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Synonymous *ABCA3* Variants Do Not Increase Risk for Neonatal Respiratory Distress Syndrome

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Abstract

Objective—To determine whether synonymous variants in the adenosine triphosphate-binding cassette A3 transporter (*ABCA3*) gene increase the risk for neonatal respiratory distress syndrome (RDS) in term and late preterm infants of European and African descent.

Study design—Using next-generation pooled sequencing of race-stratified DNA samples from infants of European and African descent at 34 weeks gestation with and without RDS (n = 503), we scanned all exons of *ABCA3*, validated each synonymous variant with an independent genotyping platform, and evaluated race-stratified disease risk associated with common synonymous variants and collapsed frequencies of rare synonymous variants.

Results—The synonymous *ABCA3* variant frequency spectrum differs between infants of European descent and those of African descent. Using in silico prediction programs and statistical strategies, we found no potentially disruptive synonymous *ABCA3* variants or evidence of selection pressure. Individual common synonymous variants and collapsed frequencies of rare synonymous variants did not increase disease risk in term and late-preterm infants of European or African descent.

Conclusion—In contrast to rare, nonsynonymous *ABCA3* mutations, synonymous *ABCA3* variants do not increase the risk for neonatal RDS among term and late-preterm infants of European or African descent.

Neonatal respiratory distress syndrome (RDS) results from insufficiency of pulmonary surfactant, a phospholipid-protein complex that is synthesized, packaged, and exocytosed by alveolar type 2 cells, decreases surface tension, and maintains alveolar expansion at end expiration.¹ RDS is generally attributed to developmental insufficiency of pulmonary surfactant production; however, genetic mechanisms also contribute to the risk for neonatal RDS.^{2–7}

Adenosine triphosphate-binding cassette A3 transporter (ABCA3) is a member of the highly conserved family of adenosine triphosphate binding cassette transporters that bind and hydrolyze adenosine triphosphate to transport substrates across cellular membranes.⁸ ABCA3 is most highly expressed in the lung and is localized to the limiting membranes of lamellar bodies, intracellular storage organelles of pulmonary surfactant.^{9,10} Rare, recessive, nonsynonymous mutations in *ABCA3* are associated with lethal neonatal RDS and chronic respiratory disease in children.^{5,11} Recently, single, rare, nonsynonymous mutations in *ABCA3* were associated with reversible RDS in term and late-preterm infants of European descent.⁷

Although nonsynonymous *ABCA3* mutations that change the amino acids coded into that protein are known to increase the risk of neonatal RDS,^{5,7,12} much less is known about synonymous variants that do not change the amino acid sequence but may alter intron–exon splicing, splicing control elements, messenger RNA stability, translation efficiency, or protein folding.^{13–18} Two synonymous *ABCA3* variants have been associated with the risk of neonatal RDS.^{19,20} The synonymous variant p.F353F, which resides in the transmembrane domain, was associated with a prolonged course of RDS in preterm Finnish infants,¹⁹ and p.P585P, which resides in the nucleotide binding domain, was overrepresented in preterm Chinese infants with RDS.²⁰

Given that mutations in *ABCA3* can cause severe neonatal RDS, the evaluation of term and late-preterm infants with progressive respiratory failure unresponsive to medical management frequently includes *ABCA3* sequencing to establish a diagnosis of ABCA3 deficiency.²¹ Because most *ABCA3* mutations are rare, private, and have not been evaluated in surrogate cell systems,^{22,23} clinicians must rely on results of in silico prediction algorithms^{24–26} and the opinions of experts. Even though synonymous variants are frequently identified with such genetic sequencing, prognostic information for these variants is limited. Thus, using high-resolution, high-throughput, next-generation exonic sequencing; computational algorithms for variant discovery; in silico programs to predict functionality; independent validation of variants; and statistical strategies to compare common synonymous variant and collapsed rare synonymous variant frequencies, we examined the associations of synonymous *ABCA3* variants with the risk of neonatal RDS in term and late-preterm infants of European and African descent.

