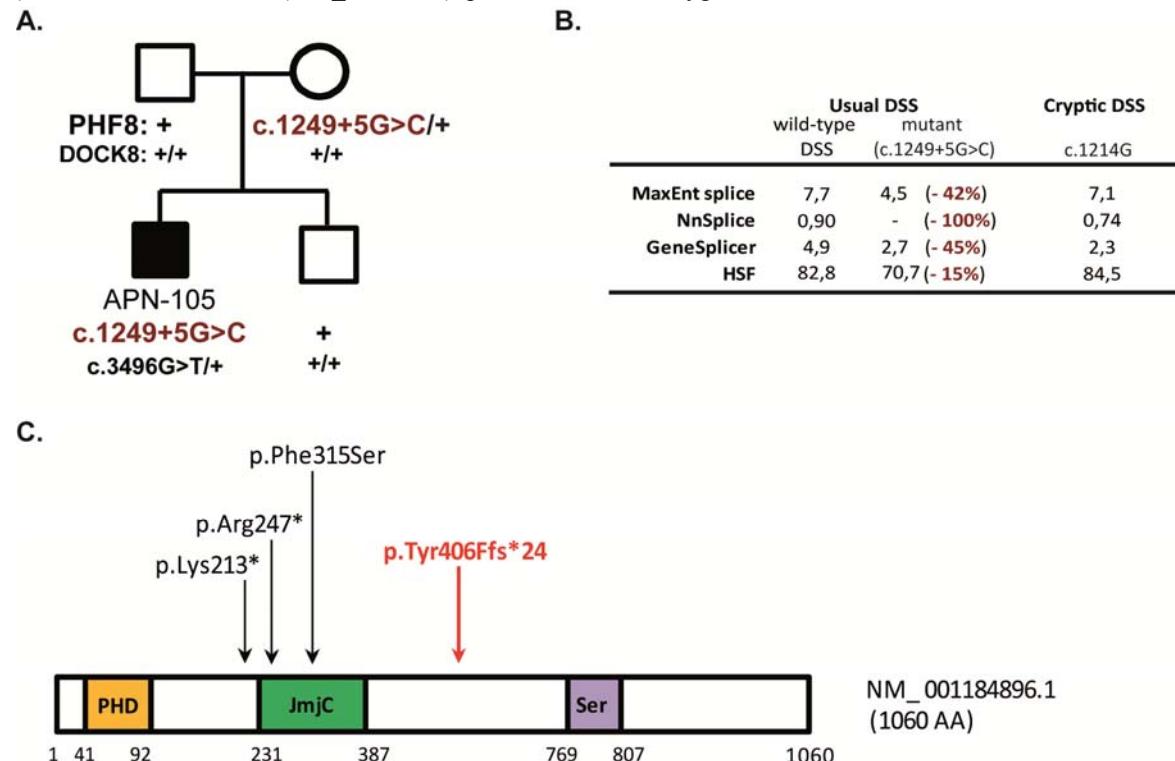
Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative; Karyotype: negative

Clinical information: Severe ID, no speech. Developmental delay followed by a stagnation and regression (3 years-old). Autistic and stereotypic features ('hand-flapping'). Measurements at birth, weight: 2640g, height: 48cm, OFC: 33cm. At 6 year-old, weight: 24.3 kg (+2SD), height: 118 cm (+1SD), OFC: 51cm.

Figure S18. PHF8, one causative mutation affecting splicing

APN-105: c.1249+5G>C (NM_001184896.1), p.Tyr406Phefs*24 hemizygous, maternally inherited
(+ DOCK8 : c.3496G>T (NM_203447.3), p.Glu1166*, heterozygous, *de novo*)



A. Pedigree of patient APN-105 carrying both the *PHF8* mutation and a *de novo* variant in *DOCK8*; **B.** Prediction scores for donor splice sites (DSS) as computed by MaxEnt, Nnsplice, GeneSplicer or Human splicing Finder (HSF): prediction scores for the normal DSS with the wild-type allele and the 1249+5G>C mutation are given as well as the percentage of score decrease created by this mutation. Prediction score for the alternative cryptic DSS is also indicated. Patient blood mRNA was analyzed and confirmed the use of this alternative cryptic DSS instead of the usual DSS in over 4/5 of the transcripts; **C.** Representation of PHF8 domains: JmjC, PHD-type and Ser-rich domains with the present (red) and previously (black) reported mutations.

Previous implication of *PHF8* in ID

Mutations in *PHF8* (a 12-bp deletion affecting exon 8 splicing and a nonsense mutation p.Arg247*) were described in two families with Siderius-Hamel syndromic ID (MIM #300263, moderate ID along with cleft/lip palate) [100]. Phenotypes vary in these families from ID with cleft lip/cleft palate to non-specific ID. Screening of additional patients with cleft lip/cleft palate identified an additional nonsense mutation (p.Lys213*) in *PHF8* and a missense (p.Phe315Ser) mutation [101-102].

Patient APN-105 (male, born in 2010)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative

MEF2C direct sequencing: negative

12p tetrasomy: negative

X-inactivation bias in APN-105's mother: 91-9%

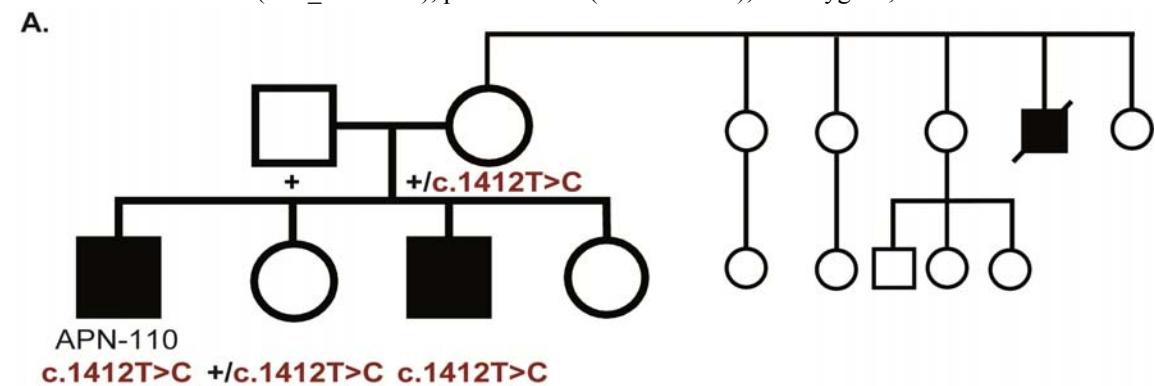
Clinical information: Mild ID, motor delay (sitting: 18 months, walk: 31 months), language delay. Hypotonia with hyperlaxity, stereotypic movements, behavioral disorders. Facial dysmorphic traits (hypertelorism, wide mouth, high hairline with golf temporal, clefts ears).

CAA, CAO blood/urine, lactate/pyruvate, glycosylation, creatine, ceruloplasmine: normal

MRI: ventricular enlargement

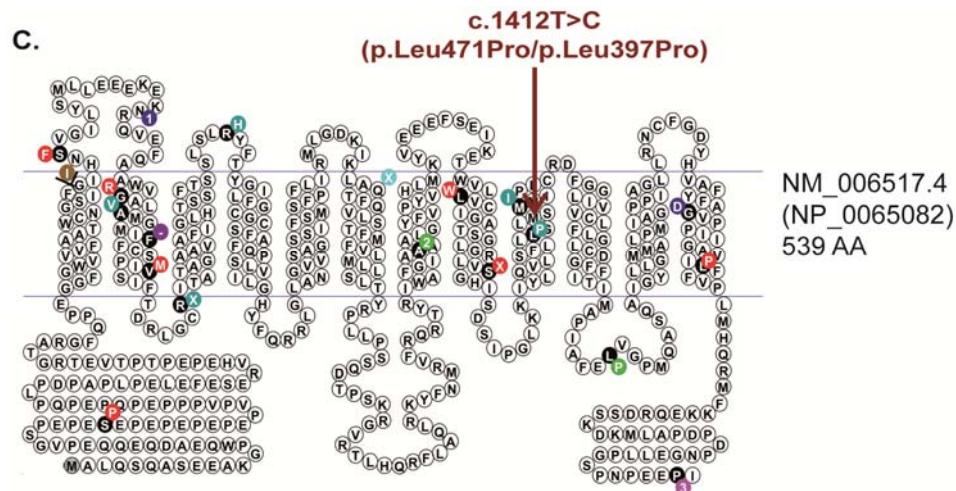
Figure S19. *SLC16A2/MCT8*, one causative missense mutation

APN-110: c.1412T>C (NM_006517.3), p.Leu471Pro (rs122455132), hemizygous, known mutation



B.

c.1412T>C p.Leu471Pro Pathogenic ([103] & this study)	
SIFT	Deleterious (0.01)
PolyPhen2	Deleterious (HumDiv: 0.997)
Mutation T@ster	Disease causing (1)
Grantham [0;215]	98
PhyloP [-14.1;6.4]	4.73
PhastCons [0;1]	1



A. Pedigree of APN-110 showing segregation of the missense mutation with the disease; **B.** Associated prediction scores of the pathogenic missense mutation identified in APN-110 in this study (initially reported in [103]), showing a predicted deleterious status and a high conservation at the nucleotide level; **C.** Representation of the protein and its transmembrane domains, and localization of the affected aminoacid within the MCT8 protein (adapted from [104]).

Previous implication of *SLC16A2/MCT8* in ID

Patients with mutations in *SLC16A2/MCT8* show severe psychomotor retardation combined with elevated level of thyroid hormone T3 (triiodothyronine) in serum. The mutation p.Leu397Pro/ *p.Leu471Pro* was initially reported in a patient with severe psychomotor retardation, axial hypotonia, spastic quadriplegia, dystonic movements and absence of speech [103]. This mutation was shown to reduce *SLC16A2/MCT8* protein levels and affect T3 uptake in JEG3 cells [105].

Patient APN-110 (male, born in April, 1986)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative

ARX, MECP2 direct sequencing: negative.

X-inactivation bias identified in patient's mother.

Clinical information: Severe ID, no sitting position (kyphosis), no walking, absence of speech. Poor autonomy. Major hypotonia without epilepsy. No facial dysmorphic traits. No dystonic movement.

Affected brother: (male, born in November, 1997): Kyphosis, no acquired walking, absence of speech. Mild growth retardation.

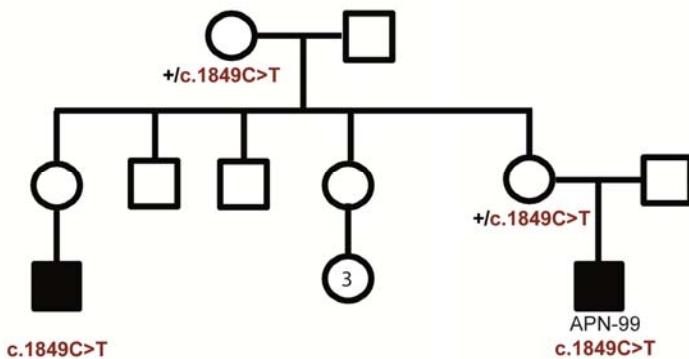
Serum thyroid hormone T3L levels confirmed the genetic diagnostic: 11.2 pmol/l (APN-110) and 14.5 pmol/l for his affected brother (normal range values: 3.1-6.8).

Maternal uncle: death at 23 year-old, with clinical features highly similar to his nephews.

Figure S20. NLGN3, one potentially-causative missense mutation

APN-99: c.1849C>T (NM_181303.1); p.Arg617Trp, hemizygous

A.



B.

c.1849C>T
(p.Arg617Trp)

NLGN3	LHIGLKPRVRDHYRAT	KVAFWKHLVPHLHNLDHM
NLGN4	LHIGLKPRVRDHYRAT	KVAFWLELVPHLHNLDNEI
NLGN1	LHIGLKPRVKEHYRANKVNLIWLELVPHLHNLDI	
NLGN2	LHIGLKPRVRDNYRANKVAFWLELVPHLHNLDHTE	

C.

	c.1849C>T p.Arg617Trp (probably pathogenic)	c.1411C>T p.Arg471Cys (pathogenic, see [106])
SIFT	Deleterious (0.00)	Deleterious (0.00)
PolyPhen2	Deleterious (HumDiv: 1)	Deleterious (HumDiv: 1)
Mutation T@ster	Disease causing (1)	Disease causing (1)
Grantham [0;215]	101	180
PhyloP [-14.1;6.4]	3.840	3.600
PhastCons [0;1]	1	1

A. Pedigree of patient APN-99 showing a probable X-linked mode of transmission of the disease; **B.** Alignment of the human neuroligin paralogs showing conservation of the affected Arg617 residue; **C.** Associated prediction scores comparing the probably pathogenic missense variant identified in this study with the initial pathogenic missense mutation described in [106], showing both a predicted deleterious status and a very high conservation at the nucleotide level.

Previous implication of NLGN3 in ID

Screening of *NLGN3* in ASD individuals led to the identification of a missense c.1411C>T (p.Arg471Cys) mutation in two brothers (one with typical autism the other with Asperger syndrome)[106] localized in the carboxylesterase domain. The *NLGN3* knock-in mouse displayed an increase in inhibitory synaptic transmission and an “autistic-like” phenotype [107 108]. Talebizadeh et al. identified an alternative transcript of *NLGN3* in lymphoblastoid cells that lacked exon 7 and encoded a new truncated protein, present in all 30 control individuals and in all but one of the 10 ASD females tested [109]. The authors speculated that the lack of the specific truncated isoform in this female patient might be implicated in her autistic phenotype.

Patient APN-99 (male, born in 2007)

Preliminary genetic tests:

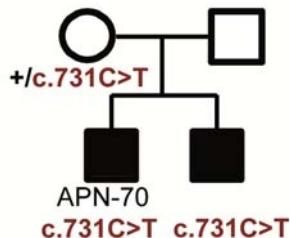
Fragile-X test: negative; Array-CGH: del 5q15 inherited from the father; Karyotype: negative

Clinical information: Severe ID with ASD and sleep disorders. Absence of speech. One maternal cousin with ID and autism, also carrying the variant.

Figure S21. *PQBP1*, one potentially-causative missense mutation in two affected brothers

APN-70: c.731C>T (NM_005710.2), p.Pro244Leu, hemizygous, maternally inherited

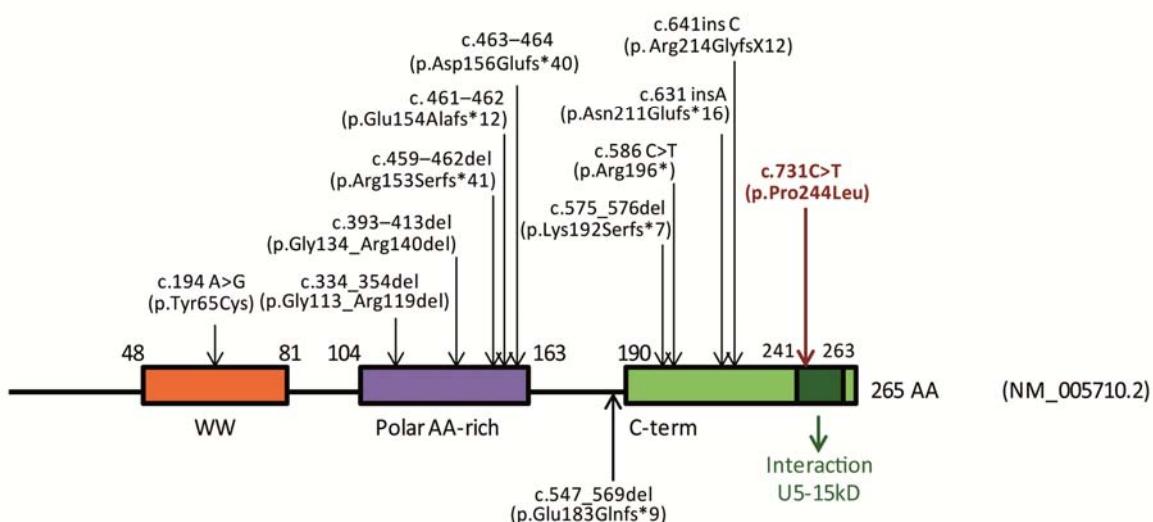
A.



B.

	c.731C>T p.Pro244Leu (probably pathogenic, this study)	c.194A>G p.Tyr65Cys (pathogenic, see [110])
SIFT	Deleterious (0.00)	Deleterious (0)
PolyPhen2	Deleterious (HumDiv: 1)	Deleterious (HumDiv: 1)
Mutation T@ster	Disease causing (1)	Disease causing (1)
Grantham [0;215]	98	194
PhyloP [-14.1;6.4]	5.13	3.76
PhastCons [0;1]	0.99	1

C.



A. Pedigree of patient APN-70; **B.** Associated prediction scores comparing the probably pathogenic missense variant identified in this study with the only pathogenic missense mutation described in [110], showing for both a similar predicted deleterious status and a high conservation at the nucleotide level; **C.** Representation of the different *PQBP1* protein domains: the WW domain is responsible for the interaction with RNA polymerase, the polar amino acid-rich domain interacts with polyglutamine-interacting proteins and the C-terminus domain contains a region involved in the interaction with the spliceosomal protein U5-15kD [111].

Previous implication of *PQBP1* in ID

Truncating mutations (and one variation leading to a missense p.Tyr65Cys) were reported in *PQBP1* in several X-chromosome-linked intellectual disability (XLID) disorders, such as Renpenning (MIM #309500), Sutherland-Haan, Hamel, Porteous, and Golabi-Ito-Hall (GIH) syndromes [110 112]. These syndromes share similar clinical features: in addition to severe intellectual disability, patients also have a lean body, short stature, microcephaly, and are frequently diagnosed with cardiac abnormalities [113].

Patient APN-70 (male, born in 1988)

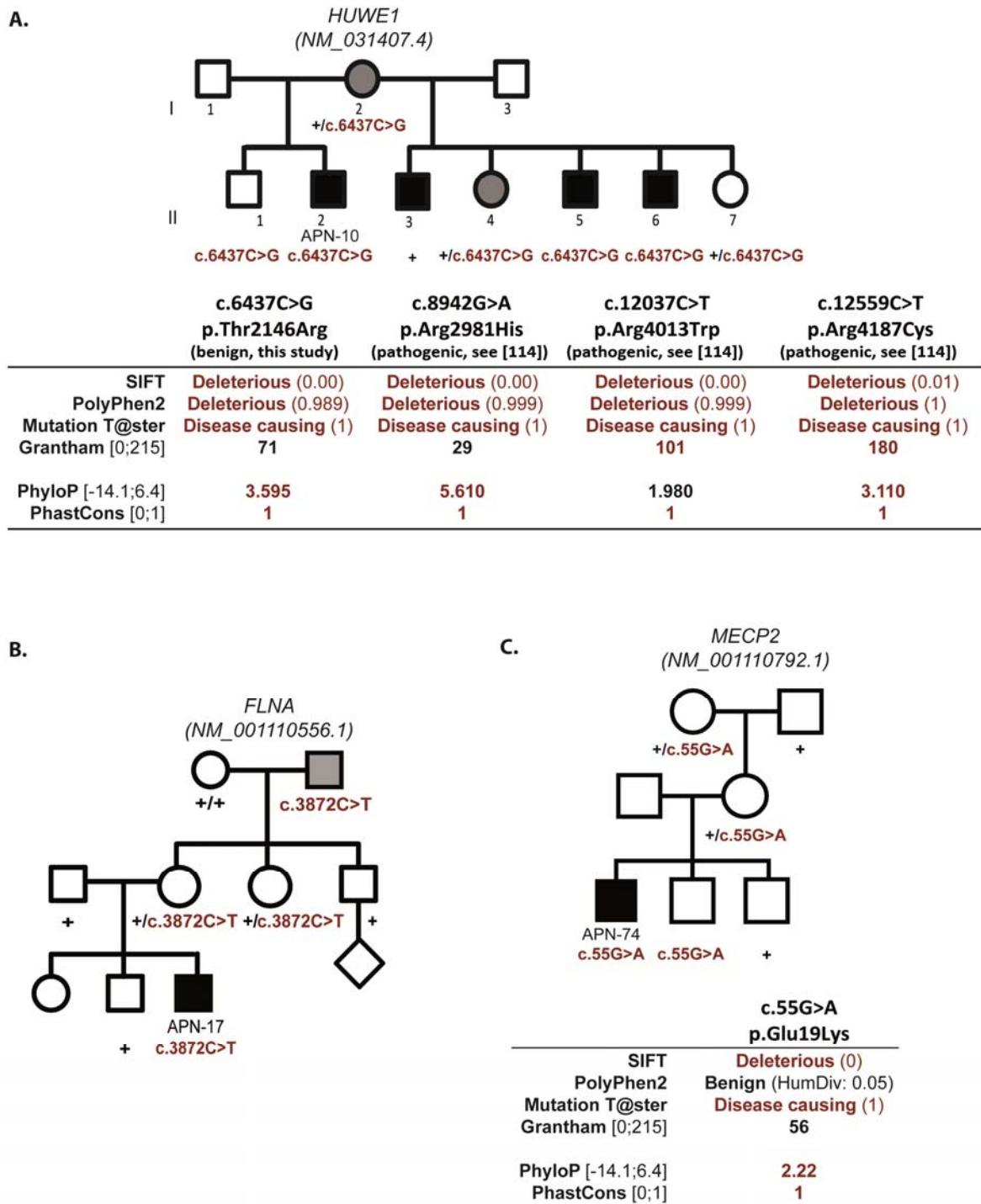
Preliminary genetic tests:

Fragile-X: negative; Array-CGH: dup 16q22.2 inherited from the father

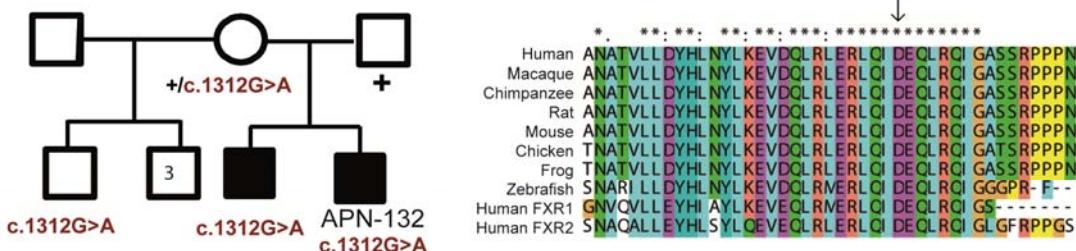
Clinical information: Moderate ID, poor autonomy (poor speech/reading/writing), communication and social interaction disorders, learning difficulties. Tip-toe walking at the beginning, autistic behavior that started at 3 years of age, stereotypic movements.

Figure S22. Other candidate variants not or ambiguously co-segregating with ID in probands' families

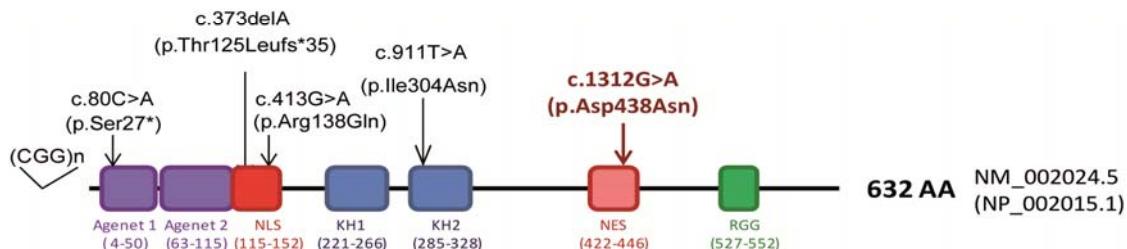
- APN-10: c.6437C>G (*HUWE1*, NM_031407.4), p.Thr2146Arg, hemizygous; reported in an unaffected brother
- APN-17: c.3872C>T (*FLNA*, NM_001110556.1), p.Pro1291Leu (known, rs137853319), hemizygous; maternally inherited, present in maternal grandfather with late onset psychiatric disorders
- APN-74: c.55G>A (*MECP2*, NM_001110792.1), p.Glu19Lys, hemizygous; reported in an unaffected brother
- APN-132: c.1312G>A (*FMRI*, NM_002024.5), p.Asp438Asn, hemizygous, maternally inherited



D.



	c.1312G>A p.Asp438Asn (potentially pathogenic)	c.911T>A p.Ile304Asn (pathogenic, see [65])
SIFT	Deleterious (0.00)	Deleterious (0.00)
PolyPhen2	Deleterious (HumDiv: 1.00)	Deleterious (HumDiv: 1.00)
Mutation T@ster	Disease causing (0.995)	Disease causing (1)
Grantham [0;215]	23	149
PhyloP [-14.1;6.4]	5.048	4.48
PhastCons [0;1]	1	1



A. Partial pedigree of patient APN-10 carrying c.6437C>G, p.Thr2146Arg variant in *HUWE1*, large family highly suggestive of an X-linked inheritance of a disease causing mutation. The prediction scores for the missense variant are also indicated, along with those associated to previously reported missense mutations [114]; **B.** Pedigree of patient APN-17 with the *FLNA* c.3872C>T variant showing the carrier status of maternal grandfather presenting with psychiatric disorder; **C.** Pedigree of patient APN-74's family showing non-segregation of the *MECP2* c.55G>A, p.Glu19Lys missense variant, affecting the first exon of the major MeCP2_e1 transcript in brain. A p.Ala2Val missense variant was reported as disease causing in two patients [115 116]; **D.** Pedigree of patient APN-132, with protein alignment of FMR1 ortholog and paralog proteins FXR1 and FXR2, showing striking conservation of the affected Asp438 residue even in FXR1 and FXR2, associated prediction scores comparing the present non-segregating missense mutation to the well-known pathogenic missense mutation [65]; and representation of FMRP and its domains, and distribution of the few point mutations that were reported in *FMR1* in patients. The herein described missense mutation is indicated in red, located at the very end of the NES (nuclear export signal) domain.

A/ Previous implication of *HUWE1* in ID

Initially, duplications of *HUWE1* were identified in families and simplex cases with intellectual disability. More recently, three missense mutations affecting highly conserved residues and cosegregating with the ID status were identified in unrelated XLID-affected families [114 117].

