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Genetic variations in *GRIA1* on chromosome 5q33 related to asparaginase hypersensitivity

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Abstract

The genetic variations that lead to asparaginase allergy are unknown. We interrogated over 500,000 single nucleotide polymorphisms (SNPs) in 485 children with acute lymphoblastic leukemia (ALL): 322 in a discovery and 163 in a validation cohort. From the top 100 SNPs associated with allergy in the discovery cohort, chromosome 5 was overrepresented compared to other chromosomes ($p = 0.00032$), hosting 10 SNPs annotated to genes. Among these 10 SNPs, one SNP (rs4958381), in *GRIA1* on chromosome 5q33, replicated in the validation cohort ($p = 1.8 \times 10^{-5}$, 2.9×10^{-3} , and 3.5×10^{-7} in the discovery, validation, and combined cohorts, respectively). Four additional SNPs annotated to *GRIA1* were also significantly associated with allergy ($p < 0.05$) in both cohorts. Chromosome 5q33 has previously been associated with asthma and atopy. These data contribute to the growing body of evidence that there is an inherited component to predisposition to drug allergy.

Keywords

pharmacogenomics; acute lymphoblastic leukemia; asparaginase; allergy

Introduction

Asparaginase is an effective antileukemic agent that has been used to treat childhood acute lymphoblastic leukemia (ALL). (1–3) Commercial formulations comprise asparaginase isolated from *Escherichia coli* or *Erwinia chrysanthemi*. Because asparaginase is a foreign

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protein, hypersensitivity reactions are common, occurring in up to 45% of patients.(4–14) Factors that may affect the incidence of allergy include the number of asparaginase injections, read ministration after a hiatus, and concurrent chemotherapy.(5,10,11,15–17) These reactions usually necessitate discontinuation of the primary formulation of asparaginase (usually *E. coli*) and subsequent substitution with another asparaginase formulation (usually *Erwinia*). However, many patients react to both formulations. Also, asparaginase hypersensitivity may attenuate its enzymatic activity and thus its antileukemic effect.(8,18,19)

Although there are numerous reported investigations of genetic predisposition to drug hypersensitivity, the vast majority of such studies have been negative.(20) Two of the most notable exceptions are the links between hypersensitivity reactions to carbamazepine and to abacavir and HLA polymorphisms.(20–24) Reactions to asparaginase occur much more commonly than do reactions to agents such as abacavir and carbamazepine, and it has not been known if there is a genetic basis to interindividual differences in asparaginase hypersensitivity. Our objective was to identify germline genetic variations contributing to the risk of asparaginase allergy in children with newly diagnosed ALL, using a genome-wide approach.

Results

Of 498 patients, 204 (41%) developed an allergic reaction to asparaginase. The majority (84%) of the allergic reactions were grade 2, 15% were grade 3, and only 1 (0.5%) was grade 4 (Supplemental Table S1). A total of 485 patients have genotyping data available and were included in the analysis.

Of the clinical variables including sex, age at diagnosis, racial ancestry, and risk arm, a low percentage of American Indian ancestry ($p = 0.016$) and assignment to the low risk treatment arm ($p = 0.041$) were significantly associated with the risk of asparaginase allergy. These features remained as independent risk factors in a multivariate model ($p = 0.014$ and 0.028 , respectively) (Table 1).

We analyzed the association between germline 364,033 SNP genotypes and asparaginase allergy in the discovery cohort of 322 patients (Supplemental Figure S1, Supplemental Table S2). From the top ranked 100 SNPs in the discovery cohort, 55 SNPs were within genes and chromosome 5 hosted 10 of these in-gene SNPs (Figure 1A and Supplemental Table S2). After normalizing for the number of SNPs interrogated on the Affymetrix SNP chip, there was a significant over-representation of chromosome 5 (Figure 1B) ($p = 0.00032$). Interestingly, all 10 SNPs on chromosome 5 (Table 2) were located at 5q33 region, a region that has been associated with asthma and atopy.(25–30)

