LYMPHOID NEOPLASIA

Genome-wide analysis links NFATC2 with asparaginase hypersensitivity

Christian A. Fernandez,1 Colton Smith,1 Wenjian Yang,1 Charles G. Mullighan,2 Chunxu Qu,2 Eric Larsen,3 W. Paul Bowman,4 Chengcheng Liu,1 Laura B. Ramsey,1 Tamara Chang,5 Seth E. Karol,5 Mignon L. Loh,6 Elizabeth A. Raetz,7 Naomi J. Winick,8 Stephen P. Hunger,9 William L. Carroll,10 Sima Jeha,5 Ching-Hon Pui,5 William E. Evans,1 Meenakshi Devidas,11 and Mary V. Relling1

1Department of Pharmaceutical Sciences, and 2Department of Pathology, St. Jude Children’s Research Hospital, Memphis, TN; 3Maine Children’s Cancer Program, Scarborough, ME; 4Department of Hematology and Oncology, Cook Children’s Medical Center, Fort Worth, TX; 5Department of Oncology, St. Jude Children’s Research Hospital, Memphis, TN; 6Department of Pediatrics, University of California School of Medicine, San Francisco, CA; 7Department of Pediatrics, University of Utah, Salt Lake City, UT; 8University of Texas Southwestern Medical Center, Dallas, TX; 9Division of Pediatric Oncology and Center for Childhood Cancer Research, Children’s Hospital of Philadelphia, Philadelphia, PA; 10Department of Pediatrics, New York University Medical Center, New York, NY; and 11Department of Biostatistics, Colleges of Medicine, Public Health & Health Professions, University of Florida, Gainesville, FL

Asparaginase is used to treat acute lymphoblastic leukemia (ALL); however, hypersensitivity reactions can lead to suboptimal asparaginase exposure. Our objective was to use a genome-wide approach to identify loci associated with asparaginase hypersensitivity in children with ALL enrolled on St. Jude Children’s Research Hospital (SJCRH) protocols Total XIIIa (n = 154), Total XV (n = 498), and Total XVI (n = 271), or Children’s Oncology Group protocols POG 9906 (n = 222) and AALL0232 (n = 2163). Germline DNA was genotyped using the Affymetrix 500K, Affymetrix 6.0, or the Illumina Exome BeadChip array. In multivariate logistic regression, the intronic rs6021191 variant in nuclear factor of activated T cells 2 (NFATC2) had the strongest association with hypersensitivity (P = 4.1 × 10−6; odds ratio [OR] = 3.11). RNA-seq data available from 65 SJCRH ALL tumor samples and 52 Yoruba HapMap samples showed that samples carrying the rs6021191 variant had higher NFATC2 expression compared with noncarriers (P = 1.1 × 10−3 and 0.03, respectively). The top ranked nonsynonymous polymorphism was rs17885382 in HLA-DRB1*07:01 allele we previously observed in a candidate gene study. The strongest risk factors for asparaginase allergy are variants within genes regulating the immune response. (Blood. 2015;126(1):69-75)

Key Points

- The rs6021191 variant in NFATC2 is associated with an increased risk of asparaginase hypersensitivity and is an expression quantitative trait locus associated with expression of NFATC2.
- Exome interrogation confirms the importance of the HLA-DRB1*07:01 allele in asparaginase hypersensitivity.
- Asparaginase is used to treat acute lymphoblastic leukemia (ALL); however, hypersensitivity reactions can lead to suboptimal asparaginase exposure. Our objective was to use a genome-wide approach to identify loci associated with asparaginase hypersensitivity in children with ALL enrolled on St. Jude Children’s Research Hospital (SJCRH) protocols Total XIIIa (n = 154), Total XV (n = 498), and Total XVI (n = 271), or Children’s Oncology Group protocols POG 9906 (n = 222) and AALL0232 (n = 2163). Germline DNA was genotyped using the Affymetrix 500K, Affymetrix 6.0, or the Illumina Exome BeadChip array. In multivariate logistic regression, the intronic rs6021191 variant in nuclear factor of activated T cells 2 (NFATC2) had the strongest association with hypersensitivity (P = 4.1 × 10−6; odds ratio [OR] = 3.11). RNA-seq data available from 65 SJCRH ALL tumor samples and 52 Yoruba HapMap samples showed that samples carrying the rs6021191 variant had higher NFATC2 expression compared with noncarriers (P = 1.1 × 10−3 and 0.03, respectively). The top ranked nonsynonymous polymorphism was rs17885382 in HLA-DRB1*07:01 allele we previously observed in a candidate gene study. The strongest risk factors for asparaginase allergy are variants within genes regulating the immune response. (Blood. 2015;126(1):69-75)