Methods

We used DNA collected from a previously reported prospectively enrolled cohort of newborn infants with and without RDS, 34 weeks gestational age, and maternally designated European or African descent recruited from the nurseries at Washington University Medical Center⁷ (Table I). We defined RDS as a requirement for supplemental oxygen (fraction of inspired oxygen > 0.3), chest radiograph findings consistent with RDS, and the need for continuous positive airway pressure or mechanical ventilation within the first 48 hours of life.^{6,7} Infants without RDS (non-RDS group) had no respiratory symptoms and were hospitalized for other neonatal problems. We assigned gestational age based on the best obstetrical estimate, and we excluded infants with cardiopulmonary malformations, pulmonary hypoplasia, culture-positive sepsis, chromosomal anomalies, known surfactant

mutations, or rapidly resolving RDS (within <24 hours of birth). We randomly excluded 1 of each set of monozygotic twins ($n = 3$) and twins in whom zygosity could not be reliably determined ($n = 2$). We extracted details of the respiratory course and outcome from the clinical chart. This study was reviewed and approved by the Washington University School of Medicine's Human Research Protection Office.

DNA Isolation and Pool Preparation

We isolated DNA from blood samples using Puregene DNA isolation kits (Qiagen, Valencia, California)^{6,7} and combined equimolar amounts from each individual into 4 race-stratified pools: infants of African descent with RDS ($n = 44$), infants of African descent without RDS ($n = 196$), infants of European descent with RDS ($n = 112$), and infants of European descent without RDS ($n = 161$).

Next-Generation Sequencing and Validation

We used an Illumina next-generation sequencing platform to sequence all exons and flanking regions (approximately 50 base pairs) of *ABCA3* (data available on request).²⁷ To optimize the selection of significance thresholds for detection of rare variants in each sequencing run, we added a 1934-bp oligonucleotide without variation and a 335-bp oligonucleotide containing 15 known insertions, deletions, and substitutions at a frequency of <1 allele per pool.²⁸ Inclusion of negative and positive controls allowed run-specific error models to achieve high sensitivity (0.99) and specificity (0.99) for detecting rare variants within each pool. We sequenced approximately 37 kb per individual, with a mean coverage of 82 $\times$.

We then used the computational algorithm SPLINTER²⁸ to detect rare (ie, minor allele frequency [MAF] <0.01) and common (MAF $\geq$ 0.01) synonymous variants. Each variant was confirmed with an independent genotyping strategy (Sequenom, TaqMan, or Sanger resequencing) and linked to its individual sample (data available on request). We had insufficient DNA for 10 infants (4 of African descent and 6 of European descent) to complete the validation studies, and thus we excluded these infants from all further analyses (Table I).

In Silico Prediction of Functionality

We used Alamut 2.3 (Interactive Biosoftware, Rouen, France), which combines the results of 7 splicing prediction algorithms— Human Splicing Finder (www.umd.be/HSF),²⁹ GeneSplicer (<http://www.cbcb.umd.edu/software/GeneSplicer>),³⁰ MaxEntScan (genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html),³¹ NNSplice,³² Splice Site Finder-Like,³³ ESE-Finder (<http://rulai.cshl.edu/tools/ESE>), and RESCUE-ESE (<http://genes.mit.edu/burgelab/rescue-ese/>)—to predict whether a synonymous variant would alter predicted intron–exon splicing patterns. We used the Genome Variation Server (<http://gvs.gs.washington.edu/GVS137/index.jsp>) to determine whether the common (MAF $\geq$ 0.01) synonymous variants were in linkage disequilibrium.

Statistical Analyses

We used χ^2 and Fisher exact tests to determine whether common *ABCA3* synonymous variants (MAF ≥ 0.01) were in Hardy-Weinberg equilibrium. We compared race-stratified frequencies of individual common (MAF ≥ 0.01) synonymous variants in infants with and without RDS using the χ^2 test (Table II). We compared collapsed frequencies of rare (MAF < 0.01) synonymous variants in infants with and without RDS using χ^2 and Fisher exact probability tests (Table II).³⁴ Because an individual is unlikely to carry more than 1 rare synonymous variant at a single gene locus, the number of rare variants in a single gene can be collapsed for statistical purposes and compared using a univariate test.³⁴ We used logistic regression to determine whether the presence of a synonymous variant increases the risk of RDS or has a gestational age-specific interaction. We used a gene dosage model³⁵ to determine whether carrying more than 1 synonymous variant imparts an additive risk for RDS. We used logistic regression to determine whether carrying a synonymous variant interacts with the presence of a single rare nonsynonymous *ABCA3* mutation to increase the risk of RDS. We used the Fisher exact test and Student *t* test to compare demographic data and severity measures between groups. We linked these data with the previously reported nonsynonymous mutation data,⁷ and performed logistic regression to determine whether synonymous variants statistically interacted with rare nonsynonymous *ABCA3* mutations to increase the risk of neonatal RDS. We used the Exome Variant Server (National Heart, Lung, and Blood Institute Exome Sequencing Project [ESP]; <http://snp.gs.washington.edu/EVS/>, release ESP6500MF, accessed March 2014), a database of more than 6500 individuals from up to 18 different US populations that participated in longitudinal cardiovascular and pulmonary-related research, to determine population-based frequencies of synonymous variants and compare these frequencies with those found in our cohorts.

