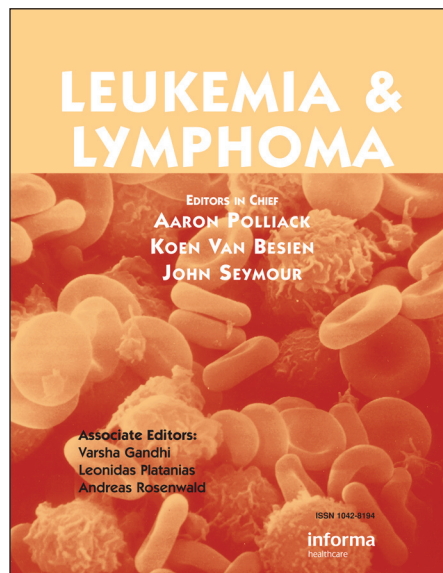




Polymorphisms in GRIA1 gene are a risk factor for asparaginase hypersensitivity during the treatment of childhood ALL

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Abstract

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Polymorphisms in GRIA1 gene are a risk factor for asparaginase hypersensitivity during the treatment of childhood ALL

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Short title: GRIA1 gene and asparaginase hypersensitivity

Abstract

L-asparaginase is an effective antineoplastic agent used in the acute lymphoblastic leukemia chemotherapy. Drug effect may be compromised by elicited immune response, resulting in the production of anti-asparaginase antibodies causing anaphylactic reaction or silent inactivation of the enzyme. To elucidate possible genetic predisposition for inter-individual differences in asparaginase hypersensitivity, we studied single nucleotide polymorphism (SNP) in GRIA1 gene in 146 paediatric patients treated with L asparaginase. Allergic reaction to L-asparaginase occurred in 49.3% of patients. We observed statistically significant association between SNPs in GRIA1 gene and occurrence of asparaginase allergy: rs4958351 with $p=0.003$, rs4958676 with $p=0.005$, rs6889909 with $p=0.005$, rs6890057 with $p=0.005$ and rs10070447 with $p=0.006$. We found statistically significant correlation between asparaginase allergy and event-free survival (p -value 0.005).

Keywords: Genetic and other predisposing conditions, Allergy and Hypersensitivity, Lymphoid Leukemia

Introduction

Long-term outcome of the lymphoblastic malignancies (acute lymphoblastic leukemia and lymphoblastic lymphoma) has improved dramatically during the last half past of the 20th century. The overall cure rate in developed countries is approximately more than 80 percent. Among the drugs used in the treatment of lymphoblastic malignancies is asparaginase. The administration of asparaginase results in hydrolyzation of the amino acid asparagine to aspartic acid and ammonia in extracellular fluid. This leads to a rapid and complete depletion of the serum asparagine which then affects the intracellular asparagine level (1 - 6). Amino acid deficiency impairs protein synthesis causing apoptosis and death of the leukemic cells. Normal cells can synthesize asparagine but leukemic cells are sensitive to the depletion of asparagine because they have low levels of asparagine synthetase and they lack the ability to up-regulate the asparagine synthetase mRNA when exposed to asparaginase (7 - 10).

Three types of asparaginase are currently available. They are derived from two different bacterial sources: native asparaginase and pegylated asparaginase which are both derived from *E. coli*, and asparaginase derived from *Erwinia chrysanthemi* (1 - 4). Asparaginase is a protein with the potential to elicit an immune response, resulting in the production of anti-asparaginase antibodies. Patients with anti-asparaginase antibodies have insufficient asparaginase for effective depletion of asparagine. Asparaginase hypersensitivity can be symptomatic with clinical signs (urticaria, rash, angioedema, bronchospasm, hypotension, anaphylactic shock), or asymptomatic without any signs. Published rates of clinical hypersensitivity vary greatly between studies. Factors that may affect the incidence of allergy include the number of asparaginase applications, route of administration, re-administration after a hiatus, and concomitant therapy (5, 6).

