Mutational Spectrum and Cellular Heterogeneity of Autism Spectrum Disorders

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Disclosure

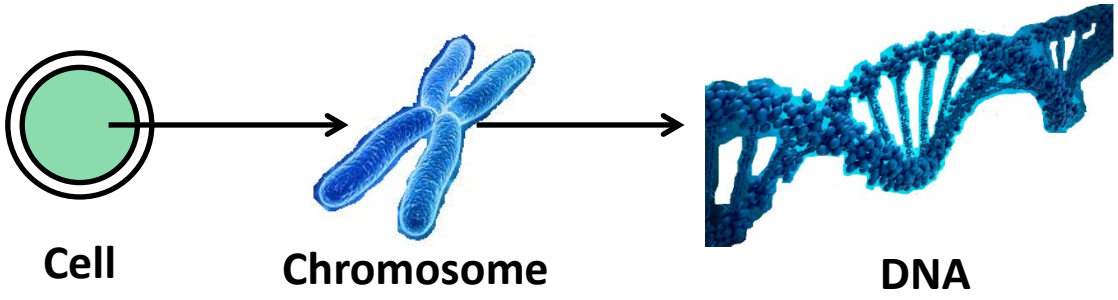
- Patent agreement with Lineagen, Salt Lake City, USA, a clinical diagnostic company.

- Chief Scientific Advisor, NeuroGen, a clinical diagnostic company.

Outline

- Background
- Genetics of Autism Spectrum Disorders
  - What type of genes impacted by mutations in autism genomes?
  - Do severity of the disease correlate with genetic mutations?
  - Do siblings have the identical genetic mutations?
  - Can cancer like somatic mutations predispose to autism?
- Genetic Diagnosis in Bangladesh
- Acknowledgements

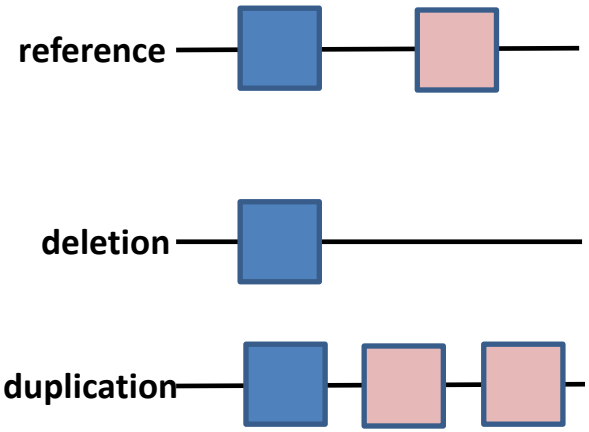
Background



Genetic Mutations:

Chromosomal Abnormalities

Copy Number Variation



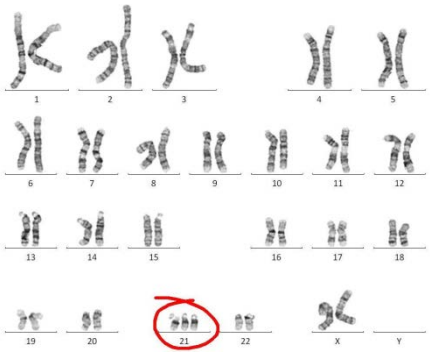
Single Nucleotide Variants (SNV)

ACGTCGCTC

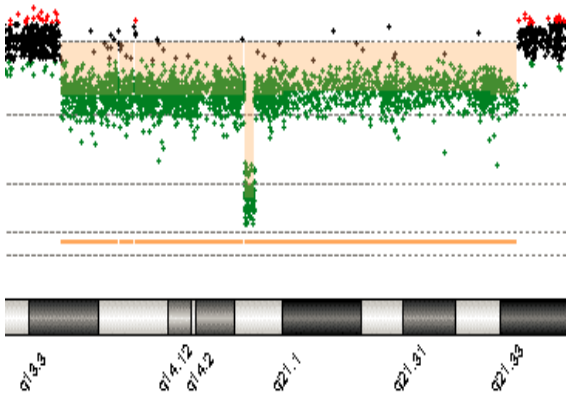
ACGTCACTC

Mutation and Disease

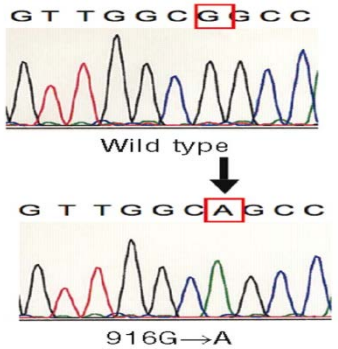
- There is strong evidence for the importance of complex genetic factors comprised of different forms of genetic variation (or architecture) in the etiology of numerous diseases.



