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Blepharospasm in a multiplex African-American pedigree

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Abstract

Background—Isolated blepharospasm (BSP) is a late-onset focal dystonia characterized by involuntary contractions of the orbicularis oculi muscles. Genetic studies of BSP have been limited by the paucity of large multiplex pedigrees. Although sequence variants (SVs) in *THAP1* have been reported in rare cases of BSP, the genetic causes of this focal dystonia remain largely unknown. Moreover, in the absence of family history and strong *in silico* or *in vitro* evidence of deleteriousness, the pathogenicity of novel SVs in *THAP1* and other dystonia-associated genes can be indeterminate.

Methods—A large African-American pedigree with BSP was phenotypically characterized and screened for mutations in *THAP1*, *TOR1A* and *GNAL* with Sanger sequencing. Whole-exome sequencing of the proband was used to examine other dystonia-associated genes for potentially pathogenic SVs. *In silico* and co-segregation analyses were performed for a novel *THAP1* SV identified in the proband.

Results—Seven family members exhibited increased blinking and/or stereotyped bilateral and synchronous orbicularis oculi spasms with age of onset ranging from early childhood to late adult life (7 to 54 years). The proband was found to harbor a novel *THAP1* SV (c.314T>C, p.L105S). However, the p.L105S SV did not co-segregate with blepharospasm in the pedigree. Moreover, *in silico* analyses suggest that p.L105S is benign. No pathogenic or likely pathogenic SVs in other dystonia-associated genes were identified with whole-exome sequencing.

Conclusions—Blepharospasm can be familial and may be hereditary in African-Americans. A comprehensive array of *in silico* tools, and, if possible, co-segregation analysis should be used to classify SVs in dystonia-associated genes.

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Keywords

Dystonia; Blepharospasm; *THAP1*; Sequence variant; African-American

1. Introduction

Isolated blepharospasm (BSP) is a late-onset focal dystonia characterized by involuntary contractions of the orbicularis oculi muscles [1]. BSP is a relatively common form of focal dystonia, with prevalence estimates ranging from 1.6 to 13.3/100,000 worldwide [2]. Mean age of onset is from 50 to 60 years and women are more commonly affected than men [1, 3]. Genetic factors are believed to play an important role in the etiopathogenesis of BSP given that 10 to 27% of affected individuals report a positive family history of dystonia [4, 5]. BSP is within the phenotypic spectrum of several genetic forms of dystonia inherited in autosomal dominant fashion (DYT1, DYT6, and DYT25) [4, 6]. Although rare cases of BSP as a singular dystonia manifestation have been linked to *THAP1* mutations, the genetic underpinnings of this important focal dystonia remain largely unknown and large pedigrees adequately powered for linkage analysis are quite uncommon [4, 7]. Although one large Italian BSP family has been described in the literature [8], there have been no reports of large BSP pedigrees in other ethnic and racial groups.

2. Patients and Methods

2.1 Human Subjects

All human studies were conducted in accordance with the Declaration of Helsinki with formal approval from the University of Tennessee Health Science Center Institutional Review Board (01-07346-FB, 05-08331-XP, and 14-03320-XP). All subjects gave written informed consent for genetic analyses and disclosure of medical and demographic information. Subjects in this pedigree (Fig. 1) were examined by a neurologist (MSL) with subspecialty expertise in movement disorders. Subjects were asked to perform specific tasks including holding their eyes open, opening and closing their eyes gently, opening and closing their eyes forcefully, along with additional verbal and postural maneuvers design to capture masticatory, laryngeal or cervical involvement. Using motor severity classification from the Unified Dystonia Rating Scale, BSP was categorized as mild, moderate, severe or extreme. Medical histories were documented for each subject. A clinical diagnosis of definite BSP was given to subjects exhibiting increased blinking and stereotyped, bilateral and synchronous orbicularis oculi spasms inducing narrowing/closure of the eyelids [9]. Subjects with isolated episodes of increased eyelid blinking were given a diagnosis of possible BSP. Each affected or possibly affected family member was queried for the presence of sensory tricks.

