ARTICLE

# *HLA-DR4* as a Risk Allele for Autism Acting in Mothers of Probands Possibly During Pregnancy

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**Objectives:** To test whether *HLA-DR4* acts in the mother, possibly during pregnancy, to contribute to the phenotype of autistic disorder in her fetus.

**Design:** Transmission disequilibrium testing in case mothers and maternal grandparents.

**Setting:** Previous studies have consistently shown increased frequency of *HLA-DR4* in probands with autism and their mothers, but not their fathers. However, this has been documented only in case-control studies and not by a more direct study design to determine whether *HLA-DR4* acts in mothers during pregnancy to contribute to autism in their affected offspring.

**Participants:** We genotyped for *HLA-DR* alleles in members of 31 families with parents and maternal grandparents. Probands with autism were tested using the Autism Diagnostic Observation Schedule–Western Psychological Services and Autism Diagnostic Interview, Revised. There was 80% power to detect an odds ratio of 3.6. Participants were all families from New Jersey and were similar in number to earlier studies of autism and *HLA-DR4*.

**Outcome Measures:** Analysis was by standard transmission disequilibrium testing. As a secondary test we examined the possibility of maternal imprinting.

**Results:** Significant transmission disequilibrium for *HLA-DR4* was seen (odds ratio, 4.67; 95% confidence interval, 1.34-16.24; $P=.008$) for transmissions from maternal grandparents to mothers of probands, supporting a role for *HLA-DR4* as an autism risk factor acting in mothers during pregnancy. Transmission disequilibrium was not seen for *HLA-DR4* transmissions from parents to probands or from mothers to probands.

**Conclusions:** The *HLA-DR4* gene may act in mothers of children with autism during pregnancy to contribute to autism in their offspring. Further studies are required to confirm these findings.

*Arch Pediatr Adolesc Med. 2009;163(6):542-546*

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In most cases in which a gene has been associated with a disorder, the disease allele acts in the affected individual. Alternatively, a disease allele may act in the mother to contribute to the phenotype of her affected child. A reasonable hypothesis is that such maternal genes act during pregnancy (postpartum action through nursing seems unlikely); however, action before conception in the ovum and the cells that influence it is also possible. So far, there are more than 30 descriptions of such maternally acting alleles, so-called teratogenic alleles.[1,2]

Children with autism show deviation from the normal developmental pattern with impaired social interactions and communication, restricted interests, and repetitive stereotyped patterns of behavior that are evident prior to 36 months of age.[3,4] Clinical genetic studies and modeling studies suggest that autism may be caused by multiple interacting gene loci[5,6] and that environmental and epigenetic factors may contribute.[6,7]

Neuropathological,[8,9] cytoarchitectonic,[10] and minicolumn studies[11,12] all support the prenatal origin of certain brain abnormalities in autism. Consequently, it is possible that maternal genes acting during pregnancy could contribute to the autism phenotype in the fetus.

A number of studies have associated *HLA-DR4* with autism, some of them suggesting its action in mothers of children with autism, possibly during pregnancy. Warren and colleagues[13] studied the frequency of a major histocompatibility complex extended haplotype that contains *HLA-DR4* in a case-control design with 21 individuals with autism, their parents, and 62 controls. Compared with controls, the major histocompatibility complex ex-

tended haplotype, *B44-SC30-DR4*, was significantly increased in both cases and mothers, but not in fathers, of individuals with autism. Daniels et al[14] confirmed this finding in a case-control study, adding 23 new individuals with autism and the parents of 18 of them and comparing them with 64 controls. All of the families in both studies were of northern European ancestry and lived in northern Utah. Subsequently, Warren et al[15] studied 50 children with autism and 79 control subjects, all of northern European ancestry and all but 2 living in northern Utah, and found that certain alleles of the third hypervariable region of *HLA-DRβ1* had a very strong association with children with autism, especially alleles within *HLA-DR4*. As an explanation of their striking maternal findings, Warren et al raised the question of whether a gene acting in the mother during pregnancy might contribute to autism in her fetus.

More recently, Torres et al[16] carried out a case-control study of individuals with autism spectrum disorder from 31 families from Oregon, 34 families from Utah, and 38 families in the Autism Genetic Resource Exchange (AGRE), all white, compared with allele frequencies in white control subjects from the National Marrow Donor Program, and found that *HLA-DR4* occurred more frequently in children with autism spectrum disorder than controls.