Patient APN-10 (male, born in 2001)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative

ARX (exon #2), *PQBP1* (exon #4), Prader-Willi: negative

Existence of an X-inactivation bias in the patient's mother

Clinical information: moderate ID, behavioral and attention disorders, myopia, facial dysmorphic traits, hyperlaxity, macrosomy. Large family with several affected males, highly suggestive of XLID.

B/ Previous implication of *FLNA* in ID

Mutations in *FLNA* can cause different disorders: loss-of-function mutations tend to cause periventricular heterotopia whereas missense mutations can lead to otopalatodigital syndrome, Melnick-Needles or fronto-metaphyseal dysplasia [118-120]. In a boy with type 2 FG syndrome, Unger *et al.* identified a hemizygous c.3872C>T, p.Pro1291Leu mutation in *FLNA* [121]. The boy presented with severe constipation, large rounded forehead, prominent ears, frontal hair upsweep, and mild delay in language acquisition. This missense is reported in one male from the Exome Variant Server, raising doubts about its pathogenicity.

Patient APN-17 (male, born in 1996)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative

Clinical information: Severe ID with minimal autonomy. Absence of speech. Autistic features with hyperactivity, behavioral and sleep disorders. Epilepsy. No regression, no microcephaly.

MRI: normal (no sign of heterotopia)

Proband's maternal grandfather also carries the variant and presented later in life with schizophrenia, behavioral disorder, speech impairment, anxiety and stereotypic behaviors. He could read and worked for part of his life as a semi-skilled worker.

C/ Previous implication of *MECP2* in cognitive disorders

See **Figure S17.**

Patient APN-74 (male, born in 1988)

Preliminary genetic tests:

Fragile-X test: negative; Array-CGH: negative; Prader-Willi: negative

Clinical information: Moderate ID with partial autonomy (no reading, no writing). Epilepsy since 11 months of age with regression of motor acquisitions. Autistic features, attention disorder, polyphagy, stereotypic movements and aggressive behavior.

D/ Previous implication of *FMR1* in cognitive disorders

See **Figure S13.**

Patient APN-132 (male, born in June, 2007) and his brother (male, born in April, 2006)

Preliminary genetic tests:

Fragile-X: negative; Array-CGH: negative; Karyotype: negative

Clinical information: Both boys present with severe ID, autistic features, behavioral and sleep disorders, and behavioral instability.

REFERENCES

1. Gecz J, Shoubridge C, Corbett M. The genetic landscape of intellectual disability arising from chromosome X. *Trends Genet* 2009;25(7):308-16 doi: S0168-9525(09)00108-5 [pii] 10.1016/j.tig.2009.05.002[published Online First: Epub Date]].
2. Lubs HA, Stevenson RE, Schwartz CE. Fragile X and X-linked intellectual disability: four decades of discovery. *Am J Hum Genet* 2012;90(4):579-90 doi: S0002-9297(12)00103-6 [pii] 10.1016/j.ajhg.2012.02.018[published Online First: Epub Date]].
3. Stevenson RE, Schwartz CE. X-linked intellectual disability: unique vulnerability of the male genome. *Dev Disabil Res Rev* 2009;15(4):361-8 doi: 10.1002/ddrr.81[published Online First: Epub Date]].
4. Piton A, Redin C, Mandel JL. XLID-causing mutations and associated genes challenged in light of data from large-scale human exome sequencing. *Am J Hum Genet* 2013;93(2):368-83 doi: 10.1016/j.ajhg.2013.06.013[published Online First: Epub Date]].
5. Kuss AW, Garshasbi M, Kahrizi K, Tzschach A, Behjati F, Darvish H, Abbasi-Moheb L, Puettmann L, Zecha A, Weissmann R, Hu H, Mohseni M, Abedini SS, Rajab A, Hertzberg C, Wieczorek D, Ullmann R, Ghasemi-Firouzabadi S, Banihashemi S, Arzhangi S, Hadavi V, Bahrami-Monajemi G, Kasiri M, Falah M, Nikuei P, Dehghan A, Sobhani M, Jamali P, Ropers HH, Najmabadi H. Autosomal recessive mental retardation: homozygosity mapping identifies 27 single linkage intervals, at least 14 novel loci and several mutation hotspots. *Hum Genet* 2011;129(2):141-8 doi: 10.1007/s00439-010-0907-3[published Online First: Epub Date]].
6. Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, Hosseini M, Behjati F, Haas S, Jamali P, Zecha A, Mohseni M, Puttmann L, Vahid LN, Jensen C, Moheb LA, Bienek M, Larti F, Mueller I, Weissmann R, Darvish H, Wrogemann K, Hadavi V, Lipkowitz B, Esmaeeli-Nieh S, Wieczorek D, Kariminejad R, Firouzabadi SG, Cohen M, Fattahi Z, Rost I, Mojahedi F, Hertzberg C, Dehghan A, Rajab A, Banavandi MJ, Hoffer J, Falah M, Musante L, Kalscheuer V, Ullmann R, Kuss AW, Tzschach A, Kahrizi K, Ropers HH. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 2011;478(7367):57-63 doi: nature10423 [pii] 10.1038/nature10423[published Online First: Epub Date]].
7. Vissers LE, de Ligt J, Gilissen C, Janssen I, Steehouwer M, de Vries P, van Lier B, Arts P, Wieskamp N, del Rosario M, van Bon BW, Hoischen A, de Vries BB, Brunner HG, Veltman JA. A de novo paradigm for mental retardation. *Nat Genet* 2010;42(12):1109-12 doi: ng.712 [pii] 10.1038/ng.712[published Online First: Epub Date]].
8. Daoud F, Angeard N, Demerre B, Martie I, Benyaou R, Leturcq F, Cossee M, Deburgrave N, Saillour Y, Tuffery S, Urtizberea A, Toutain A, Echenne B, Frischman M, Mayer M, Desguerre I, Estournet B, Reveillere C, Penisson B, Cuisset JM, Kaplan JC, Heron D, Rivier F, Chelly J. Analysis of Dp71 contribution in the severity of mental retardation through comparison of Duchenne and Becker patients differing by mutation consequences on Dp71 expression. *Human molecular genetics* 2009;18(20):3779-94 doi: ddp320 [pii] 10.1093/hmg/ddp320[published Online First: Epub Date]].
9. Courcet JB, Faivre L, Malzac P, Masurel-Paulet A, Lopez E, Callier P, Lambert L, Lemesle M, Thevenon J, Gigot N, Duplomb L, Ragon C, Marle N, Mosca-Boidron AL, Huet F, Philippe C, Moncla A, Thauvin-Robinet C. The DYRK1A gene is a cause of syndromic intellectual disability with severe microcephaly and epilepsy. *Journal of medical genetics* 2012;49(12):731-6 doi: 10.1136/jmedgenet-2012-101251[published Online First: Epub Date]].

10. Moller RS, Kubart S, Hoeltzenbein M, Heye B, Vogel I, Hansen CP, Menzel C, Ullmann R, Tommerup N, Ropers HH, Turner Z, Kalscheuer VM. Truncation of the Down syndrome candidate gene DYRK1A in two unrelated patients with microcephaly. *Am J Hum Genet* 2008;82(5):1165-70 doi: 10.1016/j.ajhg.2008.03.001[published Online First: Epub Date]|.
11. Sitz JH, Tigges M, Baumgartel K, Khaspekov LG, Lutz B. DyRK1A potentiates steroid hormone-induced transcription via the chromatin remodeling factor Arip4. *Mol Cell Biol* 2004;24(13):5821-34 doi: 10.1128/MCB.24.13.5821-5834.2004
24/13/5821 [pii][published Online First: Epub Date]|.
12. van Bon BW, Hoischen A, Hehir-Kwa J, de Brouwer AP, Ruivenkamp C, Gijsbers AC, Marcelis CL, de Leeuw N, Veltman JA, Brunner HG, de Vries BB. Intragenic deletion in DYRK1A leads to mental retardation and primary microcephaly. *Clin Genet*;79(3):296-9 doi: 10.1111/j.1399-0004.2010.01544.x[published Online First: Epub Date]|.
13. Yamamoto T, Shimojima K, Nishizawa T, Matsuo M, Ito M, Imai K. Clinical manifestations of the deletion of Down syndrome critical region including DYRK1A and KCNJ6. *Am J Med Genet A*;155A(1):113-9 doi: 10.1002/ajmg.a.33735[published Online First: Epub Date]|.
14. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee YH, Narzisi G, Leotta A, Kendall J, Grabowska E, Ma B, Marks S, Rodgers L, Stepansky A, Troge J, Andrews P, Bekritsky M, Pradhan K, Ghiban E, Kramer M, Parla J, Demeter R, Fulton LL, Fulton RS, Magrini VJ, Ye K, Darnell JC, Darnell RB, Mardis ER, Wilson RK, Schatz MC, McCombie WR, Wigler M. De novo gene disruptions in children on the autistic spectrum. *Neuron* 2012;74(2):285-99 doi: S0896-6273(12)00340-6 [pii]
10.1016/j.neuron.2012.04.009[published Online First: Epub Date]|.
15. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, Carvill G, Kumar A, Lee C, Ankenman K, Munson J, Hiatt JB, Turner EH, Levy R, O'Day DR, Krumm N, Coe BP, Martin BK, Borenstein E, Nickerson DA, Mefford HC, Doherty D, Akey JM, Bernier R, Eichler EE, Shendure J. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science*;338(6114):1619-22 doi: science.1227764 [pii]
10.1126/science.1227764[published Online First: Epub Date]|.
16. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, Turner EH, Stanaway IB, Vernot B, Malig M, Baker C, Reilly B, Akey JM, Borenstein E, Rieder MJ, Nickerson DA, Bernier R, Shendure J, Eichler EE. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature*;485(7397):246-50 doi: nature10989 [pii]
10.1038/nature10989[published Online First: Epub Date]|.
17. Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, Epstein MP, Glauser T, Goldstein DB, Han Y, Heinzen EL, Hitomi Y, Howell KB, Johnson MR, Kuzniecky R, Lowenstein DH, Lu YF, Madou MR, Marson AG, Mefford HC, Esmaeeli Nieh S, O'Brien TJ, Ottman R, Petrovski S, Poduri A, Ruzzo EK, Scheffer IE, Sherr EH, Yuskaits CJ, Abou-Khalil B, Alldredge BK, Bautista JF, Boro A, Cascino GD, Consalvo D, Crumrine P, Devinsky O, Fiol M, Fountain NB, French J, Friedman D, Geller EB, Glynn S, Haut SR, Hayward J, Helmers SL, Joshi S, Kanner A, Kirsch HE, Knowlton RC, Kossoff EH, Kuperman R, McGuire SM, Motika PV, Novotny EJ, Paolicchi JM, Parent JM, Park K, Shellhaas RA, Shih JJ, Singh R, Sirven J, Smith MC, Sullivan J, Lin Thio L, Venkat A, Vining EP, Von Allmen GK, Weisenberg JL, Widess-Walsh P, Winawer MR. De novo mutations in epileptic encephalopathies. *Nature* 2013;501(7466):217-21 doi: nature12439 [pii]
10.1038/nature12439[published Online First: Epub Date]|.

18. Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, Park AR, Spiegelman D, Dobrzeniecka S, Piton A, Tomitori H, Daoud H, Massicotte C, Henrion E, Diallo O, Shekarabi M, Marineau C, Shevell M, Maranda B, Mitchell G, Nadeau A, D'Anjou G, Vanasse M, Srour M, Lafreniere RG, Drapeau P, Lacaille JC, Kim E, Lee JR, Igarashi K, Huganir RL, Rouleau GA, Michaud JL. Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. *Am J Hum Genet*;88(3):306-16 doi: S0002-9297(11)00019-X [pii]
- 10.1016/j.ajhg.2011.02.001[published Online First: Epub Date] |.
19. Parsons CG, Stoffler A, Danysz W. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. *Neuropharmacology* 2007;53(6):699-723 doi: S0028-3908(07)00229-8 [pii]
- 10.1016/j.neuropharm.2007.07.013[published Online First: Epub Date] |.
20. Hung CC, Chen HY, Chen CH. Systematic mutation analysis of the human glutamate receptor, ionotropic, N-methyl-D-aspartate 1 gene(GRIN1) in schizophrenic patients. *Psychiatr Genet* 2002;12(4):225-30
21. Sakurai K, Toru M, Yamakawa-Kobayashi K, Arinami T. Mutation analysis of the N-methyl-D-aspartate receptor NR1 subunit gene (GRIN1) in schizophrenia. *Neurosci Lett* 2000;296(2-3):168-70 doi: S0304394000015998 [pii][published Online First: Epub Date] |.
22. Tarabeux J, Kebir O, Gauthier J, Hamdan FF, Xiong L, Piton A, Spiegelman D, Henrion E, Millet B, Fathalli F, Joober R, Rapoport JL, DeLisi LE, Fombonne E, Mottron L, Forget-Dubois N, Boivin M, Michaud JL, Drapeau P, Lafreniere RG, Rouleau GA, Krebs MO. Rare mutations in N-methyl-D-aspartate glutamate receptors in autism spectrum disorders and schizophrenia. *Transl Psychiatry*;1:e55 doi: tp201152 [pii]
- 10.1038/tp.2011.52[published Online First: Epub Date] |.
23. Asadollahi R, Oneda B, Sheth F, Azzarello-Burri S, Baldinger R, Joset P, Latal B, Knirsch W, Desai S, Baumer A, Houge G, Andrieux J, Rauch A. Dosage changes of MED13L further delineate its role in congenital heart defects and intellectual disability. *European journal of human genetics : EJHG* 2013;21(10):1100-4 doi: 10.1038/ejhg.2013.17[published Online First: Epub Date] |.
24. M MvH, G RM, Duran K, van Binsbergen E, Mpj Breur J, J CG, van Haaften G. Further confirmation of the MED13L haploinsufficiency syndrome. *European journal of human genetics : EJHG* 2014 doi: 10.1038/ejhg.2014.69[published Online First: Epub Date] |.
25. Muncke N, Jung C, Rudiger H, Ulmer H, Roeth R, Hubert A, Goldmuntz E, Driscoll D, Goodship J, Schon K, Rappold G. Missense mutations and gene interruption in PROSIT240, a novel TRAP240-like gene, in patients with congenital heart defect (transposition of the great arteries). *Circulation* 2003;108(23):2843-50 doi: 10.1161/01.CIR.0000103684.77636.CD[published Online First: Epub Date] |.
26. Elsea SH, Girirajan S. Smith-Magenis syndrome. *Eur J Hum Genet* 2008;16(4):412-21 doi: 5202009 [pii]
- 10.1038/sj.ejhg.5202009[published Online First: Epub Date] |.
27. Slager RE, Newton TL, Vlangos CN, Finucane B, Elsea SH. Mutations in RAI1 associated with Smith-Magenis syndrome. *Nat Genet* 2003;33(4):466-8 doi: 10.1038/ng1126 ng1126 [pii][published Online First: Epub Date] |.
28. Smith AC, McGavran L, Robinson J, Waldstein G, Macfarlane J, Zonona J, Reiss J, Lahr M, Allen L, Magenis E. Interstitial deletion of (17)(p11.2p11.2) in nine patients. *Am J Med Genet* 1986;24(3):393-414 doi: 10.1002/ajmg.1320240303[published Online First: Epub Date] |.

29. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsater H, Sponheim E, Goubran-Botros H, Delorme R, Chabane N, Mouren-Simeoni MC, de Mas P, Bieth E, Roge B, Heron D, Burglen L, Gillberg C, Leboyer M, Bourgeron T. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* 2007;39(1):25-7 doi: ng1933 [pii]
- 10.1038/ng1933[published Online First: Epub Date] |.
30. Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, Zwaigenbaum L, Fernandez B, Roberts W, Szatmari P, Scherer SW. Contribution of SHANK3 mutations to autism spectrum disorder. *Am J Hum Genet* 2007;81(6):1289-97 doi: S0002-9297(07)63777-X [pii]
- 10.1086/522590[published Online First: Epub Date] |.
31. Phelan K, McDermid HE. The 22q13.3 Deletion Syndrome (Phelan-McDermid Syndrome). *Mol Syndromol*;2(3-5):186-201 doi: 000334260 msy-0002-0186 [pii][published Online First: Epub Date] |.
32. Boccuto L, Lauri M, Sarasua SM, Skinner CD, Buccella D, Dwivedi A, Orteschi D, Collins JS, Zollino M, Visconti P, Dupont B, Tiziano D, Schroer RJ, Neri G, Stevenson RE, Gurrieri F, Schwartz CE. Prevalence of SHANK3 variants in patients with different subtypes of autism spectrum disorders. *Eur J Hum Genet*;21(3):310-6 doi: ejhg2012175 [pii]
- 10.1038/ejhg.2012.175[published Online First: Epub Date] |.
33. Gauthier J, Champagne N, Lafreniere RG, Xiong L, Spiegelman D, Brustein E, Lapointe M, Peng H, Cote M, Noreau A, Hamdan FF, Addington AM, Rapoport JL, Delisi LE, Krebs MO, Joober R, Fathalli F, Mouaffak F, Haghghi AP, Neri C, Dube MP, Samuels ME, Marineau C, Stone EA, Awadalla P, Barker PA, Carbonetto S, Drapeau P, Rouleau GA. De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia. *Proc Natl Acad Sci U S A*;107(17):7863-8 doi: 0906232107 [pii]
- 10.1073/pnas.0906232107[published Online First: Epub Date] |.
34. Gauthier J, Spiegelman D, Piton A, Lafreniere RG, Laurent S, St-Onge J, Lapointe L, Hamdan FF, Cossette P, Mottron L, Fombonne E, Joober R, Marineau C, Drapeau P, Rouleau GA. Novel de novo SHANK3 mutation in autistic patients. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B(3):421-4 doi: 10.1002/ajmg.b.30822[published Online First: Epub Date] |.
35. Waga C, Okamoto N, Ondo Y, Fukumura-Kato R, Goto Y, Kohsaka S, Uchino S. Novel variants of the SHANK3 gene in Japanese autistic patients with severe delayed speech development. *Psychiatr Genet*;21(4):208-11 doi: 10.1097/YPG.0b013e328341e069[published Online First: Epub Date] |.
36. Bozdagi O, Sakurai T, Papapetrou D, Wang X, Dickstein DL, Takahashi N, Kajiwara Y, Yang M, Katz AM, Scattone ML, Harris MJ, Saxena R, Silverman JL, Crawley JN, Zhou Q, Hof PR, Buxbaum JD. Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. *Mol Autism*;1(1):15 doi: 2040-2392-1-15 [pii]
- 10.1186/2040-2392-1-15[published Online First: Epub Date] |.
37. Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, Lascola CD, Fu Z, Feng G. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*;472(7344):437-42 doi: nature09965 [pii]
- 10.1038/nature09965[published Online First: Epub Date] |.
38. Wang X, McCoy PA, Rodriguez RM, Pan Y, Je HS, Roberts AC, Kim CJ, Berrios J, Colvin JS, Bousquet-Moore D, Lorenzo I, Wu G, Weinberg RJ, Ehlers MD, Philpot BD, Beaudet AL, Wetsel WC,

Jiang YH. Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. *Hum Mol Genet*;20(15):3093-108 doi: ddr212 [pii]

10.1093/hmg/ddr212[published Online First: Epub Date] |.

39. Yang M, Bozdagi O, Scattoni ML, Wohr M, Roullet FI, Katz AM, Abrams DN, Kalikhman D, Simon H, Woldeyohannes L, Zhang JY, Harris MJ, Saxena R, Silverman JL, Buxbaum JD, Crawley JN. Reduced excitatory neurotransmission and mild autism-relevant phenotypes in adolescent Shank3 null mutant mice. *J Neurosci*;32(19):6525-41 doi: 32/19/6525 [pii]

10.1523/JNEUROSCI.6107-11.2012[published Online First: Epub Date] |.

40. Suls A, Dedeken P, Goffin K, Van Esch H, Dupont P, Cassiman D, Kempfle J, Wuttke TV, Weber Y, Lerche H, Afawi Z, Vandenbergh W, Korczyn AD, Berkovic SF, Ekstein D, Kivity S, Ryvlin P, Claes LR, Deprez L, Maljevic S, Vargas A, Van Dyck T, Goossens D, Del-Favero J, Van Laere K, De Jonghe P, Van Paesschen W. Paroxysmal exercise-induced dyskinesia and epilepsy is due to mutations in SLC2A1, encoding the glucose transporter GLUT1. *Brain* 2008;131(Pt 7):1831-44 doi: awn113 [pii]

10.1093/brain/awn113[published Online First: Epub Date] |.

41. De Giorgis V, Veggiotti P. GLUT1 deficiency syndrome 2013: Current state of the art. *Seizure*;22(10):803-11 doi: S1059-1311(13)00196-9 [pii]

10.1016/j.seizure.2013.07.003[published Online First: Epub Date] |.

42. Wang D, Pascual JM, Yang H, Engelstad K, Jhung S, Sun RP, De Vivo DC. Glut-1 deficiency syndrome: clinical, genetic, and therapeutic aspects. *Ann Neurol* 2005;57(1):111-8 doi: 10.1002/ana.20331[published Online First: Epub Date] |.

43. Overweg-Plandsoen WC, Groener JE, Wang D, Onkenhout W, Brouwer OF, Bakker HD, De Vivo DC. GLUT-1 deficiency without epilepsy--an exceptional case. *J Inherit Metab Dis* 2003;26(6):559-63 doi: 5149903 [pii][published Online First: Epub Date] |.

44. Sirmaci A, Spiliopoulos M, Brancati F, Powell E, Duman D, Abrams A, Bademci G, Agolini E, Guo S, Konuk B, Kavaz A, Blanton S, Digilio MC, Dallapiccola B, Young J, Zuchner S, Tekin M. Mutations in ANKRD11 cause KBG syndrome, characterized by intellectual disability, skeletal malformations, and macrodontia. *Am J Hum Genet*;89(2):289-94 doi: S0002-9297(11)00260-6 [pii]

10.1016/j.ajhg.2011.06.007[published Online First: Epub Date] |.

45. Barbaric I, Perry MJ, Dear TN, Rodrigues Da Costa A, Salopek D, Marusic A, Hough T, Wells S, Hunter AJ, Cheeseman M, Brown SD. An ENU-induced mutation in the Ankrd11 gene results in an osteopenia-like phenotype in the mouse mutant Yoda. *Physiol Genomics* 2008;32(3):311-21 doi: 00116.2007 [pii]

10.1152/physiolgenomics.00116.2007[published Online First: Epub Date] |.

46. Berryer MH, Hamdan FF, Klitten LL, Moller RS, Carmant L, Schwartzentruber J, Patry L, Dobrzeniecka S, Rochefort D, Neugnot-Cerioli M, Lacaille JC, Niu Z, Eng CM, Yang Y, Palardy S, Belhumeur C, Rouleau GA, Tommerup N, Immken L, Beauchamp MH, Patel GS, Majewski J, Tarnopolsky MA, Scheffzek K, Hjalgrim H, Michaud JL, Di Cristo G. Mutations in SYNGAP1 cause intellectual disability, autism, and a specific form of epilepsy by inducing haploinsufficiency. *Hum Mutat*;34(2):385-94 doi: 10.1002/humu.22248[published Online First: Epub Date] |.

47. Carvill GL, Heavin SB, Yendle SC, McMahon JM, O'Roak BJ, Cook J, Khan A, Dorschner MO, Weaver M, Calvert S, Malone S, Wallace G, Stanley T, Bye AM, Bleasel A, Howell KB, Kivity S, Mackay MT, Rodriguez-Casero V, Webster R, Korczyn A, Afawi Z, Zelnick N, Lerman-Sagie T, Lev D, Moller RS, Gill D, Andrade DM, Freeman JL, Sadleir LG, Shendure J, Berkovic SF, Scheffer IE,

Mefford HC. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nat Genet*;45(7):825-30 doi: ng.2646 [pii]

10.1038/ng.2646[published Online First: Epub Date] |.

48. Hamdan FF, Daoud H, Piton A, Gauthier J, Dobrzeniecka S, Krebs MO, Joober R, Lacaille JC, Nadeau A, Milunsky JM, Wang Z, Carmant L, Mottron L, Beauchamp MH, Rouleau GA, Michaud JL. De novo SYNGAP1 mutations in nonsyndromic intellectual disability and autism. *Biol Psychiatry*;69(9):898-901 doi: S0006-3223(10)01204-7 [pii]

10.1016/j.biopsych.2010.11.015[published Online First: Epub Date] |.

49. Hamdan FF, Gauthier J, Spiegelman D, Noreau A, Yang Y, Pellerin S, Dobrzeniecka S, Cote M, Perreau-Linck E, Carmant L, D'Anjou G, Fombonne E, Addington AM, Rapoport JL, Delisi LE, Krebs MO, Mouaffak F, Joober R, Mottron L, Drapeau P, Marineau C, Lafreniere RG, Lacaille JC, Rouleau GA, Michaud JL. Mutations in SYNGAP1 in autosomal nonsyndromic mental retardation. *N Engl J Med* 2009;360(6):599-605 doi: 360/6/599 [pii]

10.1056/NEJMoa0805392[published Online First: Epub Date] |.

50. Rauch A, Wieczorek D, Graf E, Wieland T, Endele S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donato N, Dufke A, Cremer K, Hempel M, Horn D, Hoyer J, Joset P, Ropke A, Moog U, Riess A, Thiel CT, Tzschach A, Wiesener A, Wohlleber E, Zweier C, Ekici AB, Zink AM, Rump A, Meisinger C, Grallert H, Sticht H, Schenck A, Engels H, Rappold G, Schrock E, Wieacker P, Riess O, Meitinger T, Reis A, Strom TM. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 2012;380(9854):1674-82 doi: S0140-6736(12)61480-9 [pii]

10.1016/S0140-6736(12)61480-9[published Online First: Epub Date] |.

51. Hamdan FF, Daoud H, Patry L, Dionne-Laporte A, Spiegelman D, Dobrzeniecka S, Rouleau GA, Michaud JL. Parent-child exome sequencing identifies a de novo truncating mutation in TCF4 in non-syndromic intellectual disability. *Clin Genet* 2013;83(2):198-200 doi: 10.1111/j.1399-0004.2012.01890.x[published Online First: Epub Date] |.

