GLCCI1 polymorphism rs37973 and asthma treatment response to inhaled corticosteroids

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Abstract

Background. Asthma treatment response is highly variable and pharmacogenetic markers that predict treatment response would be one step closer to personalized treatment. GWAS studies have shown that polymorphisms *GLCCI1* could be associated with asthma treatment response to inhaled corticosteroids (ICS).

Materials and methods. We genotyped rs37973 of *GLCCI1* in 208 adult asthma patients treated with ICS. Change in % predicted FEV1 was analysed after short-term (3 months) and after long-term (at least 3 years) treatment. Treatment success was defined as good when FEV1 decreased less than 30 ml/year.

Results. After 3 months of treatment, change of % predicted FEV1 was higher in patients with GG genotype than in patients with AG+AA genotype, and this genotype dependent difference was only evident in non-smokers. Similar results were found after at least 3 years of treatment when all patients were analysed, in non-smokers and patients with atopy. Even though, no differences in treatment success (good vs. poor response) were observed when analysing the entire group of patients, genotype dependent treatment success was highly influenced by smoking and atopy. GG genotype was overrepresented in non-smokers and patients with atopy with good response.

Conclusions. Rs37973 was associated with short- and long-term treatment response; however, there was a great influence of smoking and atopy on pharmacogenetic association. Furthermore, we found GG genotype to be associated with better treatment response, what is contrary to results found in GWAS.

Keywords: pharmacogenetics, rs37973, *GLCCI1*, FEV1, smoking, atopy

Resumen

Introducción: La respuesta al tratamiento del asma es muy variable y los marcadores farmacogenéticos que predicen esta respuesta nos sitúan más cerca del tratamiento personalizado. Los estudios de asociación de genoma completo (GWAS) han demostrado que los polimorfismos GLCCI1 podrían estar asociados con la respuesta al tratamiento con corticosteroides inhalados (ICS).

Material y métodos. Se genotipó el polimorfismo de un solo nucleótico (SNP) rs37973 de GLCCI1 en 208 pacientes adultos con asma tratados con ICS. El cambio en el porcentual de lFEV1 predicho se analizó después de un tratamiento a corto plazo (3 meses) y después de un tratamiento a largo plazo (al menos 3 años). El éxito del tratamiento se definió como bueno cuando el FEV1 disminuyó menos de 30 ml / año.

Resultados. Después de 3 meses de tratamiento, el cambio en el porcentual del FEV1 predicho fue mayor en pacientes con genotipo GG que en pacientes con genotipo AG + AA, y esta diferencia asociada al genotipo solo fue evidente en los no fumadores. Se encontraron resultados similares, en el análisis de la muestra completa de pacientes, después de al menos 3 años de tratamiento, tanto en no fumadores y como en pacientes atópicos. A pesar de que no se observaron diferencias en el éxito del tratamiento (buena frente a mala respuesta) al analizar todo el grupo de pacientes, el éxito del tratamiento dependiente del genotipo estuvo muy influenciado por el tabaquismo y la atopia. El genotipo GG estaba sobrerrepresentado tanto en los pacientes no fumadores como en los pacientes con atopia, con buena respuesta al tratamiento.

Conclusiones: El SNP Rs37973 se asoció con una respuesta al tratamiento con corticoides inhalados a corto y largo plazo. Sin embargo, hubo una gran influencia del tabaquismo y la atopia en esta asociación farmacogenética. Además, encontramos que el genotipo GG se asocia con una mejor respuesta al tratamiento, lo que es contrario a los resultados encontrados en otros estudios de tipo GWAS.

