Am. J. Hum. Genet. 65:125–133, 1999

A Gene for Inherited Cutaneous Venous Anomalies ("Glomangiomas") Localizes to Chromosome 1p21-22

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Summary

Venous malformations (VMs) are localized defects of vascular morphogenesis. They can occur in every organ system, most commonly in skin and muscle. They can cause pain and bleeding, and in some critical locations they can be life threatening. Usually venous anomalies occur sporadically, but families with dominant inheritance have been identified. Using linkage analysis, we have established in earlier reports that some families with inherited VMs show linkage to chromosome 9p21; the mutation causes ligand-independent activation of an endothelial cell–specific receptor tyrosine kinase, TIE-2. Here we show that VMs with glomus cells (known as "glomangiomas"), inherited as an autosomal dominant trait in five families, are not linked to 9p21 but, instead, link to a new locus, on 1p21-p22, called "*VMGLOM*" (LOD score 12.70 at recombination fraction .00). We exclude three known positional candidate genes, *DR1* (depressor of transcription 1), *TGFBR3* (transforming growth factor–β receptor, type 3), and *TFA* (tissue factor). We hypothesize that cutaneous venous anomalies (i.e., glomangiomas) are caused by mutations in a novel gene that may act to regulate angiogenesis, in concert with the TIE-2 signaling pathway.

Received December 7, 1998; accepted for publication April 27, 1999; electronically published May 18, 1999.

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0002-9297/99/6501-0017$02.00

Introduction

Patients with venous malformations (VMs) are the most common referrals to centers for vascular anomalies. The prevalence of this developmental vascular anomaly is not known (for a review, see Vikkula et al. 1998). VMs (MIM 600195) are composed of dysmorphic, tortuous, spongelike veins that present as bluish swellings. The thin-walled vascular channels are composed of a deficient layer of smooth-muscle cells and are lined by quiescent endothelium (Vikkula et al. 1996, 1998). These lesions can be single or multiple, varying in size from a small bluish cutaneous spot to an extensive abnormality affecting deep soft tissues in any part of the body. Clinical management is often difficult and is best directed by an interdisciplinary team (Enjolras and Mulliken 1996). As is true for many vascular anomalies, these lesions may be obvious at birth or may appear later in life. There is often confusion in the diagnostic terminology of these lesions; typically they are mistakenly called "cavernous hemangioma" (Enjolras and Mulliken 1996; Vikkula et al. 1998). A biological classification, based on clinical and histological criteria, clearly distinguishes two major types of vascular anomalies: tumors (usually hemangioma) and malformations (Mulliken 1997).

In the past, venous anomalies were considered nonhereditary; however, there is mounting evidence that they can be inherited in a Mendelian manner (Boon et al. 1994; Vikkula et al. 1996). Typically, the affected individuals in these families have multiple small cutaneous lesions. The age at onset is usually <8 years; however, some patients first manifest these lesions during puberty. There is variable expressivity in terms of size and location of the lesions (see Boon et al. 1994).

"Glomangiomas" (MIM 138000) are a clinical and radiological subtype of VMs (Gorlin et al. 1960) (fig. 1, *top*). Their pathognomonic characteristic is the presence of undifferentiated smooth-muscle cells (glomus cells)

