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SHORT REPORT

Family-based association study of *DYX1C1* variants in autism

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DYX1C1 was recently identified as a candidate gene for developmental dyslexia, which is characterized by an unexpected difficulty in learning to read and write despite adequate intelligence, motivation, and education. It will be important to clarify, whether the phenotype caused by _DYX1C1_ extends to other language-related or comorbid disorders. Impaired language development is one of the essential features in autism. Therefore, we analyzed the allelic distribution of the _DYX1C1_ gene by family-based association method in 100 Finnish autism families. No evidence for association was observed with any intragenic marker or with haplotypes constructed from alleles of several adjacent markers. No evidence for deviated allelic diversity was either observed: the frequency of expected dyslexia risk haplotype was comparable to its frequency in Finnish controls. Thus it seems unlikely that _DYX1C1_ gene would be involved in the genetic etiology of autism in Finnish patients.

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Introduction

Autism (MIM 209850) is a severe neurodevelopmental disorder beginning before the age of 3 years and characterized by severe social and communication impairments, repetitive and ritualistic behaviors, and a restricted pattern of interests.[1] A strong genetic component has been established in autism by twin and family studies,[2,3] but the numerous genome scans performed so far have failed to produce consistent linkage signals, as reviewed elsewhere.[4] Delayed speech development represents one of the characteristic trait components of autism and has become an important focus of research in genetics of autism. Both qualitative[5–7] and quantitative[8] measures of language development have been used in attempts to improve the phenotypic homogeneity and, thus, to increase the power to identify susceptibility loci by linkage methods.

So far, only few genes have been linked to speech and language development. A monogenic severe speech and language disorder [MIM 602081] was originally mapped to 7q31 locus (SPCH1) in a large dominantly segregating KE family.[9] The location of the disease-causing gene was refined by the identification of chromosomal breakpoint on 7q31 in an unrelated patient,[10] and finally the causative gene was identified as *FOXP2*.[11] *FOXP2* is located on autism susceptibility locus 1 (AUTS1),[12,13] but the sequence and association analyses have not supported its involvement in the etiology of autism.[14–16] Noteworthy, also most samples with specific language impairment (SLI) have failed to show any linkage or association signals at the *FOXP2* locus, indicating that it is unlikely to play a

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significant role in cases of typical SLI.[14,17,18] However, one recent study showed putative evidence for association of *FOXP2* locus with SLI.[19] In a Finnish dyslexia sample, linkage for 7q31 overlapping SPCH1 and AUTS1 loci was observed, but sequencing of *FOXP2* in dyslexic individuals revealed no coding sequence mutations.[20]

Developmental dyslexia (MIM 127700) is the most common childhood learning disorder characterized by an unexpected difficulty in learning to read and write despite adequate intelligence, motivation, and education.[21] Recently, *DYX1C1* was identified as the first candidate gene for dyslexia and the second gene associated with language-related phenoptype.[22] *DYX1* locus on 15q21 was originally identified by several independent research groups,[23–25] and was further supported by the identification of a translocation t(2;15)(q11;q21), which segregated with the dyslexia phenotype in a Finnish family.[26] A novel gene entitled as *DYX1C1* was disrupted by the translocation, and subsequent analyses indicated that two *DYX1C1* variants were positively associated with dyslexia. These were −3G>A, which is located on the binding site of several transcription factors, and 1249G>T leading to a premature stop codon and deletion of four amino acids at the 3′ end of the coding sequence. *DYX1C1* protein is expressed in a subset of human glial and neuronal cells, but its precise function is yet unknown.[22]

It is important to clarify, whether the phenotype caused by *DYX1C1* is restricted to developmental dyslexia only, or whether the association on *DYX1* locus extends to other language-related or comorbid disorders. Here, we have tested association between the genetic variants of *DYX1C1* and autism, in which severe impairment of speech and language development is one of the most characteristic features.

Subjects and methods

Family material

The autism families were recruited through a nationwide search of university central hospitals of Finland. A total of 100 families with 122 affected individuals were included in the study. Three families had three affected individuals, 16 families had two affected individuals, and 81 families were trios. Thorough clinical and medical examinations were performed by an experienced child neurologist or pediatrician,[27] and the diagnoses were assessed according to ICD-10[28] or DSM-IV criteria.[1] Autism cases with associated medical conditions, such as fragile-X syndrome or chromosomal aberrations, were excluded. Only individuals with a confirmed diagnosis of infantile autism were included in the association analyses. In other words, family members with broad autism phenotype, such as Asperger syndrome, were assigned as unknown. A more detailed description of family recruitment, diagnostic instruments, and phenotypic assessment has been presented elsewhere.[27]

