Brain arteriovenous malformation (AVM) is the abnormal, undifferentiated clustering of blood vessels without an intervening capillary bed or the shunting of arterial blood in the brain parenchyma. The prevalence of brain AVM varies from 15 to 18 cases per 100,000 adults.\textsuperscript{29} The most common manifestation of brain AVM is intracranial hemorrhage with an annual risk varying from 1.3\% to 3.9\%.\textsuperscript{12} Patients with brain AVMs can also present with intractable seizures, headache, and ischemic steal syndrome.

Brain AVMs can occur sporadically or in the context of genetic disorders such as hereditary hemorrhagic telangiectasia (HHT), also known as Osler-Weber-Rendu syndrome. Patients with HHT present with telangiectasia and visceral AVM. Congenital brain AVM was reported in only 10\%–16\% of patients with HHT.\textsuperscript{16} It has been reported that mutations in 3 genes are associated with HHT: endoglin (\textit{ENG}), activin A receptor Type II-like 1 (\textit{ACVRL1}), and more recently SMAD family member 4 (\textit{SMAD4}). Familial brain AVMs without HHT have also been reported.\textsuperscript{14,18,26,30} The role of genetic variations in sporadic AVMs remains elusive. In a recent study on single nucleotide polymorphisms, a nominal association of \textit{ACVRL1} IVS3–35 A > G with sporadic AVMs has been shown.\textsuperscript{31} However, genome-wide linkage studies do not reveal the pathogenic variants. In this study, we have performed whole exome capture and Illumina sequencing in 2 siblings with brain AVM. A deleterious mutation in the \textit{ACVRL1} (p.Lys332Glu substitution) was found and confirmed by direct Sanger sequencing.

### Methods

#### Clinical Ascertainment

After institutional review board approval by the Committee for the Ethical Issues in Bahçeşehir University and affiliated hospitals was granted, written informed consent was obtained from each member of a 6-person Turkish family for participation, sample collection, and chart re-

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**ABBREVIATIONS**  
AVM = arteriovenous malformation; HHT = hereditary hemorrhagic telangiectasia; TGF-β = transforming growth factor–β.


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view. History and physical examination findings were recorded by one of the authors.

**Sample Preparation**

Peripheral blood samples were collected from the family, and DNA was extracted from the samples using the Puregene DNA isolation kit (Gentra Systems Inc).

**Whole Exome Capture and Sequencing**

The whole exome sequencings were performed on DNA extracted from blood samples from the proband F-A VM-2 (II-1) and one of his affected sisters F-A VM-5 (II-4; Table 1 and Fig. 1). NimbleGen 2.1M human exome array (Roche NimbleGen Inc.) was used according to the manufacturer’s protocol with modifications that have been described elsewhere. Exome library sequencing was performed using HiSeq2000 (Illumina Inc.) with barcoding technology, 74-bp paired-end analysis, and 6 samples per lane. Image analysis and subsequent base calling were performed using the Illumina pipeline (version 1.8).

**Exome Data Analysis**

The sequencing data were analyzed using the previously described in-house written bioinformatics pipeline. Briefly, sequence reads were aligned to the human genome reference sequence (version GRCh37, as used in phase 1 of the 1000 Genomes Project) using a hybrid of Stampy (version 1.0.16) and Burrows-Wheeler Aligner tool (BWA; version 0.5.9-r16). Variant calling of single nucleotide variants (SNVs) and small insertion-deletion events (indels) was accomplished using the Unified Genotyper algorithm from the Genome Analysis Toolkit (GATK). We annotated variant alleles using the Ensembl database (version 66) and Variant Effect Predictor (version 2.4) tool.

