Review

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Review of pharmacogenetics studies of L-asparaginase hypersensitivity in acute lymphoblastic leukemia points to variants in the *GRIA1* gene

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Abstract: Acute lymphoblastic leukemia (ALL) is a major pediatric cancer in developed countries. Although treatment outcome has improved owing to advances in chemotherapy, there is still a group of patients who experience severe adverse events. L-Asparaginase is an effective antineoplastic agent used in chemotherapy of ALL. Despite its indisputable indication, hypersensitivity reactions are common. In those cases, discontinuation of treatment is usually needed and anti-asparaginase antibody production may also attenuate asparaginase activity, compromising its antileukemic effect. Till now, six pharmacogenetic studies have been performed in order to elucidate possible genetic predisposition for inter-individual differences in asparaginase hypersensitivity. In this review we have summarized the results of those studies which describe the involvement of four different genes, being polymorphisms in the glutamate receptor, ionotropic, AMPA 1 (*GRIA1*) the most frequently associated with asparaginase hypersensitivity. We also point to new approaches focusing on epigenetics that could be interesting for consideration in the near future.

Keywords: acute lymphoblastic leukemia (ALL); hypersensitivity; L-asparaginase; pharmacogenetics; polymorphisms.

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Introduction

Acute lymphoblastic leukemia (ALL) is a major pediatric cancer in developed countries, accounting for 30% of all malignancies in children [1]. During the last few years, treatment outcome has improved substantially owing to advances in chemotherapy, with cure rates now exceeding 80% [2–4]. However, there are groups of patients who still remain refractory to therapy or experience severe toxicity affecting their quality of life during and after treatment. Consequently, there is a current interest in detecting markers which recognize, in advance, patients who will be resistant to treatment or suffer from the adverse effects of therapy, in order to adjust the treatment from the beginning.

In this context, pharmacogenetic studies can be useful in childhood ALL for several reasons, as it was mentioned in a review by Lopez-Lopez et al. [5]:

1) Chemotherapeutic drugs used in ALL treatment have a very narrow therapeutic range. This means there is a small difference between the effective dose and the dose that causes toxicity [6, 7]. Consequently, toxicity prevention is challenging because it is difficult to predict, and dose reduction is dangerous as underdosage is usually associated with decreased survival.
2) Genes involved in the metabolic pathways of the drugs used in ALL treatment are highly variable [8, 9]. In fact, several genetic variants have been described

that lead to inter-individual variability in protein levels or activity in those pathways. These variations can lead to changes in drug exposure or activity and impact treatment response.

3) Treatment protocols for ALL are standardized and well established, which allows the inclusion of large and homogeneously treated groups of patients in the studies. As a result, larger and homogeneous samples increase the statistical power of the studies and strengthen the reliability of the results.

Till date, several studies have been performed in order to search for pharmacogenetic markers of toxicity and outcome in pediatric ALL [5, 10–16]. However, the results are controversial for most of the analyzed polymorphisms. The lack of replication could be due to the characteristics of the samples included (small or mixed patients populations), to the toxicity criteria studied (different among studies), or to the differences among treatment protocols.

In Spain, the treatment of ALL was recently unified with LAL/SEHOP-PETHEMA 2013 protocol. According to this protocol, the treatment lasts an average of 2 years including induction, consolidation, re-induction and maintenance phases, in which different drugs are combined. One of the main drugs used is L-asparaginase (L-ASP), which is administered in the induction and re-induction phases, as well as in consolidation or intensification phase in high risk patients (Figure 1). Asparaginase is an enzyme that metabolizes extracellular asparagine into aspartic acid. Its antileukemic effect is based on the relative inability of leukemic cells to synthesize asparagine, as opposed to normal cells. The depletion of asparagine diminishes protein synthesis, leading to leukemic cell death [17, 18].

Several studies have demonstrated that increased dose of L-ASP improves response in patients with ALL [19, 20]. Despite this widespread success, hypersensitivity to L-ASP remains a commonly reported adverse event and often requires termination of asparaginase treatment. Therefore, the detection of markers which recognize, in advance, patients who will develop hypersensitivity to L-ASP will help to optimize the treatment.

