Diagnostic yield of exome trio analysis to identify the genetic etiology in 404 undiagnosed cases

Diez I., Rosenstone S., Martinez-Garcia M., Sanchez-Alcudia R., Rodriguez C., Perez-Carro R., Sanchez-Navarro I., Mata E., Fernandez-Tabanera E., Carcajona M., De la Vega L., Rodriguez D., Benito G., Sánchez-Bolivar N., Maietta P., Botet J., Alvarez S.
NIMGenetics, Genómica y Medicina, Madrid, Spain

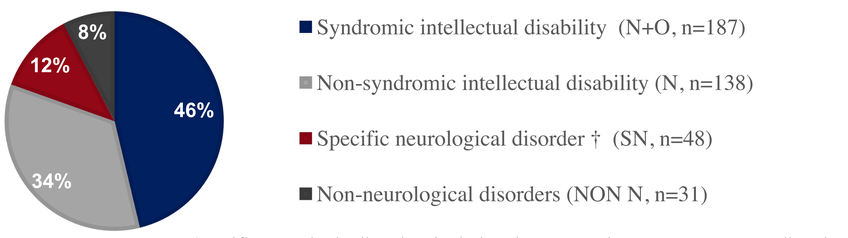
Background

Whole-exome sequencing (WES) has become an effective diagnostic method, transforming the molecular diagnosis and clinical management of many undiagnosed genetic diseases.

This approach has changed the medical practice and specifically exome trio analysis has shown to be an effective strategy in identifying *de novo,* hemizygous, newly homozygous and in compound heterozygous potential causal variants of rare genetic disorders. We present the analysis of 404 trios referred to a single institution.

Studied Cases and Methods

Samples: The study included 404 patients, distributed as 145 women (36%) and 259 men (64%), with a mean age at genetic analysis of 7 years old (range: prenatal-52y). Patients were mainly children with syndromic intellectual disability (42%) and specific neurological disorders (40%) (Figure 1).



† Specific neurologic disorders include seizures, ataxia, or any movement disorder

Figure 1. Phenotypic stratification of the patients cohort

Clinical history and pedigree were summarized into a local database. Informed consent was obtained from all family members involved.

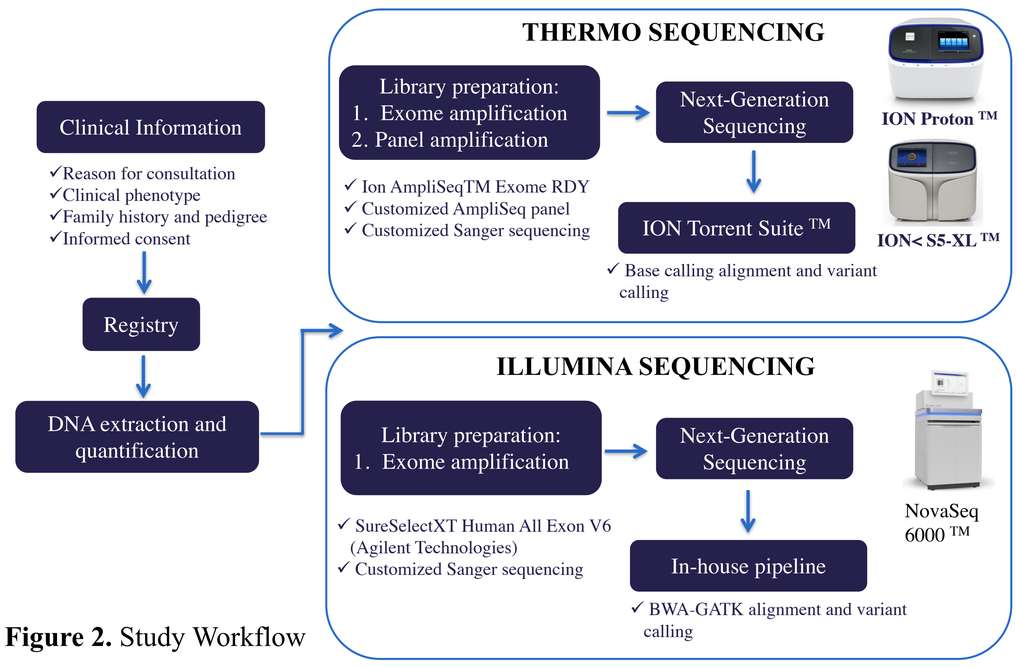


Figure 2. Study Workflow

Number of Variants (mean)			
Exome	50000	*De novo*	1
Variant Filtering	650	Newly Homozygous	1
NIMGenetics DB filtering	344	In Compound Heterizygous *in trans*	2
		Hemizygous	2

1. Exome sequencing and variant calling: An average depth of 100X and an average coverage of 98.3% in the selected genes was obtained for all the samples.
2. Variant annotation and prioritization: variants were annotated using Ion Reporter™ Software. An in-house software program and a local database (*~4000* exomes) were used for variant filtering and prioritization. Candidate variants were visualized using IGV (Integrative Genomics Viewer).
3. Evaluation, classification and reporting of candidate variants: Phenotype, genetic and technical criteria were considered. Variants were selected and classified by a board of molecular clinical geneticist, following the guidelines of the ACMG. Causal variants were discussed with the referring physician and/or clinical geneticist.
4. Segregation studies: When available, parental samples were also analyzed to determine the parental inheritance pattern.