Introduction

Current acute lymphoblastic leukemia (ALL) and lymphoma therapies depend heavily upon the use of glucocorticoids and asparaginase, among other drugs. Asparaginase is a heterologous enzyme that can influence the exposure of other drugs, including glucocorticoids.1 Moreover, allergy to asparaginase can compromise the effectiveness of both asparaginase and dexamethasone by decreasing plasma exposure to both agents, and can result in a higher risk of relapse.2 Hypersensitivity reactions to asparaginase during treatment are common, and have been associated with anti-asparaginase immunoglobulin G (IgG) antibodies rather than IgE antibodies.3,4 However, recent reports indicate that patients receiving PEGylated Escherichia coli asparaginase can develop hypersensitivity reactions to the drug without any evidence of detectable antibodies; because IgE may be bound to mast cells and may be present for only a limited time period, it is possible that IgG plays a role in asparaginase-induced reactions but eludes detection.5 Therefore, multiple pathways of asparaginase hypersensitivity are possible and currently they are not well understood.

Few studies have investigated genetic risk factors for asparaginase hypersensitivity. In a study of 485 pediatric ALL patients on St. Jude Children’s Research Hospital (SJCRH) Total XV, the rs4958351 variant in the glutamate receptor gene GRIA1 was associated with asparaginase hypersensitivity.6 Our recent study investigating the role of HLA-DRB1 genes on asparaginase hypersensitivity in 1870 patients of European ancestry identified an association with the HLA-DRB1*07:01 allele.7 However, additional agnostic genome-wide studies that encompass the racial diversity of patients with ALL are required to better understand the risk and the mechanisms of asparaginase hypersensitivity.

The present study is the largest investigation of genetic predispositions to asparaginase hypersensitivity and includes 3308 patients of diverse ancestry. We used a genome-wide association approach,
including a focus on exonic variants in addition to noncoding variants, to identify genetic loci associated with asparaginase hypersensitivity.

Materials and methods

Patients

Asparaginase hypersensitivity was assessed in children with ALL enrolled on SJCRH protocols Total XIIIA (n = 154), Total XV (n = 498), ClinicalTrials.gov #NCT00137111, and Total XVI (n = 271), ClinicalTrials.gov #NCT00549848 or Children’s Oncology Group (COG) protocols POG 9906 (n = 222, ClinicalTrials.gov #NCT00056033) and AALL0232 (n = 2163, ClinicalTrials.gov #NCT00075725). Informed consent from the patients or guardians, and consent from the patients as appropriate were obtained according to Institutional Review Board guidelines for genomic research and for treatment. The schedules and doses of asparaginase used during treatment varied by protocol and have been described elsewhere. The association between HLA variants and asparaginase allergy among patients of European ancestry enrolled in these studies has been reported previously (see supplemental Table 1, available on the Blood Web site), and we have previously reported an analysis of standard noncoding “GWAS” array single nucleotide polymorphisms (SNPs) for allergy for a subset of the Total XV patients (supplemental Table 1). Patients enrolled on protocols Total XIIIA, Total XV, and POG 9906 received native E. coli asparaginase (26%), whereas patients on Total XVI and AALL0232 received PEGylated E. coli asparaginase (74%). Hypersensitivities were graded using the scales described in the National Cancer Institute’s common toxicity criteria version 1.0 for Total XIIIA, version 2.0 for POG 9906, version 3.0 for Total XV and Total XVI, and version 4.0 for COG AALL0232. Hypersensitivity reactions of grade 2 and above were considered cases. The clinical symptoms of reactions grade 2 and above can include rash, flushing, urticaria, fever ≥38°C, symptomatic bronchospasm, and anaphylaxis.

