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Mutations in a Novel Factor, *Glomulin,* Are Responsible for Glomuvenous Malformations ("Glomangiomas")

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Glomuvenous malformations (GVMs) are cutaneous venous lesions characterized by the presence of smooth-muscle–like glomus cells in the media surrounding distended vascular lumens. We have shown that heritable GVMs link to a 4–6-cM region in chromosome 1p21-22. We also identified linkage disequilibrium that allowed a narrowing of this *VMGLOM* locus to 1.48 Mb. Herein, we report the identification of the mutated gene, *glomulin,* localized on the basis of the YAC and PAC maps. An incomplete cDNA sequence for glomulin had previously been designated "*FAP48*," for "FKBP-associated protein of 48 kD." The complete cDNA for glomulin contains an open reading frame of 1,785 nt encoding a predicted protein of 68 kD. The gene consists of 19 exons in which we identified 14 different germline mutations in patients with GVM. In addition, we found a somatic "second hit" mutation in affected tissue of a patient with an inherited genomic deletion. Since all but one of the mutations result in premature stop codons, and since the localized nature of the lesions could be explained by Knudson's two-hit model, GVMs are likely caused by complete loss of function of glomulin. The abnormal phenotype of vascular smooth-muscle cells (VSMCs) in GVMs suggests that glomulin plays an important role in differentiation of these cells—and, thereby, in vascular morphogenesis—especially in cutaneous veins.

Introduction

Venous malformations (VMs) are localized defects of vascular morphogenesis that present as multiple bluish-purple lesions, mainly in skin and mucosa. The lesions can be single or multiple, varying in size from a small blue spot to an extensive abnormality (Enjolras and Mulliken 1996). They are often present at birth and grow proportionally with the patient (Mulliken 1988). Histologically, they show enlarged endothelial-lined veinlike channels with defects in the smooth-muscle cell (SMC) layer (Vikkula et al. 1996). These lesions are commonly seen in centers that specialize in treatment of vascular anomalies. Most appear to be sporadic, but they can also be dominantly inherited (Boon et al. 1994; Gallione et al. 1995; Vikkula et al. 1996). In a previous study (Vikkula et al. 1996), we identified the endothelial-specific angiopoietin receptor TIE2/TEK, located on 9p21, as the cause of familial mucocutaneous VMs (VMCMs [MIM 600195]).

Glomuvenous malformations (GVMs [MIM 138000], also known as "venous malformations with glomus cells," or "glomangiomas") are similar to VMs (Gorlin et al. 1960; Gupta et al. 1965; Rudolph 1993; Boon et al. 1999; Calvert et al. 1999; Vikkula et al. 2001), yet, clinically, they are distinguishable: they have a cobblestone appearance (fig. 1*A* and *B*), they have a consistency harder than that of VMs, and they are painful on palpation (authors' unpublished data). Histologically, GVMs are distinguished by the presence of pathognomonic rounded cells—glomus cells—around the distended veinlike channels (fig. 1*C–F*) (Rudolph 1993; Vikkula et al. 1998; Boon et al. 1999). The term "glomus" (Latin for "ball") stems from the morphologically similar contractile cells of the Sucquet-Hoyer arteriovenous anastomoses in glomus bodies that are involved in cutaneous thermoregulation (Pepper and Lantis 1977). Glomus cells in GVMs appear to be incompletely or improperly differentiated vascular SMCs (VSMCs), since they stain positively for SMC α-actin (fig. 1*F*) and vimentin but are negative for desmin, von Willebrand factor, and S-100 (Kato et

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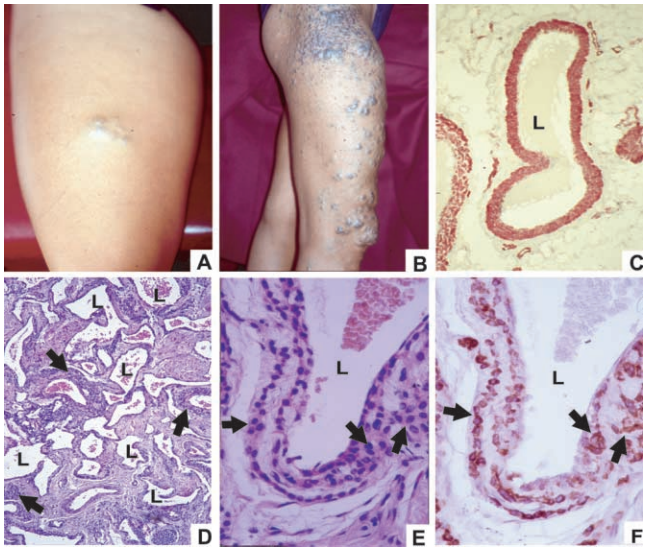

