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SLC26A9 SNP RS7512462 IS NOT ASSOCIATED WITH LUNG DISEASE SEVERITY OR LUNG FUNCTION RESPONSE TO IVACAFTOR IN CYSTIC FIBROSIS PATIENTS WITH G551D-CFTR

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Abstract

Background—The CFTR modulator ivacaftor has been variably effective in treating individuals with cystic fibrosis (CF) who harbor *CFTR* gating variants such as G551D, as well as other classes of *CFTR* variants when used with other modulators. Because *CFTR* genotype does not fully explain this variability, defining genetic modifiers of response to modulator therapy is of particular interest to the field of individualized CF drug therapy. Previous studies have proposed that a variant in *SLC26A9* (rs7512462) is associated with lung disease severity and with response to treatment with ivacaftor in 24 individuals with CF who carry G551D or gating variants.

Methods—Given the implications for CF treatment, we re-examined the reported associations in three cohorts; patients enrolled in the Twin and Siblings study at Johns Hopkins University, the CF modifier study at the University of North Carolina at Chapel Hill, and the prospective G551D Observational (GOAL) study. The GOAL study was specifically designed to measure lung function response to ivacaftor.

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Declarations of Interest: One of our co-authors, Scott Blackman, is also on the editorial board for the *Journal of Cystic Fibrosis.*

Conflict_of_Interest_Statement

One of our authors, Scott Blackman, is also on the Editorial Board of *JCF*. We have no other personal or financial conflicts of interest to declare.

Results—We find no association between *SLC26A9* (rs7512462) genotype and lung disease severity (n = 272) or change in lung function at one-, three-, and six-month intervals following ivacaftor treatment in 141 individuals enrolled in the GOAL study.

Conclusions—Our inability to replicate this association indicates that rs7512462 genotype should not be used in treatment decisions.

Introduction

Modulators targeting CFTR are highly effective in treating CF [1–5]. The first of these drugs, ivacaftor, was developed to treat patients with G551D mutations (NM_000492.4(CFTR): c.1652G>A (p.Gly551Asp) and has now been extended to those with other gating mutations [6–9]. Although ivacaftor has proven to be effective, there is a high level of inter-individual variation in response to the drug, even among those carrying G551D [6,10]. Therefore, elucidating specific factors responsible for this variation may inform individualized patient care.

SLC26A9 is a chloride transporter implicated as a modifier of cystic fibrosis related diabetes (CFRD), meconium ileus (MI, neonatal intestinal obstruction in CF patients), and newborn immunoreactive trypsinogen (IRT) levels [11–13]. Variation in *SLC26A9* was also proposed to modify lung disease severity and lung function change in response to ivacaftor treatment based on a genetic study of individuals with CF with at least one G551D allele or other gating mutation [14]. Specifically, a genetic variant in intron 5 of the *SLC26A9* gene (rs7512462; C allele) was associated with better lung function at baseline (using the Survival adjusted Kulich normalized (SaKnorm) lung phenotype, n = 70) and a larger increase in percent predicted forced expiratory volume (FEV_1 (% Pred)) in 24 individuals with CF in response to ivacaftor [14]. A subsequent study of 30 CF patients found that the C allele of *SLC26A9* (rs7512462) associated with a significant change in FEV_1 (% Pred) in response to ivacaftor, but in the opposite direction [14,15].

Given the importance of identifying and characterizing factors that affect response to CFTR modulators, we attempted to replicate the association observed between the *SLC26A9* SNP (rs7512462) and lung function in individuals with CF carrying the G551D gating mutation. To do so, we studied the association between the *SLC26A9* SNP (rs7512462) and 1) lung disease severity (SaKnorm) in 272 individuals with CF carrying at least one G551D mutation from the Twin and Sibling Study (at JHU), The CF Modifier Study (at UNC), and the prospective G551D Observational Study (GOAL) study and; 2) response of FEV_1 (% Pred) to treatment with ivacaftor in 141 G551D patients in GOAL study [9].

Materials and Methods

Study Subjects and Phenotype

To assess the association between *SLC26A9* (rs7512462) and baseline lung disease severity (SaKnorm), we assembled a cohort of 272 CF patients with at least one copy of G551D-CFTR from the prospective GOAL study (n = 141) [9], the Gene Modifier Study (at UNC; n = 66) and the Twin and Sibling Study (at JHU; n = 65) and used an allele dosage-based linear regression model to compare rs7512462 genotype and baseline lung function (*i.e.,*

without ivacaftor treatment) [16,17]. To address issues of relatedness, we selected one sibling per family for the 'unrelated' cohort or modeled related samples using linear mixed-effect models with the NLME R package [18].

