Chapter

Pharmacogenomics and Pharmacotranscriptomics of Glucocorticoids in Pediatric Acute Lymphoblastic Leukemia

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Abstract

Pharmacogenomics and pharmacotranscriptomics contribute to more efficient and safer treatment of many diseases, especially malignancies. Acute lymphoblastic leukemia (ALL) is the most common hematological malignancy during childhood. Glucocorticoids, prednisone and dexamethasone, represent the basis of chemotherapy in pediatric ALL. Therapy causes side effects in 75% of patients and 1–3% of pediatric ALL patients die because of therapy side effects rather than the disease itself. Due to this fact, pharmacogenomics and pharmacotranscriptomics have gained key positions in this field. There is a growing knowledge of pharmacogenomics and pharmacotranscriptomics markers relevant for the success of the glucocorticoid treatment of children with ALL. New technologies, such as next-generation sequencing (NGS) have created a possibility for designing panels of pharmacogenomics and pharmacotranscriptomics markers related to the response to glucocorticoid drugs. Optimization of these panels through population pharmacogenomic studies leads to new knowledge that could open the doors widely to pre-emptive pharmacogenomic testing.

Keywords: glucocorticoids, pediatric acute lymphoblastic leukemia, pharmacogenomics, pharmacotranscriptomics, population pharmacogenomics

1. Introduction

Personalized medicine has always been applied in good medical practice. Nowadays, with the development of medicine and molecular biology, personalized medicine, also known as precision medicine, has become an integral component of modern medicine. Fascinating methodological advancements, especially an improvement of high throughput "omics" analysis, has led to the conclusion that genomic and transcriptomic profiling can bring about not only knowledge concerning the causes of multiple diseases that aren't traumas or infections, but also information that could contribute to the specificities of treatment of each individual. Thus, personalized medicine is aiming to provide the most efficient and the least harmful (toxic) treatment protocol to each patient [1, 2].

Among the subfields which could contribute to the formation of these individual protocols are the subfields of pharmacogenomics and pharmacotranscriptomics.

Pharmacogenomics deals with research whose results need to show if there are any associations between the variations in the genome and the efficacy or toxicity of a certain drug. Pharmacotranscriptomics deals with research that needs to determine if there are associations between the variations in the transcriptome and the efficacy or toxicity of a drug.

Specific genes and transcripts related to metabolizing enzyme gene variants, drug transporter gene variants, and gene variants that have been related to a predisposition to certain adverse events, might influence the response of a patient to a drug.

According to the U.S. Food and Drug Administration (FDA), there are many patients who would benefit if a health care provider considered pharmacogenomic testing before prescribing an appropriate drug or drug dosage. Up to date there are more than 50 drugs for which the gene-drug interaction data support therapeutic management recommendations, and more than 30 drugs for which the gene-drug interaction data indicate a potential impact on safety or response [3].

Aside from that, pharmacogenomics and pharmacotranscriptomics also try to identify markers associated with a disease, which can be targets for new therapeutics (molecularly-targeted therapy, gene-therapy).

The ultimate goal of pharmacogenomics and pharmacotranscriptomics is to create optimal therapy strategy based on the genomic and transcriptomic profile of a patient.

2. Pharmacogenomics and pharmacotranscriptomics of glucocorticoids in pediatric acute lymphoblastic leukemia

2.1 Pediatric acute lymphoblastic leukemia

Pediatric acute lymphoblastic leukemia (ALL), a pathological increased proliferation of lymphoid progenitors, lymphoblasts, is the most common neoplasm among children and it is also the one with the highest rate of complete remission, which covers up to 85% of the patients treated with modern protocols [4, 5].

Unfortunately, unwanted treatment effects occur in about 75% of patients [6]. Studies estimate that about 1–2% of pediatric ALL patients have a lethal outcome due to treatment [7].

There are several treatment protocols for pediatric ALL, consisting of the similar phases: remission induction and early intensification, consolidation, reinduction and maintenance. Standard treatment protocols for pediatric ALL include several commonly used drugs, i.e. glucocorticoids, vincristine, asparaginase, anthracyclines, thiopurines and methotrexate [5, 8, 9].