Figure 1 Typical GVMs. *A* and *B*, Cutaneous GVMs of right lower extremity in two patients, showing phenotypic variability. *C*, Cross-section of normal vein (magnification 40 ×), stained with antibody against SMC α-actin (*red*). *D–F*, Cross-sections of GVM (magnifications 10 ×, 40 ×, and 40 ×, respectively), stained with either (*D* and *E*) hematoxylin and eosin or (*F*) antibody to SMC α-actin (*reddish brown*). Arrows indicate glomus cells; L = vascular lumens. A lesion with somatic mutation is presented in panels *A* and *D*.

al. 1990). In addition, on electron microscopy, glomus cells show smooth-muscle myofibrils and "dense bodies" (Goodman and Abele 1971).

In a previous study, we showed, in five families, that GVMs cosegregate with a 4–6-cM locus, *VMGLOM*, on chromosome 1p21-22 (Boon et al. 1999), and we excluded three candidate genes in the region: *DR1* (encoding depressor of trancription 1), *TGFBR3* (encoding transforming growth factor-β receptor, type 3), and *TFA* (encoding tissue factor). Four other families, with linkage to a 24-cM region overlapping the *VMGLOM* locus, were independently reported by another group (Calvert et al. 2001). To localize new STSs and ESTs, we generated YAC- and PAC-based physical maps (Brouillard et al. 2000). In seven additional families with linkage to the region, we performed haplotype analysis and identified linkage disequilibrium that narrowed the area to <1.48 Mb (Irrthum et al. 2001). Using STSs, we mapped three new positional candidate genes to this region: *EVI5* (the proto-oncogene), *GFI1* (the growth-factor–independence gene), and *RPL5* (encoding ribosomal protein L5) (Brouillard et al. 2000). Moreover, *FAP48* (GenBank accession number U73704), which encodes an FKBP-associated protein of 48 kD, was localized to the region on the basis of the sequence obtained from one end of clone 775d17 that was used for construction of the PAC map. Since *FAP48* was easily amplifiable from lymphoblast RNA, it was screened first. We identified several mutations but, also, important discrepancies versus the published *FAP48* sequence. Therefore, we propose to call this gene "glomulin."

Subjects and Methods

Subjects

Informed consent was obtained from all family members prior to their participation in the study and was approved by the ethical committee of the medical faculty at the Université catholique de Louvain, Brussels. Twelve families have been described elsewhere (Boon et al. 1999; Irrthum et al. 2001). Among these, family Al (presented by Irrthum et al. [2001]) was shown to have VMs, rather than GVMs, according to a recent histological diagnosis of a resected lesion. Herein, we present eight additional families with individuals affected by GVMs, as well as one sporadic case, BG, with no known family history of the disease (fig. 2). Family members with anamnestic notion of cutaneous lesions were clinically examined. For genomic-DNA extraction, buccal-cell samples were obtained from individuals Blo-52 and Blo-810, and venous blood samples were drawn from all other participants. A second blood sample was obtained from some individuals, for lymphocytic transformation with Epstein-Barr virus. Immunohistochemistry was performed as described elsewhere (Boon et al. 1999).

Northern Blots and RT-PCR

Hybridizations of the 12-Lane Human Multiple Tissue Northern (MTN) Blot (Clontech Laboratories) and Human Multiple Tissue Expression (MTE) Array 2 (Clontech Laboratories) were performed according to the MTE protocol provided by the manufacturer. Two different probes, radiolabeled by random priming with α-[^{32}]dCTP, were used: full-length *glomulin* coding sequence and a 482-bp 5′ fragment thereof (nt −23 to +459). The filters were either exposed to Biomax films (Kodak) or analyzed by phosphorimager (Molecular Dynamics). For RT-PCR, RNA extractions on eight GVM lesions and on skin, umbilical cord, and placenta were performed by standard phenol/chloroform procedure (Chomczynski and Sacchi 1987), whereas the SV Total RNA isolation system (Promega) was used on cultured SMCs. Several combinations of cDNA primers were used to amplify different fragments of *glomulin* (sequences available on request).