Karyotype
>5Mb



Copy Number Variation (CNV)
>100bp - 5Mb



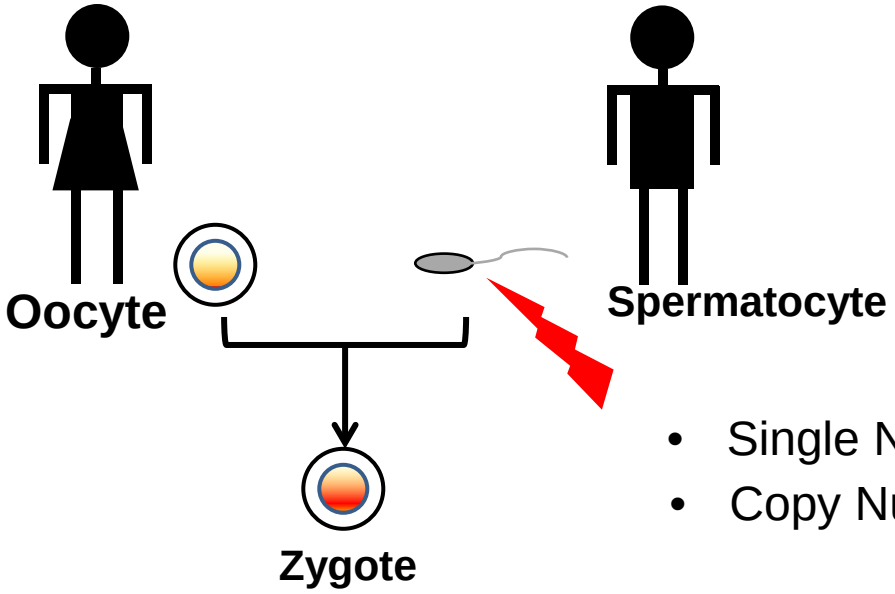
Single Nucleotide Variant (SNV) / Indel
>1 - 100bp

Clinical Microarray

Whole Genome Sequencing

de novo Mutation

- A type of mutation that arose in offspring and absent in parents.
- In each genome, there is a gene impacting *de novo* mutations that can be identified through genome sequencing.



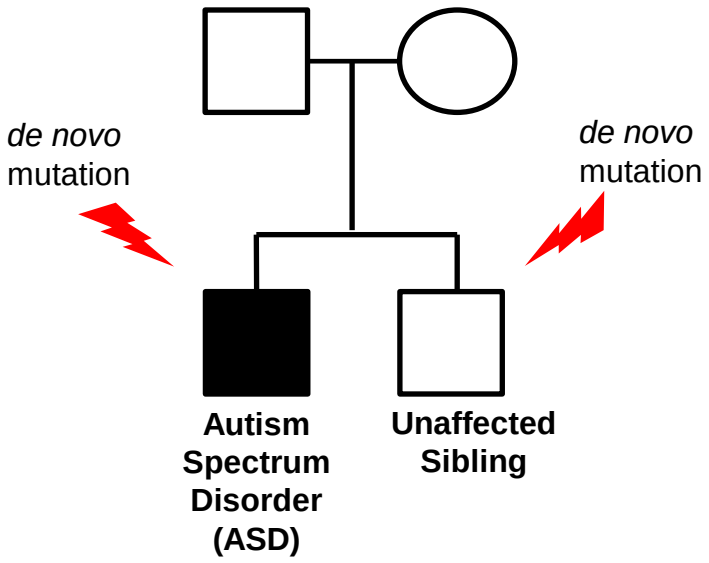
- Single Nucleotide Variant (SNV)
- Copy Number Variation (CNV)

What type of genes impacted by *de novo* mutations in autism genomes?

de novo Mutations in Autism Spectrum Disorders

- Characteristics of *de novo* mutations in autism affected and unaffected siblings:

256 families



- We have **sequenced 256 families with one affected case and one unaffected sibling**.

