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The link between some alleles on human leukocyte antigen system and autism in children

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ABSTRACT

The reason behind the initiation of autoimmunity to brain in some patients with autism is not well understood. There is an association between some autoimmune disorders and specific alleles of human leukocyte antigen (HLA) system. Thus, we examined the frequency of some HLA-DRB1 alleles in 100 autistic children and 100 healthy matched-children by differential hybridization with sequence-specific oligonucleotide probes. The risk of association between acquisition or absence of these alleles and autism and also a history of autoimmune diseases in autistic relatives was studied. Autistic children had significantly higher frequency of HLA-DRB1*11 allele than controls ($P<0.001$). In contrast, autistic children had significantly lower frequency of HLA-DRB1*03 allele than controls ($P<0.001$). Acquisition of HLA-DRB1*011 and absence of HLA-DRB1*3 had significant risk for association with autism (odds ratio: 3.21 and 0.17, respectively; 95% CI: 1.65–6.31 and 0.06–0.45, respectively). HLA-DRB1*11 had a significant risk for association with a family history of autoimmunity in autistic children (odds ratio: 5.67; 95% CI: 2.07–16.3). In conclusions, the link of some HLA alleles to autism and to family history of autoimmunity indicates the possible contributing role of these alleles to autoimmunity in some autistic children. Despite a relatively small sample size, we are the first to report a probable protective association of HLA-DRB1*03 allele with autism. It warrants a replication study of a larger sample to validate the HLA-DRB1 genetic association with autism. This is important to determine whether therapeutic modulations of the immune function are legitimate avenues for novel therapy in selected cases of autism.

1. Introduction

Increasing evidence of immune abnormalities in individuals with autism could represent the presence of altered or inappropriate immune responses in this disorder, and this immune system dysfunction may represent novel targets for treatment. Furthermore, in recent studies, antibodies directed against the fetal brain have been detected in some mothers of children with autism. These antibodies have the ability to alter the behavioral outcomes in the offspring of animal models. A better understanding of the involvement of the immune response in early brain development, with respect to autism, may have important therapeutic implications (Enstrom et al., 2009). The presence of brain-specific autoantibodies in a subgroup of autistic children (Singh et al., 1993, 1997, 1998; Vojdani et al., 2002; Singh and Rivas, 2004; Cabanlit et al., 2007; Mostafa et al., 2008; Wills et al., 2009; Goines et al., 2011; Mostafa and Al-Ayadhi, 2011a,b,c; Wills et al., 2011; Al-Ayadhi and Mostafa, 2012; Mostafa and Al-Ayadhi, 2012) may be an important clue for the possible pathogenic role of autoimmunity in autism. In addition, some autistic children have an imbalance of the T-helper (TH)1/TH2 lymphocytes toward TH2 cells which are responsible for allergic response and the production of antibodies. This predisposes autistic children to allergic and autoimmune disorders (Cohly and Panja 2005).

Inspite of the fact that the origins of abnormal immune activity in autism are unknown, the human leukocyte antigen (HLA) genes and their products might be involved (Guerini et al., 2006). HLA genes are part of the major histocompatibility complex which includes genes that are central components of the immune response. There is a substantial evidence for an association between specific antigens/alleles of the HLA system and autoimmunity. For example, HLA-DR4, a class II antigen, has been identified as one of the susceptibility markers for certain autoimmune diseases, such as rheumatoid arthritis, autoimmune hypothyroidism and insulin-dependent diabetes mellitus (Chamie 2004; Levin et al., 2004; Rosloniec et al., 2004). These disorders have a higher incidence among relatives with autism than healthy controls (Comi et al., 1999; Sweeten et al., 2003; Atladóttir et al., 2009; Mostafa and Kitchener, 2009; Mostafa et al., 2010a,b; Mostafa and Shehab, 2010; Crespi and Thiselton, 2011).

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The associations between some HLA antigens and autism have been reported in western countries, but there is a lack of such information in Arabian population. The goal of this study was the examination of the frequency of some HLA-DRB1 alleles in a group of Egyptian children with autism. The risk of association between the acquisitions or absence of these alleles and autism and also a history of autoimmune diseases in autistic relatives was studied.

2. Materials and methods

2.1. Study population

This study was conducted on 100 children with autism who were fulfilling the criteria for the diagnosis of autism according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (The American Psychiatric Association, 1994). There were 78 males and 22 females and their ages ranged between 5 and 12 years with a mean age of 8.4 ± 2.5 years. Patients were recruited from the Pediatric Neuropsychiatric Clinic, Children's Hospital, Faculty of Medicine of Ain Shams University, Cairo, Egypt, during their follow-up visits. Patients who had associated neurological diseases (such as cerebral palsy and tuberous sclerosis) and metabolic disorders (e.g., phenylketonuria) were excluded from the study.

Patients with autism were studied in comparison to 100 age- and sex-matched apparently healthy children. They included 76 males and 24 females recruited from the Outpatients Clinic, Children's Hospital, Faculty of Medicine, Ain Shams University, serving as controls. They were the sibs of the children attending this clinic because of a minor illness (e.g. common cold, tonsillitis and acute bronchitis). The control children were not related to the children with autism and had no clinical findings suggesting immune or neuropsychiatric disorders. Their ages ranged between 6 and 12 years (mean ± SD = 8.2 ± 2.6 years).