1. Introduction

Asthma is one of the most common chronic diseases worldwide, with an estimated 300 million cases [1]. First-line treatment are inhaled corticosteroids (ICS), the only therapy that suppresses airway inflammation, simultaneously inhibiting many inflammatory targets [2]. ICS decrease airway vascularity, abnormal vascular permeability, basal membrane thickness, and airway hyperresponsiveness and increase forced expiratory volume in the first second (FEV1) [3]. Despite ICS treatment efficacy, it is known that treatment response is highly variable between patients. Multiple factors including genetic, environmental and asthma related are likely to contribute to the heterogeneous response to corticosteroids. However, within the same patient, the response is highly repeatable, so it is assumed that genetic factors significantly contribute to response variability [4, 5]. Single nucleotide polymorphisms (SNPs) are increasingly studied as possible markers for predicting patient's treatment response [6]. In asthma, several genes have been associated with treatment response, like *TBX21*, *VEGFA*, *CRHR1* and others [7,8,9]. Recently, genome-wide association study (GWAS) found rs37973 in a promoter region of glucocorticoid-induced transcript 1 (*GLCCI1)* to be a major candidate associated with asthma treatment response to ICS and the results were further replicated in additional cohort [10]. *GLCCI1* gene encodes a protein with unknown function, but its expression is induced by glucocorticoids and may be an early marker for glucocorticoid-induced apoptosis. Two previous genetic association studies aimed to replicate the original findings for polymorphism rs37973, in which minor G allele was associated with worse treatment response [10]. In the first study, authors used a 2-SNP model, rs37973 in *GLCCI1* combined with rs1876828 in *CRHR1*, and were able to discriminate between very good and very bad responders [11]. In the second study performed on adult asthmatics [12] only a trend to the same direction was found as in the GWAS study [10].

To date analysed pharmacogenetic variants in asthma account for only a small portion of genetic variability in asthma treatment response. Several factors, like environmental effects and complex genetic background predispose phenotypically heterogenous disease [13], substantially complicate asthma pharmacogenetics. Litonjua *et al*. [14] found association between treatment response and polymorphism in ß2-adrenergic receptor only in non-smoking patients and furthermore, there was an association between treatment response and genotype only in atopic moderate persistent asthma patients [15]. Indeed, it is well accepted

that several asthma endotypes exists [5, 16]. The response to treatment might vary substantially between different endotypes and should be taken into consideration when performing phamacogenetic studies and even more importantly when performing replication studies.

The current study aims to identify modulating effects of rs37973 in the *GLCCI1* gene on ICS treatment responsiveness in adult patients with asthma, smokers and non-smokers, atopic and non-atopic, after short-period (three months) and long-period (at least three years) of treatment.

2. Material and Methods

(i) Patients

This study involved 208 adult Slovenian patients with atopic and non-atopic, mild to moderate persistent asthma that attended pneumological care at the outpatient pneumological practice. Clinical parameters and demographic data are listed in Table 1. At their first visit, lung function and methacholine challenge tests were performed. All patients showed a positive methacholine test defined as a decrease of baseline FEV1 of 20% with a cumulative dose of methacholine (PD20) less than 4 mg, and the great majority of them had normal or near-normal spirometry testing results. After the diagnosis was established, all patients started treatment with inhaled corticosteroids (alone or in combination with long-acting beta agonists (LABA), according to achieved asthma control). Follow-up visits with spirometry testing were made after three months and after at least three years of treatment (mean 4.6, SD 1.3 years). % predicted FEV1 was measured at all three time points. After at least three years of treatment, response was defined as good or poor. Poor response according to changes in FEV1 was defined as a decrease in FEV1 for more than 30 ml/year. All spirometry and methacholine challenge testing was performed by the same technician, with the same (dosimeter) method and equipment (Spirojet, Provojet nebulizer, Ganshorn, Germany) to avoid bias. Patients were seated and wearing nose clips. Clinical data are available from previous study and are described in more details elsewhere [7]. This study was conducted in accordance with the amended Declaration of Helsinki. Study was approved by the Slovenian ethical committee and patients gave their informed consent.

(ii) DNA isolation and SNP genotyping

Genomic DNA was extracted from EDTA-containing whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The genotypes of the analysed SNP (single nucleotide polymorphism) rs37973 in *GLCCI1* were determined using a SNP assay by design purchased from Applied Biosystems (Foster City, CA). Forward primer CTCTTTTCACTGCATAACTACAAATGTTAGG, reverse primer CTTCTGGTGATCAGGAGAAATGTCT and probe TTGTTCA[G/A]TGCAGGTTC labeled with VIC and FAM fluorescent dyes and NFQ quencher were used. For genotyping ABI 7500 Fast Real-Time PCR system using SDS v2.0.5 software (Applied Biosystems) was used.

