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Effective Diagnosis of Neurodevelopmental Disorders (NDDs) by Next Generation Sequencing gene panel



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Laboratory of Molecular genetics of Neurodevelopmental disorders

Department of Woman and Child Health

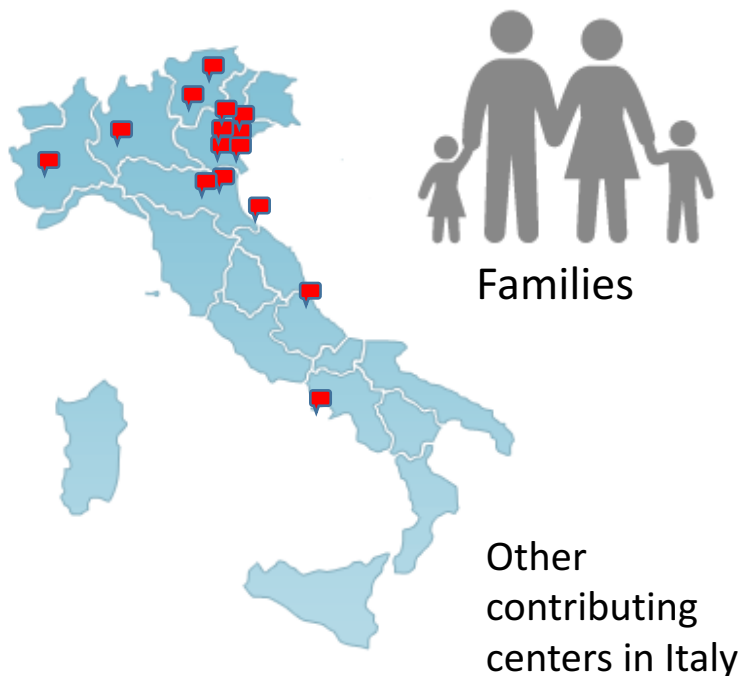
Istituto di Ricerca Pediatrica - Città della Speranza

Padua, Italy

Genetics or Pediatrics units

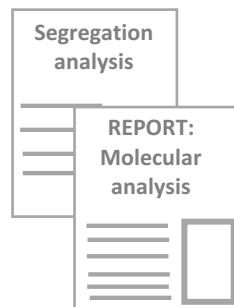
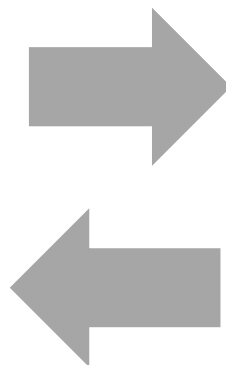