2.2 Glucocorticoid treatment of pediatric acute lymphoblastic leukemia

Synthetics glucocorticoids (GCs) are capable of inducing apoptosis in thymocytes, monocytes, and peripheral T cells. GC drugs, prednisone and dexamethasone, represent the basis of chemotherapy in pediatric ALL because of their cytotoxic and antiproliferative effect.

According to the Berlin-Frankfurt-Munster protocol, the pediatric ALL protocol specific for Europe, GCs are used in the remission induction phase of treatment. The primary goal of this phase is to use GCs to promote apoptosis in order to significantly lower the number of lymphoblasts. The number of blasts in the peripheral blood on the 8th day is an important prognostic marker. Also, GCs are used after

the consolidation phase, if a marrow relapse during or shortly following initial continuation chemotherapy is developed. GCs are administered in the first phase of reinduction (day 1–36), in order to achieve a second complete remission [10].

Inter-individual differences in the efficiency and adverse effects of GCs in children with ALL have been observed. A study on dexamethasone pharmacokinetics following treatment of children with ALL showed large inter-patient variability, with a greater than ten-fold variability in systemic drug exposure observed at a dose of 8 mg/m2/d [11]. Also, there are still 57% of patients who have poor response to prednisone, and resistance to prednisone has become one of the main obstacles to achieve successful treatment outcomes in pediatric ALL [7].

It is for this reason that pharmacogenomics and pharmacotranscriptomics became very important in GC treatment of pediatric ALL patients [12].

2.3 Pharmacogenomics markers

2.3.1 NR3C1 gene

The first pharmacogene to be studied in relation to GC sensitivity is the *NR3C1* gene that encodes the glucocorticoid receptor (GR). Four variants in this gene have been associated with variation in sensitivity to GCs, two of which contribute to a decrease in sensitivity, while the other two contribute to an increase in sensitivity [13]. Although initially these variants were shown to have no association with differences in response to GC therapy in childhood ALL, later studies with a greater number of study subjects have had different findings [14].

The variant rs56149945 (N363S) is an A > G missense variant in exon 2, which causes an asparagine to serine amino acid substitution in position 363 in the N-terminal domain of the receptor. The minor allele of this variant has been associated with increased sensitivity to GCs, resulting in increased body mass index and lower bone mineral density [15]. A proposed mechanism for this sensitivity is that the new serine residue becomes a target of phosphorylation, changing the phosphorylation state of the receptor [16]. Microarray analysis revealed a unique, variant-specific pattern of gene regulation for N363S when compared to wild-type GR [17]. In a study on childhood ALL, N363S carriers were found to be more prone to steroid-related toxicity during GC therapy, however they were also better prednisone responders overall, and had better 5-year event-free survival rates, supporting the idea that this variant causes increased sensitivity [18].

The variant rs41423247 is also associated with increased sensitivity to dexamethasone. It is a C > G single nucleotide variant in intron 2 that was discovered as a *Bcl*I restriction fragment length polymorphism [19]. It was found that the minor allele of the *Bcl*I variant was associated with good prednisone response in pediatric ALL patients [20]. Along with the *Bcl*I variant, two other variants in intron 2, rs33388 and rs33389, form a three-point ACT haplotype that is associated with increased sensitivity to GCs [21].

The linked variants rs6189/rs6190 (ER22/E23EK) have been associated with resistance to GCs as well as lower insulin, cholesterol and CRP levels [22]. Both variants are G > A single nucleotide substitutions, however variant rs62189 is silent, whereas rs6190 is a missense variant that results in an amino acid change from arginine to lysine. The variants promote expression of the GR-A isoform of GR, which is less transcriptionally active than the GR-B isoform [23]. No significant association of this variant with the therapeutic response to GCs has been found in childhood ALL [14].

Another variant associated with decreased sensitivity is rs6198. It is a A > G single nucleotide variant in exon 9β. The variant is inside an ATTTA motif and

promotes alternative splicing resulting in the expression of GRβ. This contributes to decreased sensitivity to GCs since GRβ does not bind GCs and is transcriptionally inactive [24]. It has been shown that the presence of the minor allele of variant rs6198 is associated with poor response to GCs in the initial phase of remission induction therapy in childhood ALL [25].