2.2. Genetic analyses

Blood or saliva was acquired from 12 subjects, and DNA was extracted for variant screenings. Using previously defined primers, Sanger sequencing was employed to screen the proband for sequence variants (SVs) in *THAP1*, *GNAL* and Exon 5 of *TOR1A* [4, 6, 10]. For whole-exome sequencing, the proband's genomic DNA (3 µg) was sheared to yield

100-450 bp fragments, followed by processing with an Illumina (San Diego, CA) paired-end library preparation kit. Target enrichment was performed with the Agilent SureSelect Human All Exon V5 kit (Santa Clara, CA). Enriched DNA fragments were sequenced on Illumina's HiSeq 2000 platform as paired-end 100-125 base reads (Otogenetics Co., Norcross, GA USA). Sequence reads (FASTQ) were mapped to the human reference genome (NCBI build 37.1) with NextGENe® (SoftGenetics, State College, PA, USA). An integrated query of all NCBI databases with the search term 'blepharospasm' produced the following list of genes that have been associated with BSP as an isolated dystonia or part of a more complex neurogenetic syndrome: *THAP1*, *TORIA*, *SGCE*, *ATCAY*, *CIZ1*, *GNAL*, *ANO3*, *PRRT2*, *ATM*, *PRKRA*, *GCH1*, *TAF1*, *PNKD*, *ATP1A3*, *SLC2A1*, *TH*, *SPR*, *TIMM8A*, *DRD5*, *CP*, *PANK2*, *FTL*, *FBX07*, and *DJI*. SVs in reads mapped to these genes were analyzed *in silico* with the following programs which predict pathogenicity or deleteriousness: PolyPhen-2, MutationTaster, Sorting Intolerant From Tolerant (SIFT), Likelihood Ratio Test (LRT), and Combined Annotation Dependent Depletion (CADD). The allele frequency of identified SVs was compared to reported frequencies in dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>), 1000 Genomes (1KG, www.1000genomes.org) and Exome Aggregation Consortium (ExAC, exac.broadinstitute.org).

A presumed SV in *DRD5*, detected with whole-exome sequencing, was examined with genomic DNA (gDNA) and complementary DNA (cDNA) from the proband using forward (DRD5_F, cagtccagccccgaaatgc, NM_000798.4: 383-400) and reverse primers (DRD5_R, cagcaaaaggtctgacacgg, NM_000798.4 666-647) to generate a 284 bp amplicon. PCR was performed using 40 ng of peripheral blood gDNA or 2 µl of cDNA transcribed from 10 ng of total RNA along with 200 nM of each primer in a 10-µl reaction volume with HotStarTaq® Plus DNA polymerase from Qiagen (Valencia, CA). The following cycling conditions were employed: 95°C for 15 min; 35 cycles at 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s.

3. Results

Herein, we report a large multiple African-American pedigree with BSP (Table 1, Fig. 1). Among the 12 subjects that were examined (Fig. 1), 7 were assigned a diagnosis of either definite or possible BSP (II-1, II-3, II-5, II-7, III-4, III-9, and III-12) with ages of onset ranging from 7 years to 54 years of age.

The proband (II-3) was most recently examined at 66 years of age and reported onset of BSP at 48 years of age. At age 62, he underwent an extensive dental extraction and noted the development of lower facial and jaw-opening masticatory dystonia several months later. Neurological examination showed evidence of severe BSP with increased blink frequency and prolonged spasms of the orbicularis oculi muscles. Lower facial involvement and involuntary jaw-opening were prominent. The proband has shown significant and consistent benefit from injections of incobotulinumtoxinA for treatment of BSP and lower facial dystonia. He has also received electromyographically-guided injections of incobotulinumtoxinA into the inferior head of the lateral pterygoid muscles with notable reductions in involuntary jaw opening.

Three other family members, II-5 (59 years old), III-9 (33 years old), and III-12 (27 years old) were given diagnoses of definite BSP. Subject II-5, a sister of the proband, reported episodes of increased blinking, possibly triggered by phacoemulsification of her cataracts. In agreement with our clinical examination, Subject II-5 noted that she was less severely affected than her son (Subject III-9). Subject III-12, a niece of the proband, first noticed increased eyelid blinking at the age of 7 years. Subject III-12 was diagnosed with Tourette syndrome as a child due to the presence of motor and phonic tics which largely resolved by 15 years of age. At her most recent evaluation, BSP was present with eyelid spasms and narrowing of the palpebral fissures but no motor or phonic tics were apparent. Unlike the proband, these three additional family members with definite BSP did not exhibit oromandibular or lower facial involvement. In addition, none of the individuals in this pedigree had manifest cervical or appendicular dystonia. None of these definitely affected subjects had identified any useful sensory trick. No members of the pedigree reported dry eyes. Subjects II-5, III-9 and III-12 had not sought treatment for their BSP.