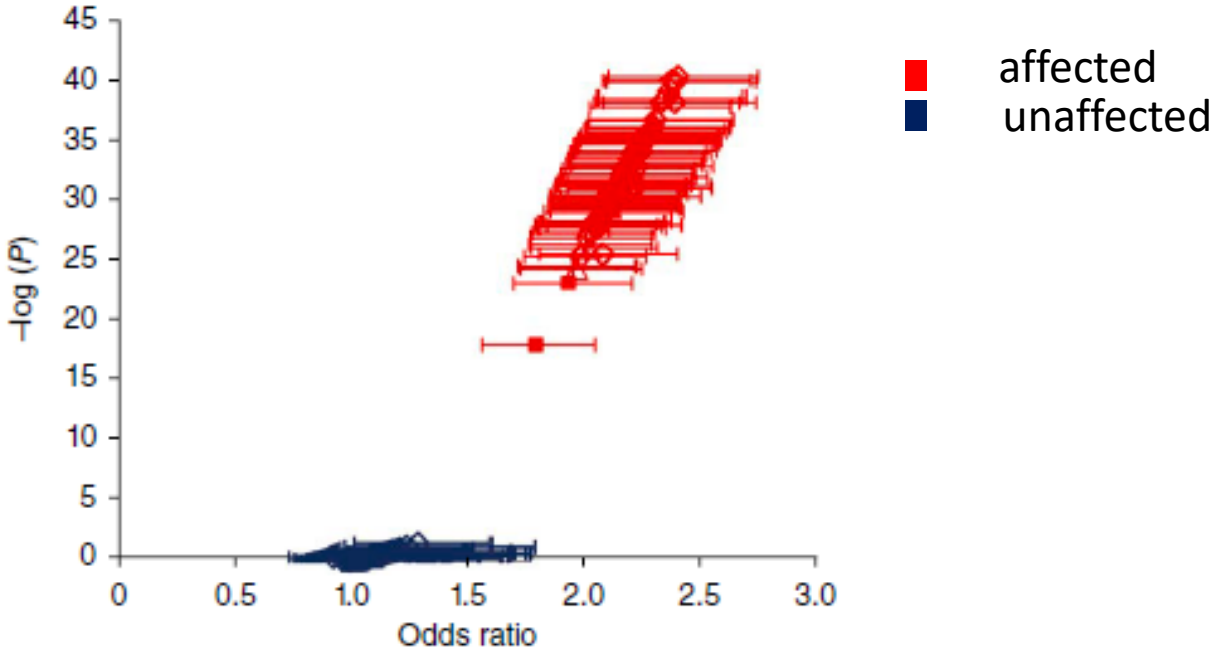
- For each autism case and unaffected member of the family, we have identified at least **one *de novo* mutation per case** that impacts a gene function.

Illumina X Ten

Uddin et al. Nature Genetics 2014

de novo Mutations in Autism Spectrum Disorders (Cont'd)

- *de novo* mutations (SNV and CNV) **significantly** impacts **"brain critical genes"** in autism genome compare to unaffected sibling.



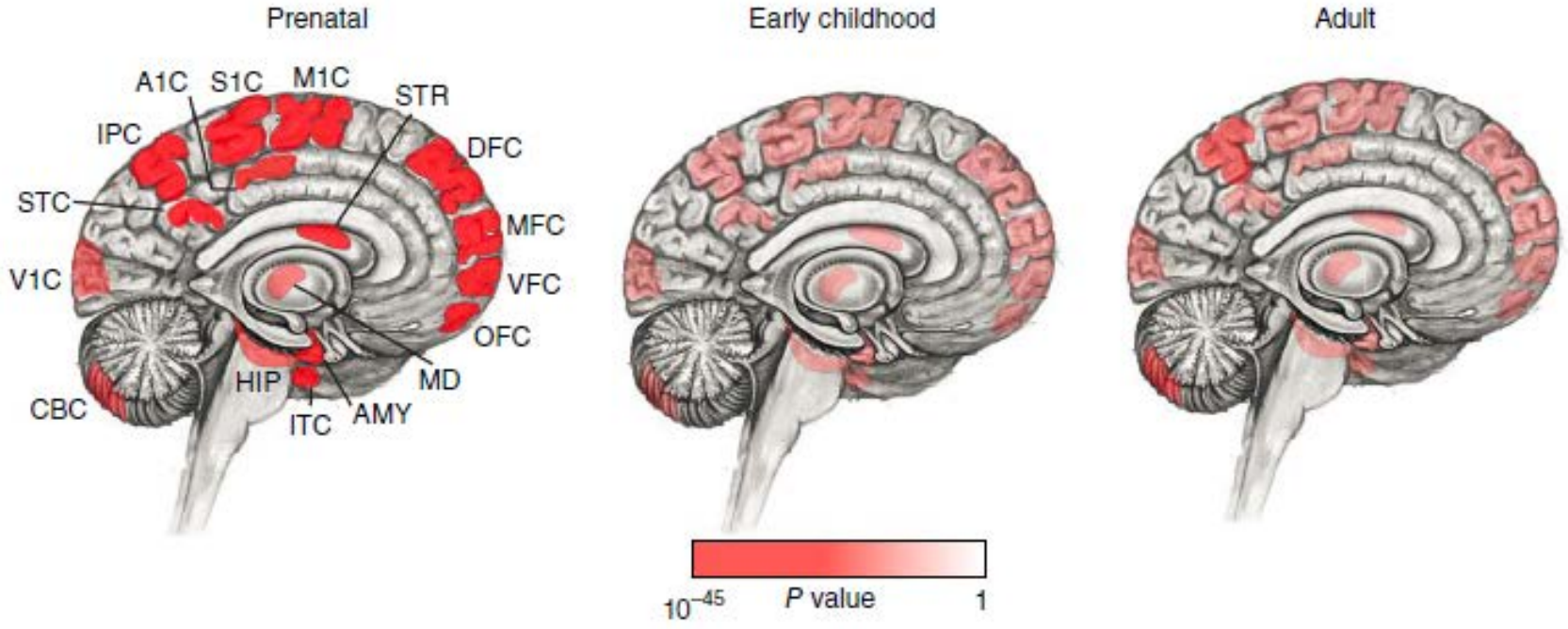
- *de novo* mutations impacts ~60% more critical genes in autism genome compare unaffected sibling genome.