To assess the response to ivacaftor by *SLC26A9* (rs7512462) allele, we obtained clinical phenotype data (including lung function) from the GOAL study [9] and the U.S. Cystic Fibrosis Foundation (CFF) Registry. Measurements of forced expiratory volume in 1 second (FEV_1) were expressed as percent predicted (% Pred) values using the Global Lung Function Initiative (GLI) equations [19] or transformed to the Kulich Normalized Mortality Adjusted CF-specific lung phenotype (SaKnorm) [20]. The SaKnorm is a quantitative lung disease phenotype which allows direct comparison of lung disease severity across ages of CF patients while accounting for differential survival.

Genotyping

Subjects in the CF gene modifier GWAS studies had rs7512462 genotyped using Illumina platforms, as described [16,17]. DNA from the GOAL subjects was genotyped using the Sequenom MassARRAY [21] for rs7512462 and 16 additional SNPs spanning the 5' region of *SLC26A9* (rs142245823, rs2036100, rs1874361, rs7555534, rs61814953, rs7415921, rs1342061, rs1342062, rs1342063, rs1342064, rs4951271, rs4077468, rs4077469, rs12047830, rs7419153, and rs7413698). The genotypes were phased using Eagle (v2.4) [22] on the University of Michigan Imputation Server [23], using the HapMap II reference panel. The phased genotypes were then used to create haplotypes using the VCFtools "—IMPUTE" function [24]. The determined haplotypes were labeled as previously published [25]. Informed consent was obtained from all participants, and approval for study of the GOAL participants was obtained from the GOAL study oversight committee and the CF Foundation.

Statistical Analysis

The primary analyses used an additive ("dose") model with respect to the rs7512462 alleles (C and T), as used in prior studies of *SLC26A9* [14, 15]. To assess whether *SLC26A9* rs7512462 is associated with lung disease severity (SaKnorm), we used a mixed-effect model for analysis, adjusted for gender and cohort (GOAL; UNC; JHU). This analysis was also repeated with the addition of CF-related diabetes (CFRD) as a covariate, since the rs7512462 C allele is associated with a lower risk of CFRD and there is a well-recognized correlation between development of CFRD and worse lung disease [12]. While MI and newborn pancreatic damage also associate with rs7512462 genotype, these phenotypes were not used as covariates in this analysis because MI does not associate significantly with FEV_1 and pancreatic damage is strongly correlated with later CFRD development [13,26,27]. Linear regression was used (as in the previously [14,15]) to determine the association between rs7512462 SNP and magnitude of change in FEV_1 (% Pred) from baseline (pre-ivacaftor) to the average of the measurements of FEV_1 made for each subject at scheduled follow-up visits (one month, three months, and six months), while being treated with ivacaftor. This analysis was adjusted for gender, age of enrollment into GOAL study, and baseline FEV_1 (% Pred). In addition, this analysis was also repeated with the addition of CFRD as a covariate. For statistical power estimation using the R package pwr [28],

standard deviation from the observed baseline or magnitude of change in FEV_1 (% Pred) were used in a balanced 2 group mean difference detection model by Student t-test with 2-sided alternative hypothesis, over a range of effect sizes, assuming 0.8 statistical power at 0.05 alpha. To estimate power of replicating prior studies [14, 15], effect size and variance were calculated from reported mean difference and SEM, and power was estimated using similar models and current replication sample size.

Results

Study Subjects

Characteristics of subjects in the SaKnorm group (UNC; JHU; GOAL, n = 272) and GOAL ivacaftor study subjects (n = 141) are summarized in Table 1. There are similar distributions across the two groups by: sex; age at study; age by *SLC26A9* rs7512462 genotype; race; and CFRD status.

