Association between reduced copy-number at T-cell receptor gamma and childhood allergic asthma: a possible role for somatic mosaicism

Kyle Walsh, Michael Bracken, William Murk, Josephine Hoh, Andrew DeWan

Center for Perinatal Pediatric and Environmental Epidemiology, Yale School of Public Health. Department of Epidemiology and Public Health, Yale University

Introduction

Asthma is a chronic inflammatory disease of the lungs which affects more than 6.5 million American children. A family-based genome-wide association study of copy-number variation identified an association between decreased copy-number at TCRγ and childhood allergic asthma1. TCRγ encodes the T-cell receptor gamma glycoprotein, a cell-surface protein found on T-cells and involved in cell-mediated immunity. Copy-number variation at these loci is well-known, but appears to be dominated by somatic mutations, as opposed to germline events2-3. Using quantitative real-time PCR, we sought to determine if copy-number variation at TCRγ, TCRβ or TCRα was associated with childhood allergic asthma in an independent cohort of 94 cases and 455 controls using DNA from buccal swabs.

Methods

Copy-number Genotyping

Copy-number genotyping was performed using real-time quantitative PCR and commercially available reagents (Applied Biosystems) following the manufacturer’s recommendations. Cases and controls were assigned to one of five plates for genotyping using 1:5 blocked randomization. A copy-number reference assay containing two primers and a VIC and TAMRA dye-labeled probe assayed copy-number at the RNaseP locus. This was amplified in a multiplex reaction with a copy-number target assay containing two primers and a FAM dye-labeled MGB probe which targeted a region in one of the TCR genes. Plates were run on the ABI 7900HT machine using the manufacturer’s recommended PCR cycling conditions. Cycle thresholds were calculated using the SDS v2.2.2 software. Wells with a cycle threshold exceeding 32.5 for either the target or the reference probe were excluded from analysis. 549 of ΔC\textsubscript{CT} values for the target and reference assay were imported into CopyCaller™ Software version 1.0 (Applied Biosystems; Foster City, CA) and analyzed using a comparative CT (ΔΔCT) relative quantification. These continuous copy-number values can be interpreted as the average copy-number within a mosaic cell population. Two different primer sets were used to genotype TCRγ, targeting positions separated by 1730 bp.

The Mann-Whitney test (a.k.a. Wilcoxon rank-sum test) ranks copy-number values and tests whether those among cases are equal to the median copy-number value among controls.

Statistical Analysis

The primary statistical test utilized to compare copy-number values was the Mann-Whitney U test. Statistical analyses were performed using SAS version 9.1 (SAS Institute; Cary, NC). All p-values reported are two-sided.

Tests of Mendelian Inheritance

To determine if the CNVs identified in this study are inherited, or if they are somatic mutations, a subset of individuals determined to have CNVs at TCR loci and the parents of these individuals were genotyped (n = 27 trios). Lack of Mendelian consistency in CNV inheritance was considered indicative of somatic mutations, as germline CNVs are known to be stably inherited and de novo germline events are quite rare4.

Results

Genotyping results indicated that copy-number variants at these genes are largely somatic mutations, as inheritance did not show Mendelian consistency. In these mosaic cell populations, copy-number was significantly reduced among asthmatic children at both TCRγ probes (p = 0.0199 and 0.0488), but was not associated at TCRβ or TCRα (p = 0.7972 and 0.8585, respectively). These findings support the association between reduced copy-number at TCRγ and childhood allergic asthma. Our study utilized DNA derived from buccal cells and demonstrates that these somatic events are not limited to blood-derived DNA. As these are somatic mutations, our results can not determine whether reduced TCRγ copy-number is a risk factor for allergic asthma, or is an immunologic response to the disease.

Discussion

Our results support the association between childhood allergic asthma and reduced copy-number at TCRγ. Further work is needed to resolve whether these mutations predispose individuals to asthma, or whether CNVs at this position arise as a result of the disease. If they do arise as a result of the disease, it will be valuable to determine if this is a coincidental marker of the asthma phenotype, or if deletion of this gene is an immunologic response which establishes a cell population that is mosaic for TCRγ copy-number. Reduced TCRγ copy-number is expected to correlate with a reduction in the proportion of total T-cells expressing a γ6 TCR, as opposed to an αβ TCR. As these T-cell subpopulations vary in their inflammatory effects, their involvement in the etiology of allergic asthma seems plausible.

References


Table 1: Statistical analyses of copy-number genotyping results at each of three TCR genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Case</th>
<th>Control</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCRγ</td>
<td>91</td>
<td>1.98</td>
<td>1.52</td>
</tr>
<tr>
<td>TCRβ</td>
<td>90</td>
<td>2.01</td>
<td>1.79</td>
</tr>
<tr>
<td>TCRα</td>
<td>94</td>
<td>1.95</td>
<td>1.81</td>
</tr>
</tbody>
</table>

*Copy-number was determined using real-time quantitative PCR. CT values for the target and reference assay were imported into CopyCaller™ Software version 1.0 (Applied Biosystems; Foster City, CA) and analyzed using a comparative CT (ΔΔCT) relative quantification. These continuous copy-number values can be interpreted as the average copy-number within a mosaic cell population.

Table 2: Parent-child trio genotypes

<table>
<thead>
<tr>
<th>Child's genotype</th>
<th>Number of trios genotyped</th>
<th>Trios showing Mendelian inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCRβ deletion</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>TCRβ duplication</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>TCRa deletion</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>TCRγ duplication</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

*For a copy-neutral child, the only parental genotype which is inconsistent with Mendelian inheritance is a homozygous deletion. Therefore, parents of children determined to be copy-neutral were not genotyped because such trios are overwhelmingly uninformative for testing Mendelian inheritance of CNVs.

*No children with TCRγ deletions had paternal DNA available. TCRγ copy-number genotypes presented here are those determined using the first TCRγ probe (ABI primer ID Hs04326912_cn).