2.3.2 ABCB1 gene

The *ABCB1* gene (previously known as *MDR1* gene) encodes a membrane transporter P-glycoprotein (PGP), which is an efflux transporter that actively pumps xenobiotics, including GCs, out of the cell. It has been shown that heightened expression of PGP can lead to resistance to GCs, making this an important pharmacogene [26].

A C > T silent variant in exon 26, rs1045642 (also called 3435C > T), has been associated with lower expression and activity of PGP *in vivo*. This leads to higher plasma levels of xenobiotics being retained [27]. The proposed mechanism of lowered expression and activity is that the minor T allele causes mRNA instability, as well as that the presence of a rare codon that it creates, affects cotranslational folding of PGP [28, 29]. The 3435CC genotype has been associated with significantly lower event-free survival and overall survival in ALL patients, showing that the presence of 3435C > T variant is associated with better treatment outcome [30].

Two other variants are in linkage disequilibrium with 3435C > T, rs1128503 (1236C > T) and rs2032582 (2677G > T/A), but it has been shown that they do not account for the change in expression, and have not shown an association with risk of relapse in ALL [31]. However, a rare CGT haplotype (rs1128503-rs2032582-rs1045642) has been associated with high blast count in the initial phase of remission induction therapy [25].

2.3.3 Glutathione S-transferase (GST) genes

Three genes, *GSTM1, GSTT1* and *GSTP1*, encode detoxification enzymes from the glutathione S-transferase family. They catalyze the conjugation of reduced glutathione and xenobiotics, which is the first step in elimination of GCs. This makes GSTs a possible pharmacogenomic marker when it comes to GCs [32].

The main *GSTM1* and *GSTT1* genotype variants are inherited homozygous deletions of the gene (null genotype), resulting in an absence of enzyme activity. The evidence on how they affect therapy outcome in ALL is conflicting. The earliest study found that *GSTT1* null, but not *GSTM1* null genotype was associated with a reduction in risk of poor response to prednisone [33]. It was also shown that the simultaneous deletion of both the *GSTM1* and *GSTT1* genes was found to be more predictive than any other parameter of early relapse of childhood B-precursor ALL [34]. A later study showed the *GSTM1* null genotype was associated with better clinical outcome within prednisone poor-responder patients, whereas the *GSTT1* null genotype was associated with worse outcome in the standard-risk group and within prednisone good responders. These findings suggest that the *GSTM1* null genotype has a protective role while the *GSTT1* null genotype has an unfavorable effect in specific subsets of ALL patients [35]. However, the largest study on 710 children with ALL, found no association between *GSTT1* and *GSTM1* null genotypes and treatment outcome [36].

GSTP1 has two most commonly studied variants - rs1695 and rs1138272. Variant rs1695 is a A > G missense variant that causes an isoleucine to valine substitution in position 105 that affects the thermal stability of the enzyme [37]. Variant rs1138272 is a nearby C > T missense variant that causes an alanine to valine substitution in position 114. It has been shown that the GC

(rs1695-rs1138272) haplotype was associated with a good response to GCs in the remission induction phase of childhood ALL [25].

2.3.4 Discovery of novel variants

Though the above-mentioned variants account for some variability in response to GC therapy, the research on GC pharmacogenomics is limited, and studies on larger cohorts and accounting for different ALL subgroups are needed. Novel pharmacogene variants could be essential for personalization of GC dose that results in minimal toxicity and maximum cancer cell death [38]. Recently, genome-wide association studies (GWAS) have attempted to discover new variants with potential impact on pharmacogenomics variation of treatment outcome.

One of the adverse effects of GCs is hypertension. A study analyzing 203 candidate polymorphisms aimed to define the genetic risk factors for steroid-induced hypertension. The strongest association was identified with the contactin-associated protein-like-2, *CNTNAP2* (7q35-q36), a gene whose impaired function has been associated with blood pressure, though the mechanism of this association is unclear. Another association with hypertension was with the missense variant rs1137101 (LEPR Gln223Arg). It is found in the *LEPR* gene, that encodes the leptin receptor, whose ligand, leptin, regulates adipose tissue mass and body weight. Three SNPs in the *CRHR1* gene were associated with hypertension. This gene encodes the corticotropin-releasing hormone receptor that mediates the release of the adrenocorticotropic hormone. Carriers of the major rs1876828 G and rs1876829 A alleles and of the minor rs242941 T allele all had a higher incidence of hypertension [39].