Asparaginase

There are currently three forms of asparaginase that are used in clinical practice: native and pegylated form derived from *Escherichia coli* (*E. coli*-ASP and PEG-ASP, respectively), and an enzyme isolated from Erwiniachrysanthemi, known as Erwiniaasparaginase (*Erwinia*-ASP), antigenically distinct from *E. coli*-derived asparaginase forms [21]. All commercially available asparaginase preparations have been reported to have the potential to elicit an immune response characterized by the development of anti-asparaginase antibodies. The presence of these L-ASP antibodies is often associated with clinical or symptomatic hypersensitivity, which is the adverse effect more frequently described. Nevertheless, L-ASP preparations do not always lead to clinical hypersensitivity, but may instead cause rapid inactivation of the asparaginase, resulting in suboptimal asparagine depletion [21]. This is commonly referred to as 'silent hypersensitivity' or 'silent inactivation' and may occur in approximately 30% of the patients and it is characterized by circulating antibodies without clinical manifestations [22].

The incidence of hypersensitivity is variable depending on three different formulations of L-ASP

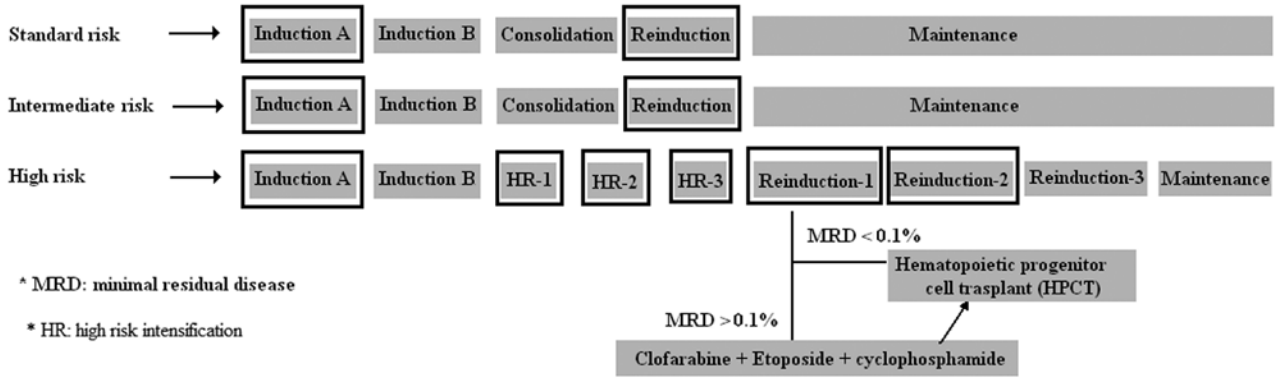

Figure 1: LAL/SEHOP-PETHEMA 2013 treatment protocol.
The phases where asparaginase is administered are pointed out.

used in clinical practice, with rates that vary from 20% to 31.5% in native *E. coli*-treated patients [23–25], from 1.8% to 9.4% in PEG-treated patients [26–28], and from 10.9% to 33% [29–31] in *Erwinia*-treated patients. Typically, patients exhibiting clinical allergy symptoms to one formulation of asparaginase are switched to another product to ensure they receive the most efficacious treatment regimen possible [22, 32]. In comparison with *E. coli*-derived asparaginase forms, *Erwinia*-ASP has a significantly reduced duration of action that permits tighter control of drug exposure should adverse effects occur. In fact, currently, hypersensitivity is the only recommended reason, as well as the only Food and Drug Administration-approved indication, for switching to *Erwinia*-ASP [33]. Nevertheless, the risk for allergic asparaginase-related adverse effects does still exist with the use of this drug. Additionally, other factors such as the number of asparaginase applications, route of administration, readministration after hiatus, and concomitant therapy can influence [34]. For example, Kutszegi et al. noticed that the highest occurrence of *E. coli* L-ASP hypersensitivity happened in the first dose of L-ASP in the reinduction, after a 3-month long break (consolidation phase) on medium risk (MR) arm [34]; in accordance with the fact that re-exposure to L-ASP after hiatus increases risk to hypersensitivity.