5′ Rapid Amplification of cDNA Ends (RACE)

5′-RACE experiments were performed with the 5′RACE System for Rapid Amplification of cDNA Ends (Life Technologies). The gene-specific primers used were 5′-GCT GAT TCC AAA GGG TAG AC-3′, 5′-TGG GAT

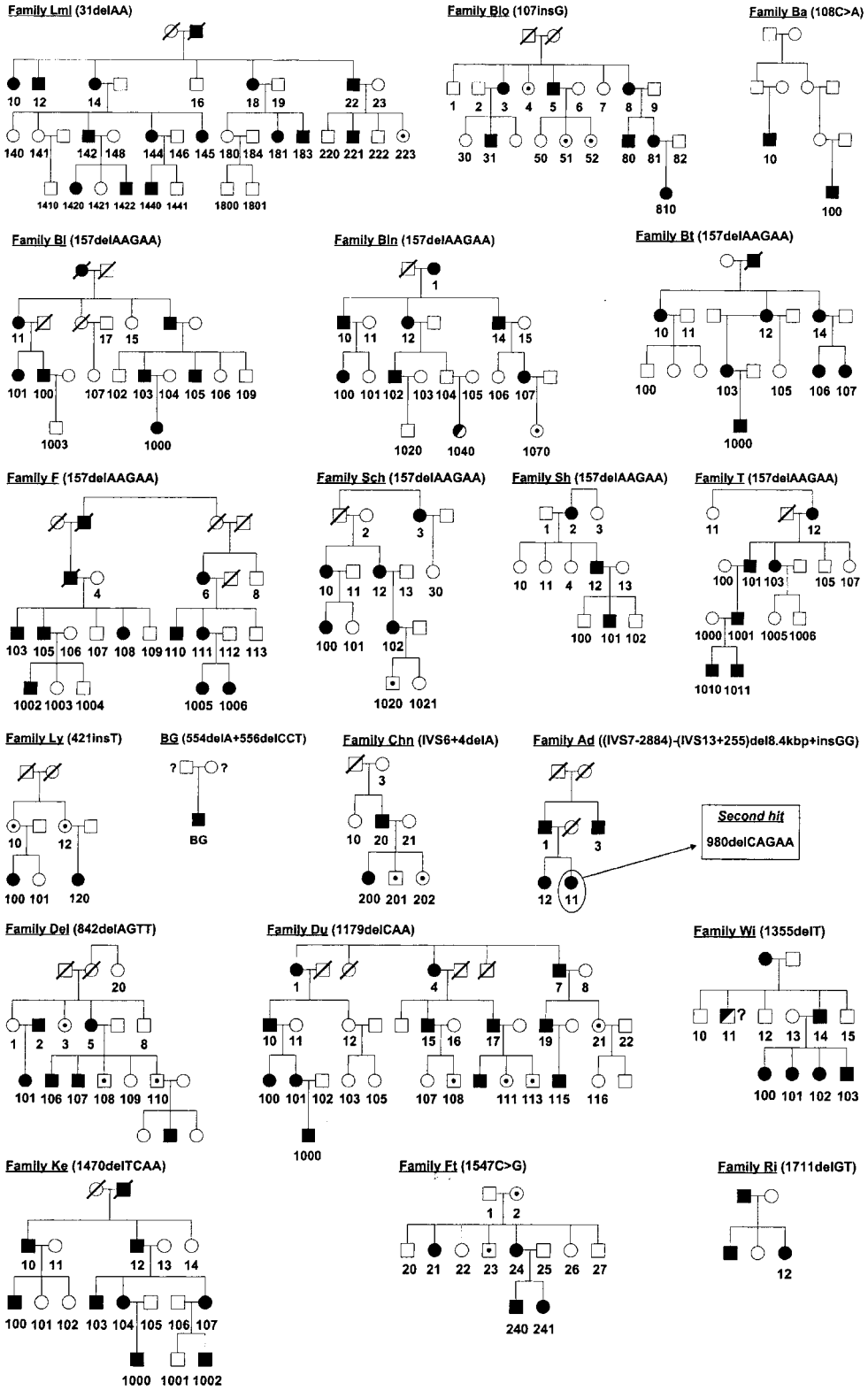

Figure 2 Pedigrees of 20 families with GVMs, presented in order of occurrence of the mutations in *glomulin*. Individuals tested are indicated by numerals. Blackened symbols denote affected patients, symbols containing a dot denote unaffected carriers, the half-blackened symbol (in families Bln and Wi) denotes a phenocopy, and question marks (?) indicate that the status is unknown. The second-hit mutation, 980delCAGAA, in family Ad, also is indicated.

ATC TGT TTT CCA GAG-3′, and 5′-CTA TCC TCT TTA TCT TTA CAC-3′.

Genomic Structure of the Glomulin *Gene*

Exon/intron boundaries for exon 4 were identified by sequencing the SP6-end of the PAC clone 775d17 (Brouillard et al. 2000). To define the remaining genomic structure, 36 primers were progressively designed on the basis of the *glomulin* cDNA sequence (sequences available on request). Different combinations of these exonic primers were used for PCR on PAC clone 775d15 and on the overlapping clone 1090n11 (Brouillard et al. 2000). To identify exon/intron boundaries, interexonic fragments obtained were completely or partially sequenced by either a DNA4000 (Li-Cor) or a CEQ2000 (Beckman Coulter) fluorescent sequencer.