Genotyping

Germline DNA was genotyped using the Affymetrix Human Mapping 500K Array Set, the Affymetrix Genome-Wide Human SNP Array 6.0, or the Illumina Exome BeadChip array. Hardy-Weinberg equilibrium tests were performed using PLINK in patients of European ancestry. SNPs with genotyping call rates <95% and SNPs that were not in Hardy-Weinberg equilibrium (P < .001 for SNPs with a minor allele frequency [MAF] ≥1%) were excluded from the association analysis. The genetic ancestry of patients was estimated using STRUCTURE as previously described, with the European-ancestry group defined as having ≥90% Northern European ancestry (CEU), the Asian-ancestry group defined as having ≥90% East Asian ancestry (CHB/JPT), the African-ancestry group defined as having ≥70% West African ancestry (YRI), the Hispanics defined as having Native American ancestry ≥10% and whose Native American ancestry was greater than their % of African ancestry, and finally, others whose ancestry was outside the above boundaries. Affymetrix genotyping was available for 94% of patients with asparaginase hypersensitivity data (supplemental Table 1). Illumina exome array genotyping was available for 95% of the patients in the combined patient cohort (supplemental Table 1). Overall, 90% of the ALL patients with asparaginase hypersensitivity data had genotyping available from both platforms (supplemental Table 1).

Nuclear factor of activated T cells 2 (NFATC2) gene expression analysis

NFATC2 gene expression was assessed in ALL tumor samples from patients enrolled on SJCRH protocols Total XV (n = 57) and Total XVI (n = 8). RNA-seq was performed as previously described. Expression levels of NFATC2 were estimated as fragment per kilobase of transcript per million mapped reads (FPKM), and gene FPKM values were computed by summing the transcript FPKM values for each gene as described previously. NFATC2 gene expression and genotyping was also available for 52 unrelated Yoruba (YRI) HapMap samples (GSE9703). NFATC2 expression levels in HapMap YRI samples were estimated by adding the hybridization signals of probes within spliced messenger RNA regions of the gene.

Data analysis

Univariate and multivariate logistic regression was used to identify clinical risk factors associated with asparaginase hypersensitivity. The association analysis between SNP genotypes and asparaginase hypersensitivity reactions was performed using logistic regression, adjusting for significant covariates and assuming an additive genetic model. Genotype and phenotype association analysis was performed in PLINK. The association analysis between NFATC2 gene expression and rs6021191 genotyping were performed using a general linear model with R statistical software (version 2.13.2).

Pathway analysis using ingenuity

Ingenuity Pathway Analysis (IPA) was used to prioritize biological pathways related to asparaginase hypersensitivity reactions within the genome-wide analysis. Genes containing interrogated SNPs with the strongest associations (lowest 0.1% P values, with P ≤ 1.1 × 10^-5) to asparaginase hypersensitivity were included in the pathway analysis (334 genes representing 499 SNPs; supplemental Table 2). Fisher’s exact test was used to determine if the genes most associated with asparaginase hypersensitivity were over-represented in each canonical pathway.

Results

Hypersensitivity reaction data to native E. coli asparaginase or PEGylated E. coli asparaginase were available from a total of 3308 pediatric ALL patients enrolled on 5 different protocols (Table 1). Their median age was 9.3 years (range, 0.17 to 30), there were slightly more males than females (56.2% vs 43.8%; Table 1), and 59.5% of the genotyped patients were of European genetic ancestry (Table 1).