Clinical hypersensitivity is characterized by an allergic reaction, and can include the following sympthoms: rash, pain around the injection site, flushing, fever, chills, dyspnea, bronchospasm and anaphylaxis (which can be life-threatening situation requiring urgent interventions [35]). Hypersensitivity reactions are classified according to gravity in grades from 1 to 5 according to Common Terminology Criteria for Adverse Events, being the most frequent grade 1 and 2 ones. Accordingly, developing clinical hypersensitivity negatively impacts outcomes in ALL due to the fact that the discontinuation of L-ASP therapy is usually needed in these cases [36], and that the presence of L-ASP antibodies can substantially reduce L-ASP activity [22, 37].

Other important adverse effects, often enhanced by concomitant use of corticoids in the induction and reinduction phases, are as following: deep vein thrombosis (5.2%, mostly in the induction), pancreatitis (5%–10% usually after first doses of L-ASP) [33, 38], hepatotoxicity (~75% in the first 2 weeks), hyperglycemia (20%–35% [39]) and severe hypertriglyceridemia (>1000 mg/dL) (7%, [40]).

Remarkably, these adverse events exhibit large inter-individual differences. Therefore, predicting which patients will suffer from L-ASP toxicity and which type of toxicity will be developed is one of the challenges of modern medicine.

Pharmacogenetics

To date, only 10 pharmacogenetics studies analyzing L-ASP have been performed in pediatric ALL [34, 41–49]. Among them, six specifically focused on the analysis of the risk to L-ASP hypersensitivity, finding the most remarkable results with 15 polymorphisms located at GRIA1, *HLA-DRB1*, *NFATC2* and *ASNS* (Table 1) [34, 41–44, 49]. Interestingly, out of these 15 significant polymorphisms, 11 were located in the *GRIA1* gene (glutamate receptor, ionotropic, AMPA 1). In 2010, Chen et al. performed a genome wide association study (GWAS) interrogating 364,033 single nucleotide polymorphisms (SNPs) in 485 children with ALL [assigned into discovery (n=322) and validation (n=163) cohorts, as well as the combined cohort (n=485)]. From the top 100 SNPs associated with allergy in the discovery cohort, they found that chromosome 5 was overrepresented with 10 significant SNPs. Among them, rs4958351 located at *GRIA1* gene was remarkably significant in the discovery, validation and combined cohorts. Focused on this gene, they found 9 SNPs (rs4958351, rs10070447, rs6890057, rs4958676, rs6889909, rs11167640, rs10072570, rs13354399 and rs17356099) significantly associated with native *E-coli*-ASP hypersensitivity in the discovery and combined cohorts. Notably, five of them (rs4958351, rs10070447, rs6890057, rs4958676 and rs6889909) also remained significant in the validation cohort [41], although no correction for multiple comparisons was applied. In 2015, Rajić et al. replicated the association of these last 5 SNPs with native *E. coli* L-ASP hypersensitivity in a cohort of 146 Caucasian children with ALL [42]. However, the association between rs4958351 and the risk to native *E. coli* L-ASP hypersensitivity was only replicated by Kutszegi et al. specifically in T-ALL subgroup, but with opposite effect. They found that A allele reduced the risk to hypersensitivity in this subgroup (n=69); whereas A allele was associated with a slightly but not significantly increased risk to hypersensitivity in [pre-B-ALL (n=402)] [34]. Kutszegi et al. explained this difference among studies because Chen et al. and Rajić et al. did not analyze specifically T-ALL due to the low number of this type of samples in their cohorts. Additionally, Kutszegi et al. found other two SNPs in *GRIA1* (rs2055083 and rs707176) associated with hypersensitivity but only in the medium risk group [34]. *GRIA1* encodes a subunit (GluR1) of the ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazole propionate

Table 1: Polymorphisms associated with L-ASP hypersensitivity in B-/T-ALL pediatric patients.