52. Amiel J, Rio M, de Pontual L, Redon R, Malan V, Boddaert N, Plouin P, Carter NP, Lyonnet S, Munnich A, Colleaux L. Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *Am J Hum Genet* 2007;80(5):988-93 doi: S0002-9297(07)60955-0 [pii]

10.1086/515582[published Online First: Epub Date] |.

53. Villard L, Fontes M. Alpha-thalassemia/mental retardation syndrome, X-Linked (ATR-X, MIM #301040, ATR-X/XNP/XH2 gene MIM #300032). *Eur J Hum Genet* 2002;10(4):223-5 doi: 10.1038/sj.ejhg.5200800[published Online First: Epub Date] |.

54. Gibbons RJ, Picketts DJ, Villard L, Higgs DR. Mutations in a putative global transcriptional regulator cause X-linked mental retardation with alpha-thalassemia (ATR-X syndrome). *Cell* 1995;80(6):837-45 doi: 0092-8674(95)90287-2 [pii][published Online First: Epub Date] |.

55. Gibbons RJ, Wada T, Fisher CA, Malik N, Mitson MJ, Steensma DP, Fryer A, Goudie DR, Krantz ID, Traeger-Synodinos J. Mutations in the chromatin-associated protein ATRX. *Hum Mutat* 2008;29(6):796-802 doi: 10.1002/humu.20734[published Online First: Epub Date] |.

56. Abidi FE, Cardoso C, Lossi AM, Lowry RB, Depetris D, Mattei MG, Lubs HA, Stevenson RE, Fontes M, Chudley AE, Schwartz CE. Mutation in the 5' alternatively spliced region of the XNP/ATR-X gene causes Chudley-Lowry syndrome. *Eur J Hum Genet* 2005;13(2):176-83 doi: 5201303 [pii]

10.1038/sj.ejhg.5201303[published Online First: Epub Date] |.

57. Guerrini R, Shanahan JL, Carrozzo R, Bonanni P, Higgs DR, Gibbons RJ. A nonsense mutation of the ATRX gene causing mild mental retardation and epilepsy. *Ann Neurol* 2000; **47**(1):117-21
58. Isidor B, Pichon O, Baron S, David A, Le Caignec C. Deletion of the CUL4B gene in a boy with mental retardation, minor facial anomalies, short stature, hypogonadism, and ataxia. *Am J Med Genet A* 2010; **152A**(1):175-80 doi: 10.1002/ajmg.a.33152 [published Online First: Epub Date].
59. Cabezas DA, Slaugh R, Abidi F, Arena JF, Stevenson RE, Schwartz CE, Lubs HA. A new X linked mental retardation (XLMR) syndrome with short stature, small testes, muscle wasting, and tremor localises to Xq24-q25. *Journal of medical genetics* 2000; **37**(9):663-8
60. Tarpey PS, Raymond FL, O'Meara S, Edkins S, Teague J, Butler A, Dicks E, Stevens C, Tofts C, Avis T, Barthorpe S, Buck G, Cole J, Gray K, Halliday K, Harrison R, Hills K, Jenkinson A, Jones D, Menzies A, Mironenko T, Perry J, Raine K, Richardson D, Shepherd R, Small A, Varian J, West S, Widaa S, Mallya U, Moon J, Luo Y, Holder S, Smithson SF, Hurst JA, Clayton-Smith J, Kerr B, Boyle J, Shaw M, Vandeleur L, Rodriguez J, Slaugh R, Easton DF, Wooster R, Bobrow M, Srivastava AK, Stevenson RE, Schwartz CE, Turner G, Gecz J, Futreal PA, Stratton MR, Partington M. Mutations in CUL4B, which encodes a ubiquitin E3 ligase subunit, cause an X-linked mental retardation syndrome associated with aggressive outbursts, seizures, relative macrocephaly, central obesity, hypogonadism, pes cavus, and tremor. *Am J Hum Genet* 2007; **80**(2):345-52 doi: S0002-9297(07)62692-5 [pii]
- 10.1086/511134 [published Online First: Epub Date].
61. Deburgrave N, Daoud F, Llense S, Barbot JC, Recan D, Peccate C, Burghes AH, Beroud C, Garcia L, Kaplan JC, Chelly J, Leturcq F. Protein- and mRNA-based phenotype-genotype correlations in DMD/BMD with point mutations and molecular basis for BMD with nonsense and frameshift mutations in the DMD gene. *Hum Mutat* 2007; **28**(2):183-95 doi: 10.1002/humu.20422 [published Online First: Epub Date].
62. Lenk U, Hanke R, Thiele H, Speer A. Point mutations at the carboxy terminus of the human dystrophin gene: implications for an association with mental retardation in DMD patients. *Hum Mol Genet* 1993; **2**(11):1877-81
63. Kerr TP, Sewry CA, Robb SA, Roberts RG. Long mutant dystrophins and variable phenotypes: evasion of nonsense-mediated decay? *Hum Genet* 2001; **109**(4):402-7 doi: 10.1007/s004390100598 [published Online First: Epub Date].
64. de Brouwer AP, Nabuurs SB, Verhaart IE, Oudakker AR, Hordijk R, Yntema HG, Hordijk-Hos JM, Voesenek K, de Vries BB, van Essen T, Chen W, Hu H, Chelly J, den Dunnen JT, Kalscheuer VM, Aartsma-Rus AM, Hamel BC, van Bokhoven H, Kleefstra T. A 3-base pair deletion, c.9711_9713del, in DMD results in intellectual disability without muscular dystrophy. *Eur J Hum Genet* doi: ejhg2013169 [pii]
- 10.1038/ejhg.2013.169 [published Online First: Epub Date].
65. De Boulle K, Verkerk AJ, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van den Bos F, de Graaff E, Oostra BA, Willems PJ. A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat Genet* 1993; **3**(1):31-5 doi: 10.1038/ng0193-31 [published Online First: Epub Date].
66. Lugenbeel KA, Peier AM, Carson NL, Chudley AE, Nelson DL. Intronucleotide loss of function mutations demonstrate the primary role of FMR1 in fragile X syndrome. *Nat Genet* 1995; **10**(4):483-5 doi: 10.1038/ng0895-483 [published Online First: Epub Date].
67. Gronskov K, Brondum-Nielsen K, Dedic A, Hjalgrim H. A nonsense mutation in FMR1 causing fragile X syndrome. *Eur J Hum Genet*; **19**(4):489-91 doi: ejhg2010223 [pii]
- 10.1038/ejhg.2010.223 [published Online First: Epub Date].

68. Collins SC, Bray SM, Suhl JA, Cutler DJ, Coffee B, Zwick ME, Warren ST. Identification of novel FMR1 variants by massively parallel sequencing in developmentally delayed males. *Am J Med Genet A*;152A(10):2512-20 doi: 10.1002/ajmg.a.33626[published Online First: Epub Date] |.
69. Behnecke A, Hinderhofer K, Bartsch O, Numann A, Ipach ML, Damatova N, Haaf T, Dufke A, Riess O, Moog U. Intragenic deletions of IL1RAPL1: Report of two cases and review of the literature. *Am J Med Genet A* 2011;155A(2):372-9 doi: 10.1002/ajmg.a.33656[published Online First: Epub Date] |.
70. Laumonnier F, Ronce N, Hamel BC, Thomas P, Lespinasse J, Raynaud M, Paringaux C, Van Bokhoven H, Kalscheuer V, Fryns JP, Chelly J, Moraine C, Briault S. Transcription factor SOX3 is involved in X-linked mental retardation with growth hormone deficiency. *Am J Hum Genet* 2002;71(6):1450-5 doi: S0002-9297(07)60866-0 [pii]
- 10.1086/344661[published Online First: Epub Date] |.
71. Lepretre F, Delannoy V, Froguel P, Vasseur F, Montpellier C. Dissection of an inverted X(p21.3q27.1) chromosome associated with mental retardation. *Cytogenet Genome Res* 2003;101(2):124-9 doi: 74167
74167 [pii][published Online First: Epub Date] |.
72. Bhat SS, Ladd S, Grass F, Spence JE, Brasington CK, Simensen RJ, Schwartz CE, Dupont BR, Stevenson RE, Srivastava AK. Disruption of the IL1RAPL1 gene associated with a pericentromeric inversion of the X chromosome in a patient with mental retardation and autism. *Clin Genet* 2008;73(1):94-6 doi: CGE920 [pii]
- 10.1111/j.1399-0004.2007.00920.x[published Online First: Epub Date] |.
73. Franek KJ, Butler J, Johnson J, Simensen R, Friez MJ, Bartel F, Moss T, DuPont B, Berry K, Bauman M, Skinner C, Stevenson RE, Schwartz CE. Deletion of the immunoglobulin domain of IL1RAPL1 results in nonsyndromic X-linked intellectual disability associated with behavioral problems and mild dysmorphism. *Am J Med Genet A*;155A(5):1109-14 doi: 10.1002/ajmg.a.33833[published Online First: Epub Date] |.
74. Behnecke A, Hinderhofer K, Bartsch O, Numann A, Ipach ML, Damatova N, Haaf T, Dufke A, Riess O, Moog U. Intragenic deletions of IL1RAPL1: Report of two cases and review of the literature. *Am J Med Genet A*;155A(2):372-9 doi: 10.1002/ajmg.a.33656[published Online First: Epub Date] |.
75. Nawara M, Klapeci J, Borg K, Jurek M, Moreno S, Tryfon J, Bal J, Chelly J, Mazurczak T. Novel mutation of IL1RAPL1 gene in a nonspecific X-linked mental retardation (MRX) family. *Am J Med Genet A* 2008;146A(24):3167-72 doi: 10.1002/ajmg.a.32613[published Online First: Epub Date] |.
76. Carrie A, Jun L, Bienvenu T, Vinet MC, McDonell N, Couvert P, Zemni R, Cardona A, Van Buggenhout G, Frints S, Hamel B, Moraine C, Ropers HH, Strom T, Howell GR, Whittaker A, Ross MT, Kahn A, Fryns JP, Beldjord C, Marynen P, Chelly J. A new member of the IL-1 receptor family highly expressed in hippocampus and involved in X-linked mental retardation. *Nat Genet* 1999;23(1):25-31 doi: 10.1038/12623[published Online First: Epub Date] |.
77. Piton A, Michaud JL, Peng H, Aradhya S, Gauthier J, Mottron L, Champagne N, Lafreniere RG, Hamdan FF, Joober R, Fombonne E, Marineau C, Cossette P, Dube MP, Haghghi P, Drapeau P, Barker PA, Carbonetto S, Rouleau GA. Mutations in the calcium-related gene IL1RAPL1 are associated with autism. *Hum Mol Genet* 2008;17(24):3965-74 doi: ddn300 [pii]
- 10.1093/hmg/ddn300[published Online First: Epub Date] |.

78. Youngs EL, Henkhaus R, Hellings JA, Butler MG. IL1RAPL1 gene deletion as a cause of X-linked intellectual disability and dysmorphic features. *Eur J Med Genet*;55(1):32-6 doi: S1769-7212(11)00110-8 [pii]
- 10.1016/j.ejmg.2011.08.004[published Online First: Epub Date].
79. Tabolacci E, Pomponi MG, Pietrobono R, Terracciano A, Chiurazzi P, Neri G. A truncating mutation in the IL1RAPL1 gene is responsible for X-linked mental retardation in the MRX21 family. *Am J Med Genet A* 2006;140(5):482-7 doi: 10.1002/ajmg.a.31107[published Online First: Epub Date].
80. Shoubridge C, Tarpey PS, Abidi F, Ramsden SL, Rujirabanjerd S, Murphy JA, Boyle J, Shaw M, Gardner A, Proos A, Puusepp H, Raymond FL, Schwartz CE, Stevenson RE, Turner G, Field M, Walikonis RS, Harvey RJ, Hackett A, Futreal PA, Stratton MR, Gecz J. Mutations in the guanine nucleotide exchange factor gene IQSEC2 cause nonsyndromic intellectual disability. *Nat Genet* 2010;42(6):486-8 doi: ng.588 [pii]
- 10.1038/ng.588[published Online First: Epub Date].
81. Tran Mau-Them F, Willems M, Albrecht B, Sanchez E, Puechberty J, Endele S, Schneider A, Ruiz Pallares N, Missirian C, Rivier F, Girard M, Holder M, Manouvrier S, Touitou I, Lefort G, Sarda P, Moncla A, Drunat S, Wieczorek D, Genevieve D. Expanding the phenotype of IQSEC2 mutations: truncating mutations in severe intellectual disability. *European journal of human genetics : EJHG* 2013 doi: ejhg2013113 [pii]
- 10.1038/ejhg.2013.113[published Online First: Epub Date].
82. Jensen LR, Amende M, Gurok U, Moser B, Gimmel V, Tzschach A, Janecke AR, Tariverdian G, Chelly J, Fryns JP, Van Esch H, Kleefstra T, Hamel B, Moraine C, Gecz J, Turner G, Reinhardt R, Kalscheuer VM, Ropers HH, Lenzner S. Mutations in the JARID1C gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. *Am J Hum Genet* 2005;76(2):227-36 doi: S0002-9297(07)62574-9 [pii]
- 10.1086/427563[published Online First: Epub Date].
83. Ounap K, Puusepp-Benazzouz H, Peters M, Vaher U, Rein R, Proos A, Field M, Reimand T. A novel c.2T > C mutation of the KDM5C/JARID1C gene in one large family with X-linked intellectual disability. *Eur J Med Genet*;55(3):178-84 doi: S1769-7212(12)00006-7 [pii]
- 10.1016/j.ejmg.2012.01.004[published Online First: Epub Date].
84. Rujirabanjerd S, Nelson J, Tarpey PS, Hackett A, Edkins S, Raymond FL, Schwartz CE, Turner G, Iwase S, Shi Y, Futreal PA, Stratton MR, Gecz J. Identification and characterization of two novel JARID1C mutations: suggestion of an emerging genotype-phenotype correlation. *Eur J Hum Genet*;18(3):330-5 doi: ejhg2009175 [pii]
- 10.1038/ejhg.2009.175[published Online First: Epub Date].
85. Santos-Reboucas CB, Fintelman-Rodrigues N, Jensen LR, Kuss AW, Ribeiro MG, Campos M, Jr., Santos JM, Pimentel MM. A novel nonsense mutation in KDM5C/JARID1C gene causing intellectual disability, short stature and speech delay. *Neurosci Lett*;498(1):67-71 doi: S0304-3940(11)00549-0 [pii]
- 10.1016/j.neulet.2011.04.065[published Online First: Epub Date].
86. Tzschach A, Lenzner S, Moser B, Reinhardt R, Chelly J, Fryns JP, Kleefstra T, Raynaud M, Turner G, Ropers HH, Kuss A, Jensen LR. Novel JARID1C/SMCX mutations in patients with X-linked mental retardation. *Hum Mutat* 2006;27(4):389 doi: 10.1002/humu.9420[published Online First: Epub Date].

87. Abidi FE, Holloway L, Moore CA, Weaver DD, Simensen RJ, Stevenson RE, Rogers RC, Schwartz CE. Mutations in JARID1C are associated with X-linked mental retardation, short stature and hyperreflexia. *J Med Genet* 2008;45(12):787-93 doi: jmg.2008.058990 [pii] 10.1136/jmg.2008.058990[published Online First: Epub Date].
88. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999;23(2):185-8 doi: 10.1038/13810[published Online First: Epub Date].
89. Clayton-Smith J, Watson P, Ramsden S, Black GC. Somatic mutation in MECP2 as a non-fatal neurodevelopmental disorder in males. *Lancet* 2000;356(9232):830-2 doi: S0140673600026611 [pii][published Online First: Epub Date].
90. Meloni I, Bruttini M, Longo I, Mari F, Rizzolio F, D'Adamo P, Denvriendt K, Fryns JP, Toniolo D, Renieri A. A mutation in the rett syndrome gene, MECP2, causes X-linked mental retardation and progressive spasticity in males. *Am J Hum Genet* 2000;67(4):982-5 doi: S0002-9297(07)63290-X [pii]
- 10.1086/303078[published Online First: Epub Date].
91. Orrico A, Lam C, Galli L, Dotti MT, Hayek G, Tong SF, Poon PM, Zappella M, Federico A, Sorrentino V. MECP2 mutation in male patients with non-specific X-linked mental retardation. *FEBS Lett* 2000;481(3):285-8 doi: S0014579300019943 [pii][published Online First: Epub Date].
92. Imessaoudene B, Bonnefont JP, Royer G, Cormier-Daire V, Lyonnet S, Lyon G, Munich A, Amiel J. MECP2 mutation in non-fatal, non-progressive encephalopathy in a male. *Journal of medical genetics* 2001;38(3):171-4
93. Zeev BB, Yaron Y, Schanen NC, Wolf H, Brandt N, Ginot N, Shomrat R, Orr-Utreger A. Rett syndrome: clinical manifestations in males with MECP2 mutations. *J Child Neurol* 2002;17(1):20-4
94. Moog U, Smeets EE, van Roozendaal KE, Schoenmakers S, Herbergs J, Schoonbrood-Lenssen AM, Schrander-Stumpel CT. Neurodevelopmental disorders in males related to the gene causing Rett syndrome in females (MECP2). *Eur J Paediatr Neurol* 2003;7(1):5-12 doi: S1090379802001344 [pii][published Online First: Epub Date].
95. Villard L. MECP2 mutations in males. *J Med Genet* 2007;44(7):417-23 doi: jmg.2007.049452 [pii] 10.1136/jmg.2007.049452[published Online First: Epub Date].
96. Maiwald R, Bonte A, Jung H, Bitter P, Storm Z, Laccone F, Herkenrath P. De novo MECP2 mutation in a 46,XX male patient with Rett syndrome. *Neurogenetics* 2002;4(2):107-8
97. Archer HL, Whatley SD, Evans JC, Ravine D, Huppke P, Kerr A, Bunyan D, Kerr B, Sweeney E, Davies SJ, Reardon W, Horn J, MacDermot KD, Smith RA, Magee A, Donaldson A, Crow Y, Hermon G, Miedzybrodzka Z, Cooper DN, Lazarou L, Butler R, Sampson J, Pilz DT, Laccone F, Clarke AJ. Gross rearrangements of the MECP2 gene are found in both classical and atypical Rett syndrome patients. *J Med Genet* 2006;43(5):451-6 doi: jmg.2005.033464 [pii]
- 10.1136/jmg.2005.033464[published Online First: Epub Date].
98. Lebo RV, Ikuta T, Milunsky JM, Milunsky A. Rett syndrome from quintuple and triple deletions within the MECP2 deletion hotspot region. *Clin Genet* 2001;59(6):406-17 doi: cge590605 [pii][published Online First: Epub Date].
99. Ravn K, Nielsen JB, Skjeldal OH, Kerr A, Hulten M, Schwartz M. Large genomic rearrangements in MECP2. *Hum Mutat* 2005;25(3):324 doi: 10.1002/humu.9320[published Online First: Epub Date].
100. Laumonnier F, Holbert S, Ronce N, Faravelli F, Lenzner S, Schwartz CE, Lespinasse J, Van Esch H, Lacombe D, Goizet C, Phan-Dinh Tuy F, van Bokhoven H, Fryns JP, Chelly J, Ropers HH, Moraine C, Hamel BC, Briault S. Mutations in PHF8 are associated with X linked mental

- retardation and cleft lip/cleft palate. *Journal of medical genetics* 2005;42(10):780-6 doi: 10.1136/jmg.2004.029439[published Online First: Epub Date] |.
101. Abidi F, Miano M, Murray J, Schwartz C. A novel mutation in the PHF8 gene is associated with X-linked mental retardation with cleft lip/cleft palate. *Clin Genet* 2007;72(1):19-22 doi: CGE817 [pii]
- 10.1111/j.1399-0004.2007.00817.x[published Online First: Epub Date] |.
102. Koivisto AM, Ala-Mello S, Lemmela S, Komu HA, Rautio J, Jarvela I. Screening of mutations in the PHF8 gene and identification of a novel mutation in a Finnish family with XLMR and cleft lip/cleft palate. *Clin Genet* 2007;72(2):145-9 doi: CGE836 [pii]
- 10.1111/j.1399-0004.2007.00836.x[published Online First: Epub Date] |.
103. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* 2004;364(9443):1435-7 doi: S0140673604172267 [pii]
- 10.1016/S0140-6736(04)17226-7[published Online First: Epub Date] |.
104. Schwartz CE, May MM, Carpenter NJ, Rogers RC, Martin J, Bialer MG, Ward J, Sanabria J, Marsa S, Lewis JA, Echeverri R, Lubs HA, Voeller K, Simensen RJ, Stevenson RE. Allan-Herndon-Dudley syndrome and the monocarboxylate transporter 8 (MCT8) gene. *Am J Hum Genet* 2005;77(1):41-53 doi: S0002-9297(07)60900-8 [pii]
- 10.1086/431313[published Online First: Epub Date] |.
105. Jansen J, Friesema EC, Kester MH, Milici C, Reeser M, Gruters A, Barrett TG, Mancilla EE, Svensson J, Wemeau JL, Busi da Silva Canalli MH, Lundgren J, McEntagart ME, Hopper N, Arts WF, Visser TJ. Functional analysis of monocarboxylate transporter 8 mutations identified in patients with X-linked psychomotor retardation and elevated serum triiodothyronine. *J Clin Endocrinol Metab* 2007;92(6):2378-81 doi: jc.2006-2570 [pii]
- 10.1210/jc.2006-2570[published Online First: Epub Date] |.
106. Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet* 2003;34(1):27-9 doi: 10.1038/ng1136
- ng1136 [pii][published Online First: Epub Date] |.
107. Chih B, Afzidi SK, Clark L, Scheiffele P. Disorder-associated mutations lead to functional inactivation of neuroligins. *Hum Mol Genet* 2004;13(14):1471-7 doi: 10.1093/hmg/ddh158
- ddh158 [pii][published Online First: Epub Date] |.
108. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Sudhof TC. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science* 2007;318(5847):71-6 doi: 1146221 [pii]
- 10.1126/science.1146221[published Online First: Epub Date] |.
109. Talebizadeh Z, Lam DY, Theodoro MF, Bittel DC, Lushington GH, Butler MG. Novel splice isoforms for NLGN3 and NLGN4 with possible implications in autism. *J Med Genet* 2006;43(5):e21 doi: 43/5/e21 [pii]
- 10.1136/jmg.2005.036897[published Online First: Epub Date] |.

110. Lubs H, Abidi FE, Echeverri R, Holloway L, Meindl A, Stevenson RE, Schwartz CE. Golabi-Ito-Hall syndrome results from a missense mutation in the WW domain of the PQBP1 gene. *J Med Genet* 2006;43(6):e30 doi: 43/6/e30 [pii]
- 10.1136/jmg.2005.037556[published Online First: Epub Date] |.
111. Takahashi M, Mizuguchi M, Shinoda H, Aizawa T, Demura M, Okazawa H, Kawano K. Polyglutamine tract-binding protein-1 binds to U5-15kD via a continuous 23-residue segment of the C-terminal domain. *Biochim Biophys Acta*;1804(7):1500-7 doi: S1570-9639(10)00085-3 [pii]
- 10.1016/j.bbapap.2010.03.007[published Online First: Epub Date] |.
112. Kalscheuer VM, Freude K, Musante L, Jensen LR, Yntema HG, Gecz J, Sefiani A, Hoffmann K, Moser B, Haas S, Gurok U, Haesler S, Aranda B, Nshedjan A, Tzschach A, Hartmann N, Roloff TC, Shoichet S, Hagens O, Tao J, Van Bokhoven H, Turner G, Chelly J, Moraine C, Fryns JP, Nuber U, Hoeltzenbein M, Scharff C, Scherthan H, Lenzner S, Hamel BC, Schweiger S, Ropers HH. Mutations in the polyglutamine binding protein 1 gene cause X-linked mental retardation. *Nat Genet* 2003;35(4):313-5 doi: 10.1038/ng1264
- ng1264 [pii][published Online First: Epub Date] |.
113. Kunde SA, Musante L, Grimme A, Fischer U, Muller E, Wanker EE, Kalscheuer VM. The X-chromosome-linked intellectual disability protein PQBP1 is a component of neuronal RNA granules and regulates the appearance of stress granules. *Hum Mol Genet*;20(24):4916-31 doi: ddr430 [pii]
- 10.1093/hmg/ddr430[published Online First: Epub Date] |.
114. Froyen G, Corbett M, Vandewalle J, Jarvela I, Lawrence O, Meldrum C, Bauters M, Govaerts K, Vandeleur L, Van Esch H, Chelly J, Sanlaville D, van Bokhoven H, Ropers HH, Laumonnier F, Ranieri E, Schwartz CE, Abidi F, Tarpey PS, Futreal PA, Whibley A, Raymond FL, Stratton MR, Fryns JP, Scott R, Peippo M, Sipponen M, Partington M, Mowat D, Field M, Hackett A, Marynen P, Turner G, Gecz J. Submicroscopic duplications of the hydroxysteroid dehydrogenase HSD17B10 and the E3 ubiquitin ligase HUWE1 are associated with mental retardation. *Am J Hum Genet* 2008;82(2):432-43 doi: S0002-9297(07)00036-5 [pii]
- 10.1016/j.ajhg.2007.11.002[published Online First: Epub Date] |.
115. Fichou Y, Nectoux J, Bahi-Buisson N, Rosas-Vargas H, Girard B, Chelly J, Bienvenu T. The first missense mutation causing Rett syndrome specifically affecting the MeCP2_e1 isoform. *Neurogenetics* 2009;10(2):127-33 doi: 10.1007/s10048-008-0161-1[published Online First: Epub Date] |.
116. Saunders CJ, Minassian BE, Chow EW, Zhao W, Vincent JB. Novel exon 1 mutations in MECP2 implicate isoform MeCP2_e1 in classical Rett syndrome. *Am J Med Genet A* 2009;149A(5):1019-23 doi: 10.1002/ajmg.a.32776[published Online First: Epub Date] |.
117. Froyen G, Belet S, Martinez F, Santos-Reboucas CB, Declercq M, Verbeeck J, Donckers L, Berland S, Mayo S, Rosello M, Pimentel MM, Fintelman-Rodrigues N, Hovland R, Rodrigues dos Santos S, Raymond FL, Bose T, Corbett MA, Sheffield L, van Ravenswaaij-Arts CM, Dijkhuizen T, Coutton C, Satre V, Siu V, Marynen P. Copy-number gains of HUWE1 due to replication- and recombination-based rearrangements. *Am J Hum Genet* 2012;91(2):252-64 doi: S0002-9297(12)00319-9 [pii]
- 10.1016/j.ajhg.2012.06.010[published Online First: Epub Date] |.
118. Fox JW, Lamperti ED, Eksioglu YZ, Hong SE, Feng Y, Graham DA, Scheffer IE, Dobyns WB, Hirsch BA, Radtke RA, Berkovic SF, Huttenlocher PR, Walsh CA. Mutations in filamin 1 prevent

- migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* 1998;21(6):1315-25 doi: S0896-6273(00)80651-0 [pii][published Online First: Epub Date] |.
119. Robertson SP, Twigg SR, Sutherland-Smith AJ, Biancalana V, Gorlin RJ, Horn D, Kenwick SJ, Kim CA, Morava E, Newbury-Ecob R, Orstavik KH, Quarrell OW, Schwartz CE, Shears DJ, Suri M, Kendrick-Jones J, Wilkie AO. Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nat Genet* 2003;33(4):487-91 doi: 10.1038/ng1119
- ng1119 [pii][published Online First: Epub Date] |.
120. Sheen VL, Dixon PH, Fox JW, Hong SE, Kinton L, Sisodiya SM, Duncan JS, Dubeau F, Scheffer IE, Schachter SC, Wilner A, Henchy R, Crino P, Kamuro K, DiMario F, Berg M, Kuzniecky R, Cole AJ, Bromfield E, Biber M, Schomer D, Wheless J, Silver K, Mochida GH, Berkovic SF, Andermann F, Andermann E, Dobyns WB, Wood NW, Walsh CA. Mutations in the X-linked filamin 1 gene cause periventricular nodular heterotopia in males as well as in females. *Hum Mol Genet* 2001;10(17):1775-83
121. Unger S, Mainberger A, Spitz C, Bahr A, Zeschnigk C, Zabel B, Superti-Furga A, Morris-Rosendahl DJ. Filamin A mutation is one cause of FG syndrome. *Am J Med Genet A* 2007;143A(16):1876-9 doi: 10.1002/ajmg.a.31751[published Online First: Epub Date] |.