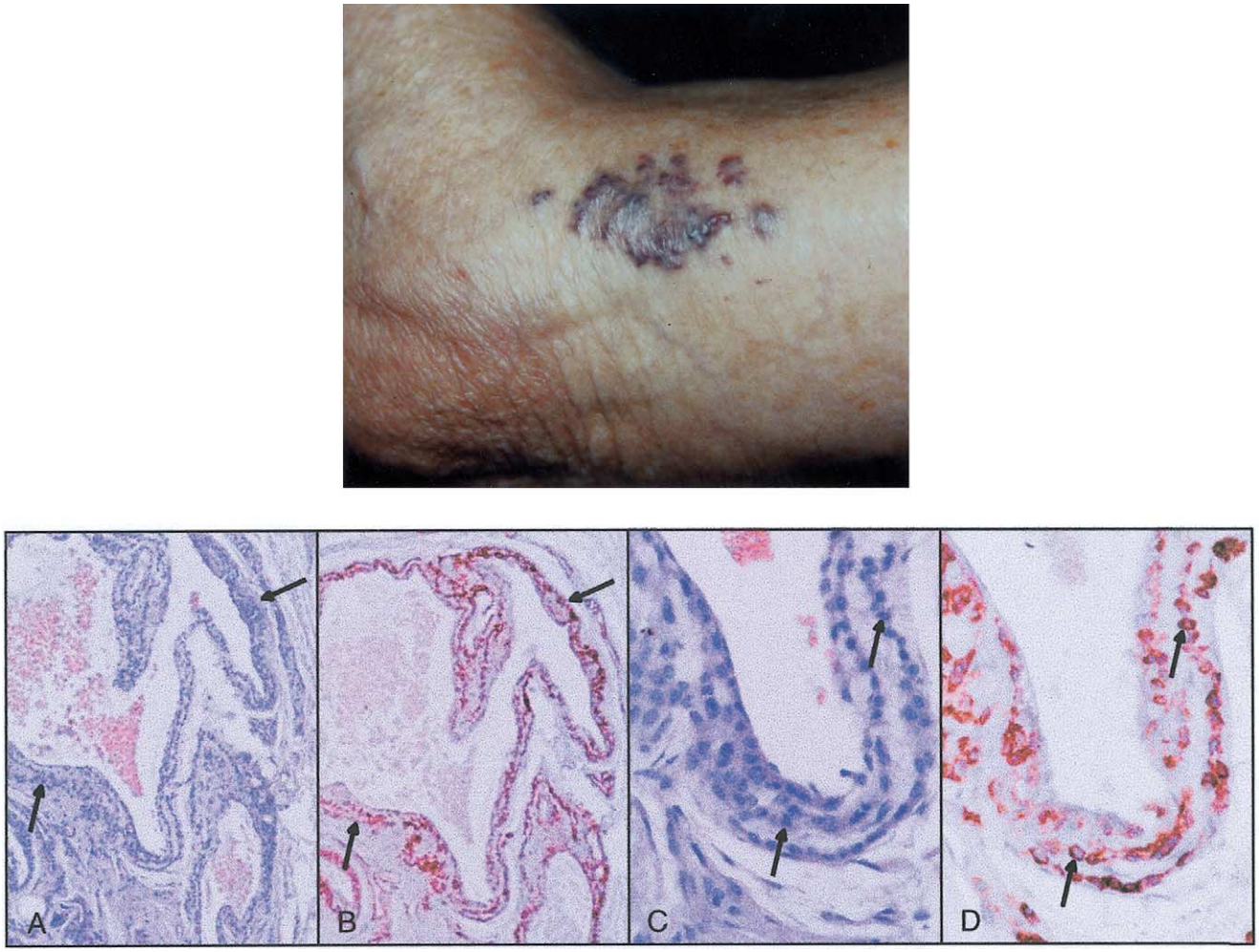

Figure 1 *Top,* Typical cutaneous venous malformation with glomus cells (i.e., glomangioma) (arm of individual F-6). *Bottom,* Histology of lesion in F-1005, showing anomalous venous channels. Poorly differentiated smooth-muscle cell–like glomus cells (*arrows*), with typical rounded nuclei (*C* and *D*), are positive for smooth-muscle α-actin (brownish color in *B* and *D*). *A* and *C,* Results of staining with hematoxylin-eosin. *B* and *D,* Results of immunohistochemical staining with antibody against SMC α-actin.

surrounding convoluted venous channels (Gorlin et al. 1960; Goodman and Abele 1971) (fig. 1, *bottom*). Clinically, glomangiomas look like any VM; however, they are more painful on palpation. In contrast to common VMs, glomangiomas are only partially compressible, and they are usually not found in mucosa. In addition, familial aggregation is more common, and several pedigrees showing autosomal dominant inheritance have been described (Touraine et al. 1936; Rudolph 1993; Iqbal et al. 1998). Penetrance is estimated to rise from 70% at age 5 years to 100% by age 30 years (Iqbal et al. 1998).

We have previously described, in a large family with autosomal dominant inheritance, cutaneous and mucosal VMs that cosegregated with markers on chromosome 9p21 (Boon et al. 1994) and that were caused by a single nucleotide change leading to an R849W substitution in the endothelial cell–specific receptor tyrosine kinase, TIE-2 (MIM 600221) (Vikkula et al. 1996). This mutation results in an increase in receptor autophosphorylation (Vikkula et al. 1996) and alters downstream signaling (Korpelainen et al. 1999). Here we show that five families with inherited cutaneous VMs with glomus cells (i.e., glomangiomas) do not show linkage to the *TIE-2* gene (*VMCM-1*) but, instead, show linkage to a new locus, *VMGLOM,* on 1p21-22 (LOD score 12.70 at recombination fraction (θ) .00). The minimal region spans 4–6 cM and is estimated to contain 200 genes. Six of these genes are already known, and we have excluded three of the latter: *DR1* (depressor of transcription 1), *TGFBR3* (transforming growth factor–β receptor, type 3), and *TFA* (tissue factor). We propose that the *VMGLOM* gene is important for the proper development/maintenance of veins and that it may act in con-

cert with TIE-2–dependent signaling. We conclude that separate loci are involved in the ethiopathogenesis of familial cutaneomucosal VMs and glomangiomas.

Families and Methods

Families and Histochemistry of Lesions

After informed consent was obtained, a clinical history was taken, and physical examination was performed on all family members participating in the study. Venous-blood samples were drawn for extraction of DNA. An additional sample was drawn, for lymphocytic transformation, from each of individuals Bl-11, Bl-103, Bl-105, Bt-12, Bt-103, Sh-2, Sh-12, and Sh-101. The pedigrees are shown in figure 2. Family F has been described in detail elsewhere (Rudolph 1993).

