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Novel single nucleotide polymorphisms in the superoxide dismutase 1 and 2 genes among children with myelomeningocele

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Abstract

Objective—Excessive oxidative stress has been demonstrated as a mechanism for neural tube defects (NTDs). The current exploratory study sought to examine sequence variations in the superoxide dismutase 1 (*SOD1*) and 2 (*SOD2*) genes in patients with myelomeningocele and to identify variants altering risk for myelomeningocele.

Study Design—We sequenced deoxyribonucleic acid from 96 patients with myelomeningocele. The 11 exons were amplified by polymerase chain reaction, and the products were sequenced with the Sanger method. Results were compared with reference sequences (NM_000454, NM_000636, and NM_001024466) obtained from University of California Santa Cruz Genome Browser. Observed alleles that differed from the reference sequences were considered novel variants.

Results—We found 1 novel variant and 1 variant only recently described in phase 1 of the 1000 Genomes Project but not yet validated. The novel variant is located in the 3'-untranslated region (UTR) of *SOD2* and is present in 2 of 96 patients (1.0% allele frequency). The other variant is located in the 3'-UTR of *SOD1* and is present in 2 of 96 patients (1.0% allele frequency). Minor allele frequencies of known single nucleotide polymorphisms were compared with unaffected population controls.

Conclusion—We identified 1 novel variant and made the second report of an additional variant in the *SOD* genes studied. The variant located in the 3'-UTR of *SOD1* is predicted to alter microribonucleic acid (miRNA) binding. The variant located in the 3'-UTR of *SOD2* is predicted to alter 2 miRNA binding sites and potentially affects messenger ribonucleic acid production. We also identified 2 known single-nucleotide polymorphisms that occur in significantly different frequency compared with the unaffected population controls.

Keywords

myelomeningocele; single-nucleotide polymorphism; superoxide dismutase

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Neural tube defects (NTDs) are heterogeneous congenital malformations of the brain and spinal cord resulting from failure of normal neural tube to closure by 28 days after conception.¹ NTDs (both anencephaly and spina bifida) have a worldwide incidence at birth of approximately 0.5-1 in 1000 and are the most common structural malformations of the central nervous system in humans.²⁻⁴ A common and severe NTD phenotype, myelomeningocele, represents more than 90% of live-born infants with spina bifida.⁵ Most individuals living with myelomeningocele have a multiple disabilities and a shortened life expectancy.⁶ However, in spite of a high prevalence and major morbidity, the etiology of NTDs is not well understood.

The exact cause of an individual NTD is typically not known, but clustering within families as well as associations with specific genetic syndromes suggests that these phenotypes are related and are caused by an underlying genetic susceptibility.^{2,5,7} NTDs exhibit a multifactorial inheritance pattern, with genetic and environmental influences. Identifying the hereditary basis of NTDs and the contributing genetic factors is critical for characterizing the interactions between genes and the environment.⁸

The role of folate metabolism in the development of NTDs prompted the US Food and Drug Administration to mandate fortification of all cereal grains with folate in 1998. The result was a significant decrease in the rate of NTDs by 20-30%.^{9,10} However, in spite of folate supplementation, NTDs still occur with a prevalence of 3.5 per 10,000, likely involving other genetic and environmental influences.³

It has been demonstrated that increased oxidative stress (such as seen with maternal diabetes) influences a change in embryonic gene expression and enzymatic pathways that contribute to key developmental processes, resulting in an increased risk of congenital defects.¹¹ The immature antioxidant defense mechanism characteristic of early embryonic development compounds the impact of reactive oxygen species on the developing embryo.^{12,13}

The superoxide dismutase (SOD) enzymes, which produce hydrogen peroxide from superoxide radicals, are key protective mechanisms in the body's defense against reactive oxygen species. Studies have found that that addition of SOD or antioxidants to diabetic culture medium reduces the frequency of embryonic defects.¹⁴ Dietary copper deficiency has also been associated with malformed embryos and considerably reduced SOD activity, supporting a contributing environmental influence on NTD formation.¹⁵ Transgenic murine studies have also demonstrated that overexpression of SOD enzymes results in reduced neural malformation rates in embryos exposed to high levels of oxidative stress.^{16,17} Aberrations of SOD function are therefore a possible mechanism by which oxidative stress may contribute to the risk of congenital anomalies, including NTDs.