Table S1: Technical sequencing results on all 106 samples

Batch#	Sample ID	# input reads	Read mapped (%)	Median coverage	>1X (%)	>10X (%)	>40X (%)	>80X (%)	Multi-mapped reads (%)	Duplicate reads (%)	Reads left after filtering (%)	Reads in target regions (%) before filtering	Reads in target regions (%)			Nb known SNV	Nb new SNV	%New SNVs
					>1X (%)	>10X (%)	>40X (%)	>80X (%)					Nb SNV	Nb indels				
1	APN-01	23 927 282	99.6%	158	99.8%	99.5%	97.5%	88.7%	0.5%	82.5%	17.0%	70.4%	64.5%	602	139	578	24	4%
	APN-02	30 366 258	99.6%	227	99.9%	99.6%	98.5%	95.1%	0.5%	80.4%	19.1%	70.0%	65.0%	562	146	543	19	3%
	APN-03	26 100 034	99.6%	209	99.8%	99.5%	98.1%	93.5%	0.6%	78.3%	21.1%	70.5%	63.4%	617	146	605	12	2%
	APN-04	35 432 234	99.6%	270	99.9%	99.6%	99.0%	97.3%	0.6%	78.3%	21.1%	67.5%	60.2%	623	152	605	18	3%
	APN-07	28 382 460	99.7%	181	99.8%	99.4%	97.6%	90.6%	0.4%	83.2%	16.4%	71.9%	65.1%	619	147	608	11	2%
	APN-08	32 866 324	99.7%	265	99.9%	99.6%	99.0%	97.0%	0.6%	78.3%	21.1%	70.9%	63.5%	562	148	549	13	2%
	APN-09	28 392 354	99.5%	207	99.8%	99.5%	98.1%	93.8%	0.6%	79.3%	20.1%	67.1%	60.4%	596	152	584	12	2%
	APN-10	25 786 264	99.6%	194	99.9%	99.6%	98.4%	94.0%	0.5%	79.2%	20.3%	69.9%	61.5%	603	150	587	16	3%
	APN-11	19 842 144	99.7%	169	99.8%	99.5%	98.1%	91.7%	0.7%	75.8%	23.6%	68.5%	59.8%	588	141	572	16	3%
	APN-12	31 237 990	98.6%	198	99.8%	99.4%	98.1%	93.8%	0.9%	71.4%	27.7%	47.0%	34.7%	615	149	595	20	3%
	APN-13	18 916 942	99.2%	155	99.8%	99.4%	97.3%	88.1%	0.6%	78.0%	21.3%	68.4%	63.9%	583	150	572	11	2%
2 ^a	APN-15	55 437 506	97.9%	91	99.8%	99.1%	92.6%	60.5%	1.8%	57.2%	41.0%	43.3%	68.4%	607	142	600	7	1%
	APN-16	69 337 832	98.7%	145	97.5%	91.6%	84.4%	75.1%	0.7%	82.7%	16.5%	55.8%	20.5%	548	142	533	15	3%
	APN-19	13 620 090	97.5%	79	99.1%	93.3%	70.5%	49.7%	2.1%	48.0%	49.9%	41.7%	20.6%	543	125	535	8	1%
3	APN-24	52 250 914	97.2%	184	99.9%	99.8%	99.4%	97.0%	0.5%	83.3%	16.2%	60.8%	27.8%	620	185	604	16	3%
	APN-25	35 420 424	97.7%	232	99.9%	99.8%	99.4%	98.4%	0.6%	77.5%	21.9%	62.4%	37.6%	634	169	623	11	2%
	APN-28	46 303 338	97.2%	402	99.9%	99.8%	99.6%	99.1%	0.7%	72.6%	26.7%	61.1%	45.2%	584	163	571	13	2%
	APN-29	44 522 830	97.5%	333	99.9%	99.7%	99.5%	98.8%	0.6%	77.0%	22.5%	63.2%	45.8%	605	167	588	17	3%
	APN-30	37 719 174	97.5%	295	99.9%	99.7%	99.2%	98.0%	0.6%	74.7%	24.7%	60.6%	44.3%	627	170	614	13	2%
	APN-31	35 946 982	97.6%	279	99.9%	99.7%	99.3%	98.1%	0.6%	74.9%	24.5%	63.0%	44.3%	638	180	620	18	3%
	APN-32	48 462 058	97.4%	454	99.9%	99.8%	99.6%	99.2%	0.6%	72.6%	26.7%	63.3%	48.9%	574	179	559	15	3%
	APN-33	41 053 574	97.4%	371	99.9%	99.8%	99.6%	99.2%	0.6%	72.8%	26.5%	62.8%	46.1%	674	179	653	21	3%
	APN-34	36 702 446	97.4%	232	99.9%	99.7%	99.2%	97.8%	0.5%	80.2%	19.3%	63.5%	44.1%	596	168	581	15	3%
	APN-35	51 145 142	96.5%	230	99.9%	99.8%	99.5%	98.3%	0.7%	77.1%	22.2%	55.6%	26.7%	596	180	582	14	2%
	APN-36	48 399 084	97.1%	241	99.9%	99.8%	99.5%	98.6%	0.5%	82.0%	17.5%	62.9%	37.0%	584	180	575	9	2%
4	APN-37	44 564 444	97.1%	356	99.9%	99.8%	99.6%	99.2%	0.7%	75.0%	24.3%	60.5%	44.4%	619	170	608	11	2%
	APN-39	40 672 474	97.6%	480	99.9%	99.8%	99.6%	99.4%	0.8%	68.1%	31.1%	63.4%	51.8%	600	154	580	20	3%
	APN-40	44 111 500	97.2%	417	99.9%	99.8%	99.6%	99.2%	0.7%	72.8%	26.5%	63.7%	49.8%	565	167	554	11	2%
	APN-41	52 875 660	97.3%	425	99.9%	99.8%	99.6%	99.4%	0.6%	75.6%	23.8%	63.3%	44.3%	624	176	614	10	2%
	APN-42	41 040 590	97.4%	428	99.9%	99.8%	99.6%	99.2%	0.7%	69.8%	29.4%	62.2%	49.1%	573	173	560	13	2%
	APN-43	49 583 266	97.4%	145	99.9%	99.8%	99.3%	88.9%	0.4%	88.3%	11.4%	64.7%	32.1%	656	190	642	14	2%
	APN-46	47 255 380	97.2%	180	99.9%	99.7%	99.1%	95.4%	0.5%	83.4%	16.1%	60.2%	32.9%	596	182	584	12	2%
	APN-47	36 227 960	97.2%	178	99.9%	99.7%	99.2%	96.0%	0.5%	82.0%	17.5%	61.5%	37.7%	614	167	602	12	2%
	APN-48	43 920 858	96.9%	183	99.9%	99.8%	99.3%	96.5%	0.5%	82.9%	16.7%	59.9%	33.6%	630	169	617	13	2%
	APN-49	50 099 296	97.0%	223	99.9%	99.8%	99.3%	97.7%	0.5%	82.6%	17.0%	62.6%	36.5%	609	169	599	10	2%
5	APN-50	42 289 892	97.0%	148	99.9%	99.7%	98.6%	90.8%	0.4%	85.7%	13.9%	61.6%	35.1%	629	169	616	13	2%
	APN-45	31 253 880	97.4%	112	99.9%	99.8%	98.6%	67.2%	0.6%	79.6%	19.8%	61.7%	22.8%	633	180	624	9	1%
	APN-51	31 867 712	97.6%	225	99.9%	99.8%	99.4%	98.3%	0.8%	73.1%	26.1%	62.3%	34.8%	596	182	585	11	2%
	APN-53	24 884 362	97.6%	161	99.9%	99.7%	99.3%	94.2%	0.7%	75.1%	24.1%	61.8%	34.4%	588	181	573	15	3%
	APN-54	28 353 188	97.5%	97	99.9%	99.7%	97.5%	57.6%	0.6%	81.6%	17.8%	62.3%	23.9%	599	182	589	10	2%
	APN-56	30 468 964	97.3%	102	99.9%	99.7%	98.0%	61.5%	0.6%	81.0%	18.4%	59.3%	23.1%	567	179	560	7	1%
	APN-57	28 507 966	97.5%	74	99.9%	99.6%	90.8%	45.4%	0.5%	84.0%	15.5%	61.5%	22.0%	572	184	560	12	2%
	APN-58 ^b	33 477 982	97.6%	35	99.9%	98.6%	41.5%	0.9%	0.3%	90.8%	8.9%	68.9%	16.3%	621	212	574	47	8%

	APN-60	27 830 732	97.4%	80	99.9%	99.6%	93.6%	50.5%	0.5%	83.3%	16.2%	61.3%	23.6%	591	169	576	15	3%
	APN-63	25 668 896	97.4%	109	99.9%	99.7%	98.3%	66.1%	0.6%	81.7%	17.7%	62.5%	30.5%	587	177	569	18	3%
	APN-65	34 420 082	97.3%	115	99.9%	99.7%	98.6%	68.8%	0.6%	82.6%	16.8%	61.8%	24.8%	590	180	583	7	1%
6	APN-14 ^c	23 144 314	99.2%	196	99.9%	99.6%	99.0%	95.0%	0.4%	84.4%	15.2%	77.7%	73.6%	608	158	599	9	1%
	APN-68	19 810 224	99.2%	151	99.9%	99.6%	98.7%	90.6%	0.4%	84.9%	14.7%	77.7%	68.7%	592	164	582	10	2%
	APN-70	3 444 990	99.2%	114	99.8%	99.4%	96.4%	75.9%	1.4%	38.6%	60.0%	75.2%	73.1%	618	150	605	13	2%
	APN-73	23 056 048	99.4%	238	99.9%	99.7%	99.4%	98.2%	0.6%	77.4%	21.9%	73.4%	62.6%	613	154	610	3	0%
	APN-74	22 070 772	99.2%	130	99.9%	99.6%	98.1%	84.5%	0.3%	88.4%	11.3%	75.0%	69.1%	616	154	606	10	1%
	APN-76	21 962 484	99.2%	258	99.9%	99.7%	99.4%	98.3%	0.5%	77.2%	22.3%	76.0%	69.9%	603	164	589	14	2%
	APN-77	18 575 362	96.5%	250	99.8%	99.6%	99.2%	98.0%	1.4%	47.0%	51.6%	38.3%	32.0%	645	164	629	16	2%
	APN-80	21 129 530	99.3%	184	99.9%	99.6%	98.9%	94.4%	0.4%	83.3%	16.3%	76.6%	71.1%	595	148	584	11	1%
	APN-81	18 074 502	99.2%	174	99.9%	99.6%	98.9%	93.2%	0.4%	82.0%	17.6%	77.0%	72.3%	547	161	542	5	1%
	APN-82	20 574 088	99.3%	193	99.9%	99.7%	99.0%	95.0%	0.4%	82.5%	17.1%	77.3%	72.5%	573	151	564	9	1%
	APN-83	25 008 458	99.2%	295	99.9%	99.8%	99.4%	98.9%	0.6%	75.6%	23.8%	75.5%	65.8%	585	163	575	10	1%
	APN-84	23 851 690	99.1%	376	99.9%	99.8%	99.5%	99.1%	0.9%	67.1%	32.1%	73.9%	65.6%	597	163	585	12	2%
	APN-86	16 885 790	99.1%	241	99.9%	99.7%	99.4%	98.3%	0.8%	68.5%	30.7%	75.5%	61.1%	586	172	580	6	1%
	APN-87	21 682 206	99.1%	264	99.9%	99.8%	99.4%	98.7%	0.7%	72.6%	26.7%	74.9%	60.1%	626	170	618	8	1%
7	APN-17 ^c	18 692 086	99.1%	299	99.9%	99.7%	99.3%	98.5%	0.9%	66.9%	32.2%	75.6%	66.3%	582	172	574	8	1%
	APN-18 ^c	25 957 694	98.6%	251	99.9%	99.6%	99.2%	97.4%	1.1%	68.8%	30.1%	65.3%	44.1%	577	156	570	7	1%
	APN-26	25 012 706	98.9%	208	99.8%	99.6%	98.8%	95.6%	0.7%	77.9%	21.3%	71.1%	52.5%	617	163	606	11	1%
	APN-38	21 900 976	98.2%	151	99.8%	99.5%	98.2%	90.6%	1.1%	68.1%	30.8%	58.7%	30.2%	612	161	598	14	2%
	APN-69	23 433 926	97.7%	184	99.9%	99.5%	98.7%	94.2%	1.4%	59.9%	38.7%	50.6%	27.8%	615	166	605	10	1%
	APN-72	27 072 904	97.5%	248	99.9%	99.7%	98.9%	96.8%	1.3%	62.3%	36.5%	53.6%	34.5%	607	158	600	7	1%
	APN-114	17 241 784	95.5%	188	99.8%	99.3%	97.0%	89.3%	2.7%	9.7%	87.6%	18.3%	17.4%	563	140	559	4	1%
	APN-115	19 560 280	97.1%	433	99.9%	99.6%	99.0%	97.7%	2.2%	24.7%	73.0%	45.7%	41.5%	617	147	606	11	1%
	APN-116	17 207 384	98.0%	590	99.8%	99.5%	99.1%	98.2%	1.7%	23.5%	74.8%	59.1%	56.3%	599	153	586	13	2%
	APN-117	13 374 526	99.1%	609	99.8%	99.6%	99.0%	98.1%	1.5%	20.5%	78.0%	72.2%	71.3%	626	159	618	8	1%
	APN-118	20 137 422	98.2%	686	99.8%	99.6%	99.2%	98.5%	1.7%	25.8%	72.6%	60.6%	57.4%	650	152	638	12	1%
	APN-119	20 227 292	98.8%	645	99.9%	99.7%	99.2%	98.5%	1.9%	30.7%	67.3%	66.2%	63.9%	633	156	624	9	1%
	APN-120	24 984 952	98.5%	664	99.9%	99.6%	99.3%	98.7%	1.7%	36.9%	61.3%	63.0%	58.6%	609	158	601	8	1%
	APN-121	18 167 438	98.9%	563	99.9%	99.6%	99.2%	98.4%	1.8%	35.3%	62.9%	69.3%	66.4%	620	154	610	10	1%
	APN-122	20 241 724	99.0%	737	99.8%	99.6%	99.3%	98.7%	1.5%	34.9%	63.7%	72.0%	68.9%	617	165	607	10	1%
8	APN-123	19 947 194	97.7%	372	99.8%	99.5%	98.6%	96.6%	1.9%	39.2%	59.0%	50.7%	42.8%	603	151	591	12	2%
	APN-124	23 381 352	97.4%	504	99.9%	99.6%	99.2%	98.3%	2.0%	24.3%	73.7%	45.0%	40.2%	585	156	570	15	2%
	APN-125	22 035 142	99.1%	874	99.8%	99.6%	99.3%	98.8%	1.4%	29.6%	69.0%	71.6%	69.4%	600	155	594	6	1%
	APN-126	21 898 210	98.7%	791	99.8%	99.6%	99.3%	98.8%	1.4%	33.5%	65.1%	70.5%	67.7%	630	156	617	13	2%
	APN-127	26 850 456	98.8%	696	99.9%	99.7%	99.4%	98.8%	1.5%	43.8%	54.8%	68.4%	63.7%	617	167	605	12	2%
	APN-99	19 156 392	98.3%	365	99.9%	99.6%	98.8%	97.3%	1.3%	57.7%	41.0%	64.5%	62.7%	626	183	615	11	1%
	APN-100	14 919 436	98.7%	80	99.7%	98.5%	84.4%	50.0%	0.3%	89.8%	9.9%	74.1%	73.0%	596	175	581	15	2%
	APN-101	20 575 066	98.5%	417	99.9%	99.6%	98.9%	97.6%	1.1%	58.2%	40.7%	68.4%	67.1%	625	168	613	12	2%
	APN-102	24 463 598	98.7%	294	99.9%	99.5%	98.5%	95.9%	0.7%	76.3%	23.1%	71.8%	70.0%	632	173	619	13	2%
	APN-103	23 568 632	98.8%	325	99.9%	99.5%	98.4%	95.6%	0.7%	73.3%	25.9%	73.9%	72.1%	610	178	605	5	1%
	APN-104	19 208 764	97.3%	404	99.8%	99.6%	98.7%	97.1%	1.7%	39.6%	58.7%	51.1%	49.0%	629	171	620	9	1%
	APN-105	23 921 986	97.6%	402	99.9%	99.5%	98.7%	97.0%	1.3%	54.8%	43.9%	53.5%	52.6%	610	168	599	11	1%
	APN-106	19 424 216	97.1%	556	99.8%	99.6%	99.1%	98.2%	2.6%	14.9%	82.4%	49.9%	47.4%	599	176	592	7	1%
	APN-107	27 343 616	96.7%	612	99.9%	99.7%	99.2%	98.3%	2.4%	25.8%	71.8%	45.8%	42.5%	607	155	592	15	2%
	APN-108	20 657 638	96.6%	499	99.9%	99.6%	98.9%	97.6%	2.6%	6.0%	91.4%	37.0%	36.8%	603	158	596	7	1%
	APN-109	30 928 180	95.9%	554	99.8%	99.6%	99.1%	98.1%	2.7%	14.2%	83.1%	31.7%	29.8%	587	172	577	10	1%
	APN-110	23 887 290	98.6%	839	99.9%	99.7%	99.4%	98.8%	1.9%	26.2%	72.0%	67.6%	65.7%	637	168	623	14	2%
	APN-111	24 851 496	98.5%	540	99.9%	99.7%	99.3%	98.6%	1.5%	53.2%	45.3%	69.7%	64.7%	648	173	640	8	1%
	APN-112	17 718 676	98.2%	492	99.8%	99.6%	99.0%	97.9%	1.7%	35.4%	62.9%	63.1%	60.1%	617	171	604	13	2%

	APN-113	27 627 002	98.5%	775	99.9%	99.7%	99.3%	98.7%	1.7%	38.4%	59.9%	67.4%	63.2%	609	185	595	14	2%	
9	APN-128	19 785 578	99.0%	607	99.9%	99.6%	99.2%	98.3%	1.8%	34.2%	64.1%	68.3%	64.8%	590	166	580	10	1%	
	APN-130	19 853 062	99.2%	726	99.9%	99.6%	99.2%	98.5%	1.3%	35.0%	63.7%	71.9%	68.5%	667	159	651	16	2%	
	APN-131	23 072 640	99.1%	609	99.9%	99.7%	99.3%	98.6%	1.5%	42.5%	56.0%	67.9%	63.4%	580	162	574	6	1%	
	APN-132	29 360 264	98.2%	238	99.8%	99.5%	98.6%	96.0%	0.8%	77.0%	22.2%	74.0%	49.2%	614	167	607	7	1%	
	APN-134	25 278 984	99.1%	751	99.9%	99.7%	99.4%	98.8%	1.6%	35.5%	62.9%	67.6%	63.6%	599	153	592	7	1%	
	APN-135	25 325 698	99.0%	660	99.9%	99.7%	99.3%	98.6%	1.6%	43.7%	54.7%	68.7%	63.9%	652	156	640	12	1%	
	APN-137	24 441 638	98.9%	826	99.9%	99.7%	99.3%	98.7%	1.9%	25.6%	72.5%	65.9%	62.8%	624	162	615	9	1%	
	APN-138	23 128 034	99.1%	456	99.8%	99.6%	99.0%	98.0%	1.2%	54.7%	44.1%	69.1%	60.5%	622	160	609	13	2%	
	APN-139	26 354 740	99.1%	632	99.9%	99.6%	99.3%	98.6%	1.3%	48.6%	50.1%	70.4%	64.3%	573	162	558	15	2%	
	APN-141	26 997 926	99.1%	633	99.9%	99.6%	99.3%	98.7%	1.3%	50.0%	48.7%	71.0%	64.3%	710	161	702	8	1%	
	APN-142	21 163 728	99.1%	941	99.9%	99.6%	99.2%	98.7%	1.5%	21.1%	77.4%	71.9%	69.7%	624	164	612	12	2%	
		Mean	28 458 084	98.2%	353	99.8%	99.5%	97.7%	91.7%	1.0%	61.8%	37.1%	63.7%	51.2%	607	164	595	12	2%
		SD	11 060 265	1.0%	218	0.2%	1.0%	6.6%	15.2%	0.6%	22.8%	22.2%	10.5%	16.4%	27	13	27	5	1%

a : Capture reaction step was performed on a pool of two equimolar DNA samples (such as previously described in Redin et al.), what may explain the drop of coverage and of capture efficiency observed in those samples

b: APN-58 did not pass our quality criteria for sequencing data but it is however indicated in the table since a causative mutation was detected in *DYRK1A* in spite of general lower-coverage data.

c: Re-sequenced samples because of poor coverage results obtained initially with batch 2 sequencing

Table S2: List of benign or pathogenic CNVs detected in patients using a depth of coverage comparison method

Gene	Patient ID	Sex	Inheritance	Segregation	Gene MIM#	CNV type	Confirmation	Conclusion
X-Linked genes								
<i>MECP2</i>	APN-3	M	XL	inherited (M)	300005	inversion, hemz	Sanger	Pathogenic
<i>FMR1</i>	APN-26	M	XL	inherited (M)	309550	deletion, hemz	Sanger, CGH (1 deleted probe)	Pathogenic
<i>MAGT1</i>	APN-141	M	XL	?	300715	duplication, htz	-	Probably benign
Autosomal recessive genes								
<i>MCPH1</i>	APN-42	M	AR	inherited (M)	607117	deletion, htz	CGH (in report)	Benign
Others								
<i>CYFIP1</i>	APN-14	M	?	?	606322	duplication, htz	CGH (in report)	Benign