Immunohistochemical analysis of 5-μm sections was performed as described elsewhere (Vikkula et al. 1996; Stratmann et al. 1998), by means of a primary monoclonal antibody against human SMC $\alpha-$actin (1:300; Dako) and a biotinylated secondary antibody (sheep anti-mouse IgG, 1:500 dilution; Boehringer Mannheim). After incubation with streptavidin-peroxidase complex, 3-amino-9-ethyl-carbazole (Boehringer Mannheim) was used for color detection of immunoreactivity.

Linkage Analysis

Genomic DNA was extracted from the buffy coat (Qiagen DNA extraction kit). Genotyping of individuals was performed as described elsewhere (Boon et al. 1994). Polymorphic markers *D9S161, D9S169,* and *D9S171* were used for analysis of chromosome 9. For chromosome 1, several markers were selected either from the Weber 6.0 primer set (Research Genetics) or on the basis of their map positions (Centre d'Étude du Polymorphisme Humain and Cooperative Human Linkage Center) (synthesized by Gibco-BRL). Linkage analyses were performed under the following assumptions: dominant mode of inheritance, 1/10,000 disease-allele frequency, and equal allele frequencies for markers. For marker *D1S188,* calculations were also done under the assumption of 1/100,000 disease-allele frequency with published allele frequencies. Penetrances of 90% and 100% were used for individuals >16 years of age. The three unaffected individuals <16 years of age were assigned to liability classes with either 80% penetrance (individuals Sh-100, age 13 years; and T-1006, age 11 years) or 70% penetrance (T-1005, age 5 years). Calculations were performed with Linkage Package version 5.1 (Web Resources of Genetic Linkage Analysis) (Lathrop et al. 1984).

Candidate-Gene Analysis

To screen the *DR1* and *TFA* genes for mutations, primers (synthesized by Gibco-BRL) were designed according to known intronic sequences. Primer pairs P1 and P8, P9 and P10, and P11 and P6 were used for the *DR1* gene (Rozet et al. 1996), and 13 primers were designed for the *TFA* gene, according to the published sequence (GenBank J02846).

The size of the amplification products was 206–301 bp. For SSCP and heteroduplex analysis, both PCR primers were end-labeled with γ-[^{32}P] with use of polynucleotide kinase (TAKARA), according to manufacturer's recommendations. The PCR reactions were divided into two aliquots before the latter were loaded onto nondenaturing polyacrylamide gels (MDE gel solution; FMC). EDTA (final concentration 5 mM), and nondenaturing loading buffer (according to FMC) was added to the reactions for heteroduplex analysis, whereas a denaturing loading buffer (according to FMC) was added to the SSCP samples. After heat denaturation, the samples for SSCP analysis were immediately loaded onto SSCP gels. The samples for heteroduplex analysis were first cooled from 95°C to 37°C, at 1°C/min, to increase the formation of heteroduplexes. Both gels were run for 14–16 h, with SSCP gels at constant power (6–8 W) and with heteroduplex gels at constant potential (700 V). Gels were vacuum-dried and were exposed, for 12–24 h, to KODAK X-Omat film. Fragments showing abnormal migration were reamplified, purified (Qiagen PCR columns), and cycle sequenced (Gibco cycle-sequencing kit).

To screen the *TGFBR3* gene for mutations, cDNA was synthesized from total RNA extracted from Epstein-Barr virus–transformed lymphoblasts, as described elsewhere (Vikkula et al. 1996). Nested PCR reactions were performed in a total volume of 10 μl. Forty PCR primers were designed (on the basis of the published sequence [GenBank L07594]). The first-round PCRs amplified the cDNA in two overlapping pieces, and the second-round PCR amplified 15 overlapping fragments of 220–288 bp. PCR conditions were as described elsewhere (Vikkula et al. 1995).

Southern blot hybridizations were performed with amplified fragments of the *DR1* and *TGFBR3* genes as probes (prepared with primer pair P1 and P6, for the *DR1* gene, and primer pairs TGFBR3-1 and -7 and TGFBR3-4 and -40, for the *TGFBR3* gene). The DNA fragments were radiolabeled by random priming with Klenow (GIBCO-BRL), with α-[^{32}P] (ICN), to a specific activity of 1–2 $\times$ 10^8. Unincorporated nucleotides were removed with Micro Bio-Spin columns (Bio-Rad). Five micrograms each of genomic DNA from Bl-103, Bl-105, Bt-12, Bt-103, Sh-2, Sh-12, F-105, F-108, T-101, T-1010, and an unaffected control were digested with ei-