Results

Infants of European Descent

We identified synonymous *ABCA3* variants in 181 of the 267 infants, with 54 infants carrying more than 1 synonymous variant (Table III; available at www.jpeds.com). Four synonymous variants (p.A227A, p.F353F, p.P585P, and p.S1372S) were common, and none deviated from Hardy-Weinberg equilibrium or were in linkage disequilibrium (maximum $R^2 = 0.053$; data available on request). Using Alamut, we found that none of the synonymous variants was predicted to disrupt intron–exon splicing.

There were no differences in the individual common synonymous *ABCA3* variant frequencies or the collapsed rare synonymous variant frequencies between infants of European descent with RDS and those without RDS (Table II). We used logistic regression models that included gestational age, sex, and mode of delivery, 3 covariates known to be associated with the risk of neonatal RDS, to determine that neither the presence of a single synonymous variant ($P = .37$) nor the number of synonymous variants ($P = .26$) was significantly predictive of RDS status. Although gestational age was an independent predictor of RDS among infants of European descent ($P < .001$), further modeling did not reveal a statistically significant interaction between the presence of a synonymous *ABCA3* variant and gestational age ($P = .15$).

Even though the infants of European descent with RDS had a lower mean gestational age compared with those without RDS, there was no difference in mean gestational age ($P = .069$) or birth weight ($P = .56$) between infants with and without synonymous *ABCA3* variants (Tables I and IV; Table IV available at www.jpeds.com). Moreover, we found no differences in measures of disease severity between infants of European descent with RDS with an *ABCA3* mutation and those without an *ABCA3* mutation, including duration of the need for mechanical ventilation, supplemental oxygen, pneumothorax, need for extracorporeal membrane oxygenation, need for home oxygen, and death (Table V; available at www.jpeds.com). The lack of statistically significant interactions between the presence of a synonymous variant ($P = .80$) or number of synonymous variants ($P = .56$) and rare nonsynonymous mutations in *ABCA3* suggests that nonsynonymous mutations act independently to increase the risk of neonatal RDS in term and late-preterm infants of European descent.⁷

Infants of African Descent

We found synonymous variants in 158 of 236 infants of African descent, 67 of whom carried more than 1 synonymous variant (Table III). In addition to the 3 synonymous variants (p.F353F, p.P585P, and p.S1372S) commonly seen in infants of European descent, p.V150V and p.V623V were common in the infants of African descent as well (Table VI). None of these common variants deviated from Hardy-Weinberg equilibrium or were in linkage disequilibrium (maximum $R^2 = 0.024$; data available on request). Using Alamut, none of the synonymous variants identified in our infants was predicted to disrupt intron-exon splicing.

We found no differences in the individual common synonymous *ABCA3* variant frequencies or the collapsed rare synonymous variant frequencies between infants of African descent with RDS and those without RDS (Table VI). We applied the same logistic regression models used in the European descent cohort and found that neither the presence of a single synonymous variant ($P = .87$) nor the number of synonymous variants ($P = .47$) was significantly predictive of RDS status. Similar to the European descent cohort, there was no statistically significant interaction between the presence of a synonymous *ABCA3* variant and gestational age ($P = .80$), and we found no differences in disease severity between infants of African descent with RDS with synonymous *ABCA3* variants and those without synonymous *ABCA3* variants (Table VII; available at www.jpeds.com). We also found no statistically significant interactions between the presence ($P = .75$) or number ($P = .78$) of synonymous variants and rare nonsynonymous *ABCA3* mutations to increase the risk of neonatal RDS.