Gene	Polymorphism	Allele[a]	Study SNPs	Samples[b]	Ethnicity	p-Value	Risk (95% CI)	Ref.
GRIA1 (Chr.5)	rs4958351	G/A	GWAS	322	W/B/H/A/O[c]	1.8 E–5	HZ = 1.72 (1.34–2.20)	[41]
			GWAS	163	W/B/H/A/O[d]	0.0029	HZ = 1.93 (1.25–2.98)	[41]
			5	146	Caucasian	0.003	OR = 1.74 (1.18–2.57)	[42]
			20	464	Hungarian	NS	NS	[34]
	rs10070447	C/T	GWAS	322	W/B/H/A/O[c]	1.3E–4	HZ = 1.59 (1.25–2.02)	[41]
			GWAS	163	W/B/H/A/O[d]	0.0023	HZ = 1.96 (1.27–3.01)	[41]
			5	146	Caucasian	0.006	OR = 1.65 (1.11–2.45)	[42]
	rs6890057	C/T	GWAS	322	W/B/H/A/O[c]	4.3 E–4	HZ = 1.64 (1.24–2.15)	[41]
			GWAS	163	W/B/H/A/O[d]	0.0035	HZ = 1.80 (1.21–2.68)	[41]
			5	146	Caucasian	0.005	OR = 1.59 (1.17–2.17)	[42]
	rs4958676	G/A	GWAS	322	W/B/H/A/O[c]	0.0014	HZ = 1.57 (1.19–2.08)	[41]
			GWAS	163	W/B/H/A/O[d]	0.0066	HZ = 1.66 (1.15–2.40)	[41]
			5	146	Caucasian	0.005	OR = 1.59 (1.17–2.17)	[42]
	rs6889909	C/T	GWAS	322	W/B/H/A/O[c]	0.0020	HZ = 1.53 (1.17–2.00)	[41]
			GWAS	163	W/B/H/A/O[d]	0.016	HZ = 1.58 (1.09–2.29)	[41]
			5	146	Caucasian	0.005	OR = 1.59 (1.17–2.17)	[42]
	rs11167640	C/T	GWAS	322	W/B/H/A/O[c]	0.0025	HZ = 1.72 (1.21–2.45)	[41]
			GWAS	163	W/B/H/A/O[d]	NS	NS	[41]
			20	490	Hungarian	NS	NS	[34]
	rs10072570	G/A	GWAS	322	W/B/H/A/O[c]	0.0038	HZ = 1.47 (1.13–1.91)	[41]
			GWAS	163	W/B/H/A/O[d]	NS	NS	[41]
	rs13354399	G/A	GWAS	322	W/B/H/A/O[c]	0.0069	HZ = 1.41 (1.10–1.80)	[41]
			GWAS	163	W/B/H/A/O[d]	NS	NS	[41]
	rs17356099	T/C	GWAS	322	W/B/H/A/O[c]	0.017	HZ = 1.85 (1.12–3.07)	[41]
			GWAS	163	W/B/H/A/O[d]	NS	NS	[41]
	rs2055083	G/A	20	490	Hungarian	0.104	OR = 0.66 (0.39–1.09)	[34]
	rs707176	T/C	20	477	Hungarian	0.041	OR = 1.90 (1.02–3.48)	[34]
HLA-DRB1 (Chr.6)	HLA-DRB1*07 : 01		54	1870	European	0.002	OR = 1.64 (1.28–2.09)	[43]
	rs17885382	C/T	GWAS	3308	E/Af/H/A/O[e]	3.5E-5	OR = 1.66 (1.3–2.1)	[44]
NFATC2 (Chr.20)	rs6021191	A/T	GWAS	3308	E/Af/H/A/O[e]	5.4E-6	OR = 3.07 (1.87–4.94)	[44]
ASNS (Chr.7)	Haplotype*1		14	285	Caucasian	0.01	OR = 0.4 (0.2–0.8)	[49]
			14	248	Caucasian	0.002	OR = 0.3 (0.1–0.6)	[49]

HZ, hazard ratio; OR, odds ratio; CI, confidence interval; NS, no significant. [a]Alleles are listed with the risk allele as the second allele. [b]Number of individuals with genotyping data for the SNP of interest. [c]White 65.5%/Black 12.5%/Hispanic 14.3%/Asian 1.2%/Other 6.5%. [d]White 60.7%/Black 20.3%/Hispanic 14.7%/Asian 1.2%/Other 3.1%. [e]European 59.5%/Hispanic 22.0%/African 7.7%/Asian 1.6%/Other 9.2%.