We recently reported that 5 single-nucleotide polymorphisms (SNPs) in the *SOD1* and *SOD2* genes were implicated in myelomeningocele risk.⁷ Four of the 5 SNPs were located in the intronic regions remote from the exons, and one (rs2070424) is approximately 260 bp upstream from exon 4 of *SOD1*. As a follow-up study, we sequenced the 11 exons of the *SOD1* and *SOD2* genes of deoxyribonucleic acid (DNA) obtained from a selected population of children born after 1998 with myelomeningocele to develop a more comprehensive understanding of variants in the *SOD1* and *SOD2* genes potentially altering myelomeningocele risk.

Materials and Methods

The children with a myelomeningocele in our study are a subset from a larger cohort of 864 myelomeningocele patients enrolled in ongoing genetic studies in our laboratory. The study

was approved by the institutional review board of the University of Texas Health Science Center at Houston. The study population characteristics are further described in detail elsewhere.¹⁸

The 96 children with nonsyndromic myelomeningocele tested in this study included 48 whites of European descent and 48 Hispanics of Mexican descent living in the United States or Canada. Hispanics of Mexican descent are of particular importance because they are underrepresented in genetic databases and have the highest prevalence of NTDs in the United States (approximately 4 per 10,000).³

A sample size of 96 was utilized because of the configuration of the standard polymerase chain reaction (PCR) plate (8 rows $\times$ 12 columns = 96). Additionally, each child has 2 alleles ($96 + 96 = 192$ total alleles) so that rare variants with an approximately 0.5% population frequency (1 of 192) maybe detected. Patients were selected with the goal of having an equal representation of white and Hispanic individuals and to maximize those patients in whom DNA was also available from both parents. We included only myelomeningocele affected patients born after the 1998 mandatory folic acid fortification of the US diet to select those patients whose mothers were more likely to have adequate dietary folate.

Blood and saliva samples were obtained from the patients and their parents. Genomic DNA was extracted from whole blood using the Puregene DNA purification kit (Gentra Systems, Minneapolis, MN). DNA from saliva was extracted using the Oragene DNA preparation kit (DNA Genotek; Kanata, Ontario, Canada). Stock DNA was then stored at -80°C . Working DNA aliquots of 10 ng/ μL were prepared for PCR at the start of the study following whole genome amplification (GenomiPhi; GE Healthcare, Piscataway, NJ).

PCR and nested-sequencing primers were designed based on the reference genomic sequences of *SOD1* (NM_000454) and *SOD2* (NM_000636, NM_001024466) extracted from the University of Santa Cruz Genome Browser (University of California Santa Cruz Genome Browser; Available at: <http://genome.ucsc.edu/cgi-bin/hgGateway>. Accessed DATE). We included approximately 50-100 bases flanking the boundaries of exons to also examine the splice donors and acceptors. A total of 6 PCR primer pairs were designed for *SOD1* and 9 primer pairs for *SOD2* (available upon request) synthesized by Integrated DNA Technologies USA (Commercial Park, Coralville, IA).

PCR amplification of exons was performed using the MJ Research PTC-100 Programmable Thermal Cycler (MJ Research, Waltham, MA). We confirmed the PCR product sizes by 1.8% agarose gel electrophoresis. The amplified exon DNA was then treated with T7 exonuclease and shrimp alkaline phosphatase (United States Biochemicals, Affymetrix, Cleveland, OH) to remove excess primers and nucleotides. The digested product was subjected to Sanger sequencing using the BigDyeTerminator (Applied Biosystems, Foster City, CA) reagents with nested sequencing primers and resolved on the ABI3100 Genetic Analyzer (Life Technologies Inc, Grand Island, NY).

In cases in which new SNPs were identified, we utilized custom TaqMan SNP genotyping assays with fluorescently labeled probes (Life Technologies) following the manufacturer's protocol to interrogate our total cohort of 864 patients. We added as controls 92 Mexican-American volunteers recruited from the Houston area and 93 white individuals from the Human Variation Panel-Caucasian Panel of 100 (HD100CAU; Coriell Institute for Medical Research, Camden, NJ) with no personal or family history of NTDs. The genotyping results were read using the 7900HT Fast realtime PCR system (Life Technologies) and analyzed using the 7900HT sequence detection system software.