Although the mean gestational age was lower in the infants of African descent with RDS compared with those without RDS, there was no difference in mean gestational age ($P = .48$) or birth weight ($P = .44$) between infants with synonymous variants and those without synonymous variants (data available on request). In addition, there were fewer female infants of African descent with RDS than males, likely related to our consecutive enrollment strategy and the lower incidence of RDS among females of African descent compared with males of similar gestational age.³⁶ Infants of African descent with RDS were more likely to

be delivered via cesarean delivery compared with those without RDS. Although transient tachypnea of the newborn is associated with cesarean delivery,³⁷ we only included infants with respiratory symptoms persisting for >24 hours.

Using the Exome Variant Server, we found no difference in the frequencies of common synonymous *ABCA3* variants between our cohort and individuals of European and African descent (Tables II, VIII, and IX; Tables VIII and IX available at www.jpeds.com). In addition, the collapsed frequency of rare synonymous variants in the ESP European descent cohort was similar to that in our infants of European descent without RDS (4.2% vs 7.0%; $P = .098$). More rare synonymous variants were present in the ESP African descent cohort compared with our infants of African descent without RDS (7.6% vs 3.1%; $P = .022$). This difference may reflect the greater number of individuals of African descent in the ESP, which enhances the ability to detect rare variants.³⁸

Discussion

ABCA3 mediates the transport of phospholipids into lamellar bodies and is required for their formation.^{39–41} Functional studies in surrogate cell systems have suggested that *ABCA3* mutations alter intracellular trafficking, impair adenosine triphosphate hydrolysis,^{22,23,26} or induce endoplasmic reticulum stress.⁴² Functional studies in other genes have suggested that synonymous variants can affect splicing, alter messenger RNA stability, affect translation rates by altering codon usage, and change protein folding patterns.¹⁴

Whereas previous studies identified associations with p.F353F and p.P585P, 2 of the most common synonymous variants in *ABCA3*, we did not find a statistically significant association for either of these variants with the risk of neonatal RDS.^{19,20} Possible explanations for our failure to replicate those previously reported findings include population differences (gestational age and ethnicity) between the cohorts. For example, to reduce confounding of developmental disruption of surfactant function, we studied more mature infants (>34 weeks gestation), whereas the previous studies evaluated preterm infants (25–34 weeks gestation).^{19,20} A gene-by-development interaction specific for more immature infants might account for their results. Our study focused on infants of European and African descent, whereas the previous studies involved infants of Chinese and Finnish descent. Finally, their large cohort size ($n = 503$) decreased the risk of type II error, which can confound studies of disease associations of common variants (MAF > 0.01) that are not under selection pressure and remain in the population.

Our finding that no common (MAF > 0.01) synonymous variants deviated from Hardy-Weinberg equilibrium suggests stable genetic variation and lack of selection. The similarity between synonymous *ABCA3* variant frequencies in our infants without RDS and individuals in the ESP suggests no loss of alleles between the neonatal period and adulthood. Our failure to find a statistically significant interaction between common synonymous variants and rare deleterious *ABCA3* mutations suggests the absence of modifying effects on *ABCA3* function; however, in vitro studies would permit more reliable, functional testing of variant–mutation interaction.

Alterations in splicing caused by synonymous variants are well described in numerous disease states.⁴³ We used the in silico splice site predictors embedded within Alamut to determine whether synonymous variants disrupted intron–exon splicing patterns. Likely related to the distance of most synonymous variants (>30 base pairs) from the intron–exon junction, no synonymous variants in *ABCA3* were predicted to affect splicing with high certainty.⁴⁴ This lack of splicing effect is consistent with the observation that synonymous variants do not increase the risk of RDS; however, because these predictions are based on computer modeling and not on studies in model systems, we may be misestimating their effects on splicing.

In addition to synonymous and nonsynonymous variants, other genetic alterations can affect gene expression. For example, variants within the promoter, introns, and 5′ and 3′ untranslated regions,⁴⁵ as well as neighboring deletions⁴⁶ involving key regulatory elements, can alter gene transcription and translation.