SLC26A9 (rs7512462) genotype distributions

For the analysis of lung function association with *SLC26A9* rs7512462 genotype, using the HardyWeinberg package in R [29], we calculated a minor allele frequency (MAF; C allele) of 0.46 (n = 272) and confirmed that our cohort does not diverge significantly from Hardy-Weinberg equilibrium (HWE) (χ^2 goodness-of-fit test p-value = 0.77). Similarly, the distribution of rs7512462 genotypes do not deviate from HWE for: the unrelated subjects (n = 243, MAF = 0.46, HWE χ^2 p-value = 0.69); the GOAL cohort (n = 141, MAF = 0.49, HWE χ^2 p-value = 0.42); and the subset of the GOAL cohort with FEV_1 values between 40 and 90 (% Pred) (n = 71; MAF of 0.45, HWE χ^2 p-value = 0.12).

SLC26A9 (rs7512462) genotype and lung disease severity

In the mixed-effect model of additive (“dose”) analysis, there was no evidence of an association between *SLC26A9* (rs7512462) genotype and lung disease severity (SaKnorm) in 272 CF patients with at least one G551D allele (Figure 1), whether CFRD was (p = 0.28) or was not (p = 0.25) included as a covariate. A Forest plot of the 3 cohorts (UNC; JHU; GOAL) by rs7512462 C dose is provided (Figure S1), demonstrating no heterogeneity in association. There was also no evidence of an association when we analyzed the 243 unrelated subjects, whether CFRD was used as a covariate (p = 0.21), or not (p = 0.27).

Baseline lung function and magnitude of response to ivacaftor

To maximize the opportunity to replicate association, we used intervals for lung function response to ivacaftor that were consistent with those employed in the prior two studies [14,15]. In the initial study, two intervals were used for determining lung function response: within 55 days (n = 21), and within 400 days of beginning ivacaftor treatment (n = 24) [14]. Similarly, a later study used two timeframes: between 15 and 75 days after initiation of treatment (n = 14 with G551D, 9 with other gating mutations) and during the first year of treatment with ivacaftor (n = 16 with G551D, 14 with other gating mutations) [15]. In 141 subjects from the GOAL study, the magnitude of change in FEV_1 (% Pred) was not different between baseline (pre-ivacaftor) and follow-up (post-ivacaftor) measures at one month, or three months, or six months (p = 0.22) (Figure S2). Thus, for our analyses, the measure for

response to ivacaftor was the mean change in FEV_1 (% Pred) from baseline (pre-ivacaftor) to each of the three time points over six months.

Since individuals with higher baseline values inherently have a reduced range for improvement than those with lower baselines, we examined the relationship between baseline FEV_1 (% Pred) and magnitude of mean change in FEV_1 (%Pred) during treatment with ivacaftor in all 141 GOAL subjects. The response was highly correlated with baseline lung function ($r = -0.078$, $p = 0.0051$) (Figure 2). As previously noted in clinical trials [9], there was a smaller improvement in FEV_1 (% Pred) in response to ivacaftor in subjects with a higher baseline FEV_1 (% Pred). Further, the magnitude of the response to ivacaftor was small in subjects with $FEV_1 < 40$ (% Pred) (Figure 2).

SLC26A9 (rs7512462) genotype and response to ivacaftor

Based on the correlation noted above, the standard study design used in therapeutic clinical trials, and the FEV_1 enrollment criteria in one study of *SLC26A9* [14,15], our primary analysis of the response to ivacaftor focused on 71 GOAL subjects with baseline (pre-ivacaftor) FEV_1 between 40 and 90 (% Pred). In these 71 subjects, there was no evidence of an association between rs7512462 genotype and the magnitude of the change in FEV_1 (% Pred) following ivacaftor treatment (Figure 3, $p = 0.90$ without CFRD as covariate, and $p = 0.93$ with CFRD as covariate). There was also no association when we analyzed 115 GOAL subjects with baseline FEV_1 between 40 and 110 (% Pred) (Figure S3A) ($p = 0.56$ without CFRD as a covariate and $p = 0.62$ with CFRD as a covariate). Further, there was no association when we analyzed all 141 GOAL subjects (Figure S3B) ($p = 0.65$ without CFRD as a covariate and $p = 0.69$ with CFRD as a covariate). The 141 subjects in GOAL had 80% power at an alpha of 0.05 to detect a 4% or greater difference in the predicted FEV_1 (%Pred) response to ivacaftor. More importantly, we estimate that our analysis of the 141 GOAL subjects has >97% power to replicate the previously reported association [14,15]. To test a recessive model, we analyzed by *SLC26A9* (rs7512462) genotype (*e.g.*, CC vs CT/TT or TT vs TC/CC) and saw no association with magnitude of FEV_1 (% Pred) response to ivacaftor in any of the 3 groups of subjects ($n = 71$; $n = 115$; $n = 141$ (see Figure S4 and above)), as p-values ranged from 0.25 to 0.93. We also saw no association between ivacaftor response and *SLC26A9* haplotypes encompassing the sixteen SNPs from 5', intron 1 and the intron 5 SNP (rs7512462) among the 141 individuals in the GOAL study. We saw no difference in baseline FEV_1 (%Pred) ($p = 0.79$; $n = 268$) or SaKnorm ($p = 0.24$; 270) when comparing Hopkins + UNO + GOAL subjects with one or two copies of the haplotypes associated with earlier onset of CF related diabetes and lower *SLC26A9* expression (HR) to those with one or two copies of the haplotypes associated with later onset CFRD and higher *SLC26A9* expression (LR) (Figure S5A) [25]. Finally, we saw no difference in ivacaftor response when we compared 141 GOAL subjects homozygous for LR haplotypes to those homozygous for the HR haplotype ($p = 0.14$; Figure S5A) or when individuals with one or two LR haplotypes were compared with those with one or two HR haplotypes (Figure S5B).