Sequence results were analyzed using the DNA Sequencing Analysis Software version 5.1 from Applied Biosystems. To identify variants in sequences, we manually compared results with the reference sequences (NM_000454, NM_000636, NM_001024466) obtained from the University of California Santa Cruz (UCSC) Genome Browser. The white reference population (CEU) includes unaffected whites from Utah included in the Centre d'Étude du Polymorphisme Humain Collection for mapping genetic markers. The Mexican American (MEX) reference population includes unaffected Mexican Americans from Los Angeles, CA, used in the International HapMap project, the single-nucleotide polymorphism database (dbSNP). These reference populations have been shown to be genetically very similar to the 2 patients group in our study.¹⁷

Allele frequencies of variants were tallied, and any variant not reported in the dbSNP was considered a novel variant. Potential variants were subjected to a repeat PCR, and a reverse sequencing reaction to confirm the validity of the variant. The allele frequencies of known SNPs among myelomeningocele patients were compared with the SNP frequencies among ethnically matched unaffected population controls reported in the dbSNP and compared with a Fisher's test (when possible). A 2-tailed $P < .05$ was considered significant.

Results

In sequencing the exons of the *SOD1* and *SOD2* genes among 96 children with myelomeningocele, we found a novel variant at c.*68G>A within the 3'-untranslated region (UTR) of *SOD2* not previously reported in public SNP databases (Figure 1, A). Aligning sequences of the *SOD2* 3'-UTR variant (NM_001024466; c.*68G>A) in humans to 23 different available vertebrates using the UCSC Genome Browser (Available at: <http://genome.ucsc.edu/index.html>; Accessed DATE) shows the reference allele c.*68G to be highly conserved, particularly among primates (Figure 1, B).

We additionally noted a variant only recently described in the phase 1 of the 1000 Genomes Project, and not yet validated at c.*153T>G (rs188029963), located in the 3'-UTR of *SOD1* (Figure 2, A).¹⁸ Aligning sequences of the *SOD1* 3'-UTR of 30 different vertebrate species using the UCSC Genome Browser shows the T allele (reference allele) at c.*153T>G to be highly conserved and only different in the lizard and pika (Figure 2, B). In both cases, DNA from each affected individual's parents was available for sequencing, and this variant was present in 1 parent of each affected child.

We evaluated 25 SNPs within or flanking the exons of *SOD1* and 35 SNPs within or flanking the exons of *SOD2*. We report those SNPs in which a rare allele was identified among our cohort (Tables 1 and 2). Sixteen of the 25 SNPs evaluated in *SOD1* do not have population frequencies reported for the Caucasian reference population (CEU), and 24 of 25 do not have frequencies reported for the reference Mexican American (MEX) populations. Only 3 SNPs and 1 insertion/deletion demonstrated heterozygosity among white American children, and only 1 SNP demonstrated heterozygosity among Mexican American children for *SOD1* (Table 1). Thirteen of the 35 SNPs studied in *SOD2* do not have population frequencies reported for the CEU reference population, and 26 of the 35 SNPs studied do not have frequencies reported for the reference MEX populations.

Heterozygosity was demonstrated in 15 SNPs among white American children and in 12 SNPs among Mexican American children for *SOD2*. All SNPs are in Hardy-Weinberg equilibrium except for rs4880 and rs2758332 among the white children.

We compared the allele frequencies for the SNPs between affected children with the ethnically matched reference population and observed a nominal statistically significant higher minor allele frequency for rs5746134 (C/T) in exon 5 of the *SOD2* gene among

Mexican American children ($P = .004$). Additionally, a significantly lower minor allele frequency was observed for rs5746141 in exon 5 of the *SOD2* gene among white children ($P = .04$), when compared with the reference population (Table 1). No affected children carried both heterozygous genotypes of the 2 SNPs (rs5746134 and rs5746141) with nominal significance.

When we compared allele frequencies between ethnicities among our cohort, there was a significant difference between the whites and Mexican Americans for 2 SNPs (rs4880, rs5746140). Only the reference allele was demonstrated for 21 of 25 SNPs for *SOD1* and 20 of 35 SNPs for *SOD2* for the 11 sequenced exon regions in our cohort.

Lastly, we determined the frequency of the 2 newly identified SNPs (rs188029963 and NM_001024466; c.*68G>A) in our overall cohort of 864 patients. Fluorescently labeled probes designed using custom SNP genotyping TaqMan assays (Life Technologies) were used to subsequently genotype all of the patients at the 2 alleles of interest (available upon request).

Overall, there were 8 patients who were heterozygous at the *SOD1* 3'-UTR SNP (rs188029963) for a rare allele frequency of 0.4% (8 of 1728), all of whom were white. Among white patients ($n = 358$), the rare allele frequency was 1.1% (8 of 716). Four of the 8 white patients were born after 1991 of a total of 102 white patients born after 1991, resulting in a similar rare allele frequency (1.96%; 4 of 204) as the discovery cohort (Table 1).