We tested whether these 10 polymorphisms were associated with asparaginase allergy in the validation cohort. Only one SNP (rs4958381), located in *GRIAI*, was significantly associated with asparaginase allergy ($p = 2.9 \times 10^{-3}$) (Table 2, Supplemental Table S3). In the combined cohort, the SNP genotypes at rs4958381 were significantly associated with asparaginase allergy at $p = 3.5 \times 10^{-7}$. Focusing on *GRIAI*, there were 11 additional SNPs in *GRIAI* that were significantly associated with asparaginase allergy at $p < 0.05$ in the discovery cohort, 4 of which (rs10070447, rs6890057, rs4958676, and rs6889909) were significantly associated in the validation cohort (Table 3, Supplemental Figure S2). In the combined cohort, 9 SNPs in *GRIAI* were significantly associated with asparaginase allergy ($p < 0.05$, Table 3, Supplemental Table S3). An example is shown for rs4958381 (Figure 2); there was a monotonic relationship between the number of copies of the A allele and the risk of asparaginase allergy in patients on the low risk and standard/high risk treatment arms.

Overall, the cumulative incidence of asparaginase allergy was 74%, 44%, and 32% for patients with the AA, AG, or GG genotypes, respectively. There was not an association between these genotypes and the severity of asparaginase allergy ($\geq$ grade 2 vs. $<$ grade 2, $p = 0.61$, by linear regression model).

Discussion

Asparaginase is an effective drug in the treatment of ALL. A major toxicity of L-asparaginase is allergy, which may attenuate the pharmacological effect of the drug. In the present study, we used a genome-wide interrogation that identified germline genetic variations in *GRIA1*, located at 5q33, that were associated with asparaginase allergy in both discovery and validation cohorts. The 5q33 locus has been previously associated with asthma and atopy.(25–30)

Clinical symptoms of asparaginase allergy include localized pain, fever, skin rash, urticaria, respiratory distress, and anaphylaxis. In the present study, 172 patients (84%) had grade 2 reaction, 31 (15%) had grade 3 reaction, and only 1 (0.5%) had grade 4 reaction. Many of the asparaginase allergic reactions are typical of type I hypersensitivity, which is also a hallmark of asthma. In the present study, we found that SNPs on chromosome 5q33 were overrepresented as associated with asparaginase allergy (Figure 1). Chromosome 5q31–33 contains a cluster of cytokine and other immune-related genes such as interleukin (IL)-4, IL-13, IL-5; this region has been mapped as a susceptibility locus for several inflammatory or autoimmune diseases, including asthma.(25–30) Our findings may support that drug allergy and asthma share a range of candidate genes.(31)

GRIA1 encodes a subunit of the AMPA receptor, a tetrameric ligand-gated ion channel that transmits glutamatergic signals in the brain. The same polymorphisms of *GRIA1* that we found associated with asparaginase allergy are associated with schizophrenia and bipolar disease.(32,33) All the genetic variations we identified in *GRIA1* were in intronic sequences. Interestingly, intronic polymorphisms in the glutamate receptor subunit *GluR2* direct RNA editing of the *GluR2* coding sequence,(34) suggesting a mechanism by which intronic variations could affect gene function. Furthermore, it has recently been shown that glutamate not only has a role as a neurotransmitter, but also as an immunomodulator.(35,36) To our knowledge, this is the first direct link between *GRIA1* polymorphisms and an immune-related phenotype.

Forty-one percent of the 498 patients who were treated with Total Therapy XV protocol had a hypersensitivity reaction to asparaginase, which is consistent with the reported frequency in children (up to 45%).(4–14) The incidence of allergic reactions was very low within 100 days after treatment was initiated (Figure 2), consistent with exposure to a relatively small number of doses, but reactions could have been attenuated by immunosuppression associated with early concomitant chemotherapy,(5,10,15–17) particularly steroids. The majority of the reactions occurred during continuation or reinduction therapy. Interestingly, the occurrence of reactions differed between low risk arm and standard/high risk arm, which may be related to intermittent use in the low-risk arm compared to the weekly administration of asparaginase in the standard/high risk arm (Supplemental Table S3). Our findings support that the risk of hypersensitivity reactions may increase with read ministration after a period of no asparaginase therapy for at least a month.(14,15)

We found that the frequency of asparaginase allergy was significantly associated with racial ancestry (Table 1), consistent with a previous report that white children had a higher frequency of allergic reactions than either black or Hispanic patients.(37) Our catchment area is racially and ethnically diverse, and for this study, we used genomic variation to

define ancestral background of all patients. Involvement of variations in *HLA* genes or other immunoregulatory factors was suggested.(37) Genetic variation in *HLA-B*5701* has indeed been shown to be significantly associated with hypersensitivity to an unrelated medication, abacavir, (22–24) and there are some data indicating racial differences for the association of *HLA-B*5701* and abacavir hypersensitivity.(38,39) Interestingly, the prevalence of asthma has been shown to be higher among black children and among children from certain Hispanic ethnic subgroups.(40–42) In genetic studies of asthma, some genetic variations were found to be associated with certain population subgroups,(43–46) which indicates that the genetic risk factors in allergic diseases can vary with race and ethnicity.