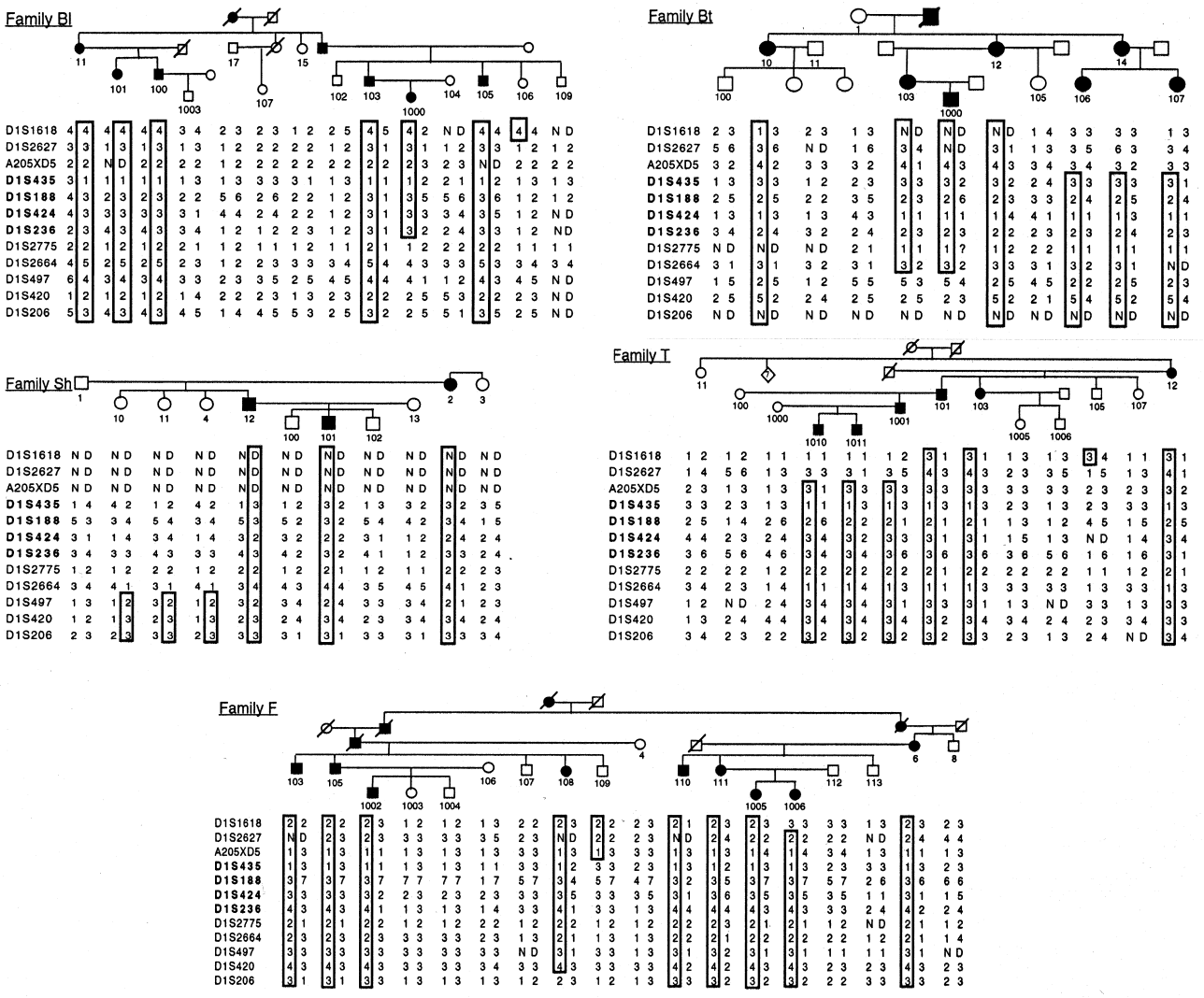

Figure 2 Pedigrees of five families with inherited VMs with glomus cells. Genotypes for markers in the *VMGLOM* region are depicted beneath the symbols representing individuals in the pedigrees. The haplotype cosegregating with the disorder is boxed, and markers in the linked region are boldface. Circles represent females, and squares denote males; affected individuals are denoted by blackened symbols, and unaffected individuals are denoted by unblackened symbols. A diagonal slash (/) through a symbol indicates that the individual is deceased. ND = not determined.

ther *Eco*RI, *Pvu*II, or *Hin*dIII, according to the manufacturer's recommendations (Pharmacia), were separated on 0.7% agarose gels, and were transferred, for Southern blot hybridization, onto Hybond N^+ membrane (Amersham). The membrane was hybridized at 42°C (in a Techne HB-1D oven), with 50 ml of hybridization solution containing 6 × SSC, 50 mM Na_2HPO_4/NaH_2PO_4 (pH 6.8), 50% deionized formamide, 50 mg dextran sulfate/ml, 25 μg ssDNA/ml, 1 mM EDTA (pH 8.0), 0.5% SDS, and 1 × Denhardt's solution (0.02% BSA, 0.02% Ficoll, and 0.02% polyvinylpyrrolidone). After being washed with 0.1 × SSC and 0.1% SDS at room temperature (four times for 5 min each) and at 55°C (three times for 30 min each), the filters were exposed, for 12–24 h to KODAK X-OMAT film.