XL: X-linked, AR: Autosomal Recessive, ?: unclear implication in ID

Table S3: List of regions consistently poorly-covered in our assay

Gene	Exon/Intron #	Chrom	Start	End	Length of the region coding uncoding total (pb)			GC content	Mean depth Batch 1	Mean depth Batch 2	Mean depth Batch 3	Mean depth Batch 4	Mean depth Batch 5	Mean depth Batch 6	Mean depth Batch 7	Mean depth Batch 8	Mean depth Batch 9	Max Depth All	Mean Depth All	Mean depth ^a (Percentile: 90%)
<i>DEAF1</i>	E1 (5'UTR)	11	695 133	695 176	0	44	44	79%	8	3	35	40	24	21	16	13	13	58	20	39
<i>NRXN2</i>	E2 (5'UTR + coding)	11	64 481 117	64 481 332	55	161	216	81%	4	0	18	23	11	7	5	4	5	37	9	14
<i>RAIL</i>	E6 (coding + 3' UTR)	17	17 713 292	17 713 385	4	90	94	77%	12	4	30	35	24	26	17	15	18	66	21	29
<i>GAMT</i>	E1 (coding + 5'UTR)	19	1 401 471	1 401 599	5	124	129	80%	5	1	21	26	12	15	8	8	8	48	12	18
<i>SHANK1</i>	E22	19	51 172 026	51 172 224	199	0	199	79%	11	2	27	29	17	22	9	8	10	44	15	31
<i>AFF3</i>	I2	2	100 721 046	100 721 084	0	39	39	87%	14	1	22	26	17	26	20	17	22	52	20	27
<i>PRODH</i>	E2 (5'UTR + coding) + I2	22	18 923 494	18 923 831	273	65	338	79%	4	0	8	10	6	9	7	6	7	19	7	10
<i>SHANK3</i>	E1 + I1	22	51 113 030	51 113 174	63	82	145	85%	0	0	2	3	3	2	1	1	1	6	1	2
<i>SHANK3</i>	I10	22	51 135 785	51 135 802	0	18	18	71%	14	4	20	25	14	30	32	29	26	78	24	40
<i>SHANK3</i>	I10 + E11 + I11	22	51 135 837	51 136 183	152	195	347	79%	3	2	4	6	3	6	3	3	4	10	4	5
<i>SYNGAP1</i>	E1 (5' UTR + coding) + II	6	33 387 996	33 388 117	67	55	122	66%	2	0	1	1	2	2	1	1	0	5	1	2
<i>LAMC3</i>	E1 (5' UTR + coding)	9	133 884 469	133 884 618	17	133	150	83%	3	0	28	34	13	6	4	5	4	62	11	17
<i>RALGDS</i>	I1 + E1	9	135 996 336	135 996 385	27	23	50	78%	10	2	25	25	16	25	19	17	20	46	19	26
<i>RALGDS</i>	E1 (coding + 5'UTR)	9	135 996 436	135 996 553	106	12	118	77%	7	1	20	21	13	18	11	10	15	36	14	19
<i>NHS</i>	E1 (5'UTR + coding)	X	17 393 847	17 394 146	266	34	300	76%	5	1	22	24	12	9	8	6	8	40	11	15
<i>RPS6KA3</i>	E1 (upstream + coding)	X	20 284 705	20 284 772	46	22	68	75%	13	0	20	24	11	24	20	14	16	62	18	29
<i>ARX</i>	I2+E2	X	25 031 435	25 031 939	481	24	505	81%	7	1	15	15	10	17	11	8	12	34	12	16
<i>ATP6AP2</i>	E1 (5' UTR + coding) + II	X	40 440 226	40 440 443	37	181	218	77%	4	1	11	12	7	9	8	6	6	23	7	12
<i>IQSEC2</i>	E1 (5' UTR)	X	53 350 457	53 350 483	0	27	27	81%	5	0	37	39	15	8	9	6	9	74	14	23
<i>FGD1</i>	E1 (5' UTR)	X	54 521 880	54 521 891	0	12	12	91%	10	3	39	37	15	24	21	12	20	69	21	38
<i>SRPX2</i>	I10	X	99 924 410	99 924 449	0	40	40	64%	16	1	29	40	17	17	17	12	18	52	19	25
<i>LAMP2</i>	I1 + E1 (coding + 5'UTR)	X	119 602 936	119 603 057	64	58	122	69%	9	4	24	27	13	13	16	11	19	46	16	22
<i>OCRL</i>	E1 (5' UTR + coding) + II	X	128 674 326	128 674 543	39	179	218	76%	3	1	7	8	4	10	5	3	5	16	6	8
<i>SOX3</i>	E1 (3'UTR + coding)	X	139 585 853	139 585 902	18	32	50	67%	10	1	23	25	14	24	20	13	17	43	18	25
<i>MECP2</i>	I1 + E1	X	153 362 925	153 363 118	58	136	194	80%	1	0	2	2	1	2	2	2	3	6	2	3
		Total		1 977	1 786	3 763	77%													

a: Regions where 90% of patients harbor a coverage <40X

Table S4: List of the variants annotated as pathogenic mutations according to dbSNP but unlikely associated to ID

Gene	Transcript#	Variation nomenclature	Clinical Significance in dbSNP	EVS carriers	EVS MAF	Predictions	phyloP	Grantham Dist	All Count (/106)	Responsible for ID	Reason for questioning
X-Linked genes											
<i>FGD1</i> (XL)	NM_004463.2	chrX:g.54496615G>A c.935C>T p.Pro312Leu (rs28935498)	pathogenic	7 (2M hemz, 5 F htz)	0.07%	Deleterious (Sift), Neutral (Polyphen2)	1.34	98	1 hmz	No	Already discussed in Piton et al., AJHG, 2013
<i>OTC</i> (XL)	NM_000531.5	chrX:g.38268220A>G c.809A>G p.Gln270Arg (rs1800328)	pathogenic	351 (93 M hemz, 6 F hmz, 246 F htz)	3.32%	Deleterious (Sift, Polyphen2)	4.48	43	6 hmz, 2 htz) ⁽⁴⁾	No	Frequency too high in males in control cohort
Autosomal recessive genes											
<i>ACYI</i> (AR)	NM_000666.2	chr3:g.52022837C>T c.1057C>T p.Arg353Cys (rs121912698)	pathogenic	33 (33 htz)	0.25%	Deleterious (Sift, Polyphen2)	2.47	180	1 htz	Unlikely	Frequency high in control cohort
<i>ACYI</i> (AR)	NM_000666.2	chr3:g.52023042G>A c.1178G>A p.Arg393His (rs121912701)	pathogenic	59 (1 hmz, 58 htz)	0.46%	Tolerated (Sift), Deleterious (Polyphen2)	0.13	29	1 htz	Unlikely	Frequency too high in control cohort
<i>GAMT</i> (AR)	NM_000156.4	chr19:g.1399056C>T c.460-31G>A p.? (rs55776826)	pathogenic	1989 (139 hmz, 1711 htz)	15.29%	NA	-3.83	NA	23 (2 hmz, 21 htz)	No	Frequency too high in control cohort
<i>PRODH</i> (AR*)	NM_016335.4	chr22:g.18905964C>T c.1292G>A p.Arg431His (rs2904552)	pathogenic	1061 (48 hmz, 965 htz)	8.16%	Deleterious (Sift, Polyphen2)	1.42	29	17 hmz, 16 htz) ⁽¹⁾	No	Frequency too high in control cohort
<i>PRODH</i> (AR*)	NM_016335.4	chr22:g.18905899G>A c.1357C>T p.Arg453Cys (rs3970559)	pathogenic	119 (2 hmz, 115 htz)	0.91%	Deleterious (Sift, Polyphen2)	1.01	180	4 htz	No	Frequency too high in control cohort
<i>PRODH</i> (AR*)	NM_016335.4	chr22:g.18909902A>T c.865T>A p.Leu289Met (rs137852934)	pathogenic	60 (60 htz)	0.46%	Tolerated (Sift), Neutral (Polyphen2)	0.37	15	1 htz	Unlikely	Frequency too high in control cohort
<i>PRODH</i> (AR*)	NM_016335.4	chr22:g.18905934A>G c.1322T>C p.Leu441Pro (rs2904551)	pathogenic	76 (76 htz)	0.58%	Deleterious (Sift, Polyphen2)	3.92	98	1 htz	Unlikely	Frequency too high in control cohort
<i>PMM2</i> (AR)	NM_000303.2	chr16:g.8905010G>A c.422G>A p.Arg141His (rs28936415)	pathogenic	50 (50 htz)	0.39%	Deleterious (Sift, Polyphen2)	5.69	29	2 htz	Unlikely	Frequency too high in control cohort

Some variants are annotated as 'pathogenic' in databases such as dbSNP but their frequency in our cohort or in a large general population is incoherent with penetrant pathogenicity

EVS: Exome Variant Server; MAF: minor allele frequency; htz: heterozygous; hemz: hemizygous; hmz: homozygous

Table S5: List of initial candidate variants with excluded/questioned pathogenicity

Gene	Patient ID	Transcript #	Variation nomenclature	Predictions	phyloP	Grantham Dist	Count in cohort (/106)	Responsible for ID	Reason for questioning
Autosomal dominant genes									
<i>DEAF1</i>	APN-109	NM_021008.2	chr11:g.691601G>C c.290-3C>G p.?	100% splice site disruption (MES, NNS). Alters splicing in vitro	2,55	-	1 htz	Unlikely	Inherited from unaffected mother
<i>DOCK8</i>	APN-105	NM_203447.3	chr9:g.197171G>T c.3496G>T p.Glu1166*	-	NA	-	1 htz	Unlikely	Proband also carries a causative splice site mutation in <i>PHF8</i> , and the implication of <i>DOCK8</i> in ID most probably substantial
<i>ZNF599</i>	APN-31, APN-42, APN-100	NM_001007248.2	chr19:g.35250691_35250692del c.1014_1015del p.Tyr339* (rs148227520)	-	-	-	3 htz	No	Reported in 3 patients of the cohort, among which one (APN-42) also carries a frameshift mutation in <i>DMD</i>
X-linked genes									
<i>SHROOM4</i>	APN-86	NM_020717.3	chrX:g.50345803G>A c.3772C>T p.Gln1258*	-	4,08	NA	1 hemz	No	Present in 2 unaffected brothers. Implication of this gene already questionned in 12
<i>SRPX2</i>	APN-13	NM_014467.2	chrX:g.99920309del c.602del p.Ala201Valfs*10	-	-	NA	1 hemz	Unlikely	Present in 3 maternal aunts. Absent from maternal grandmother, probably inherited from unaffected (deceased) grandfather. Implication of this gene already questionned in (12)
<i>FLNA</i>	APN-17	NM_001110556.1	chrX:g.153588207G>A c.3872C>T p.Pro1291Leu (rs137853319)	Deleterious (Sift), Neutral (Polyphen2)	1,74	98	1 hemz	Unlikely	Described as pathogenic but present in a control male (EVS). Patient has inconsistent phenotype compared to the initial patient with this mutation (46)
<i>HUWE1</i>	APN-10	NM_031407.4	chrX:g.53596663G>C c.6437C>G p.Thr2146Arg	Deleterious (Sift, Polyphen2)	3,6	71	1 hemz	Unlikely	Present in an unaffected brother
<i>MECP2</i>	APN-74	NM_001110792.1	chrX:g.153363068C>T c.55G>A p.Glu19Lys	Deleterious (Sift), Neutral (Polyphen2)	2,22	56	1 hemz	Unlikely	Present in an unaffected brother
<i>FMRI</i>	APN-132	NM_002024.5	chrX:g.147024687G>A c.1312G>A p.Asp438Asn	Deleterious (Sift, Polyphen2)	5,05	23	1 hemz	Unlikely	Present in the unaffected maternal half-brother

htz: heterozygous, hemz: hemizygous

MES: MaxEntScan, NNS: NNSplice.

Table S6: Comparison of advantages and drawbacks of trio-genome, trio-exome and targeted sequencing strategies for the diagnosis of intellectual disability

Compared features	full-genome	exome	targeted
Trio analysis	required	needed (or preferable) ^a	not needed
Cost	very expensive (for required depth)	expensive ^a	4-5x cheaper ^a
Variant analysis	highly complex, very time-consuming (several millions of variants to analyze, of which >22,000 coding)	complex, time-consuming (>20,000 variants to analyze)	more straightforward (several hundred variants to analyze)
# of variant detected	4.4x10 ⁶ SNVs	~ 24,324 ² ; ~21,100 ³	~700
Depth of coverage (mean)	80X ¹	64X ² ; 112X ³ ; 130X ⁴	373X
% covered regions	92% >40X ¹	87%>10X ² ; 90%>20X ³ ; 95%>20X ⁴	97.6%>40X
Sensitivity	very high NA ¹	high 97.4% ² ; 97.5% ³	very high 100%
Diagnostic efficiency ^b	42% ¹ (CI: 28-56%)	16% ² (updated to 27% in ¹); 31% ³ ; 33% ⁴ ; 24.5% (updated to 30% ¹) in pooled data on 211patients (+/-6% CI)	25% (CI: 17-33%)
Gene discovery	yes	yes	no
Data re-analysis with new knowledge	yes	yes	yes/no
Incidental findings	yes	yes	no
Best suited application	research	research/diagnosis	diagnosis

NA: non available, SNVs: single nucleotide variants

^a three exomes for the exome strategy vs only proband for the targeted sequencing strategy, less samples pooled per sequencing lane in cases of exome vs targeted sequencing. Prices highly vary upon the mean sequencing depth to be reached. While Yang et al. performed proband only exome seq, they had to check further by Sanger 5-8 variants/sample, and reported a 14% false positive rate of NGS calls, ^b confidence interval (CI)

¹ Gilissen et al, Nature, 2014; ² de Ligt et al., NEJM, 2012; ³ Rauch et al., Lancet, 2012 ; ⁴ Yang et al., NEJM, 2013

Table S7: Genes in which certainly-causative mutations were reported in the previous trio-exome studies

Rauch et al., Lancet, 2012		De Ligt et al., NEJM, 2012	
Gene	Mutation	Gene	Mutation
Autosomal dominant genes			
<i>SATB2</i>	Missense, de novo	<i>CTNNB1</i>	Frameshift, de novo
<i>SCN2A</i>	Frameshift, de novo	<i>DYNC1H1</i>	Missense, de novo
<i>SCN2A</i>	Frameshift, de novo	<i>GATAD2B</i>	Nonsense, de novo
<i>SNC2A</i>	Missense, de novo	<i>GRIN2A</i>	Missense, de novo
<i>SCN8A</i>	Missense, de novo	<i>GRIN2A</i>	Missense, de novo
<i>SETBP1</i>	Nonsense, de novo	<i>GRIN2B</i>	Missense, de novo
<i>SLC2A1</i>	Missense, de novo	<i>SCN2A</i>	Nonsense, de novo
<i>STXBP1</i>	Missense, de novo	<i>SYNGAP1</i>	Splice mutation, de novo
<i>STXBP1</i>	Splice mutation, de novo	<i>TCF4</i>	Missense, de novo
<i>STXBP1</i>	Missense, de novo	<i>TUBA1A</i>	Frameshift, de novo
<i>SYNGAP1</i>	Frameshift, de novo		
<i>SYNGAP1</i>	Frameshift, de novo		
<i>TCF4</i>	Missense, de novo		
Autosomal recessive genes			
None	-	<i>LRP2</i>	frameshift de novo + missense inherited
X-Linked genes			
<i>IQSEC2</i>	Nonsense, de novo	<i>ARHGEF9</i>	Missense, inherited
<i>MECP2</i>	Frameshift, de novo	<i>ARHGEF9</i>	Splice mutation, inherited
<i>NAA10</i>	Missense, de novo	<i>PDHA1</i>	Frameshift, de novo
		<i>PDHA1</i>	Missense, inherited
		<i>SLC6A8</i>	Frameshift, de novo
Initially candidate genes for dominant ID that have now been relicated			
<i>CHD2</i>	Frameshift, de novo	<i>EEF1A2</i>	Missense, de novo
<i>DEAF1</i>	Missense, de novo	<i>KIF5C</i>	Missense, de novo
<i>KCNQ3</i>	Missense, de novo		
<i>SETD5</i>	Nonsense, de novo		

bold: genes not included in our panel of 217 genes

Data from the study from Yang et al., NEJM, 2013 could not be included since although it is indicated that 60 patients had been recruited for neurological disorders (developmental delay,speech delay, ASD or ID), genes in which certainly-causative mutations have been detected for such category of patients are not clearly specified.

Table S8: List of the 217 targeted genes with associated mode of inheritance, presence of truncating variants in Exome Variant Server, nature of mutations reported in patients, and side-symptoms.

Official gene symbol	Gene MIM #	Associated syndrome MIM #)	(Phenotype	Associated traits	Truncating mutations in EVS	Nature of mutations reported in OMIM (Truncating, Missenses, other)	Total size of targeted regions (bp)
X-Linked genes							
<i>ACSL4</i>	300157	Mental retardation, X-linked 63 (MIM 300387)	NS-ID	-	1T, 2M	2 785	
<i>AFF2</i>	300806	Mental retardation, X-linked, FRAXE type (MIM 309548)	ADHD, autistic traits or NS-ID	-	1 trinucleotide expansion, 1 large exon deletion	4 867	
<i>APIS2</i>	300629	Mental retardation, X-linked syndromic, Friedreich type (MIM 300630)	Hypotonia, walking delay, aggressivity, DF or NS-ID	-	5T	634	
<i>ARHGEF6</i>	300267	Mental retardation, X-linked 46 (MIM 300436)	NS-ID	-	1T, 1 translocation	3 211	
<i>ARHGEF9</i>	300429	Epileptic encephalopathy, early infantile, 8 (MIM 300607)	Hyperekplexia or NS-ID	-	1T, 1M, 1 translocation, 1 large deletion encompassing 2 other genes	2 021	
<i>ARX</i>	300382	Epileptic encephalopathy, early infantile, 1 (MIM 308350); Hydranencephaly with abnormal genitalia/Lissencephaly, X-linked 2 (MIM 300215); Mental retardation, X-linked 29 and others (MIM 300419); Partington syndrome (MIM 309510), Proud syndrome (MIM 300004)	Epileptic encephalopathy, lissencephaly, hydranencephaly with abnormal genitalia, or NS-ID	-	10T, 7M, 1 polyAla expansion, 2 large exon deletions	1 889	
<i>ATP6AP2</i>	300556	-	Epilepsy	1 splice hemz	1 synonymous variant, slightly affecting splicing	1 413	
<i>ATP7A</i>	300011	Menkes disease (MIM 309400); Occipital horn syndrome (MIM 304150)	Growth retardation, cerebellar degeneration, seizures; hyperelastic skin, hyperextensible joints, skeletal anomalies	-	9T, 5M, 2 large exon deletions	5 383	
<i>ATRX</i>	300032	Alpha-thalassemia syndrome (MIM 300448, 301040)	DF, genital abnormalities, microcephaly, hypotonia, anemia, or NS-ID	1 stop htz	8T, 18M	8 879	
<i>BCOR</i>	300485	OFCD syndrome (MIM 300166)	Microphthalmia, cataract, cardiac anomalies, DF, dental anomalies	1 splice htz	6T, 1M, 2 large exon deletions	5 828	
<i>BRWD3</i>	300553	Mental retardation, X-linked 93 (MIM 300659)	Macrocephaly, DF	-	2T, 1M	7 295	
<i>CACNA1F</i>	300110	Congenital stationary night blindness (MIM 300071)	Nystagmus, autistic traits	-	5T, 2M, 1 large exon deletion	8 387	

<i>CASK</i>	300172	FG syndrome 4 (MIM 300422) MICPCH syndrome (MIM 300749)	Hypotonia, constipation, behavioral disturbances, nystagmus, microcephaly, pontine & cerebellar hypoplasia, speech & walk delay, DF	-	4T, 6M, 1 large exon deletion, 1 splice site decreased	3 927
<i>CCDC22</i>	300859	-	Hypoplastic phalanges, DF, genital anomalies	-	1M	2 957
<i>CDKL5</i>	300203	Angelman syndrome-like (MIM 105830); Early Infantile Epileptic Encephalopathy-2 (MIM 300672)	Ataxia, hypotonia, epilepsy, abnormal behaviors, speech delay, autistic traits, DF	-	7T, 7M	3 893
<i>CLCN4</i>	302910	-	Infantile epileptic encephalopathy, microcephaly, regression, hypotonia	-	1M	2 804
<i>CNKS2</i>	300724	-	Epilepsy, microcephaly	-	1 large deletion	4 007
<i>CUL4B</i>	300304	Mental retardation, X-linked, syndromic 15 (Cabezas type, MIM 300354)	Short stature, hypogonadism, abnormal gait, speech delay, tremor	-	2T, 1M	3 595
<i>DCX</i>	300121	Lissencephaly, X-linked (MIM 300067)	Seizures, growth retardation, hypogenitalism	-	4T, 10M	1 624
<i>DKC1</i>	300126	Dyskeratosis congenita (MIM 305000); Hoyeraal-Hreidarsson syndrome (MIM 300240)	Abnormal skin pigmentation, hypogenitalism, anemia, microcephaly, growth retardation, spastic paresis, ataxia, anemia, immunodeficiency, cerebellar hypoplasia	-	1T, 10M, 1 in frame deletion, 1 large exon deletion, 1 promoter mutation, 1 intronic variant	2 145
<i>DLG3</i>	300189	Mental retardation, X-linked 90 (MIM 300850)	NS-ID	1 stop hemz in an alternative exon	4T	3 610
<i>DMD (DP71)</i>	300377	Becker muscular dystrophy (MIM 300376); Duchenne muscular dystrophy (MIM 310200)	Cardiac anomalies	1 stop htz, 3 splices in 9 htz and 1 hemz)	69T, 10M, 6 large exon deletions, 1 alu insertion	12 142
<i>EIF2S3</i>	300161	-	Microcephaly, short stature, DF	-	1M (Borck et al., Mol. Cell., 2012)	1 932
<i>FGD1</i>	300546	Aarskog-Scott syndrome (MIM 305400)	ADHD, short stature, shawl scrotum, brachydactyly	-	5T, 6M, 1 large exon deletion	3 757
<i>FLNA</i>	300017	FG syndrome 2 (MIM 300321); Periventricular Heterotopia (MIM 300049); Melnick-Needles syndrome (MIM 309350)	Hypotonia, speech delay, macrocephaly, constipation, epilepsy, cleft palate, skeletal anomalies, deafness, urogenital defects, DF	-	15T, 14M	11 265
<i>FMR1</i>	309550	Fragile-X syndrome (MIM 300624)	Macroorchidism, speech delay, DF	-	3T, 1M, 1 trinucleotide expansion	2 674
<i>FRMPD4</i>	300838	-		-	1 partial duplication (Honda et al., J. Hum. Genet., 2010)	4 632
<i>FTSJ1</i>	300499	Mental retardation, X-linked 9 (MIM 309549)	Aggressive behaviour or NS-ID	-	3T, 1 large exon deletion	1 689

<i>GDI1</i>	300104	Mental retardation, X-linked 41 (MIM 300849)	NS-ID	-	2T, 2M	2 007
<i>GK</i>	300474	Glycerol kinase deficiency (MIM 307030)	Gastrointestinal symptoms	-	2T, 3M, 2 large exon deletions, 1 alu insertion	2 590
<i>GPC3</i>	300037	Simpson-Golabi-Behmel syndrome 1 (MIM 312870)	Overgrowth, congenital cardiac anomalies, cleft palate, DF	-	5T, 4M, 2 large exon deletions	2 172
<i>GRIA3</i>	305915	Mental retardation, X-linked 94 (MIM 300699)	Macrocephaly, seizures, autistic traits or NS-ID	-	3M, 1 entire gene deleted, 1 translocation, 1 submicroscopic duplication, 2 partial duplication	3 440
<i>HCCS</i>	300056	Microphthalmia, syndromic 7 (MIM 309801)	Hyperpigmented skin	-	1T, 1M, 1 large exon deletion	1 047
<i>HCFC1</i>	300019	Mental retardation, X-linked 3 (methylmalonic acidemia and homocysteinemia, cbIX type, MIM 309541)	Epilepsy, choreoathetosis, microcephaly, growth retardation or NS-ID	-	4M, 1 5' UTR variant	7 411
<i>HPRT1</i>	308000	Lesch-Nyhan syndrome (MIM 300322)	Spastic cerebral palsy, choreoathetosis, uric acid urinary stones, self-destructive behaviour, megaloblastic anemia	-	16T, 16M, 1 in frame deletion, 1 in frame insertion, 6 large exon deletions, 1 large inversion, 1 large exon duplication, 1 entire gene deletion	1 017
<i>HSD17B10</i>	300256	17-beta-hydroxysteroid dehydrogenase X deficiency (MIM 300438)	Choreoathetosis, developmental regression, epilepsy, visual anomalies	-	5M	1 157
<i>HUWE1</i>	300697	Mental retardation, X-linked syndromic, Turner type (MIM 300706)	Macrocephaly, macroorchidism or NS-ID	-	3M, 6 microduplications	16 786
<i>IDS</i>	300823	Mucopolysaccharidosis II (MIM 309900)	Airway obstruction, skeletal anomalies, cardiomyopathy	-	7T, 9M, 1 entire gene deletion	2 079
<i>IGBP1</i>	300139	Corpus callosum, agenesis of, with mental retardation, ocular coloboma and micrognathia (MIM 300472)	-	-	1 5' UTR variant	1 260
<i>IL1RAPL1</i>	300206	Mental retardation, X-linked 21/34 (MIM 300143)	Autistic traits, synophrys, hyperextensible joints, or NS-ID	-	2T, 2 large exon deletions	2 491
<i>IQSEC2</i>	300522	Mental retardation, X-linked 1 (MIM 309530)	Epilepsy, speech delay, brachycephaly, strabismus, or NS-ID	-	4M	5 269
<i>KDM5C</i>	314690	Claes-Jensen type XLID (MIM 300534)	Spastic paraparesis, short stature, microcephaly, epilepsy, facial hypotonia, maxillary hypoplasia	-	3T, 6M	3 915
<i>KIAA2022</i>	300524	Mental retardation, X-linked 98 (MIM 300912)	NS-ID	-	1 pericentric inversion	4 671
<i>KLF8</i>	300286	-	Hypotonia	-	1 translocation (Lossi et al., J; Med. Genet., 2002)	1 320

<i>L1CAM</i>	308840	CRASH syndrome (MIM 303350); MASA syndrome (MIM 303350); Hydrocephalus with Hirschsprung disease (MIM 307000)	Spastic paraparesis, aphasia, shuffling gait, speech delay	-	6T, 10M, 1 large exon duplication, 1 synonymous variant leading to an in-frame deletion	5 613
<i>LAMP2</i>	309060	Danon disease (MIM 300257)	Cardiac anomalies, muscle weakness	-	10T, 2M	1 956
<i>MAGT1</i>	300715	Mental retardation, X-linked 95 (MIM 300716)	NS-ID	1 stop (1 hemiz)	1M	1 504
<i>MAOA</i>	309850	Brunner syndrome (MIM 300615)	Impulsive aggressiveness or NS-ID	-	1T	2 184
<i>MBTPS2</i>	300294	IFAP/BRESHECK syndrome (MIM 308205)	Ichthyosis follicularis, atrichia, photophobia, hirschsprung disease, kidney dysplasia, cryptorchidism, cleft palate, skeletal anomalies	-	5M	2 000
<i>MECP2</i>	300005	Angelman syndrome (MIM 105830); Severe neonatal encephalopathy (MIM 300673); Rett syndrome (MIM 312750)	Gait anomalies, abnormal behavior, epilepsy, speech limitation, microcephaly, hypotonia, respiratory anomalies, constipation, growth retardation, developmental regression, autistic traits, DF	-	22T, 14M, 1 large in frame deletion, 1 entire gene duplication	1 683
<i>MED12</i>	300188	Lujan-Fryns syndrome (MIM 309520); Opitz-Kaveggia syndrome (MIM 305450); Ohdo syndrome (MIM 300895)	Marfanoid habitus, underweight, cryptorchidism, joint laxity, clinodactyly, skin pigmentation, deafness, feeding disorder, macrocephaly, hypotonia, constipation, agenesis of the CC, DF	-	5M	8 805
<i>MIDI</i>	300552	Opitz GBBB syndrome (MIM 300000)	Hypospadias, cleft palate, laryngotracheoesophageal abnormalities, imperforate anus, cardiac defects	-	4T, 2M, 1 exon duplication, 1 in-frame deletion, 1 in-frame duplication	2 827
<i>MIR222</i>	300569	-	-	-	-	150
<i>NDP</i>	300658	Norrie disease (MIM 310600)	Early blindness, deafness, seizures, growth retardation	-	3T, 13M	482
<i>NDUFA1</i>	300078	Mitochondrial complex I deficiency (MIM 252010)	Macrocephaly, leukodystrophy, encephalopathy, cardiomyopathy, myopathy, parkinson disease, liver disease, optic neuropathy	-	3M	333
<i>NHS</i>	300457	Nance-Horan syndrome (MIM 302350)	Congenital cataract, dental anomalies, DF	-	6T	5 390
<i>NLGN3</i>	300336	Asperger syndrome susceptibility, X-linked 1 (MIM 300494); Autism susceptibility, X-linked 1 (MIM 300425)	ASD, Asperger	-	1M	2 827