(AMPA) receptor, a tetrameric ligand-gated ion channel that transmits glutamatergic signals in the brain. Glutamate has been recently shown to have a role not only as a neurotransmitter, but also as an immunomodulator [50, 51]. In 2003, AMPA-activated ionotropicGluRs were detected in human lymphocytes [52]. Moreover, glutamate was shown to be able to activate and modulate T cell activity by itself [50, 53–55]. In addition, *GRIA1* gene is located in chromosome 5q31-33, which has been considered as a susceptibility locus for several inflammatory or autoimmune diseases, including asthma. These results suggest that drug allergy and asthma share a range of candidate genes [56–61].

Regarding the *HLA-DRB1* (major histocompatibility complex, class II, DR β 1) gene, Fernandez et al. identified the polymorphism HLA-DRB1*07 : 01 allele associated with L-ASP hypersensitivity. These authors analyzed two cohorts of 541 and 1329 Caucasian patients, and suggested that risk allele conferred high-affinity binding to asparaginase epitopes leading to a higher frequency of hypersensitivity reactions [43]. In line with these results, other studies linked variants in *HLA* genes on chromosome 6 with autoimmune diseases [62, 63]. Additionally, several *HLA-B* alleles were associated with small molecule drug allergy and adverse reactions [64–69]. Specifically, HLA-DRB1*07 : 01 allele was associated with elevated levels of serum alanine transferase during ximelagatrn and lapatinib treatment through a drug-induced adaptative immune response [70, 71]. In 2015, the same group performed a genome-wide approach and found significant the nonsynonymous polymorphism rs17885382 in the exon 2 of *HLA-DRB1* (Arg > Gln, 54), which is in complete

linkage disequilibrium with the HLA-DRB1*07 : 01 allele previously mentioned. In addition, they pointed out another gene, nuclear factor of activated T cells 2 (*NFATC2*), as a possible candidate gene implicated in the risk of hypersensitivity. They found that the intronic rs6021191 polymorphism was associated with higher risk of L-ASP hypersensitivity increasing NFATC2 expression [44]. *NFATC2* gene encodes a cytoplasmatic component of the NFAT transcription factor family. Upon T-cell receptor stimulation, cytoplasmatic *NFATC2* is dephosphorylated and traslocated to the nucleus where it participates in gene regulation. So far, diverse studies have shown that *NFATC2* can influence the development and function of regulatory T cells and can have either negative or positive regulatory influence on the immune response, depending on the antigen [72, 73]. Nevertheless, other studies have demonstrated that inhibition of the NFAT pathway can attenuate an immune response [74–79].

Finally, in 2015 Ben Tanfous et al. studying 14 SNPs in *ATF5* (activating transcription factor 5), *ASNS* (asparagine synthetase) and *ASS1* (argininosuccinate synthase) genes in 285 Caucasian pediatric ALL, discovered that homozygosity for haplotype*2 (3R allele) in *ASNS*, conferred higher risk of pancreatitis and hypersensitivity in the discovery cohort, while haplotype*1 (defined by C-181 of rs3757676 and 2R alleles) was associated with protective effect for hypersensitivity in the discovery (n = 285) and replication (n = 248) cohorts [49].

Noncoding RNAs

To date, most of pharmacogenetic research of L-ASP hypersensitivity in ALL has focused on the analysis of variants in coding genes. Nevertheless, these regions correspond only to approximately 1.5% of the entire genome [80]. Nowadays, it is known that regions that do not codify proteins (98.5%), such as microRNAs (miRNAs), may have an important regulatory function. MiRNAs can regulate the expression of 50% of the genes, including those involved in the administration, distribution, metabolism and excretion (ADME) of drugs (ADME genes). Therefore, deregulation or polymorphisms of miRNAs, by regulating the expression of drug-related genes, can play a pivotal role in drug response or side effects. In fact, a new field in pharmacogenetics has been proposed: the mir-pharmacogenetics [81].