Discussion

Identification of genetic modifiers of CF provides an opportunity to develop therapies targeted to key components of disease modulating pathways. A single nucleotide polymorphism (rs7512462) in the *SLC26A9* gene was reported to modify lung function and lung function response to ivacaftor in individuals who carry the G551D and other gating variants. The idea that SLC26A9 may play a role in lung function is not without precedent, particularly in relation to chloride transport and mucous production. Increased airway mucous blockage due to loss of chloride secretion following exposure to IL-13 was observed in *Slc26a9* knockout mice [30]. That group also reported that a polymorphism (rs2282430) in the 3' UTR of SLC26A9 was associated with childhood asthma [30]. However, alleles of the 3' SNP are not co-inherited (*i.e.,* not in linkage disequilibrium) with the alleles of rs7512462 under study here, indicating a separate genetic effect.

Three prior studies analyzed association between lung function (SaKnorm or FEV_1) in individuals with CF with at least one G551D or other gating allele in *CFTR* and the rs7512462 genotype. The initial association [14] did not replicate in the two subsequent studies [15,31]. One group found no association between rs7512462 allele and FEV_1 in 127 CF patients ($p = 0.19$) [31] and the other found no association between rs7512462 allele and SaKnorm in 49 patients with G551D ($p = 0.40$) and 93 patients with other gating mutations ($p = 0.27$) [15]. Our results also failed to detect an association between lung disease severity (SaKnorm) and rs7512462 genotype in 272 CF patients with at least one G551D allele.

Two prior studies have reported a dose-dependent association between rs7512462 genotype and lung function change (FEV_1 (% Pred)) following ivacaftor treatment in individuals with at least one G551D or other *CFTR* gating mutation [14,15]. While these papers both report an association, the direction of change in FEV_1 (% Pred) are in opposite directions, which was attributed to the differing population structures of the study participants between the two studies [15]. We used similar timeframes (one, three, and six months post initiation of ivacaftor treatment) in the prospective GOAL subjects as in the prior studies, and found no difference in the magnitude of response. Furthermore, we documented a negative linear relationship between baseline FEV_1 (% Pred) and magnitude of ivacaftor treatment response in the GOAL study, likely due to the greater potential for improvement in response observed in those with lower lung function, than in those individuals with normal or near normal lung function. Consequently, we performed association using two subgroups with constrained baseline values and we also tested the entire group of GOAL subjects regardless of baseline value. In each case, there was no association between *SLC26A9* (rs7512462) genotype and change in FEV_1 (% Pred) after treatment, regardless of the cohort studied or whether CFRD status is used as a covariate or not.

The variant examined in this study (rs7512462) is part of a cluster of SNPs on chromosome 1 that demonstrate association with MI, CFRD, and neonatal pancreatic damage at the genome-wide level [11–13]. The SNPs in the cluster are in high linkage disequilibrium (LD), with co-inherited haplotypes showing a strong correlation with age at onset of diabetes and confer a 12-20% difference in reported gene expression [25]. We found no association between the same diabetes risk haplotypes and lung disease severity or response to ivacaftor.