The proband's son (III-4, 30 years old) and two sisters (II-1, 69 years old, and II-7, 50 years old) were assigned diagnoses of possible BSP due to increased blink frequency. The exact ages of onset are unknown for these subjects. Subject II-1, a diabetic, reported eye spasms which were less severe than those experienced by the proband and other affected individuals, but indicated that her spasms were worse at night and when fatigued. Neither Subject III-4 nor II-7 reported eye spasms, although episodes of increased eyelid blinking were provoked in all three of these subjects during examinations.

Sanger sequencing of *THAPI*, *GNAL*, and Exon 5 of *TORIA* exposed a *THAPI* missense SV (c.314T>C, p.L105S). This SV was not present in dbSNP or 1KG. In addition, c.314T>C was not detected in 200 African-American alleles or 1000 Caucasian control alleles in our own laboratory database. However, in the ExAC database, c.314T>C was found in one African-American subject (Minor Allele Frequency [MAF] = 0.000008). In our pedigree, this SV was detected in only 5 of 7 affected subjects (II-1, II-3, II-7, III-4, and III-12) and 1 normal subject (II-4). Therefore, c.314T>C did not co-segregation with BSP in this family. *In silico* analyses with PolyPhen-2, MutationTaster, SIFT and LRT indicated that this variant is not pathogenic with scores of 0, 1, 0.75, and 0.009, respectively. Moreover, CADD, a robust tool for scoring the deleteriousness of single nucleotide variants as well as insertion/deletion variants in the human genome, generated a PHRED score of 0.476 which suggests that *THAPI* c.314T>C is benign.

Whole-exome data from the proband showed that over 98.6% of exons were covered at 10× and 97.5% were covered at 20× (Table 2). As shown in Table 3, all dystonia-associated genes were adequately covered to detect coding SVs. SVs in genes previously reported to be associated with dystonia were analyzed for pathogenicity/deleteriousness using CADD, SIFT, PolyPhen-2, and MutationTaster. However, we did not identify a single potential candidate SV based on *in silico* analyses of pathogenicity/deleteriousness and associated MAFs within 1KG and ExAC (Table 4). Although a few SVs had high CADD PHRED scores, they were not deemed to be viable candidate SVs due to high MAFs in 1KG and ExAC.

Bidirectional Sanger sequencing of gDNA and cDNA showed that the presumed SV in *DRD5* (Table 3, c.116C>T) was, in fact, a three nucleotide change (c.115TCA>GTG) present within *DRD5* pseudogenes (*DRD5P1* and *DRD5P2*), and, hence, a next-generation mapping error. Although *DRD5* pseudogene transcription has been reported in brain, we did not detect c.115TCA>GTG in leukocyte cDNA.

4. Discussion

Though BSP is a seemingly common form of focal dystonia seen by neurologists and ophthalmologists, the reported prevalence of BSP has been highly variable, with crude estimates ranging from 16 to 133 cases per million, mainly in studies of Caucasian populations [1, 2]. BSP has been described in African-Americans [6, 11] but to our knowledge no large multiplex BSP pedigrees have been published in the English literature. However, one subject from a large African-American pedigree with dystonia due to a *GNAL* SV did manifest BSP as part of her segmental craniocervical dystonia [6]. While cervical dystonia is reportedly less common in African-Americans than Caucasian-Americans, the relative prevalence of BSP in African-Americans and other racial groups has not been well characterized [12].

Age and female gender are risk factors for developing BSP [1, 13]. Among the 7 definitely or probably affected subjects in our pedigree, 4 were female and 3 were male. However, the 2 most severely affected individuals were male. Previous studies have also shown that there is an increased risk of developing BSP in subjects with a family history of dystonia or postural tremor, prior head and face trauma, and prior eye disease [1, 13]. None of the patients in our pedigree had a manifest action tremor or history of head trauma. One affected female subject reported the onset of BSP several weeks after bilateral phacoemulsification of cataracts. No associations have been found with age-related medical conditions such as hypertension and diabetes which were present in two of our patients. Patients with BSP often show spread of dystonia to contiguous craniocervical anatomical segments, most commonly within 5 years, as seen our proband [3].