A total of 589 patients had asparaginase hypersensitivity reactions (17.8%; Table 2). By univariate analysis both treatment protocol (P = 2.1 × 10^-51; Table 2) and asparaginase formulation (P = 2.1 × 10^-50; Table 2) were associated with hypersensitivity. Protocols including native E. coli asparaginase had a higher frequency of hypersensitivity than those using PEGylated E. coli asparaginase (35.1% vs 11.6%; Table 2). Age <10 years, ancestry, and B-cell ALL were also associated with the risk of hypersensitivity (Table 2). In multivariate analysis including treatment protocol, gender, age group, ancestry, and ALL lineage (Table 2), only protocol (P = 2.3 × 10^-46), gender (P = .04), and ALL lineage (P = .003) were associated with asparaginase hypersensitivity. Patients with T-cell ALL were only enrolled on SJCRH protocols; within the standard/high-risk arms of these studies, there were more asparaginase hypersensitivity reactions among patients with B-lineage compared with those with T-cell ALL receiving identical treatment (supplemental Figure 1; P = .001). Asparaginase preparation was confounded with protocol, but it also associated with hypersensitivity in multivariate analysis (P = 7.2 × 10^-45) when removing protocol as a covariate from the regression model. To identify genomic variants associated with asparaginase hypersensitivity, a multivariate logistic regression model adjusted for treatment arm, ancestry (as a categorical covariate), gender, age, and ALL lineage was used. The minor allele of an intronic polymorphism in NFATC2 (rs6021191) was associated with a higher risk of hypersensitivity at the genome-wide significance threshold (P = 4.1 × 10^-8), odds ratio [OR] = 3.11; Figure 1A-B). Focusing on nonsynonymous SNPs, the minor allele at rs17885382 in the HLA-DRB1 gene (P = 3.23 × 10^-26, OR = 1.63; Figure 1A,C) had the strongest association with asparaginase hypersensitivity.
Table 1. Demographics of ALL patients with asparaginase hypersensitivity data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SJCRH total XIIIA (%)</th>
<th>SJCRH total XV (%)</th>
<th>SJCRH total XVI (%)</th>
<th>POG 9906 (%)</th>
<th>COG AALL0232 (%)</th>
<th>Combined (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>154 (4.7%)†</td>
<td>498 (15.1%)†</td>
<td>271 (8.2%)†</td>
<td>222 (6.7%)†</td>
<td>2163 (65.4%)†</td>
<td>3308†</td>
</tr>
<tr>
<td>Age (n ≥ 10, mean age)</td>
<td>7.1</td>
<td>7.0</td>
<td>7.2</td>
<td>11.2</td>
<td>10.1</td>
<td>9.3</td>
</tr>
<tr>
<td>Gender (n, Male)</td>
<td>90 (58.4%)†</td>
<td>279 (56.0%)†</td>
<td>156 (57.6%)†</td>
<td>150 (67.6%)†</td>
<td>1183 (54.7%)†</td>
<td>1858 (56.2%)†</td>
</tr>
<tr>
<td>Ancestry</td>
<td>126 (25.3%),†</td>
<td>73 (26.9%),†</td>
<td>146 (66.8%),†</td>
<td>1372 (63.4%),†</td>
<td>1760 (53.2%),†</td>
<td>9.3</td>
</tr>
<tr>
<td>European</td>
<td>99 (75.6%)†</td>
<td>322 (66.1%)†</td>
<td>187 (69.5%)†</td>
<td>122 (55.0%)†</td>
<td>1,215 (56.2%)†</td>
<td>1959 (59.5%)†</td>
</tr>
<tr>
<td>Hispanic</td>
<td>11 (8.4%)†</td>
<td>66 (13.6%)†</td>
<td>21 (7.8%)†</td>
<td>64 (28.8%)†</td>
<td>560 (25.9%)†</td>
<td>725 (22.0%)†</td>
</tr>
<tr>
<td>African</td>
<td>19 (17.6%)†</td>
<td>72 (14.8%)†</td>
<td>11 (4.1%)†</td>
<td>14 (6.3%)†</td>
<td>134 (6.2%)†</td>
<td>254 (7.7%)†</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0%)†</td>
<td>6 (1.2%)†</td>
<td>1 (0.4%)†</td>
<td>4 (1.8%)†</td>
<td>42 (1.9%)†</td>
<td>53 (1.6%)†</td>
</tr>
<tr>
<td>ALL lineage§</td>
<td>130 (85.5%)†</td>
<td>423 (84.9%)†</td>
<td>225 (83.0%)†</td>
<td>222 (100.0%)†</td>
<td>2153 (100.0%)†</td>
<td>3153 (95.6%)†</td>
</tr>
</tbody>
</table>

*The percentage of patients enrolled within each protocol relative to the total number of patients enrolled across all protocols.
†The percentage of patients with the characteristic described in the first column.
‡The genetic ancestry of patients with available genotyping was estimated using STRUCTURE, with numbers of patients indicated for those in the European-ancestry group defined as >90% Northern European ancestry (CEU), Asian-ancestry group as >90% East Asian ancestry (CHB/JPT), and African-ancestry group as >70% West African ancestry (YRI), Hispanics as >10% Native American ancestry and greater than % of African ancestry, and others as those outside the above boundaries.
§ALL lineage refers to the ALL immunophenotype; patients without B-lineage ALL had T-cell ALL and were only enrolled on SJCRH treatment protocols.