It is not clear whether there is a genetic basis for inter-individual differences in asparaginase hypersensitivity. The genome-wide association study of Chen et al. identified germ line genetic variations (single nucleotide polymorphism (SNP)) in *GRIA1* gene, located at 5q33, which were associated with asparaginase hypersensitivity (11).

GRIA1 gene encodes the formation of GluR1 subunit, which is a member of the AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid) ionotropic glutamate receptor family. Ionotropic glutamate receptors (iGluRs) form Ca^{2+} permeable ion channels and mediate fast excitatory glutamate response. *GRIA1* spans over 320 kb and it is composed of 16 small exons transcribed in a 3242 bp mRNA. Like all the other members of the AMPA receptor family, GluR1 occurs in two isoforms (flip and flop) (12). Alternative splicing generates the flip (long) and flop (short) variants encoded by exons 14 and 15, respectively, that differ by a 38-amino acid insertion into the region that forms part of the extracellular ligand-binding domain and is localised before membrane-spanning M3 domain. The flip and flop variant differs in the kinetic properties, especially in the time of desensitization and the time of recovery from desensitization (13 - 15). Nucleotide sequences of the mRNAs of AMPA receptors subunits are enzymatically modified by the deaminase ADAR2, introducing amino acids that are not encoded by the genome. This post-transcriptional modification, known as RNA editing, occurs in two locations in the AMPA receptor subunits

(R/G and Q/R site). Thus, it has been shown that RNA editing can modify function and ion flow in glutamate receptor channels, and that different molecular products produced by RNA editing have distinct trafficking, maturation, and gating properties (16 - 19).

Glutamate-mediating signaling has not only been described in the central nervous system, but in the recent years also in T cell-mediated immunity (20 – 22). In 2003, AMPA activated iGluRs were detected in human lymphocytes (23). It had been shown that glutamate has ability to activate and modulate T cell activity by itself (20, 21, 22, 24). Asparaginase allergic reactions belong to type I hypersensitivity reactions or TH2 response orchestrated by T-helper 2 cells (25). Ca^{2+} signals are critical for T cell function. A number of ion channels regulate Ca^{2+} influx from the extracellular space in T cells, either by conducting Ca^{2+} ions or by modulating the membrane potential that provides the driving force for Ca^{2+} influx (26).

Intronic polymorphisms in the glutamate receptor subunit GluR2 are the ones that direct RNA editing with consequential impacts on Ca^{2+} permeability and influx rate (26, 27).

We hypothesize that SNPs (rs4958351, rs10070447, rs6890057, rs4958676, rs6889909) in GRIA1 gene can determine clinical picture and severity of symptomatic asparaginase hypersensitivity. We tested this hypothesis in a cohort of paediatric patients treated with chemotherapy for acute lymphoblastic leukemia and lymphoblastic lymphoma.

Methods and material

Study design and patient population

We studied a cohort of 146 Caucasian pediatric patients treated for acute lymphoblastic leukemia and lymphoblastic lymphoma at the Oncology Unit at the University Children's Hospital in Ljubljana, Slovenia, between 1990 and 2012. All were exposed to asparaginase during treatment. All of our patients were treated according to the Berlin-Frankfurt-Münster (BFM) backbone protocols (BFM86, BFM90, BFM95, ALLICBFM2002, and ALLICBFM2009). Risk assignment (standard, intermediate and high) was performed according to the protocol criteria (29). Standard and intermediate risk group patients received treatment with native *E. coli* asparaginase 5,000IU/m² IV every 3 days for 8 doses in induction followed by 10,000IU/m² IV for 4 doses in re-induction. High risk group patients received native *E. coli* asparaginase 25,000IU/m² IV twice in each of the three blocks. In case of a hypersensitivity reaction to the native *E. coli* asparaginase, which led to discontinuation of treatment, the following options were offered: pegylated *E. coli* asparaginase and *Erwinia chrysanthemi* asparaginase. Pegylated *E. coli* asparaginase was used in a dose of 1,000IU/m² IV (once or twice, two weeks apart) depending on the time point of this adverse event. One dose of pegylated *E. coli* asparaginase substituted 4 doses of the native *E. coli* asparaginase. *Erwinia chrysanthemi* asparaginase was used in a dose of 10,000IU/m²/dose every other day. Each 6 doses of *Erwinia chrysanthemi* asparaginase substituted 4 doses of native *E. coli* asparaginase.