At the time of this report, only one of the family members with BSP was receiving medical treatment for this disorder. The other affected individuals in the family related that were not motivated to pursue treatment due to a variety of factors including fear of botulinum toxin injections, lack of insurance coverage, concerns related to insurance co-payments, and modest functional disability associated with their BSP. This information suggests that racial and socioeconomic factors can exert important effects on epidemiological studies of BSP, other dystonias, and other movement disorders, and BSP may be more common in the African-American population than commonly thought.

THAP1 dystonia often begins in an arm during the late teen years or early-adult life [4]. Involvement of the neck, larynx and lower cranial musculature is common. BSP is uncommon in *THAP1* dystonia (18.9% of patients with missense mutations) and typically occurs in combination with involvement of other craniocervical anatomical segments and the arms [4]. BSP as the site of dystonia onset is seen in less than 1% of subjects with missense mutations in *THAP1* [4, 7]. Non-coding variants in the 5'-untranslated region

(UTR, c.-42C>T) and Intron 1 (c.71+9C>A) of *THAP1* were reported in two patients with sporadic BSP [4]. The same 5'UTR SV (c. -40T>C) was also reported by another group in a subject with BSP [7]. In the same report, a novel missense SV (c.208 A>G, p.K70E) was described in a 65-year old woman diagnosed with sporadic BSP at the age of 55 [7]. The *THAP1* SV reported herein was predicted to be benign by multiple *in silico* analyses and did not co-segregate with BSP (c.314T>C, p.L105S).

Whole-exome sequencing in the proband did not identify another candidate SV in a dystonia-associated gene. The presumed *DRD5* SV identified with whole-exome sequencing was actually localized to *DRD5* pseudogenes [14, 15]. As shown in our proband, paralogs, read errors, and mapping errors can complicate interpretation of next-generation sequencing data [16, 17].

Although we showed that a rare *THAP1* SV did not co-segregate with the BSP phenotype in our African-American pedigree, we did confirm that BSP can be familial and may be hereditary in African-Americans. The distribution of the BSP phenotype in our pedigree is most consistent with autosomal dominant inheritance as has been the case for most isolated dystonia genes (*TOR1A*, *THAP1*, *GNAL*, and *CIZ1*) [18]. Although *in silico* tools such as CADD provide useful information on the deleteriousness of missense variants, they do not reliably predict pathogenicity. Therefore, pedigrees should be expanded and subjected to co-segregation analysis if novel SVs are identified in probands. Moreover, next-generation sequencing data must be confirmed with bidirectional Sanger sequencing and bioinformatic analyses to exclude mislocalization to paralogous sequences in the genome.

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Highlights

Blepharospasm can be familial in African-Americans

Blepharospasm may be hereditary in African-Americans

If possible, co-segregation analysis should be used to classify sequence variants

Paralogous genes can complicate interpretation of whole-exome sequencing data

Fig 1.

Family pedigree. Arrow = proband. Filled symbols = affected. Half-filled symbols = possibly affected. +/+, homozygous wild-type allele. +/-, heterozygous for *THAP1* c. 314T>C.

Table 1

Phenotypes and *THAP1* genotypes

Patient ID	Age (years)	Gender	Age of onset (years)	BSP Severity	BSP Diagnosis	<i>THAP1</i> c.314T>C	Other Medical Disorders
II-1	69	F	unknown	Mild	Possible	Heterozygote	Diabetes
II-3	66	M	48	Severe	Definite	Heterozygote	Hypertension
II-5	59	F	54	Moderate	Definite	Normal	Phacoemulsification cataracts
II-7	50	F	unknown	Mild	Possible	Heterozygote	None
III-4	30	M	unknown	Mild	Possible	Heterozygote	None
III-9	33	M	30	Severe	Definite	Normal	None
III-12	27	F	7	Moderate	Definite	Heterozygote	None

Table 2
Exome sequencing quality metrics and sequence variant filtering

Sample ID	Phenotype	Mapped reads	Reads in exons +/-18bp (% of mapped)	Read depth (% of exons)			Total # variants	dbSNP filter
				10×	20×	50×		
II-3	Affected	47,084,143	34,487,065 (73.25%)	183,169 (98.67%)	181,017 (97.51%)	164,543 (88.63%)	38,497	3,162