The MAF for the NFATC2 rs6021191 variant was highest in patients of non-European ancestry (0.062; supplemental Table 3), and only 0.001 among patients of European ancestry, MAFs that are consistent with those reported in the general population (supplemental Table 3). The rs6021191 variant was associated with hypersensitivity across all non-European ancestries (supplemental Table 3), with the largest effect size seen in those with primarily Asian ancestry (OR = 8.71; supplemental Figure 2A), followed by those with African ancestry (OR = 4.45; supplemental Figure 2A). The association was also present across all protocols (supplemental Figure 2B) and with both asparaginase preparations (supplemental Figure 2C).

The NFATC2 rs6021191 variant is located in an intron of the gene and it is in a region with weak enhancer activity in HUVEC and K562 cell lines, suggesting that the SNP may influence the expression of NFATC2. We found higher NFATC2 gene expression in both ALL tumor samples and in YRI HapMap lymphoblastoid cell line samples carrying the rs6021191 variant allele (P = 1.1 × 10−3 and 0.03, respectively; Figure 2A-B), indicating that the SNP is an expression quantitative trait locus (eQTL).

Table 2. Analysis of covariates associated with asparaginase hypersensitivity

<table>
<thead>
<tr>
<th>Covariate</th>
<th>% Asparaginase allergy*</th>
<th>Univariate P value</th>
<th>Multivariate P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol (%) of cases</td>
<td>Total XV: 204 (41.0%)</td>
<td>2.1 × 10−51</td>
<td>2.3 × 10−46</td>
</tr>
<tr>
<td></td>
<td>Total XIII: 50 (32.5%)</td>
<td>21.6 (13.6%)</td>
<td>64 (28.8%)</td>
</tr>
<tr>
<td></td>
<td>POG 9906: 53 (23.9%)</td>
<td>122 (55.0%)</td>
<td>1,215 (56.2%)</td>
</tr>
<tr>
<td></td>
<td>SJCRH total XVI: 38 (14.0%)</td>
<td>14 (6.3%)</td>
<td>134 (6.2%)</td>
</tr>
<tr>
<td></td>
<td>COG AALL0232: 244 (11.3%)</td>
<td>6 (1.2%)</td>
<td>4 (1.8%)</td>
</tr>
<tr>
<td></td>
<td>Combined: 589 (17.8%)</td>
<td>42 (1.9%)</td>
<td>212 (9.8%)</td>
</tr>
<tr>
<td>Asparaginase preparation‡</td>
<td>E coli asparaginase: 307 (35.1%)</td>
<td>2.1 × 10−50</td>
<td>7.2 × 10−45</td>
</tr>
<tr>
<td></td>
<td>PEGylated E coli asparaginase: 282 (11.6%)</td>
<td>.06</td>
<td>.04</td>
</tr>
<tr>
<td>Gender (%) of cases</td>
<td>Male: 351 (18.9%)</td>
<td>.06</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>Female: 238 (16.4%)</td>
<td>.06</td>
<td>.04</td>
</tr>
<tr>
<td>Age (&lt;10, ≥10) (%) of cases</td>
<td>&lt;10: 314 (20.3%)</td>
<td>3.3 × 10−4</td>
<td>.11</td>
</tr>
<tr>
<td></td>
<td>≥10: 273 (15.5%)</td>
<td>2.1 × 10−4</td>
<td>.07</td>
</tr>
<tr>
<td>Ancestry§ (%) of cases</td>
<td>African: 51 (20.1%)</td>
<td>2.1 × 10−4</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>European: 385 (19.7%)</td>
<td>2.1 × 10−4</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>Hispanic: 102 (14.1%)</td>
<td>2.1 × 10−4</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>Other: 40 (13.2%)</td>
<td>2.1 × 10−4</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>Asian: 4 (7.5%)</td>
<td>2.1 × 10−4</td>
<td>.07</td>
</tr>
<tr>
<td>ALL lineageⅨ (%) of cases</td>
<td>B-lineage: 363 (33.8%)</td>
<td>5.3 × 10−4</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>T-lineage: 27 (18.8%)</td>
<td>5.3 × 10−4</td>
<td>.003</td>
</tr>
</tbody>
</table>