Uddin et al. Nature Genetics 2014

de novo Impacts 'Critical-Genes' in Human Developmental Brain

- Using **'postmortem brain samples'** from prenatal to adult, we have mapped expression (gene activity) patterns of genes that were impacted by *de novo* mutations in autism spectrum disorder and unaffected siblings.

- **Genes that are critical for prenatal brain development and synaptogenesis were impacted in autism genome (by *de novo* mutations) compare to unaffected siblings.**



Summary

- Our results suggest that ‘critical genes’ that are active in early human brain development are highly enriched for *de novo* mutations in autism spectrum disorder genomes.

- Using postmortem brain, I have patended the method of characterizing genes that are critical for developing human brain.

nature genetics

Brain-expressed exons under purifying selection are enriched for *de novo* mutations in autism spectrum disorder

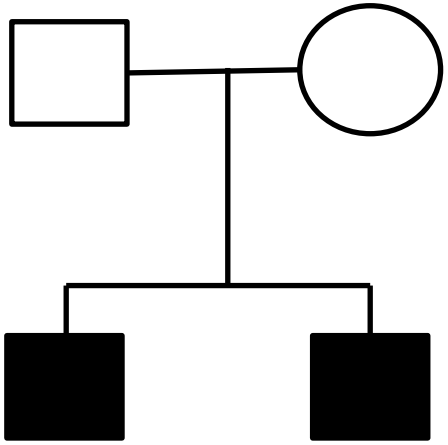
Mohammed Uddin[1], Kristiina Tammimies[1,2], Giovanna Pellecchia[1], Babak Alipanahi[3], Pingzhao Hu[1], Zhuozhi Wang[1], Dalila Pinto[4–6], Lynette Lau[1], Thomas Nalpathamkalam[1], Christian R Marshall[1,7], Benjamin J Blencowe[8,9], Brendan J Frey[3,8], Daniele Merico[1], Ryan K C Yuen[1] & Stephen W Scherer[1,7,9]

- **Quantifying Critical-exon method has been patented and licensed to a private company in USA.**

Do the affected siblings in a family share the same genetic cause?

Affected Sibling and Genetic Mutation

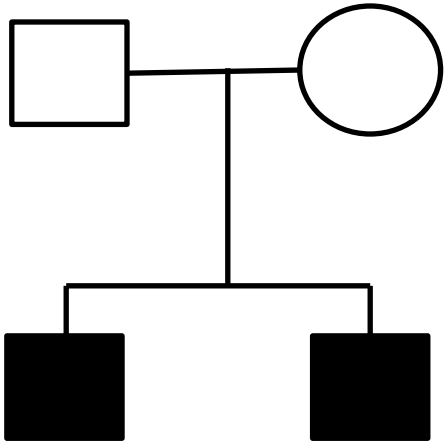
- We used whole-genome sequencing (WGS) of 85 quartet families (parents and two ASD-affected siblings), consisting of 170 individuals with ASD,



- For each affected siblings we have identified possible causal *de novo* mutations.

Affected Sibling and Genetic Mutation (Cont'd)

- **80% of affected siblings have a different genetic mutation that is causal for autism spectrum disorder.**



- 20% of affected siblings share a causal genetic mutation due to gonadal mosaicism. Even in the case of identical mutations, disease severity can be different between siblings.

Summary

- Genetic diagnosis is critical for both affected siblings.

- Since, shared mutation is less common, this has wide implication on

Do severity of the disease correlate with genetic mutations?