Pediatric Clinic, University of Padua

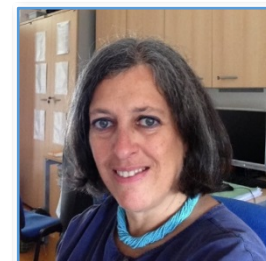


Families

Other
contributing
centers in Italy



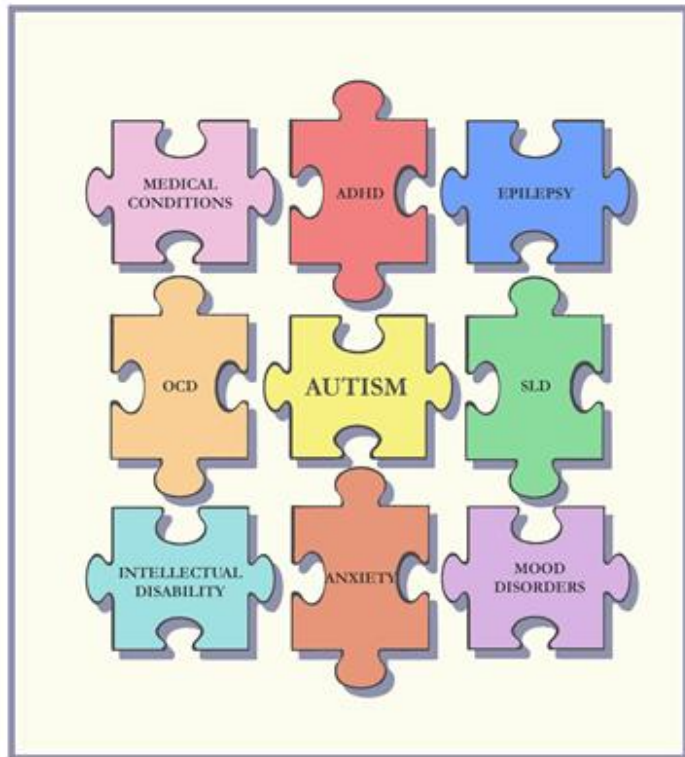
Genetics of NDDs Lab



Prof. Alessandra Murgia

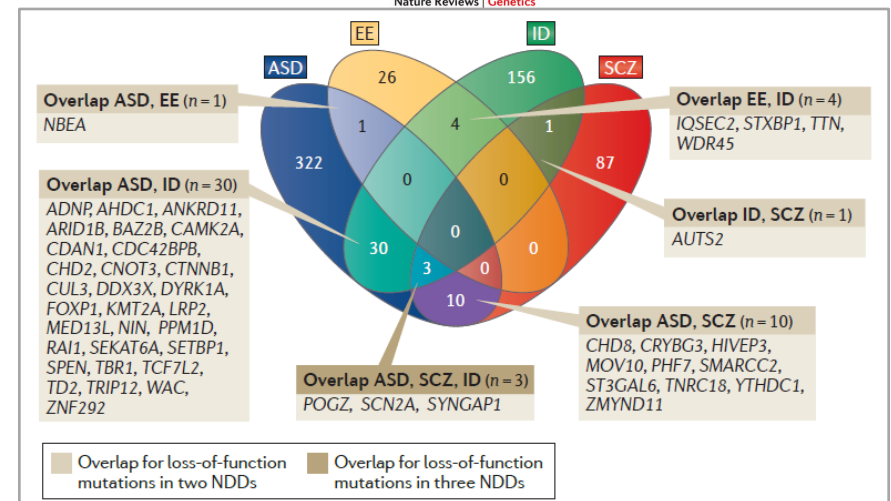
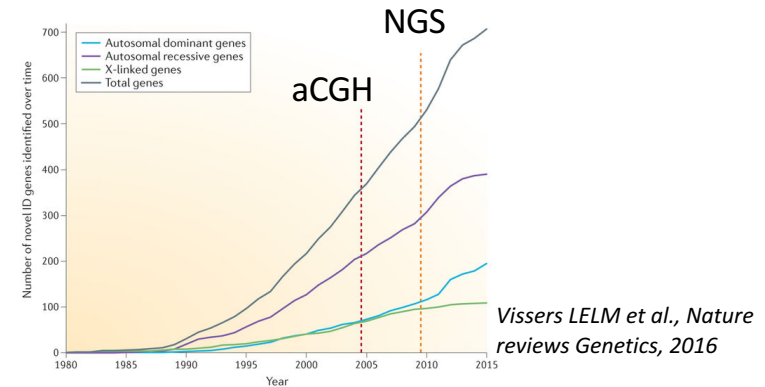
Department of Women's and Children's Health
University of Padua – Hospital of Padua

Neurodevelopmental disorders (NDDs) are a group of clinically and genetically heterogeneous conditions



