Characterization of copy number variations of genomic regions containing long noncoding RNA in children with neurological phenotypes

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INTRODUCTION

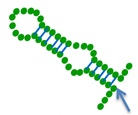
Children with neurological disorders comprise 5-10% of the general pediatric population and represent the largest group referred for genetic evaluation. Microarray analysis provides a diagnostic yield of ~10-15% for patients with such conditions. The diagnostic yield from whole exome and genome sequencing is ~25-34%.

There remain a significant proportion of affected children who are undiagnosed. Their phenotypic features are not specific and the majority do not have clinically identifiable variants or copy number variations (CNVs) of known clinically relevant genes, thus a definitive genetic diagnosis remains elusive.

In recent years comprehensive analysis using RNA deep sequencing showed that a large number of long non-coding RNAs (lncRNAs) display neuronal-specific expression, suggesting a role in neurological disorders[1-5]. Several groups reported aberrant expression of lncRNAs in patients with autism spectrum disorders[4,5]. Despite this explosion of data, little is known about how many different types of lncRNAs exist, how lncRNAs function and their neurobiological significance.

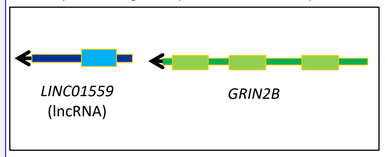
In this study we screened for CNVs containing lncRNAs in children with neurological disorders referred for clinical microarray analysis in order to identify new targets for further research.

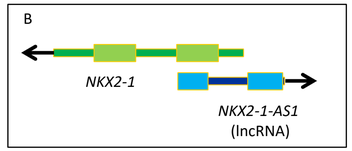
Biology of long non-coding RNAs (lncRNAs)[6,7]
- ≥200 nucleotides in length; possess functional domains
- Non-coding = does not encode protein
- Transcribed by RNA polymerase II
- Contain canonical splice sites (GU/AG)
- Like mRNA have introns, exons and are similar in size
- Lack Open Reading Frames



Functional domain
Long non-coding RNA secondary structure

Select positioning examples of lncRNA compared to nearby coding genes[7]



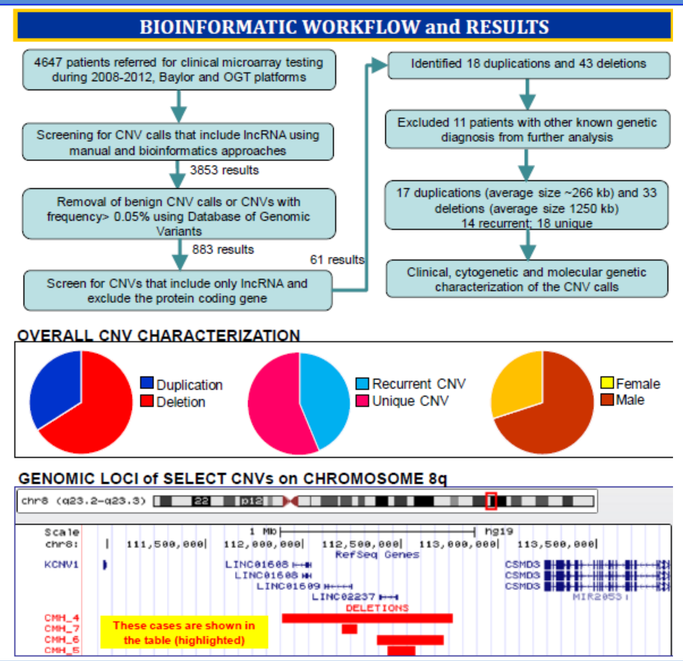


A. lncRNAs may reside adjacent to genes. These lncRNAs are thought to be transcribed along with the adjacent coding gene, oftentimes sharing that gene's promoter. **B**. An anti-sense position, with respect to a nearby coding gene, is also a common position for lncRNAs throughout the human genome.

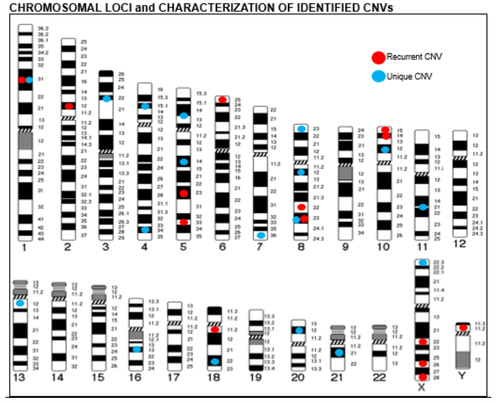
Select known uses of lncRNA functional domains

Functions of select well-known lncRNAs:
- *XIST*: inactive X-chromosome silencing in females[7] - *HAR1F*: Primate forebrain development[8]
- *BDNF-AS1*:inhibition of BDNF expression, repress neuronal outgrowth and differentiation[1]
- *17A*: Impairs GABAβ signaling by reducing GABAβ receptor transcription[2]