Table 3

Gene-specific coverage metrics

Gene	Chr.	Total Exons	Total Reads*	Mean Reads/CDS	CDS with Lowest Coverage	Coordinates of CDS with Lowest Coverage*	% of Mean Reads in CDS with Lowest Coverage
<i>ADCY5</i>	3	21	3470	145	10	123,039,555-769	38.04
<i>ANO3</i>	11	27	4291	165	14	26,581,060-384	26.66
<i>ATCAY</i>	19	13	1695	121	9	39,17,589-800	46.25
<i>ATM</i>	11	63	9213	158	52	108,203,470-699	25.18
<i>ATP1A3</i>	19	23	3904	260	1	42,498,140-402	13.06
<i>CIZ1</i>	9	17	2987	175	16	130,928,403-644	19.35
<i>CP</i>	3	19	2708	142	13	148,901,256-500	14.73
<i>DJI</i>	1	7	650	108	2	8025392-597	47.07
<i>DRD5</i>	4	1	248	82	1	9784627-782	84.67
<i>FBXO7</i>	22	9	2171	241	8	32891360-589	21.14
<i>FTL</i>	19	4	638	212	1	49468803-088	71.01
<i>GCHI</i>	14	6	1056	132	4	55313778-025	35.60
<i>GNAL</i>	18	13	2056	171	10	11872202-449	37.35
<i>PANK2</i>	20	7	1850	231	5	3897593-813	54.91
<i>PNKD</i>	2	10	1705	106	6	219206110-375	44.10
<i>PRKRA</i>	2	8	1100	138	1	179315570-959	22.55
<i>PRT2</i>	16	4	704	352	2	29825675-123	45.73
<i>SGCE</i>	7	12	1797	119	8	94228829-067	30.88
<i>SLC2A1</i>	1	10	1877	268	9	43393251-504	21.25
<i>SPR</i>	2	3	492	164	1	73114543-950	23.17
<i>TAF1</i>	X	38	3502	100	1	70586135-459	12.99
<i>TH</i>	11	14	1528	138	1	2192955-178	39.59
<i>THAPI</i>	8	3	801	267	1	42698140-384	73.40
<i>TMM8A</i>	X	2	131	66	2	100601587-742	32.06
<i>TOR1A</i>	9	5	1497	374	1	132586210-552	9.88

CDS, coding sequence. Each gene is divided into multiple CDSs which only approximate exonic boundaries.

* Genomic coordinates are based on Human Genome version 19.

Table 4
Sequence variants identified in dystonia-associated genes and *in silico* analyses