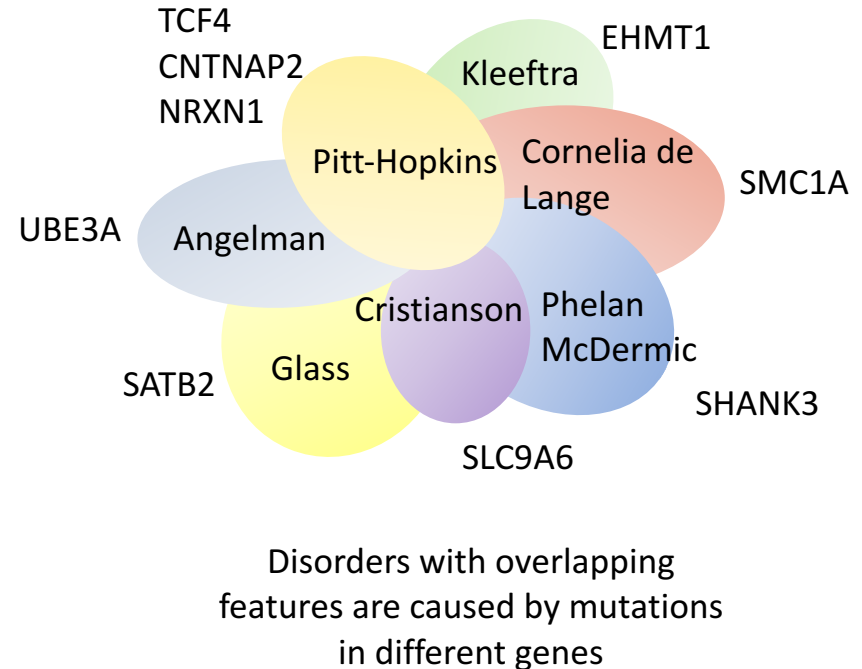
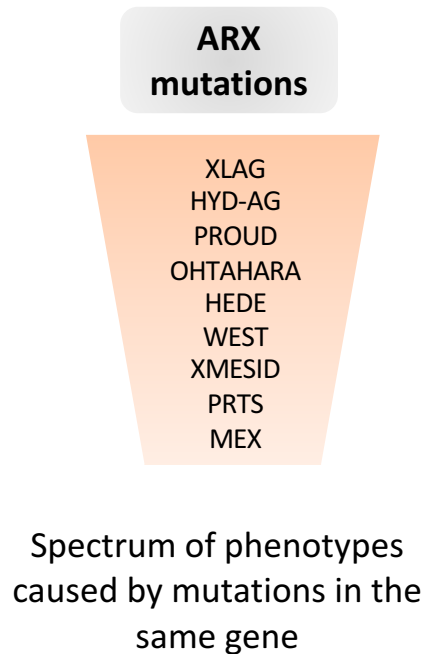
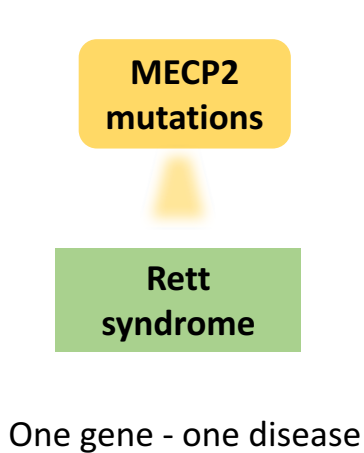
ASD is the most severe manifestation among NDDs, affecting 1 in around 88 individuals. 70% of ASD patients have ID.

ID is reported in 1.5-2% of children. 25% of ID patients have **epilepsy** and 6% are **macrocephalic**.



Genes are shared among these disorders and converge on common functional pathways

Targeted high-throughput sequencing: a new strategy for the molecular diagnosis of NDDs



Our Laboratory changed molecular approach for the diagnosis of NDDs: from single gene to gene panel testing

AS742	BRP1	DMRTA5	FOXP2	FKBP	MYH9	RAB29	SPPL2	USP49
AA19	USP49	BLAC4	GAL3	MYH9A	NRN	TM6SF2	CHST14	KIF22L
AA19	CHST14	RHBB	LIN1	MYH9B	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19A	CHST14	CHST14	OPD1	AP3D1A	NFI	ROB1		
AA19B	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19C	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19D	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19E	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19F	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19G	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19H	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19I	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19J	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19K	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19L	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19M	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19N	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19O	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19P	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19Q	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19R	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19S	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19T	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19U	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19V	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19W	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19X	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19Y	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A
AA19Z	CHST14	CHST14	OPD1	AP3D1A	MYO10	SLC39A3	SLC39A3	AP3D1A