**Exome Data Filtering and Candidate Selection**

One thousand genomes and Single Nucleotide Polymorphism Database (build 131) as well as our group private exome data set (approximately 5000 chromosome) were predicted to test the novelty of variations. Variants in common with the F-A VM-2 and F-A VM-5 samples were prioritized. A loss of function (LOF) mutation followed by the missense mutations were predicted as deleterious. Missense mutations were prioritized using the following criteria: CADD_PHRED > 20 (Combined Annotation–Dependent Depletion), functional effect scores calculated by SIFT, PolyPhen2, Mutation Taster, GERP, and PhyloP. Hereditary hemorrhagic telangiectasia Type 2 (HHT2) can be caused by mutations in ENG, ACVRL1, and SMAD4. Therefore, a deleterious mutation in any of these genes has been considered as the highest ranked.

**Sanger Sequencing**

Direct Sanger sequencing of the ACVRL1 mutation identified by the exome sequencing was performed on all family members using the standard methods following polymerase chain reaction (PCR) amplification using specific primers. Sequences of primers used for sequencing the ACVRL1 mutation are as follows: forward primer, ACVRL1-F (5’-GCTCCCTCTACGACTTT CTG-3’); reverse primer, ACVRL1-R (5’-GATGGTATG GTGGAAGGAATC-3’).

**Results**

A 6-person Turkish family, including 3 siblings with brain AVMs, was evaluated. One of the 3 siblings with brain AVM also had spinal AVM. The 20-year-old proband (II-1) initially presented with an episode of paraparesis at the age of 17 years, and he was diagnosed with a high-flow AVM at the level of the mid to lower thoracic spine by using spinal MRI and subsequent spinal angiography. The diagnosis was confirmed after surgical treatment of the spinal AVM. A 2–cm, left-sided thalamic AVM was detected on cranial MRI during his routine follow-up. His 24- and 31-year-old siblings (II-3 and II-4) were also diagnosed with brain AVMs using cranial MRI even though they had no signs or symptoms of brain AVM. The 61- and 56-year-old parents (I-1 and I-2) and the 37-year-old fourth sibling (II-2) had no AVMs on cranial MRI (Table 1). We performed exome sequencing on DNA extracted from blood samples of the proband F-A VM-2 (II-1) and his affected sister F-A VM-5 (II-4). We identified a heterozygous ACVRL1 A > G mutation in both F-A VM-2 (II-1) and F-A VM-5 (II-4) patients (Supplementary Tables 1 and 2). Sanger sequencing of the ACVRL1 mutation identified by the exome sequencing was performed on all family members, which confirmed the same observed variations. Subsequently, the third affected sibling and the unaffected mother were found to be heterozygous for the mutation by direct Sanger sequencing (Supplementary Fig. 1).

The variant affects all 3 transcripts and changes the amino acid lysine into glutamic acid (hg19: ENSP00000373574.4: p.Lys332Glu, ENSP00000392492.2: p.Lys346Glu, ENSP00000447884.1: p.Lys346Glu). This variant, p.Lys332Glu, was absent from the Yale Exome data set as well as publically available databases including the Exome Aggregation Consortium (ExAC) data set.

The p.Lys332Glu variant is predicted to be among the top 0.1%–1% most deleterious variants (CADD_PHRED > 20). Moreover, all functional effect–predicting methods used in our analyses calculate the variant to be deleterious (Supplementary Fig. 2). It is worth nothing that amino acid Asp330 is the proton acceptor active site and that p.Ser333Ile, a variant previously reported in a patient with HHT2, is known to render the protein nonfunctional since the variant protein is retained in the endoplasmic reticulum.

**Discussion**

In this study, we performed whole exome capture and Illumina sequencing in 2 siblings with brain AVM. A deleterious mutation in ACVRL1, a p.Lys332Glu substitution, was found and confirmed by direct Sanger sequencing.