<i>NLGN4X</i>	300427	Mental retardation, X-linked (MIM 300495); Asperger syndrome susceptibility, X-linked 2 (MIM 300497); susceptibility, X-linked 2 (MIM 300495)	ASD, Asperger or NS-ID	-	2T, 1 large exon deletion	2 651
<i>NSDHL</i>	300275	CHILD syndrome (MIM 308050); CK syndrome (MIM 300831)	Hemidysplasia, ichthyosiform erythroderma, limb defects, seizures, microcephaly, cortical malformations, DF	-	4T, 3M, 1 in-frame deletion	1 402
<i>NXF5</i>	300319	-	Short stature, pectus excavatum, epilepsy, hypotonia, autistic traits, DF	2 stops in 8 hemz individuals	1 large inversion, 1 large deletion, 2 large duplications (reviewed in Piton et al., AJHG, 2013)	2 017
<i>OCRL</i>	300535	Lowe syndrome (MIM 309000); Dent disease (MIM 300555)	Hydrophthalmia, cataract, aminoaciduria, hypotonia, renal defects	-	3T, 6M	3 770
<i>OFDI</i>	311200	Joubert syndrome 1 (MIM 300804); Oral-facial-digital syndrome 1 (MIM 311200); Simpson-Golabi-Behmel syndrome, type 2 (MIM 300209)	Hypotonia, cerebellar ataxia, breathing defects, retinal dystrophy, polydactyly, dental anomalies, renal defects, macrocephaly, gastrointestinal and genitourinary anomalies, DF	-	8T, 1M, 1 large exon deletion	4 006
<i>OPHNI</i>	300127	Mental retardation, X-linked, with cerebellar hypoplasia and distinctive facial appearance (MIM 300486)	Hypotonia, strabismus, seizures, DF	-	4T, 2 large exon deletions, 1 translocation	3 329
<i>OTC</i>	300461	Ornithine Transcarbamylase Deficiency (MIM 311250)	Encephalopathy, hyperammonemia, respiratory alkalosis	-	6T, 20M, 2 intronic variants, 1 entire gene deletion	1 505
<i>PAK3</i>	300142	Mental retardation, X-linked 30/47 (MIM 300558)	Microcephaly, hypotonia, drooling, psychiatric features	-	2T, 3M	2 383
<i>PCDH19</i>	300460	Epileptic encephalopathy, early infantile, 9 (MIM 300088)	Psychiatric features	-	6T, 2M	3 687
<i>PDHA1</i>	300502	Leigh syndrome (MIM 308930); Pyruvate dehydrogenase E1-alpha deficiency (MIM 312170)	Breathing disorder, hypotonia, spasticity, lactic acidosis; hypotonia, seizures, spasticity, cerebellar anomalies	-	7T, 12M, 1 in-frame deletion, 3 in-frame insertions	1 788
<i>PHF6</i>	300414	Borjeson-Forssman-Lehmann syndrome (MIM 301900)	Epilepsy, hypogonadism, hypometabolism, obesity, DF	-	4T, 6M	1 563
<i>PHF8</i>	300560	Siderius mental retardation (MIM 300263)	DF, cleft lift/palate	-	2T, 1M, 1 in-frame deletion	4 332
<i>PLP1</i>	300401	Pelizaeus-Merzbacher disease (MIM 312080)	Nystagmus, spastic paraplegia, ataxia, spasticity, seizures	-	5T, 19M, 1 in 5' UTR, 1 entire gene deletion, 1 entire gene duplication	1 114
<i>PORCN</i>	300651	Focal dermal hypoplasia (MIM 305600, lethal in males)	Skin pigmentation, digit anomalies, visual defects (coloboma)	-	3T, 2M	2 243

<i>PQBP1</i>	300463	Renpenning syndrome (MIM 309500)	Microcephaly, short stature, hypogonadism, coloboma, DF	-	6T, 1M	1 130
<i>PRPS1</i>	311850	Arts syndrome (MIM 301835); Charcot-Marie-Tooth (MIM 311070); Phosphoribosylpyrophosphate Synthetase superactivity (MIM 300661)	Hypotonia, ataxia, deafness, optic atrophy, polyneuropathy, gout, hyperuricemia	-	11M	1 237
<i>PTCHD1</i>	300828	-	Autistic traits, NS-ID	-	6M, 1 in-frame indel	2 787
<i>RAB39B</i>	300774	Mental retardation, X-linked 72 (MIM 300271)	Seizures, autistic traits	-	2T	722
<i>RBM10</i>	300080	TARP syndrome (MIM 311900)	Cardiac anomalies, clubfoot, DF	-	2T	4 481
<i>RPL10</i>	312173	Autism, susceptibility to, X-linked 5 (MIM 300847)	ASD	-	2M	970
<i>RPS6KA3</i>	300075	Coffin-Lowry syndrome (MIM 303600)	Skeletal defects, growth retardation, hearing defects, paroxysmal movement disorders, DF or NS-ID	-	9T, 9M, 2 in-frame deletions, 1 large exon duplication	3 144
<i>SHROOM4</i>	300579	Stocco dos Santos syndrome (MIM 300434)	Short stature, hip luxation	1 stop htz	1M	4 842
<i>SLC16A2</i>	300095	Allan-Herndon-Dudley syndrome (MIM 300523)	Hypotonia, muscular atrophy, joint contractures, spastic paraplegia	-	3T, 5M, 2 large exon deletions, 1 in-frame deletion	2 082
<i>SLC6A8</i>	300036	Cerebral creatine deficiency syndrome 1 (MIM 300352)	Speech delay, seizures, behavioral abnormalities	-	3T, 5M, 2 in-frame deletions	2 729
<i>SLC9A6</i>	300231	Christianson mental retardation (MIM 300243)	Microcephaly, severe speech delay, hypotonia, seizures, impaired ocular movements, drooling	1 splice htz	3T, 2 in-frame deletions	2 746
<i>SMC1A</i>	300040	Cornelia de Lange syndrome 2 (MIM 300590)	Limb defects, growth retardation, epilepsy, cerebellar anomalies, DF	-	3M, 2 in-frame deletions, 1 large exon deletion	5 249
<i>SMS</i>	300105	Snyder-Robinson mental retardation syndrome (MIM 309583)	Hypotonia, marfanoid habitus	-	1T, 2M	1 541
<i>SOX3</i>	313430	Isolated growth hormone deficiency (MIM 300123)	Hypopituitarism	-	3T	
<i>SRPX2</i>	300642	Rolandic epilepsy, mental retardation, and speech dyspraxia (MIM 300643)	-	-	2M	1 798
<i>SYN1</i>	313440	Epilepsy, X-linked, with variable learning disabilities and behavior disorders (MIM 300491)	Autistic traits	-	2T, 2M	2 830
<i>SYP</i>	313475	Mental retardation, X-linked 96 (MIM 300802)	Epilepsy, or NS-ID	-	3T, 1M	1 182
<i>TIMM8A</i>	300356	Jensen syndrome (MIM 311150); Mohr-Tranebjærg Syndrome (MIM 304700)	Progressive deafness, blindness and dystonia	-	7T, 1M, 1 entire gene deletion	520

<i>TSPAN7</i>	300096	Mental retardation, X-linked 58 (MIM 300210)	NS-ID	-	2T, 1M	1 030
<i>UBE2A</i>	312180	XLID, Nascimento-type (MIM 300860)	DF, synophrys, hypogenitalism	-	1T, 2M	699
<i>UPF3B</i>	300298	Mental retardation, X-linked, syndromic 14 (MIM 300676)	Autistic traits, slender build or NS-ID	1 splice htz	3T, 1M	1 892
<i>ZDHHC15</i>	300576	Mental retardation, X-linked 91 (MIM 300577)	Hypotonia, seizures, limb anomalies, obesity, DF	-	1 translocation	1 534
<i>ZDHHC9</i>	300646	Mental retardation, X-linked syndromic, Raymond type (MIM 300799)	Marfanoid habitus, arachnodactyly, strabismus		2T, 2M	1 455
<i>ZMYM3</i>	300061	-	NS-ID	-	1 translocation	5 296
<i>ZNF41</i>	314995	Mental retardation, X-linked 89 (MIM 300848)	Epilepsy, speech delay or NS-ID	1 splice hemz, 1 stop htz	1T, 1M, 1 translocation	2 500
<i>ZNF674</i>	300573	Mental retardation, X-linked 92 (MIM 300851)	NS-ID	2 stops, in 19 hemz, 5 htz individuals	1T	1 906
<i>ZNF711</i>	314990	Mental retardation, X-linked 97 (MIM 300803)	NS-ID	-	2T	2 566
<i>ZNF81</i>	314998	Mental retardation, X-linked 45 (MIM 300498)	NS-ID	1 stop hemz	1M	2 146
Autosomal Recessive genes						
<i>ACY1</i>	104620	Aminoacylase 1 deficiency (MIM 609924)	Encephalopathy, seizures, hypotonia	-	2T, 4M	2 162
<i>ADRA2B</i>	104260	-	NS-ID	1 stop htz	1M (Najmabadi et al., Nature, 2011)	1 383
<i>ADSL</i>	608222	Adenylosuccinase deficiency (MIM 103050)	Autistic traits, epilepsy, hypotonia	-	7M, 1 5' UTR variant	1 975
<i>ALDH18A1</i>	138250	Cutis laxa, autosomal recessive, type IIIA (MIM 219150)	Visual defects, skin elasticity, hypotonia, joint laxity	2 splices htz	1T, 2M	3 179
<i>ALG6</i>	604566	Congenital disorder of glycosylation, type Ic (MIM 603147)	Psychomotor delay, hypotonia, ataxia, seizures	-	2T, 3M, 2 in-frame deletions	2 170
<i>ANK3</i>	600465	Mental retardation, autosomal recessive, 37 (MIM 615493)	Autistic traits, ADHD	1 splice htz	1T, 4M, 1 translocation	15 633
<i>AP4B1</i>	607245	Spastic paraplegia 47, autosomal recessive (MIM 614066)	Hypotonia progressing into hypertonia, speech and psychomotor delay	3 stops htz	1T, 1 in-frame insertion	2 610
<i>AP4E1</i>	607244	Spastic paraplegia 51, autosomal recessive (MIM 613744)	Hypotonia progressing into hypertonia, speech and psychomotor delay, seizures	-	2T, 1 large exon deletion	4 368
<i>AP4M1</i>	602296	Spastic paraplegia 50, autosomal recessive (MIM 612936)	Hypotonia progressing into hypertonia, strabismus, speech and psychomotor delay	1 stop htz, 1 splice htz	1T, 1M	2 307
<i>AP4S1</i>	607243	Spastic paraplegia 52, autosomal recessive (MIM 614067)	Hypotonia progressing into hypertonia, speech and psychomotor delay	2 stops, 3 splices in 6 htz individuals	1T	843
<i>ASPM</i>	605481	Microcephaly 5, primary, autosomal recessive (MIM 608716)	Speech delay, seizures	5 stops, 2 splices in 113 htz, 2 hmz individuals	11T	11 796

<i>C12orf57</i>	615140	Temptamy syndrome (MIM 218340)	Coloboma, DF, cerebellar defects, seizures	1 splice htz	2M	498
<i>CACNA1G</i>	604065	-	Cataract	1 splice htz	1T (Najmabadi et al., Nature, 2011)	9 272
<i>CC2D1A</i>	610055	Mental retardation, autosomal recessive 3 (MIM 608443)	NS-ID	2 splices in 14 carriers	1T	4 777
<i>CDK5RAP2</i>	608201	Microcephaly 3, primary, autosomal recessive (MIM 604804)	-	4 stops, 1 splice in 5 htz	2T	7 202
<i>CENPJ</i>	609279	Microcephaly 6, primary, autosomal recessive (MIM 608393); Seckel syndrome 4 (MIM 613676)	Joint stiffness, growth retardation, DF	7 stops in 9 htz	3T, 2M	4 821
<i>CNTNAP2</i>	604569	Cortical dysplasia-focal epilepsy syndrome (MIM 610042); Pitt-Hopkins like syndrome 1 (MIM 610042)	Speech regression, aberrant social interactions, hyperactivity	1 stop htz	4T, 1M, 2 large exon deletions	4 956
<i>CRBN</i>	609262	Mental retardation, autosomal recessive 2 (MIM 607417)	NS-ID	1 stop htz	1T	1 769
<i>ELP2</i>	-	-	NS-ID	4 stops in 5 htz, 1 splice htz	2M (Najmabadi et al., Nature, 2011)	3 661
<i>FOLR1</i>	136430	Neurodegeneration due to cerebral folate transport deficiency (MIM 613068)	Developmental regression, epilepsy, movement disturbances, leukodystrophy	2 splices in 28 htz	3T, 1 in-frame duplication	930
<i>FTCD</i>	606806	Glutamate formiminotransferase deficiency (MIM 229100)	Megaloblastic anemia	6 stops in 24 htz, 1 hertz	1T, 2M	2 283
<i>GAMT</i>	601240	Cerebral creatine deficiency syndrome 2 (MIM 612736)	Developmental delay/regression, speech impairment, seizures	1 stop htz	2T, 3M	1 252
<i>GRIK2</i>	138244	Mental retardation, autosomal recessive, 6 (MIM 611092)	NS-ID	-	1 large deletion/inversion	3 612
<i>HAL</i>	609457	Histidinemia (MIM 235800)	NS-ID or no ID	4 stops in 49 htz, 4 splices in 8 htz & 1 hertz	4M	3 078
<i>HIST1H4B</i>	602829	-	Microcephaly, strabismus	1 stop htz	1T (Najmabadi et al., Nature, 2011)	351
<i>KCNJ10</i>	602208	SESAME syndrome (MIM 612780)	Seizures, deafness, ataxia	1 stop htz	2T, 11M	1 180
<i>KDM5A</i>	180202	-	DF	-	1M (Najmabadi et al., Nature, 2011)	6 165
<i>LAMA1</i>	150320	-	Strabismus	3 stops htz, 2 splices in 2 htz & 1 hertz	1T (Najmabadi et al., Nature, 2011)	11 772
<i>LAMC3</i>	604349	Cortical malformations, occipital (MIM 614115)	Seizures associated with transient loss of vision	2 stops htz, 1 splice in 2 htz	3T, 1M	5 941
<i>LINS</i>	610350	-	Microcephaly	2 stops in 3 htz	1T (Najmabadi et al., Nature, 2011)	2 649
<i>MANIB1</i>	604346	Mental retardation, autosomal recessive 15 (MIM 614202)	NS-ID, overweight	-	1T, 2M	2 663
<i>MANBA</i>	609489	Mannosidosis, beta (MIM 248510)	Developmental delay	2 stops htz, 4 splices in 5 htz	7T, 1M	3 320

<i>MCPH1</i>	607117	Microcephaly 1, primary, autosomal recessive (MIM 251200)	-	6 stops htz, 2 splices in 3 htz	3T, 3M, 1 large exon deletion	3 076
<i>MED23</i>	605042	Mental retardation, autosomal recessive 18 (MIM 614249)	NS-ID	2 stops htz	1M	5 386
<i>MPI</i>	154550	Congenital disorder of glycosylation, type Ib (MIM 602579)	Diarrhea, vomiting, hypoglycemia, convulsions, liver defects	-	1T, 4M	1 592
<i>MTHFR</i>	607093	Homocystinuria due to MTHFR deficiency (MIM 236250)	Psychiatric matters, muscle weakness, seizures	-	3T, 7M	2 411
<i>NPC2</i>	601015	Niemann-pick disease, type C2 (MIM 607625)	Neurodegeneration, respiratory defects, viscera defects	4 stops htz	7T, 3M	656
<i>NRXN1</i>	600565	Pitt-Hopkins-like syndrome 2 (MIM 614325)	Hyperventilation, autistic traits, developmental delay/regression, constipation, DF	-	1T, 3 large exon deletion	5 863
<i>NSUN2</i>	610916	Mental retardation, autosomal recessive 5 (MIM 611091)	Poor postnatal growth, DF, microcephaly, developmental delay	1 splice htz	4T, 1M	3 133
<i>PARP1</i>	173870	-	Spasticity, hyperreflexia, pes cavus	1 stop htz	1M (Najmabadi et al., Nature, 2011)	3 942
<i>PDHX</i>	608769	Lacticacidemia due to PDX1 deficiency (MIM 245349)	Psychomotor retardation, hypotonia, ataxia, lactic acidosis	2 stops htz (1 in alternative isoform), 1 splice htz	6T, 1M, 1 in-frame deletion, 2 large exon deletions	2 089
<i>PIGV</i>	610274	Hyperphosphatasia with mental retardation syndrome 1 (MIM 239300)	Hypotonia, seizures	1 stop htz	5M	1 602
<i>PMM2</i>	601785	Congenital disorder of glycosylation, type Ia (MIM 212065)	Psychomotor retardation, hypotonia, hypotonia, ataxia, strabismus	1 stop htz, 1 splice in 2 htz	2T, 20M, 1 large exon deletion	1 061
<i>PRKRA</i>	603424	Dystonia 16 (MIM 612067)	Dystonia, parkinsonism, bradykinesia	-	1T, 1M	1 286
<i>PRSS12</i>	606709	Mental retardation, autosomal recessive 1 (MIM 249500)	NS-ID	3 stops htz, 3 splices in 8 htz	1T	3 148
<i>RABL6</i>	610615	-	NS-ID	1 stop htz	1M (Najmabadi et al., Nature, 2011)	3 188
<i>RALGDS</i>	601619	-	NS-ID	-	1M (Najmabadi et al., Nature, 2011)	3 504
<i>RELN</i>	600514	Lissencephaly 2 (Norman-Roberts type, MIM 257320)	Microcephaly, DF, postnatal growth deficiency, seizures, hypertension	2 stops htz, 1 splice htz	2T	13 154
<i>SARS</i>	607529	-	-	1 stop htz	1M (Puettmann et al., abstract, 15th MRX workshop, Berlin, 2011)	1 974
<i>SCAPER</i>	611611	-	NS-ID	3 stops htz, 1 in an alternative isoform, 1 splice htz	1T (Najmabadi et al., Nature, 2011)	5 554
<i>SLC46A1</i>	611672	Folate malabsorption, hereditary (MIM 229050)	Megaloblastic anemia, diarrhea, immune deficiency, infections, convulsions	-	4T, 6M	1 581
<i>SOBP</i>	613667	Mental retardation, anterior maxillary protraction, and strabismus (MIM 613671)	-	-	1T	2 862

<i>SRD5A3</i>	611715	Congenital disorder of glycosylation, type Ig (MIM 612379); Kahrizi syndrome (MIM 612713)	Colobomas, ichthyosis, cerebellar anomalies, kyphosis, DF	1 splice htz	7T	1 152
<i>STIL</i>	181590	Microcephaly 7, primary, autosomal recessive (MIM 612703)	Strabismus, ataxia, seizures	-	3T	4 507
<i>TECR</i>	610057	Mental retardation, autosomal recessive 14 (MIM 614020)	NS-ID	-	1M	1 747
<i>TMEM135</i>	-	-	Microcephaly, congenital cataract	1 stop htz	1M (Najmabadi et al., Nature, 2011)	1 962
<i>TRAPPC9</i>	611966	Mental retardation, autosomal recessive 13 (MIM 613192)	Microcephaly, white matter abnormalities, obesity, autistic traits (hand-flapping)	3 stops htz	4T	4 661
<i>TTI2</i>	614426	Mental retardation, autosomal recessive 39 (MIM 615541)	NS-ID	1 splice htz	1M	1 800
<i>TUSC3</i>	601385	Mental retardation, autosomal recessive 7 (MIM 611093)	NS-ID	1 splice in 3 htz	2T	1 503
<i>UPB1</i>	606673	Beta-ureidopropionase deficiency (MIM 613161)	Hypotonia, microcephaly, seizures	1 stop in 2 htz, 2 splices in 30 htz	2T, 2M	1 555
<i>UROCI</i>	613012	Urocanase deficiency (MIM 276880)	Atypical behaviour, ataxia	1 stop htz	2M	3 111
<i>WDR45L</i>	609226	-	Microcephaly	1 stop htz	1M (Najmabadi et al., Nature, 2011)	1 425
<i>WDR62</i>	613583	Microcephaly 2, primary, autosomal recessive, with or without cortical malformations (MIM 604317)	Psychomotor delay	1 stop htz, 4 stops htz	8T, 5M	6 185
<i>ZC3H14</i>	613279	-	NS-ID	-	1T, 1 large deletion (Pak et al., PNAS, 2011)	3 316
<i>ZNF526</i>	614387	-	NS-ID	1 stop htz	2M (Najmabadi et al., Nature, 2011)	2 052
<i>ZNF697</i>	-	-	NS-ID	-	1M (Puettmann et al., abstract, 15th MRX workshop, Berlin, 2011)	1 716
Autosomal Dominant genes						
<i>AFF3</i>	601464	-	Cerebellar anomalies	-	1 large deletion (Steichen-Gersdorf et al., Clin Genet, 2008)	4 744
<i>ANKRD11</i>	611192	KBG syndrome (MIM 148050)	Macrodontia, DF, short stature, skeletal anomalies, seizures	-	3T	8 517
<i>ARHGEF4</i>	605216	-	ADHD, epilepsy, behavioral abnormalities	2 stops htz	1 recurrent large deletion or reciprocal duplication in 2q21.1 (Dharmadhikari et al., Hum Mol Genet, 2012)	2 662
<i>ARID1B</i>	614556	Mental retardation, autosomal dominant 12 (MIM 614562)	Psychomotor delay, hypotonia, short stature	-	10T	7 550
<i>CACNG2</i>	602911	Mental retardation, autosomal dominant 10 (MIM 614256)	NS-ID	-	1M	1 132

<i>CDH15</i>	114019	Mental retardation, autosomal dominant 3 (MIM 612580)	Limb anomalies, DF	1 frequent stop (in 11 hHz, 451 hHz), 1 splice hHz	3M, 1 translocation	3 005
<i>CIC</i>	612082	-	Development delay	-	1M (Vissers et al., Nat genet, 2010)	6 225
<i>CREBBP</i>	600140	Rubinstein-Taybi syndrome (MIM 180849)	Talon cusps, glaucoma, broad thumbs and toes, DF	1 stop hHz	4T, 4M	8 569
<i>DEAF1</i>	602635	-	NS-ID, speech impairment	-	3M (Vissers et al., Nat genet, 2010; Rauch et al., Lancet, 2012)	2 178
<i>DISC1</i>	605210	Schizophrenia, susceptibility (MIM 181500 & 604906)	Schizophrenia	4 stops hHz, 1 in an alternative isoform	-	4 154
<i>DLG2</i>	603583	-	Epilepsy, ASD	1 stop hHz, 1 stop loss hHz	1 large exon deletion (Nillesen et al., 15th MRX workshop, Berlin, 2011)	4 642
<i>DYRK1A</i>	600855	Mental retardation, autosomal dominant 7 (MIM 614104)	Epilepsy, microcephaly, speech delay, feeding anomalies, stereotypic features	-	4T, 1 large exon deletion	2 805
<i>EPB41LI</i>	602879	Mental retardation, autosomal dominant 11 (MIM 614257)	Hypotonia	-	1M	3 486
<i>EHMT1</i>	607001	Kleefstra syndrome (MIM 610253)	Hypotonia, brachy/microcephaly, epilepsy, synophrys, cardiac defects, DF	-	3T, 1M	5 062
<i>FOXG1</i>	164874	Rett syndrome, congenital variant (MIM 613454)	Progressive microcephaly, hypotonia, unresponsiveness, apraxia, autistic traits, no speech, corpus callosum hypoplasia	-	9T, 1M	1 510
<i>FOXP1</i>	605515	Mental retardation with language impairment and autistic features (MIM 613670)	-	-	1T, 1 large exon deletion	3 037
<i>GRIN1</i>	138249	Mental retardation, autosomal dominant 8 (MIM 614254)	Epilepsy, speech delay	1 stop hHz	1M, 1 in-frame duplication	4 235
<i>GRIN2A</i>	138253	Epilepsy, focal, with speech disorder and with or without mental retardation (MIM 245570)	Hypotonia, behavioral abnormalities	-	1T, 3M, 1 translocation	4 667
<i>GRIN2B</i>	138252	Mental retardation, autosomal dominant 6 (MIM 613970)	NS-ID	-	6T, 3M, 2 translocations	4 935
<i>HDAC4</i>	605314	Brachydactyly-mental retardation syndrome (MIM 600430)	Short stature, eczema, behavioral abnormalities	-	2T	4 338
<i>HRAS</i>	190020	Congenital myopathy with excess of muscle spindles (MIM 218040); Costello syndrome (MIM 218040)	Short stature, feeding difficulties, growth retardation, cardiac defects, DF	2 stops in 3 hHz	13M, 2 in-frame duplications	887
<i>KCNK9</i>	605874	Birk-Barel mental retardation dysmorphism syndrome (MIM Birk-Barel mental retardation dysmorphism syndrome)	Hypotonia, hyperactive, feeding difficulties, DF	-	1M	1 205
<i>KCNQ2</i>	602235	Epileptic encephalopathy, early infantile, 7 (MIM 613720)	Hypotonia, dystonia, spasticity	-	1T, 5M	3 363