In conclusion, we identified genetic variations in the glutamate receptor *GRIA1* on chromosome 5q33 was associated with risk of asparaginase allergy. These findings add to these indicating a role of 5q genes in immune responses, and contribute to the data showing the importance of inherited variation on risk of drug allergy.

Materials and Methods

Patients and treatment regimen

Of the 498 patients treated on St. Jude Children's Research Hospital treatment protocol Total Therapy XV for newly diagnosed childhood ALL, we included all patients who were evaluable for asparaginase allergy and who had evaluable DNA (n=485). The study had approval of the institutional review board. Informed consent from the parent or guardian and age-appropriate assent from the patient were obtained at the time of study enrollment. Risk assignment (low, standard, or high risk) and the treatment regimen were described previously.(47) In brief, the remission induction therapy contained daily prednisone for 28 days, weekly vincristine for 4 doses, weekly daunorubicin for 2 doses, thrice weekly native *E. coli* asparaginase (Elspar, 10,000 IU/m²) for 6 doses, and triple intrathecal therapy. Additional asparaginase (Elspar for 3 doses, 10,000 IU/m²) was administered based on status of minimal residual disease on day 19. Subsequent induction therapy consisted of cyclophosphamide, mercaptopurine, and cytarabine. Consolidation therapy consisted of high-dose methotrexate, mercaptopurine, and triple intrathecal therapy. Asparaginase treatment differed markedly by risk arm in the first 20 weeks of continuation therapy (Supplemental Table S4). Standard/high risk cases received asparaginase (25,000 IU/m²) weekly during week 1–20; low risk cases only received asparaginase (10,000 IU/m²) thrice weekly during both reinductions (week 7–9 and week 17–20).

Phenotyping of asparaginase allergy

Hypersensitivity reactions to asparaginase were characterized by local manifestations at the injection site and systemic manifestations (erythema, swelling urticaria, rash, pruritus, tachypnea, and wheezing). Asparaginase allergy was graded (grade 1, 2, 3, or 4) using the National Cancer Institute Common Toxicity Criteria version 2.0. The phenotype of interest was the first episode of allergy to asparaginase (which was always to native *E. Coli*).

Genotyping and Quality Control

DNA was extracted from normal peripheral blood leukocytes, digested with restriction enzymes, amplified, labeled, and hybridized to the Affymetrix GeneChip Human Mapping 500K sets or SNP 6.0 (Affymetrix, Santa Clara, California) as described.(48) SNPs with call rates less than 95% and minor allele frequency less than 5% were filtered out.

SNP genotypes were coded according to the number of B alleles in the genotype call, with the AA, AB, and BB genotype calls coded as 0, 1, and 2, respectively. SNPs were annotated to genes if they were within 5Kb of the transcription start site and transcription 3' end.

Genome-wide analysis for asparaginase allergy

We assigned all the patients into 2 cohorts – discovery and validation cohorts – by using a balanced stratified approach,(49) balancing age, sex, treatment arm, race, and the presence of asparaginase allergy. The discovery cohort comprised 67% of patients, and the validation cohort comprised 33% of patients (Supplemental Table S5). Using this approach, given our sample size, we performed post-hoc power calculations to estimate our power to detect a polymorphism associated with the risk of allergy (details in Supplemental Table S6); for example, with a hazard ratio of 1.8, and a minor allele frequency of 0.27, power is estimated at 99.7% in the discovery cohort and 67% in the validation cohort, at an alpha level of 0.005. Population ancestry was estimated using STRUCTURE(50) based on SNP genotypes with HapMap phase II samples and publicly available genotype data of American Indians(51) as reference populations. Population ancestries were included as covariates, treated with continuous values (percentage of European, African, Asian or American Indian ancestries) in the association analysis between asparaginase allergy and genotypes.