Regarding childhood ALL therapy, several studies have already reported variations in miRNA expression or miRNA-related SNPs associated with the response to treatment as well as with drug-related adverse effects [10–12, 82–85]. However, to date, there are no studies analyzing miRNAs involved in L-ASP hypersensitivity. Nevertheless, three studies have shown deregulated miRNAs expression associated with L-ASP response in childhood B-ALL [86–88]. In 2011, Schotte et al. found that miR-454 was expressed at a 1.9-fold lower level in L-ASP-resistant cases [86]. In another study, Mei et al. in 2014 observed that low levels of miR-210 in B-ALL patients were associated with a lower response to L-ASP treatment and higher risk of relapse. Moreover, *in vitro* analysis also showed that low levels of mir-210 in cell lines increased L-ASP resistance [87]. Finally, MesrianTanha et al., in 2016 found miR-491-5p and miR-93-5p deregulated in patients who presented L-ASP resistance [88].

Therefore, taking into account that all the four genes associated with L-ASP hypersensitivity have both predicted and validated miRNA binding sites in miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk/), it is tempting to speculate that changes in miRNAs could be also associated with L-ASP hypersensitivity. (Supplemental table 1 gives characteristics of the 6 pharmacogenetic studies analyzing the L-ASP hypersensitivity in pediatric ALL patients).

Conclusions and future perspective

During the last few years, several studies have been performed in the context of ALL pharmacogenetics. Some of them have focused on L-ASP induced hypersensitivity pharmacogenetics, pointing out the involvement of four genes *GRIA1*, *HLA-DRB1, NFATC2* and *ASNS*. However, the low number of studies performed and the lack of replication have failed to produce consistent results until now.

In this review, considering all the published data together, we show up that there is growing evidence of the involvement of *GRIA1* in L-ASP hypersensitivity, for which SNPs located in this gene were significantly associated in three different studies [34, 41, 42]. Thus, those SNPs could be new potential predictors of L-ASP hypersensitivity, but further studies are required. Indeed, the majority of the pharmacogenetic studies analyzing L-ASP hypersensitivity have been conducted in ALL patients treated with native *E. coli* L-ASP (removed from the market in some countries [89], although it continues being used in the first line of ALL therapy in many others), so it would be interesting to analyze the effects of other formulations in the future. In this context, we think that measurement of antibody assessment and asparaginase levels could become useful tools to predict future allergic reaction or to alert physicians to the possibility of silent hypersensitivity, so further pharmacogenetic studies analyzing

L-ASP induced hypersensitivity, including clinical and also silent forms, could be also of interest. Moreover, as ALL is a complex and heterogeneous disease, the subtype of ALL should be also taken into account in these studies. Similarly, information about when hypersensitivity occurs, number of asparaginase injections and co-administrations should be available in the different studies with the goal of optimizing the therapy schedule and limiting this toxicity.

Additionally, data about treatment outcomes and resistance to asparaginase therapy are also interesting. In this line, recently, it has been demonstrated that KMT2E-ASNS fusion transcript contributes to acquired chemotherapy resistance and relapse in ETP-ALL through ASNS over-expression [90].

On the other hand, considering that polymorphisms in miRNA genes have been already associated with other childhood B-ALL treatment-related toxicities, but there are no studies in L-ASP toxicity, it would be interesting to analyze polymorphisms in miRNAs regulating *GRIA1, HLA-DR1, NFATC2* and *ASNS* genes. In fact, these genes contain miRNA binding sites, so miRNAs regulating them could also be L-ASP hypersensitivity markers. This field of miRNA pharmacogenomics is very promising; especially if we consider that miRNA expression could be exogenously controlled by blocking the expression of upregulated miRNAs or by restoring the expression of downregulated miRNAs. Finally, other epigenetic regulators, such as DNA methylation, histone modifications and long non-coding RNAs could also have a role in the development of L-ASP hypersensitivity. In this line, it has been already demonstrated that ALL *ETV6-RUNX1* subtype displays hypermethylation of *ASNS* [91], which was associated with a higher sensitivity of leukemic cells to L-ASP [92] in *ETV6-RUNX1* patients [93]. This developing field of pharmacoepigenetics has started to produce promising results and epigenetic variants have great potential to be used as biomarkers for personalized therapy. Therefore, future studies in this area could be a breakthrough in L-ASP pharmacogenetics.

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