There were 5 patients who were heterozygous at the novel SNP (NM_001024466; c.*68G>A), for an allele frequency of 0.3% (5 of 1728). Of the 5 patients with the novel SNP, 3 are white, 1 is Mexican American, and 1 patient is of unknown ethnicity. Two of the 3 white patients were born after 1991. Because this is a novel SNP, there is no population reference to make comparisons. None of the 185 control samples had the rare allele of either SNP.

Comment

The principal findings of our study were the discovery of a novel SNP in the 3'-UTR of *SOD2* with predicted detrimental functional consequences and the second report of a new SNP in the 3'-UTR of *SOD1* (rs188029963), which also is predicted to have deleterious functional consequences. We also demonstrated that 2 known SNPs (rs5746141 and rs5746134) in the 3'-UTR of *SOD2* were present in significantly different frequencies among our myelomeningocele cohort compared with healthy ethnic-matched population controls.

The first SNP, rs5746141, was found to be significantly less frequent among white children in our cohort compared with the healthy unaffected population controls ($P = .028$) so that the rare allele may confer a protective effect in association with other genetic or environmental factors. The second SNP (rs5746134) was found to be significant among Hispanics of Mexican descent at a $P = .004$. After using the most stringent correction to adjust for the multiple comparisons (Bonferroni, 9 comparisons, $P < .0056$), the P value remains significant.

We additionally quantified allele frequencies of 60 known SNPs, including those without known population frequencies. We originally identified that a SNP located in exon 5 of *SOD1* was novel because it is not present in the UCSC genome browser or the National Heart, Lung, and Blood Institute Exome Sequencing Project database. However, the latest release of data from the phase 1 of the 1000 Genomes Project recently described this SNP, and it has been given the name rs188029963 but has not yet been validated.¹⁹ Additional

population-based studies will be needed to validate and provide allele frequency information.

The novel variant (NM_001024466, c.*68G>A) in the 3'-UTR (exon 6) of *SOD2* was identified in 2 white children with myelomeningocele. One unaffected parent of each child also has the variant. This same variant was identified in 3 additional patients using the Taqman assay, yielding a rare allele frequency of 0.3% in this cohort of patients with myelomeningocele. However, the rare allele appears to be enriched among 102 white patients born after 1991 in our study cohort (2 of 102, 0.98%).

The frequencies for the reference CEU population or the MEX reference patients are not available. As noted in the previous text, the reference allele (G) is highly conserved throughout evolution and implies a biological importance of this allele site. The variant occurs in the UTR, which does not code for proteins but consists of an important regulatory site for messenger ribonucleic acid (mRNA) stability or protein translation efficiency. Specifically, this variant is predicted to alter recognition of 2 microribonucleic acids (miRNAs), hsa-mir-1324 and hsa-mir-4273, using the online miRBase search function (Available at: <http://www.mirbase.org/>. Accessed DATE; Figure 3).

The recently described SNP in the 3'-UTR (exon 5) of *SOD1* (rs188029963) was initially detected in 2 white children. One unaffected parent of each child also has the variant. An additional 6 patients were found to carry this variant using the Taqman assay, yielding an overall frequency of 0.4% in our cohort of patients with myelomeningocele. However, the rare allele appears to be enriched among 102 white patients born after 1991 in our study cohort (4 of 102, 1.96%).

This variant is also in the 3'-UTR of *SOD1* and is predicted to alter the recognition site of 1 miRNA, hsa-miR20a-3p, using the miRBase search function (Available at: <http://www.mirbase.org/> Accessed DATE; Figure 4). Alteration in miRNA recognition may potentially alter the stability of the corresponding mRNA and subsequently affect the expression of the coding protein. However, the significance of these variants will need to be verified through functional molecular studies of cloned DNA or assays for SOD activity in the affected children and parents. For this SNP, the frequency among the CEU and MEX healthy control populations is not available and is listed only as 0.1% for the entire 1000 Genomes Project population.¹⁹

There are several strengths of our study. We utilized Sanger sequencing, which is considered the gold standard for DNA sequencing and novel DNA variant discovery, but it is both time intensive and costly. Sanger sequencing allows for the sequencing of exon flanking regions, UTRs, and regions with high guanine and cytosine content, which newer modalities such as massively parallel shotgun sequencing of the exome will often miss. We additionally had parental DNA available from all children in whom new variants were found and verified the genotype of the unaffected parents. Although all of the variant alleles were inherited from unaffected parents, this does not imply that they are not significant but that environmental influences may have a greater contribution to the lesion in the genetically susceptible individual.