Gene	Genomic location	cDNA	AA	dbSNP	MAF				SIFT	LRT	Polyphen-2	Mutation Taster	CADD score	
					IKG	ExAC ALL	ExAC AF	Raw					Raw	PHRED
<i>ATM</i>	11:108106443	c.378T>A	p.D126E	rs2234997	0.062	0.019	0.19	0.195	Tolerated	Neutral	Benign	Polymorphism	0.195	4.63
<i>ATM</i>	11:108114840	c.657T>C	p.C219C	rs2235003	0.028	0.008	0.09	0.007				-1.19	0.007	
<i>ATM</i>	11:108143299	c.3118A>G	p.M1040V	rs3092857	0.014	0.004	0.04	-0.961	Tolerated	Neutral	Benign	Polymorphism	-0.961	0.019
<i>ANO3</i>	11:26656563	c.1989C>T	p.F663F	rs7946841	0.341	0.381	0.23	0.197					0.197	4.65
<i>ANO3</i>	11:26568966	c.1158A>G	p.L386L	rs2663168	0.324	0.686	0.58	2.201					2.201	17.52
<i>ANO3</i>	11:26677947	c.2682C>T	p.P894P	rs10835051	0.441	0.634	0.35	1.666					1.666	14.22
<i>ATCAY</i>	19:3905421	c.137-11T>C		rs73919387	0.021	0.013	0.12	-0.137					-0.137	1.46
<i>ATP1A3</i>	19:42471050	c.*39C>G		rs919390	0.281	0.637	0.77	0.027					0.027	2.85
<i>ATP1A3</i>	19:42489516	c.666T>G	p.T222T	rs2217342	0.098	0.889	0.98	-1.382					-1.382	0.003
<i>CIZ1</i>	9:130941377	c.1109A>G	p.E370G	rs45554035	0.012	0.002	0.03	3.486	Tolerated	Neutral	Benign	Polymorphism	3.486	23.10
<i>CIZ1</i>	9:130948018	c.396C>T	p.L132L	rs45545033	0.032	0.011	0.12	1.1566					1.1566	11.52
<i>CIZ1</i>	9:130941451	c.1035G>A	p.A345A	rs45536439	0.034	0.012	0.13	2.618					2.618	20.30
<i>CP</i>	3:148916235	c.1632A>T	p.E544D	rs701753	0.137	0.916	0.69	0.305	Tolerated	Neutral	Benign	Polymorphism	0.305	5.75
<i>CP</i>	3:148896430	c.2662-12T>C		rs16861582	0.342	0.333	0.47	-0.130					-0.130	1.51
<i>DRD5</i>	4:9783917	c.264T>C	p.L88L	rs76288744	NA	NA	NA	-1.106					-1.106	0.01
<i>DRD5</i>	4:9784631	c.978C>T	p.P326P	rs6283	0.391	0.631	0.59	1.184					1.184	11.67
<i>DRD5</i>	4:9783905	c.252C>T	p.A84A	rs2227848	NA	NA	NA	1.795					1.795	14.96
<i>DRD5</i>	4:9785134	c.*47T>C		rs1967551	0.425	0.571	0.39	-0.341					-0.341	0.53
<i>DRD5</i> [†]	4:9783769	c.116C>T	p.S39L	NA	NA	NA	NA	-0.351	Tolerated	Unknown	Benign	Polymorphism	-0.351	0.50
<i>FTL</i>	19:49469087	c.163T>C	p.L55L	rs2230267	0.461	0.498	0.48	1.121					1.121	11.33
<i>GCHI</i>	14:55369421	c.-40C>T		rs28458175	0.061	0.004	0.14	1.082					1.082	11.12
<i>GCHI</i>	14:55309593	c.*1142G>A		rs10151500	0.046	NA	NA	0.280					0.280	5.50
<i>PANK2</i>	20:3903980	c.*39G>A		rs71647859	0.005	0.002	0.01	2.173					2.173	17.33
<i>PANK2</i>	20:3870124	c.377G>C	p.G126A	rs3737084	0.121	0.865	0.97	0.812	Tolerated	Neutral	Benign	Polymorphism	0.812	9.541
<i>PRRT2</i>	16:29825126	c.751T>C	p.L251L	rs11150573	0.008	0.998	0.97	-0.829					-0.829	0.03
<i>SGCE</i>	7:94227276	c.1294A>C	p.S432R	rs10247562	0.238	0.695	0.66	-0.931	Tolerated	Unknown	Benign	Polymorphism	-0.931	0.02
<i>SLC2A1</i>	1:43392250	c.*462G>C		rs4658	0.383	NA	NA	-0.947					-0.947	0.02

Gene	Genomic location	cDNA	AA	dbSNP	MAF					LRT	Polyphen-2	Mutation Taster	CADD score	
					IKG	ExAC ALL	ExAC AF	SIFT	Raw				PHRED	
<i>SLC2A1</i>	1:43394666	c.1011C>T	p.H337H	rs2229681	0.092	0.025	0.24					0.590	8.09	
<i>SLC2A1</i>	1:43394887	c.966C>T	p.V322V	rs2229680	0.081	0.021	0.23					0.824	9.61	
<i>SLC2A1</i>	1:43408966	c.45C>T	p.A15A	rs1385129	0.241	0.244	0.18					2.230	17.71	
<i>SLC2A1</i>	1:43424519	c.-197A>C		rs11537640	0.232	NA	NA					0.612	8.24	
<i>TH</i>	11:2185556	c.1494C>T	p.D498D	rs3842724	0.028	0.008	0.08					0.427	6.85	
<i>THAP1</i>	8:42693433	c.314T>C	p.L105S		8.0E-06		9.7E-05	Tolerated	Neutral	Benign	Polymorphism	-1.843	0.002	

[†]Next-generation read error. AA, amino acid change. ExAC ALL, all subjects in the ExAC database. ExAC AF, African-American subjects in the ExAC database.