# Detection of Clinically Relevant Copy Number Variation of *SEZ6L2* Gene in a Bangladeshi Autism Spectrum Disorder Cohort

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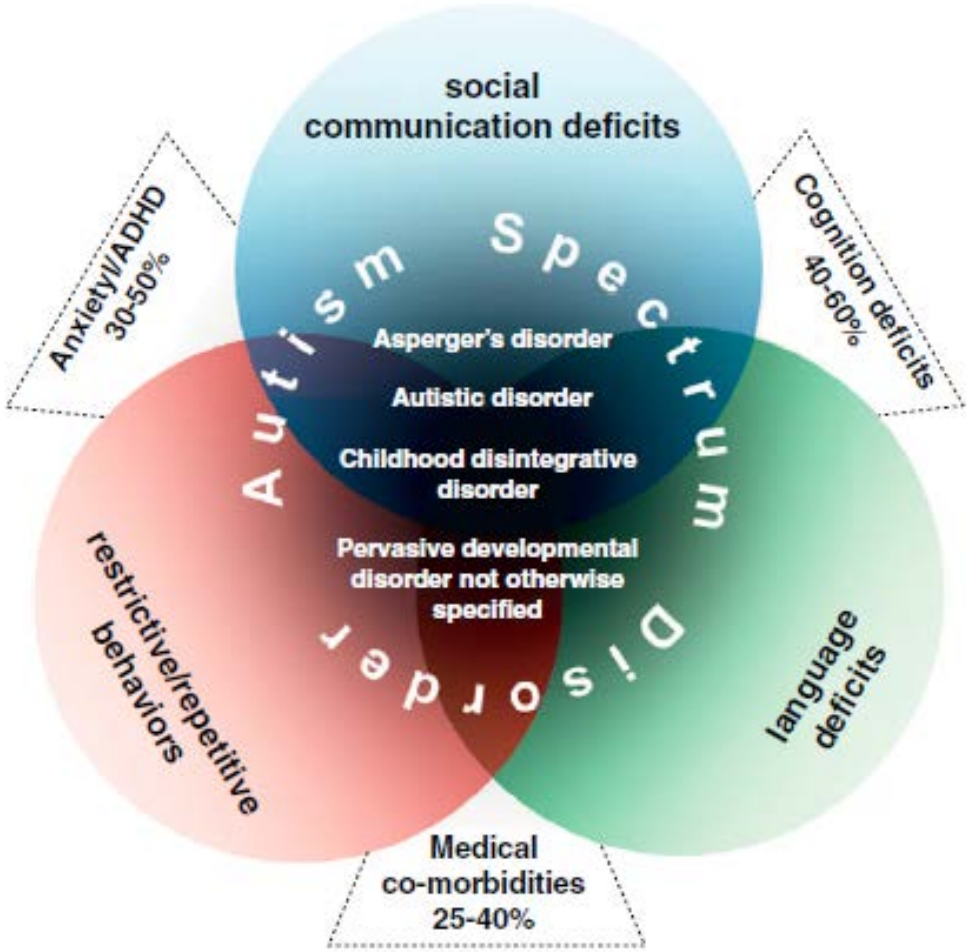
# Autism Spectrum Disorder (ASD)

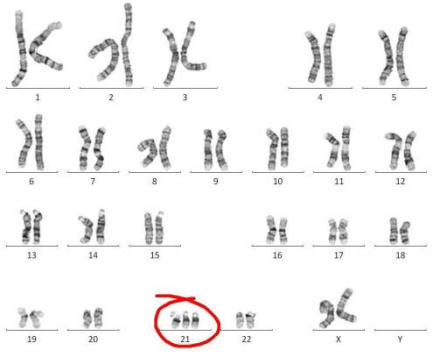
- ASD impacts 1 in every 68 children[2].
- ASD impacts 1 in every 42 boys and 1 in every 89 girls[2].
- There is strong evidence for the importance of complex genetic factors comprised of different forms of genetic variation (or architecture) in the etiology of ASD [1].
- The most convincing evidences are from rare/de novo mutations[1].



