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Autism Spectrum Disorder: Linked-Read Sequencing Reveals New and Undetected Variants

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Autism Spectrum Disorder (ASD): epidemiology and etiology

Epidemiology:

- It occurs within the first 3 years of life
- The prevalence is of 1:68 with male:female ratio=5:1

Etiology not completely understood

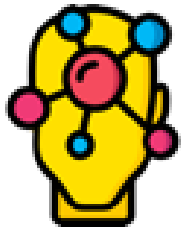
- Genetics play a key role
- Causative or predisposing genetics variants in 30% of patients
- Thousands of genes are involved
- Gene-environment interaction has been proposed

Objective and study design

- **Hypothesis**: variations in non coding and/or unaccessible regions could be involved in ASD

- **Study design**: linked-read WG-NGS of patients with ASD is used to access and discover phasing disease associated regions

- **Expectations:** new variants can:
  - be integrated in interactome studies
  - represent new biomarkers useful:
    - to design diagnostic protocols and kits
    - in patient stratification and personalized medicine



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Methods

- Ten patients with ASD were involved: 7 males and 3 females, including 2 couples of male siblings and 1 couple of male-female siblings

- HMW DNA was submitted to 10Xgenomics microfluidics partitioning and barcoding technology and then to Illumina library preparation and WG-NGS

- 10X Long Ranger pipelines and Loupe Genome browser were used to find and phase variants across >10Mb haplotype blocks in comparison to reference genomes.

- Genes affected by variants were compared with those already associated with ASD

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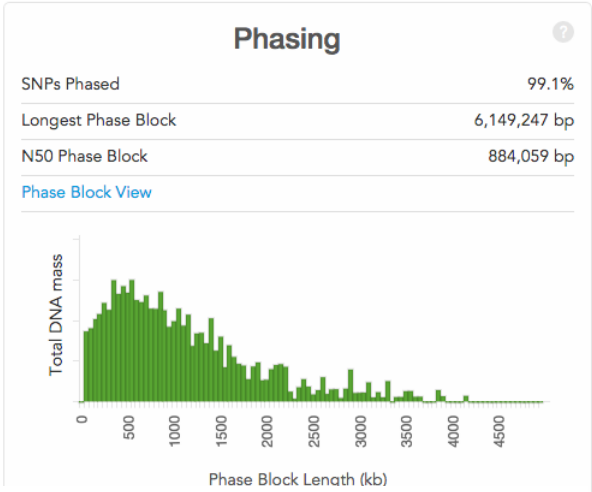
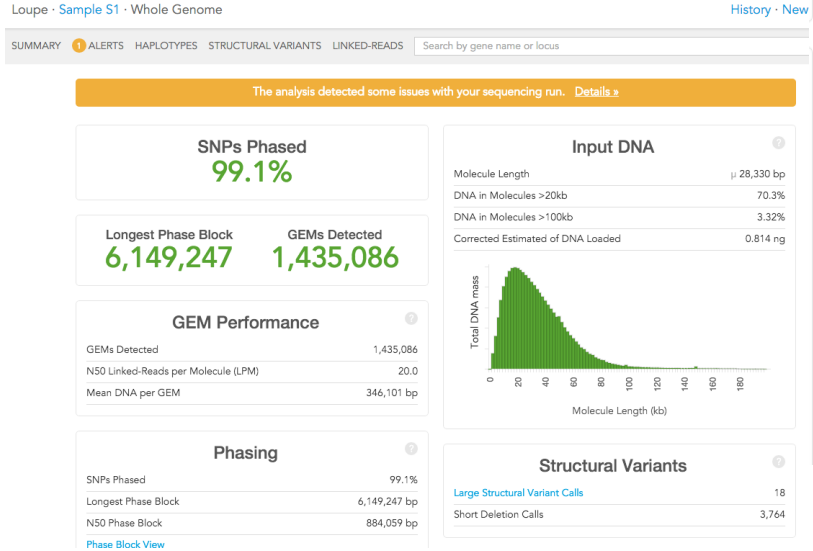
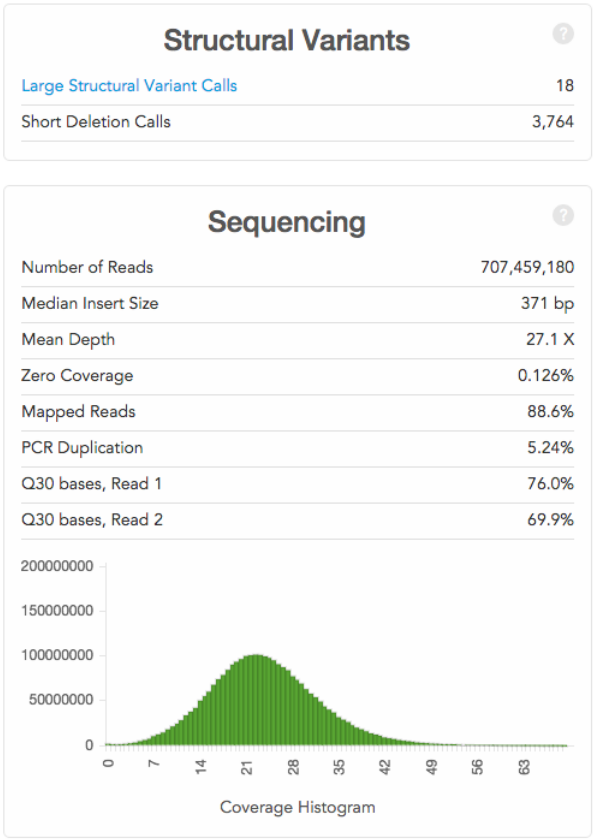
Results:
quality of linked read analysis