(iii) Statistical analysis

The Hardy-Weinberg equilibrium was tested using the chi-square test for the goodness-of-fit (one degree of freedom) model. Data distribution was evaluated by the D'Agostino & Pearson test. Parametric statistics (paired and unpaired *t* test) were used on normally distributed data, and non-parametric statistics (Mann-Whitney, Wilcoxon test and Kruskal-Wallis test) were used if the distribution deviated from normal. Genotypic distribution and allelic frequencies were compared by Fisher's exact test calculated on contingency tables. Odds ratios with 95% confidence intervals (95% CI) were calculated using the same test. We used the GraphPad PRISM program (version 5.0 for Windows, GraphPad Software, San Diego, CA). A P value less than .050 was accepted as statistically significant. We calculated power using R [17]. The proportion of high risk genotype (GG) among cases/controls was estimated using a risk allele frequency of 0.41, risk trait population prevalence of 0.8 and high risk genotype relative risk estimation of 1.3 (recessive inheritance model). The evaluation of the proportion of the high risk genotype among cases/controls using the above parameters was 0.208 and 0.0083 respectively. Afterwards the stimulation method was used to calculate power (1000 stimulated samples with sample sizes concordant the with number of cases/controls for each individual property of the studied group and the type I error of less than .050).

3. Results

(i) Treatment response and GLCCI1

Overall, asthma patients treated with ICS, had improvement in lung function, as average % predicted FEV1 increased from 84% before treatment to 90% after 3 months (P < .001) and 89% after at least three years of treatment (P < .001). However, change in % predicted FEV1 ranged from 28% decline to 49% improvement, showing large variability between patients treatment response (Fig. 1). Also, according to criteria defined in Methods, 17% of patients had poor response after at least three years of ICS treatment.

We genotyped for rs37973 in the *GLCCI1* gene and found 71 (34%) of patients were major AA homozygotes, 101 (49%) were heterozygotes and 36 (17%) were minor GG homozygotes, similar to the CEU population. The genotype distribution of the polymorphism analysed was in Hardy–Weinberg equilibrium. There was no genotype dependent difference in % predicted FEV1 before treatment.

(ii) Short-term treatment and GLCCI1

We analysed association between rs37973 in *GLCCI1* and change in lung function, defined as change in % predicted FEV1, in patients with asthma, after ICS short-term treatment (3 months). To assess the effect of environment and disease phenotypes on ICS asthma pharmacogenetics, we performed additional analysis where patients were stratified according to smoking and atopic status. After three months of ICS treatment, change in % predicted FEV1, was higher in patients with GG genotype compared to AG+AA, precisely 7.5% vs. 4% (P = .049). When patients were divided according to smoking status, this association was confirmed only in non-smokers (8% vs. 4%, P = .038). There was no genotype dependent changes in % predicted FEV1 when patients were stratified according to atopy (Table 2, Fig. 2).

(iii) Long-term treatment and GLCCI1

To assess whether there is a difference in pharmacogenetics of rs37973 in *GLCCI1* after short- or long-term period of treatment (at least three years) we also analysed changes in %

predicted FEV1 and treatment response (good vs. poor) after at least three years of treatment. Similarly as for short term response, also after at least three years of treatment, patients with atopy and smokers were analysed as separate subgroups. After at least three years of treatment genotype specific differences in treatment outcome were even more evident than after three months (Fig. 2). Patients with GG genotype had 7% improvement of % predicted FEV1 compared to 3.5% in AG+AA (P = .041) (Fig. 2) and similar genotype dependent changes were found only in non-smokers (7% vs. 3%, P = .035). Even though no difference in treatment success (good vs. poor response) was observed when analysing the entire group of patients, genotype specific treatment success was highly evident in non-smokers. As 21% of non-smokers with good response had GG genotype, but only 4% of patients with poor response (P = .030, OR 6.95, 95%CI 0.90−53.58). On contrast, 49% of smokers with good response had AA genotype and there were none (0%) patients with poor response and AA genotype (P = .015, OR 16.28, 95%CI 0.89−299.10). However, there were no differences treatment outcome between smokers and non-smokers when they were not stratified according to genotype. Furthermore, atopic patients with GG genotype had 12% increase in % predicted FEV1 compared to 3% in patients with AG+AA genotype (P < .001) and all atopic patients with GG genotype had good response to treatment compared to 75% of AG+AA patients (P = .035, OR 9.84, 95%CI 0.56−172.80), whereas there was no genotype specific difference in non-atopic patients (Table 2).

(iv) ICS only or ICS+LABA and GLCCI1

To test modifying effect of LABA on ICS pharmacogenetics, patients were further divided into two groups. In the first group, patients were treated only with ICS (n=121) and in second, patients received combination of ICS and LABA (n=87). We found an association between genotype and change of % predicted FEV1 after at least three years of treatment only in patients receiving only ICS; patients with GG had 8.5% increase vs. 3% in AG+AA (P = .001) and all patients with GG genotype had good response to treatment, compared to 72% of AG+AA patients (P = .007, OR 15.90, 95%CI 0.93−271.90). On the other hand, there was no genotype associated response in patients who received combined treatment ICS+LABA.