Recently, Lee et al[17] carried out a case-control study of *HLA-DR4* in autism in 16 families from eastern Tennessee and 33 families from AGRE who were selected from all parts of the United States and in which multiple male participants had autism and compared them with a control group of 475 healthy unrelated adults who were evaluated at the Johns Hopkins Hospital as potential bone marrow donors. All families with individuals with autism and control families were white. Compared with controls, children with autism and their mothers, but not their fathers, for the east Tennessee group had a significantly higher frequency of *HLA-DR4* alleles than control subjects; specifically, the mothers were 5.54 times (95% confidence interval [CI], 1.74-18.67) and their children with autism were 4.20 times (95% CI, 1.37-13.27) more likely to have *HLA-DR4* than control individuals.[17] The *HLA-DR4* frequencies in children with autism in the AGRE group, their mothers, and their fathers were not significantly different from controls.[17] The authors interpreted their findings in the eastern Tennessee group as consistent with a hypothesis that maternal-fetal immune interaction in utero could affect fetal brain development; such an immune interaction could conceivably involve both *HLA* and related genes in both genetic and epigenetic mechanisms.

Although these studies suggested a maternal effect of *HLA-DR4* for autism, all of them compared mothers of individuals with autism with controls in case-control study designs and none of them carried out a more direct test such as the maternal transmission/disequilibrium test (TDT)[1,2,18-20] that could document the presence of a maternal allele acting during pregnancy to contribute to the autism phenotype. Because *HLA-DR4* had been associated with both individuals with autism and the mothers of affected children compared with controls, we tested the association of these alleles with autism using a case-parent study design with mothers of children with autism and maternal grandparents and analysis by standard TDT.[21] Finding the action of *HLA-DR4* during pregnancy to contribute to autism in offspring could be important because the *DR4* data for autism show one of the highest odds ratios (OR), 4.20, of any marker associated with autism so far.[17] Also, the association of *DR4* with autism would raise the possibility of an autoimmune component to autism and thus the possibility for therapy. Identifying *HLA-DR4* as a teratogenic allele for autism would be important because pregnancy may be the earliest opportunity for therapeutic intervention in autism.

Increased frequency of an allele in both cases and mothers in a case-control study could also occur if the allele were imprinted in the mothers and acted in the cases. Therefore, as a secondary hypothesis, we tested for maternal imprinting of these alleles.

## METHODS

Thirty-one families with mothers and maternal grandparents as well as probands and fathers were ascertained with the help of the Center for Outreach and Services for the Autism Community, a New Jersey autism support group. All but 1 of the families were also part of a separate study of *GSTP1*.[22] Family members were genotyped for *HLA-DR* by the Center for Blood Research Laboratories and BioSciences Research Associates at Harvard University. The *HLA-DR* alleles were determined by polymerase chain reaction amplification of genomic DNA and dot-blot analysis using sequence-specific oligonucleotide probes.[23] All probands were tested using the Autism Diagnostic Observation Schedule–Western Psychological Services (ADOS-WPS) and Autism Diagnostic Interview, Revised (ADI-R). Using "mother of child with autism" as the affected phenotype, we applied the standard TDT, comparing the transmissions and nontransmissions of *HLA-DR4* against the binned set of all other alleles. Overtransmission of an hypothesized risk allele to mothers would suggest that the allele genuinely is a risk allele for autism that acts in mothers.

An additional explanation for the observed increased allele frequency in mothers and probands, but not fathers, in families with a child with autism would be that the allele is imprinted in the mothers but acts in probands, both mothers and probands being enriched for that allele. To examine this possibility, as a secondary test we tested for maternal imprinting with the method of Weinberg[24] and also applied the TDT to the offspring with autism.[21] Because these additional tests were secondary tests, a correction for multiple comparisons was not applied; even if a correction had been applied, the maternal TDT data would have remained statistically significant.

At the observed allele frequencies in founders, the study had sufficient power to detect large effects, namely 80% power to detect an OR of 3.6. The study was approved by the institutional review board of UMDNJ–Robert Wood Johnson Medical School and informed consent was obtained from the participants.

## RESULTS

Clinical diagnoses by ADOS-WPS and ADI-R were autistic disorder for 30 of the probands and pervasive developmental disorder not otherwise specified (PDD-NOS) for 1. In 25 families, both parents were non-Hispanic white; in the other 6 families, 1 parent in each was non-Hispanic white

**Table. Transmission Disequilibrium Testing at the *DRβ1* Locus for Mothers of Probands With Autism and, as a Secondary Test, for Probands With Autism**

| Locus | Hypothesized Risk Allele | Transmissions | Nontransmissions | *P* Value | OR (95% CI) |
|---|---|---|---|---|---|
| DRβ1 | *DR4* | 14 to mother | 3 | .008 | 4.67 (1.34-16.24) |
| DRβ1 | *DR4* | 12 to cases | 9 | .39 | 1.33 (0.56-3.16) |
| DRβ1 | *DR4* | 6.5 to cases from mothers | 6.5 | .99 | 1.00 (0.34-2.97) |

Abbreviations: CI, confidence interval; OR, odds ratio.

and the other was non-Hispanic Asian (3), Hispanic white (2), or non-Hispanic black (1).