First, we computed the statistical significance using Gray's test for each SNP genotype's association with asparaginase allergy in the discovery cohort, stratifying for risk arm and adjusting for racial ancestry. Among the top significant SNPs (ranked by *p* value), we examined whether there was overrepresentation of any chromosomes by dividing the number of in-gene SNPs from the top 100 ranked SNPs by the number of in-gene SNPs on each chromosome interrogated by the Affymetrix SNP chip. Second, we tested the significance of the genotypes of SNPs of interest from the discovery cohort in the validation cohort. Third, we analyzed the significance of the relationship between the cumulative incidence of asparaginase allergy for the SNPs of interest in the combined cohort by Gray's test. Time at risk was censored at the end of re-induction II treatment (last asparaginase exposure) or at the date of allergic reaction. Patients who underwent bone marrow transplant or were off-study before they reached re-induction II were censored at their off study date. Patients who relapsed or died were censored at time of that event, which was treated as a competing event in the estimation of cumulative incidence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. The number of in-gene SNPs ($n = 55$) in each chromosome among the top 100 SNPs associated with asparaginase allergy in the discovery cohort (A) unadjusted and (B) after normalization for the number of SNPs interrogated on each chromosome on the Affymetrix SNP chip.

Figure 2.

The cumulative incidence of asparaginase allergy was compared by Gray test (stratified for treatment arms) for each genotype at rs4958381 in *GRIA1* in the combined entire cohort.

Table 1

Multivariate analysis of factors associated with asparaginase allergy

Clinical features	No. (%)	Hazard ratio (95% CI)	p value
Age at diagnosis			
≤ 10 years old	361 (74)	–	
>10 years old	124 (26)	1.14 (0.78 – 1.67)	0.51
Sex (male vs. female)			
Female	216 (45)	–	
Male	269 (55)	1.20 (0.89 – 1.61)	0.24
Risk arm (low risk vs. standard/high risk)*			
Standard/High Risk	249 (51)	–	
Low Risk	236 (49)	1.45 (1.04 – 2.03)	0.028
Racial ancestry [#]			
White		–	
Black		0.74 (0.45 – 1.22)	0.25
Asian		0.35 (0.05 – 2.41)	0.29
American Indian ^{\$}		0.20 (0.06 – 0.72)	0.014

[#] Determined by the STRUCTURE program.^{*} Also significant in univariate analysis ($p = 0.041$, hazard ration = 1.36).^{\$} Also significant in univariate analysis ($p = 0.016$, hazard ration = 0.20).

Table 2

In-gene SNPs from chromosome 5 (n = 10) from the top 100 SNPs associated with asparaginase allergy in the discovery and validation cohorts.

SNP#	Chromosome	Location [§]	Gene	Alleles [%]	Risk Allele Frequency	Discovery cohort (n = 322) p value ^{&}	Hazard ratio (95% CI) [*]	Validation cohort (n = 163) p value ^{&}	Hazard ratio (95% CI) [*]
rs4958351	5	153150567	GRIA1	G/A	0.27	1.8×10^{-5}	1.73 (1.35 – 2.21)	0.0029	1.93 (1.25 – 2.98)
rs11167667	5	153634138	GALNT10	T/C	0.58	2.1×10^{-5}	1.81 (1.38 – 2.38)	0.29	0.82 (0.57 – 1.18)
rs12523441	5	153634860	GALNT10	T/C	0.58	2.1×10^{-5}	1.81 (1.38 – 2.38)	0.29	0.82 (0.57 – 1.18)
rs707184	5	153466019	C5orf3, MFAP3	T/C	0.37	3.5×10^{-5}	1.77 (1.35 – 2.31)	0.35	1.19 (0.82 – 1.71)
rs7737215	5	153625371	GALNT10	C/T	0.54	7.2×10^{-5}	1.69 (1.31 – 2.18)	0.40	0.85 (0.59 – 1.24)
rs7717132	5	150634674	SLC36A3	G/T	0.46	6.1×10^{-5}	1.61 (1.27 – 2.03)	0.39	1.16 (0.82 – 1.64)
rs10875583	5	153593909	GALNT10	C/G	0.57	9.7×10^{-5}	1.72 (1.32 – 2.24)	0.23	0.80 (0.56 – 1.15)
rs1438588	5	153425143	C5orf3, MFAP3	G/A	0.35	7.7×10^{-5}	1.65 (1.29 – 2.11)	0.22	1.25 (0.87 – 1.79)
rs6890748	5	153668293	GALNT10	G/A	0.39	9.7×10^{-5}	1.66 (1.29 – 2.13)	0.047	0.71 (0.51 – 0.99)
rs6863455	5	153665437	GALNT10	G/A	0.64	1.1×10^{-4}	1.7 (1.3 – 2.23)	0.22	0.81 (0.57 – 1.14)

rs numbers according to the NCBI SNP database.