<i>KIRREL3</i>	607761	Mental retardation, autosomal dominant 4 (MIM 612581)	DF	-	3M, 1 translocation	3 124
<i>MBD5</i>	611472	Mental retardation, autosomal dominant 1 (MIM 156200)	Developmental delay, seizures, autistic traits, ataxia	-	1 large exon deletion	4 885
<i>MEF2C</i>	600662	Chromosome 5q14.3 deletion syndrome / Mental retardation, stereotypic movements, epilepsy, and/or cerebral malformations (MIM 613443)	ADHD, autistic traits, poor eye contact, speech delay, hypotonia, DF	-	3T, 2M	2 019
<i>NRXN2</i>	600566	-	ASD	2 splice htz	1T (Gauthier et al., Hum Genet, 2011)	6 324
<i>PACS1</i>	607492	Mental retardation, autosomal dominant 17 (MIM 615009)	Cranofacial anomalies, genital abnormalities	-	1M	3 966
<i>PAFAH1B1</i>	601545	Lissencephaly 1, Subcortical laminar heterotopia (MIM 607432)	Spasticity, seizures, hypotonia, microcephaly, DF	-	3T, 7M	1 633
<i>PGA5</i>	169730	-	Hypotonia, developmental delay	1 splice htz	1M (Vissers et al., Nat genet, 2010)	1 589
<i>RAI1</i>	607642	Smith-Magenis syndrome (MIM 182290)	Hypotonia, speech delay, hearing loss, sleep disturbances, DF	-	5T, 2M	5 881
<i>SCNIA</i>	182389	Dravet syndrome (MIM 607208); Epilepsy, generalized, with febrile seizures plus, type 2 (MIM 604403)	Absences, ataxia, mental decline	-	5T, 15M, 2 large exon deletions	7 070
<i>SCN2A</i>	182390	Epileptic encephalopathy, early infantile, 11 (MIM 613721)	Seizures, spasticity, developmental delay	-	1T, 3M	7 245
<i>SHANK1</i>	604999	-	ASD with high functioning	-	2 large exon deletions (Sato et al., AJHG, 2012)	7 383
<i>SHANK2</i>	603290	Autism susceptibility 17 (MIM 613436)	ASD	-	1T, 2 large exon deletions	6 328
<i>SHANK3</i>	606230	Phelan-McDermid syndrome (MIM 606232)	Neonatal hypotonia, developmental delay, speech delay, autistic traits	-	3T, 1M	6 403
<i>SLC2A1</i>	138140	GLUT1 deficiency syndrome 1 (MIM 606777); GLUT1 deficiency syndrome 2 (MIM 612126)	Epileptic encephalopathy, developmental delay, microcephaly, ataxia, spasticity, seizures, sleep disturbances	-	2T, 10M, 1 entire gene deletion, 1 in-frame deletion, 1 in-frame insertion	1 931
<i>STXBP1</i>	602926	Epileptic encephalopathy, early infantile, 4 (MIM 612164)	Seizures, spasticity, brain malformations	-	3T, 4M	2 695
<i>SYNGAP1</i>	603384	Mental retardation, autosomal dominant 5 (MIM 612621)	Developmental delay, seizures, autistic traits	-	8T, 2M	4 811
<i>TCF4</i>	602272	Pitt-Hopkins syndrome (MIM 610954)	Hyperventilation, sleep apneas, intestinal abnormalities, clubbed toes & fingers, DF	2 stops htz	3T, 3M	
<i>UBE3A</i>	601623	Angelman syndrome (MIM 105830)	Gait disorder, abnormal behavior, epilepsy, speech limitations, DF	-	9T, 2M	3 189
<i>YY1</i>	600013	-	Microcephaly, developmental delay	-	1M (Vissers et al., Nat genet, 2010)	1 445

ZBTB20	606025	-	ASD, NS-ID	-	2M, 1 translocation, 1 large deletion (Srivastava et al., abstract, ICHG, Montreal, 2011 and ASHG, Boston, 2013)	2 382
ZEB2	605802	Mowat-Wilson syndrome (MIM 235730)	Developmental delay, epilepsy, neurocristopathy	-	12T, 3M, 1 in-frame deletion, 1 large exon deletion	4 005
ZNF599	-	-	Hypotonia, developmental delay	4 stops in 47 htz, 1 hmez; 1 splice htz	1M (Vissers et al., Nat genet, 2010)	1 927
Others						
DOCK8	611432	Mental retardation, autosomal dominant 2 (MIM 614113) ; Hyper-IgE recurrent infection syndrome, autosomal recessive (MIM 243700)	Developmental delay, poor speech	1 stop htz, 1 splice htz	1 large deletion, 1 translocation	8 220
KIF1A	601255	Mental retardation, autosomal dominant 9 (MIM 614255); Spastic paraparesia 30, autosomal recessive (MIM 610357)	Hypotonia, spasticity, cerebellar anomalies, hyperreflexia,	2 stops htz	1M ; 2M	7 036
MED13L	608771	Transposition of the great arteries, dextro-looped 1, autosomal dominant (MIM 608808); ID with cardiac anomalies, autosomal dominant; NS-ID, autosomal recessive;	Hypotonia, cardiac defects, ataxia and DF; NS-ID;	-	1 translocation, 3M (Munckle et al., circulation, 2003); 2 large exon deletions, 1 triplication (Asadollahi, EJHG, 2013) 1 M (Najmabadi et al., Nature, 2011);	7 842
PRODH	606810	Hyperprolinemia, type I (MIM 239500); Schizophrenia, susceptibility to, 4 (MIM 600850)	Renal abnormalities, hearing loss, epilepsy	-	8M, 1 large exon deletion	2 466
PRRT2	614386	Convulsions, familial infantile, with paroxysmal choreoathetosis (MIM 602066) autosomal dominant; Episodic kinesigenic dyskinesia 1 (MIM 128200) autosomal dominant; BFIS/PKD with ID; autosomal recessive	Ataxia, absences	-	1T (Labate et al., Epilepsia, 2012)	1 140
DIP2B	611379	Mental retardation, FRA12A type (MIM 136630)	NS-ID	-	1 CGGn 5'UTR expansion	6 347
CYFIP1	606322	-	-	2 stops in 17 htz	-	

T: truncating mutation, M: missense mutation

Table S9: list of variants passing our filtering criteria in each patient with corresponding annotations by VaRank

DNA ID	Sex	MOI	Segregation	Gene	Omim #	Transcript#	Coverage (X)	Reads variation (%)	Nomenclature (g.)	Nomenclature (c.)	Nomenclature (p.)	rs #	Clinical Significance	EVs carriers	EVs MAF	DMES (%)	DSSF (%)	DNNS (%)	Local splice effect (prediction)	Sift	PPH2	phyloP	Phast Cons	Grant ham	All Count	Conclusion
APN-1	M	AR	AD	SHANK1	604999	NM_016148.2	24	58	g.51171270C>T	c.394T>A	p.Gly1316Asp	-	-	-	0	0	0	-	Tolerated	neutral	1.174	0.992	94	1 hz	Possibly benign	
			LAMA1	150320	NM_005595.2	273	44	g.7017272C>T	c.2808>G	p.?	rs201030108	-	24 (hz)	0.18%	-63.5	-14.8	-88.3	-	NA	3.837	1.00	NA	1 hz	VOUS		
			SLC46A1	611672	NM_080669.3	139	48	g.6137A>T	c.623A>T	p.Tyr208Phe	rs201837257	-	-	0	0	0	0	-	Deleterious	neutral	NA	0.098	22	1 hz	VOUS	
APN-2	M	AR	CFYIP1	606322	NM_00130328.1	310	47	g.22956361A>G	c.305A>G	p.His102Arg	-	-	-	0	0	0	-	Tolerated	neutral	-0.44	0.00	29	1 hz	Possibly benign		
			XL inherited (Mo)	IQSEC2	300522	NM_00111125.1	163	99	g.53265591G>A	c.3364C>T	p.Arg1122Cys	-	-	-	0	0	0	-	Tolerated	deleterious	3.03	1.00	180	1 hemz	VOUS	
			ALDH18A1	138250	NM_002860.3	311	46	g.97393376C>G	c.589G>C	p.Glu197Gln	rs201691969	-	-	0	0	0	0	-	Deleterious	deleterious	5.371	1.00	29	1 hz	VOUS	
APN-3	M	AR	RELN	600514	NM_007045.3	110	52	g.103629379C>A	c.65G>T	p.Arg22Met	rs139532757	-	1	0.01%	0	0	0	-	Deleterious	neutral	1.739	1.00	91	1 hz	VOUS	
			LAMA1	150320	NM_005595.2	412	46	g.69825197C>A	c.5867A>G	p.Asn1956Ser	rs117433399	-	16 (hz)	0.12%	0	0	0	-	Tolerated	neutral	0.044	0.00	46	1 hz	Possibly benign	
			TUSC3	601385	NM_006765.3	142	54	g.15397977C>T	c.38C>T	p.Ala13Val	rs20080372	-	4 (hz)	0.03%	0	0	0	-	Deleterious	neutral	0.69	0.976	64	2 hz	VOUS	
APN-4	M	AR	ZNF526	614387	NM_133444.1	176	43	g.42729310_42729312del	c.755_757del	p.Glu252del	rs138669870	-	-	0	0	0	-	NA	NA	NA	NA	2 hz	VOUS			
			XL	MID1	300522	NM_00119327.1	223	99	g.10469476C>T	c.8800G>A	p.Glu294Lys	rs111428432	-	-	0	0	0	-	Notscored	neutral	-1.409	0.00	56	1 hemz	VOUS	
			ADRHEF4F	605216	NM_032995.1	144	51	g.131802007G>A	c.1735G>A	p.Ala579Thr	-	-	1	0.01%	0	0	0	-	Tolerated	neutral	-1.328	0.00	58	1 hz	Possibly benign	
APN-5	M	AR	AP4E1	607244	NM_007347.3	368	49	g.51260460G>A	c.1852G>A	p.Val618Le	rs142210428	-	4	0.03%	-23.7	-4.8	-11.7	-	Tolerated	neutral	4.483	1.00	29	1 hz	Possibly benign	
			HAL	609457	NM_002108.2	219	49	g.96388732G>A	c.287C>T	p.Ser96Phe	rs142371886	-	12 (hz)	0.09%	NA	0	0	-	Deleterious	neutral	2.95	0.858	155	1 hz	VOUS	
			NRXN1	600565	NM_00135659.1	281	53	g.50847195G>A	c.1405C>T	p.Pro469Ser	rs78540316	-	34 (hz)	0.28%	0	0	0	-	NA	deleterious	6.42	1.00	74	1 hz	VOUS	
APN-6	M	AR	TRAPP9C	611966	NM_031466.5	229	51	g.14146133C>T	c.434G>A	p.Arg145Gln	rs147182402	-	7 (hz)	0.05%	0	0	0	-	Tolerated	neutral	3.111	0.992	43	1 hz	Possibly benign	
			TT2	614246	NM_025115.3	135	50	g.33361318G>A	c.1063C>T	p.Arg355Cys	rs138108276	-	50 (hz)	0.38%	0	0	0	-	Deleterious	deleterious	2.788	1.00	180	1 hz	VOUS	
			PMM2	601785	NM_000303.2	475	48	g.89050147G>A	c.426T>G	p.Ile142Met	-	-	0	0	0	-	Tolerated	neutral	-0.198	0.937	10	1 hz	Possibly benign			
APN-7	M	AD	KDMSA	180202	NM_00142603.1	365	40	g.432809G>A	c.2107C>T	p.Pro703Ser	-	-	0	0	0	-	Tolerated	neutral	2.385	1.00	74	1 hz	Possibly benign			
			CACNA1G	604065	NM_198396.1	360	49	g.48655394G>T	c.1831G>T	p.Ala611Ser	-	-	0	0	0	-	Tolerated	neutral	3.111	0.992	99	1 hz	Possibly benign			
			CREBBP	600140	NM_004380.2	405	45	g.3827646G>A	c.2126C>T	p.Ser709Phe	rs147740753	other	-	-	0	0	-0.7	-	Deleterious	deleterious	5.371	1.00	155	1 hz	Benign	
APN-8	M	AR	CIC	612082	NM_015125.3	173	47	g.42797352C>G	c.3685C>G	p.Pro1229Ala	-	-	0	0	0	-	Tolerated	neutral	0.69	0.677	27	1 hz	Possibly benign			
			DEAF1	602635	NM_021008.2	329	52	g.679660G>A	c.1126_1128C>T	p.?	-	-	0	0	0	-	Cryptic ASS	NA	NA	-1.247	0.00	NA	1 hz	Possibly benign		
			LAMA1	150320	NM_005595.2	263	34	g.6947145_6947161del	c.8844_8844+17del	p.?	-	-	-100	-100	-100	-	NA	NA	NA	NA	NA	1 hz	VOUS			
APN-9	M	AR	TRAPP9C	611966	NM_031466.5	278	41	g.140922432C>T	c.3217G>A	p.Gly1073Ser	-	-	NA	NA	NA	-	Tolerated	neutral	2.546	0.984	56	1 hz	Possibly benign			
			SCAPER	616161	NM_00145923.1	593	47	g.76994121C>T	c.1748G>A	p.Arg53His	-	-	0	0	0	-	Tolerated	NA	5.694	0.992	29	1 hz	VOUS			
			XL inherited (Mo)	NLG3N	300336	NM_181303.1	86	97	g.70367619C>T	c.20C>T	p.Pro7Leu	rs199925687	-	3 (2F, 1M)	0.03%	0	0	0	-	Tolerated	neutral	-0.117	0.016	98	1 hemz	Possibly benign
APN-10	M	AR	MBDS	611472	NM_018328.4	415	46	g.1492261602C>T	c.590T>C	p.Val197Ala	-	-	0	0	0	-	Deleterious	neutral	0.125	0.976	64	1 hz	Benign			
			CENPJ	609279	NM_018451.3	509	51	g.25478104T>C	c.2785A>G	p.Lys929Glu	rs141844033	-	9 (hz)	0.07%	0	0	0	-	Deleterious	deleterious	4.483	1.00	56	1 hz	VOUS	
			PARP1	173870	NM_001618.3	180	48	g.22657074G>T	c.1148C>A	p.Ser383Trp	rs3219062	-	27 (hz)	0.21%	0	0	0	-	Deleterious	neutral	4.887	0.362	144	1 hz	VOUS	
APN-11	M	AD	CACNA1G	604065	NM_198397.1	231	42	g.48683469G>A	c.4507G>T	p.Gly1503Ser	-	-	0	0	0	-	New DSS	Deleterious	-1.651	0.00	56	1 hz	Possibly benign			
			RAK1B1	610615	NM_00173988.1	234	50	g.139737632K>G	c.1592C>T	p.Pro531Leu	rs143011384	-	38 (hz)	0.30%	0	0	0	-	Tolerated	NA	0.851	0.00	98	1 hz	VOUS	
			XL inherited (Mo)	HUWE1	300697	NM_014074.7	158	96	g.53566633G>C	c.6437C>G	p.Thr214Arg	rs199925687	-	-	0	0	0	-	Deleterious	deleterious	3.595	1.00	71	1 hemz	Possibly benign	
APN-12	M	AD	Absent from Mo	GRIN2A	138253	NM_00134407.1	178	48	g.9827479C>T	c.2554G>A	p.Val852Met	rs150316865	-	-	0	0	0	-	Deleterious	deleterious	2.788	1.00	21	1 hz	VOUS	
			PMM2	601785	NM_000303.2	349	44	g.8905010G>A	c.422G>A	p.Arg141His	rs28936415	pathogenic	50 (hz)	0.39%	0	0	0	-	Deleterious	deleterious	5.694	1.00	29	2 hz	VOUS	
			LAMA1	150320	NM_005595.2	346	51	g.65939349G>A	c.7724C>T	p.Thr2575Met	rs76482057	-	42 (hz)	0.32%	0	0	0	-	Cryptic ASS	Deleterious	-0.44	0.00	81	1 hz	VOUS	
APN-13	M	AR	RAGLD5	601619	NM_00104268.1	247	45	g.135983956G>T	c.613_104A>G	p.Arg1040Asn	rs199846522	-	3 (hz)	0.02%	0	0	0	-	New DSS	Deleterious	0.36	0.00	52	2 hz	VOUS	
			de novo	MED12L	608370	NM_018355.4	239	35	g.116408345_11640852del	c.6118_6123del	p.Glu204ArgAsn*32	-	-	0	0	0	0	-	NA	NA	NA	NA	NA	1 hz	Pathogenic	
			AD	ARID1B	614556	NM_020732.3	83	45	g.157100380G>C	c.1317G>C	p.Glu439Asp	-	-	0	0	0	0	-	Tolerated	neutral	-0.117	0.937	45	1 hz	Possibly benign	
APN-14	M	AR	YY1	600013	NM_003403.3	285	51	g.890705788_10075970del	c.222_224del	p.His80del	-	-	0	0	0	0	-	NA	NA	NA	NA	NA	1 hz	VOUS		
			PMM2	601785	NM_000303.2	283	51	g.8905010G>A	c.422G>A	p.Arg141His	rs28936415	pathogenic	50 (hz)	0.39%	0	0	0	-	Deleterious	deleterious	5.694	1.00	29	2 hz	VOUS	
			HRAS	190200	NM_005343.2	222	50	g.532627T>A	c.5*4A>T	p.?	-	-	-37	-	-11.2	-20.3	-	NA	NA	2.707	1.00	NA	1 hz	VOUS		
APN-15	M	AR	CC2D1A	610055	NM_017221.4	153	52	g.14030696G>A	c.1288G>A	p.Glu430Lys	-	-	0	0	0	0	-	Tolerated	neutral	3.514	1.00	56	1 hz	Possibly benign		
			XL	NSDHL	300275																					

APN-30	M	AR	CC2D1A	610055	NM_017221.4	269	46	g.14040420G>A	c.2657G>A	p.Ara886His	rs201921029	-	12	0.10%	0	0	0	-	Deleterious	neutral	1.013	1.00	29	1 hz	VOUS		
APN-31	M	AR	inherited (Fa)	FTCD	606806	NM_006657.2	324	53	g.47571578C>A	c.530G>T	p.Gly177Val	-	-	-	0	0	0	-	Deleterious	deleterious	5.532	1.00	109	1 hz	Benign		
			inherited (Fa)	FTCD	606806	NM_006657.2	170	52	g.47566194C>G	c.954G>C	p.Lys318Asn	-	-	-	0	0	0	-	Deleterious	deleterious	0.205	0.472	94	1 hz	Benign		
			SCAPER	611611	NM_001145923.1	509	48	g.77067254T>C	c.239A>G	p.Glu80Gly	rs186407258	-	29	0.24%	0	0	0	-	Cryptic ASS	Deleterious	NA	2.304	0.583	98	1 hz	VOUS	
APN-32	M	AD	SOBP	613667	NM_018103.3	202	48	g.10795577C>T	c.1729C>T	p.Pro577Ser	rs200685194	-	8	0.07%	0	0	0	-	Tolerated	neutral	0.69	0.417	74	1 hz	Possibly benign		
			RELN	600514	NM_000545.3	414	45	g.103214642C>T	c.4408G>A	p.Val1470Ile	rs143213152	-	33	0.25%	0	0	0	-	Tolerated	neutral	1.981	1.00	29	1 hz	Possibly benign		
			inherited (Fa)	HDAC4	605314	NM_006037.3	556	51	g.24003689G>A	c.1627C>T	p.Arg543Trp	rs140814864	-	1	0.01%	0	0	0	-	Deleterious	deleterious	3.837	1.00	101	1 hz	Benign	
APN-33	M	AR	inherited (Mo)	SHANK2	606230	NM_001080420.1	243	47	g.51169586..51169588del	c.5090..5092del	p.His1697Leu	-	-	-	0	0	0	-	Deleterious	neutral	NA	NA	NA	1 hz	VOUS		
			SHANK3	606230	NM_000545.3	726	52	g.10315136G>A	c.812C>T	p.Arg273Trp	rs202166176	-	-	-	0	0	0	-	Deleterious	deleterious	1.577	1.00	101	1 hz	VOUS		
			RELN	600514	NM_000545.3	726	52	g.10315136G>A	c.812C>T	p.Arg273Trp	rs202166176	-	-	-	0	0	0	-	Deleterious	deleterious	1.577	1.00	101	1 hz	VOUS		
APN-34	M	AD	XL	KDM5C	314690	NM_004187.3	162	100	g.53228255G>C	c.211C>T	p.Arg718Pro	-	-	-	0	0	0	-	Deleterious	deleterious	0.967	1.00	27	1 hz	Pathogenic		
			CDH15	611409	NM_004932.3	312	48	g.89253943C>A	c.770C>A	p.Ala257Asp	-	-	-	0	0	0	-	Deleterious	neutral	2.627	0.961	126	1 hz	VOUS			
			DOCK8	611432	NM_204474.3	299	49	g.17646717A>G	c.279A>G	p.Ile927Val	-	-	-	11	4.7	7.2	-	Deleterious	neutral	NA	0.024	29	1 hz	VOUS			
APN-35	M	AD	AR	CC2D1A/PODNL1	610055	NM_017221.4	144	51	g.14040915A>G	c.2735A>G	p.Glu191Arg	-	-	-	0	0	0	-	Deleterious	neutral	3.03	0.992	43	1 hz	VOUS		
			AR	RAB16	610615	NM_00173988.1	173	39	g.397347499..139734801del	c.2018..2020del	p.Lys567Ile	rs1432189	-	-	-	0	0	0	-	Cryptic DSS	NA	NA	NA	1 hz	VOUS		
			inherited (Mo)	CREB3P	600141	NM_004380.2	243	51	g.5778520G>A	c.5728C>T	p.g.22243Val	rs190153828	-	1	0.01%	0	0	0	-	Deleterious	neutral	2.047	0.992	64	1 hz	VOUS	
APN-36	M	AD	inherited (Mo)	SHANK2	605114	NM_012039.3	263	53	g.103207403C>T	c.4747..5G>A	p.?..?	-	-	-	NA	NA	NA	-	Deleterious	neutral	NA	NA	NA	1 hz	Benign		
			inherited (Fa)	RELN	600514	NM_000545.3	289	50	g.103207403C>T	c.4747..5G>A	p.?..?	-	-	-	NA	NA	NA	-	Cryptic ASS	NA	NA	NA	1.416	0.11	NA	1 hz	VOUS
			XL	TSPAN7	300096	NM_004615.3	188	99	g.48530697G>A	c.338G>A	p.Arg131His	-	-	-	0	0	0	-	Tolerated	neutral	3.999	1.00	29	1 henz	Possibly benign		
APN-37	M	AR	XL	ANK3	600465	NM_200987.3	335	48	g.61898843C>T	c.2615G>A	p.Gly872Asp	-	-	-	-3.8	-4.4	-16.2	-	Deleterious	deleterious	4.241	1.00	94	1 hz	Benign		
			ACV1	104620	NM_0006662	259	49	g.52012045C>T	c.625C>T	p.Arg209Cys	rs140738368	-	5	0.04%	0	0	0	-	Deleterious	deleterious	1.497	0.00	180	1 hz	VOUS		
			CACNA1G	604065	NM_198396.1	269	46	g.5856462G>A	c.1879G>A	p.Gly627Arg	rs200317339	-	4	0.03%	0	0	0	-	New DSS	Tolerated	0.932	0.354	125	1 hz	Possibly benign		
APN-38	M	AD	AD	ZBTB20	606028	NM_015642.4	463	51	g.114057962C>T	c.1897G>A	p.Val633Met	rs190153828	-	1	0.01%	0	0	0	-	Tolerated	deleterious	3.514	1.00	21	1 hz	Benign	
			PRODH	606010	NM_016335.5	284	50	g.189105859G>A	c.1397C>T	p.Thr466Met	rs2870984	pathogenic	34	0.26%	0	0	0	-	Deleterious	neutral	1.577	1.00	81	1 hz	VOUS		
			AR	SLC46A1	611672	NM_080669.3	491	51	g.602611A>G	c.5121T>A	p.Val171Asp	rs19105810	-	-	-	0	0	0	-	Deleterious	deleterious	NA	0.00	152	1 hz	VOUS	
APN-39	M	AD	XL	TCF4	602272	NM_001083962.1	680	43	g.53017662..53017625del	c.514..517del	p.Lys172Phe*61	-	-	-	0	0	0	-	New DSS	NA	NA	NA	0.198	0.00	NA	1 henz	Possibly benign
			AR	SYNAPG1	603384	NM_006772.2	308	36	g.3341366..334136419del	c.3357..3365del	p.Arg2381Cys	rs142063208	-	16	0.12%	0	0	0	-	Deleterious	deleterious	3.353	1.00	180	1 hz	VOUS	
			AR	RABL6	610615	NM_00173988.1	552	49	g.139734210C>T	c.1826C>T	p.Arg224Lys	rs142411vs	-	17	0.13%	0	0	0	-	Tolerated	deleterious	4.079	1.00	26	1 hz	VOUS	
APN-40	M	AD	XL	MDI1	608771	NM_015335.4	659	50	g.116466341G>A	c.1877C>T	p.Pro626Leu	rs186297695	-	1	0.01%	0	0	0	-	Tolerated	neutral	0.69	0.031	98	1 hz	Possibly benign	
			AR	GAMT/NDUF57	601240	NM_000156.4	410	48	g.1397462G>A	c.607C>T	p.Arg2031Phe	rs142311704	-	-	-	0	0	0	-	Deleterious	neutral	1.013	0.984	101	1 hz	VOUS	
			AD	HUWE1	300697	NM_013107.4	310	99	g.536007507G>A	c.6272A>G	p.Asn209Phe	rs142311704	-	-	-	0	0	0	-	New DSS	Tolerated	1.9	1.00	46	1 henz	Pathogenic	
APN-41	M	AD	XL	TCF4	602272	NM_001083962.1	680	43	g.53017662..53017625del	c.514..517del	p.Lys172Phe*61	-	-	-	0	0	0	-1	NA	NA	NA	NA	NA	1 hz	Pathogenic		
			AR	SYNAPG1	603384	NM_006772.2	308	36	g.3341366..334136419del	c.3357..3365del	p.Arg2381Cys	rs142063208	-	16	0.12%	0	0	0	-	Deleterious	deleterious	3.353	1.00	180	1 hz	VOUS	
			AR	HAL	609457	NM_002108.2	523	49	g.96370339G>T	c.1801G>A	p.Glu601Lys	rs14096011s	-	1	0.01%	NA	NA	NA	-	Deleterious	neutral	6.016	1.00	56	1 hz	VOUS	
APN-42	M	AD	XL	DMD	300377	NM_004010.3	275	94	g.3116444del	c.10520del	p.Arg350Glnfs*27	-	-	-	0	0	0	-	NA	NA	NA	NA	NA	1 henz	Pathogenic		
			AD	inherited (Mo)	KIRREL3	607761	NM_032531.3	200	49	g.126319042G>A	c.1406C>T	p.Thr469Met	-	-	-	1	0.01%	0	0	-	Deleterious	deleterious	5.371	1.00	81	1 hz	Benign
			AR	AP4M1/TAF6	602296	NM_004722.3	673	63	g.99702938G>A	c.803G>A	p.Arg268Gln	-	-	-	0	0	0	-	Deleterious	deleterious	4.967	1.00	43	1 hz	VOUS		
APN-43	M	AD	XL	RELN	600514	NM_000545.3	724	51	g.103180699G>T	c.6875G>A	p.Arg229His	rs142311704	-	-	-	0	0	0	-	Deleterious	deleterious	5.694	1.00	29	1 hz	Possibly benign	
			AR	WDR62	615838	NM_001083961.1	471	49	g.36595425C>A	c.4159C>A	p.Leu138Ile	rs147652186	-	5	0.04%	0	0	0.3	-	Tolerated	neutral	0.69	0.00	5	1 hz	Possibly benign	
			inherited (Mo)	SLC9A6	300231	NM_00142537.1	84	90	g.13508258..13508262del	c.5269..526del	p.Gly778Glnfs*7	-	-	-	-32	-10.4	-77.8	-	NA	NA	NA	NA	NA	1 henz	Pathogenic		
APN-44	M	AD	AD	RAII	607642	NM_030665.3	212	39	g.17698594..17698598del	c.233..236del	p.Gly778Glnfs*7	-	-	-	0	0	0	0	Deleterious	deleterious	0.367	0.969	101	1 hz	Pathogenic		
			inherited (Mo)	KIRREL3	607761	NM_032531.3	200	46	g.126319042G>A	c.859C>T	p.Arg287Trp	-	-	-	0	0	0	-8.2	Deleterious	deleterious	3.837	1.00	58	1 hz	Benign		
			AR	ELP2	614242	NM_025115.3	209	48	g.33361016C>T	c.71C>T	p.Ala24Val	-	-	-	0	0	0	-	Tolerated	neutral	1.255	0.992	98	2 hz	Possibly benign		
APN-45	M	AD	XL	ELP2	614242	NM_025115.3	321	48	g.33734736G>C	c.1126..1127G>C	p.Thr247Ala	-	-	-	0	0	0	-	Deleterious	neutral	0.044	0.00	32	1 hz	Possibly benign		