The local Ethical Committee of the Faculty of Medicine, Ain Shams University, approved this study. In addition, an informed written consent of participation in the study was signed by the parents or the legal guardians of the studied subjects.

2.2. Study measurements

2.2.1. Clinical evaluation of the children with autism

This was based on a clinical history taken from the caregivers, clinical examination and neuropsychiatric assessment. Disease severity was assessed by using the Childhood Autism Rating Scale (CARS) (Schopler et al., 1986) which rates the child on a scale from one to four in each of the fifteen areas (relating to people; emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal communication; non-verbal communication; activity level; level and consistency of intellectual response; adaptation to change; visual response; taste, smell and touch response and general impressions). According to the scale, children who have scored 30–36 have mild to moderate autism (n = 60), while those with scores ranging between 37 and 60 points have severe autism (n = 40).

In addition, a family history of autoimmune diseases in healthy children and autistic patients was ascertained. Parents were asked to fill out a questionnaire regarding which first- or second-degree relatives had received a diagnosis of specified autoimmune disorders. The questionnaire was formulated with the assistance of an experienced immunologist. A list of autoimmune diseases with descriptions was provided. There was a verification of the diagnosis of autoimmune diseases via medical record review or direct clinical examination. The disorders inquired about in the questionnaire included rheumatoid arthritis (RA), juvenile rheumatoid arthritis, systemic lupus erythematosus (SLE), insulin-dependent diabetes mellitus (IDDM), rheumatic fever, vasculitis, ankylosing spondylitis, dermatomyositis, polymyositis, scleroderma, uveitis, Sjogren's syndrome, polyarteritis nodosa, Wegener's granulomatosus, Takayasu's arteritis, psoriasis, multiple sclerosis, vitiligo, myasthenia gravis, amyotrophic lateral sclerosis, Crohn's disease, ulcerative colitis, autoimmune thyroiditis, idiopathic thrombocytopenic purpura, Addison's disease, pemphigus and Guillain–Barre syndrome. These disorders were chosen because all have a known or a suspected autoimmune cause.

2.2.2. HLA-DRB1 typing

This was accomplished by differential hybridization with sequence-specific oligonucleotide probes. Three ml of venous blood was collected from each child on EDTA containing tubes.

2.2.2.1. DNA extraction. DNA extraction was done using Wizard^R genomic DNA purification kit (Promega Corporation, USA). The kit uses chaotic agent to extract nucleated blood cells and facilitate the binding of genomic DNA to a glass fiber matrix contained in a microscopic column. The purified DNA is eluted in a low ionic strength buffer ready for use. The kit provides an accurate, reliable and fast method for detecting amino acid motives QKRAA, QRRAA and RRRAA that characterize the SE of all known HLA-DRB1 alleles. Genomic DNA is concentrated and desalted by isopropanol precipitation. HLA-DRB typing was then done by using Dynal RELI™ SSO HLA-DRB Typing Kit (Dynal Biotech Ltd., UK).

2.2.2.2. HLA-DRB1 typing. The principle of the HLA-DRB typing is based on three major processes:

1. Plymerase chain reaction (PCR) target amplification, in which a multiplex of 5 primers is used, one pair for amplification of specific DNA target sequences in the polymorphic second exon of DRB1, DRB3, DRB4 and DRB5 genes. The additional 3 primers are used for amplification of specific sequences within intron one of DRB1*15 and DRB1*18. PCR was done by applying the following protocol: denaturation at 95 °C for 15 s, primer annealing at 60 °C for 45 s and extension at 72 °C for 15 s. This process was repeated for 35 cycles. DNA amplification technique by PCR increases the number of copies of target DNA in the sample, theoretically yielding more than a billion-fold amplification, prior to hybridization.
2. Hybridization of the amplified product to nylon membrane-immobilized sequence specific oligonucleotide (SSO) probes. The biotin-labeled amplicons then bind (hybridize) to those SSO probes that contain a complementary target sequence and thus are "captured" onto the membrane strip. A stringent wash step after hybridization ensures the specificity of the reaction and removes all unbound amplicon and only hybrids with 100% complementary sequences survive. Detection is subsequently achieved by the sequential addition of streptavidin-coupled alkaline phosphatase.
3. Detection of the probe bound amplified product by color formation after addition of a streptavidin horseradish peroxidase conjugate that binds the biotin-labeled amplicons captured by the membrane bound probe. Addition of hydrogen peroxide and tetramethylbenzidine substrate, results in the formation of a blue color complex. The resulting probe signals are compared to the control probe intensity and the samples hit pattern recorded for interpretation. Interpretation of results was done using the Dynal RELI™ SSO pattern matching program (product no. 811.00).

2.3. Statistical analysis

The results were analyzed by commercially available software package (StatView, Abacus Concepts, Inc., Berkeley, CA, USA). Chi-square (X^2) test was used for comparison between qualitative variables of the studied groups. For all tests, a probability (P) of less than 0.05 was considered significant. Risk estimation for association of HLA-DRB1 alleles with autism was done by using logistic regression analysis to calculate the odds ratio and its 95% confidence interval (CI).