In conclusion, we found no statistical associations between individual common or collapsed rare synonymous *ABCA3* variants and the risk of neonatal RDS. We have outlined a computational strategy for assessment of individual synonymous variants discovered during the evaluation of infants with progressive respiratory failure. Future experiments in human model systems may be helpful in evaluating the contributions of individual synonymous variants to disruption of protein function.

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Glossary

ABCA3	Adenosine triphosphate-binding cassette A3 transporter
ESP	Exome Sequencing Project
MAF	Minor allele frequency
RDS	Respiratory distress syndrome

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Table 1

Characteristics of European and African descent disease-based groups (n = 503)

	European descent			African descent		
	RDS (n = 109)	Non-RDS (n = 158)	P	RDS (n = 44)	Non-RDS (n = 192)	P
Sex, n (%)						
Female	45 (41)	72 (46)	.49	10 (0.23)	99 (0.52)	<.001
Male	64 (59)	86 (54)		34 (0.77)	93 (0.48)	
Gestational age, wk, mean $\pm$ SD	36.9 $\pm$ 1.7	38.2 $\pm$ 1.6	<.001	37.6 $\pm$ 2.7	38.9 $\pm$ 1.7	.003
Birth weight, kg, mean $\pm$ SD	3.1 $\pm$ 0.6	3.1 $\pm$ 0.7	.50	2.9 $\pm$ 1.0	3.1 $\pm$ 0.4	.07
Route of delivery, n (%)						
Vaginal	54 (0.49)	71 (0.45)	.53	15 (0.34)	131 (0.68)	<.001
Cesarean	55 (0.51)	87 (0.55)		29 (0.66)	61 (0.32)	

Table II

Synonymous variants identified among infants of European descent

Variant	RDS (n = 109)	Non-RDS (n = 158)	P	ESP (n = 4300)	Distance from splice site, bp
V150V	1 (0.0046)	5 (0.016)	.41	6 (0.00070)	3
A227A	5 (0.023)	8 (0.025)	1.0	192 (0.022)	68
F353F	26 (0.12)	41 (0.13)	.79	847 (0.098)	53
P585P	40 (0.18)	61 (0.19)	.82	1702 (0.20)	14
A928A	0	1 (0.0032)	1.0	0	84
L982L	1 (0.0046)	0	.41	1 (0.00012)	59
A1027A	0	1 (0.0032)	1.0	0	77
S1372S	18 (0.083)	36 (0.11)	.25	729 (0.085)	49
E1618E	0	1 (0.0032)	1.0	0	56
V1648V	0	1 (0.0032)	1.0	27 (0.0063)	35
P1653P	0	2 (0.0063)	.52	0	25
Total synonymous variant frequency	91 (0.42)*	157 (0.50)*	.078	3652 (0.42)*,†	
Collapsed rare (MAF <0.01) synonymous variant frequency‡	2 (0.018)§	11 (0.070)§	.081	182 (0.042)‡,§	

* Number (carrier allele frequency, all synonymous variants).

† Includes additional synonymous variants detected only in the ESP, not in our cohort.

‡ MAF based on ESP allele frequency results.

§ Number (carrier rare allele frequency, assuming 1 rare synonymous variant per individual).

Table IIINumber of patients with synonymous *ABCA3* variants

Group	0 variants	1 variant	2 variants	3 variants	4 variants	Rare nonsynonymous <i>ABCA3</i> mutation
European descent	86	127	41	13	0	22
African descent	78	91	47	16	4	5

Table IVClinical characteristics of infants of European descent with and without synonymous *ABCA3* variants

Characteristic	Variant present (n = 181)	Variant absent (n = 86)	<i>P</i>
Sex			
Female	80 (44)	37 (43)	.86
Male	101 (56)	49 (57)	
Gestational age, wk, mean SD	37.8 ± 1.7	37.4 ± 1.7	.069
Birth weight, kg, mean SD	3.1 ± 0.7	3.1 ± 0.6	.56
Route of delivery, n (%)			
Vaginal	83 (0.46)	42 (0.49)	.65
Cesarean	98 (0.54)	44 (0.51)	

Table V

Disease severity measurements among infants of European descent with RDS with and without synonymous *ABCA3* variants

Characteristic	Variant present (n = 68)	Variant absent (n = 41)	P
Duration of ventilation, d, mean SD	11 ± 16	9 ± 11	.36
Duration of oxygen, d, mean SD	26 ± 65	15 ± 14	.17
Pneumothorax, %	25	10	.18
ECMO, %	2	3	.36
Home oxygen therapy, %	6	6	.35
Death, %	2	4	.20

ECMO, extracorporeal membrane oxygenation.