Mutational Screening

cDNA.—RNA was extracted from a resected lesion in individuals Ad-11, F-6, Sch-100, Ba-10, Ft-24, Ri, and BG and/or from cultured, Epstein-Barr virus–transformed lymphoblasts, by the SV Total RNA isolation system (Promega). cDNA synthesis, SSCP, and heteroduplex analyses were performed as described elsewhere (Boon et al. 1999). PCR primers were synthesized to cover the coding sequence of *glomulin,* in overlapping fragments of ~250 bp (sequences available on request).

Genomic DNA.—Thirty-eight intronic primers (sequences available on request) were synthesized from the obtained genomic sequences, to amplify the 19 exons of *glomulin*. Genomic DNA was screened by radioactive SSCP and heteroduplex analysis for Ad-3, Ad-11 (tissue), Al-14, Ba-10, BG, Chn-200, Del-2, Du-10, Ft-21, Ke-10, Ly-100, Ri-12, Wi-14, and several control individuals, as described elsewhere (Boon et al. 1999). Amplified fragments were also loaded onto denaturing 5%-acrylamide sequencing gels, to identify potential insertions or deletions. Fragments with abnormal migration were reamplified, purified, and sequenced on a CEQ2000 capillary sequencer (Beckman Coulter). The amplification product for somatic mutation 980delCAGAA was cloned into pBLSK+ (Stratagene), for sequencing (fig. 3). Since the *glomulin* RT-PCR for patients from family Ad showed several aberrant bands, Elongase enzyme mix (Life Technologies) was used for long-range genomic PCRs for deletion mapping. The amplicon obtained with primers in introns 7 and 13 was smaller than normal. It was sequenced to identify the breakpoints (fig. 3). Since mutation 157delAAGAA was identified in samples from families F and Sch, families Bl, Bln, Bt, Sh, and T, which share the same haplotype (Irrthum et al. 2001), were checked for that mutation only.

Segregation of Mutations

Since most mutations create size differences, we used sequencing gels to assess inheritance in the families. Mutations modifying a restriction-enzyme cutting site were checked by appropriate digestion: destruction of a *Nsi*I site for mutations 107insG and 108 C→A, creation of a *Spe*I site for 554delA+556delCCT, and destruction of a *Xmn*I site for 1711delGT. Since mutation 1547C→G did not change either the amplicon size or any restriction site, we synthesized wild-type and mutant primers (5′-CTG CTT CAT AAT GTG CTT TT(C/G)- 3′) for allele-specific PCR. These were used in combination with the forward primer (5′-AGT AGG CAA TCA ATC ATT GTT G-3′) of exon 17. The annealing temperature was 58°C. A reverse primer (5′-AAT GGC TTA GCT GTT ATG GTC-3′) of exon 17 was added to the reaction to serve as an internal positive control and as competitor to improve the specificity of the reaction. The 8.4-kb deletion in family Ad was checked by PCR using a primer (5′-TTG AGC ATG TTT ATA GCT GTT G-3′) 4 kb upstream of exon 8 and a reverse primer (5′-TAG AGA TAG AGC AAT AAC TCA C-3′) of exon 14. This produced a band of 2.7 kb, instead of 11.1 kb.

Computational Analyses

Nucleotide- and protein-database searches were performed through the Web site of the National Council of Biotechnology Information by BLAST searches blastn, blastx, and tblastn, with nonredundant databases, dbEST, species-specific databases, high-throughput genomic sequences, and genome-survey sequences. SMART and PredictProtein were used to identify functional domains.

Results

Identification of the Glomulin *Gene*

During our positional cloning efforts, we sequenced the ends of several PAC clones of the haplotype-shared area (Brouillard et al. 2000), and, by a BLAST search with the sequences from the *SP6* end of PAC 775d17, we identified some ESTs and the cDNA for *FAP48*. *FAP48* had been cloned from a Jurkat-cell library by a yeast–two-hybrid screen, by use of the immunosuppressant drug-binding domain of a rabbit immunophilin, FKBP59, as bait (Chambraud et al. 1996). We amplified and sequenced *FAP48* from lymphoblasts, various tissues, and Jurkat cells. In all the cDNA samples tested, an 85-bp exon (position 1215 downstream of the ATG), which was not present in the published *FAP48* sequence, and a single G at position 1479–1480 were observed (shown in fig. 4). Both of these changes modify the open reading frame, resulting in a larger protein, comprising 594 amino acids instead of the reported 417 amino acids (i.e., 68 kD vs.

