

Supplemental Data

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Supplement to: Santiago-Sim T, Fang X, Hennessy ML, et al. THSD1 Mutation as a Cause of Intracranial Aneurysm.

Mutations in *THSD1* as a Cause of Intracranial Aneurysm

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Investigators:

Teresa Santiago-Sim, Ph.D.; Xiaoqian Fang, Ph.D.; Morgan L. Hennessy; Stephen V. Nalbach, M.D.; Steven R. DePalma, Ph.D.; Ming Sum Lee, M.D., Ph.D., Steven C. Greenway, M.D.; Barbara McDonough, R.N.; Georgene W. Hergenroeder, R.N., M.H.A.; Kyla J. Patek, M.S., C.G.C.; Sarah M. Colosimo, M.S., C.G.C.; Krista J. Qualmann, M.S.; John P. Hagan, Ph.D.; Dianna M. Milewicz, M.D., Ph.D.; Calum A. MacRae, M.D., Ph.D.; Susan Dymecki, M.D., Ph.D.; Christine E. Seidman, M.D.; J.G. Seidman, Ph.D.; Dong H. Kim, M.D.

Full Study Methods

STUDY PARTICIPANTS

Studies were approved by the Institutional Review Boards at the University of Texas Health Science Center at Houston, Texas and the Brigham and Women's Hospital in Boston, Massachusetts.

Starting in 2000, IA patients treated at the University of Texas Houston or the Brigham and Women's Hospital had thorough phenotypic characterization and screening to identify affected family members. Over 500 probands, and 100 affected families were enrolled. DNA samples were obtained; those with a family history underwent genetic counseling, tracing of pedigrees with review of medical records, and screening of unaffected members using brain Magnetic Resonance Angiography (MRA). We only designated as affected those patients with a *do novo* weakening of the arterial wall. We excluded fusiform dilatations caused by atherosclerosis or dissection, arterial ectasia, fibromuscular dysplasia, or other cerebrovascular abnormalities; and all subjects with a defined or suspected genetic condition (ADPKD, Ehlers-Danlos syndrome type IV, neurofibromatosis type 1, Loeys-Dietz syndrome). We also excluded subjects with small, possible aneurysms not validated on subsequent imaging, and one subject who developed IA following cranial radiation (which has been associated with increased incidence of IA ¹).

All positive subjects had an aneurysmal dilatation in a normal artery with a risk for rupture. By anatomical shape, they were classified as “saccular aneurysm” – arising from an arterial branch point – or “fusiform aneurysm” – arising from the side wall of an artery (Fig. I). Both were considered equivalent findings. We analyzed 35 family members from family IA001 (Fig. IA). The affection status of individuals with clinical signs of intracranial aneurysm was confirmed with medical records or through radiological or surgical examinations. For individuals with positive MRA screening results, cerebral angiography was performed to determine need for treatment. Individuals with negative MRA results and spouses of family members were scored as unaffected.

We also analyzed 507 unrelated IA patients and their families, and 305 controls with no intracranial disease by medical history. The 507 IA patients consisted of 367 (72.4%) females and 140 (27.6%) males. The ethnic background was European-American in 321 (63.3%), Hispanic in 88 (17.4%), African-American in 80 (15.8%), Asian in 13 (2.6%) and unknown in 5 (1.0%). Among 421 individuals with data on aneurysm shape, the aneurysm was saccular in 410 (97.4%), fusiform in 10 (2.4%) and both saccular and fusiform in 1 (0.2%). Among 421 individuals with data on family history, 109 (25.9%) reported having a relative (up to 3rd degree) with intracranial aneurysm or spontaneous SAH, while the other 312 (74.1%) reported the absence of familial predisposition.

The control cohort consisted of 305 individuals recruited at the University of Texas Medical School at Houston or the Brigham and Women's Hospital in Boston. These controls had no known intracranial disease by medical history. No diagnostic tests were performed to exclude the presence of intracranial aneurysms. This control group consisted of 152 (49.8%) females and 153 (50.2%) males. The ethnic background was European-American in 290 (95.1%), Hispanic in 10 (3.3%), and African-American in 5 (1.6%).

DETECTION OF MUTATIONS

Blood or saliva samples were collected from study participants and genomic DNA was extracted using standard methods or using DNA purification kits (Flexigene or Oragene).

Genome-wide whole exome sequencing and copy number analysis were performed in family IA001 in which a susceptibility locus in 13q14-21 was identified.² For copy number analysis, genomic DNA (2.5 µg) from subjects III-9 and IV-14 were each analyzed alongside with human reference DNA consisting of a pool of DNA from normal lymphocytes from three healthy females, on a NimbleGen Human CGH 385K Whole-Genome Tiling microarray (Roche NimbleGen, Inc) ,which provided measurements from 385,000 unique genomic loci. All sample and data processing were performed by NimbleGen Systems, Inc.

Genomic breakpoints were mapped using the segMNT v1.1 CGH segmentation analysis algorithm. Three window sizes (approximately 60,000; 120,000 and 300,000 base pairs) were further used for analysis. Thresholds for averaged log₂ ratio data were set to 0.1 and -0.1 for gains and losses, respectively.

Exome sequencing was performed at the Partners Center for Personalized Genomic Medicine core and at the Harvard Medical School Biopolymers Facility. Exome capture and next generation sequencing were carried out for IA001 subjects IV-6 and IV-15. Genomic DNA was captured on the NimbleGen Sequence Capture 2.1 Exome Array, which contained 2.1 million probes that capture ~180,000 coding exons and 551 miRNA sequences. Captured DNA was sequenced on an Illumina Genome Analyzer using manufacturer protocols. Reads were aligned with Burrows-Wheeler Alignment tool (BWA)³, processed with GATK and Picard Tools (see URLs), and called with GATK Unified Genotyper and IndelGenotyperV2. Public data from the National Heart, Lung, and Blood Institute (NHLBI), Exome Sequencing Project (ESP), and the Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: <http://exac.broadinstitute.org>) [accessed April 2015] were used to assess allelic frequency of identified variants. Exome variants were filtered for autosomal variants that were shared by the two affected relatives, resulted in altered amino acid sequences, and had minor allele frequencies (MAF) less than 0.1% in the EVS database. After the filtering process,

one variant fell under the linkage peak- a nonsense variant in *THSD1* (National Center for Biotechnology Information [NCBI] reference sequence NM_018676.3) which was validated by sequencing and subjected to segregation analysis.