Results

Families and Histochemistry of Lesions

Affected individuals in all five families had localized cutaneous venous lesions, clinically similar to those in affected members of the two families showing linkage to the *TIE-2* gene on 9p21 (fig. 1, *top*). The size of the lesions varied from small blue spots (1–5 mm in di-

ameter) to larger spongy masses (as large as 20 cm in diameter). The lesions were scattered, and the number per affected individual was 1–17. In 50% of patients, the lesions were painful, especially on palpation and/or at pubertal onset. There were no mucosal lesions. The age at appearance of the first lesion varied, from birth to puberty. Thirty percent of the affected individuals had noted gradual enlargement of their lesions, and 40% reported an increase in number. Eleven patients had undergone resection of one or more painful lesions. We obtained two of these tissue samples, one from individual Bl-1000 and one from individual F-1005. Histologically, the lesions were composed of malformed veins with variable numbers of glomus cells (Rudolph 1993) (fig. 1, *bottom*). The presence of glomus cells in such cutaneous venous anomalies is pathognomonic for the diagnosis of "multiple glomangiomas" or "glomus tumors" (Gorlin et al. 1960).

Exclusion of TIE-2 *Gene* (VMCM-1)

Because these cutaneous venous lesions were similar to those in the families with a TIE-2 mutation (Boon et al. 1994; Vikkula et al. 1996), we first tested the five families for linkage to chromosome 9p. In each of the families, one or more affected individuals were recombinant for markers on both sides of the *TIE-2* gene. The combined LOD scores ($\theta = .00$, penetrance 90%) were -16.1 for *D9S171,* -24.1 for *D9S169,* and -23.4 for *D9S161;* the θ values for combined LOD scores <-2.00 were .20, .20, and .10, respectively. The order of these markers was *D9S171, TIE-2, D9S169, D9S161*. These results clearly exclude a mutation in the *TIE-2* gene in these families and indicate that there must be locus heterogeneity for familial VMs.

Linkage to 1p21-22

Having excluded the *TIE-2* gene in the five families, we next tested the *TIE-2* homologue *TIE-1* on human chromosome 1p33-34. TIE-1 is an orphan receptor also known to be important for angiogenesis, because Tie-$1^{-/-}$ murine embryos exhibit severe mesenchymal hemorrhage by embryonic day 18.5 (Sato et al. 1995). However, markers around the *TIE-1* gene excluded this locus (*D1S193, D1S447, D1S451,* and *D1S213* had combined two-point LOD scores <-2.00). Therefore, we started random screening of the genome, with polymorphic markers on both the short arm and the centromeric portion of the long arm of chromosome 1, and identified a region in which several markers for all five families gave combined LOD scores >3.0 (see table 1). Thus, the random screening was discontinued, and no other chromosomes were tested. Changing the disease-allele frequency (1/10,000 or 1/100,000), marker-allele frequencies (equal or published), and penetrances (90% or 100%) did not significantly alter the LOD scores. The highest combined LOD score was for marker *D1S188:* with 90% penetrance and 1/10,000 disease-allele frequency, it reached 12.70 ($\theta = .00$) with published allele frequencies and 12.42 ($\theta = .00$) with equal allele frequencies. If 100% penetrance and published allele frequencies were used for all individuals, the LOD score increased to 13.58 ($\theta = .00$). Three of the five families gave maximum positive LOD scores (all informative meioses) with this marker at $\theta = .00$. The LOD scores (penetrance 90%) were 2.97 (family Bl), 4.27 (family F), and 1.82 (family Sh). The other two families also had positive LOD scores with this marker: 1.91, maximum 2.21 (family Bt); and 1.45, maximum 2.39 (family T). Several other markers on both sides of *D1S188* also showed positive LOD scores in all five families, although not all individuals were informative for all markers (table 1).