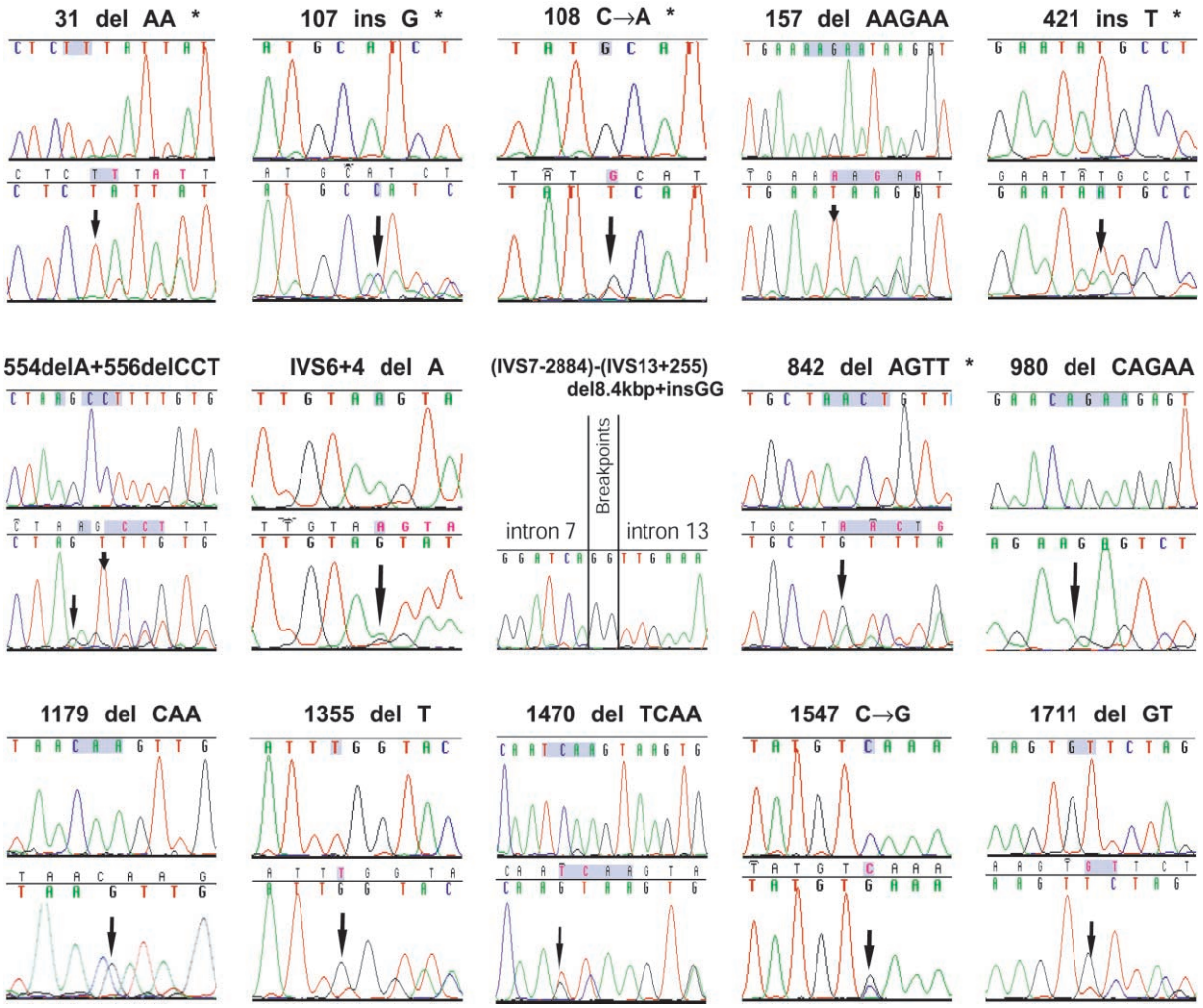

Figure 3 *Glomulin* mutations. Control sequence (*upper chromatograms*) is compared with mutant sequence (*lower chromatograms*). Asterisks (*) indicate chromatograms showing reverse-strand sequence. Sequences presented for large genomic deletions and for the second-hit mutation, 980delCAGAA, were obtained from cloned fragments. Arrows indicate site(s) of mutation.

48 kD). Since the new, larger protein is significantly different from FAP48 i.e., it is 32% longer, and the 12 most–carboxy-terminal amino acids of FAP48 are discordant, and since we here show that mutations in the sequence encoding the 68-kD protein are responsible for GMVs, we propose to name this novel factor "*glomulin*" (GenBank accession number AJ302735).