*Percent asparaginase allergy refers to the percentage of cases within each category that had asparaginase hypersensitivity reactions.
†Multivariate P values refer to the association of each covariate with treatment arm, gender, age, ancestry, and ALL lineage included in the model as categorical covariates.
‡Asparaginase preparation was confounded with protocol, therefore the multivariate P value for asparaginase was estimated by removing protocol from the model.
§Ancestry was estimated using genotyped SNPs.
ⅨALL lineage refers to the ALL immunophenotype.
ⅨUnivariate P value for ALL lineage was calculated only for the SJCRH cohort because T-ALL patients were only enrolled within those protocols; all COG protocols were B-lineage ALL only; see also supplemental Figure 1.
The MAF for the *HLA-DRB1* rs17885382 variant was highest in patients of European ancestry (MAF = 0.125; supplemental Table 4) and present in all non-European ancestries at a MAF $\geq 0.05$ (supplemental Table 4). The effect size was most pronounced in patients of European ancestry ($P = 5.9 \times 10^{-5}$, OR = 1.68; supplemental Figure 3A), but elevated in other ancestries, for all treatment protocols, and for both asparaginase preparations (supplemental Figure 3A-C).

The rs17885382 variant is located in exon 2 of the *HLA-DRB1* gene (Arg $\rightarrow$ Gln, 54). Our previous candidate gene analysis of HLA alleles associated with asparaginase allergies in patients of European ancestry identified an association with 10 amino acids in *HLA-DRB1*, and position 54 (amino acid position 25 when excluding the leader signal sequence) was among those implicated. The rs17885382 variant is in linkage disequilibrium with the *HLA-DRB1* *rs70191* allele ($R^2 = 0.94$); therefore, it is likely that the haplotype is driving the association between rs17885382 and asparaginase hypersensitivity.

In a multivariate analysis, including both *rs6021191* (*NFATC2*) and rs17885382 (*HLA-DRB1*) genotype, both variants remained associated with asparaginase hypersensitivity ($P_{rs6021191} = 1.8 \times 10^{-8}$, $P_{rs17885382} = 3.2 \times 10^{-10}$), and modest increases in effect size were seen after
Other variants in activity because a multiethnic cohort was available for our investigation. (supplemental Table 3); nevertheless, we were able to identify the as-phenotypes, such as narcolepsy, self-reported allergies (cat, pollen, NFATC2 associated with higher NFATC2 messenger RNA expression (Figure 2A-B). 20 pathways included, T-cell activation, or T-cell disorders (Figure 4A). Genes within the top hypersensitivity, 13 pathways involved T-cell apoptosis, T-cell signal-play a role in the development of asparaginase hypersensitivity. Moreover, among individuals with Down syndrome, the inhibition of NFAT transcription factor family. Upon T-cell receptor stimula-tion, cytoplasmic NFATC2 is dephosphorylated and translocated to the nucleus where it participates in gene regulation. The role of NFATC2 on the risk of drug-induced allergy is unknown, yet studies have shown that NFATC2 can influence the development and function of regulatory T cells and can have either a negative or positive regulatory influence on the immune response, depending on the antigen. Nevertheless, several studies using NFAT inhibitors for different immune-related disease models support that inhibition of the NFAT pathway can attenuate an immune response. Moreover, among individuals with Down syndrome, the inhibition of