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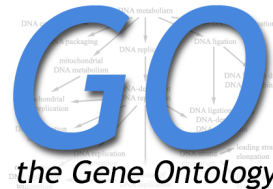
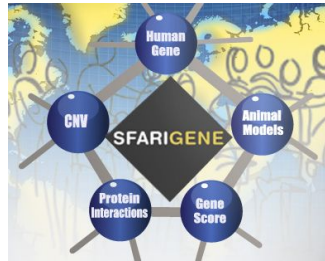
Diagnostic yield for different ID gene panels

Disease	N° genes	Patients	Diagnostic Yield	Authors
non-specific ID	90	52	21%	Tan et al. 2015
ID	217	106	25%	Redin et al., 2014
XLD ID	107	150	26% familial and 5% sporadic cases	Tzschach et al., 2015
ID	565	968 in absence of parental DNA	11%	Grozeva et al., 2015
Syndromic ID	1256	92	39%	Martinez et al., 2017
NDD	OMIM referenced genes	216	25.9%	Cherot et al., 2017

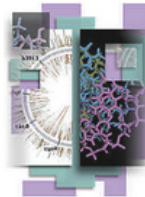
Over the last 3 years ca. 400 patients requested an ID-ASD gene panel analysis in our lab;
ca. 250 have been done; 150 patients are awaiting results
We hope to reduce the time needed to produce results!!