Structure and Expression Profile of Glomulin

Prior to screening for mutations, we established the genomic structure of *glomulin* (fig. 4). The gene is composed of 19 exons and extends over 55 kb (GenBank accession numbers AJ302727–AJ30273); exon 1 is noncoding, exon 2 contains the translation start site, and exon 19 contains the TGA stop codon. Expression of *glomulin* is ubiquitous, on the basis of northern blot hybridizations (fig. 5). One major transcript of ~2 kb, corresponding to the size (1,785 nt) of *glomulin* coding sequence, was observed in all 12 human tissues on the MTN filter (fig. 5). An additional band, of ~3 kb, was present in most of the tissues. Since we found a single 5′-RACE product, which contained an in-frame stop codon 81 nt upstream of ATG, the second band is unlikely to result from splice variations in the 5′ region. *Glomulin* was also detected in all 75 tissues present on the MTE filter (data not shown). In addition, with RT-PCR, transcripts were found in all eight GVM lesions tested, as well as in cultured SMCs, normal skin, umbilical cord, and placenta (data not shown).

Inherited Mutations

Screening for mutations in *glomulin* was performed on genomic DNA extracted from blood and/or on

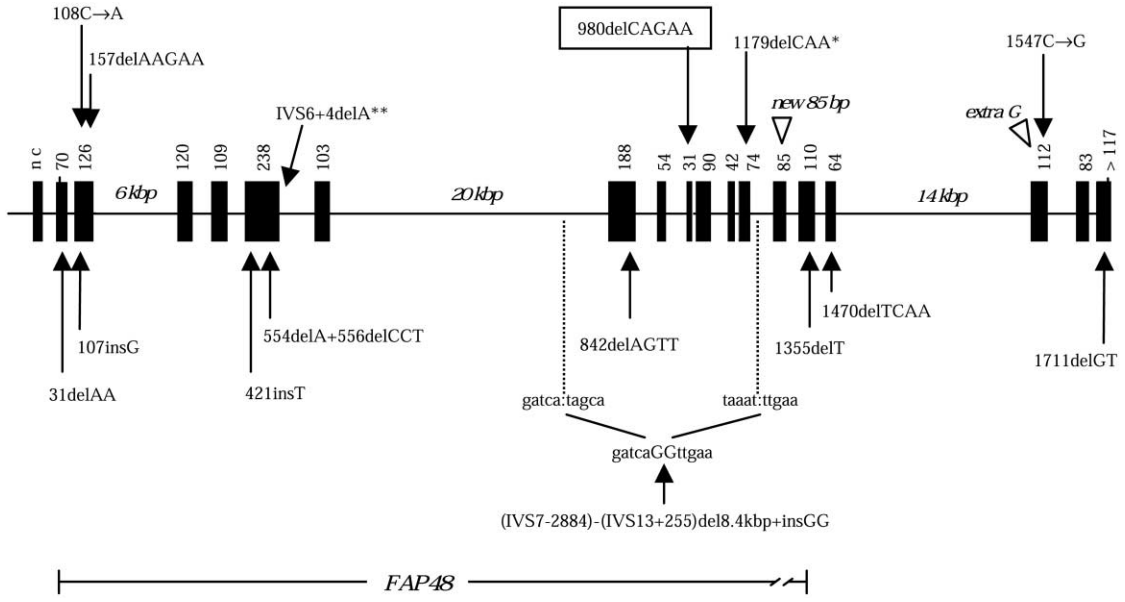

Figure 4 *Glomulin* gene—structure and mutations. The sizes of exons and of the three largest introns are given; other introns are to scale. Exon 1 is noncoding (n c), exon 2 contains the translation start site, and exon 19 contains the TGA stop codon. Above the sequence line, the white arrowheads indicate differences versus *FAP48* cDNA (i.e., a new, 85-bp exon and an extra G), the single asterisk (*) indicates a single amino acid deletion, the double asterisks (**) indicate a splice-site mutation, and the box indicates the second-hit mutation, 980delCAGAA; the other three mutations cause immediate stop codons. Below the sequence line, frameshift mutations leading to premature stop codons are indicated, as are sequences of breakpoints of 8.4-kb deletion with GG insertion. The "*FAP48*" line indicates exons encoding *FAP48*.