All coding exons (2 to 5) and closely-flanking intronic regions in *THSD1* were sequenced in 507 intracranial aneurysm probands and 305 controls by means of standard methods. *THSD1* was also sequenced in the proband from IA001 family as positive control. DNA amplifications were carried out using *THSD1*-specific primers and bidirectional sequencing was by performed using Big Dye chemistry. The products were purified using CleanSEQ magnetic beads (Agencourt) and analyzed on the ABI3730xl Genetic Analyzer (Applied Biosystems). Mutation analyses were done by visual inspection of aligned sequences in comparison with published genomic DNA sequences (SeqMan, Lasergene). Variants were filtered for those that resulted in altered amino acid sequences and had MAF less than 0.2% in the ExAC database.

Bioinformatic analysis of rare variants was done to assess extent of conservation of disrupted amino acids in orthologs, as well as to predict the effect of variants on protein structure and function. Amino acid numbering shown in Supplementary Table I is based on the NP_061146.1 isoform. The domain location of altered amino acids was defined with the NCBI protein database. The Polyphen2 and SIFT packages were used to predict functional effects of amino acid alterations.

IMMUNOFLUORESCENCE and HISTOPATHOLOGIC ANALYSES IN MICE

Immunofluorescence and histopathologic analyses were performed on brain sections from a Venus reporter knock-in mouse for *Thsd1* (also known as Transmembrane molecule with thrombospondin module or *Tmtsp*)⁴ and wild type littermates. All mouse studies were performed in accordance with the approved animal protocols at all involved institutions. *Thsd1* heterozygous null mice, in C57BL/6 background, were generously provided by H. Nakauchi (University of Tokyo). The *Thsd1* locus was targeted by replacing DNA sequence corresponding to 2 *Thsd1* translational start codons with *Venus*, a variant of yellow fluorescent protein and neomycin-resistant gene⁴. Mutant mice (*Thsd1*^{Venus/+} and *Thsd1*^{Venus/Venus}) and littermate wild-type controls were bred from heterozygous matings.

Thsd1^{Venus/Venus} animals were anesthetized and intracardially perfused with ice-cold phosphate buffered saline (PBS) followed by ice-cold 4 % paraformaldehyde (PFA), followed by overnight fixation in 4 % PFA at 4 °C. Brains were cryoprotected with sucrose, embedded and frozen. 40µm floating coronal sections were collected and stained with 4',6-diamidino-2-phenylindole (DAPI), chicken anti-GFP (that detects Venus) 1:5,000 (Jackson Immuno Labs) and either rat anti-CD31 1:200 (BD Pharmingen) or mouse anti-smooth muscle actin 1:800 (Sigma). Sections were imaged on a Zeiss LSM 780 confocal microscope and background subtracted using FIJI.

For histopathologic analysis, 16 week old *Thsd1*^{Venus/Venus} *Thsd1*^{Venus/wt} and wild type animals were anesthetized and intracardially perfused with PBS, followed by 10% neutral buffered formalin. Following perfusion, brains were harvested carefully and stored in 10% formalin at 4°C overnight. The brains were paraffin embedded and sectioned into 5µm sections. Sections were stained with hematoxylin and eosin (H&E) and images were acquired using Olympus DP-71 Digital Camera and BX-60 Microscope (Olympus America Inc. Center Valley, PA18034).

ZEBRAFISH MORPHOLINO KNOCKDOWN

A morpholino (MO) antisense oligo (*LOC797520*-MO: 5'-GCAAGCATGCATTCTTACCAAATCC-3') designed against the splice acceptor site of exon 2 in the zebrafish *thsd1* ortholog and a random 25'-mer control MO from within the gene (*Control*-MO:5'-AGACTTTCTGAGAAACTGGGCCTCT-3') were used in this study (Gene Tools). These oligos showed no significant similarities to any other zebrafish sequences in a directed BLAST search. The MOs were resuspended in Danieau buffer (58 mM NaCl, 0.7 mM KCl, 0.4 mM MgSO₄, 0.6 mM Ca(NO₃)₂, 5.0 mM HEPES pH 7.6) and injected into one-cell stage embryos (2-8 ng/embryo) from TuAB fish using a microinjector. Different dosages of MO (0, 250, 500 and 2000 pg) were injected in independent zebrafish and embryos were allowed to develop at 28.5 °C until 48 h post fertilization. Images of live embryos were obtained and documented at 20X magnification using a Nikon TE2000 microscope. For each condition, 53–70 embryos were analyzed.

MAGNETIC RESONANCE IMAGING IN MICE

All imaging was performed in accordance with the approved animal protocols at all involved institutions. All magnetic resonance imaging (MRI) was performed with a 9.4 Tesla Biospec 94/20 spectrometer (Bruker Instruments, Billerica, MA, USA). The system was equipped with an actively shielded gradient system and a quadrature radio-frequency volume coil with an inner-diameter of 23 mm. Mice were kept sedated under 1-2% isoflurane gas anesthesia and respiration was monitored during the entire imaging procedure. To evaluate the cerebral ventricle size a Rapid Acquisition with Relaxation Enhancement (RARE) pulse sequence was used with the following parameters: repetition time (TR) = 4000 ms, effective echo time = 27 ms, echo train length = 8, number of averages (NEX) = 4, acquisition matrix = 256 x 256, field-of-view (FOV) = 25.6 x 25.6 mm² yielding an in-plane resolution of 0.1 x 0.1 mm. 30 contiguous image slices were acquired with a slice thickness of 0.5 mm.