Notably, the same variants do not show association with lung disease severity in a meta-analysis of genome wide studies primarily involving subjects carrying the common F508del variant (NM_000492.3(CFTR): c. 1521_1523delCTT (p.Phe508del)) (n = 6,365) [16]. These findings may reflect that this cluster of SNPs does not affect *SLC26A9* expression in the lungs, as opposed to the pancreas, as suggested by expression quantitative trait locus analysis [25]. We acknowledge that sample sizes were considerably reduced when using haplotypes which may have reduced power to detect a significant association. However, we were unable to detect association in larger sample sizes achieved by grouping individuals with one or two of the common diabetes risk haplotypes.

Subject stratification, genotyping error, relatedness may lead to false-positive or false-negative association. One way to address these issues is to determine if genotypes distribute as expected for the frequency of the DNA variant. Our cohorts for both lung function and ivacaftor response did not diverge significantly from Hardy-Weinberg equilibrium, as shown above. Likewise, the rs7512462 genotype distributions did not deviate from HWE in one prior study (MAF = 0.41, HWE χ^2 p-value = 0.49) [15]. Although a MAF of 0.38 was reported in the second prior study, the number of individuals with each genotype was not described precluding comparison with HWE [14]. Genotype distributions associated with response to ivacaftor presented in a figure shows only one individual with the CC genotype (Figure 2) [14]. Given the MAF and 21 subjects measured in the first 55 days or 24 subjects measured within the first 400 days of ivacaftor treatment, one would have expected about three individuals with this genotype. Thus, it is possible that the relatively small sample of individuals used to demonstrate association between *SLC26A9* and response to ivacaftor in the initial report was missing individuals with the most informative genotype (CC). Hardy-Weinberg equilibrium conformance also could not be determined for the full cohort of 70 individuals because genotype could not be deduced from the information given.

We recognize that a larger study might reveal subtle evidence of association between the variation in *SLC26A9* and lung function and/or response to ivacaftor. For example, association was observed between *SLC26A9* SNPs and peak expiratory flow (PEF) in 307,638 healthy UK Biobank participants [32]. While PEF and FEV_1 (%Pred) do correlate to some degree, the relationship between FEV_1 and PEF is poor and there are significant sex effects [33,34]. It is not immediately apparent that the association observed between *SLC26A9* SNPs and PEF in healthy individuals would translate to a modifier effect in individuals with CF. Nevertheless, we cannot exclude the possibility that variation in *SLC26A9* has a small, but biologically meaningful influence on lung function in CF. However, we had ample power to replicate the associations reported by two prior studies [14,15], as the relatively small number of subjects in each study implied a large effect size for the rs7512462 SNP. Based on the implied effect size, the current study had ample power (>97%) to replicate the previously reported association. Therefore, all CF subjects carrying a G551D allele should be eligible for treatment with ivacaftor, and not denied approval by health insurance on the basis of SLC26A9 genotype.

Multiple studies have demonstrated that CFTR and SLC26A9 interact and that F508del-CFTR can alter the stability, function, and localization of SLC26A9 [14,35–40]. These cell-based studies indicate that the strategies for therapeutic targeting of SLC26A9 should

account for *CFTR* genotype. However, the data presented here do not support a role for variation in *SLC26A9* mediating lung function response to ivacaftor in individuals with *CFTR* bearing gating variants. Consequently, rs7512462 genotype should not be used at this time to individualize ivacaftor treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Baseline FEV_1 (%Pred) correlates inversely with ΔFEV_1 (%Pred) on ivacaftor.
- *SLC26A9* genotype does not correlate with lung disease severity in G551D patients.
- *SLC26A9* genotype does not correlate with ivacaftor lung response in G551D patients.