Another important adverse effect of GC administration is osteonecrosis. A GWAS study of SNPs in a cohort comprising 2285 children with ALL, found that the presence of minor allele at SNP rs10989692, near the glutamate receptor *GRIN3A* locus, was associated with osteonecrosis. The second highest osteonecrosis-associated ranked variant was in a similar gene, *GRIK1*. These findings point to the involvement of the glutamate pathway in the pathogenesis of GC-induced osteonecrosis [40].

In a GWAS study, 440 044 SNPs were scanned on whether they contributed to the risk of relapse in 2535 childhood ALL patients. Dexamethasone plasma clearance was associated with 4 out of 134 SNPs associated with relapse, 2 of which were within the above mentioned *ABCB1I gene,* and both associated with higher dexamethasone clearance and a higher relapse risk [41].

The Cortisol Network (CORNET) consortium undertook a GWAS meta-analysis for plasma cortisol in 12,597 Caucasian participants, and found that individual differences in morning plasma cortisol levels amongst Europeans can be attributed to genetic variation within a region on chromosome 14. This locus includes *SERPINA6*, which encodes the corticosteroid binding globulin (CBG), the major cortisol-binding protein in plasma, as well as *SERPINA1*, which encodes α1-antitrypsin, a protein that inhibits cleavage of the reactive center loop that releases cortisol from CBG. Three SNPs were identified, some of which were associated with total CBG concentration, while the top hit, rs12589136, was found to influence the immunoreactivity of the reactive center loop of CBG [42]. Research like this gives insight into possible new candidate-gene targets that could be included in an expanding pharmacogenomics panel.

2.4 Pharmacotranscriptomics markers

Aside from the above-mentioned variants that directly affect expression levels, research in the field of pharmacotranscriptomics markers of GC response is still new and insufficient. Recently, the expression level of certain RNAs has been

associated with drug response, trying to establish the measuring of RNA expression as a marker of drug response that could guide therapy individualization [12]. A new area of focus is non-coding RNAs (ncRNAs) - transcripts which are not translated into proteins, but whose expression profile is widely altered in many malignancies. Two types of ncRNAs have been studied in relation to GC resistance, long non-coding RNAs (lncRNAs), which are non-coding transcripts longer than 200 nucleotides, and micro-RNAs (miRNAs), which are short transcripts with an important role as post-transcriptional regulators [43].

One lncRNA, GAS5, has been shown to be associated with a poor GC response in childhood ALL during the phase of remission induction therapy [44]. GAS5 is a regulatory tumor-suppressor lncRNA whose expression was first detected in growth-arrested cells. One of the mechanisms by which GAS5 achieves its role is molecular mimicry of the glucocorticoid response element (GRE). This causes GAS5 to compete with the genomic GREs for binding of the GC-GR complex [45]. ALL patients whose number of blasts on day 8 after the start of treatment was below 100 per μL of peripheral blood had a higher GAS5 expression at diagnosis, and those who had a higher ratio of GAS5 expression on day 15 versus after the start of treatment had a higher number of blasts on day 8. This suggests that the expression level of GAS5 could be a potential marker of therapy response in remission induction therapy [44].

One study that used a computational approach based upon emerging biomedical and biological ontologies and semantic technologies was used to investigate the roles of miRNA regulation on GC resistance in childhood ALL. It was found that hsa-miR-142-3p and hsa-miR-17-5p are the two most promising miRNAs related to GC resistance in pediatric ALL [46]. In another study, it was reported that T-cell ALL patients with high expression of hsa-miR-142-3p had a shorter survival time than those with low expression. This was explained by the oncogenic role of hsa-miR-142-3p that was mediated by inducing resistance to GC treatment through targeting GC receptor-α [47]. Down-regulated hsa-miR-17-5p was related to apoptosis induced by dexamethasone in primary *ex vivo* ALL cells. Therefore, hsa-miR-17-5p might play a role in GC-induced cell death and GC resistance in B-cell ALL [48].