Time of Flight-MRA data were recorded with a 3D gradient echo pulse sequence with the following parameters: TR = 40 ms, echo time = 2.3 ms, flip angle = 30°, FOV = 25.6 x 25.6 x 20 mm³, acquisition matrix = 256 x 192 x 200, NEX = 1. All data were zero-filled to 256 x 256 x 200. A magnetization transfer (MT) pulse was employed to suppress the intensity of the brain tissue signal relative to the flowing blood thus enhancing the blood vessel contrast in the MR images. The MT pulse had the following parameters: duration = 10 ms, peak amplitude = 5 μT and a frequency offset of 2,500 Hz with respect to the water resonant frequency. An angiogram was generated using these data by maximum-intensity projection (MIP) using Paravision v5.1 software (Bruker Instruments, Billerica, MA, USA).

Coronal MRI images covering the entire brain were imported into ImageJ (NIH) for processing and segmentation. Ventricle area was calculated as the sum of lateral and 3rd (dorsal and ventral) ventricular areas. Ventricular volume per slice was calculated by multiplying area with slice thickness. Total ventricular volume per animal was calculated as the sum of ventricular volumes from all 30 slices. In

parallel, the volume of total brain parenchyma (excluding brain stem and brain ventricles) was calculated for each animal.

STUDIES IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

Human umbilical vein endothelial cells (HUVEC, Life Technologies, Carlsbad, CA) were maintained in M200 medium supplemented with LSGS (low serum growth supplement, Life Technologies) at 37°C in 5% CO₂. HUVECs were used between passage 2 and passage 5. Cell density was 10,000 cells per cm² for immunofluorescence staining, and 52,000 cells per cm² for immunoblotting and adhesion assays.

A pCR3.1 expression vector containing full length human *THSD1* cDNA was generously provided by M. L. Lung (Hong Kong University of Science and Technology, Hong Kong, China). Using the QuickChange lightning mutagenesis kit (Stratagene, La Jolla, CA), two silent mutations (G993 and G996) were introduced in the *THSD1* cDNA sequence corresponding to the target of a *THSD1*-specific siRNA (described below). This would allow expression of an siRNA-resistant transcript. Using the same mutagenesis kit, single *THSD1* missense mutations (L5F, R450X, R460W, E466G, G600E, P639L, T653I, S775P) were then introduced. The entire *THSD1* cDNA sequence was verified in each construct by standard DNA sequencing.

HUVECs were transfected with a *THSD1*-specific siRNA (Stealth™ RNAi siRNA, HSS148179, Life Technologies) or a control siRNA [Stealth™ RNAi Negative Control siRNA, 12935-300) together with a *THSD1*-expressing pCR3.1 vector or an empty pCR3.1 vector. Transfection was accomplished using Lipofectamine 2000 transfection reagent (Life Technologies) according to the manufacturer's instruction with minor modification. One day before transfection, cells were plated in 6-well plates or on coverslips pre-coated with 100 µg/ml collagen I (Sigma-Aldrich, Saint Louis, MI). On the day of transfection, cells were incubated in Optima medium containing transfection mixture and the medium was replaced with supplemented M200 4 hours later. About 10 pmol of siRNA and 0.3 µg of pCR3.1 DNA were used per reaction (10⁵ cells). Subsequent experiments were carried out after 48 hours.

For western analyses, HUVECs were lysed in NuPAGE LDS sample buffer (Life Technologies). Proteins were resolved in 4-12% NuPAGE gels (Life Technologies), transferred to polyvinylidene difluoride membranes, and hybridized with primary and horseradish peroxidase-conjugated secondary antibodies. Primary antibodies used were N and C terminal-targeting anti-THSD1 antibodies recognizing amino acids 65-344 (Santa Cruz Biotechnologies, Santa Cruz, CA) and amino acids 504-613 (Novus, Littleton, CO) respectively, and an anti-GAPDH antibody (Santa Cruz Biotechnologies).

Coimmunoprecipitations were performed as follows. Prior to immunoprecipitation, 50 μ l of protein G Dynabeads was washed with PBS and incubated with anti-THSD1 (Novus, Littleton, CO) or anti-Talin (Sigma Aldrich, St. Louis, MO) antibody for 1 hour at room temperature. HUVECs were lysed with a lysis buffer containing (mM): HEPES (20), NaCl (150), 0.1% (v/v) Triton X-100, EDTA (2), 10% (v/v) glycerol, protease inhibitor and phosphatase inhibitor cocktail. Following cell sonication, solubilized proteins were incubated with antibody-conjugated Dynabeads at 4°C overnight. After three times washing with PBS, the immunoprecipitates were eluted in LDS elution buffer by heating the beads at 70°C for 10 minutes and subjected to western analysis. Two percent of input and 60% of the immunoprecipitates were loaded.

For immunofluorescence analyses, HUVECs were fixed in 4% paraformaldehyde for 10 minutes, washed three times with phosphate buffered saline solution and permeabilized in 0.5% Tween-20 at room temperature. Cells were then blocked with 1% Bovine Serum Albumin (BSA) for 1 hour and then incubated at 4°C with primary antibody overnight followed by Alexa Fluor 488-conjugated or Alexa Fluor 568-conjugated secondary antibody (1:200, Life technologies, Carlsbad, CA) for 1 hour at room temperature. Stained cells were embedded in Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Immunofluorescence images were taken under inverted microscope and analyzed using ImageJ software (NIH). Primary antibodies used in cell staining were C-terminal targeting anti-THSD1, 1:50 (Novus, Littleton, CO), anti-paxillin, 1:100 (BD Biosciences, San Jose, CA), anti-phospho-FAK (Y397), 1:50 (Millipore, Temecula, CA), and anti-vinculin-FITC, 1:50 (Sigma Aldrich, St. Louis, MO).