To summarize, rs37973 in *GLCCI1* is associated with treatment response to ICS in patients with asthma, especially in non-smokers and atopic patients. Furthermore, rs37973 is strongly associated with treatment response to ICS compared to combination of ICS and LABA.

4. Discussion

ICS are one of the most commonly used asthma treatment. Despite their effectiveness, treatment response is highly variable and is likely to be multifactorial, with significant genetic background [18]. Numerous genes were associated with asthma treatment response [19], but the first asthma pharmacogenomic GWAS discovered *GLCCI1* gene, located on 17p21.3, as the major candidate associated with ICS treatment response. AA genotype in polymorphism rs37973, located in the promoter of *GLCCI1*, was associated with better lung function improvement [10].

We performed a study on well characterised 208 adult asthma patients treated with ICS and analysed whether there is an association between treatment response and *GLCCI1* polymorphism rs37973. We have found genotype dependent differences in treatment response. Change in % predicted FEV1 was higher in patients with GG genotype when a recessive genetic model was used. This association was only evident in non-smokers, whereas in smokers we found no genotype dependent changes in % predicted FEV1. GG genotype was overrepresented in non-smokers with good response and on contrary, AA genotype in smokers with good response. These results show that smoking modifies the association between rs37973 in *GLCCI1* and asthma treatment response to ICS. Further analysis showed genotype GG is associated with higher improvement of lung function only in atopic patients and association in same direction was evident only in patients receiving ICS alone. Factors that cause variations in drug response are multifold and complex [20]. Smoking and atopic status data included in our analysis revealed the importance of environmental and phenotype variables on pharmacogenetic effect of analysed polymorphism. However, despite an association between rs37973 and treatment response, our results are contrary to the association found in GWAS, where they found three times better response in patients with AA genotype [10]. Because of the conflicting results, further studies are needed.

GLCCI1 gene is highly expressed in lymph nodes, leukocytes, breast, testis, adrenal, lungs, kidneys and others [21]. Because of high glomerul expression [22] *GLCCI1* rs37973 was also analysed on patients with nephrotic syndrome receiving oral steroids, however no pharmacogenetic association was found [23]. On lymphoblastoid B cells variants in *GLCCI1* were shown to be associated with decreased expression of *GLCCI1*, both at baseline and in response to dexamethasone treatment [10]. Using bioinformatics tool, we tried to highlight the mechanism by which polymorphism rs37973 could modify gene expression. rs37973 and *GLCCI1* polymorphisms in strong linkage disequilibrium (r ≥ .99), rs37969, rs37971 and rs37972, are located in enhancer histone marks, DNAse and protein binding sites and in regulatory motifs [24]. Methyltransferases, H3K4Me1 and H3K4Me3, are located directly in rs37973 [25]. These data suggests that there could be a functional cause for association between rs37973 and asthma treatment response. It could be hypothesized, that altered methylation because of this variation (SNP), results in changed gene expression. Methylation has an important role in corticosteroids action, as it was shown that its effectiveness is reduced by methyltransferase inhibitor [26].

To date analysed pharmacogenetic variants in asthma account for only a small number of genetic variability in asthma treatment response, so further studies are needed to resolve the environmental effect and nevertheless, the possible role of genetic interactions that are particularly important when each involved feature only demonstrates a minor effect [27]. This is the first study that analysed how environment and atopy influence on *GLCCI1* asthma pharmacogenetics. Our results show the importance of disease phenotype and analysis in pharmacogenetics, especially in heterogeneous disease like asthma.

In conclusion, rs37973 in *GLCCI1* is associated with treatment response in asthma; however this association is highly modified by smoking status and atopy. Because our results are in contrast with those found in GWAS [10] further studies need to be done, to elucidate the role of rs37973 in ICS asthma pharmacogenetics.

Acknowledgements

Conflicts of interests

All authors declare no conflict of interest.

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Table 1. Clinical characteristics and genotype of the GLCCI1 polymorphism of patients with asthma.