Methods



Results



Results

Select Cases with CNVs Involving lncRNA

Age and sex	Clinical features	CNV	lncRNA	Cytoband	Other negative genetic test results
5 yo; F	Dev. delay, hypotonic; mild cognitive deficits; microcephaly; seizure; Abnormal EEG	Deletion	*LINC01788*	Chr 1p31.1	Angelman/Prader Willi Syndrome; *MECP2*; N-glycan and serum carbohydrate transferrin
3 yo; M	Developmental Delay	Deletion	*LOC105374820*	Chr 2p12	Fragile X
2.5 yo; F	Non-progressive; long-standing neurodevelopmental disorder	Deletion	*LOC101928135*	Chr 3p22.3	46,XY; 22q11.2 FISH
3.8 yo; F	Developmental/speech delay	Deletion	*LOC102723828, LOC105377651*	Chr 4p15.1	None
3 yo; M	Autistic features/global dev. delay	Duplication	*LINC01098, LINC01099*	Chr 4q34.3	46,XY; Fragile X
5 yo; M	Developmental delay	Deletion	*LOC101929645, LINC02109, LINC02064*	Chr 5p14.1p13.3	Fragile X; 46,XY,ins(18;2)(q21.33;p21p23).ish ins(18;2)(WCP2+)
6 yo; M	Hypotonia; seizure; gliosis; dev. delay; balance/communication deficits	Deletion	*MEF2C-AS1*	Chr 5q14.3	46,XY;Angelman/Prader Willi Syndrome; *COX* mutation analysis
15 yo; F	Dev. delay; left temporal focal epilepsy	Deletion	*LINC01470*	Chr 5q33.2	46,XY
7 mo; M	Failure to thrive; R/O Russell Silver Synd.	Duplication	*LINC02214*	Chr 5q23.1	Methylation (RSS)
2 yo; M	Choanal atresia; speech delay; facial nerve palsy	Duplication	*LOC285768*	Chr 6p25.3	*CHD7* mutation analysis
9 yo; M	Dev. Delay; ADHD; polymicrogyria; R-side hemiplagia	Duplication	*LINC01287*	Chr 7q36.2	None
4 yo; M	Developmental disorder	Deletion	*LINC01608, LINC01609, LINC02237*	Chr 8q23.2q23.3	Fragile X
4 yo; M	Autism; dev. delay	Deletion	*LINC02237*	Chr 8q23.2	None
11 yo; M	Autism; dev. delay	Deletion	*LINC02237*	Chr 8q23.2	Fragile X; 46,XY
8 yo; M	Autism; dev. delay	Deletion	*LINC01609*	Chr 8q23.2	Fragile X; 46,XY
6 yo; M	Dev. delay; seizures; delivered at 25 week gestation	Deletion	*LOC731789*	Chr 10p12.1	46,XY
7 yo; F	Dev. delay; Autism; aggressive and impulsive behavior; epilepsy; obesity	Deletion	*LOC105755953*	Chr 10p14	Angelman/Prader Willi Syndrome
3 yo; F	Dev. delay; failure to thrive; short stature	Deletion	*JRKL-AS1, LOC105369443*	Chr 11q21	Fragile X
4 yo; F	Cognitive delay	Deletion	*LINC00442*	Chr 13q12.11	None
2 mo; F	Toe polydactyly and syndactyly; hyperteloric; frontal bossing; macrocephalic	Duplication	*LINC00922, LINC02126*	Chr 16q21	46,XX
9 yo; M	Single palmer crease; hypospadias; skeletal anomalies; macrocephalic; frontal bossing	Deletion	*LOC101927571*	Chr 18q11.2	None
19 yo; M	Seizures; microcephalic; cognitive impairment	Deletion	*LOC101929413, C20orf187, LINC01752*	Chr 20p12.2	Fragile X; X-linked disability panel (92 genes)
18 yo; M	Seizure; autism; Von Willebrand disease; dev. delay	Deletion	*LINC01687, LINC00308*	Chr 21q21.1	None
13 yo; F	Developmental delay	Duplication	*FIRRE*	Chr Xq26.2	Fragile X
8 yo; M	Dev. Delay; seizure; hearing loss	Duplication	*RBMY3AP, TTTY8B, TTTY7/B, TTTY1B*	Chr Yp11.2	Fragile X; *GJB2* mutation analysis

Conclusion

- 96% of patients with a lncRNA CNV presented with a range of neurological disorders including autism, epilepsy, and developmental delay, compared to 58% of all patients evaluated
- 16 CNVs containing lncRNAs have not been reported as benign in the Database of Genomic Variants (DGV)
- Another 18 of the identified CNVs were reported in DGV at a frequency of ≤ 0.06%
- The highest frequency of CNV occurrence was observed on chromosomes 5, 8, 21, and X
- The lncRNAs described herein may contribute to human neuronal development

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