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PPP2R5D variants in patients with variable neurodevelopmental phenotype

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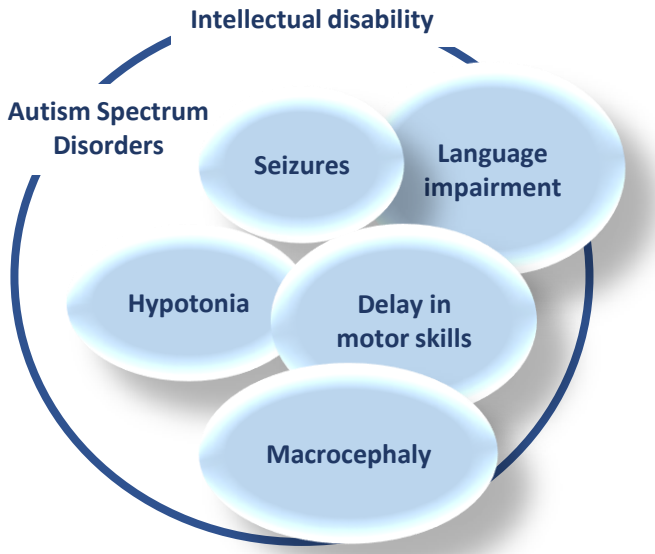
INTRODUCTION

- The *PPP2R5D* gene encodes for the Protein Phosphatase 2 (PP2A) Regulatory Subunit B', Isoform Delta
- The identification of heterozygous pathogenic variants were associated to Mental Retardation, autosomal dominant 35 (OMIM#616355), characterized by mild to severe Intellectual Disability (ID), Autism Spectrum Disorder (ASD), pronounced hypotonia and other significant comorbidities
- From 2015 to date only 25 individuals with *PPP2R5D* mutations were reported in the published literature

In this study we report two novel cases of ***PPP2R5D*** with **Pathogenic mutations** already described in literature (Shang L. et al., 2016 Neurogenet; Loveday C. et al., 2015 Hum Mol Genet; Hunge G. et al., J Clin Inv) and two other ***PPP2R5D*** **Likely Pathogenic** variants in a population of unrelated individuals affected with ID and ASD.



METHODS

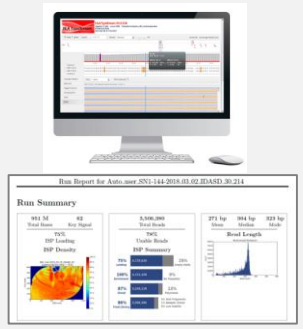
We performed amplicon-based next generation sequencing of 74 **ID-ASD genes** (Aspromonte MC et al., 2019 Hum Mut) including ***PPP2R5D* gene**, in a cohort of **618** unrelated individuals, affected with ID/ASD, previously resulted negative for Fragile-X and array-CGH testing.

Laboratory and Bioinformatic workflow

Sequencing with Ion Torrent PGM using ION 326v.2 – 3178v.2 chips

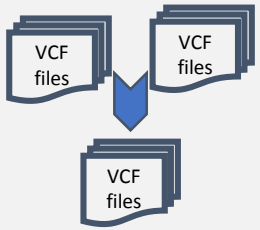
PGM system

Raw data processing



Ion Torrent Suite Software

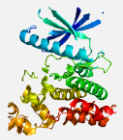
ANNOVAR **functional annotation and frequencies calculation** of SNVs on tested individuals