Sample and laboratory analysis

We isolated DNA for most of normal peripheral blood leukocytes and archive bone marrow smears. DNA was extracted with QIAmp[®] DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's recommendation. GRIA1 single nucleotide polymorphisms

were determined by real-time PCR (ABI PRISM 7500 Sequence Detection system, Applied Biosystems). Custom TaqMan[®] SNP genotyping assays (Applied Biosystems, Foster City, CA) were used for the determination of genotypes. For studied SNPs in GRIA1 gene, we used the following TaqMan[®] SNP Genotyping Assays (Table 1). Real-time PCR genotyping was conducted on AB 7000 Real Time PCR System (Applied Biosystems). Differently labeled fluorescent wild-type and mutant specific probes were used for allelic discrimination upon amplification.

Phenotyping of allergy to asparaginase

We graded asparaginase allergy as grade 1, 2, 3, or 4 according to the common terminology criteria for adverse events v3.0.

(http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v3.pdf)

Statistical analysis

All analyses were conducted using SPSS version 22.0 (SPSS Inc, Chicago, IL). We used χ^2 test, univariate, and multivariate analyses for determining the influence of gene polymorphism on asparaginase allergy. Results were considered statistically significant for values $p < 0.05$.

Results

Patient characteristics including risk group assignment and applied protocols of treatment are shown in the Table 2. Gender distribution in our cohort showed more male patients than female (77 vs. 69). Mean age at diagnosis was 7.2 years. Majority of the patients (50%) had common B immunophenotype of the leukaemic cells and were assignment in the standard risk group (47.3%). Most of patients (51.4%) were treated according to the protocol ALL ICBFM2002.

Asparaginase allergy occurred in 72 patients (49.3%). Interestingly, the rate of occurrence of reactions differs between risk groups: in the standard risk group 44.9% experienced allergy, in the intermediate 44.6%, and in the high risk group 76.2%. The most of allergic reactions were presented as grade 2 (63.9%), and grade 3 (23.6%), (Table 3).

In 69.5 % of patients allergy occurred during the post-induction phase of treatment.

Population diversity of studied GRIA1 SNPs in Caucasian population are the following: for rs4958351: GG 45.8%, GA 44.1%, and AA 10.2%; for rs4958676: GG 65.7%, GA 32.2%, and AA 2.1%; for rs6889909: CC 65.7%, CT 32.2%, and TT 2.1%; for rs6890057: CC 65.7%, CT 31.9%, and TT 2.4%; and for rs10070447: CC 38.8%, CT 47.8%, and TT 13.5% (source <http://www.ensembl.org>). The diversity of studied SNPs in our cohort is concomitant with the diversity in the general Caucasian population (Table 4).

In our study we found a statistically significant correlation between all studied SNPs in GRIA1 gene and asparaginase allergy occurrence: rs4958351 with $p=0.003$, rs4958676 with $p=0.005$, rs6889909 with $p=0.005$, rs6890057 with $p=0.005$, and rs10070447 with $p=0.006$ (all p -values < 0.05 ; Table 5).

The association between the severities of allergic reaction with SNPs is shown in the Table 6. In our cohort individuals carrying the SNPs rs4958351 GA genotype or rs10070447 CT

genotype are associated with allergy grade 2, while individuals carrying SNPs rs4958676, rs6889909 and rs6890057 GG or CC genotype are associated with allergy grade 2 and 3.

We performed multivariate analysis that included the following covariates: age, gender, treatment protocol, immuno-phenotype of the acute lymphoblastic leukemia/lymphoblastic lymphoma, risk group, and body mass index (BMI). Only the risk group showed a statistically significant correlation with asparaginase allergy (trend p -value 0.041). Those assignment to the high group showed p -value 0.017 (OR: 3.9; 95% confidence interval: 0.081 – 0.783).