cDNA produced from RNA extracted from either resected GVMs or cultured lymphoblasts. We identified 14 different germline mutations in patients from 20 unrelated families (figs. 2–4). One mutation, (IVS7−2884)–(IVS13+255)del8.4kb+insGG, is an 8.4-kb genomic deletion removing exons 8–13. Ten of the mutations were deletions or insertions that cause frameshifts resulting in premature stop codons. Of these 10 mutations, 157delAAGAA was present in all seven families (i.e., families Bl, Bln, Bt, F, Sch, Sh, and T; fig. 2) in which we previously had found strong evidence for linkage disequilibrium, proving the ancestral origin of the identified haplotype (Irrthum et al. 2001). Another deletion, IVS6+4delA, affects an adenine at the +4 position of the consensus donor splice-site sequence of intron 6. This should interfere with splicing of exon 6 and probably results in skipping of the 238-bp exon, which would modify the reading frame and result in a premature stop codon. However, no RNA was available from this family to confirm this. Two nonsense mutations were also detected: 108C→A in a TGC codon and 1547C→G in a TCA codon. The only mutation that would not cause a premature stop codon was a deletion of 3 nt (1179delCAA), equivalent to the removal of an asparagine at position 394.

No mutation was found in family Al, which we had reported elsewhere (Irrthum et al. 2001). This result is in accordance with the histological analysis of a recently resected lesion in which no glomus cells were identified: since the mutations in families Ad, Ba, and Del (fig. 2), all three of which share with family Al a putative haplotype B in the *VMGLOM* locus, are different, this finding confirms the hypothesis that a similar haplotype is shared by chance in these four families (Irrthum et al. 2001). Overall, we have identified a mutation for all five pedigrees (i.e., Bl, Bt, F, Sh, and T) reported by Boon et al. (1999), for all six families (Ad, Ba, Bln, Del, Lml, and Sch) with GVM (of the seven families studied) reported by Irrthum et al. (2001), and for all seven of the pedigrees newly reported here (fig. 2).

Penetrance and Somatic Second Hit

Cosegregation experiments in all pedigrees allowed the detection of a total of 18 unaffected carriers and 2 phenocopies (i.e., affected individuals without the inherited mutation) (fig. 2). Patient Wi-11 is considered to be "affected" on the basis of anamnestic information only, since no clinical examination was performed, and patient Bln-1040 had only a single small (i.e., <5-mm) lesion in the pubic area. Among the carriers, a bluish cutaneous lesion in patient Du-21 was resected when the

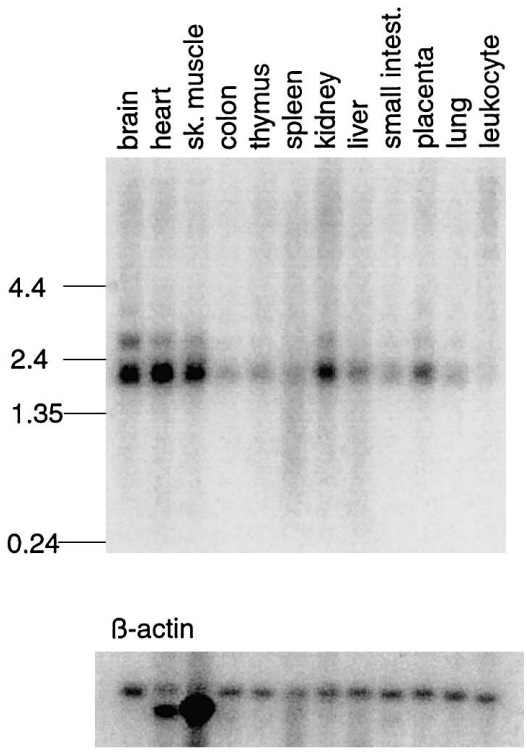

Figure 5 *Glomulin* northern blot analysis—human MTN blot (Clontech) hybridized with a 482-bp 5′ probe of *glomulin.* Each lane contains 1 μg polyA+ RNA from adult tissue. Similar results were obtained with a full-length *glomulin* probe. *Lower gel,* β-actin control.

individual was young, but no histological results were available; thus, she could be affected. Moreover, patients Ly-10 and Ly-12 had extensive varicose veins that could hide a GVM. Unaffected carriers Lml-223, Blo-4, and Ft-2 were not examined. Overall, the observed penetrance of the mutations was 88.6% and rose to 92.7% at age 20 years.

Interestingly, in addition to the inherited large genomic deletion found in family Ad (figs. 2–4), a 5-bp deletion in the unlinked allele was identified in DNA from a lesion in patient Ad-11 (figs. 1*A* and *D,* 2–4). This change was present only in the DNA and RNA extracted from the resected tissue, not in cells from blood, and, moreover, none of the other members of family Ad had this 5-bp deletion; thus, this mutation must represent a somatic "second hit."