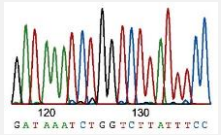
In-house database

Variants filtering and interpretation

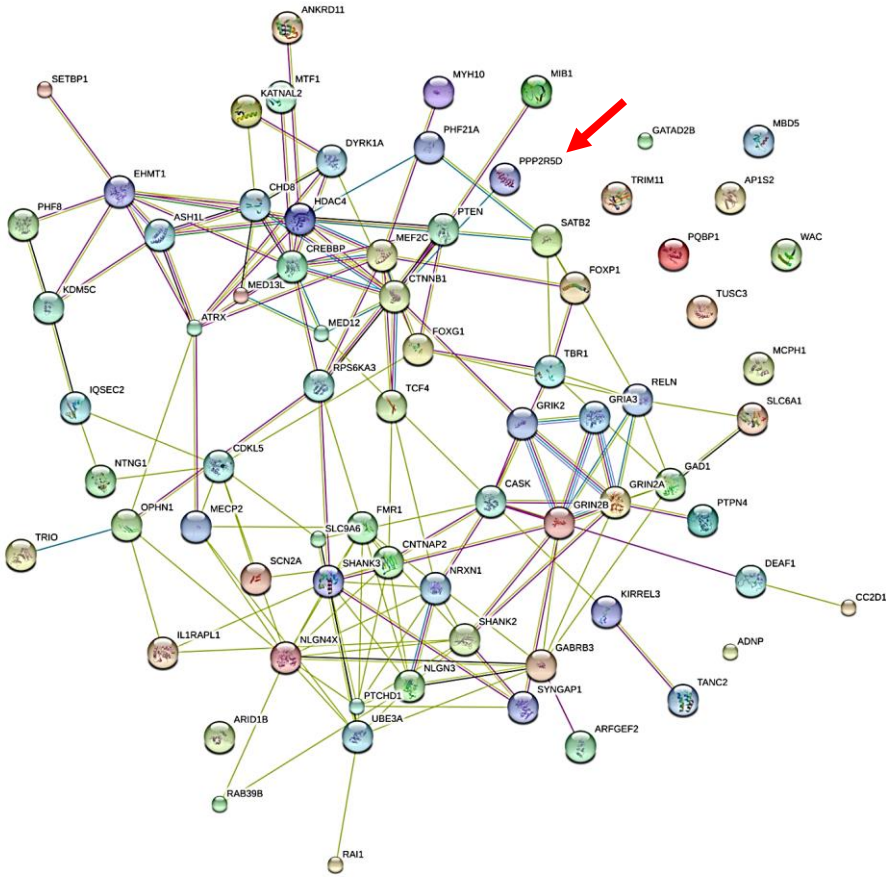
- Consensus of pathogenicity predictors (>6/12)
- CADD score (>15)
- I*n silico* protein structural / functional analysis of the mutation effects



Sanger Validation
Segregation analysis



Variant classification: InterVar System based on American College Medical Genetics guidelines (ACMG)



The **74 panel genes** are more connected in an interaction network than a set of randomly selected genes. The red arrow showed the *PPP2R5D* included in the panel. The network imaging was performed with **STRING**

GENE PANEL RESULTS

PPP2R5D SNVs

A total of 6 rare single nucleotide variants (SNVs) (Table 1) (Fig. 1), either not yet reported or with low frequency in public databases, have been identified:

- **2 Likely Gene Disrupting** variants located in a conserved Intra-Loop 2 domain and involved in the catalytic subunit binding;
- **2 new Likely Pathogenicmissense variants:** one of the two p.Arg219Cys alters a high conserved residue mapping in the alpha-helix, and the other one p.Thr536Ser alters a conserved residue in a disorder C-terminal region;
- **2 variants of Uncertain Significance**: one intronic variant predicted altering splicing mechanism and one new nonframeshift deletion in a Gln-Pro rich region.

Patient	Sex	Mutation	Variant segregation	dbSNP	gnomAd AC/AN	CADD
1	M	*c.598G>A; p.Glu200Lys	n/a	rs863225079	//	33
2	M	c.655C>T; p.Arg219Cys	n/a	//	//	34
3	F	*c.589G>A; p.Glu198Lys	*de novo*	rs863225081	//	32
4	F	c.1606A>T; p.Thr536Ser	n/a	//	//	19
5	M	c.115_132del p.39_44del	n/a	//	//	//
6	F	c.917+9C>T	n/a	rs199597619	3/251478	//

Table 1. Rare SNVs identified in individuals with ID and ASD (NM_006245.3; NP_006236.1) *Mutations report as pathogenic in literature.

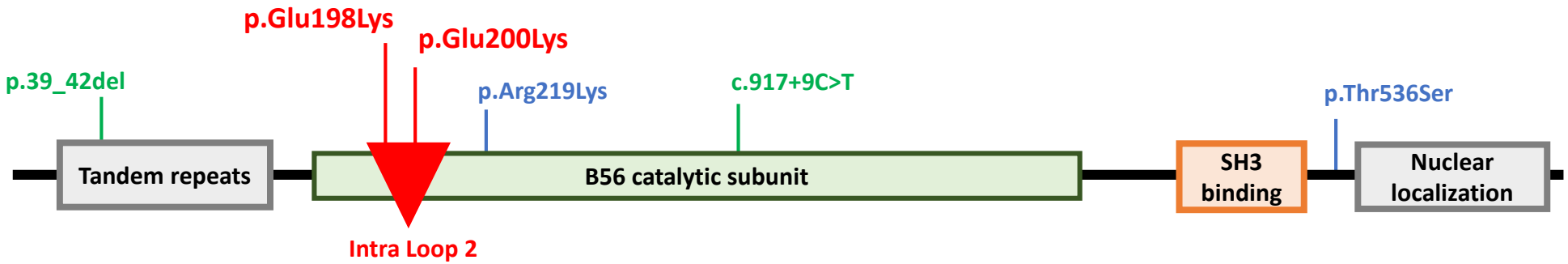


Figure 1. Representation of PP2A-substrate binding regulatory subunit (Isoform B56δ). SNVs are colored on the bases of their classification: LGD (red); Likely Pathogenic (blue); Uncertain Signifcance variants (green)

Clinical features of individuals with *PPP2R5D* Pathogenic and Likely Pathogenic variants