Even though most brain AVMs occur sporadically, their association with some genetic disorders is well documented. Brain AVMs were reported in patients with HHT, an autosomal dominant disorder caused by mutations in ENG (OMIM:131195), ACVRL1 (OMIM: 601284), and
SMAD4 (OMIM: 600993) in HHT1, HHT2, and juvenile polyposis, respectively. All 3 genes encode proteins involved in the transforming growth factor-β (TGF-β) signaling pathway. Furthermore, at least 2 more unidentified genes are associated with classic HHT: HHT3 is associated with chromosome 5q between D5S201127 and D5S2490, whereas HHT4 is associated with chromosome 7q between D7S2252 and D7S510. Other genetic syndromes associated with vascular malformations of the central nervous system include capillary malformation–arteriovenous malformation syndrome, Bannayan–Riley–Ruvalcaba syndrome, and Sturge-Weber syndrome, among others.

In the literature, 55 patients with brain AVMs from 26 families including 2 or more affected relatives without HHT or hereditary neurocutaneous angiomatous malformations have been reported. The familial clustering of brain AVMs shows that genetic factors may play a role in the development of brain AVMs. However, it is important to realize that the number of reported familial brain AVMs is very small. Most of the studies reporting brain AVMs without HHT failed to exclude the genetic diseases. Furthermore, genome-wide linkage studies of additional family members have not yet revealed the pathogenic variant. Therefore, direct Sanger sequencing for known AVM-related genes may not be able to reveal disease-associated variants. Hence, we decided to perform exome sequencing on the proband and one of his affected sisters.

Exome sequencing, also known as targeted exome capture, is an efficient tool to sequence coding regions of the genome selectively. Exons are short, functionally important sequences of DNA that represent the regions in genes that are translated into protein. In the human genome there are approximately 180,000 exons: these constitute approximately 1% of the human genome, which translates to about 30 megabases (Mb) in length. It is estimated that the protein-coding regions of the human genome

### TABLE 1. Demographics and characteristics of the family members

<table>
<thead>
<tr>
<th>Family Member</th>
<th>Age (yrs)/Sex</th>
<th>Presentation</th>
<th>Radiological Findings</th>
<th>Intervention</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1 (father)</td>
<td>61/M</td>
<td>—</td>
<td>Normal</td>
<td>None</td>
<td>A/A</td>
</tr>
<tr>
<td>I-2 (mother)</td>
<td>56/F</td>
<td>—</td>
<td>Normal</td>
<td>None, only follow-up</td>
<td>A/G</td>
</tr>
<tr>
<td>II-1 (proband)</td>
<td>20/M</td>
<td>Episode of paraparesis at 17 yrs of age</td>
<td>AVM at level of mid- to lower thoracic spine &amp; lt thalamic AVM</td>
<td>Spinal AVM treated surgically &amp; cerebral AVM subsequently treated w/ Gamma Knife radiosurgery</td>
<td>A/G</td>
</tr>
<tr>
<td>II-2</td>
<td>37/F</td>
<td>—</td>
<td>Normal</td>
<td>None</td>
<td>A/A</td>
</tr>
<tr>
<td>II-3</td>
<td>24/F</td>
<td>No apparent signs or symptoms</td>
<td>AVM in posterior fossa</td>
<td>Gamma Knife radiosurgery</td>
<td>A/G</td>
</tr>
<tr>
<td>II-4</td>
<td>31/F</td>
<td>No apparent signs or symptoms</td>
<td>AVM in rt occipital lobe</td>
<td>Gamma Knife radiosurgery</td>
<td>A/G</td>
</tr>
</tbody>
</table>

A = adenine; G = guanine.

![FIG. 1. Pedigree chart. I-1 (father) and II-2 (unaffected sibling) wild-type (Lys332), I-2 (mother) heterozygous-unaffected (Lys332Glu), and other siblings heterozygous-affected (Lys332Glu). Arrow indicates the proband. Lys = lysine. Figure is available in color online only.](image-url)
consist of about 85% of the disease-causing mutations. Recently, we reported the novel mutations in non-NF2 meningiomas using exome sequencing in meningiomas and glioblastomas. The clinical utility of whole exome sequencing has also been shown by many other studies in both the identification of molecular defects—not only for family screening, but also in patients with neuosurgical-pathologies—and the clinical diagnosis of complex genetic disorders. Improvements in exome sequencing technology in terms of its reduced costs, as compared with the costs of various genetic diagnostic tests and standardized analysis pipelines, has led to tremendous potential for exome sequencing in brain AVMs. Our study is a promising example of using whole exome sequencing for family screening to identify a possible hereditary disorder.