Although the BMI value was in the overweight zone (mean 26.3 kg/m² and expected with corticosteroid treatment), we did not found statistically significant association with asparaginase allergy occurrence (p -value 0.621). In our cohort we found statistically significant correlation between asparaginase allergy and event-free survival (EFS) (p -value 0.005) (Figure 1). The relapse of the disease was assigned as an event, and patients with asparaginase allergy occurrence experienced significantly more events. Correlation between asparaginase allergy and overall survival (OS) was not statistically significant (p -value 0.722) (Figure 2).

Discussion

Hypersensitivity reactions remain an important side-effect of the asparaginase treatment and result in a number of patients not receiving the full treatment course. Published rates of clinical hypersensitivity vary greatly between the different studies. For example, Woo et al. report hypersensitivity reactions in 33% of the patients, Pieters et al. in up to 60% of patients (5, 7). In our cohort asparaginase allergy occurred in the 49.3% of patients. The most of allergic reactions were presented as grade 2 (63.9%) and grade 3 (23.6%).

Clinical hypersensitivity occurs mostly during the post-induction (intensification, re-induction) phase of treatment (6,8). Almost 70% patients in our cohort experienced allergy during the post-induction phase of treatment which is in accordance with published data. There are several possible explanations for the rarity of allergic reaction during induction phase of treatment, despite measurable antibody production in this phase. Because of the delay in an effective immune response due to the time taken for production of antibodies, the allergy symptoms may be masked by intensive corticosteroids treatment during the induction (prednisone 60 mg/m²/day for 38 days) and re-induction (dexamethasone 10 mg/m²/day for 21 days), and the frequency of drug application during the induction may have a desensitizing effect (5,9).

During the consolidation phase of treatment, B-cell lymphocytes, which were exposed to asparaginase during induction, proliferate and produce antibodies against the asparaginase. In response to allergens, B-cells produce either IgG or IgE antibodies. Anti-asparaginase antibodies (mainly IgG4) lead to allergic reactions on re-exposure of the drug (10). The frequency of anti-asparaginase antibodies is variable, with reports ranging from 79% in adults and up to 70% in children after the administration of native asparaginase. Individuals with anti-asparaginase antibodies have insufficient asparaginase for effective depletion of

asparagine (5). As we already mentioned, asparaginase hypersensitivity can be symptomatic or asymptomatic without any signs (silent inactivation).

Multivariate analysis showed a clear correlation between allergy occurrence and assignment to the high risk (HR) group (p -value 0.017). Those patients received native *E. coli* asparaginase 25,000IU/m² IV twice in each of the three blocks. At the same time they received concurrent chemotherapy including dexamethason. Regardless, a certain number of HR patients experienced asparaginase allergy. The possible explanation could be the dose (higher in HR than in standard and intermediate risk group), regimen, and route of the asparaginase administration in HR blocks. Nevertheless, it is also important to mention the possible influence of the concomitant therapy. Yang et al. showed in their study that the serum albumin and dexamethasone clearance were significantly greater in patients who had developed an allergy to asparaginase than in those who had not. They also showed that the dexamethasone clearance paradoxically increased with the use of CYP3A4 inhibitors (i.e. fluconazol, the drug used as fungal infection prophylaxis in HR patients) (55).

We found a statistically significant correlation between asparaginase allergy and EFS (p -value 0.005). Zalewska-Szewczyk et al. suggested that the children who developed anti-asparaginase antibodies in the induction phase of treatment showed lower EFS as well as OS in comparison with children without antibodies (53). In our cohort we do not confirm a statistically significant correlation between asparaginase allergy and OS.

Silverman et al. reported that stopping therapy with asparaginase or delivering less than 80% of the planned asparaginase dose might lead to decreased EFS (54). Therefore, it would seem wise to confirm and meticulous document any allergic reactions prior to discontinuing the drug because patients are often considered “allergic” to asparaginase, regardless of the severity of the reaction.