§ Physical location of a SNP based on March 2006 human genome assembly (hg18).

% Alleles were listed with the second allele as the risk allele.

& p values from Gray's test.

* For example, patients harboring an additional copy of the risk allele (A allele) of the SNP rs4958351 are 1.73 times more likely to have an allergic reaction compared to those without the risk allele in the discovery cohort.

Table 3

The *p* values for 12 SNPs in *GRIA1* associated ($p < 0.05$) with asparaginase allergy in the discovery cohort. Also listed are the *p* values & hazard ratio these 12 SNPs in the validation and combined cohorts.

SNP#	Location [§]	Risk Allele	Alleles %	Frequency	Discovery cohort		Validation cohort		Combined cohort	
					<i>p</i> value ^{&}	Hazard ratio (95% CI) [*]	<i>p</i> value ^{&}	Hazard ratio (95% CI) [*]	<i>p</i> value ^{&}	Hazard ratio (95% CI) [*]
rs4958351	153150567	G/A	0.27	0.27	1.8×10^{-5}	1.72 (1.34–2.20)	0.0029	1.93 (1.25–2.98)	3.5×10^{-7}	1.75 (1.41–2.17)
rs10070447	153159007	C/T	0.27	0.27	1.3×10^{-4}	1.59 (1.25–2.02)	0.0023	1.96 (1.27–3.01)	2.0×10^{-6}	1.66 (1.35–2.05)
rs6890057	153103681	C/T	0.17	0.17	4.3×10^{-4}	1.64 (1.24–2.15)	0.0035	1.80 (1.21–2.68)	2.1×10^{-5}	1.63 (1.30–2.05)
rs4958676	153132470	G/A	0.17	0.17	0.0014	1.57 (1.19–2.08)	0.0066	1.66 (1.15–2.40)	9.2×10^{-5}	1.56 (1.25–1.95)
rs6889909	153103626	C/T	0.18	0.18	0.0020	1.53 (1.17–2.00)	0.016	1.58 (1.09–2.29)	2.3×10^{-4}	1.51 (1.21–1.87)
rs11167640	153112499	C/T	0.74	0.74	0.0025	1.72 (1.21–2.45)	0.84	1.04 (0.71–1.52)	0.014	1.39 (1.07–1.81)
rs10072570	153159960	G/A	0.22	0.22	0.0038	1.47 (1.13–1.91)	0.21	1.32 (0.85–2.05)	0.0035	1.40 (1.12–1.75)
rs4424038	153100457	G/A	0.73	0.73	0.0056	1.62 (1.15–2.28)	0.27	0.83 (0.59–1.16)	0.125	1.21 (0.95–1.55)
rs13354399	153169624	G/A	0.22	0.22	0.0069	1.41 (1.10–1.80)	0.21	1.32 (0.85–2.05)	0.0055	1.36 (1.09–1.69)
rs17356099	153123241	T/C	0.06	0.06	0.017	1.85 (1.12–3.07)	0.43	1.37 (0.63–3.01)	0.019	1.66 (1.09–2.54)
rs7711124	153123087	C/G	0.65	0.65	0.021	1.40 (1.05–1.86)	0.90	0.98 (0.66–1.45)	0.084	1.22 (0.97–1.53)
rs7708391	153127393	A/G	0.65	0.65	0.022	1.39 (1.05–1.85)	0.85	0.96 (0.64–1.44)	0.089	1.22 (0.97–1.53)

[#] rs numbers according to the NCBI SNP database.

[§] Physical location of a SNP based on March 2006 human genome assembly (hg18).

[%] Alleles are listed with the risk allele as the second allele.

[&] *p* values from Gray's test.

^{*} For example, patients harboring an additional copy of the risk allele (A allele) of the SNP rs 4958351 are 1.72 times more likely to have an allergic reaction compared to those without the risk allele in the discovery cohort.