Individual	1	2	3	4
Sex	**M**	**M**	**F**	**F**
Age	**4 years**	**7 years**	**6 years**	**17 years**
Intellectual disability	**mild**	**yes**	**severe**	**moderate**
Speech	**n/a**	**speech disorder in production and understanding**	**absent**	**n/a**
Macrocephaly	**yes**	**yes**	**macrocrania**	**no**
Hypotonia	**no**	**no**	**yes**	**n/a**
Autism spectrum disorders	**no**	**yes**	**yes**	**yes**
Behavioural abnormalities	**n/a**	**self-injurious behavior and difficulty in relationship**	**n/a**	**stereotypies, anxiety and mood swings**
Seizures	**n/a**	**yes**	**n/a**	**n/a**
Dysmorphic features	**snub noses, malocclusion, strabismus**	**n/a**	**hypotonic face, down slanting palpebral fissures**	**nose with enlarged base and bulbous tip, protruding ears**
Motor dysfunction	**n/a**	**difficulty with fine motor skills**	**delay**	**delay**

Dual diagnosis case

In a girl (Patient 3) a *de novo* Glu198Lys mutation was identified by whole-exome sequencing (WES). She also carried an *FMR1* mutation with size mosaicism, with both full (>200 CGG triplets) and premutation (73 CGG triplets) (Fig.2)

The methylation analysis of *FMR1* gene demonstrated also:

1. a ratio of 53% of methylation for the normal allele
2. a complete methylation for the allele full mutated
3. the fragment with premutation completely unmethylated

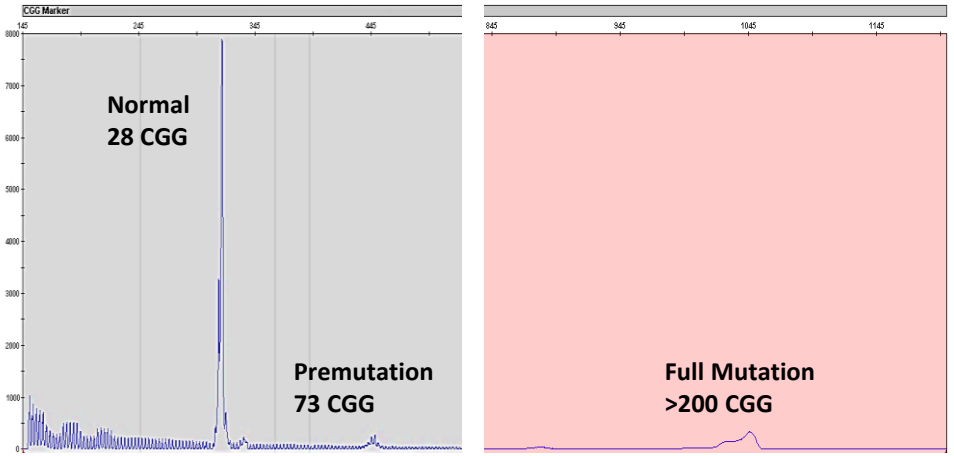


Figure 2. For detection of CGG repeats AmplideX™ PCR Kit (Asuragen) was used according to the manufacture's protocol. Fragment analysis was performed with ABI Prism 3130 POP-7 polymer and the elaboration data with GeneMapper 4.0 Software.

CONCLUSIONS

- We recommend that analysis of the *PPP2R5D* gene be included in a molecular genetic testing approach, such as a gene-targeted panel
- We describe for the first time a **Dual-diagnosis**: a girl carrying a de novo ***PPP2R5D* mutation** also carries a mutation with size mosaicism of the ***FMR1*** gene**.** In this individual the fragile X phenotype seemed to be overshadowed by the severe PPP2R5D clinical features
- The clinical profile of the subject who has the missense mutation **p.Glu200Lys** is less severe than that with **p.Glu198Lys** and those reported in literature; he does not show any hypotonia and autism spectrum disorders
- We report **two novel missense variants** that are not mapped in the PP2A-catalytic subunit interaction interface, but are classified as Likely Pathogenic by InterVar System; segregation analysis and in silico evaluations need to be carried out in order to confirm the pathogenicity of the new variants described

References

Aspromonte MC et al. (2019) Characterization of Intellectual Disability and Autism Comorbidity Through Gene Panel Sequencing. Human Mutation

Houge G. et al. (2015) B56δ-related Protein Phosphatase 2A Dysfunction Identified in Patients With Intellectual Disability. Journal of Clincal Investigation

Loveday C. et al. (2015) Mutations in the PP2A Regulatory Subunit B Family Genes PPP2R5B, PPP2R5C and PPP2R5D Cause Human Overgrowth. Human Molecular Genetics

Shang L. et al. (2016) De Novo Missense Variants in PPP2R5D Are Associated With Intellectual Disability, Macrocephaly, Hypotonia, and Autism. Neurogenetics

The authors declare no conflict of interest