Genotyping

The DNA samples were genotyped using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (SEQUENOM Inc., San Diego, CA, USA).[29] Four SNPs (−3G>A, rs3743204, 572G>A, and 1249G>T) were genotyped in a single multiplex reaction. PCR assays and associated extension reactions were designed using the SpectroDESIGNER software (Sequenom Inc., San Diego, CA, USA) and primers, for which the sequences are available on request, were obtained from Metabion GmbH (Planegg-Martinsried, Germany). All amplification reactions were run in the same conditions in a total volume of 5 μl with 2.5 ng of genomic DNA, 1 pmol of each amplification primer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$ and 0.2 U of HotStarTaq DNA Polymerase (Qiagen). Reactions were heated at 95°C for 15 min, subjected to 45 cycles of amplification (20 s at 94°C, 30 s at 60°C, 30 s at 72°C) before a final extension of 7 min at 72°C. Extension reactions were conducted in a total volume of 9 μl using 5 pmol of allele-specific extension primer and the Mass EXTEND Reagents Kit before being cleaned using SpectroCLEANER (Sequenom Inc., San Diego, CA, USA) on a MULTIMEK 96 automated 96-channels robot (Beckman Coulter, Fullerton, CA, USA). Clean primer extension products were loaded onto a 384-elements chip with a nanoliter pipetting system (SpectroCHIP, SpectroJet, Sequenom) and analyzed by a MassARRAY mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The resulting mass spectra were analyzed for peak identification using the SpectroTYPER RT 2.0 software (Sequenom). For each SNP, two independent scorers confirmed all genotypes.

Statistical analyses

The statistical analyses were performed using FBAT program (http://www.biostat.harvard.edu/~fbat/fbat.htm),[30] which is capable of using information from both single markers and haplotypes constructed from the alleles of several adjacent markers. The empirical variance option was used, as appropriate in the presence of linkage and when data of multiple sibs in a family are available.[31] The extent of linkage disequilibrium between SNPs at the *DYX1C1* gene was tested using the tail probability (P-value) of Fisher's exact test implemented by Genepop 3.4 (http://wbiomed.curtin.edu.au/genepop/).

Results and discussion

DYX1C1 consists of 10 exons and encodes a 420 amino-acid protein with three tetratricopeptide repeat (TPR) domains. We genotyped four intragenic SNP markers flanking ~68 kb in a set of 100 autism families with 122 affected family members: −3G>A located on 5'UTR (exon 2), rs3743204 in the second intron, 572G>A (G191E) in exon 5, and 1249G>T (E417X) in exon 10. SNPs were

Table 1 Results from the association analyses

Variant	*Minor allele frequency*	*Single markers*			*Two-marker haplotypes*			*Three-marker haplotypes*			*Four-marker haplotype*		
		df	χ^2	*P-value*	*df*	χ^2	*P-value*	*df*	χ^2	*P-value*	*df*	χ^2	*P-value*
−3G>A	0.04	—	—	—									
					2	4.455	0.108						
rs3743204	0.09	1	0.000	1.000				2	1.919	0.383			
					2	1.167	0.558				2	1.059	0.589
572G>A	0.42	1	0.696	0.404				3	1.256	0.740			
					2	2.053	0.358						
1249G>T	0.07	1	0.727	0.394									

The analyses were performed by FBAT program using empirical variance option.

chosen on the basis of their previously shown association with risk of dyslexia in the Finnish population[22] (−3G>A and 1249G>T) or because of reported frequency of about 30–50% in the population and use as control SNPs in our assays (572G>A with ~50% and rs3743204 with 25–75% in NCBI database, http://www.ncbi.nlm.nih.gov/). We calculated the family-based association for each individual marker as well as for two-, three-, and four-marker haplotypes constructed from the genotypes of the adjacent markers. The analyses were restricted only to families with confirmed diagnoses of infantile autism to ascertain that all the patients assigned as affected had marked problems in language development and communication. Consistently with the original dyslexia study,[22] all the patients were of Finnish origin. This type of study sample where families are collected from the isolated Finnish population should maximize genetic, environmental and diagnostic homogeneity of this complex trait.[32]

None of the tested associations yielded positive results as shown in Table 1. We also calculated the frequency of a two-marker haplotype of −3A/1249T shown to be positively associated with dyslexia. In the original report, 13% of cases with dyslexia were shown to carry this haplotype *versus* 5% of controls, yielding an odds ratio of 2.8 ([CI 95%] = 1.2–6.5).[22] The corresponding frequency of the expected risk haplotype in our family-based autism material was 3% both in the affected and in the founder individuals. We were not able to conduct a family-based association analysis of this haplotype because only one major allele was present in the autism families. Altogether, 17 out of 100 families in our study sample had at least one family member affected with developmental dysphasia ($n_{\text{affected}} = 20$), but this sample was not sufficient for association analysis. Significant LD ($P < 0.001$) was observed between all marker pairs, excluding markers rs3743204 and 572G>A. Consistently with the association data, we did not observe linkage evidence with the SNP markers analyzed here (data not shown) or with the flanking microsatellites analyzed in our previous genome scan.[27]

Based on our data, it seems unlikely that the *DYX1C1* gene would have a major role in the etiology of autism in the Finnish families. It was earlier hypothesized that *DYX1C1* might be involved in the broad spectrum of common childhood neuropsychiatric disorders involving learning and acquisition of specific competences.[33] Autism is an extreme example of such disorder. On the other hand, autism is sometimes associated with extraordinarily advanced skills involving for example, special abilities in memory, mathematics or music. In some cases, autistic children display hyperlexic reading skills, and are able to identify printed words even at 18 months of age without instruction.[34] This indicates that common, although opposite, physiological mechanisms might sometimes underlie behind dyslexia and autism.[35] In future, it is important to analyze *DYX1C1* also in other autism samples, as well as in disorders involving impaired speech and language development, especially in specific language impairment (SLI), and in comorbid disorders, such as attention-deficit hyperactivity disorder (ADHD).[33]

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