Autism Severity and Mutational Landscape

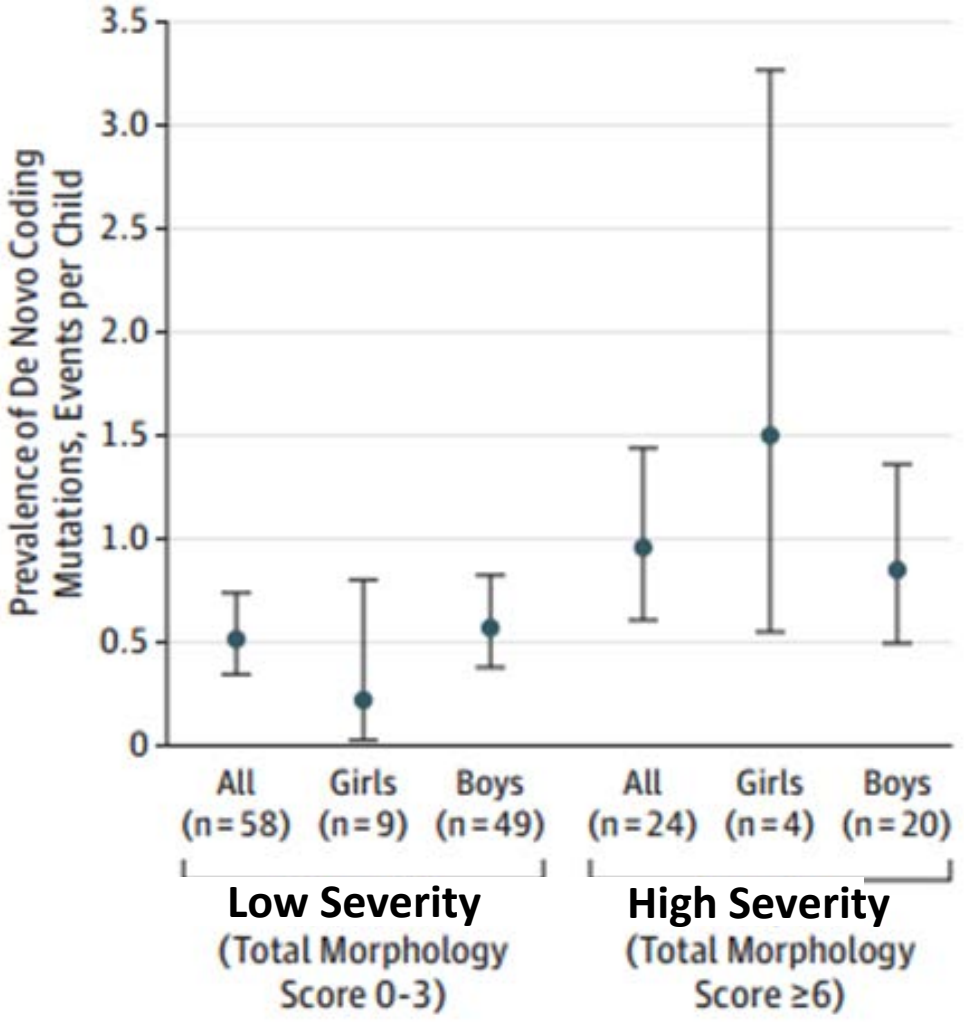
- We have designed a cohort of f 258 consecutively ascertained unrelated children with ASD. We have screened whole genome of each family members.

- As a measure of severity, each case underwent detailed assessments to define morphology scores based on the presence of major congenital abnormalities and minor physical anomalies.

- More score means more severe and vice-versa.

Autism Severity and Mutational Landscape (Cont'd)

- We have identified disease severity is positively correlated with genetic mutations.
- High severity means high number of penetrating genetic mutations.



Tamimmies et al. 2015 JAMA

Summary

- Genetic diagnostic is important for both low and high severity autism cases.

- High severity (with physical morphology) will have higher genetic mutational outcome.

Can cancer like somatic mutations predispose to autism?

Somatic Mosaic Mutation in Surgically Resected Epilepsy Brain

- A 6-year old proband (male) diagnosed with refractory focal-onset epilepsy with global developmental disorder in SickKids. MRI imaging showed evidence of focal cortical dysplasia type 1 in the left anterior temporal cortex.
- We have identified (targeted sequencing) **a *de novo* 4.9Kb heterozygous deletion** impacting exon 3-4 of the *STXBP1* gene from blood.
- **The patient underwent left anterior temporal resective surgery and achieved complete seizure remission.**

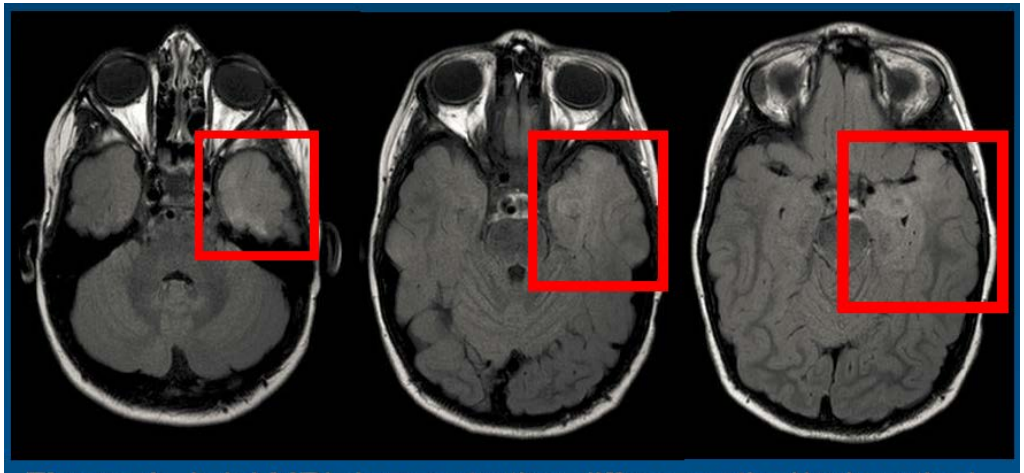
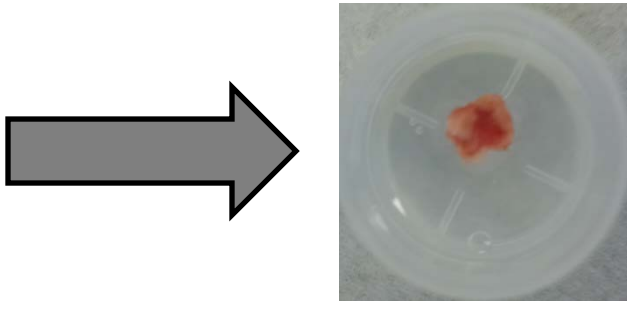