Discussion

Asparaginase is a mainstay in the treatment of ALL, but immune re sponses to asparaginase can decrease the systemic exposure to aspar aginase and result in a higher risk of relapse. In this study, we used a genome-wide association approach to identify genetic loci associated with asparaginase hypersensitivity within a racially diverse set of pediatric patients with ALL. We found that the rs6021191 variant is associated with a higher risk of asparaginase hypersensitivity at the genome-wide significance level (Figure 1A-B), and the variant is associated with higher NFATC2 messenger RNA expression (Figure 2A-B). The NFATC2 variant was rare in patients of European ancestry (supplemental Table 3); nevertheless, we were able to identify the association between the NFATC2 variant and asparaginase hypersensitivity because a multietnic cohort was available for our investigation. Other variants in NFATC2 have also been related to immune-mediated phenotypes, such as narcolepsy, self-reported allergies (cat, pollen, and dust-mite allergy), and thiazolidinedione-induced edema. Interestingly, both self-reported allergies and narcolepsy also have associations with HLA genetic factors. The NFATC2 gene encodes a cytoplasmic component of the NFAT transcription factor family. Upon T-cell receptor stimula-tion, cytoplasmic NFATC2 is dephosphorylated and translocated to the nucleus where it participates in gene regulation. The role of NFATC2 on the risk of drug-induced allergy is unknown, yet studies have shown that NFATC2 can influence the development and function of regulatory T cells and can have either a negative or positive regulatory influence on the immune response, depending on the antigen. Nevertheless, several studies using NFAT inhibitors for different immune-related disease models support that inhibition of the NFAT pathway can attenuate an immune response. Moreover, among individuals with Down syndrome, the inhibition of

Figure 3. Patients carrying both the HLA-DRB1 rs17885382 and NFATC2 rs6021191 variant have a higher risk of developing asparaginase hypersensitivity compared with patients with no risk variant. The risk of developing hypersensitivity was determined for patients carrying a single-risk variant (HLA-DRB1 rs17885382 or NFATC2 rs6021191) or for patients carrying both risk variants (NFATC2 rs6021191 and HLA-DRB1 rs17885382). The risk of hypersensitivity was higher in patients carrying a single variant (ORrs6021191 = 3.07, CIrs6021191 = 1.87-4.94, ORrs17885382 = 5.4, CIrs17885382 = 1.2-10) compared with patients with no risk alleles. The associations were determined using a general linear model adjusted for treatment, ALL immuno-phenotype, gender, age group, and ancestry.

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hypersensitivity by showing that HLA-DRB1*07:01 is also a risk allele in non-European patients (supplemental Figure 3). Furthermore, we show that the association between the HLA-DRB1 rs17885382 variant is independent from the NFATC2 rs6021191 variant and that patients carrying both alleles have a higher risk of developing asparaginase hypersensitivity compared with patients with no risk alleles or with a single risk allele (Figure 3). Interestingly, the GRIA1 rs4958351 variant that we previously found to associate with asparaginase hypersensitivity6 was also associated with the risk of asparaginase hypersensitivity in our combined patient cohort (P = .03; OR = 1.2). However, the risk of allergy was stronger in patients receiving native E coli asparaginase (OR = 1.55) than in patients receiving PEGylated E coli asparaginase (OR = 1.02).

In conclusion, we have identified that the NFATC2 rs6021191 variant is an eQTL that is associated with the risk of developing asparaginase hypersensitivity. This is the first study to identify an association between NFATC2 and a drug-induced hypersensitivity.

Figure 4. Genes involved in T-cell function may contribute to the risk of developing asparaginase hypersensitivity reactions. IPA was used to determine if genes most associated with asparaginase hypersensitivity were overrepresented in specific biological pathways. A total of 334 genes involved by 499 SNPs associated with asparaginase hypersensitivity annotated to 234 distinct IPA pathways. (A) The top 20 pathways identified, all with P < 3 × 10^{-2}, were enriched for genes involved in T-cell apoptosis, T-cell signaling, T-cell activation, or T-cell disorders. (B) A total of 33 genes containing SNPs associated with hypersensitivity were included within the top 20 canonical pathways (shown on x-axis). Many of these genes were included in multiple pathways. The number of pathways (out of 20) in which each of these 33 genes is involved is plotted on the y-axis. For example, HLA-DRB1 is involved in 14 out of the 20 top canonical pathways. GPCR, G-protein coupled receptor; nNOS, neuronal NOS; IL, interleukin; iCOS, inducible T-cell costimulator and its ligand, iCOSL.
Moreover, the results from our single SNP and pathway analyses suggest that T cells play an important role in the development of asparaginase hypersensitivity, likely due to their role in B-cell activation and differentiation.

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Correspondence: Mary V. Relling, St. Jude Children’s Research Hospital, 262 Danny Thomas Place, Mail Stop 313, Memphis, TN 38105; e-mail: mary.relling@stjude.org.

References


Genome-wide analysis links \textit{NFATC2} with asparaginase hypersensitivity