2.5 Panel of pharmacogenes and pharmacogenomic variants of glucocorticoid response

In order to design a panel of pharmacogenes and pharmacogenomics variants related to GC therapy, several approaches have been used to identify pharmacogenes and pharmacogenomics markers whose pharmacogenomics potential could be relevant for application in clinical practice [49].

Using the database PharmGKB (www.pharmgkb.org) and searching the literature on the PubMed database, 22 pharmacogenes have been selected in order to create a panel of genes for which there is evidence of their influence on the effects of GCs (**Table 1**).

Further searching of databases and literature has resulted in selecting 18 pharmacovariants for which there is evidence of influence on the effects of GCs (**Table 2**).

Three criteria were applied to evaluate the potential of variants to be pharmacogenomics markers of GCs. First, the classification was performed by the level of evidence according to PharmGKB. Then, only variants with high minor allele frequencies (MAF) were considered, and finally, the third criterion was the assessment of the functional effect of the variant using *in silico* prediction algorithms that estimate the potential influence of an amino acid substitution on the functioning of the proteins which they encode [49].

Assignment of a level of evidence by the PharmGKB annotation scoring system for clinical and variant annotations enables easier identification of significant

ABCB1	*FCER2*	*HSPA4*
ADRB2	*FKBP5*	*NCOA3*
CREBBP	*GSTM1*	*NR3C1*
CRHR1	*GSTP1*	*SERPINA6*
CYP3A4	*GSTT1*	*ST13*
CYP3A5	*HSD11B2*	*STIP1*
CYP3A7	*HSP90AA1*	*TBP*
		TBX21

Table 1.
List of pharmacogenes related to GC therapy.

pharmacovariants. The clinical annotation score represents the sum of the scores of all attached variant, guidelines and drug label annotations. Variant annotations are scored depending on: phenotype category, p-value, cohort size, effect size and weighting by study type or by association and significance. Clinical annotation scores do not rank or compare clinical annotations within a given level of evidence. Level 1A clinical annotations designate variant-drug combinations with variant-specific prescribing guidance in a current clinical guideline or an FDA-approved drug label annotation, while level 1B supports the association but without variant-specific prescribing guidance in an annotated clinical guideline or FDA drug label. Variants level 2A belong to known pharmacogenes, listed in PharmGKB's Very Important Pharmacogenes (VIPs), and describe variant-drug combinations with a substantial evidence to support their importance. Variants level 2B clinical annotations describe variant-drug combinations with a moderate level of evidence supporting the association and not listed in PharmGKB's VIPs. Level 3 clinical annotations describe variant-drug combinations based on a single study or on preliminary results. In level 4 clinical annotations, variant-drug combinations total score is negative with no evidence to support an association between the variant and the drug phenotype [50].

For the evaluation of the pharmacogenomic potential of the selected variants, the level of evidence that correspond to association of each variant to drug response is extracted from PharmGKB database. Evidence level 1 corresponds to highest degree of certainty, while higher numbers correspond to lower degree of evidence for a variant-drug pair. Only variants with MAF higher than 10% have been considered as good candidates for pharmacovariants. Also, only exon variants, whose pharmacogenomics potential in GC therapy has already been confirmed in earlier studies, have been considered.

However, none of the selected variants have enough evidence to support the claim that they have sufficient pharmacogenomics potential in order to be included in the protocols of treatment where GCs are used. The PharmGKB level of evidence was 3 for several variants, but most of them had only variant annotation scores. Even among variants that are in exons, only a few have been predicted to impact the structure and/or function of encoded proteins (probably damaging). Some of the variants have a high MAF. However, none of them completely fulfilled the criteria for a pharmacogenomics variant. Therefore, there is no basis to include any of these variants in clinical practice.

To conclude, up to now, there is not enough indication for any pharmacogenomics marker to be recommended for pre-emptive genetic testing when GCs are administered, and they cannot be introduced in clinical practice.