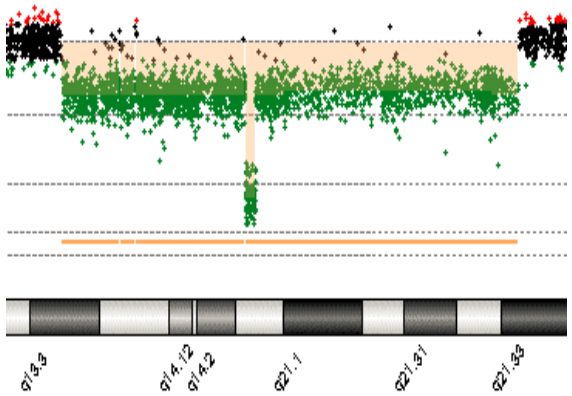

1. Devlin & Scherer, Gen. ad Dev. 2012.
2. CDC, March, 2014.
Devlin & Scherer, Gen. ad Dev. 2012.

# Genetics of Autism Spectrum Disorder

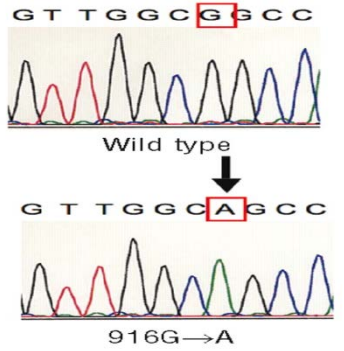
- There is strong evidence for the importance of complex genetic factors comprised of different forms of genetic variation (or architecture) in the etiology of autism spectrum disorder.