To define the borders of the linked interval, we created haplotypes based on the database order of the markers (Centre d'Étude du Polymorphisme Humain and Cooperative Human Linkage Center). This haplotypic analysis identified obligatory recombinations at the telomeric border of the region between markers *D1S2627* and *D1S1618* (Bl-106; unaffected), between markers *D1S226* and *D1S464* (Sh-10 and -11; both unaffected), between *AFMA205XD5* and *D1S435* (F-109; unaffected), and between *D1S2627* and *D1S435* (T-1001; affected) (see fig. 2). Obligatory recombinations at the centromeric end of the linked interval were observed between markers *D1S236* and *D1S2775* (Bl-1000; affected), between markers *D1S2664* and *D1S497* (Sh-10, -11, and -12; all unaffected), between markers *D1S2775* and *D1S497* (Bt-103; affected), and between markers *D1S420* and *D1S206* (F-108; affected) (fig. 2). Since we did not have DNA from the deceased affected grandfather (Bt-2) in family Bt, the three affected daughters (Bt-10, -12, and-14) were not fully informative for any of the markers in the region. However, since Bt-14 was recombinant for marker *AFMA205XD5* and markers telomeric to this marker, the region linked to the phenotype reached, at a maximum, to *AFMA205XD5*. In the aggregate, the haplotype data defined a minimal region of overlap between markers *D1S2775* and *AFMA205XD5* (fig. 3). On the basis of the linkage maps (Centre d'Étude du Polymorphisme Humain and Cooperative Human Linkage Center) and the MIT YAC-contig map (Whitehead Institute for Biomedical Research/MIT Center for Genome Research), this interval is ~4–6 cM, approximately equivalent to 4–6 Mb.

Exclusion of Positional Candidate Genes

The region under study is estimated to contain 200 genes. On the basis of the human linkage, physical, and

Table 1

LOD Scores for Markers at *VMGLOM,* for Disease–Allele Frequency .0001, Equal Allele Frequencies, and 90% Penetrance

	$\theta = .00$	$\theta = .01$	$\theta = .10$	$\theta = .20$	$\theta = .30$	$\theta = .40$
MARKER	Two-Point LOD Score for Family F					
D1S1618	−3.76	−1.06	−.13	.06	.11	.07
D1S2627	1.67	1.63	1.29	.90	.53	.22
AFMA205XD5	1.65	1.64	1.45	1.12	.73	.35
D1S435	4.07	3.40	3.30	2.46	1.56	.65
D1S188	4.27	4.20	3.49	2.66	1.76	.83
D1S424	2.01	1.98	1.63	1.22	.78	.36
D1S236	2.86	2.82	2.40	1.87	1.27	.63
D1S2775	1.88	1.85	1.57	1.21	.81	.40
D1S2664	2.70	2.63	2.01	1.33	.72	.25
D1S497	1.83	1.81	1.53	1.15	.75	.35
D1S420	3.77	3.70	3.04	2.25	1.41	.57
D1S206	−2.52	.43	1.01	.83	.53	.24
	Combined LOD Scores for Five Families with Inherited VMs with Glomus Cells[a]					
D1S1618[b]	−7.90	−1.58	.44	.68	.52	.26
D1S2627[b]	−.08	5.00	4.89	3.77	2.40	1.04
AFMA205XD5[b]	−.60	1.38	2.09	1.82	1.27	.62
D1S435	9.62	9.47	7.92	5.95	3.79	1.60
D1S188	12.42	12.22	10.23	7.73	5.01	2.26
D1S424	8.26	8.13	6.80	5.09	3.22	1.39
D1S236	8.23	8.12	6.92	5.33	3.55	1.65
D1S2775	−1.07	2.18	2.58	2.06	1.36	.62
D1S2664	4.68	7.47	6.58	4.72	2.79	1.10
D1S497	−.82	4.86	5.43	4.31	2.87	1.33
D1S420	1.60	4.73	5.34	4.24	2.74	1.18
D1S206[c]	−5.33	.62	1.99	1.71	1.08	.42

[a] Penetrance for T-1005 was 70%; penetrance for Sh-100 and T-1006 was 80%.
[b] No data were available for family Sh.
[c] No data were available for family Bt.

transcript maps, six of these are known; one of the latter, *ABCR* (ATP-binding–cassette transporter) is mutated in Stargardt disease (MIM 248200) and thus was excluded as a candidate gene for VMs with glomus cells. Because of their known function, we also considered the genes encoding ribosomal protein L5 (*RPL5*) and γ–glutamylcysteine synthetase (*GLCLR*) to be unlikely candidates. The three remaining genes could not be excluded as positional candidates, on the basis of current information. Therefore, we screened the coding sequences of these genes, for rearrangements and nucleotide changes, by SSCP, heteroduplex analysis, and Southern blot hybridizations in families Bl, Bt, and Sh.

Southern blot hybridization of the three positional candidate genes—*DR1, TFA,* and *TGFBR3*—showed no evidence for genomic rearrangements or deletions. SSCP screening for the *DR1* and *TFA* genes revealed only a known polymorphism, in exon 3 in the *DR1* gene (Rozet et al. 1996), and polymorphism of an extra G, in the sequence of intron 5 of the *TFA* gene (position 10262; GenBank J02846).

SSCP screening of the full-length cDNA of the *TGFBR3* gene showed three nucleotide polymorphisms: G563→A (*Pst*I), G1548→A, and C2370→T (GenBank L07594). The three positional candidate genes were also screened for altered nucleotides by heteroduplex analysis, but only one additional DNA sequence change was identified, a G2918→A (*Msp*I) polymorphism in the *TGFBR3* gene. None of these changes either cause an amino acid substitution or, even if identified in patients, segregate with the phenotype, implying that they reside on the unlinked allele.