Results

The genetic etiology was potentially elucidated in 129 probands harboring 82 causal variants and 47 likely causal variants (Figure 3).

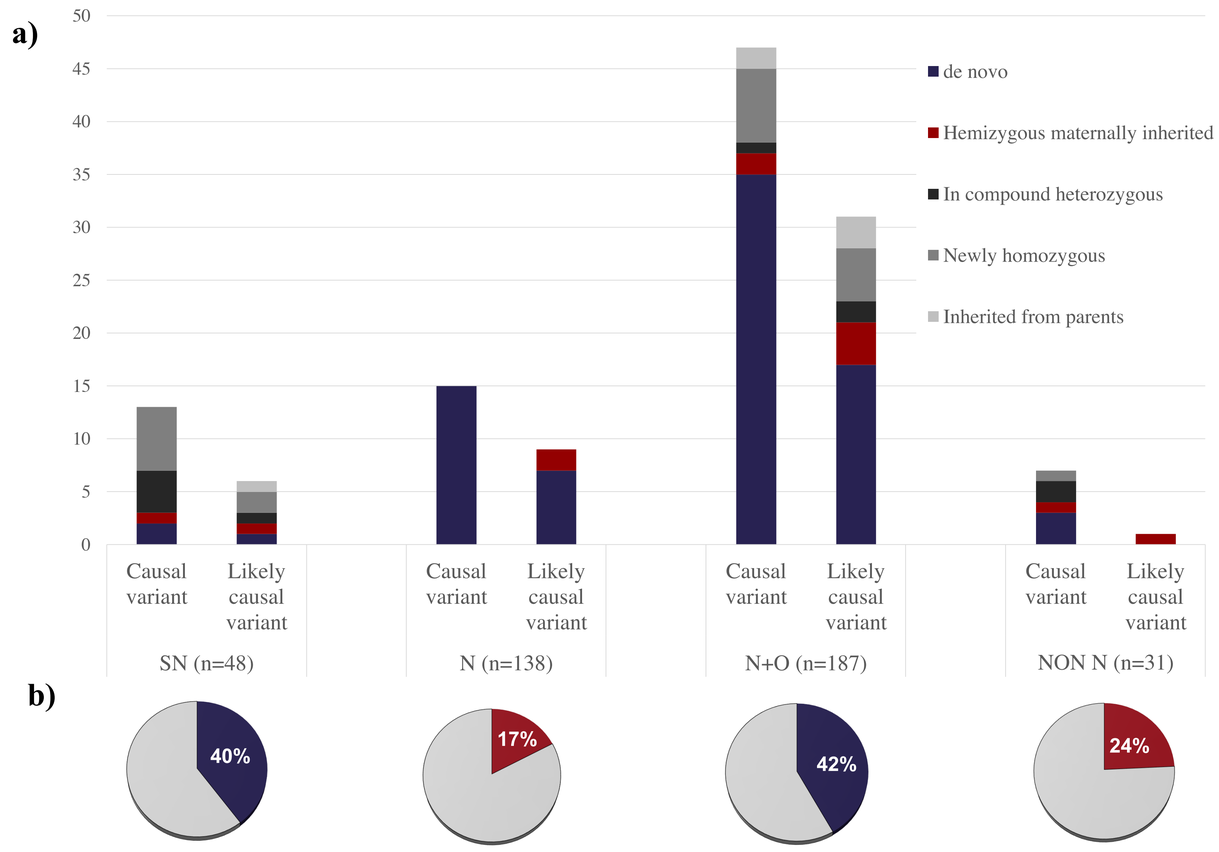


Figure 3. Inheritance patterns and diagnostic yield. **a)** Distribution of the identified variants: 80 *de novo*, 21 newly homozygous, 12 hemizygous maternally inherited, 10 in compound heterozygous and 6 inherited variants. **b)** Diagnostic yield in the four different groups of patients: specific neurological disorders (SN), non-syndromic intellectual disability (N), syndromic intellectual disability (N+O) and without a neurological disorder (NON N) patients.