Karyotype
>5Mb



Copy Number Variation (CNV) 100bp - 5Mb

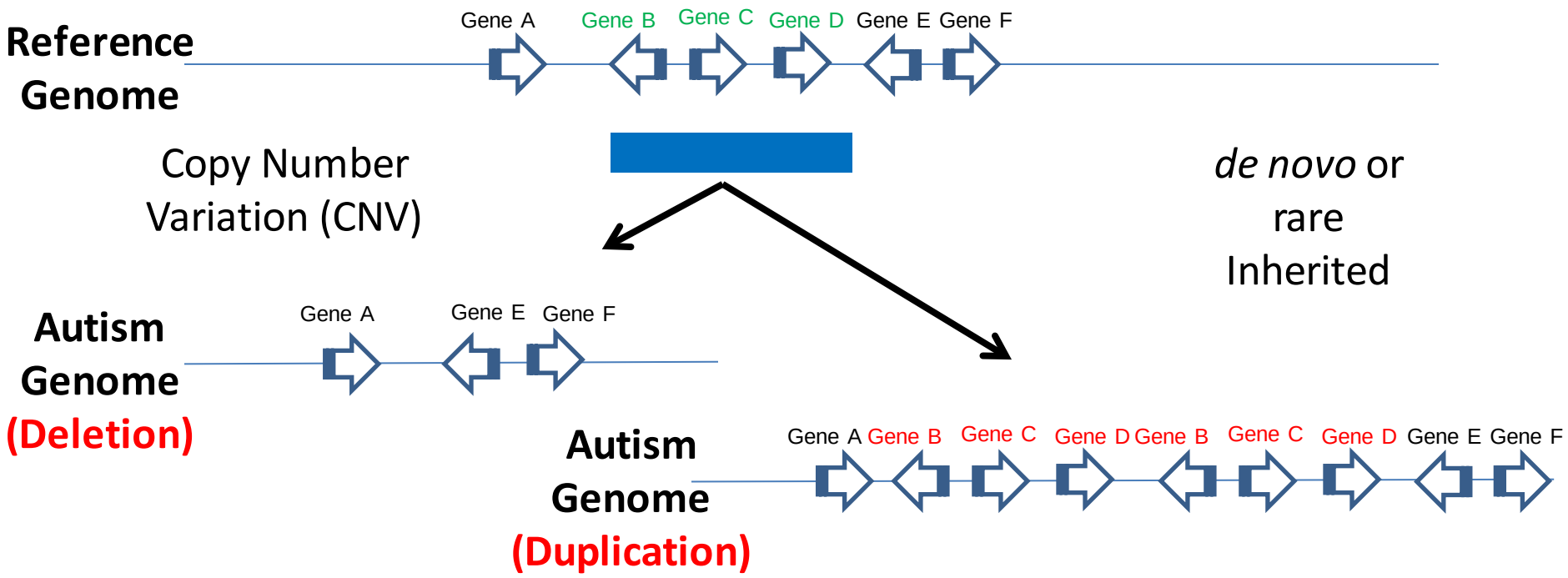



Single Nucleotide Variant (SNV) / Indel
1 - 100bp

**Clinical Microarray**

**Whole Genome Sequencing**

# Genetics of Autism Spectrum Disorder (Cont'd)




- **Copy Number Variation (CNV) in ASD:**
  - A higher burden of rare/*de novo* CNVs identified in ASD cases (7 to 10%) compare to controls[1].
  - These variants within ASD cases are typically large and comprised with multiple genes.

1. Pinto *et al.* AJHG, 2014

# Objective

- To find out the copy number variation (CNV) at chromosome 16p11.2 by targeting *SEZ6L2* (Seizure Related 6 Homolog-Like 2, **Breakpoint:** chr16:29606852-30199855 **Length:** 593 KB) gene among the Bangladeshi cohort with Autism Spectrum Disorder.

# Goals

1. To detect CNV in the candidate *SEZ6L2* gene and to quantify the association of *SEZ6L2* impacting CNVs with Bangladeshi ASD cases
2. To describe the phenotypic characterization of autistic individual
3. To describe the phenotypic characterization of CNV positive ASD patients in comparison to CNV negative patients.

# Methods

(inclusion criteria)

- Age: Between 3-19 years of age.
- Diagnosed case of ASD, based on DSM-IV criteria supported by Autism Diagnostic Observation Schedule (ADOS).
- IQ equivalent>35determined by the Wechsler Intelligence Scale for Children-Revised (WISC-R) which reduce the likelihood of including individuals with severe mental retardation only.

## **Methods** (exclusion criteria)

- Significant Hearing, vision problems.
- Significant motor problems.
- Significant birth complications such as Cerebral Palsy
- ASD related disorder by clinical examination Such as Tuberous Sclerosis, Rett Syndrome, Down syndrome and Fragile-X Syndrome.

## **Methods** (recruitment of cases)

- Data base of IPNA and ICMH
- Screening of the patients by Clinical Psychologist using the Autism Diagnostic Check List (ADCL)

*for screen positive patients:*

1. Autism Diagnostic Observation Schedule (ADOS; DSM-IV criteria) and Wechsler Intelligence Scale for Children-Revised (WISC-R; *for IQ*)
2. Neurodevelopment & physical assessment by neurologist
3. Speech and language by speech therapist

***SEZ6L2* Primer:**

Forward primer sequence is 5′-CCTCTCTCTTCCCCACAAAGG-3′;

Reverse primer sequence is 5′ TGGACAGCCTGGTTCTCTCT-3′;

Primer3 software v. 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/)