Gene panel: how it was designed

Ca. 1,000 genes



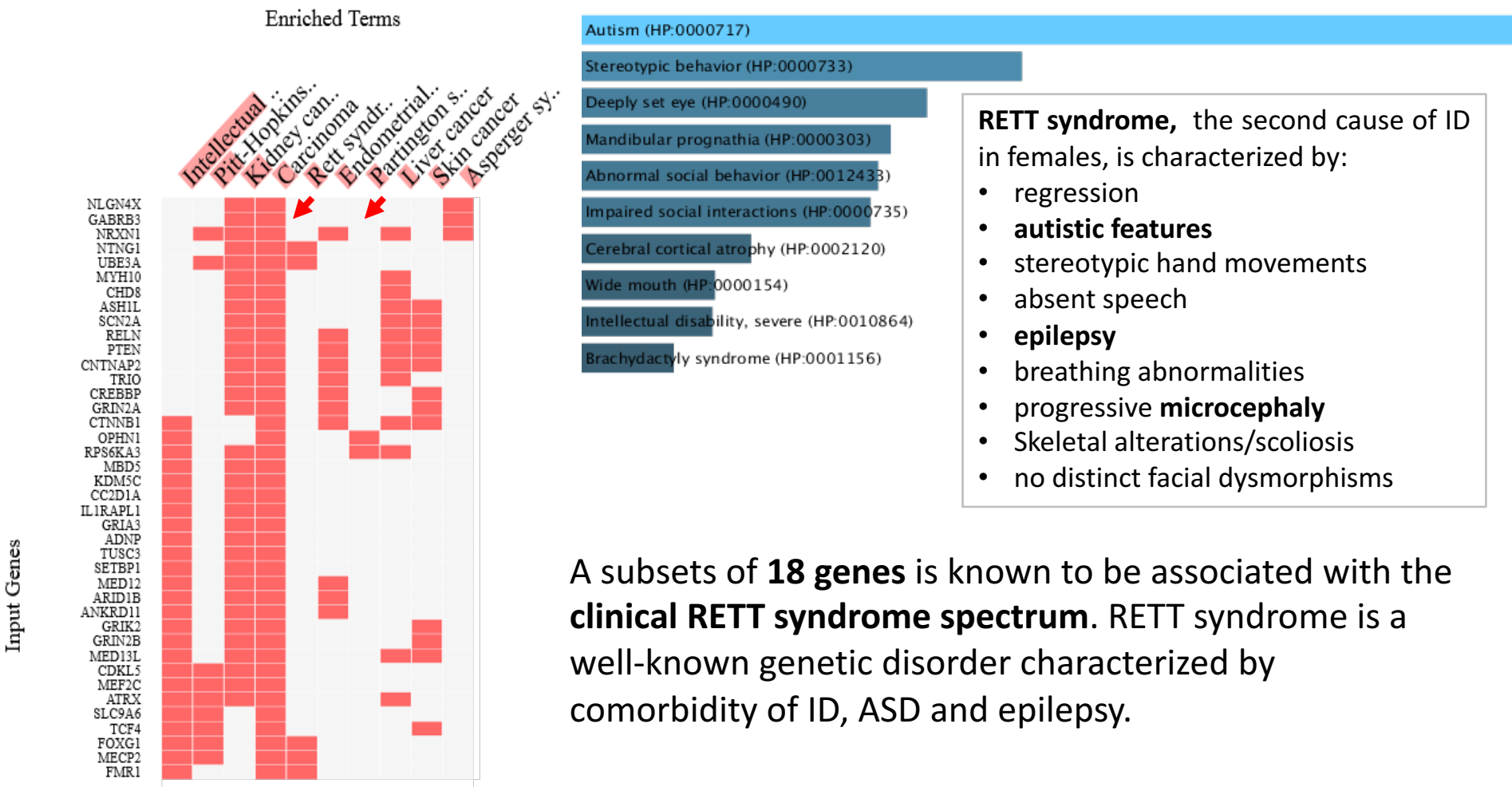
ENDEAVOUR



*74 genes
associated with
ID and ASD*



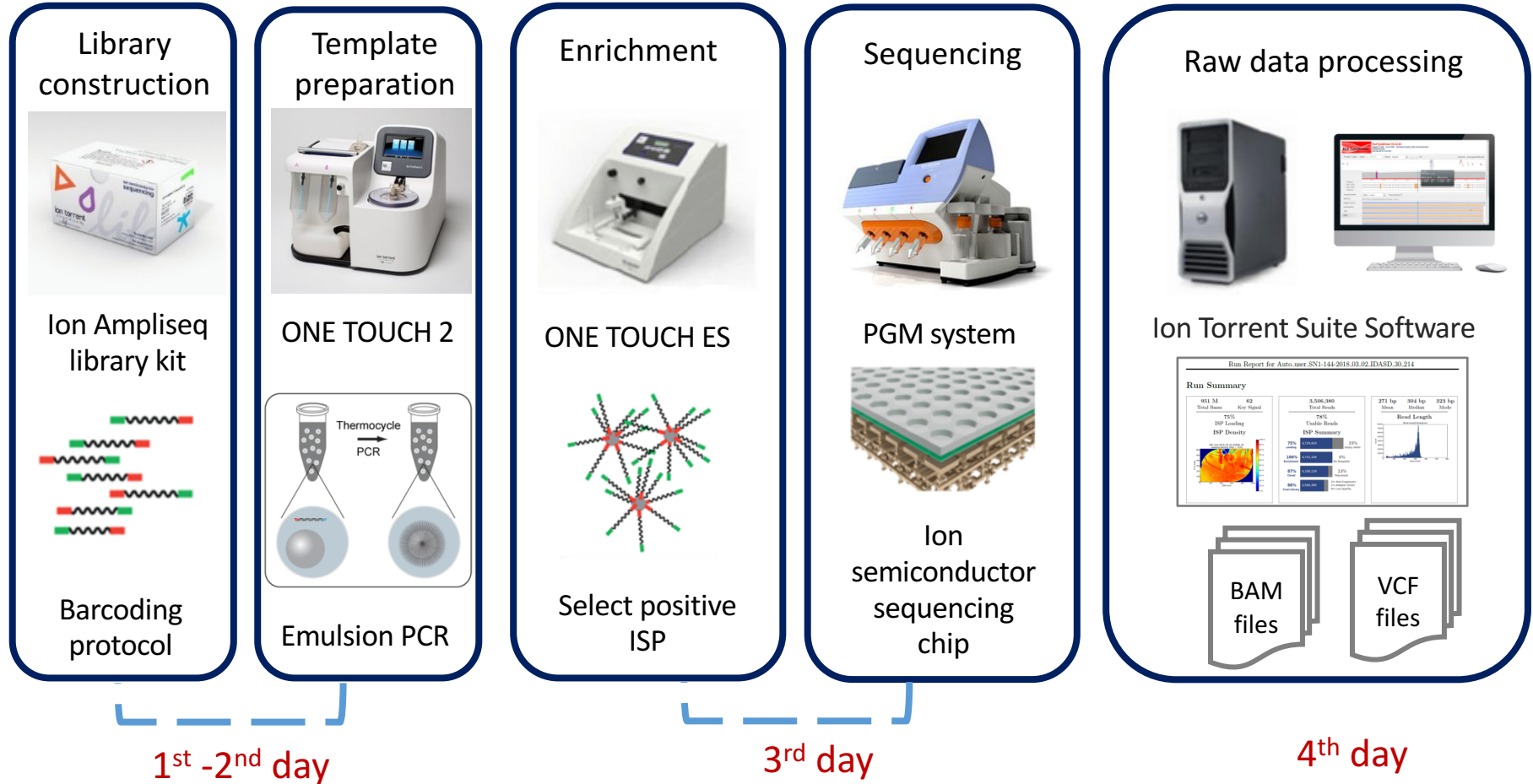
74 genes panel: enriched in HPO terms for Rett-like disorders