The transcriptome consists of various coding mRNAs and non-coding RNAs. The content of the transcriptome is inconsistent. It depends on alternative splicing, RNA editing and alternative transcription. It can vary with environmental conditions and the time point of transcriptome profiling has to be considered for the establishment of the transcriptome data. Therefore, the introduction of

rs number[1]	Gene	Variant	PharmGKB[2]	MAF[3]	Effect[4]
rs2229109	*ABCB1*	c.1199G > A (p.Ser400Asn)	Level 3	3.3%	Benign
rs1128503	*ABCB1*	c.1236 T > C (p.Gly412=)	VA	41.6%	NA
rs2032582	*ABCB1*	c.2677G > T/A (p. Ser893Ala/Thr)	Level 3	42.7%	Benign
rs1045642	*ABCB1*	c.3435 T > C/A (p.Ile1145Met/Ile)	Level 3	51.8%	Prob.* Damag.
rs1042713	*ADRB2*	c.46G > A (p.Gly16Arg)	Level 3	38.6%	Benign
rs1695	*GSTP1*	c.313A > G (p.Ile105Val)	VA	66.9%	Benign
rs1138272	*GSTP1*	c.341C > T (p.Ala114Val)	VA	7.1%	Benign
rs10873531	*HSP90AA1*	c.282 C > T (p. Thr94=)	VA	88.1%	NA
rs6195	*NR3C1*	c.1088A > G (p.Asn363Ser)	VA	1.8%	Benign
rs104893913	*NR3C1*	c.1433G > A (p.Arg478His)	VA	0.00039%	Prob. Damag.
rs6194	*NR3C1*	c.1767C > T (p.His589=)	VA	0.2%	NA
rs138896520	*NR3C1*	c.1899G > A (p.Gln633=)	VA	0.0014%	NA
rs72558023	*NR3C1*	c.198A > G (p.Pro66=)	VA	0.00079%	NA
rs6196	*NR3C1*	c.2301 T > C (p.Asn767=)	VA	14.9%	NA
rs6189	*NR3C1*	c.66G > A (p.Glu22Asp)	VA	3%	Benign
rs72542742	*NR3C1*	c.685G > A (p.Ala229Thr)	VA	0.1%	Benign
rs6190	*NR3C1*	c.68G > A (p.Arg23Lys)	VA	3%	Benign
rs2240017	*TBX21*	c.99C > G (p.His33Gln)	Level 3	2%	Benign

[1]rs number: a reference SNP ID number of SNPs that map to an identical location assigned by NCBI.
[2]PharmGKB Level of Evidence: score of pharmacogenomics variant relevance that includes both clinical and variant annotation scores. Level 1: the highest, level 4: the lowest variant-drug evidence association. VA: variant annotation.
[3]MAF: minor allele frequency.
[4]Effect is estimated using PolyPhen-2; a tool for prediction of possible impact of an amino acid substitution on the structure and function of a human protein based on a number of features comprising the sequence, phylogenetic and structural information characterizing the substitution.
**Refers to minor allele C; NA – non applicable.*

Table 2.
Pharmacovariants related to GC therapy.

pharmacotranscriptomics markers in the panel possibly used for pre-emptive testing, in order to optimize GC use at the point of prescribing, will be very challenging.

2.6 Population pharmacogenomics

The more investigations were performed in the field of pharmacogenomics, the more prominent became the differences between different ethnical groups when it came to drug response. Due to this fact, it was no longer possible to use data gained

from investigating one population in order to apply it to another population [51]. A good example of variability between populations, which has pharmacogenomics significance is the deficiency of the enzyme glucose-6-phosphate-dehydrogenase (G6PD), responsible for the response to unwanted, toxic effects of the drug primaquine. There is a significantly higher number of G6PD-deficient carriers in the population the dark-skinned people of Africa, compared to the white skinned population of America. The prevalence of this genetic marker in Africa is a result of selective pressure, since the carriers of this variant cannot contract malaria, a common cause of death on this continent [52].