In the 31 families with an autistic child genotyped for *HLA-DRβ1*, statistically significant transmission disequilibrium for *HLA-DR4* was seen by TDT (OR, 4.67; 95% CI, 1.34-16.24; *P*=.008) (**Table**) for transmissions to mothers of individuals with autism from maternal grandparents, supporting a role for *HLA-DR4* as a risk factor for autism acting in the mothers in this group of families. There were 14 copies transmitted and 3 untransmitted, as opposed to 35 and 46, respectively, for other alleles. The mother of the child with PDD-NOS had an *HLA-DR4* transmission; when that family was dropped, the result remained statistically significant (OR, 4.33; 95% CI, 1.23-15.21; *P*=.01).

To examine an alternative possible explanation for the significantly increased frequency of *HLA-DR4* reported in mothers of individuals with autism, that the *HLA-DR4* allele is a risk allele in the child through maternal imprinting and that the mothers are, of necessity, enriched for this allele, we followed up with a secondary test in the children with autism and their parents, again using the standard TDT along with a test for maternal imprinting. Statistically significant transmission disequilibrium was not seen for transmissions from parents to individuals with autism themselves (OR, 1.33; 95% CI, 0.56-3.18; *P*=.39) (Table), nor from mothers specifically (OR, 1.00; 95% CI, 0.34-2.97; *P*=.99) (Table). The Weinberg test for maternal imprinting was also not statistically significant (*P*=.79). These findings did not support action of *HLA-DR4* as a risk factor for autism acting in children with autism themselves either directly or through imprinting.

## COMMENT

As discussed above, earlier case-control studies suggested the possibility of a maternal effect in autism originating from *HLA-DR4*, but they did not clarify what sort of a maternal effect, if any, might be operating. Nor did they use a more direct study design to prove this.

The present study describes the action of *HLA-DR4* during pregnancy in mothers of children with autism that contributes to autism in their offspring. The known possible reasons for increased frequency of an allele repeatedly observed in mothers of affected individuals include (1) the allele is a teratogenic allele, (2) the allele acts by imprinting and is imprinted in the mother, (3) it acts in the affected individual and hence will have increased frequency in the parents—sometimes by chance the allele will have increased frequency in mothers but not fathers, and (4) the allele is a mitochondrial allele and hence is transmitted only by mothers to affected individuals; therefore, it has increased frequency in mothers. Our study excluded all of these possibilities except the first. Our tests for imprinting were negative. Case TDT was not supported. Mitochondrial alleles do not show segregation, but *DR4* did show segregation in transmissions from maternal grandparents to mothers; in any case, *DR4* is known not to be the product of a mitochondrial gene. The study has several novel or unusual features. First, it provides an answer to the long-standing question of what sort of maternal effect originating from *HLA-DR4* contributes to autism. Second, it supports the possibility of an immune component to autism pathogenesis acting in mothers during pregnancy. Third, it adds to the small number of gene alleles shown to act in mothers, probably during pregnancy, to contribute to a disorder in their offspring. Because nearly all of these disorders have turned out to be neurodevelopmental,[1,2] it is possible that this could be a mechanism of more general importance for these disorders, especially if it were more widely known and more frequently looked for. The genetic architecture of autism can not be adequately understood if maternally acting genes are not looked for. Fourth, our findings encourage additional studies to address the pathogenesis of autism that could ultimately contribute to prevention or therapy for the disorder.

Our study was a small one, but was not underpowered for this purpose. At the observed allele frequencies among founders, the study had sufficient power to detect large effects, namely 80% power to detect an OR of 3.6. The effect that we found for *HLA-DR4* gave an OR of 4.67 (95% CI, 1.34-16.24; *P*=.008). This OR is comparable with those in other studies of the effect that did not determine the genetic mechanism. Participants in the present study were all families from New Jersey, and the number of families was similar to those of geographically defined areas in the earlier studies of autism and *HLA-DR4*. This report should encourage larger studies to confirm this effect in autism. Unlike the case-control study design, the case-parent study design is resistant to population stratification,[21] which was, in any case, minimal in our families.