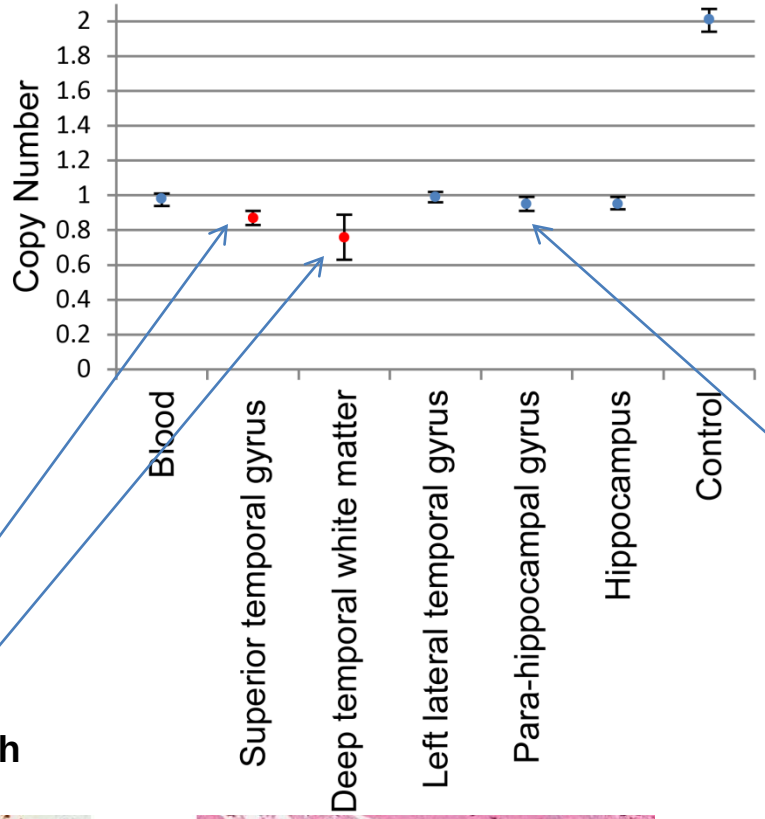
Figure 1: Axial MRI demonstrating diffuse cortical/subcortical high FLAIR signal (red boxes) confined to anterior left superior temporal gyrus and mesial temporal structures.

Resected Brain Tissue

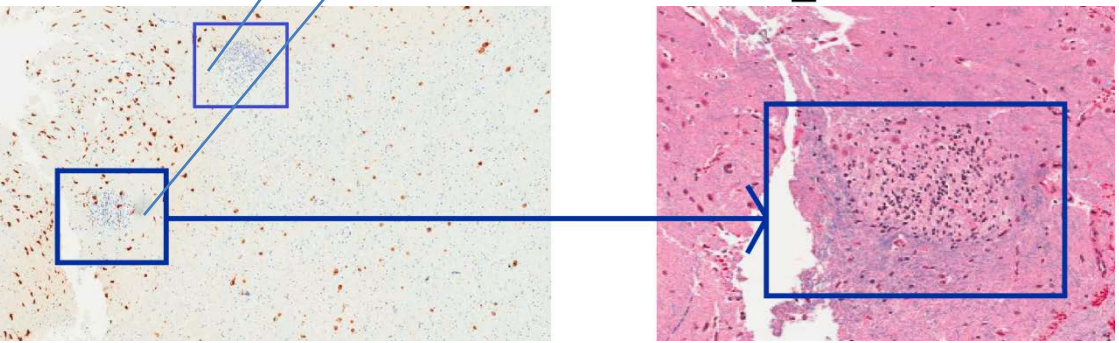


Uddin et al. Manuscript in Preparation.

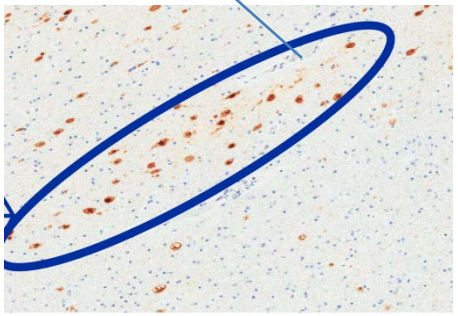
Somatic Mosaic Mutation in Surgically Resected Epilepsy Brain (Cont'd)



Dysmorphic Tissue with clusters of neurons.