In addition to these polymorphisms, we found three nucleotides (C at location 555 and GG at location 564) missing from the published *TGFBR3* sequence (GenBank L07594), both an extra G (position 2909) and a missing G (position 2955) in the same *TGFB3R* sequence, and 52 bp of intronic sequence that is not identified in the published *DR1* sequence (Rozet et al. 1996). These changes were also found in control individuals and therefore represent mistakes in the published sequences.

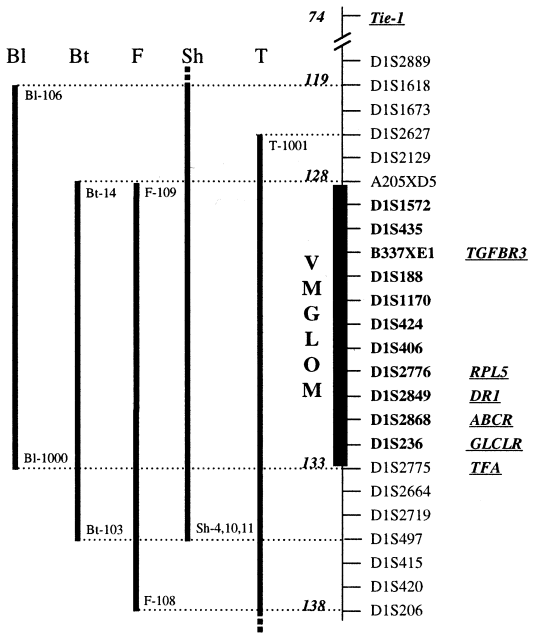

Figure 3 Schematic map of *VMGLOM* region. Marker order is based on the MIT physical map (Whitehead Institute for Biomedical Research/MIT Center for Genome Research). Positions of the *TIE-1* gene and five known genes in *VMGLOM* are also shown: *TGFBR3, RPL5, DR1, ABCR, GLCLR,* and *TFA*. Numbers in boldface italic are distances (in cM) from 1pter. The vertical bars with identification numbers for recombinant individuals (fig. 2) show the linked regions in the families.

Discussion

VMs are developmental in origin, and, if extensive, they are difficult to treat (Mulliken and Young 1988). They are composed of convoluted thin-walled vascular channels with deficient intramural smooth muscle. A mutation in the *TIE-2* gene causes one familial type of mucocutaneous VM (Vikkula et al. 1996). The identified substitution, R849W in two families, causes ligand-independent autophosphorylation of the receptor (Vikkula et al. 1996). In addition, the mutant receptor activates STAT1 signaling, normally not activated by wild-type TIE-2 (Korpelainen et al. 1999).

The affected individuals in the five families described in the present study had lesions clinically similar to the cutaneous VMs in the families showing linkage to TIE-2, although they were typically painful on palpation. No mucosal lesions were noted in these five families. The distinctive glomus cells also differentiated these lesions. As in patients with the R849W TIE-2 mutation, lesions in the five families described in the present study evidenced variable expressivity in size, location, and number. A high (98%) penetrance was observed for the mutation in the *TIE-2* gene (Vikkula et al. 1996). Since the phenotype never skipped a generation in the five families and since 34/61 (55.7%) of the children of affected individuals had venous lesions, the penetrance can be assumed to be high in these families too. Of the 38 affected individuals, 18 (47.3%) were males, and male-to-male transmission was found in four of the pedigrees. Thus, the inheritance seems to be autosomal dominant, with (nearly) 100% penetrance and without sex influence.

Using linkage analysis, we identified a locus, called "*VMGLOM,*" on chromosome 1p21-22. Several markers showed high combined positive LOD scores (table 1). Varying gene frequencies and allele frequencies in the linkage calculations did not significantly alter the LOD scores. With three liability classes, of 90%, 80%, and 70% penetrance, respectively, the maximum LOD score was 12.70 ($\theta = .00$), and with a single liability class of 100% penetrance it was 13.58 ($\theta = .00$). These results give strong statistical evidence that this chromosomal region contains a gene that, when mutated, causes VMs with glomus cells (i.e., glomangiomas).

Haplotype analysis defined the smallest linked interval at 4–6 cM, between markers *AFMA205XD5* and *D1S2775*. Possible positional candidate genes are *DR1, TFA,* and *TGFBR3*. DR1 is a depressor of transcription and could be involved in TIE-2 signaling. In knockout mice, tissue factor has been shown to be important for both extraembryonic recruitment of pericytes and vascular development (Bugge et al. 1996; Toomey et al. 1996). TGFβ-binding proteins are implicated in another vascular disorder, hereditary hemorrhagic telangiectasia (McAllister et al. 1994; Johnson et al. 1996). However, none of these genes was found mutated in the families showing linkage to *VMGLOM*.