The possible mode of action of *DR4* in mothers is unknown. Maternal *DR4* could contribute to a subset of autism cases by interacting with other risk alleles for autism and with environmental factors to perturb pathways affecting brain development in autism. A possible environmental factor could be the maternal infections during pregnancy (urinary tract, respiratory, and vaginal) described previously as more common in the mothers of

children with autism than controls, although that increase was not statistically significant.[25] The *HLA-DR4* gene (along with *DR3*) is a risk allele for type I diabetes mellitus and appears to modulate the humoral immune response to enterovirus antigens.[26]

Interestingly, in mice, maternal immune stimulation during gestation may affect the developmental outcome of offspring. For example, maternal immune stimulation reportedly ameliorated malformations induced by chemical teratogens,[27] perhaps through maternal immune regulation of fetal gene expression including cell cycle and/or apoptotic genes.[28] Maternal stimulation with interferon gamma decreased the severity of fetal cleft palate caused by urethane, while stimulation with Freund's complete adjuvant reduced both the incidence and severity of the lesion.[29] Maternal immune stimulation inducing inflammation increased fetal brain cytokine response, decreased the number of reelin-immunoreactive cells in certain areas of postnatal brain in offspring, and altered behavior in adult offspring.[30] Reelin gene polymorphisms have been associated with autism,[31,32] and reelin protein levels are decreased in autism in blood[33] and cerebellum.[34]

Immune responses to infections generate products of oxidative stress, which could be a contributing factor to altered brain development in autism. We recently reported that a biomarker of oxidative stress is elevated postnatally in children with autism,[35] a finding that has been confirmed.[36] Interestingly, recent work suggests that the immune system participates in the shaping of brain synaptic circuits during childhood development.[37]

The present findings do not exclude the possibility of a contribution to autism susceptibility by an allele of *HLA-DQβ1*, a major histocompatibility complex locus closely linked to *HLA-DRβ1*, or alleles at other loci within a conserved extended haplotype containing *HLA-DR4*.[38] Further studies are needed to confirm and extend the present findings, including a larger maternal TDT study, a study of other DR alleles, a study of fine structure subtypes of *DR4*, and a study of whether *HLA-DQβ1* alleles contribute to autism.

**Accepted for Publication:** October 24, 2008.
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**Author Contributions:** Dr Johnson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Johnson, Stenroos, Williams, and Lambert. *Acquisition of data:* Johnson, Mars, Stenroos, Williams, Stein, and Lambert. *Analysis and interpretation of data:* Johnson, Buyske, Sreenath, Stenroos, Williams, and Lambert. *Drafting of the manuscript:* Johnson, Buyske, Stein, and Lambert. *Critical revision of the manuscript for important intellectual content:* Johnson, Buyske, Mars, Sreenath, Stenroos, Williams, and Lambert. *Statistical analysis:* Buyske. *Obtained funding:* Johnson and Lambert. *Administrative, technical, and material support:* Sreenath, Stenroos, Williams, Stein, and Lambert. *Study supervision:* Johnson.

**Financial Disclosure:** Drs Johnson and Mars report being members of the Professional Advisory Board of The New Jersey Center for Outreach and Services for the Autism Community (COSAC).
**Funding/Support:** This study was supported by grants R21-44244 and K25-AA015346 from the National Institutes of Health (NIH); grant 2R25 GM55145 from the Initiative to Maximize Student Diversity in Biomedical Sciences (Dr Williams); The New Jersey Governor's Council; the National Alliance for Autism Research; and grants P50-ES09598 and P01-ES11256 from the NIH United States Environmental Protection Agency Center of Excellence.
**Previous Presentations:** These data were presented as a poster at the International Meeting for Autism Research; May 16, 2008; London, England; and the Child Neurology Society; November 6, 2008; Santa Clara, California.
**Additional Contributions:** The authors are grateful for the participation of the individuals with autism and their family members and for the assistance of COSAC (Ewing, New Jersey), which helped with recruitment of participants. The authors wish to thank Zuheir Awdeh, PhD, of CBR Laboratories for reading the manuscript.

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***Announcement***

**Submissions.** The Editors welcome contributions to Picture of the Month. Submissions should describe common problems presenting uncommonly, rather than total zebras. Cases should be of interest to practicing pediatricians, highlighting problems that they are likely to at least occasionally encounter in the office or hospital setting. High-quality clinical images (in either 35-mm slide or electronic format) along with parent or patient permission to use these images must accompany the submission. The entire discussion should comprise no more than 750 words. Articles and photographs accepted for publication will bear the contributor's name. There is no charge for reproduction and printing of color illustrations. For details regarding electronic submission, please see: http://archpedi.ama-assn.org.