Sample 1:
input 1,25ng of HMW-DNA







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Results

Number of variants	
Total genes	23'740
Total variants	13’297
Annotations within genes	759'163
Annotations out of genes	338'078
Existing variants	942'744
New variants	154'497

Type of variants	
3_prime_UTR_variant	splice_donor_variant
5_prime_UTR_variant	start_lost
coding_sequence_variant	stop_gained
downstream_gene_variant	stop_lost
frameshift_variant	stop_retained_variant
inframe_deletion	synonymous_variant
inframe_insertion	TF_binding_site_variant
intergenic_variant	transcript_ablation
intron_variant	upstream_gene_variant
missense_variant	feature_truncation
non_coding_transcript_exon_variant	non_coding_transcript_variant
protein_altering_variant	splice_region_variant
regulatory_region_variant	regulatory_region_ablation
splice_acceptor_variant	TFBS_ablation

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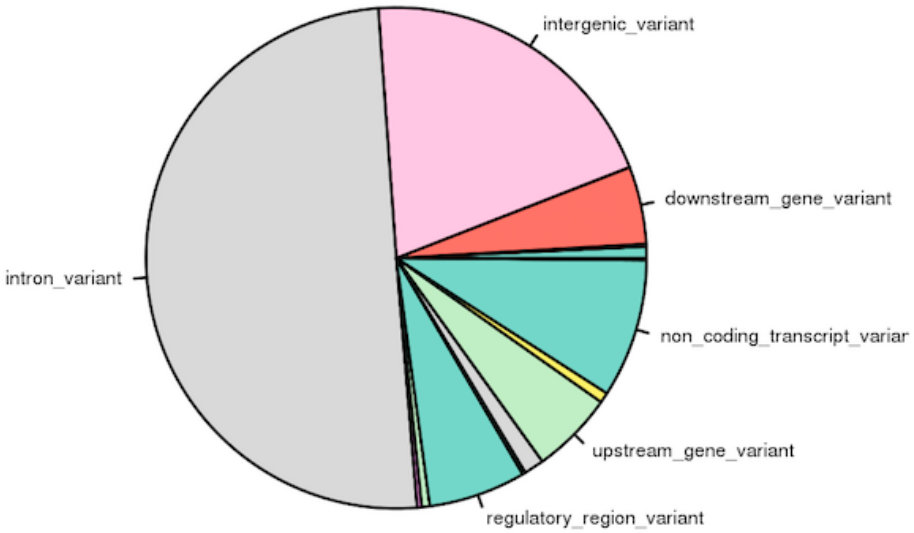
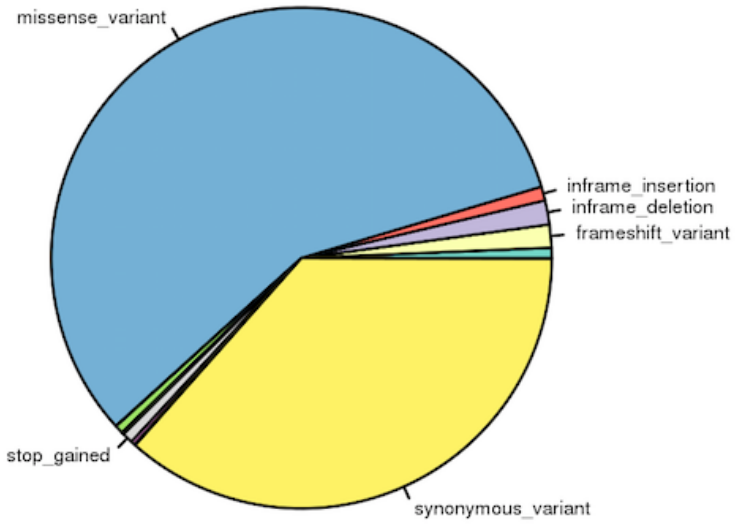
Results: variant consequences