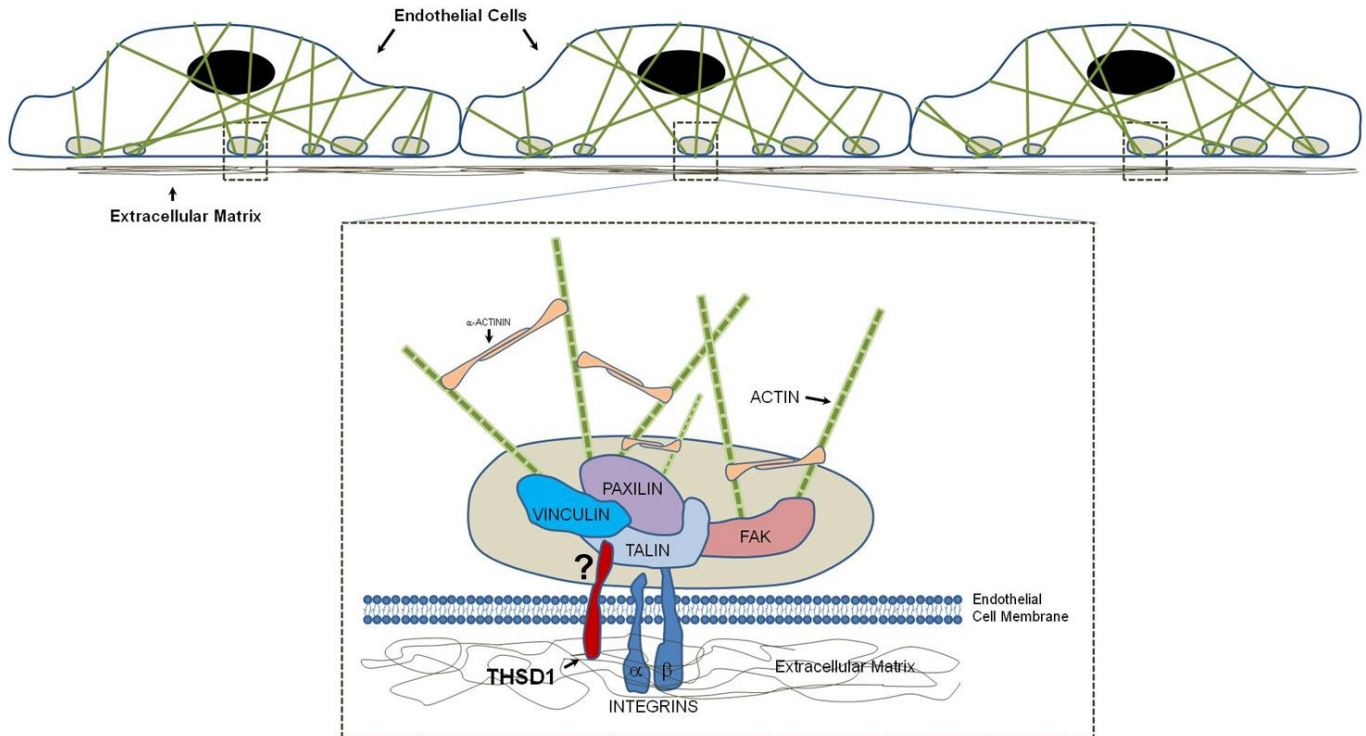
For the cell adhesion assay, cells were trypsinized and resuspended in culture medium. 45,000 cells were plated on 96-well plates pre-coated with 40 $\mu\text{g/ml}$ of collagen I and incubated at 37°C for 30 minutes. Following incubation, unattached cells were gently washed away with PBS containing $\text{Ca}^{2+}/\text{Mg}^{2+}$. Attached cells were then stained with crystal violet at room temperature for 5 minutes. The number of adherent cells was measured by dissolving the stained cells in solubilization buffer (25% ethanol and 50 mM NaH_2PO_4) then recording the absorbance at 570 nm. The measurements detected in BSA-coated wells were used to determine non-specific cell adhesion.

STATISTICAL ANALYSES

The binomial test was used to compare the frequency rare *THSD1* loss-of-function variants (nonsense, frameshift, splice site variants with MAF <0.2% in ExAC) between European cases and controls (ExAC controls and controls sequenced in the laboratory). Differences in frequency of intracranial hemorrhage between control MO and *LOC797520*-MO injected zebrafish were performed by Chi squared test.

Differences in cerebral ventricular and whole brain volumes between the *Thsd1*-mutant and wild-type mouse groups were analyzed using the Welch Two Sample t-test. All *in vitro* experiments were performed in triplicates and repeated at least 3 times. Data are expressed as means and standard errors. The unpaired t-test was used to perform between-group comparisons.

Genome-wide Copy Number analysis results. Copy number analysis in IA1 identified 4 autosomal Copy Number Variants (CNVs) that were shared by III-9 and IV-14. Located in chromosomes 2p11, 8q24, 10q11, and 15q11, all 4 CNVs have been reported with high population frequency in the Database of Genomic Variants and were therefore regarded as benign polymorphisms.



Supplementary Figure S1. Proposed involvement of THSD1 in focal adhesions. Focal adhesions are large, specialized, dynamic structures where actin filament bundles are anchored to transmembrane integrin receptors through an assembly of proteins. Integrins bind to extracellular proteins and within the cell, the intracellular domain of integrins bind to the actin filament bundles via adapter proteins such as talin, α -actinin and vinculin. Aside from anchoring the cell, focal adhesions function as signal carriers that can affect cellular behavior. Many intracellular signalling proteins, including focal adhesion kinase (FAK), bind to the complex to initiate downstream signaling events that result in reorganization of the actin cytoskeleton, changes in cell shape and motility and gene expression. We propose that THSD1 is expressed in the plasma membrane, is a component of focal adhesions where it interacts with talin and perhaps other proteins, and is important for the maintenance of endothelial cell morphology and anchorage to the basement membrane .