The limitations of our study include the small sample size and a low minor allele frequency of many SNPs, which limits the power of the study to compare genotype distributions between the patient and reference populations.

Our study provides additional evidence that the *SOD1* and *SOD2* genes may have a role in myelomeningocele susceptibility. Given the overall low frequency of these SNPs in the population, they are not expected to play a major role in myelomeningocele development.

Future studies in our laboratory will include cell culture with cloned DNA from affected children with these variants to evaluate the effect on SOD activity, compared with reference alleles of the unaffected parental controls. Although there have been tremendous advances in the prevention of NTDs with preconceptional folate supplementation and treatment of NTDs following diagnosis, there remains a pressing need to, improve our understanding of the genetic basis and mechanisms for development of NTDs.²⁰

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Figure 1. Sanger sequencing of reference allele c.*68G and sequence alignment

A, Sanger sequencing result of reference allele c.*68G (arrow) is shown on the *left panel*, and the result for the patient with the variant allele c.*68G>A (arrow) is shown on the *right panel*. **B**, The sequence alignment of the *SOD2* 3'-UTR for 23 vertebrate species listed on the *left-hand side* is generated from the UCSC Genome Browser (Available at: <http://genome.ucsc.edu/index.html>; Accessed DATE) with the c.*68G allele enclosed in a *box*. *SOD2*, superoxide dismutase 2.

Figure 2. Sanger sequencing of reference allele c.*153T and sequence alignment

A, Sanger sequencing result of reference allele c.*153T (arrow) is shown on the *left panel*, and the result for the patient with the variant allele c.*153T>G (arrow) is shown on the *right panel*. **B**, The sequence alignment of the *SOD2* 3'-UTR for 30 vertebrate species listed on the *right-hand side* is generated from the UCSC Genome Browser (Available at: <http://genome.ucsc.edu/index.html>; Accessed DATE) with the c.*153T allele enclosed in a *box*. *SOD2*, superoxide dismutase 2.

c.*68G 61 aagaugu**g**cau-caagccugguac 37
 || ||||| || ||| |
hsa-mir-1324 84 cuuucacguaucuaagacagacc -61

c.*68A 61 aagaugu**a**cau-caagccugguac 37
 || || ||| || ||| |
hsa-mir-1324 84 cuuucacguaucuaagacagacc -61

Note: The G>A change removes one G:C bond potentially weakening the interaction with hsa-mir-1324.

c.*68G 53 ugucuucuaagaugu**g**cauc 72
 || |||| |||| ||
hsa-mir-4273 11 guguucucugaugg**a**cag 28

c.*68A 53 ugucuucuaagaugu**a**cauc 72
 || |||| |||| |||
hsa-mir-4273 11 guguucucugaugg**a**cag 28

Note: The G>A change adds a matching nucleotide potentially increase the similarity to hsa-mir-4273.

Figure 3. Predicted miRNA binding site change with variant c.*68G>A of SOD2

The variant is removes 1 G:C bond in a region in which hsa-mir-1324 is predicted to bond, potentially weakening the interaction. Additionally, the G>A change adds a matching nucleotide, which may increase the similarity to hsa-mir-4273.

c. *153T	147	agaaac <u>u</u> gauuuauaucacuuggaa	173
hsa-miR20a-3p	30	gaugga <u>c</u> gugauauucgugaaau	8
c. *153G	147	agaaac <u>g</u> gauuuauaucacuuggaa	173
hsa-miR20a-3p	30	gaugga <u>c</u> gugauauucgugaaau	8

Note: The T>G change removes a U:A bond potentially weakening the interaction with hsa-miR20a-3p.

Figure 4. Predicted miRNA binding site change with variant rs188029963 of *SOD1*

Note that the T>G change removes a U:A bond, potentially weakening the interaction with hsa-miR20a-3p.