Amplicon size- 67 bp

BLAST tool- *SEZ6L2 specificity*

# Results

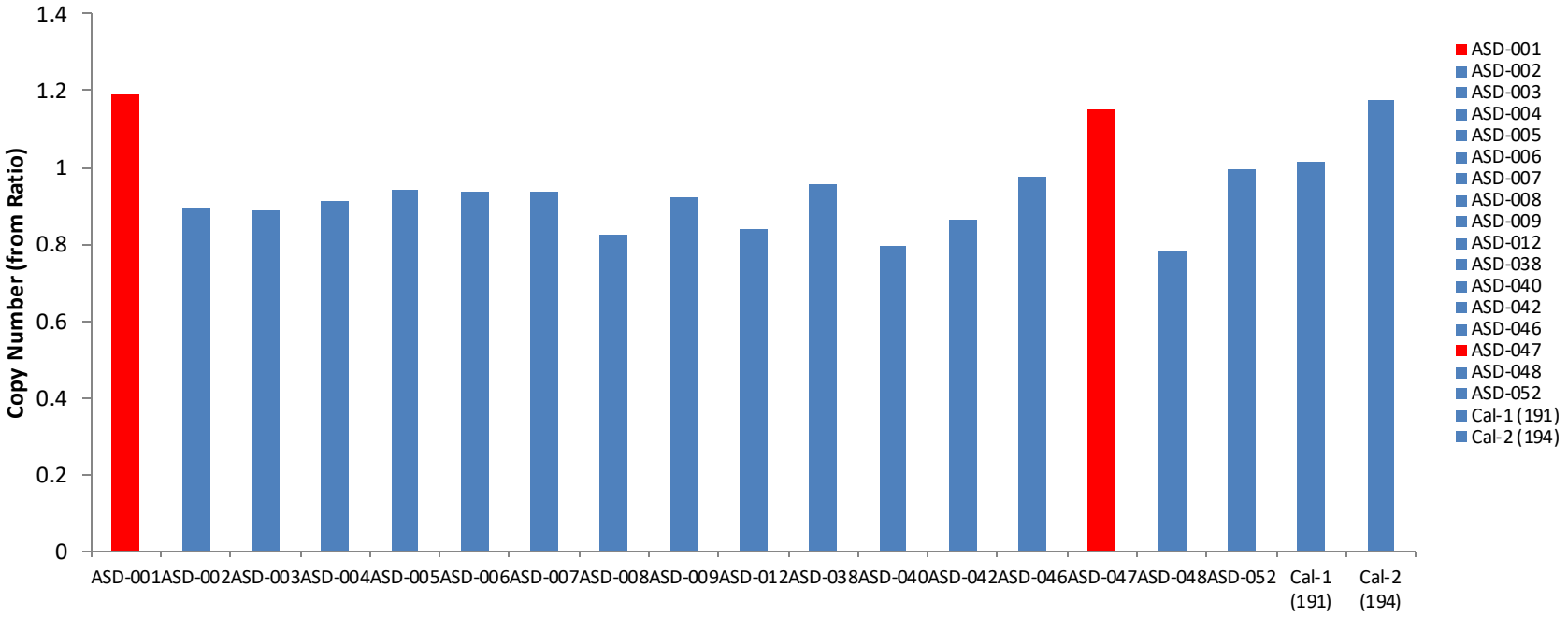



**Figure 9:** Showed the Copy Number Variation on sample ID (ASD-001 and ASD-047)

Table: Base line clinical characteristics of ASD

| Characteristics | N (%) | ASD with CNV; n (%) | ASD without CNV; n (%) | P value |
|---|---|---|---|---|
| **Mean age (SD) in years** | - | 11.7 (0.9) | 8.8 (4.5) | Ns |
| **Gender** | | | | |
| **Male** | 20 (80) | 2 (100) | 18 (78) | Ns |
| **Female** | 5 (20) | 0 (0) | 5 (22) | |
| **Autistic sibling** | 3 (12) | 0 (0) | 3 (14) | - |
| **Consanguinity present** | 2 (8) | 0 (0) | 2 (9) | Ns |
| **Peri-natal asphyxia** | 4 (16) | 0 (0) | 4 (17) | Ns |
| **Postnatal infection** | 6 (24) | 2 (100) | 4 (17) | **0.05** |
| **Development milestones delayed** | 16 (64) | 1 (50) | 4 (29) | Ns |
| **Speech language** | 12 (48) | 2 (100) | 10 (44) | Ns |
| **Language regression** | 6 (24) | 1 (50) | 5 (22) | Ns |
| **Epileptic seizure** | 2 (8) | 2 (100) | 0 (0) | **0.003** |
| **Macrocephaly** | 4 (16) | 0 (0) | 4 (17) | - |