Consequences (all)



Consequences (coding)



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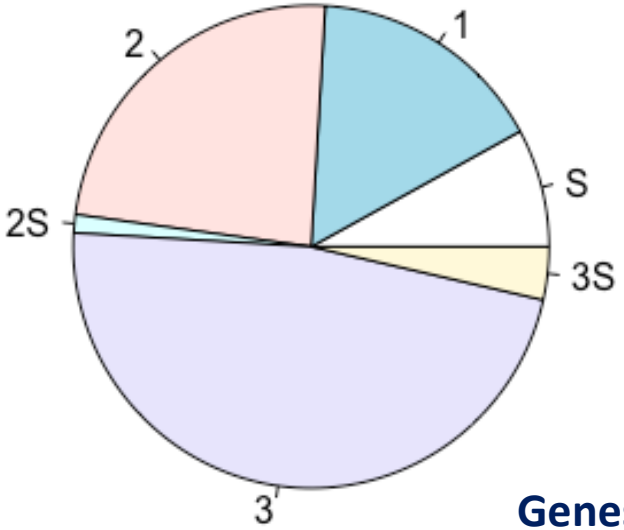
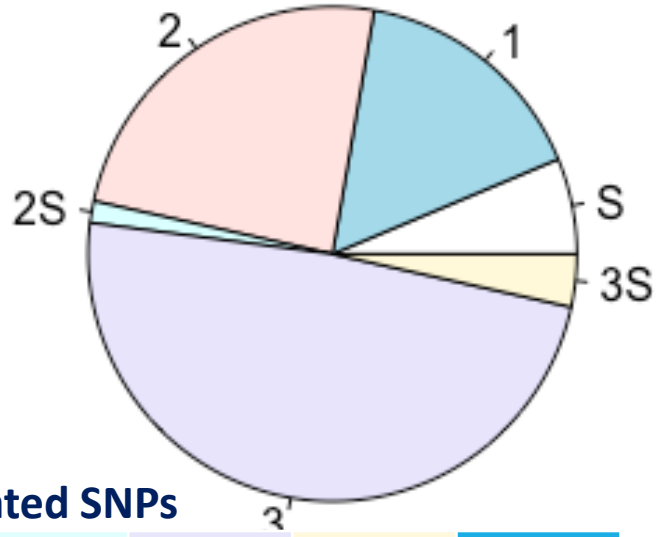
Results: genes containing SFARI annotated/not annotated SNPs distributed into SFARI categories

Genes containing SFARI annotated SNPs distributed into SFARI categories

Sfari modules	S	1	2	2S	3	3S	Tot
#genes	65	132	195	10	388	29	819





Genes containing SFARI not annotated SNPs

Sfari modules	S	1	2	2S	3	3S	Tot
#geni	31	79	120	7	240	17	494

SFARI database https://gene.sfari.org/

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Conclusions

- Linked-read sequencing technology performed at CNR-ITB has allowed to decipherer the genomics heterogeneity in ADS
- Homozygous variants common to 3 couples of affected siblings was identified.
- Further studies will be performed to identify haploblocks carrying the risk alleles and to validate the new ASD variations
- The new biomarkers will be used:
    - to stratify ASD patients population
    - to identify pathways and gene networks involved in the disorder
    - design target-driven personalized treatment

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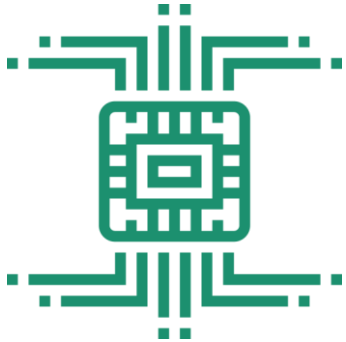
Perspectives

- Further sophisticated bioinformatics analyses of the results can predict new ASD associated genes

- Possible development and application of dedicated AI, performed at CNR-ITB,
can accelerate these analyses



- These linked-read WG-NGS based approaches can be applied to many other pediatric diseases with unknown genetic causes

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Acknowledgements

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Projects:

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