SNPs with demonstrated rare alleles in the *SOD1* gene of children with myelomeningocele

Table 1

Variable	Minor allele (A2) frequency					CEU/MEX
	A1/A2	All MM	MM	Referent	MM	
SNP						P value
<i>SOD1</i>						
21:33036248	A/ATAAACAG	0.50% (1/190)	1.00% (1/96)	0.44% (36/8218) ^b	0.00%	ND
rs2234694	A/C	2.10% (4/190)	3.20% (3/94)	4.20% (5/120) ^c	1.00% (1/96)	ND
rs18802963	T/G	1.00% (2/192)	2.10% (2/96)	ND	0.00%	ND
rs17880487	C/T	1.60% (3/190)	1.00% (1/96)	0.40% (1/226) ^b	2.10% (2/94)	4.20% (5/116)
						.44/.46

Variants without rs number are represented by the hg19 genomic location with chromosome number followed by the number of the nucleotide.
A1, reference allele; *A2*, variant allele; *CEU*, Centre d'Etude du Polymorphisme; *MEX*, Mexican American; *MM*, myelomeningocele; *ND*, no data for reference population; *P*, Fisher exact test *P*-value (2 tailed); *SNP*, single-nucleotide polymorphism; *SOD1*, superoxide dismutase 1.

^aThe MEX population consists of Mexican ancestry from Los Angeles, CA (International HapMap Project);

^b1000 Genomes Project;¹⁸

^cThe CEU population consists of Utah residents with northern and western European ancestry from the CEPH collection (International HapMap Project).

Table 2
SNPs with demonstrated rare alleles in the *SOD2* gene of children with myelomeningocele

Variable	Minor allele (A2) frequency						CEU/MEX
	CEU			MEX			
SOD2							
SNP	AI/A2	All MM	MM	Referent	MM	Referenta	P value
rs5746092	G/C	20.1% (37/184)	22.90% (22/96)	25.80% (31/120) ^b	17.00% (15/88)	ND	.63/–
rs5746093	C/G	0.54% (1/188)	1.00% (1/96)	ND	0.00%	ND	–/–
rs5746094	T/C	20.21% (38/188)	22.9% (22/96)	25.00% (30/120) ^b	17.40% (16/92)	ND	.75/–
rs4880	A/G	52.63% (100/190)	42.71% (41/96)	43.94% (145/330) ^c	62.77% (59/94)	63.53% (108/170)	.14/1.00
rs4987023	C/T	0.00%	0.00%	0.00%	0.00%	1.70% (2/116)	–/–
rs2758332	G/T	53.13% (102/192)	47.92% (46/96)	41.70% (50/120) ^b	58.33% (56/96)	ND	.33/–
rs5746133	G/C	0.00%	0.00%	2.30% (4/174) ^c	0.00%	ND	–/–
rs5746134*	C/T	4.17% (8/192)	1.04% (1/96)	0.30% (1/330) ^c	7.29% (7/96)	0.60% (1/166)	.24/.004
rs5746135	G/A	4.17% (8/192)	1.04% (1/96)	0.30% (1/328) ^c	7.29% (7/96)	13.53% (23/170)	.40/.15
rs5746136	G/A	25.52% (49/192)	29.17% (28/96)	31.82% (105/330) ^c	21.88% (21/96)	25.58% (44/172)	.71/.55
rs5746137	A/T	1.04% (2/192)	2.08% (2/96)	1.70% (2/120) ^b	0.00%	ND	1.00/–
rs5746138	A/G	4.17% (8/192)	3.13% (3/96)	7.50% (9/120) ^b	5.21% (5/96)	ND	.23/–
rs5746140	C/A	7.29% (14/192)	1.04% (1/96)	ND	13.54% (13/96)	9.87% (15/152)	–/.41
rs5746141*	G/A	2.63% (5/190)	2.08% (2/96)	9.39% (31/330) ^c	3.19% (3/94)	2.91% (5/172)	.028/1.00
rs7855	T/C	5.21% (10/192)	6.25% (6/96)	6.06% (20/330) ^c	4.17% (4/96)	1.74% (3/172)	1.00/.43
6:160100282	G/A	1.04% (2/192)	2.08% (2/96)	ND	0.00%	ND	–/–
rs2842980	A/T	16.15% (31/192)	20.80% (20/96)	24.70% (81/328) ^c	11.46% (11/96)	11.31% (19/169)	.50/1.00

Variaants without rs number are represented by the hg19 genomic location with chromosome number followed by the number of the nucleotide.

AI, reference allele; A2, variant allele; CEU, Centre d'Etude du Polymorphisme; MEX, Mexican American; MM, myelomeningocele; ND, no data for reference population; P, Fisher exact test P value (2 tailed); SNP, single-nucleotide polymorphism; SOD1, superoxide dismutase 1.

^aThe MEX population consists of Mexican ancestry from Los Angeles, CA (International HapMap Project);

^b1000 Genomes Project;¹⁸

^cThe CEU population consists of Utah residents with northern and western European ancestry from the CEPH collection (International HapMap Project).