A subsets of **18 genes** is known to be associated with the **clinical RETT syndrome spectrum**. RETT syndrome is a well-known genetic disorder characterized by comorbidity of ID, ASD and epilepsy.

At the time of the design, **10 genes** were **candidates for ASD**, with a single piece of evidence in the literature.

Ion Torrent work flow



ID-ASD gene panel of 74 genes (~520Kb):
1834 amplicons (range 125-375bp)
99% average coverage per gene

Mean coverage depth of 250x
for each individual

Gene panel data analysis and validation

1-2 months

Filtering

General population frequency

gnomAD browser

Variant class (exonic or splicing)

Reported pathogenic

Inheritance mode (AD, AR, XLD, XLR)

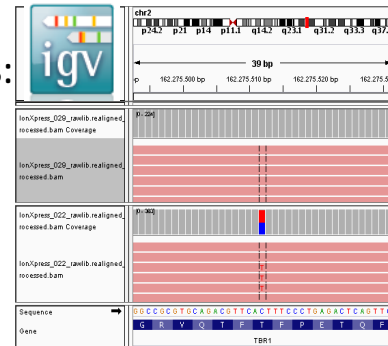
Clinical phenotype



Validation

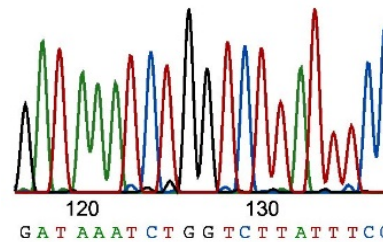
Evaluate **sequencing and alignment errors**:

- Genotype Quality (GQ)
- Read depth (DP)
- Allele Frequency (AF)

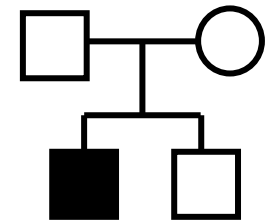


Sanger Validation

Only for variants with DP<100x



Variant classification



Segregation analysis



American College of Medical Genetics and Genomics guidelines 2015,
based on 28 criteria

InterVar: Classify System

Conclusions

Our work demonstrates how the combination of a NGS gene panel, with accurate gene-selection, coupled with a thorough clinical evaluation allows to efficiently investigate the molecular causes of ID and ASD.

Acknowledgements



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