Possible association of the extended MHC haplotype B44-SC30-DR4 with autism

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Abstract. We previously reported that the complement C4B null allele appears to be associated with infantile autism. Since the C4B null allele is known to be part of the extended or ancestral haplotype [B44-SC30-DR4], we investigated the incidence of [B44-SC30-DR4] in 21 autistic children and their parents. This extended haplotype was increased by almost six-fold in the autistic subjects as compared with healthy controls. Moreover, the total number of extended haplotypes expressed on chromosomes of autistic subjects was significantly increased as compared with those expressed on chromosomes of healthy subjects. We conclude that a gene related to, or included in, the extended major histocompatibility complex may be associated with autism.

Introduction

The etiology and pathogenesis of autism is unknown. Recent investigations, however, suggest that this neurodevelopmental disorder shares many characteristics of established autoimmune disorders including: a) genetic predisposition (Folstein and Rutter 1977; Smalley et al. 1988); b) immune abnormalities of helper T-cell deficiency and cell-mediated immunity and/or antibodies against brain antigens (Stubbs et al. 1977; Warren et al. 1986; Warren et al. 1990; Yonk et al. 1990; Stubbs 1977; Weizman et al. 1982; Todd and Ciarenello 1985; Singh and Warren); c) possible microbial triggers, i.e., viral infections (Chess 1977; Stubbs 1978); d) sex differences (autism is 4–5 times more common in boys than in girls).

Materials and methods

Subjects. This study included 21 autistic subjects (19 males and two females) and all of their biologic parents (all of northern European ancestry) who were living in northern Utah, USA. The autistic children ranged from 3–27 years with a mean age of 11.0 years. Study of the parents was done concurrent with that of the patients to assist in the assignment of the various haplotypes. Written, informed consent was obtained from all study subjects or their parents. "Autistic haplotypes" were defined as all HLA haplotypes which occurred in the probands. "Family healthy" haplotypes were those present in the parents but not in the patients. The diagnosis of autism satisfied DSM-IIIR criteria for infantile autism as ascertained by at least two psychiatrists or psychologists. None of the autistic subjects had an identifiable cause of their disease and all were living at home at the time of study. Also included in the investigation were 62 randomly chosen healthy subjects also of northern European ancestry, unrelated to the autistic subjects, who were
living in the same geographical area as the autistic subjects. Forty-eight
of the healthy subjects were parents of children with idiopathic mental
retardation.

**HLA Typing.** HLA-A, B, C, and HLA-DR antigens were typed using the
standard 2-stage National Institutes of Health (NIH) microlymphocytotoxicity assays. Lymphocytes were isolated by Ficoll-Hypaque
centrifugation and B cells for HLA-DR typing were isolated by a panning
technique using plates carrying F(ab)2 goat anti-human IgG, M, A.
Many of the later assays for HLA-DR were performed using the magnetic
bead system (Dynabeads II; Robbins Scientific, Mountain View, CA)
for separation of B cells, followed by dual fluorescence with acridine
orange and ethidium bromide. Reagents for tissue typing were ob-
tained from One Lambda (Los Angeles, CA and Gen Trak, Plymouth
Meeting, PA).

**Complotyping.** The genetic typing for the complement proteins was per-
formed by The Center for Blood Research Laboratories (Boston, MA)
under the direction of D. H. Bing using previously described techniques
(Awdeh et al. 1983). Briefly, typing for the C4 phenotypes was carried
out by incubating samples with neuraminidase from *Clostridium perfr-
ingens* overnight at room temperature with continuous dialysis against
0.1 M phosphate buffer, pH 7.0, containing 0.005 M ethy-
lenediaminetetraacetate (EDTA)-Na2. The desialated samples were sub-
jected to electrophoresis and immunofixation in 1% agarose and the
bands were visualized with goat anti-human C4 (Atlantic Antibodies,
Scarborough, ME). Some samples, processed as above, were developed
with a C4 complement overlay consisting of antibody-sensitized sheep
red blood cells (SRBC) and C4-deficient guinea pig serum incorporated
into a gel and layered onto a C4 agarose gel. The presence of null alleles
(QO) was determined by inspection of immunofixation patterns or by
crossed immunoelectrophoresis. BF typing was carried out with frozen
plasma in agarose gel and immunofixation with goat-antiserum to human
factor B (Atlantic Antibodies) as previously described. Typing for the
C2 complement proteins was performed by isoelectric focusing of the
samples in polyacrylamide gel and an overlay agarose gel containing
antibody-sensitized SRBCs and diluted fresh healthy human serum.

**Statistical Analyses.** Statistical significance was determined by chi-
square analysis using 2×2 contingency tables.

**Results**

Assignment of the HLA A, B, DR alleles and the complement
BF, C2, C4A, C4B alleles in the 21 autistic subjects is given in Table 1. Twelve of probands had chromosomes carrying a C4B null allele. Of these 12 chromosomes, six
also expressed B*44, DR*4, BF*S, C2*C, and C4A*3 (the other alleles making up the extended MHC haplotype [B44-SC30-DR4]). The gene frequency (.142) of [B44-
SC30-DR4] in the autistic subjects (Table 2) was signifi-
cantly (p =0.0035) elevated as compared with that (.24)
of the healthy control subjects. This extended haplotype
was also significantly (p = 0.016) increased in the mothers
but not in the fathers of the autistic subjects. A chro-
mosome of one autistic subject carried [B35-FC (3,2)
0-DR1], another extended haplotype (with a duplicated
C4A allele) which also carries the
C4B null allele. Five
of the proband chromosomes had C4B null alleles which
were not part of a complete extended haplotype, however
three of the five were found on fragments of extended

**Table 1. HLA alleles and Complotypes in autistic subjects.**

<table>
<thead>
<tr>
<th>Probands</th>
<th>Maternal origin</th>
<th>Paternal origin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HLAA-B-DR</td>
<td>BR C2 C4A C4B</td>
</tr>
<tr>
<td>1</td>
<td>33-14-1</td>
<td>SC2 (2, 1)*</td>
</tr>
<tr>
<td>2</td>
<td>24-51-2</td>
<td>SC30</td>
</tr>
<tr>
<td>3</td>
<td>29-44-4</td>
<td>SC30</td>
</tr>
<tr>
<td>4</td>
<td>30-13-7</td>
<td>SC30</td>
</tr>
<tr>
<td>5</td>
<td>28-7-2</td>
<td>SC31</td>
</tr>
<tr>
<td>6</td>
<td>2-44-4</td>
<td>SC30</td>
</tr>
<tr>
<td>7</td>
<td>1-8-3</td>
<td>SC01</td>
</tr>
<tr>
<td>8</td>
<td>1-57-7</td>
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</tr>
<tr>
<td>21</td>
<td>24-7-2</td>
<td>SC31</td>
</tr>
</tbody>
</table>

* Indicates duplicated allele.
† Extended haplotypes are shown in bold type.
‡ Marker not determined is designated with an X.