Supplemental Table I. Spectrum of rare THSD1 Mutations in IA Patients^a

Variant	Consequence	PolyPhen-2 Prediction	Kindred	Familial/ Sporadic	Ethnicity	ID	Gender	Age at diagnosis	Aneurysm site(s)	Shape	Ruptured/ Nonruptured	Other Cerebrovascular Anomalies Noted
c.1348 C>T	R450X	NA	IA001	Familial (in 9 affected members)	EurAm	III-9	F	71	Right OA	Sac	R	
						IV-6	F	51	Left MCA Left OA	Sac Sac	NR NR	Dissection of the right ICA
						IV-9	F	46	Right MCA	Fus	NR	
						IV-10	M	48	ACoA	Sac	NR	
						IV-11	M	42	Right PCA	Fus	NR	
						IV-12	F	47	Right PCA-PCoA	Sac	NR	
						IV-14	F	53	Right OA Right A2 Right colossal-marginal Right MCA Right MCA ACoA Left AChA Left MCA Left PCA	Sac Sac Sac Sac Sac Sac Sac Sac Sac Sac	NR NR NR NR NR NR NR NR NR NR	FMD in cervical ICA and VA
						IV-15	M	35	Unk	Sac	R	
						IV-17	M	27	ACoA Right MCA	Sac Sac	R R	
c.15 G>C	L5F	D	IA002	Sporadic	EurAm	NR0782	F	66	Left MCA	Sac	NR	
c.15 G>C	L5F	D	IA003	Sporadic	EurAm	NR2464	F	59	Right ICA Right OA Right PCoA	Sac Sac Sac	NR NR NR	
c.1378 C>T	R460W	D	IA004	Sporadic	EurAm	MG2815	F	56	Right PCoA	Sac	R	
c.1397 A>G	E466G	D	IA005	Familial (in 2 affected members)	EurAm	NR0033	F	62	ACoA	Sac	R	
						Daughter of NR0033	F	47	Right ICA Left ICA	Sac Sac	NR NR	
c.1799 G>A	G600E	B	IA006	Sporadic	EurAm	MG05135	F	51	Unk	Unk	Unk	
c.1916 C>T	P639L	B	IA007	Sporadic	EurAm	NR0756	F	53	Right MCA	Fus	NR	
c.1958 C>T	T653I	D	IA008	Sporadic	EurAm	NR0660	F	54	Right MCA ACoA Left SCA Left MCA	Sac Sac Sac Sac	NR NR NR NR	
c.2323 T>C	S775P	B	IA009	Sporadic	AfAm	NR0773	F	45	Left ICA ACoA Left MCA	Sac Sac Sac	R NR NR	FMD in left VA

^a The protein and complementary DNA annotations are based on National Center for Biotechnology Information reference sequence numbers NP_061146.1 and NM_018676.3, respectively. Rare variants were defined as those having a minor allele frequency in controls of less than 0.2%. IA denotes intracranial aneurysm, NA not applicable, D probably damaging, B benign, EurAm European-American, AfAm African-American, F female, M male, OA ophthalmic artery, MCA middle cerebral artery, ACoA anterior communicating artery, PCA posterior cerebral artery, ACA anterior cerebral artery, AChA anterior choroidal artery, ICA internal carotid artery, VA vertebral artery, PCoA posterior communicating artery, SCA superior cerebellar artery, Sac saccular, Fus fusiform, R ruptured, NR nonruptured, FMD fibromuscular dysplasia.

Supplementary Table II. Allele Counts of Rare *THSD1* Variants in Intracranial Aneurysm Cases and Controls ^a

hg19 position	Ptein Consequence	Transcript Consequence	Annotation	Cases ^{bc}				Lab Controls ^{bc}			ExAC individuals ^{bcd}		
				EurAm (644)	AfAm (160)	Hisp (176)	Others (36)	EurAm (580)	AfAm (10)	Hisp (20)	Non-Finnish Eur (66518)	African (10350)	Latino (11562)
13:52976734	p.M1?	c.2T>C	initiator codon	0	0	0	0	0	0	0	2	0	0
13:52976721	p.L5F	c.15G>C	missense	2	0	0	0	0	0	0	0	0	0
13:52976708	p.N10D	c.28A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52976699	p.L13M	c.37T>A	missense	0	0	0	0	0	0	0	1	0	0
13:52976680	p.Y19C	c.56A>G	missense	0	0	0	0	0	0	0	8	0	0
13:52972317	p.E23del	c.68_70delAAG	inframe deletion	0	0	0	0	0	0	0	1	0	0
13:52972260	p.Y43F	c.128A>T	missense	0	0	0	0	0	0	0	3	0	0
13:52972236	p.G51D	c.152G>A	missense	0	0	0	0	0	0	0	0	1	0
13:52972231	p.N53D	c.157A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52972212	p.V59YfsX14	c.175delG	frameshift	0	0	0	0	0	0	0	1	0	0
13:52972174	p.V72I	c.214G>A	missense	0	0	0	0	0	0	0	0	1	0
13:52972174	p.V72L	c.214G>C	missense	0	0	0	0	0	0	0	0	0	1
13:52972167	p.T74I	c.221C>T	missense	0	0	0	0	0	0	0	0	0	1
13:52972141	p.Q83E	c.247C>G	missense	0	0	0	0	0	0	0	1	0	0
13:52972116	p.F91S	c.272T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52972112	p.Y92X	c.276T>A	stop gained	0	0	0	0	0	0	0	1	0	0
13:52972107	p.K94R	c.281A>G	missense	0	0	0	0	0	0	0	0	2	0
13:52972081	p.M103L	c.307A>T	missense	0	0	0	0	0	0	0	1	0	0
13:52972081	p.M103V	c.307A>G	missense	0	0	0	0	0	0	0	2	0	0
13:52972060	p.N110D	c.328A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52972046	p.F114L	c.342C>G	missense	0	0	0	0	0	0	0	2	0	0
13:52972040	p.W116C	c.348G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52972037	p.W117C	c.351G>T	missense	0	0	0	0	0	0	0	4	0	0
13:52972036	p.E118X	c.352G>T	stop gained	0	0	0	0	0	0	0	1	0	1
13:52972032	p.K119T	c.356A>C	missense	0	0	0	0	0	0	0	1	0	0
13:52972024	p.F122L	c.364T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52971994	p.V132F	c.394G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52971984	p.N135S	c.404A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52971981	p.R136M	c.407G>T	missense	0	0	0	0	0	0	0	0	1	0
13:52971961	p.G143S	c.427G>A	missense	0	0	0	0	0	0	0	0	0	1
13:52971945	p.G148V	c.443G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52971936	p.T151I	c.452C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52971928	p.P154S	c.460C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52971924	p.L155R	c.464T>G	missense	0	0	0	0	0	0	0	1	0	0
13:52971924	p.L155P	c.464T>C	missense	0	0	0	0	0	0	0	24	0	1