Evenly distributed neurons.



Detection of Somatic Mosaic Mutations in Autism

- An active collaborative projects between Harvard and SickKids hospital.
- A total of ~6,000 trios were whole exome sequenced (18,000 samples) and anayzed in collaboration with The Autism Sequencing Consortium and the Simons Simplex Collection.
    - 4,035 families (mostly trios) with affected autism spectrum disorder probands and
    - 1,918 trios with unaffected siblings

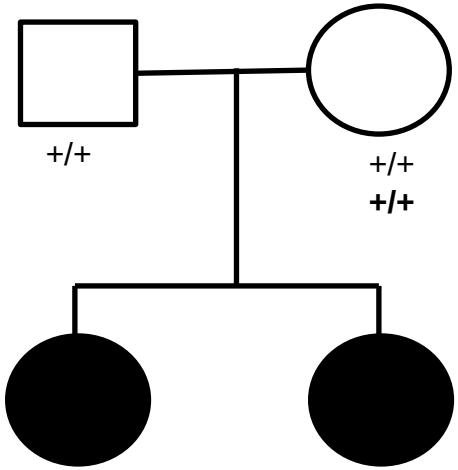
Elaine Lim, Mohammed Uddin et al. Nature Neuroscience (revising manuscript)

Detection of Somatic Mosaic Mutations in Autism (Cont'd)

- ~7% of cases harbor a pathogenic somatic mosaic mutation.

***PARP1* stopgain in 20% of the blood cells**

Mosaic *SCN2A* 1.7kb deletion



***SCN2A* 1.7kb deletion**

***SCN2A* 1.7kb deletion**

***PARP1 de novo* stopgain mutation**

***RELN* damaging *de novo* missense in 60% of blood cells**

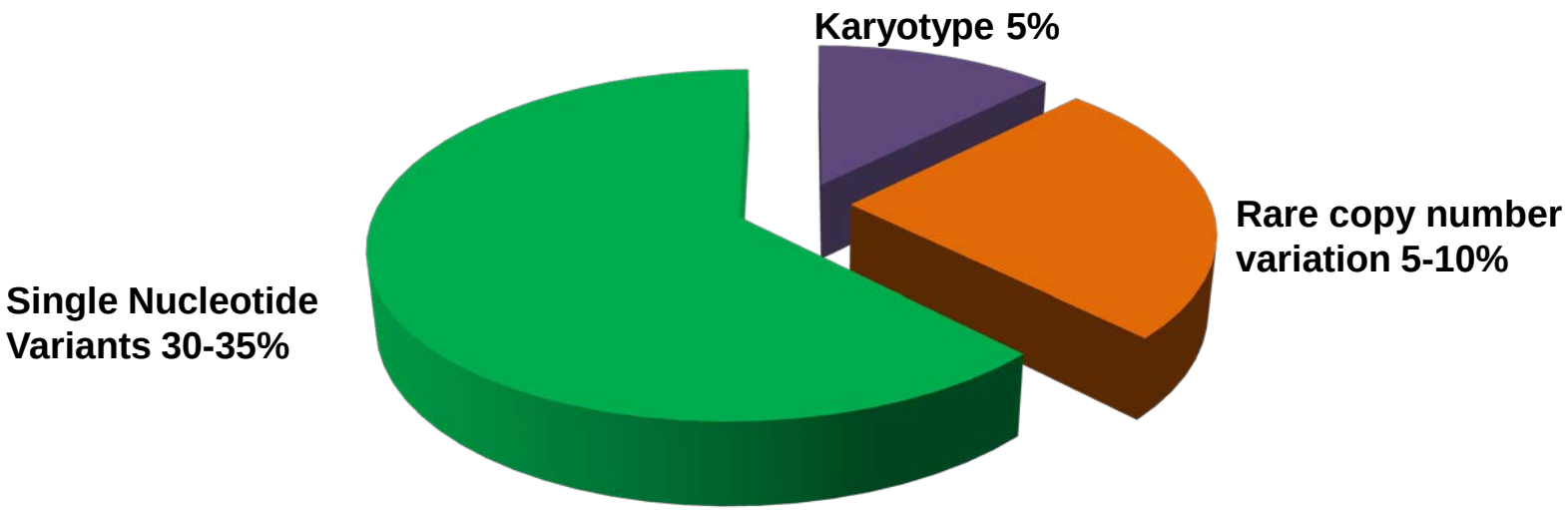
Summary

- Somatic mutations not only predispose to cancer, it can also impact neurodevelopment and manifest as a disease.

Clinical Diagnosis Genome Sequencing

Genetic Diagnosis of Autism Spectrum Disorder

- What is the genetic diagnosis yield in autism spectrum disorder?