Three other known genes are located in the *VMGLOM* locus: *ABCR, GLCLR,* and *RPL5*. However, these are unlikely to be the *VMGLOM* gene(s). The *ABCR* gene is mutated in Stargardt macular dystrophy (Allikmets et al. 1997). The γ-glutamylcysteine synthetase light subunit is the inhibitory part of a two-subunit enzyme complex needed for glutathione synthesis; and a deficiency in this catalytic activity has been linked to hemolytic anemia (Meister 1974). The *RPL5* gene is not a strong candidate either, because ribosomal proteins usually have important functions in a large number of different cell types.

Since cutaneous VMs and VMs with glomus cells resemble one another, it might be assumed that their ethiopathogenic mechanisms are similar. Therefore, genes that interact with the TIE-2 signaling pathway are possible candidates for *VMGLOM*. The TIE-2 receptor may signal via SH-PTP2, GRB2 (Huang et al. 1995), Dok-R (Jones and Dumont 1998), or AKT, via activation by p85/PI3-kinase (Kontos et al. 1998) and by STAT3 and

STAT5 (signal transducers and activators of transcription) (Korpelainen et al. 1999). In addition, the R849W mutant receptor appears to activate STAT1 and, thereby, p21 (Korpelainen et al. 1999). However, none of the genes encoding these intracellular substrates localize to *VMGLOM*.

Three TIE-2 ligands are known: angiopoietin-1 (MIM 601667), -2, and -4 (ANG-1, -2, and -4) (Davis et al. 1996; Suri et al. 1996; Maisonpierre et al. 1997; Valenzuela et al. 1999). However, the genes are located on 8q22.3-q23, 8p23, and 20p13, respectively (Cheung et al. 1998; Valenzuela et al. 1999). TIE-2–receptor signaling could involve another receptor subunit. This possibility is supported by the observation that ANG-2 and ANG-4 exert a cell-type specific effect on TIE-2. They do not induce TIE-2 autophosphorylation in endothelial cells, but they do cause TIE-2 activation in fibroblasts (Maisonpierre et al. 1997; Witzenbichler et al. 1998; Valenzuela et al. 1999). These findings suggest that fibroblasts either express a protein that converts the ANG-2/ANG-4 effect from negative to positive or they lack a protein, expressed in endothelial cells, responsible for the endothelial-specific inhibitory effect of ANG-2 and ANG-4. A loss-of-function mutation in such a protein would render TIE-2 susceptible to the activating effects of ANG-2 and ANG-4. This would be similar to the activating R849W mutation, in TIE-2, that is responsible for familial cutaneomucosal VMs (Vikkula et al. 1996).

In summary, there is locus heterogeneity for inherited cutaneous VMs. Affected individuals in five families with VMs and glomus cells (i.e., glomangiomas) do not have mutations in the *TIE-2* gene. Instead, the disorder in these families is linked, with a maximum combined LOD score of 12.70 ($\theta = .00$), to a second locus, called "*VMGLOM*," on chromosome 1p21-22, located between markers *D1S2775* and *AFMA205XD5*. Three known positional candidate genes were normal in affected family members. Although the identity of the *VMGLOM* gene remains to be discovered, histological observations suggest that *VMGLOM* mutations cause decreased differentiation of smooth-muscle cells in affected veins. Furthermore, the presence of glomus cells helps to distinguish between families showing linkage to the *TIE-2* gene and families showing linkage to *VMGLOM*. The high penetrance observed in these families underscores the likelihood of affected progeny of affected individuals. This report provides a framework for testing other families for linkage to the *VMGLOM* locus and for positional cloning of the causative gene.

Acknowledgments

The authors thank all the families for their invaluable participation. We are indebted to H. P. W. Kozakewich and C. Godfraind for histopathological assessment. Studies from the authors' laboratories were supported, in part, by Organogenesis Inc. and National Institutes of Health grants HL33014 and AR36820 (both in support of B.R.O.) and by the Human Frontier Science Program; Fonds de Développement Scientifique–Université Catholique de Louvain; the Belgian Federal Service for Scientific, Technical and Cultural Affairs; and European Commission grant ERB4001GT963858 (all in support of M.V.). P.B. and A.I. are supported by fellowships from F.R.I.A. (Fonds pour la formation à la recherche dans l'industrie et dans l'agriculture), and L.K. is supported by the Academy of Finland. The authors are also grateful to Ms. Ana Gutierrez for expert technical assistance.

Electronic-Database Information

Centre d'Étude du Polymorphisme Humain, http://www.cephb.fr

Cooperative Human Linkage Center, http://chlc1.fccc.edu/ChlcMarkerMaps.html

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for J02846 and LL07594)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for ANG-1, -2, and -4 [MIM 601667]; glomangiomas [MIM 138000]; Stargardt disease [MIM 248200]; TIE-2 [MIM 600221]; and VM [MIM 600195])

Web Resources of Genetic Linkage Analysis, http://linkage.rockefeller.edu

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www-genome.wi.mit.edu

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