13:52971918	p.P157Q	c.470C>A	missense	0	0	0	0	0	0	0	0	1	0
13:52971895	p.I165V	c.493A>G	missense	0	0	0	0	0	0	0	1	1	1
13:52971862	p.P176T	c.526C>A	missense	0	0	0	0	0	0	0	0	1	0
13:52971861	p.P176L	c.527C>T	missense	0	0	0	0	0	0	0	0	1	0
13:52971859	p.E177K	c.529G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52971838	p.Q184X	c.550C>T	stop gained	0	0	0	0	0	0	0	0	0	1
13:52971837	p.Q184R	c.551A>G	missense	0	0	0	0	0	0	0	5	0	0
13:52971822	p.R189K	c.566G>A	missense	0	0	0	0	0	0	0	2	0	0
13:52971796	p.Q198E	c.592C>G	missense	0	0	0	0	0	0	0	13	0	1
13:52971795	p.Q198R	c.593A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52971793	p.G199S	c.595G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52971766	p.P208S	c.622C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52971730	p.K220E	c.658A>G	missense	0	0	0	0	0	0	0	0	0	11
13:52971720	p.G223E	c.668G>A	missense	0	0	0	0	0	0	0	0	0	1
13:52971718	p.R224X	c.670C>T	stop gained	0	0	0	0	0	0	0	1	0	0
13:52971705	p.I228T	c.683T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52971688	p.I234V	c.700A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52971687	p.I234T ^e	c.701T>C	missense	3 ^e	0	0	0	1 ^e	0	0	290 ^e	13	4
13:52971666	p.G241A	c.722G>C	missense	0	0	0	0	0	0	0	0	0	1
13:52971655	p.V245M	c.733G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52971652	p.M246V	c.736A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52971625	p.G255R	c.763G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52971624	p.G255E	c.764G>A	missense	0	0	0	0	0	0	0	2	0	0
13:52971622	p.V256L	c.766G>T	missense	0	0	0	0	0	0	0	3	0	0
13:52971591	p.T266I	c.797C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52971555	p.A278V	c.833C>T	missense	0	0	0	0	0	0	0	2	0	1
13:52971532	p.T286P	c.856A>C	missense	0	0	0	0	0	0	0	0	0	1
13:52971505	p.P295S	c.883C>T	missense	0	0	0	0	0	0	0	0	0	2
13:52971498	p.G297E	c.890G>A	missense	0	0	0	0	0	0	0	0	2	0
13:52971486	p.R300del	c.899_901delG GA	inframe deletion	0	0	0	0	0	0	0	1	0	0
13:52971483	p.I302T	c.905T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52971477	p.N304T	c.911A>C	missense	0	0	0	0	0	0	0	1	0	0
13:52971460	p.M310V	c.928A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52971458	p.M310I	c.930G>A	missense	0	0	0	0	0	0	0	2	0	0
13:52971456	p.G311E	c.932G>A	missense	0	0	0	0	0	0	0	0	1	0
13:52971406	p.S328P	c.982T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52971393	p.E332G	c.995A>G	missense	0	0	0	0	0	0	0	0	0	1
13:52971392	p.E332D	c.996G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52960306	p.W346X	c.1037G>A	stop gained	0	0	0	0	0	0	0	1	0	0
13:52960298	p.W349R	c.1045T>C	missense	0	0	0	0	0	0	0	2	0	0
13:52960255	p.R363H	c.1088G>A	missense	0	0	0	0	0	0	0	4	1	0
13:52960252	p.R364H	c.1091G>A	missense	0	0	0	0	0	0	0	2	1	0
13:52960247	p.V366M	c.1096G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52960220	p.P375S	c.1123C>T	missense	0	0	0	0	0	0	0	0	18	0
13:52960194	p.E383D	c.1149G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52960186	p.L386P	c.1157T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52960178	p.L389V	c.1165C>G	missense	0	0	0	0	0	0	0	2	0	0
13:52960169	p.C392G	c.1174T>G	missense	0	0	0	0	0	0	0	1	0	0
13:52960165	p.A393G	c.1178C>G	missense	0	0	0	0	0	0	0	1	0	0
13:52960163	p.A394P	c.1180G>C	missense	0	0	0	0	0	0	0	1	0	0
13:52952901	p.P402S	c.1204C>T	missense	0	0	0	0	1	0	0	0	0	0
13:52952894	p.Q404R	c.1211A>G	missense	0	0	0	0	0	0	0	5	0	0
13:52952888	p.Q406R	c.1217A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52952883	p.P408A	c.1222C>G	missense	0	0	0	0	0	0	0	1	0	0
13:52952840	p.L422S	c.1265T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52952811	p.L432F	c.1294C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952783	p.R441L	c.1322G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952757	p.R450X	c.1348C>T	stop gained	1	0	0	0	0	0	0	0	0	0
13:52952727	p.R460W	c.1378C>T	missense	1	0	0	0	0	0	0	2	0	0
13:52952720	p.N462T	c.1385A>C	missense	0	0	0	0	0	0	0	1	0	0
13:52952711	p.E465G	c.1394A>G	missense	0	0	0	0	0	0	0	0	0	1
13:52952709	p.E466K	c.1396G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952708	p.E466G	c.1397A>G	missense	1	0	0	0	0	0	0	16	0	0
13:52952697	p.E470K	c.1408G>A	missense	0	0	0	0	0	0	0	27	1	0
13:52952687	p.E473V	c.1418A>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952681	p.R475H	c.1424G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952678	p.G476V	c.1427G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952664	p.G481R	c.1441G>C	missense	0	0	0	0	0	0	0	1	0	0
13:52952655	p.G484R	c.1450G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952630	p.T492I	c.1475C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952622	p.P495S	c.1483C>T	missense	0	0	0	0	0	0	0	0	0	1
13:52952622	p.P495T	c.1483C>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952609	p.R499K	c.1496G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952573	p.S511F	c.1532C>T	missense	0	0	0	0	0	0	0	0	1	0
13:52952571	p.G512S	c.1534G>A	missense	0	0	0	0	0	0	0	0	0	3
13:52952568	p.S513G	c.1537A>G	missense	0	0	0	0	0	0	0	2	0	0
13:52952565	p.E514K	c.1540G>A	missense	0	0	0	0	0	0	0	2	0	0
13:52952563	p.E514D	c.1542G>T	missense	0	0	0	0	0	0	0	0	1	0
13:52952559	p.F516L	c.1546T>C	missense	0	0	0	0	0	0	0	1	0	0
13:52952552	p.S518Y	c.1553C>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952547	p.A520T	c.1558G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952520	p.S529C	c.1585A>T	missense	0	0	0	0	0	0	0	0	0	1
13:52952507	p.A533V	c.1598C>T	missense	0	0	0	0	0	0	0	0	1	0
13:52952484	p.K541E	c.1621A>G	missense	0	0	0	0	0	0	0	1	0	0