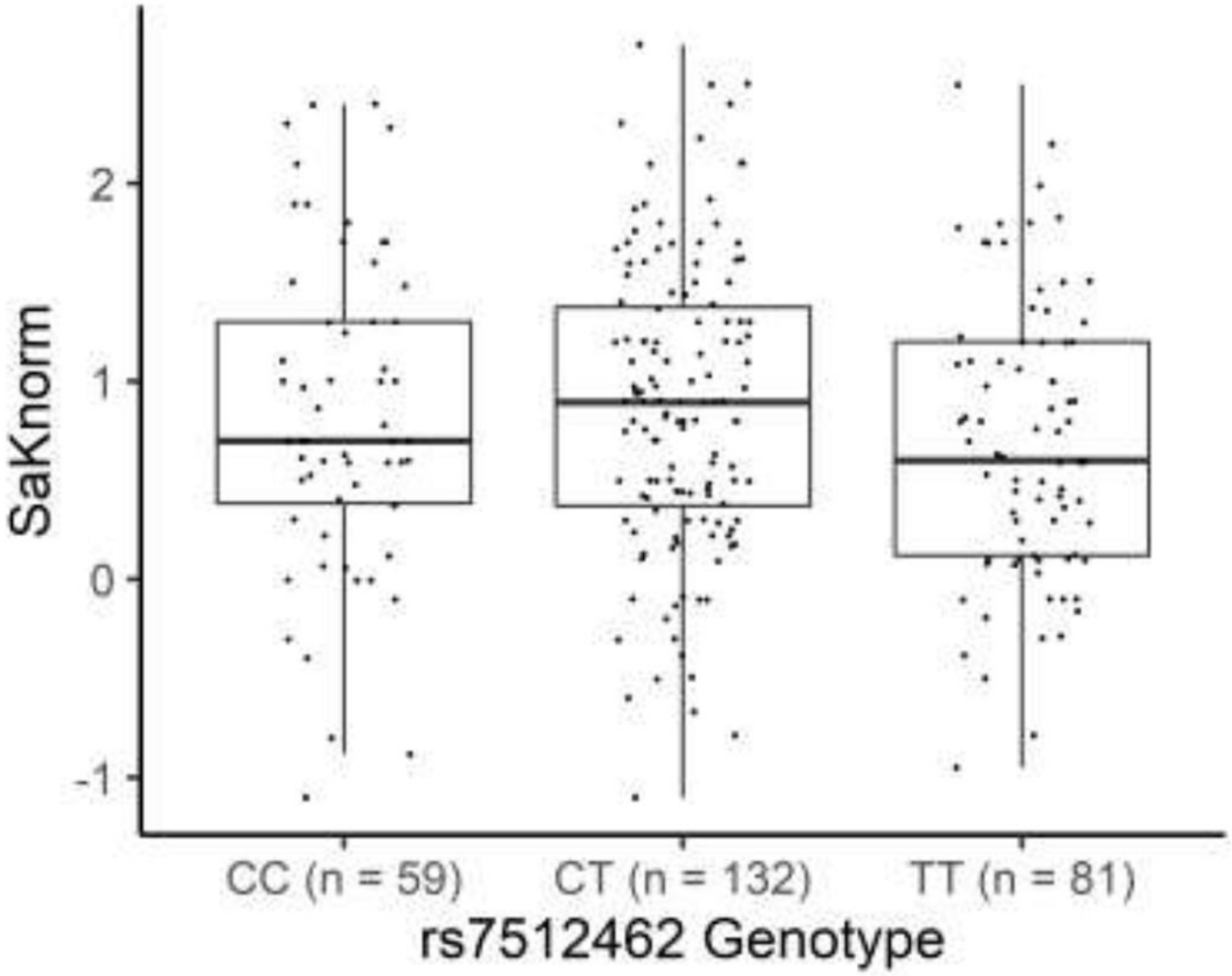

Figure 1.

Boxplot of SaKnorm by *SLC26A9* rs7512462 genotypes for 272 CF patients with at least one G551D allele. There was no significant association (p = 0.25 without CFRD as a covariate, p = 0.28 with CFRD as a covariate).