Asparaginase allergic reactions belong to type I (immediate) hypersensitivity reactions or TH2 response orchestrated by T-helper 2 cells. The development of TH2 cells is stimulated by IL-4 and suppressed by TGF- β . TH2 cells produce IL-4, IL-5, IL-10 and IL-13, thereby stimulating activity of mast cells, eosinophils, and stimulate production of IgE by B-cells. (25)

Chen et al. used GWAS to identify a single nucleotide polymorphism (SNP) in GRIA1 gene, located at 5q33, which were associated with asparaginase hypersensitivity (11). In our study we found a statistically significant correlation between all studied SNPs in GRIA1 gene and asparaginase allergy occurrence: rs4958351 with $p=0.003$, rs4958676 with $p=0.005$, rs6889909 with $p=0.005$, rs6890057 with $p=0.005$, and rs10070447 with $p=0.006$.

GRIA1 gene encodes the formation of GluR1 subunit which is a member of the AMPA ionotropic glutamate receptor family, forming Ca²⁺ permeable ion channels and mediate fast excitatory glutamate response. The role of glutamate and glutamate receptors in synaptic transmission in the nervous system is well established. Recent evidence has emerged that glutamatergic mechanisms also exist in a variety of non-neural cells (12,13). In the year 2003, Ganor et al. detected AMPA-activated iGluRs in human lymphocytes (23). Glutamate interact directly with T-cell expressed glutamate receptors leading to the activation or

suppression of various T-cell functions (cytokine secretion, proliferation, integrin-mediated adhesion, and migration) (20,21,50). Pacheco et al. showed that resting T-cells express mGluR5 and iGluR3, which induce increases in cAMP and intracellular Ca^{2+} , K^{+} and Na^{+} . Stimulation of iGluR3 is involved in adhesion and chemotactic migration (48).

All studied SNPs are in intronic sequences of the GRIA1 gene. Precise mechanism of intronic SNPs influence on allergy occurrence is not yet clear. RNA editing is a term for introducing sequence changes in gene transcripts. The study of Egebjerg et al. (1994) demonstrated that a region in the GluR2 intron B is essential for editing (27). Editing of mammalian nuclear transcripts is catalyzed by deaminases (adenosine deaminases acting on RNA – ADARs)(46). The editing created codon changes in GluRs that affect amino acid positions of critical impact on properties of the glutamate activated cation channels. This change, as consequence, influences the gated AMPA channel's permeability for Ca^{2+} ions. The presence of positively charged amino acid arginine in the inner channel pore makes the receptor channel impermeable to Ca^{2+} , reduces its ion conductance, and alters its current/voltage relationship (46,47,49). Thus, the possible mechanism of intronic SNPs influence on T-cell function and TH2 response development may be by Ca^{2+} influx changes. Ca^{2+} signals are critical for T-cell function (26).

Interestingly, the chromosome 5q region also contains the genes for IL-3, IL-4, IL-5, IL-9, and IL-13. IL-4 is a key cytokine involved in the regulation of IgE synthesis and in the modulation of the TH2 response which is, as we already mentioned, the main pathway of asparaginase allergic reactions. The studies of Noguchi (50) and Beyer (51) had reported evidence of linkage between asthma, atopy phenotype, and atopic dermatitis with the markers (including IL-4) on chromosome 5q31 – q33.

Fernandez et al. identified a higher incidence of hypersensitivity and asparaginase antibodies in patients with HLA-DRB1*07:01 alleles, suggesting a mechanism of allergy, whereby HLA-DRB1 alleles confer high affinity binding asparaginase epitopes with a higher frequency of reactions (52).

The study supports our initial hypothesis that SNPs in GRIA1 gene are associated with asparaginase hypersensitivity and that they can determine clinical picture and severity of symptomatic asparaginase hypersensitivity. Our study also provides evidence that genetic variability in AMPA receptors may have influence on clinical presentation of the asparaginase hypersensitivity reactions. The results of our study, similarly to the results of the study by Fernandez (52), suggest that there may be a genetic basis for inter-individual differences in asparaginase hypersensitivity, and further prospective studies will be needed to elucidate the different aspects of the complex relation between asparaginase, immune system and asparaginase allergy occurrence.