13:52952468	p.T546M	c.1637C>T	missense	0	0	0	0	0	0	0	1	0	1
13:52952453	p.V551E	c.1652T>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952452	p.Y552VfsX14	c.1652_1653ins A	frameshift	0	0	0	0	0	0	0	1	0	0
13:52952449	p.Y552X	c.1656T>A	stop gained	0	0	0	0	0	0	0	0	2	0
13:52952445	p.V554M	c.1660G>A	missense	0	0	0	0	0	0	0	2	0	0
13:52952439	p.Q556E	c.1666C>G	missense	0	0	0	0	0	0	0	7	0	4
13:52952434	p.S557R	c.1671T>G	missense	0	0	0	0	0	0	0	0	0	1
13:52952414	p.I564T	c.1691T>C	missense	0	0	0	0	0	0	0	2	0	0
13:52952406	p.A567T	c.1699G>A	missense	0	0	0	0	0	0	0	0	2	0
13:52952403	p.P568S	c.1702C>T	missense	0	0	0	0	0	0	0	2	0	0
13:52952394	p.P571S	c.1711C>T	missense	0	0	0	0	0	0	0	0	1	0
13:52952368	p.E579D	c.1737A>T	missense	0	0	0	0	0	0	0	21	0	0
13:52952363	p.A581V	c.1742C>T	missense	0	0	0	0	0	0	0	5	1	6
13:52952357	p.N583I	c.1748A>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952356	p.N583K	c.1749C>G	missense	0	0	0	0	0	0	0	0	0	2
13:52952348	p.R586Q	c.1757G>A	missense	0	0	0	0	0	0	0	2	0	0
13:52952327	p.E593G	c.1778A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52952318	p.A596V ^e	c.1787C>T	missense	0	0	0	0	0	0	0	1	30 ^e	0
13:52952316	p.V597I	c.1789G>A	missense	0	0	0	0	0	0	0	0	0	6
13:52952306	p.G600E	c.1799G>A	missense	1	0	0	0	1	0	0	42	0	2
13:52952297	p.P603R	c.1808C>G	missense	0	0	0	0	0	0	0	0	0	1
13:52952294	p.P604L	c.1811C>T	missense	0	0	0	0	0	0	0	0	0	1
13:52952282	p.D608G	c.1823A>G	missense	0	0	0	0	0	0	0	0	0	1
13:52952282	p.D608A	c.1823A>C	missense	0	0	0	0	0	0	0	0	0	1
13:52952249	p.S619I	c.1856G>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952238	p.T623S	c.1867A>T	missense	0	0	0	0	0	0	0	2	0	0
13:52952231	p.I625N	c.1874T>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952214	p.R631G	c.1891A>G	missense	0	0	0	0	0	0	0	3	0	0
13:52952192	p.G638V	c.1913G>T	missense	0	0	0	0	0	0	0	0	0	1
13:52952189	p.P639L	c.1916C>T	missense	1	0	0	0	0	0	0	0	0	0
13:52952177	p.S643N	c.1928G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952154	p.R651W	c.1951A>T	missense	0	0	0	0	0	0	0	6	1	0
13:52952147	p.T653I	c.1958C>T	missense	1	0	0	0	1	0	0	4	1	0
13:52952145	p.A654T	c.1960G>A	missense	0	0	0	0	0	0	0	0	3	0
13:52952135	p.H657P	c.1970A>C	missense	0	0	0	0	0	0	0	1	0	0
13:52952130	p.A659T	c.1975G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952118	p.R663G	c.1987C>G	missense	0	0	0	0	0	0	0	0	0	2
13:52952117	p.R663Q	c.1988G>A	missense	0	0	0	0	0	0	0	0	0	2
13:52952114	p.P664L	c.1991C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952096	p.M670R	c.2009T>G	missense	0	0	0	0	0	0	0	1	0	0
13:52952093	p.S671F	c.2012C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952079	p.R676W	c.2026C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52952078	p.R676Q	c.2027G>A	missense	0	0	0	0	0	0	0	0	2	1
13:52952064	p.Y681H	c.2041T>C	missense	0	0	0	0	0	0	0	0	1	0
13:52952051	p.T685K	c.2054C>A	missense	0	0	0	0	0	0	0	5	1	0
13:52952048	p.R686Q	c.2057G>A	missense	0	0	0	0	0	0	0	0	1	0
13:52952043	p.C688S	c.2062T>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952040	p.E689K	c.2065G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52952023	p.R694S	c.2082A>C	missense	0	0	0	0	0	0	0	1	0	0
13:52952016	p.P697S	c.2089C>T	missense	0	0	0	0	0	0	0	0	4	0
13:52952010	p.S699G	c.2095A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52952004	p.G701C	c.2101G>T	missense	0	0	0	0	0	0	0	0	0	1
13:52951977	p.L709RfsX18	c.2126_2127del TG	frameshift	0	0	0	0	0	0	0	0	3	0
13:52951976	p.E710G	c.2129A>G	missense	0	0	0	0	0	0	0	0	1	0
13:52951959	p.S716C	c.2146A>T	missense	0	0	0	0	1	0	0	11	3	0
13:52951957	p.S716R	c.2148T>A	missense	0	0	0	0	0	0	0	1	0	0
13:52951944	p.P721A	c.2161C>G	missense	0	0	0	0	0	0	0	1	0	0
13:52951939	p.L722F	c.2166A>T	missense	0	0	0	0	0	0	0	1	0	0
13:52951934	p.P724R	c.2171C>G	missense	0	0	0	0	0	0	0	0	1	0
13:52951934	p.P724H	c.2171C>A	missense	0	0	0	0	0	0	0	6	0	0
13:52951928	p.P726L	c.2177C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52951917	p.I730P	c.2188A>C	missense	0	0	0	0	0	0	0	1	0	0
13:52951908	p.Q733K	c.2197C>A	missense	0	0	0	0	0	0	0	1	0	0
13:52951898	p.R736K	c.2207G>A	missense	0	0	0	0	0	0	0	0	1	0
13:52951895	p.K737T	c.2210A>C	missense	0	0	0	0	0	0	0	0	2	0
13:52951886	p.L740H	c.2219T>A	missense	0	0	0	0	0	0	0	0	1	0
13:52951886	p.L740P	c.2219T>C	missense	0	0	0	0	0	0	0	0	0	1
13:52951886	p.L740R	c.2219T>G	missense	0	0	0	0	0	0	0	1	0	0
13:52951881	p.D742Y	c.2224G>T	missense	0	0	0	0	0	0	0	0	0	1
13:52951877	p.H743R	c.2228A>G	missense	0	0	0	0	0	0	0	2	0	0
13:52951868	p.G746E	c.2237G>A	missense	0	0	0	0	0	0	0	1	0	0
13:52951866	p.L747I	c.2239T>A	missense	0	0	0	0	0	0	0	1	0	0
13:52951854	p.I751V	c.2251A>G	missense	0	0	0	0	0	0	0	0	1	0
13:52951853	p.I751S	c.2252T>G	missense	0	0	0	0	0	0	0	0	0	2
13:52951847	p.R753T	c.2258G>C	missense	0	0	0	0	0	0	0	2	0	0
13:52951836	p.H757Y	c.2269C>T	missense	0	0	0	0	0	0	0	4	0	0
13:52951827	p.R760C	c.2278C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52951826	p.R760P	c.2279G>C	missense	0	0	0	0	0	0	0	0	3	0
13:52951826	p.R760H	c.2279G>A	missense	0	0	0	0	0	0	0	0	0	1
13:52951824	p.R761W	c.2281C>T	missense	0	0	0	0	0	0	0	1	0	0
13:52951817	p.P763Q	c.2288C>A	missense	0	0	0	0	0	0	0	1	0	0
13:52951809	p.S766C	c.2296A>T	missense	0	0	0	0	0	0	0	0	0	2
13:52951799	p.S769T	c.2306G>C	missense	0	0	0	0	0	0	0	0	2	0