Table VI

Synonymous variants identified among infants of African descent

Variant	RDS (n = 44)	Non-RDS (n = 192)	P	ESP (n = 2198)	Distance from splice site, bp
V150V	13 (0.15)	50 (0.13)	.61	498 (0.11)	3
A227A	1 (0.011)	1 (0.0026)	.33	11 (0.0025)	68
A279A	1 (0.011)	0	.18	0	37
I318I	1 (0.011)	1 (0.0026)	.33	20 (0.0045)	37
P343P	0	1 (0.0026)	1.0	6 (0.0014)	39
F353F	4 (0.045)	30 (0.077)	.37	383 (0.087)	53
P585P	7 (0.080)	33 (0.084)	1.0	329 (0.075)	14
V623V	1 (0.011)	3 (0.0077)	.56	59 (0.013)	28
Y754Y	0	2 (0.0051)	1.0	0	2
H780H	1 (0.011)	1 (0.0026)	.33	19 (0.0043)	75
S1372S	21 (0.24)	77 (0.20)	.38	968 (0.22)	49
Total synonymous variant frequency	41 (0.47)*	199 (0.51)*	.50	2404 (0.55)*,†	
Collapsed rare (MAF <0.01) synonymous variant frequency§	4 (0.091)‡	6 (0.031)‡	.089	167 (0.076)‡,‡	

* Number (carrier allele frequency, all synonymous variants).

† Includes additional synonymous variants detected only in the ESP, not in our cohort.

‡ Number (carrier rare synonymous variant frequency, assuming 1 rare variant per individual).

§ MAF based on ESP allele frequency results.

Table VII

Disease severity measurements among infants of African descent with RDS with and without *ABCA3* synonymous variants

Characteristic	Variant present (n = 30)	Variant absent (n = 14)	<i>P</i>
Duration of ventilation, d, mean $\pm$ SD	11 $\pm$ 30	4 $\pm$ 5	.29
Duration of oxygen, d, mean $\pm$ SD	17 $\pm$ 31	9 $\pm$ 8	.21
Pneumothorax, %	4	0	.29
ECMO, %	0	0	NA
Home oxygen therapy, %	2	0	1.0
Death, %	2	0	1.0

NA, not applicable.

Table VIII

Synonymous variants among infants of European descent in the ESP

Variant	ESP (n = 4300)*	Distance from splice site, bp
T25T	1 (0.00012)	21
L31L	1 (0.00012)	39
P58P	1 (0.00012)	116
T92T	1 (0.00012)	44
A131A	16 (0.0019)	55
V150V	6 (0.00070)	3
Y158Y	1 (0.00012)	27
T168T	1 (0.00012)	57
P193P	1 (0.00012)	35
A227A	192 (0.022)	68
V240V	1 (0.00012)	107
P248P	1 (0.00012)	130
P249P	6 (0.00070)	127
I251I	5 (0.00058)	121
P254P	2 (0.00023)	112
L268L	1 (0.00012)	70
T277T	1 (0.00012)	43
V282V	3 (0.00035)	28
L317L	1 (0.00012)	40
I318I	1 (0.00012)	37
A319A	1 (0.00012)	34
A336A	3 (0.00035)	18
P343P	1 (0.00012)	39
L351L	2 (0.00023)	61
F353F	847 (0.098)	53
F377F	1 (0.00012)	20
P394P	1 (0.00012)	71
A499A	1 (0.00012)	30
A528A	1 (0.00012)	28
T574T	1 (0.00012)	20
P585P	1702 (0.20)	14
T586T	1 (0.00012)	17
S593S	1 (0.00012)	38
D600D	1 (0.00012)	59
V602V	1 (0.00012)	65
R663R	3 (0.00035)	64
L679L	1 (0.00012)	16
D724D	2 (0.00023)	92

