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HLA polymorphisms in Italian children with autism spectrum disorders: Results of a family based linkage study

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ABSTRACT

To verify correlations between HLA and autism spectrum disorders (ASD) we studied 61 Italian families with an ASD child; results showed such correlation in 65% of cases. Case–control and TDT analysis of intrafamilial transmission of SNPs, Msats, and HLA markers surrounding the α and β blocks, indicated significant positive associations for MOGc*131 and D6S2239*105 alleles in ASD, and a negative association of MIB *332 allele in healthy siblings. Polymorphism haplotype analysis demonstrated that two haplotypes comprising the TNF-238(G)-TNF-308(G)-MIB*332-HLA-B*38-HLA-Cw*12 and the D6S265*218-HLA-A*23-MOGc*131-rs2857766 (G) alleles are more frequently transmitted to ASD. MOGc and MIB loci are linked with ASD in Italian patients.

1. Introduction

Autism spectrum disorders (ASD) are neurodevelopmental syndromes thought to be associated with brain abnormalities (Amaral et al., 2008) and characterised by early childhood onset. ASD include Autistic Disorder, Asperger's syndrome, Rett's syndrome, Childhood Disintegrative Disorder (CDD), and Pervasive Developmental Disorders Not Otherwise Specified (PDD-NOS), as per the DSM-IV-TR classifications (Anon, 1994). The incidence of ASD has dramatically risen from 2–5 to 15–60/10,000 children in the past two decades; broader diagnostic criteria and increased medical awareness have likely contributed to this perceived increase (Rutter, 2005; Persico and Bourgeron, 2006). The aetiology of ASD is still unclear; nevertheless, both genetic and environmental causes are believed to contribute to the risk for these conditions. Recent evidence suggests that environmental factors such as exposure to toxic compounds, teratogens, perinatal insults, and prenatal infections, may be responsible directly and/or secondarily for the immune mechanisms that mediate the impairments of the central nervous system seen in ASD (Muhle et al., 2004; Lauritsen et al., 2005). Family studies report a disease risk in siblings of ASD children that is much higher than in the general population but is nevertheless lower than what would be expected in single-gene diseases (Bolton et al., 1994; Gillberg et al., 1992; Ritvo et al., 1989; Szatmari et al., 1993; Bailey et al., 1995; Folstein and Rutter, 1977). These data, as well as the wide phenotypic variability of ASD, suggest that diverse genes, gene–gene interactions, and gene–environment interactions play a role in these diseases (Pinto et al., 2010; Freitag, 2007; Rutter et al., 1999; Pickles et al., 1995; Santangelo and Folstein, 1999; Risch et al., 1999).

The possible associations between particular human leukocyte antigen (HLA) alleles and autism have been investigated in several articles that either supported (Lee et al., 2006; Torres et al., 2006, 2002; Warren et al., 1996) or denied such associations (Rogers et al., 1999). Recent data (Guerini et al., 2006, 2009; Johnson et al., 2009) reinforced earlier findings supporting the role of an extended haplotype in ASD that is made up of a relatively constant sequence of DNA over the Major Histocompatibility Complex (MHC) region, inclusive of, but not limited to, the region from HLA-B to HLA-DR. The 44.1 haplotype was observed to occur more frequently in autism (Daniels et al., 1995; Warren et al., 1992; Odell et al., 2005); this

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haplotype contains the complement C4B null allele in the class III region, DRβ1*04 (DR4) in the class II region, and A2 and B44 alleles in the class I region. Although HLA-A- and HLA-B-encoded alleles have similar functions, they are in linkage disequilibrium with different genetic loci in two different polymorphic blocks that are conserved in the HLA region (Dawkins et al., 1999). HLA-A2, which is suggested to play an important role in ASD, is located within the α block; this block borders the ß block, which is where the HLA-B locus maps (Torres et al., 2006). It is therefore possible that any gene within these regions may be associated with ASD. This could be important as HLA class I molecules were recently demonstrated to play significant roles in brain development (Boulanger and Shatz, 2004).

We recently showed a linkage of HLA loci with ASD development in a Sardinian population (Guerini et al., 2006); in particular, we calculated a strong association between the α block region and ASD (Guerini et al., 2009). Since our observations were limited to Sardinians, which are a genetically homogenous group of Italians, we wanted to confirm these results in ASD patients from peninsular Italy. To this end, we analyzed the HLA region for connections to ASD development in 76 children with a diagnosis of ASD from peninsular Italy. Additionally, we analysed a 6 Mb region spanning the HLA region that ranged from the HLA-DR to the Hemochromatosis (HFE) gene, focusing our study on the α and ß blocks by microsatellite (Msat) and single-nucleotide polymorphism (SNP) analysis as previously explained (Guerini et al., 2009). Finally, the HLA-A, HLA-B, HLA-Cw and HLA-DR genes were systematically typed in ASD children and their relatives; both stratification and haplotype-based analysis were performed in order to take into account their potential linkage disequilibrium (LD) and co-segregation.