13:52951784	p.Q774R	c.2321A>G	missense	0	0	0	0	0	0	0	1	0	0
13:52951782	p.S775P	c.2323T>C	missense	0	1	0	0	0	0	0	0	0	0
13:52951778	p.S776C	c.2327C>G	missense	0	0	0	0	0	0	0	1	0	0
13:52951772	p.I778T	c.2333T>C	missense	0	0	0	0	0	0	0	5	0	0
13:52951769	p.S779Y	c.2336C>A	missense	0	0	0	0	0	0	0	6	0	0
13:52951751	p.Q785delinsL X	c.2353_2354ins TCT	stop gained	0	0	0	0	0	0	0	6	0	0
13:52951747	p.Q785RfsX5	c.2354_2357del AGAG	frameshift	0	0	0	0	0	0	0	6	0	0
13:52951719	p.R796G	c.2386A>G	missense	0	0	0	0	0	0	0	1	0	0
Total rare variant allele count				8	1	0	0	4 ^f	0	0	387	86	76

^a A rare variant is defined here as that having a minor allele frequency of less than 0.2% in controls.

^b EurAm denotes European-American, AfAm African-American, Hisp Hispanic, As Asian, Unk unknown, Eur European.

^c In parentheses are average numbers of alleles sequenced.

^d ExAC denotes Exome Aggregation Consortium.

^e I234T is not a rare variant in the European-American population; A596V is not a rare variant in Africans.

^f One European American Lab Control is heterozygous for both G600E and T653I.

Supplementary Appendix References

1. Sciubba DM, Gallia GL, Recinos P, Garonzik IM, Clatterbuck RE. Intracranial aneurysm following radiation therapy during childhood for a brain tumor. Case report and review of the literature. *J Neurosurg.* 2006;105:134-9.
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4. Takayanagi S, Hiroyama T, Yamazaki S, et al. Genetic marking of hematopoietic stem and endothelial cells: identification of the Tmtsp gene encoding a novel cell surface protein with the thrombospondin-1 domain. *Blood.* 2006;107:4317-25.