Variant	ESP (n = 4300)*	Distance from splice site, bp
I733I	8 (0.00093)	65
Y754Y	1 (0.00012)	2
A756A	17 (0.0020)	5
H780H	1 (0.00012)	75
V839V	1 (0.00012)	4
D882D	3 (0.00035)	55
D952D	6 (0.00070)	149
L982L	1 (0.00012)	59
D988D	2 (0.00023)	41
A1055A	1 (0.00012)	114
H1069H	2 (0.00023)	72
F1077F	7 (0.00081)	48
P1078P	1 (0.00012)	45
E1093E	6 (0.00070)	1
N1103N	1 (0.00012)	31
D1168D	1 (0.00012)	21
T1173T	1 (0.00012)	36
T1211T	1 (0.00012)	71
I1222I	1 (0.00012)	38
L1226L	1 (0.00012)	26
Y1265Y	1 (0.00012)	68
V1279V	1 (0.00012)	26
S1299S	2 (0.00023)	35
L1328L	2 (0.00023)	52
C1337C	2 (0.00023)	25
S1372S	729 (0.085)	49
P1373P	1 (0.00012)	46
L1402L	1 (0.00012)	42
L1404L	1 (0.00012)	48
A1420A	1 (0.00012)	96
L1499L	1 (0.00012)	53
R1513R	1 (0.00012)	9
I1530I	1 (0.00012)	43
P1547P	1 (0.00012)	78
S1638S	1 (0.00012)	5
V1648V	27 (0.0031)	35
G1674G	1 (0.00012)	39
P1697P	1 (0.00012)	25
Total synonymous variant frequency	3652 (0.42)*	
Collapsed rare (MAF < 0.01) synonymous variant frequency	182 (0.042) [†]	

* Number (carrier allele frequency, all synonymous variants).

[†]Number (carrier rare synonymous variant frequency, assuming 1 rare variant per individual).

Table IX

Synonymous variants identified among infants of African descent in the ESP

Variant	ESP (n = 2198) *	Distance from splice site, bp
L9L	1 (0.00023)	27
F71F	1 (0.00023)	107
A131A	4 (0.00091)	55
V150V	498 (0.11)	3
A227A	11 (0.0025)	68
A229A	1 (0.00023)	74
L256L	1 (0.00023)	106
T274T	10 (0.0023)	52
V282V	1 (0.00023)	28
V283V	1 (0.00023)	25
L311L	1 (0.00023)	58
I318I	20 (0.0045)	37
A319A	1 (0.00023)	34
P343P	6 (0.0014)	39
F353F	383 (0.087)	53
F449F	1 (0.00023)	62
T574T	14 (0.0032)	20
P585P	329 (0.075)	14
V623V	59 (0.013)	28
C744C	2 (0.00045)	32
S747S	2 (0.00045)	23
A756A	3 (0.00068)	5
P770P	1 (0.00023)	47
H780H	19 (0.0043)	75
A792A	3 (0.00068)	39
S866S	3 (0.00068)	85
L897L	1 (0.00023)	10
L931L	1 (0.00023)	93
V932V	16 (0.0036)	96
T966T	1 (0.00023)	107
L1001L	2 (0.00045)	2
V1037V	1 (0.00023)	107
L1065L	8 (0.0018)	84
E1093E	3 (0.00068)	1
A1119A	1 (0.00023)	79
H1137H	2 (0.00045)	73
T1211T	1 (0.00023)	71
H1247H	1 (0.00023)	38
A1280A	1 (0.00023)	23

Variant	ESP (n = 2198)*	Distance from splice site, bp
S1299S	1 (0.00023)	35
A1311A	2 (0.00045)	71
C1315C	2 (0.00045)	83
S1372S	968 (0.22)	49
P1373P	2 (0.00045)	46
A1398A	2 (0.00045)	30
A1405A	1 (0.00023)	51
S1516S	1 (0.00023)	1
D1539D	1 (0.00023)	70
A1623A	1 (0.00023)	41
V1648V	5 (0.0011)	35
S1684S	2 (0.00045)	64
T1699T	1 (0.00023)	19
Total synonymous variant frequency	2404 (0.55)*	
Collapsed rare (MAF < 0.01) synonymous variant frequency	167 (0.076) [†]	

* Number (carrier allele frequency, all synonymous variants).

[†] Number (carrier rare synonymous variant frequency, assuming 1 rare variant per individual).