APN-104	M	ΔΔΔ												ΔΔΔ														
		AR	ADRA2B	NA	NM_0006825	544	46	g.60575T>C	c.1055T>C	p.Lys940Arg	rs3219145	-	13	0.10%	0	0	0	0	-	Tolerated	deleterious	5.129	1.00	26	1 hz	VOUS		
APN-106	M	AD	AFF3	601464	NM_0022852	981	49	g.100194796C>T	c.2911G>A	p.Asp971Asn	-	-	-	0	0	0	0	-	Tolerated	deleterious	2.304	1.00	23	1 hz	VOUS			
		AR	KIF1A	NA	NM_001240081	823	49	g.24170418C>T	c.1311G>A	p.-	-	-	0	0	0	0	-	New ASS	NA	NA	-1.005	0.472	NA	1 hz	VOUS			
		AD	CENPQ	609279	NM_0184513	1022	50	g.25481285A>C	c.1021UT>G	p.Tyr341Asp	rs143258862	33	0.25%	0	0	0	0	-	Deleterious	deleterious	1.416	1.00	160	2 hz	Possibly damaging			
		inherited (Fa)	KCNQ2	602235	NM_1721072	678	51	g.62039761C>T	c.1887+5G>A	p.-	-	-	-	-	-	-	-63.5	-15.3	-69.6	-	NA	NA	2.788	0.26	NA	1 hz	Benign (does not	
		inherited (Mo)	EHMT1	607001	NM_0247574	654	45	g.10674130G>A	c.2236G>A	p.Ala746Thr	-	-	-	0	0	0	0	-	Deleterious	deleterious	4.241	0.992	58	1 hz	Benign			
APN-107	M	AR	ASPM	605481	NM_0181364	1054	47	g.197060113T>C	c.9503A>G	p.Lys3168Arg	-	-	-	0	0	0	0	-	Deleterious	neutral	0.286	0.00	26	1 hz	VOUS			
		ASPM	605481	NM_0181364	1403	49	g.197071579G>C	c.6802C>G	p.Leu2268Val	-	-	-	0	0	0	0	-	Tolerated	neutral	-0.924	0.00	32	1 hz	Possibly benign				
APN-108	M	AD	inherited (Mo)	DEAF1	602635	NM_0210082	697	48	g.691601G>C	c.290-3C>G	p.-	-	-	-	-	-	-100	-12.1	-99.8	-	NA	NA	2.546	1.00	NA	1 hz	VOUS	
		AR	NSUN2	610916	NM_0177555	1223	50	g.6605449C>A	c.1674G>T	p.Arg558Ser	-	-	-	0	0	0	0	-	Deleterious	deleterious	0.448	1.00	110	1 hz	VOUS			
		GRBN	609262	NM_0163023	1146	46	g.19157525C>A	c.860G>C	p.Cys287Phe	-	-	-	0	0	0	0	-	Tolerated	deleterious	5.613	1.00	205	1 hz	VOUS				
		XL	inherited (Mo)	SLC76A2	300495	NM_0065170	422	100	g.23734996T>C	c.1412T>C	p.Leu471Ile	rs122455132	pathogenic	-	0	0	0.9	-	Deleterious	deleterious	4.725	1.00	98	1 hemz	Pathogenic			
		AR	RELN	600514	NM_0004543	664	47	g.10312418R>C	c.10093G>A	p.Val1336Ile	rs15035120	8	0.06%	0	0	0	0	-	Deleterious	deleterious	2.062	0.984	29	1 hz	VOUS			
		BRAL6	NA	NM_0247184	1358	50	g.13973417G>A	c.1789G>A	p.Asn597Asn	rs141843004	-	-	0	0	0	0	-	Cryptic DSS	Tolerated	4.322	0.913	23	1 hz	VOUS				
APN-110	M	AD	UPBL	606673	NM_0163273	342	45	g.24911958G>C	c.917L>G	p.Ile159Pro	rs143493067	28	0.22%	-100	-100	-100	-	NA	NA	5.855	1.00	NA	1 hz	Possibly benign				
		AD	CIC/PAPAH1B3	612082	NM_0151253	91	48	g.27992997G>C	c.4783T>C	p.Ser1595Pro	-	-	-	0	0	0	0	-	Deleterious	neutral	2.627	0.102	74	1 hz	VOUS			
		LAMA1	150320	NM_0055929	998	49	g.70232070G>A	c.2657G>T	p.Ala886Val	rs144738522	34	0.26%	0	0	0	0	-	Tolerated	deleterious	1.981	0.953	64	1 hz	VOUS				
		AP4M1/TAF6	602294	NM_0047223	367	51	g.99704084C>T	c.1084C>T	p.Ars362Cys	-	-	-	0	0	0	0	-	Deleterious	neutral	1.9	0.331	180	1 hz	VOUS				
		inherited (Mo)	ATRX	300032	NM_0004893	826	100	g.76939735G>C	c.1013C>G	p.Ser338Cys	-	-	-	0	0	0	0	-	Deleterious	deleterious	1.981	0.425	112	1 hemz	Possibly pathogenic			
		FTCD	605806	NM_0065727	867	49	g.47557244G>A	c.1484C>T	p.Ala483Val	rs145609043	3	0.02%	0	0	0	-6.6	-	Deleterious	deleterious	2.223	0.992	64	1 hz	VOUS				
APN-113	M	XL	inherited (Mo)	EPB41LI	602879	NM_0121562	73	56	g.34782048C>T	c.1301-86C>T	p.?	rs139014428	-	-	0	0	0	0	-	New DSS	NA	NA	0.125	0.00	NA	2 hz	VOUS	
		AR	PRSS12	606709	NM_0036193	433	50	g.1929162127T>C	c.2179A>G	p.Ile727Val	rs146362932	3	0.02%	0	0	0	0	-	Deleterious	deleterious	2.304	1.00	29	1 hz	Possibly damaging			
		BRAL6	610615	NM_001739881	145	51	g.139734624C>T	c.1952C>T	p.Pro651Leu	rs199511338	5	0.04%	0	0	0	-0.5	-	Tolerated	NA	NA	1.416	0.331	98	1 hz	Possibly benign			
		AD	ARHGEF6	300267	NM_0048842	365	98	g.135754192T>G	c.2122A>C	p.Ile708Leu	-	-	-	0	0	0	0	-	Tolerated	neutral	0.77	0.976	5	1 hemz	Possibly benign			
		KAIA2022	300524	NM_0010085372	532	98	g.73962391C>T	c.1501G>A	p.Gly501Ser	-	-	-	0	0	0	0	-	Tolerated	neutral	1.82	1.00	56	1 hemz	Possibly benign				
		AD	SHANK1	604994	NM_0161482	45	42	g.5117345C>T	c.3872G>A	p.Gly1291Asp	-	-	-	0	0	0	0	-	Tolerated	deleterious	3.514	1.00	94	1 hz	VOUS			
		ANK3	600465	NM_0209873	1077	49	g.61831298A>G	c.9341T>C	p.Val1314Ala	rs181031970	-	-	0	0	0	0	-	Deleterious	neutral	2.223	0.819	64	1 hz	VOUS				
		inherited (Mo)	PRRT2	614384	NM_1452392	451	39	g.29825042dup	c.649dup	p.Arg217Profs*	-	-	-	0	0	0	0	-	NA	NA	NA	NA	NA	1 hz	VOUS			
		AR	MCPH1	607117	NM_00172574	216	45	g.62642111G>A	c.22+1G>A	p.?	-	-	-	-100	-100	-100	-	NA	NA	1.013	0.969	NA	1 hz	VOUS				
		PIGV	610274	NM_00120544	594	46	g.1185525561-1185455del	c.1277C>T	p.Pro426Leu	rs146969255	20	0.15%	0	0	0	-0.5	-	Deleterious	neutral	2.062	0.969	98	1 hz	VOUS				
		XL	SLC6A8	300036	NM_0056293	299	51	g.1529600226C>G	c.1649C>G	p.Thr550Ser	rs199635059	1	0.01%	0	0	0	0	-	Tolerated	neutral	1.9	1.00	58	1 hz	Possibly benign			
		AD	EHMT1	607001	NM_0247574	281	48	g.14061140G>A	c.148G>A	p.Ala50Thr	rs78104547	-	-	0	0	0	0	-	Tolerated	neutral	0.286	0.00	58	1 hz	Possibly benign			
		AR	RELN	600514	NM_0004543	771	47	g.103136238C>G	c.9301G>C	p.Asp310His	-	-	-	0	0	0	0	-	Deleterious	deleterious	6.016	1.00	81	1 hz	VOUS			
		?	DIPZB	611379	NM_1736022	1017	50	g.511082691C>T	c.2741T>C	p.Ile914Thr	-	-	-	0	0	0	0	-	Tolerated	neutral	4.806	1.00	89	1 hz	Possibly benign			
		XL	MBTPS2	300294	NM_018843	789	45	g.21896786C>T	c.1237C>T	p.His413Trp	rs200298161	3 (2F, 1M)	0.03%	0	0	0	0	-	Tolerated	neutral	1.739	1.00	83	1 hz	Possibly benign			
		AD	TCF4	602272	NM_001083962	944	49	g.53017619G>A	c.520C>T	p.Arg174*	-	-	-	0	0	0	0	-	NA	NA	0.932	1.00	NA	1 hz	Pathogenic			
		ADAC	HDAC4	605314	NM_0060373	599	47	g.24036871C>T	c.1654G>A	p.Ala552Thr	-	-	1	0.01%	0	0	0	0	-	Tolerated	neutral	-0.521	0.00	58	1 hz	Possibly benign		
		AR	MCPH1	607117	NM_0245963	595	49	g.6479014C>G	c.2254C>G	p.Arg752Gly	rs146325050	-	-	0	0	0	0	-	Deleterious	deleterious	4.564	0.992	125	1 hz	VOUS			
		AR	ASPM	605481	NM_0181364	947	47	g.19711206G>A	c.676C>T	p.Pro226Ser	rs147830520	1	0.01%	0	0	0	0	-	Tolerated	neutral	1.82	0.772	74	1 hz	Possibly benign			
		MTIFHR	607093	NM_0059574	578	40	g.1185525561-1185455del	c.1204T>C	p.Cys206del	rs1206del	-	-	0	0	0	0	-	NA	NA	NA	NA	NA	1 hz	VOUS				
		XL	RPS6KA3	300075	NM_0045862	682	50	g.20213116T>C	c.10C>T	p.?>Arg546	rs143982014	-	17	0.13%	0	0	0	0	-	Tolerated	neutral	0.528	0.00	74	2 hz	Possibly benign		
		AD	ANKRDI1	611192	NM_002516812	478	51	g.89347214G>T	c.5736C>A	p.Arg1912Glu	-	-	-	0	0	0	0	-	Deleterious	neutral	0.044	0.992	45	1 hz	VOUS			
		FOLR1	136430	NM_0167242	805	46	g.71906438C>T	c.493+2T>C	p.?	rs144637717	-	27 (hz)	0.21%	-100	-10.9	-100	-	NA	NA	NA	NA	NA	1 hz	Possibly benign				
		AR	LAMC3	604349	NM_0060593	65	38	g.133884674C>T	c.71C>T	p.Ala24Val	rs200969079	3	0.02%	0	0	0.5	-	-	Tolerated	neutral	3.192	1.00	64	1 hz	VOUS			
		?	AP4S1	607243	NM_0070773	870	47	g.3153981A>G	c.373A>G	p.Ile125Val	rs20994885	-	-	0	0	0	0.5	-	Tolerated	deleterious	-0.844	0.00	29	1 hz	Possibly benign			
		CFEP1	606322	NM_0146082	670	51	g.22998442G>A	c.3118G>A	p.Gly1040Arg	rs1404024del	-	-	0	0	0	0.5	-	Tolerated	deleterious	4.402	1.00	125	1 hz	VOUS				
		APN-125	F	XL	inherited (Fa)	SCAPER	611611	NM_001459231	919	49	g.76914159T>A	c.1919A>T	p.Tyr640Phe	rs184003295	-	45	0.38%	0	0	0.1	-	Tolerated	neutral	1.416	1.00	22	1 hz	Possibly benign
		AD	MPI	154550	NM_0024351	186	43	g.75182424C>T	c.10C>T	p.Pro45Ser	rs143																	

APN-132	M	AR	<i>AP4B1</i>	607245	NM_006594.2	528	59	g.114442885A>G	c.755T>C	p.Val252Ala	rs141417436	-	25	0.19%	0	0	0	-	Deleterious	neutral	2.465	1.00	64	2 hz	VOUS	
			<i>LINS</i>	610350	NM_001040616.2	595	78	g.1011152697>C	c.554A>G	p.Asn815Ser	rs146704559	-	4	0.03%	0	0	0	-	Tolerated	neutral	0.448	0.00	46	1 hz	Possibly benign	
		?	<i>CYFIP1</i>	606322	NM_014608.2	469	53	g.23969215G>A	c.2441G>A	p.Arg814Gln	rs147972810	-	2	0.02%	0	0	0	-	Tolerated	neutral	1.658	1.00	43	1 hz	Possibly benign	
APN-134	M	AR	<i>RAB16</i>	NA	NM_001173989.2	599	64	g.13972302G>T	c.736T>G	p.Cys246Gly	rs187468519	-	-	-	0	0	0	-	Tolerated	neutral	0.448	0.051	159	1 hz	Possibly benign	
XL	inherited (Mo)	<i>KDM5C</i>	314690	NM_004187.3	707	91	g.53240784dup	c.1296dup	p.Glu433*	-	-	-	-	0	0	0	-	NA	NA	NA	NA	NA	1 henz	Pathogenic		
APN-135	M	AD	<i>DISC1</i>	600563	NM_018662.2	582	83	g.231935893A>G	c.1729A>G	p.Lys577Glu	-	-	-	-	0	0	0	-	Deleterious	deleterious	1.174	0.26	51	1 hz	VOUS	
		AR	<i>UPB1</i>	606673	NM_016327.2	545	46	g.24919670C>G	c.1000C>T	p.Arg334Trp	-	-	1	0.01%	0	0	0	-	Deleterious	deleterious	1.497	0.00	56	1 hz	VOUS	
XL	inherited (Mo)	<i>CUL4B</i>	300304	NM_003588.3	798	95	g.119681099_119681101del	c.81L_81del	p.Glu271Aspfs*11	-	-	-	-	NA	NA	NA	-	NA	NA	NA	NA	NA	1 henz	Pathogenic		
APN-137	M	AR	<i>LAMA1</i>	150320	NM_005559.2	594	85	g.6978321C>T	c.6064G>A	p.Ile2022Thr	rs140764072	-	13	0.10%	0	0	0	-	Tolerated	deleterious	2.707	0.827	58	1 hz	VOUS	
		<i>NRXN1</i>	600565	NM_001135659.1	547	60	g.51255909G>A	c.322C>T	p.Pro108Ser	rs199784029	-	18	0.14%	0	0	0	-	NA	neutral	2.788	0.984	74	1 hz	Possibly benign		
		<i>RALGDS</i>	601619	NM_001042368.1	327	52	g.13597712G>A	c.2075C>T	p.Pro692Leu	-	-	-	-	0	0	0	-	Tolerated	neutral	2.304	1.00	98	1 hz	VOUS		
		<i>NPC2</i>	601015	NM_006432.3	579	90	g.74951269T>C	c.212A>G	p.Lys71Arg	rs142075589	-	2	0.02%	0	0	0	-	Tolerated	neutral	0.932	0.764	26	1 hz	Possibly benign		
XL	inherited (Mo)	<i>ATRX</i>	300302	NM_006489.3	604	110	g.76972632G>A	c.109C>T	p.Arg337*	rs122445108	pathogenic	-	-	0	0	0	-	NA	NA	NA	NA	NA	1 henz	Pathogenic		
	inherited (Mo)	<i>CDH15</i>	114019	NM_004932.3	303	46	g.89251647G>C	c.569G>C	p.Arg190Pro	-	-	-	-	0	0	0	-	Deleterious	deleterious	2.062	0.984	103	1 hz	Benign		
APN-138	M	AD	<i>DISC1</i>	605210	NM_001164550.1	572	87	g.231856789G>T	c.1214G>T	p.Gly405Val	-	-	-	-	0	0	0	-	New DSS	Notscored	-0.037	0.02	109	1 hz	VOUS	
		<i>MED13L</i>	608771	NM_015335.4	603	70	g.116446347G>A	c.1871C>T	p.Ser624Leu	rs200545513	-	1	0.01%	0	0	0	-	Tolerated	neutral	0.528	0.969	145	1 hz	Probably benign		
		<i>LAMA1</i>	150320	NM_005559.2	562	79	g.6978147G>C	c.6257A>C	p.Lys208Thr	rs142934543	-	8	0.06%	0	0	0	-	Tolerated	deleterious	4.402	1.00	78	1 hz	VOUS		
		<i>KDM5A</i>	180202	NM_001042603.1	583	84	g.402151G>C	c.1472A>C	p.Glu1491Ala	rs201998974	-	1	0.01%	0	0	0	-1.3	Tolerated	neutral	3.595	1.00	107	1 hz	Possibly benign		
XL	inherited (Mo)	<i>T0SEC2</i>	300522	NM_015075.1	474	104	g.53272587C>A	c.2301G>T	p.Arg734Leu	-	-	-	-	0	0	0	-	Tolerated	deleterious	5.532	1.00	102	1 henz	VOUS		
		<i>KIAA2022</i>	300524	NM_00108537.2	580	120	g.73951127G>A	c.365C>T	p.His89Tyr	-	-	-	-	0	0	0	-	Deleterious	neutral	1.129	1.00	83	1 henz	VOUS		
APN-139	M	AD	de novo	<i>SIN3GAP1</i>	603384	NM_006772.2	100	100	g.3444346G>A	c.3583G>A	p.Arg1136Gln	-	-	-	-	-93.6	-100	NA	New DSS	NA	NA	2.869	1.00	NA	1 henz	Pathogenic
	inherited (Mo)	<i>MED13L</i>	608771	NM_015335.4	572	91	g.116413113G>T	c.5594G>A	p.Arg1865Gln	-	-	-	-	0	0	0.1	-	Deleterious	deleterious	4.241	1.00	43	1 hz	Benign		
		<i>ARID1B</i>	NA	NM_021073.2	293	35	g.157099186dupCGCGCGGCAC	c.133_141dup	p.Ala45_Ala47dup	-	-	-	-	0	0	0	-	NA	NA	NA	NA	NA	1 hz	Possibly benign		
		<i>CC2D1A</i>	610055	NM_017721.4	596	87	g.14020663G>T	c.88G>T	p.Asp30Tyr	-	-	-	-	0	0	0	-	Deleterious	deleterious	4.564	0.976	160	1 hz	VOUS		
		<i>CC2D1A</i>	610055	NM_017721.4	587	87	g.14020723G>T	c.148G>T	p.Ala50Ser	-	-	1	0.01%	0	0	0	-	Tolerated	neutral	3.03	0.992	99	1 hz	Possibly benign		
XL	inherited (Mo)	<i>CUL4B</i>	300304	NM_003588.3	314	106	g.119694175G>C	c.373C>G	p.Leu125Val	-	-	-	-	0	0	0	-	Tolerated	neutral	0.125	0.992	32	1 henz	Possibly benign		
		<i>DOCK8</i>	NA	NM_203447.3	588	78	g.197135C>T	c.3460C>T	p.Arg1154Cys	rs34390308	-	-	-	0	0	0	-	Deleterious	deleterious	NA	1.00	180	1 hz	VOUS		
		<i>PGAS5</i>	169730	NM_014224.2	475	53	g.61015898A>C	c.664A>C	p.Lys222Gln	rs200053998	-	-	-	0	0	5.5	-	Tolerated	neutral	-1.812	0.00	53	1 hz	Possibly benign		
APN-141	M	AD		<i>DISC1</i>	605210	NM_001164537.1	388	49	g.231881156G>T	c.1124C>T	p.Thr375Met	-	-	-	0	0	-0.4	-	Notscored	neutral	-0.682	0.004	81	1 hz	VOUS	
		<i>NRXN1</i>	600565	NM_001135659.1	595	75	g.51254885G>A	c.529C>T	p.Arg171Irp	-	-	-	-	0	0	0	-	NA	neutral	2.062	1.00	101	1 hz	VOUS		
		<i>LAMC3</i>	604349	NM_006095.3	546	53	g.133951317G>C	c.3594G>C	p.Arg198Ser	rs199612934	-	-	-	0	0	0	-	Tolerated	neutral	-2.297	0.00	110	1 hz	Possibly benign		
		<i>MPI</i>	154550	NM_002435.1	356	54	g.75182424C>T	c.10C>T	p.Pro45Ser	rs143982014	-	17	0.13%	0	0	0	-	Tolerated	neutral	0.528	0.00	74	2 hz	Possibly benign		
		<i>CENPJ</i>	609279	NM_018451.3	600	82	g.25486989T>C	c.175A>G	p.Thr59Ala	rs138732534	-	26	0.20%	0	0	0	-	Tolerated	neutral	-0.36	0.00	58	1 hz	Possibly benign		
de novo		<i>MECP2</i>	300005	NM_004992.3	596	84	g.15329677G>A	c.502C>T	p.Arg168*	rs61748421	-	-	-	0	0	0	-	New DSS	NA	NA	1.82	1.00	NA	1 hz	Pathogenic	
XL		<i>FLNA</i>	300017	NM_001105561.1	289	48	g.153599601G>A	c.13C>T	p.His17Y	-	-	-	-	0	0	0	-	Deleterious	neutral	4.564	1.00	83	1 hz	VOUS		
		<i>IDS</i>	300823	NM_000202.5	589	82	g.148579167C>T	c.530A>G	p.Glu177Gly	-	-	-	-	0	0	0	-	Tolerated	neutral	0.286	0.984	98	1 hz	Possibly benign		
APN-142	F		<i>SLC9A6</i>	300231	NM_001042537.1	589	83	g.135076986C>T	c.367C>T	p.Pro123Ser	-	-	-	-	0	0	0	-	Tolerated	neutral	5.048	1.00	74	1 hz	Possibly benign	
		<i>OCRL</i>	300535	NM_000276.3	609	83	g.128696622C>A	c.1103C>A	p.Thr368Asn	-	-	-	-	0	0	NA	-	Tolerated	neutral	3.514	1.00	65	1 hz	Possibly benign		
		<i>ANK3</i>	600465	NM_001204403.1	128	56	g.62493041C>T	c.37G>A	p.Glu13Lys	-	-	-	-	0	0	0	-	Deleterious	neutral	-0.521	0.00	56	1 hz	VOUS		
		<i>RELN</i>	600514	NM_005045.3	586	74	g.103197603G>A	c.5618C>T	p.Thr1873Ile	rs41275239	-	30	0.23%	0	0	0.1	-	Tolerated	neutral	2.869	1.00	89	2 hz	Possibly benign		

MOI: mode of inheritance; XL: X-linked; AD: autosomal dominant; AR: autosomal recessive

M: Male; F: Female

Fa: father; Mo: mother

VOUS: Variant of unknown significance

possibly causative mutation

certainly causative mutation