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Conflicts of interest

None of the authors have any conflicts financial or otherwise related to the publication of this manuscript to disclosure.

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Table 1. GRIA1 SNPs considered in this study.

SNP	Position	HWE	Allels	Location
rs4958351	153170374	0.43	c. 2271-3807 G>A	Intron 13
rs4958676	153152277	0.22	c. 2270-2302 G>A	Intron 13
rs6889909	153123433	0.23	c. 1824-20561 C>T	Intron 11
rs6890057	153123488	0.25	c. 1824-20506 C>T	Intron 11
rs10070447	153178814	0.44	c. 2386-3102 C>T	Intron 14

HWE: Hardy-Weinberg equilibrium (Ref. 28)

Table 2. Patient characteristics.

Characteristics	Value
Gender (Number of pts. (%)):	
Male	77 (52.7)
Female	69 (47.3)
Age at diagnosis (years):	
Range	0.4 – 20.7
Mean $\pm$ SD	7.2 $\pm$ 5.6
Immunophenotype of the leukaemic cells (Number (%)):	
Common B	73 (50)
Pre B	41 (28.1)
Pro B	8 (5.5)
Pre B Ph+	1 (0.7)
Pre B with myeloid marker co-expression	1 (0.7)
T cell	17 (11.5)
Pro T	1 (0.7)
Pre T	1 (0.7)
Biphenotypic	3 (2.1)
Risk group assignment (Number (%)):	
Standard	69 (47.3)
Intermediate	56 (38.3)
High	21 (14.4)
Treatment protocols (Number (%)):	
BFM86	1 (0.7)
BFM90	21 (14.4)
BFM95	36 (24.6)
ICBFM2002	75 (51.4)
ICBFM2009	10 (6.8)
Interfant06	3 (2.1)

Table 3. Occurrence and distribution of asparaginase allergy in standard, intermediate and high risk group patients treated for acute lymphoblastic leukemia and lymphoblastic lymphoma.

Asparaginase allergy	Grade of allergy	Risk group assignment (Number)			Total (Number (%))	
		Standard	Intermediate	High		
No	0	38	31	5	74 (50.7)	
Yes	1	1	2	0	3 (4.2)	72 (49.3)
	2	22	14	10	46 (63.9)	
	3	5	8	4	17 (23.6)	
	4	3	1	2	6 (8.3)	

Table 4. Single nucleotide polymorphisms allele distribution and asparaginase allergy in cohort of 146 subjects treated for acute lymphoblastic leukemia and lymphoblastic lymphoma.

SNP	Variant	Asparaginase allergy		Total
		Yes	No	
rs4958351 (Number of patients (%))	GG	21 (14.4)	40 (27.4)	61 (41.8)
	GA	39 (26.7)	23 (15.8)	62 (42.5)
	AA	12 (8.2)	11 (7.5)	23 (15.7)
rs4958676 (Number of patients (%))	GG	41 (28.1)	58 (39.7)	99 (67.8)
	GA	28 (19.2)	14 (9.6)	42 (28.8)
	AA	3 (2.0)	2 (1.4)	5 (3.4)
rs6889909 (Number of patients (%))	CC	41 (28.1)	58 (39.7)	99 (67.8)
	CT	28 (19.2)	14 (9.6)	42 (28.8)
	TT	3 (2.0)	2 (1.4)	5 (3.4)
rs6890057 (Number of patients (%))	CC	41 (28.1)	58 (39.7)	99 (67.8)
	CT	28 (19.2)	14 (9.6)	42 (28.8)
	TT	3 (2.0)	2 (1.4)	5 (3.4)
rs10070447 (Number of patients (%))	CC	20 (13.8)	37 (25.3)	57 (39.1)
	CT	40 (27.4)	24 (16.4)	64 (43.8)
	TT	12 (8.2)	13 (8.9)	25 (17.1)

SNP. single nucleotide polymorphism

Table 5. Results of the statistical analyses of relation between asparaginase allergy and studied single nucleotide polymorphism variables.