Table 1. Representative reported causal variants on some recurrently genes

Candidate Gene	Number of Cases	Disease (MIM#)	Inheritance pattern	Variants	Mutation Type	Classification	Patient's Phenotype
ANKRD11	*2*	KBG syndrome (#148050)	AD	p.(Lys635Glnfs*26) p.(Arg1311*)	*Frameshift* *Nonsense*	Pathogenic Likely Pathogenic	Neurodevelopmental disorder, short stature and peculiar phenotype.
ATRX	*2*	Mental retardation-hypotonic facies syndrome (#309580)	XL	p.(Met536Val) p.(Thr1358Ile)	*Missense* *Missense*	VUS VUS	Psychomotor delay, mainly motor, impaired social interactions and macrocephaly
CDKL5	2	Epileptic encephalopathy, early infantile 2 (#300672)	XL	p.(Tyr24Cys) p.(Gln860Ter)	*Missense* *Nonsense*	Likely Pathogenic Likely Pathogenic	Lennox-Gastaut syndrome, ID and retinal abnormalities
DDX3X	*2*	Mental retardation 102 (#300160)	XL	p.(Arg475Pro) p.(Gly539Ser)	*Missense* *Missense*	Likely Pathogenic VUS	ID, obesity, peculiar phenotype, scoliosis and abnormal gait
EHMT1	*2*	Kleefstra syndrome 1 (#610253)	AD	p.(Cys1111Tyr) p.(Arg948Trp)	*Missense* *Missense*	Likely Pathogenic VUS	West syndrome, peculiar phenotype, Autism and language delayed
FBN1	*2*	Weill-Marchesani syndrome 2 (#608328)	AD	p.(Pro1424Ala) p.(Arg1596*)	*Missense* *Nonsense*	Pathogenic Pathogenic	Developmental delay, Williams phenotype and joint stiffness
FLNA	*3*	Melnick-Needles syndrome (#309350)	XL	p.(Thr1232Ile) p.(Ser61Thr) p.(Val1309Ile)	*Missense* *Missense* *Missense*	Likely Pathogenic VUS VUS	Peculiar phenotype, macrocephaly, congenital cardiomyopathy and recurrent bronchitis
MBD5	*2*	Mental retardation 1 (#156200)	AD	p.(Gln230Ter) p.(Ile1130Asn)	*Nonsense* *Missense*	Likely Pathogenic VUS	Autism, seizures and language delay
NPC1	*2*	Niemann-Pick disease type C1(#257220)	AR	p.(Gly1240Arg) p.(Arg372Trp) p.(Pro1007Ala)^	*Missense* *Missense* *Missense*	Pathogenic Pathogenic Pathogenic	Spinocerebellar ataxia, bipolar disorder, obesity, sensorineural hearing loss and leukodystrophy
PACS1	*3*	Schuurs-Hoeijmakers syndrome (#615009)	AD	p.(Arg203Trp)	*Missense*	Pathogenic	Sagittal synostosis, language developmental delay, autism, ID and peculiar phenotype
SATB2	*2*	Glass syndrome (#612313)	AD	p.(Gln419*) p.(Val476Glyfs*21)	*Nonsense* *Frameshift*	Likely Pathogenic Likely Pathogenic	Developmental delay, peculiar phenotype, language deficit, and ADHD
SMARCA2	*2*	Nicolaides-Baraitser syndrome (#601358)	AD	p.(Arg525Cys) p.(Gln491Pro)	*Missense* *Missense*	Likely Pathogenic VUS	ID, joint stiffness, intrauterine growth retardation and Poor speech
SPTAN1	*2*	Epileptic encephalopathy, early infantile 5 (#613477)	AD	p.(Gly2341Asp) p.(Asp2303_Leu2305dup)	*Missense* *Duplication*	Likely Pathogenic Pathogenic	Gross motor delay, West syndrome, moderate ID, peculiar phenotype and Optic atrophy
SYNE1	*3*	Spinocerebellar ataxia, 8 (#610743)	AR	p.(Ala3150Gly) p.(Asn4617His) p.(Gln189*)^	*Missense* *Missense* *Nonsense*	VUS VUS Pathogenic	Muscular dystrophy, gait ataxia and abnormal eye movements

^ Homozygous variant

Conclusions

- Trio analysis provided globally a diagnostic yield of 32% in patients previously studied with conventional strategies and without a molecular diagnosis. These results are in concordance with previously published, despite the difference in sequencing technology.[1,2,3]
- Our results highlight the clinical value of trio exome sequencing approach in the identification of causal mutations among previously undiagnosed patients, providing unprecedented opportunities in the discovery of novel disease-causing genes.
- Patients with non-neurological disorders and non-syndromic intellectual disability showed lower diagnostic rates, suggesting a larger fraction of unknown genes or non-genetic underlying mechanisms. The implementation of WES as a first-tier diagnostic approach would provide a higher diagnostic yield and a cost-efficient option particularly in rare syndromic intellectual disabled patients.

Achlowledgments

We would like to thank our collaborators and patients.

References

1. Farwell KD, *et al*. Genet Med. 2015 Jul;17(7):578-86.
2. Lee H, *et al*. JAMA. 2014 Nov 12;312(18):1880-7.
3. Moroe GR, *et al*. Genet Med. 2016 Feb 4.

The authors declare no conflict of interest