Here, we reported the results of a whole exome sequencing analysis on 2 siblings with brain AVM. One of those siblings also had spinal AVM, whereas 2 others had only brain AVMs. A comprehensive assessment of this family’s members did not reveal the characteristic findings of HHT such as nasal bleeding, multiple visceral AVMs, or telangiectasias in the hands, face, or oral cavity. As previously mentioned, brain AVM is mostly detected as an isolated finding. The brain AVMs in the family in our study appear to be inherited in an autosomal dominant fashion with reduced penetrance, which is genetically defined as a measure of the proportion of individuals carrying a particular variant of a gene (allele or genotype) that also expresses an associated trait (phenotype). From a clinical viewpoint, the penetrance of a disease-causing mutation is the proportion of individuals with the mutation who exhibit clinical symptoms. In the present example, reduced or incomplete penetrance is more likely to have occurred because the mother failed to express the trait even though she was an allele carrier.

Endothelial cells express ACVRL1 on the cell surface, and it interacts with TGF-β superfamily proteins. The ACVRL1 product is a Type I cell surface receptor, which is a transmembrane protein kinase that generates diverse heteromeric serine/threonine kinase complexes of different signaling capacities. Smad 1 and Smad 5 pathways, which have a role in cell growth differentiation and development, are activated by ACVRL1 signaling. ACVRL1 mutations causing structural modifications or misfolded proteins lead to a degradation of protein and lack of protein expression on the cell surface. In our study, whole exome sequencing analysis revealed a shared, novel ACVRL1 mutation (p.Lys332Glu) in 2 patients that was consequently confirmed by direct Sanger sequencing. The Sanger sequencing also identified the ACVRL1 mutation in other family members. The mutation proved to be heterogeneous in all family members except for the father and the unaffected daughter who did not exhibit the mutation. The recurrent p.S333I variations in ACVRL1 were reported in families with HHT2. The p.S333I variant was retained in the endoplasmic reticulum as opposed to the wild-type variant, which is targeted and expressed on the cell surface. The amino acid at position 330 of the protein constitutes the active site of the protein. PolyPhen-2 software (Polymorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph/) predicted the p.K332E substitution to be deleterious with a high possible score (Supplementary Fig. 2). Similar to the p.S333I mutation, a p.K332E mutation may also cause improper folding of the ACVR1L protein. Since the p.K332E mutation domain is located in the protein acceptor site on the protein, which is a highly conserved region among Type I receptors, mutations in these locations are critical to protein-ligand interactions (Supplementary Fig. 1).

Regarding limitations of this study, further functional studies should be done to determine the regulation of downstream SMAD signaling pathway, protein misfolding, and intracellular degradation mechanisms and to explain the lack of surface expression of mutant proteins.

Conclusions

The role of genetic variations in sporadic AVMs remains elusive. Even though single nucleotide polymorphisms have depicted nominal association of ACVRL1 with sporadic AVMs, genome-wide linkage studies do not reveal the pathogenic variants. Our results suggest that whole exome sequencing analysis is particularly useful in cases of locus heterogeneity and uncertain diagnostic classification schemes in patients with hereditary brain AVMs.

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Novel ACVRL1 mutation in arteriovenous malformation


Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Conception and design: Yılmaz, Işık, Bilguvar, Kılıç, Günel. Acquisition of data: Yılmaz, Toktaş, Akakin, Işık, Bilguvar, Kılıç. Analysis and interpretation of data: Yılmaz, Toktaş, Akakin, Işık, Bilguvar, Günel. Drafting the article: Yılmaz, Akakin, Işık, Bilguvar, Günel. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Yılmaz. Administrative/technical/material support: Toktaş. Study supervision: Kılıç, Günel.

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