- **Total diagnosis yield of autism spectrum disorder from whole genome sequencing is 40-50%.**

Uddin et al. Sce.Rep 2016

Bona fide Autism Genes and Locus

- *SCN2A*
- *POGZ*
- *CHD8*
- *ANK2*
- *UBE3A*
- *DMD*
- *KATNAL2*
- *SHANK1-3*
- *16p11.2 microdeletion*
- *15q13.3 microdeletion*

Uddin et al. Nature Gen. 2014

Genetic Test in Bangladesh

NeuroGen

....a road to early intervention

NeuroGen

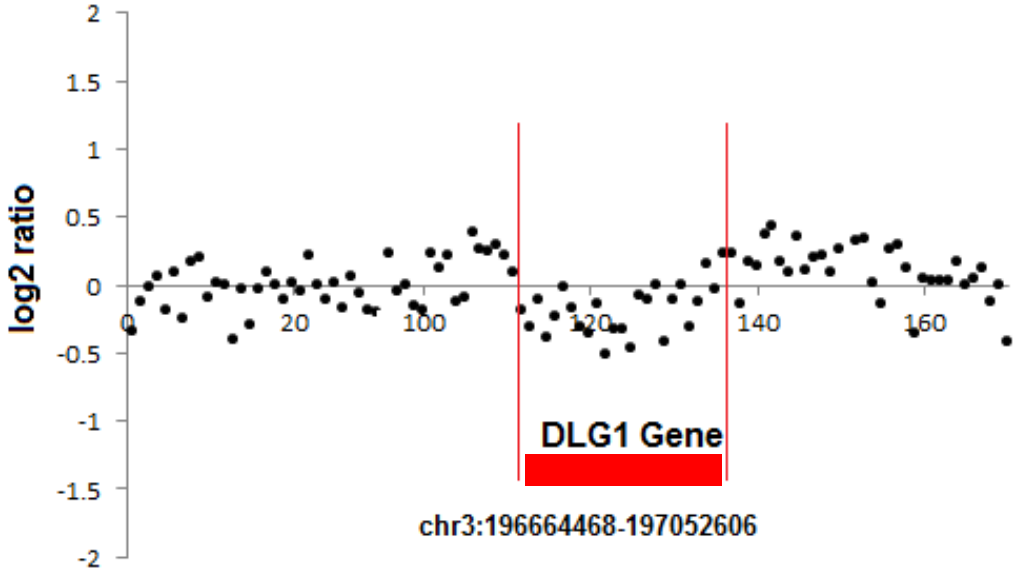
- **Neuropsychiatry Genetic Diagnosis:**
  - Autism Spectrum Disorder Genome Screen
  - Intellectual Disability and Global Developmental Delay Genome Screen

- **Cancer Genetic Diagnosis:**
  - Breast/Ovarian Cancer
  - Lung Cancer
  - Colorectal Cancer

- **Rare Disease Genetic Diagnosis:**
  - Cystic Fibrosis (CFTR Gene)
  - Types of Muscular Dystrophy
  - Cardio Vascular Diseases

NeuroGen

- *DLG1* deletion detection through genome screen at NeuroGen laboratory.



***DLG1* gene is primarily responsible for synaptogenesis.**

NeuroGen

Banting Postdoctoral Fellowships

SickKids® THE HOSPITAL FOR SICK CHILDREN

Acknowledgments

- Stephen W. Scherer
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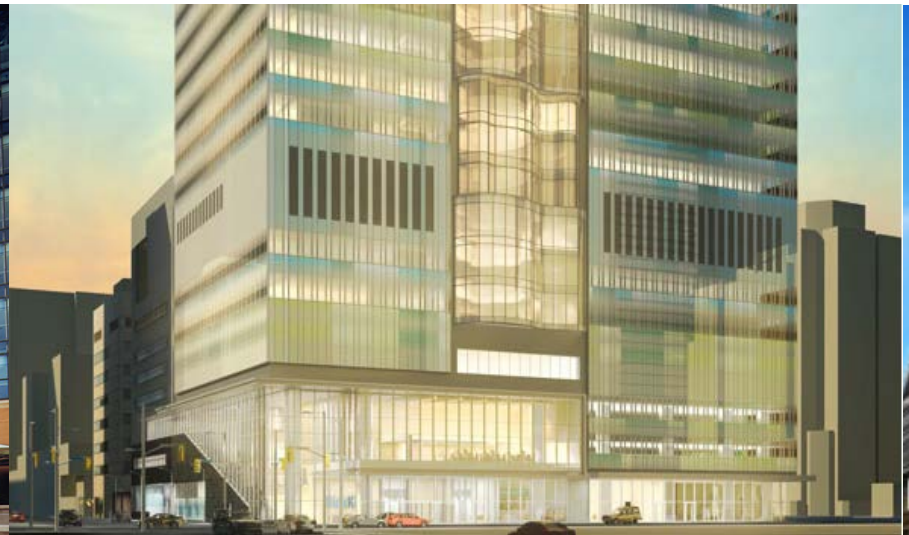
- Ada Chan
- Thomas Nalpathankalam

- Brain Span
- 1000 Genomes Project
- Human Protein Map