2. Materials and methods

2.1. Study population

Two hundred twenty-five individuals were enrolled in the study. Seventy-six of these individuals (17 females and 59 males; mean age: 10 ± 4 years) were children with a diagnosis of ASD according to DSM-IV-TR criteria; 61 of them were enrolled together with their parents. One hundred-fourteen parents were available for this study, because in eight families one of the two parents was unavailable. Whenever possible, healthy siblings were enrolled as well; a total of 35 of such healthy siblings (23 females and 12 males; mean age: 13 ± 7 years) were recruited in the study. The remaining 15 ASD individuals were children whose families were unavailable for typing, but their data were included in the case–control analysis. All subjects were born in peninsular Italy from families without Sardinian ancestry and were of Italian descent.

The 61 families participating in the study were characterised as follows: 31 had 2 or more children, 1 of whom was autistic while the rest were healthy, and 30 families had only 1 child, who was autistic. All ASD children underwent in-depth clinical, neurological, and neuropsychological evaluations, as well as examinations of mental status that covered the social interaction, imaginative play, language, and communication domains and neuropsychological aspects (using the Leiter-R, WISC-R, Raven and Vineland Adaptive Behavior Scales). Other diagnostic tools employed in the evaluation included the Modified Checklist for Autism in Toddlers (MCHAT) and the Childhood Autism Rating Scale (CARS). Karyotype and DNA analysis for fragile X and MECP2, screens for inborn errors of metabolism (phenylketonuria (PKU), amino and organic acidopathies), electro-encephalogram (EEG), brain-stem acoustic evoked potentials (BAEP), visual evoked responses, computerised tomography (CT) or magnetic resonance imaging (MRI) were also used to identify and eventually exclude from the study those children presenting an ASD-associated encephalopathy. The diagnosis of ASD was made according to DSM-IV-TR criteria (Anon, 1994; children were classified as follows: autism disorders (71 cases), PDD-NOS (5 cases). Informed consent was obtained from all participants or the legal guardians prior to inclusion in the study.

2.2. Genotyping

Genomic DNA was isolated from peripheral blood by phenol-chloroform extraction using standard procedures.

2.2.1. MSat typing

Genotyping of four Msat markers, spanning the HLA region (Fig. 1) was performed by an automatic method analysing fluorescently labelled PCR fragments on an ABI PRISM 310 Genetic Analyser. For MIB, Myelin Oligodendrocyte Glycoprotein c (MOGc) and D6S2239, previously described PCR primers were used (Grimaldi et al., 1996; Roth et al., 1995; Foissac et al., 2000); whereas for D6S265, primers were newly designed (forward :5′ACGTTCGTACCCATTAACCT, reverse: 3′GAACTGGAGGTTCTGATATTA). Alleles were named according to PCR product length. The CEPH-134702 reference sample carried the following alleles: D6S265, 214–218 bp; MIB, 326–336 bp; MOGc, 126 bp; and D6S2239, 106–110 bp.

Size range, repeated pattern, and number of alleles observed for each Msat are shown in Table 1.

2.2.2. SNP typing

SNPs were typed using the Taqman SNP Genotyping Assays (Applied Biosystem) on an ABI PRISM 7000 Sequence Detection System. Primers and probes for tumor necrosis factor α (TNFα)-308 and 238 were synthesised by Applied Biosystems. For rs2857766 and C_25474376, the Human Pre-Designed Assay (Applied Biosystems, Foster City, CA U.S.A.) was used.

Msats and SNPs were chosen taking into account their location within the MHC; marker location is shown in Fig. 1. MIB maps 25 Kb centromeric of the HLA-B gene; D6S265, 115 Kb centromeric of HLA-A; MOGc, 21 kb upstream of the MOG gene (262 Kb telomeric of HLA-A); and D6S2239, 23 kb telomeric of the HFE gene. Two SNPs map at the position −308 and −238 in the tumor necrosis factor α (TNFα) promoter located in the HLA class III region, approximately 250 kb centromeric of the HLA class I region and 850 kb telomeric of the HLA class II region gene (Nedwin et al., 1985). Finally, rs2857766 (V142L) is located in exon 3 of the MOG gene.

2.2.3. HLA polymorphism

HLA typing of class I HLA-A, HLA-B and HLA-Cw and class II DRB1 loci was performed by standard sequence specific primer polymerase chain reaction (SSP-PCR) (Olerup and Zitterquist, 1992), using Histo Type DNA well plates (BAG, Formedic diagnostici, Milan, Italy) according to the manufacturer's instructions. Detection of the alleles recognised by the specific primers was possible after amplification in a GeneAmp PCR 9700 thermocycler (Applied Biosystems, Foster City, CA U.S.A.) and gel electrophoresis on 2% agarose gel.

2.3. Statistical analysis

Linkage analysis was performed using LINKAGE software (share-ware software developed by J. Ott at the Rockefeller University of New York) (Terwilliger and Ott, 1994); a model of recessive inheritance of autism with complete penetrance was fitted. A LOD score >3 is considered significant, that is, indicative of linkage.

Since autism is a multifactorial and polygenic disorder, we adopted a model that allowed for heterogeneity among the families. We tested for linkage between 2 loci in a model that allows the presence of heterogeneity in which the locus is linked or unlinked to the disease. HOMOG, part of the LINKAGE software (Terwilliger and Ott, 1994), calculates log likelihoods under the assumption that in one proportion (α) of families, autism is linked to a marker, and in another proportion (1-α) of families, it is unlinked to the marker.