Individualization of therapy, as a practical application of knowledge from pharmacogenomics, has been based on studies performed on the populations of white skinned people. When other ethnical groups were included in the clinical investigations, the data led to the conclusion that an individual's ethnic background can influence the response of the individual to different therapeutics. Since the metabolism of drugs is population-specific, data gained from studies performed on one population cannot be extrapolated on the rest. Understanding the pharmacogenomics differences between populations can be of great importance for the pharmaceutical industry and for reducing costs of treatment and overall performances of health systems of any country [53].

Population pharmacogenomics studies enable the integration of pharmacogenomics into health care systems around the world and give a strong support to pre-emptive pharmacogenomics testing [54]. Transcriptome variation in the human population has rarely been studied and there is no evidence on the studies of its application in pharmacotrascriptomics.

2.7 Discovery of new potential pharmacogenomic markers of glucocorticoid response

Novel high-throughput methodology for genomic profiling, especially next-generation sequencing (NGS), has provided a great amount of data that can be a source for bioinformatics analysis. New knowledge can be gained using these modern approaches. They can also be used for the discovery of new potential pharmacogenomics markers.

Analysis of known pharmacogenes related to GC therapy for potential novel pharmacogenomics markers can be performed using two criteria: a prediction algorithm (such as Polyphen-2) showing that the variant affects the protein function, and the frequency of the altered (minor) allele being high.

Population pharmacogenomics study can be helpful in this effort because if the MAF is considerably high in a certain population for some potential new pharmacogenomics marker, validation and clinical studies are strongly encouraged.

One of the most comprehensive human genome database, "1000 genomes" has been searched, and two variants in known pharmacogenes related to GC therapy that could be interesting for validation studies and clinical association studies, have been found: *FCER2* rs28364072 and *NCOA3* rs2230782. Validation and clinical association studies are needed in order to confirm their pharmacogenomics potential.

Variant *FCER2* rs28364072 is located in the splice-site region and the mutations in that intronic region could influence protein function. Its MAF is around 30% in European populations, but as high as 60% in the population of Africa (**Figure 1**). Therefore, this variant is a candidate pharmacogenomics marker in the African population and further validation and clinical studies are recommended in that population.

The effect of the variant *NCOA3* rs2230782 is probably damaging, according to PolyPhen-2 prediction tool. Its MAF in European population is 10–14% and it is a candidate pharmacogenomics marker in this population. However, MAF for this

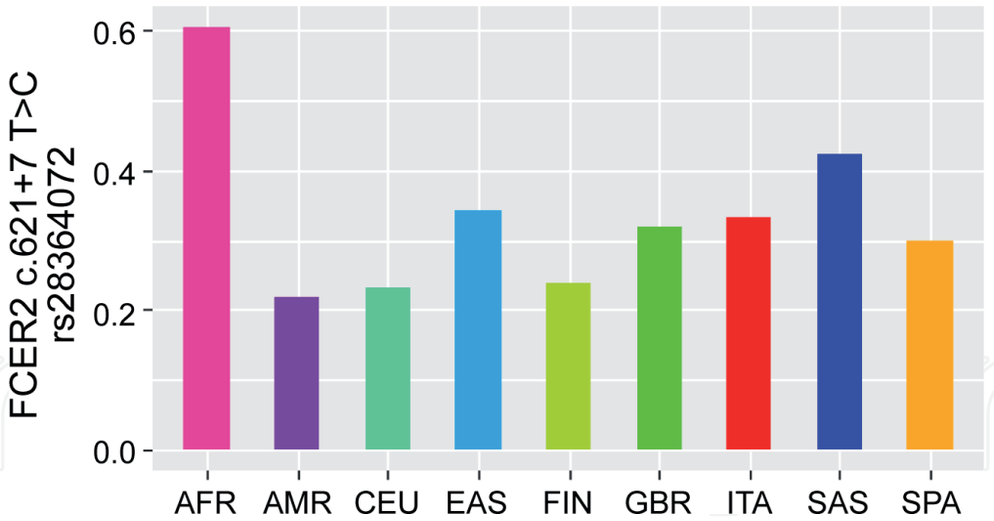

Figure 1.
Distribution of FCER2 rs28364072 MAF in world populations.

