Identification of a genomic homozygous deletion of ZNF277 in a child with SLI


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Introduction

Specific language impairment (SLI) is a common neurodevelopmental disorder affecting 3-7% of English-speaking preschool children. Mixed SLI affects both expression and comprehension of language in the absence of explanatory environmental, intellectual or medical conditions such as hearing loss. During a CNV scan using a multi-algorithm approach, we identified a homozygous deletion of 21.379 bp in the ZNF277 gene, overlapping exon 5, in an individual with severe mixed SLI. The ZNF277 gene maps to a region on chromosome 7q31.1, which has repeatedly shown linkage to autism (AUTS1).

Given the phenotypic overlaps between SLI and autistic spectrum disorders (ASDs) and the genomic position of this deletion, we investigated the frequency of ZNF277 microdeletions in cohorts of individuals with SLI or ASD and their effects upon gene expression levels in the AUTS1 region.

Materials and Method

Identification and Validation in the Discovery Pedigree.

During a genome-wide CNV screen, performed using SNP data from the Illumina Human OmniExpress beadchip and the algorithms Nexus4, PennCNV and QuantiSNP5, a homozygous microdeletion of exon 5 of ZNF277 was predicted to be present in a child with SLI.

Quantitative PCR (qPCR) and Sanger sequencing were performed to validate the presence of the microdeletion and to examine cosegregation in the discovery pedigree.

Screening of SLI and ASD Cohorts.

Primers spanning the ZNF277 deletion breakpoints were used for a PCR-based screening of 3 separate cohorts.

- The SLI cohort: 1234 individuals from 322 families (545 parents, 318 SLI probands, 371 sibs), part of the SLI Consortium cohort.
- The ASD cohort: 1021 individuals from 252 families (454 parents, 412 affected children, 155 sibs), part of the International Molecular Genetic Study of Autism Consortium (IMGSAC) cohort.
- The control cohort: 224 ECAC non-related UK Caucasian blood donors.

Gene Expression Evaluation

The expression levels of ZNF277, DOCK4 and IMPM2L were evaluated by qPCR in DNA derived from:
- lymphoblastoid cell lines (LCLs) from ten non-independent individuals from IMGSAC families, five of whom carried a heterozygous ZNF277 microdeletion;
- blood from two individuals from a Dutch multiplex ASD family with a DOCK4/IMPM2L deletion, previously described by Pagnoto et al. (2010).

Results: validation in the discovery pedigree

All three children presented a similar pattern of language deficit.

- The proband (4) had a clinical diagnosis of mixed SLI.
- The younger sister (5) also had a diagnosis of SLI, although she was less severely affected than the proband.
- The older brother (3) reported an early language delay, but did not have a formal diagnosis of SLI.
- Both parents reported a family history of speech or language problems.

The microdeletion was confirmed in the parents and the proband with two independent methods:

- (A) qPCR and (B) Sanger sequencing.
- The two siblings were confirmed to have two normal copies of this genomic region.
- The genomic position of the deletion is indicated by a red rectangle.

B) The size of the ZNF277 deletion was determined by Sanger sequencing using primers flanking the predicted deletion boundaries.

The absence of exon 5 causes a frameshift in the ORF of the gene, introducing a premature stop codon in exon 7.

Results: Screening of SLI and ASD Cohorts

We screened cohorts of children with SLI or ASD and control subjects for the presence of deletions involving exon 5 of ZNF277.

<table>
<thead>
<tr>
<th></th>
<th>Related individuals</th>
<th>ZNF277 deletions</th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLI families</td>
<td>1234*</td>
<td>19</td>
<td>1.5%</td>
</tr>
<tr>
<td>ASD families</td>
<td>1021</td>
<td>7</td>
<td>0.7%</td>
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*including the discovery pedigree

With the exception of the discovery pedigree, all the deletions were identified in the heterozygous form.

In the families where DNA of both parents was available, we observed none that of ZNF277 microdeletions were de novo.

The co-segregation with SLI phenotype was incomplete.

Results: Gene Expression Evaluation

ZNF277 flanks the DOCK4 and IMPM2L genes, which have been previously implicated in ASD and dyslexia.

A) We performed qPCR analyses of the expression of IMPM2L, DOCK4 and ZNF277 in LCLs from families carrying a heterozygous ZNF277 microdeletion (indicated in red).

B) Blood transcript levels of ZNF277 were also analysed in the parents of a family,1 where the reading-impaired mother (indicated in green) carried a heterozygous deletion that removes exons 27-52 of DOCK4 and the first three exons of IMPM2L. The father instead (indicated in purple) had two normal copies of these genes.

In the graph on the left, normalised ratios are calculated as an average of 5 samples.

We found that, while ZNF277 microdeletions affect the expression of ZNF277, they do not alter the levels of DOCK4 or IMPM2L transcripts. Similarly, a deletion in the 3’ end of DOCK4 does not affect the expression level of ZNF277.

Conclusion

We identified a homozygous deletion of exon 5 in ZNF277, predicted to result in a complete lack of functional protein. The same CNV in the heterozygous form causes a decrease in the expression of ZNF277 and shows an increased frequency in individuals affected by SLI compared to controls and individuals with ASD, suggesting that the disruption of ZNF277 may play a role in SLI susceptibility that is distinct from the autism risk loci described in the AUTS1 region.

The function of the human gene ZNF277 has not yet been studied, but its expression in the neocortex and hippocampus in early mid-fetal development indicates that it might be a promising candidate gene for SLI.

Further studies will be required to replicate these findings and characterize the potential function of the human protein ZNF277, clarifying its potential implication in language development.

References

1. Darie OS. Application of Nexus Copy Number Software for CNV Detection and Analysis. Curr Protoc Hum Genet 2010; Chapter 4 Unit 4.13.28.