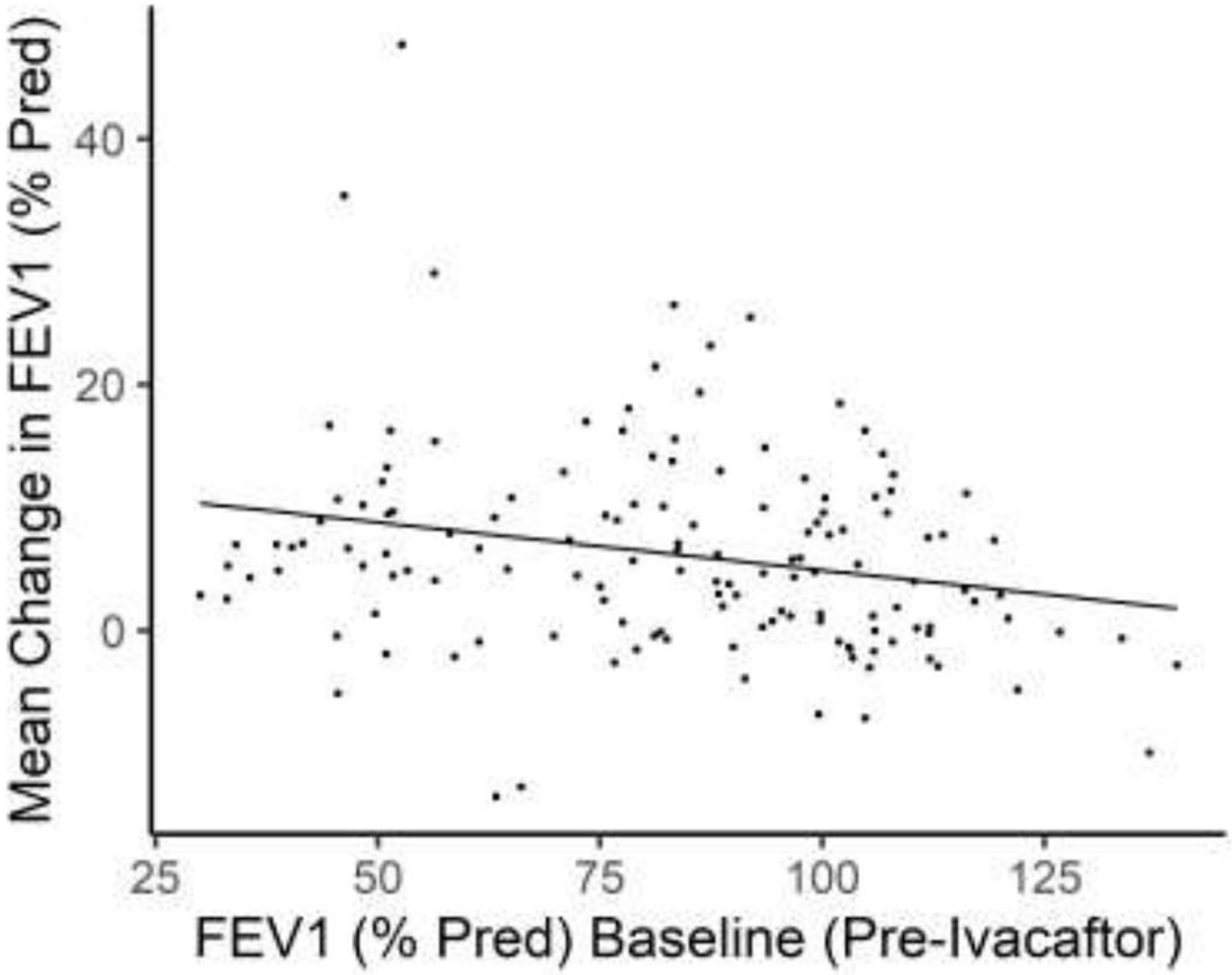

Figure 2.

Plot of mean change in FEV_1 (% Pred) from baseline in 141 GOAL subjects during treatment with ivacaftor. Overall, there was a strong inverse relationship (p = 0.0051).