	All patients	Atopics	Non-atopics	Smokers	Non-smokers	P value ‡
Subjects n (%)	208	90 (43)	118 (57)	53 (25)	155 (75)	
Age, years mean (SD)	43 (14)	36 (13)	49 (12)	44 (14)	43 (15)	
Male sex n (%)	70 (43)	41 (46)	29 (24)	25 (47)	45 (29)	
% predicted FEV1 median (IQR):						
At baseline	84 (18)	88 (19)	83 (20)	79(17)	87 (19)	
Change after 3 months of treatment *	5 (9)	5 (10)	6 (9)	5 (7)	5 (9)	$P < .001$
Change after >3 years of treatment *	4 (9)	4 (11)	4 (8)	4 (10)	4 (9)	$P < .001$
PD20 at baseline (mg) † median(IQR)	0.65 (1.09)	0.56 (1.10)	0.69 (1.09)	0.48 (0.87)	0.71 (1.20)	$P < .001$
Genotype n (%)						
AA	71 (34)	29 (32)	42 (36)	22 (42)	49 (32)	
AG	101 (49)	47 (52)	54 (46)	22 (42)	79 (51)	
GG	36 (17)	14 (16)	22 (19)	9 (17)	27 (17)	

*: The change from baseline in the % predicted FEV1 after 3 months and after >3 years of treatment; †: The change from baseline PD20 in mg after 3 years of treatment; ‡: Significance of changes from baseline for the entire group of 208 patients (Wilcoxon matched-pairs signed rank test).

Table 2. Association of *GLCCI1* polymorphism rs37973 with change of % predicted FEV1 after 3 months and >3 years of treatment and patients response.

rs37973 *GLCCI1*	Genotype	Change of % predicted FEV1 after 3 months	P value	Change of % predicted FEV1 after >3 years	P value	Patients with good response (%)	P value, OR 95%CI
All patients	GG	7.5	.050	7.0	.041	92	> .99
	AG + AA	4.0		3.5		81	
Smokers	GG	6.0	> .99	7.0	> .99	78	> .99
	AG + AA	5.0		4.0		86	
Non-smokers	GG	8.0	.038	7.0	.035	96	.030, 6.95
	AG + AA	4.0		3.0		79	0.90−53.58 *
Atopics	GG	7.0	> .99	12.0	< .001	100	.035, 9.86
	AG + AA	4.0		3.0		75	0.56−172.80 **
Non-atopics	GG	7.5	> .99	4.5	> .99	86	> .99
	AG + AA	4.0		3.0		85	
ICS † only	GG	6.0	> .99	8.5	.001	100	.007, 15.90
	AG + AA	4.0		3.0		72	0.93−271.90 ***
ICS+LABA †	GG	4.5	> .99	8.5	> .99	81	> .99
	AG + AA	5.0		5.0		93	

†: ICS; inhaled corticosteroids; ‡: long-acting beta agonists; *: Statistical power to detect the association was 92%; **: Statistical power to detect the association was 56%; ***: Statistical power to detect the association was 87%.

Figure 1. Change in % predicted FEV1 after at least 3 years of treatment in patients not stratified to genotype and in patients stratified according to rs37973 in *GLCCI1* genotype. A clear shift towards better response can be seen in GG patients.
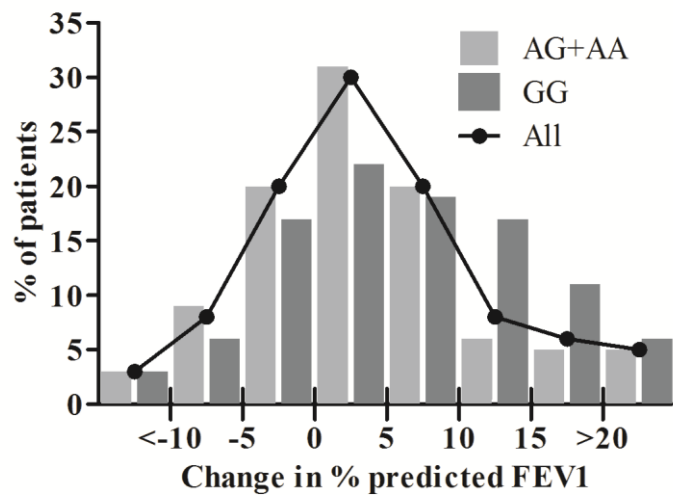
Figure 2. rs37973 in *GLCCI1* is associated with treatment response in asthma, however this association is modified by smoking status and atopy. Data are presented as median values with IQR. *, P < .05; **, P < .01.