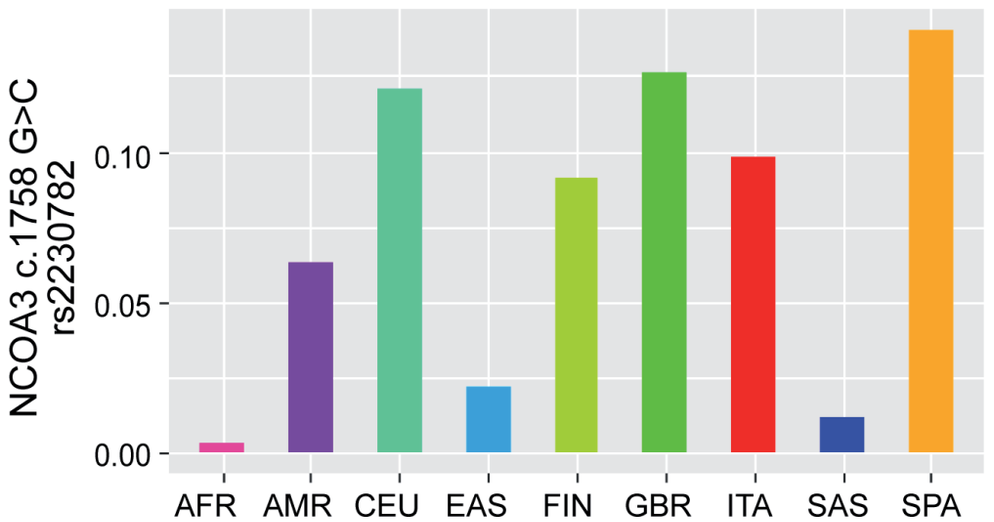

Figure 2.
Distribution of NCOA3 rs2230782 MAF in world populations.

variant is very low in other populations (**Figure 2**). Validation and clinical association studies regarding *NCOA3* rs2230782 pharmacogenomics are recommended only for European populations.

3. Conclusion

Big data in pharmacogenomics and pharmacotranscriptomics was produced so far, but their implementation in clinical practice is poor. Particularly, no pharmacogenomics marker related to GC therapy is reliable enough to be recommended for pre-emptive genetic testing.

A population specific pharmacogenomics landscape relevant for GC therapy could contribute to better understanding of the inconsistency in therapy response and could be helpful in predicting a higher risk of developing adverse reactions in patients that need to be treated with GCs.

Research efforts in the field of pharmacogenomics and pharmacotranscriptomics ought to be directed to data analysis and design of prediction models using machine learning algorithms. Bioinformatics tools and implementation of artificial

intelligence are expected to open the door wide for personalized treatment of children with ALL.

Acknowledgements

This work was supported by Ministry of Education, Science and Technological Development Republic of Serbia, EB: 451-03-9/2021-14/200042.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ABCB1	ATP binding cassette subfamily B member 1
ALL	Acute lymphoblastic leukemia
BclI	*Bacillus caldolyticus* I restriction enzyme
BFM	Berlin-Frankfurt-Munster
CBG	Corticosteroid binding globulin
CNTNAP2	Contactin-associated protein-like-2
CRHR1	Corticotropin-releasing hormone receptor 1
CRP	C-reactive protein
FDA	Food and Drug Administration
FCER2	Fc Fragment Of IgE Receptor II
G6PD	Glucose-6-phosphate-dehydrogenase
GAS5	Growth arrest-specific 5
GC	Glucocorticoid
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
GRIK1	Glutamate Ionotropic Receptor Kainate Type Subunit 1
GRIN3A	Glutamate Ionotropic Receptor NMDA Type Subunit 3A
GST	Glutathione S-transferase
GWAS	Genome wide association study
LEPR	Leptin receptor
lncRNA	Long non-coding RNA
MAF	Minor allele frequency
miRNA/miR	Micro RNA
NCBI	National Center for Biotechnology
NCOA3	Nuclear Receptor Coactivator 3
ncRNA	Non-coding RNA
NGS	Next generation sequencing
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
PGP	P-glycoprotein
PharmGKB	Pharmacogenomics Knowledge Base
SNP	Single nucleotide polymorphism
VA	Variant annotation

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