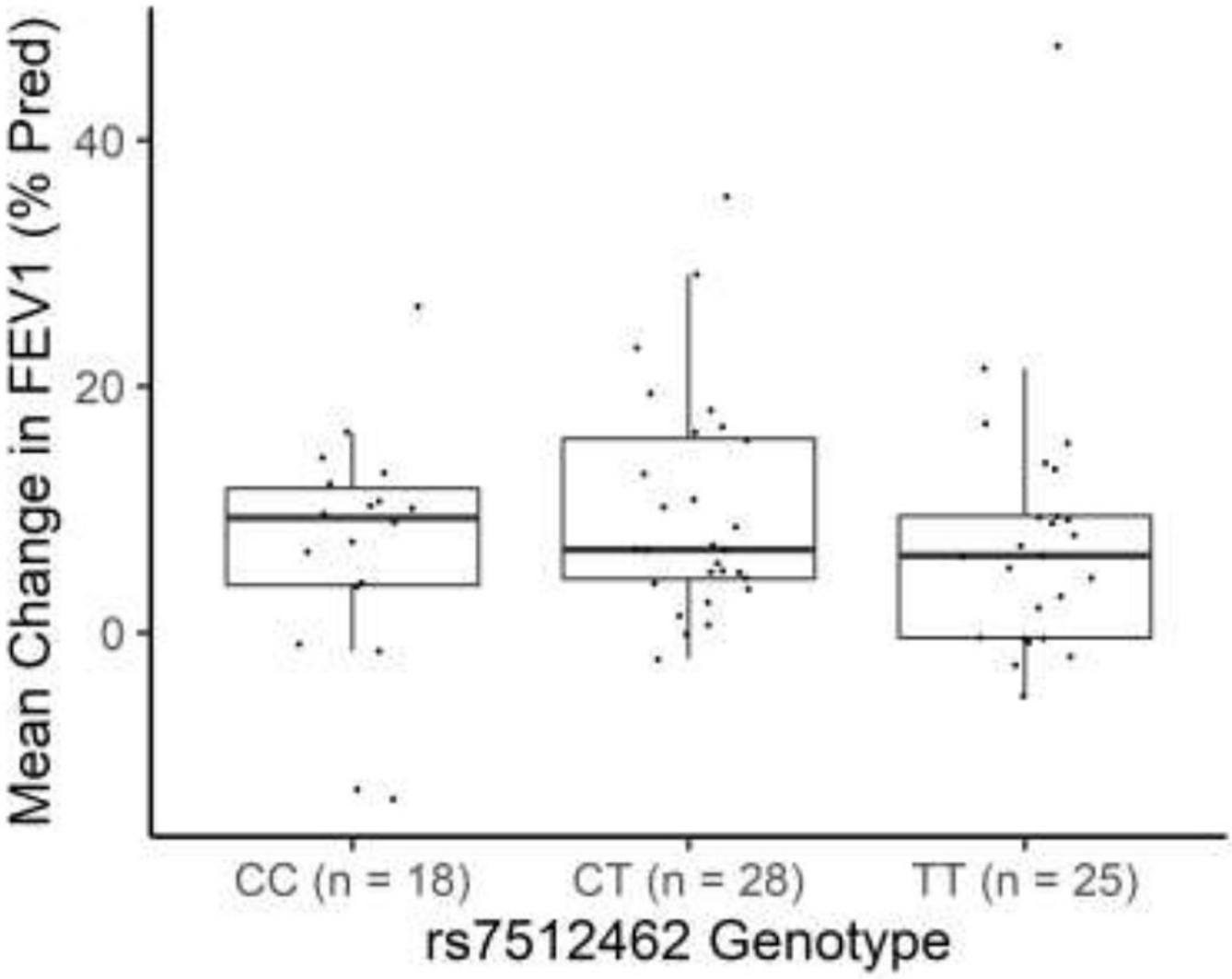

Figure 3.

Boxplot of mean change in FEV_1 (% Pred) by *SLC26A9* rs7512462 genotypes in 71 CF patients on ivacaftor in GOAL study with baseline FEV_1 between 40-90 (% Pred). There was no significant association (p = 0.90 and p = 0.93 without and with CFRD as covariate, respectively).

Table 1.

Demographics and Baseline (Pre-ivacaftor) Characteristics of G551D-CFTR Subjects.

	SaKnorm Group* (n = 272)**	GOAL Subjects (n =141)
Female, n (%)	129 (47.4)	66 (46.8)
Age at study (years); mean (SD)	20.7 (11.7)†	20.4 (11.7)‡
Age by genotype (years); mean (SD)		
SLC26A9, rs7512462		
CC	19.1 (10.6)†	20.4 (11.4)‡
CT	21.3 (12.3)†	20.6 (12.5)‡
TT	20.9 (11.4)†	20.1 (10.7)‡
Race, n (%)		
White	263 (96.7)	137 (97.2)
Hispanic	4 (1.5)	2 (1.4)
Black/Afr. Am.	5 (1.8)	2 (1.4)
Lung disease severity; SaKnorm (SD)	0.81 (0.75)†	N/A
Lung function; FEV_1, % Pred (SD)	75.6 (26.5) †	83.7 (24.8)‡
CFRD//, n (%)	77 (28.3)#	41 (29.1)

*Includes 141 GOAL subjects (9)

**Unrelated, n = 243

†At calculation of SaKnorm, pre-ivacaftor usage

‡At baseline (pre-ivacaftor), GOAL study

//CFRD = CF-related diabetes

#Based on available data (n = 263)