# Phenotypic characterization (Patient A)

- Eleven years old boy from a non-consanguineous family
- epileptic seizure from seven months of age which was stopped at the age of 8 year.
- Partial delay in developmental milestones-delay in walking (at 2yr) and speech delay.
- Height: 155 cm ($75^{th}$percentile) and Overweight 62 kg ($95^{th}$ percentile, BMI=25.9)
- Occiputo frontal circumference (OFC) is 52 cm and overweight
- Motor movements and sensory is normal
- During psychological assessment at three year of age, cognition (or mental age) corresponded to the age of 24 months old.

# Phenotypic characterization (Patient B)

- Twelve years old boy from a non-consanguineous family
- Epileptic seizure at the age of 9 years
- Speech delay which regressed at some extent of his age.
- Underweight and weight: 36 kg (between $10^{th}$ to $25^{th}$percentile)
- Height of 154 cm ($75^{th}$percentile) and OFC is 53 cm
- Motor movements and sensory is normal
- During psychological assessment at 3 year of age, cognition (or mental age) corresponded to the age of 15 month.

# CONCLUSION

- Prevalence of CNVs might be more frequent among the Bangladeshi cohort (8.3%).
- Epilepsy and post-natal infection might be more common among autism patients with CNV.

# LIMITATION

- Small size of the sample
- With only two cases harboring pathogenic copy number variants, it is difficult to conclude anything about phenotype genotype correlations with any mathematical certainty
- Another very important limitation comes from the uncertainty when it comes to interpreting the clinical significance of any copy number variant.
- Variants reported in the Database of Genomic Variants (DGV) were not followed up in this study, but some of them might be linked to autism susceptibility in the future, and such CNV genome wide association studies will be needed to understand the role of so-called benign CNVs in disease

# RECOMMENDATION

- These CNVs should be preferentially screened in ASD affected individuals in particular for those having history of epilepsy and post-natal infection.
- Microarray analysis performed on these patients with 16p11.2 microdeletion/microduplication is recommended which could increase diagnostic yield.
- Should include investigations at the RNA and protein expression levels, the creation of mouse models, and bioinformatics analyses of pathways involved, which could lead to the identification of other candidate genes that could be involved in ASD pathogenesis.
- Furthermore, each pathogenic CNV should be investigated for its possible

# Acknowledgements

Dr. Nasima Sultana
Prof. & Head, Department of Biochemistry, Dhaka Medical College

Dr. Md. Rubel Amin
Assoc Prof. Department of Medicine
Dhaka Medical College & Hospital

Dr. Md. Mizanur Rahman
Prof. of Institute of Paediatric Neurodisorder & Autism (IPNA), BSMMU

Dr. Muhammad Abdul Aleem
Research Associate icddr'b

# Acknowledgements

Dr. Wahida Khanam
Prof. of Institute of Child and Mother Health (ICMH), Matuail

Dr. Laila Anjuman Banu
Prof. & Chairman, Department of Anatomy, BSMMU

Dr. Shaheen Akhter
Prof. of Institute of Paediatric Neurodisorder & Autism (IPNA), BSMMU

Narsis Rahman
Clinical Psychologist, Institute of Child and Mother Health (ICMH), Matuail

# Acknowledgements

Dr. Kazi Ashraful Islam

Asst. Prof. of Institute of Paediatric Neurodisorder & Autism (IPNA)

Dr. Md. Abdul Baqui

Asst. Prof. Biochemistry, Holy family Red Crescent Medical College

Dr. Mohammad Uddin

Hospital for Sick Children (SickKids), Toronto University, Canada.

# Acknowledgements

NeuroGen Technology

Panthopath, Dhaka

*Thank you*