SNP	p-value	Odds ratio	95% Confidence interval	
rs4958351	0.003	1.74	0.389	0.845
rs4958676	0.005	1.59	0.460	0.857
rs6889909	0.005	1.59	0.460	0.857
rs6890057	0.005	1.59	0.460	0.857
rs10070447	0.006	1.65	0.408	0.899

SNP. single nucleotide polymorphism

Table 6. Association between the studied SNPs and grade of allergy in 72 subjects with asparaginase allergy.

SNP	Variant	Grade of the asparaginase allergy			
		1	2	3	4
rs4958351 (Number of patients (%))	GG	0	12 (16.7)	7 (9.6)	2 (2.8)
	GA	3 (4.2)	26 (36.1)	6 (8.3)	4 (5.6)
	AA	0	8 (11.1)	4 (5.6)	0
rs4958676 (Number of patients (%))	GG	2 (2.8)	23 (31.9)	12 (16.7)	4 (5.6)
	GA	1 (1.4)	22 (30.4)	3 (4.2)	2 (2.8)
	AA	0	1 (1.4)	2 (2.8)	0
rs6889909 (Number of patients (%))	CC	2 (2.8)	23 (31.9)	12 (16.7)	4 (5.6)
	CT	1 (1.4)	22 (30.4)	3 (4.2)	2 (2.8)
	TT	0	1 (1.4)	2 (2.8)	0
rs6890057 (Number of patients (%))	CC	2 (2.8)	23 (31.9)	12 (16.7)	4 (5.6)
	CT	1 (1.4)	22 (30.4)	3 (4.2)	2 (2.8)
	TT	0	1 (1.4)	2 (2.8)	0
rs10070447 (Number of patients (%))	CC	0	11 (15.3)	7 (9.7)	2 (2.8)
	CT	3 (4.2)	27 (37.5)	6 (8.2)	4 (5.6)
	TT	0	8 (11.1)	4 (5.6)	0

SNP, single nucleotide polymorphism

Figures

Figure 1. Event free survival rate in relation to asparaginase allergy

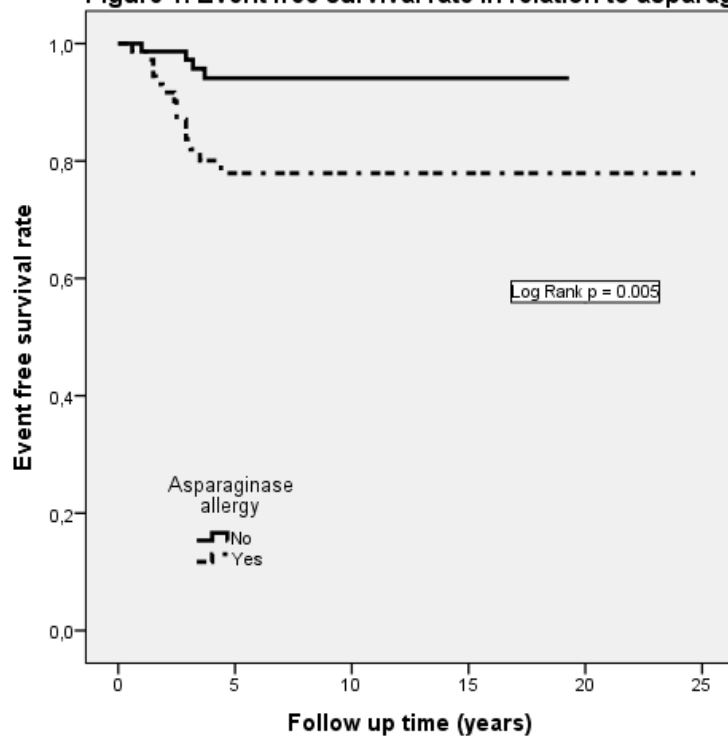


Figure 2. Overall survival rate in relation to asparaginase allergy