In Silico *Analysis of Glomulin*

Database searches with the glomulin-coding sequence did not identify either a signal sequence or any other functional or conserved domain. Partial sequence identity with exons 6–11 was found in DNA from human chromosome 21, but no corresponding ESTs could be retrieved. Thus, this partial paralogue is likely a pseudogene. In human, mouse, and rat, only ESTs with a high degree of similarity to *glomulin* were found. We retrieved putative genes with significant homologies in *Drosophila melanogaster, Danio rerio, Xenopus laevis, Tetraodon nigroviridis,* and *Arabidopsis thaliana,* but no significant alignments were found in the complete genomic sequences of lower organisms, such as *Caenorhabditis elegans, Saccharomyces cerevisiae,* or *Escherichia coli.* In the putative promoter of the human *glomulin* gene (deduced from sequences in the public databases), we identified a CArG-like element (Shimizu et al. 1995), often present in promoters of genes expressed in SMCs.

Discussion

The data that have been presented clearly demonstrate that mutations in *glomulin* are the cause of GVMs. Since the first truncating mutation occurs as early as at the first 31 bp of coding sequence, the disease is very likely due to loss of glomulin function; however, the expressivity of the phenotype is variable for the same mutation, and not all individuals harboring a mutation are affected (fig. 2). Thus, haploinsufficiency is not sufficient to explain the ethiopathogenesis of GVMs. In fact, we have shown that a mutation on the second allele is needed, suggesting that GVM lesions are caused by complete, *localized* loss of glomulin function. The defined nature and the variability, in both number and size, of the lesions (authors' unpublished data) could be explained by the frequency and the time of appearance of somatic mutations; however, additional tissue samples must be collected in order to prove this promising hypothesis. Indeed, cellular heterogeneity of GVMs may be important for the detection of somatic mutation. The lesion in family Ad contained a high number of glomus cells (fig. 1*D*), which may explain the fact that, to date, a somatic mutation has been identified only in that tissue. It also remains to be revealed why, despite the ubiquitous tissue distribution of *glomulin,* only cutaneous lesions are seen in patients with GVMs.

Since only individuals who reported having cutaneous lesions were examined, it could be that many of the unaffected carriers have a small, asymptomatic lesion. However, since it is known that lesions may not be obvious at birth and may appear later in life (Enjolras and Mulliken 1996) and that the condition may express as only a single tiny blue lesion anywhere on the body (Boon et al. 1999), this could explain the unaffected status of the younger carriers, such as Sch-1020 (examined at age 1 year), Bln-1070 (examined at age 2 years), Blo-52 (examined at age 12 years), Blo-51 (examined at age 14 years), Lml-223 (examined at age 14 years), and Chn-201 (examined at age 18 years).

To date, little is known about glomulin. Since no

paralogues were identified on the basis of database searches, *glomulin* seems to be unique and not a member of a family of homologous genes. Glomulin seems to be present only in higher organisms, such as vertebrates and the fly. One possible partial homologue in humans is FAP48; however, in the tested cDNAs, we have not found any transcript specific for this protein, nor have we found any FAP48-specific EST in the databases. Since mutations in the *glomulin* gene result in GVMs regardless of whether they would affect the coding sequence of FAP48, the development of GVMs is independent of FAP48.

The primary cellular defect in GVMs is the presence of the SMC-like glomus cells. As mesenchymal cells differentiate and acquire an elongated cell shape, they express markers such as SMC α-actin, desmin, calponin, SM22, and SM myosin (Yang et al. 1999). Glomus cells express many SMC markers, and, as shown by electron microscopy, they display a cellular structure reminiscent of that of SMCs (Goodman and Abele 1971; Miettinen et al. 1983). However, glomus cells are round or polygonal (Goodman and Abele 1971; Kato et al. 1990). In addition, whereas SMCs of normal veins express desmin (Nanaev et al. 1991), glomus cells do not (Miettinen et al. 1983). This partial expression of VSMC markers, associated with an abnormal morphology and mural organization, suggests that the important role of glomulin is played at a late stage in VSMC maturation.

Although the cell type(s) expressing glomulin remain(s) to be defined, loss of function seems to be the cause of the specific VSMC phenotype. This effect can be direct, since we found that VSMCs express *glomulin*. Furthermore, the *glomulin* gene contains a CArG-like element in its promoter (i.e., one A/T is replaced by a G or a C). The same motif is present in SMC-expressed genes, such as α-actin and myosin heavy chains (Hill and Treisman 1995; Miano et al. 2000). Thus, glomulin could have a cell-type–specific expression pattern that would explain why mutations in glomulin affect only VSMCs.

If glomulin has interactions similar to those shown for FAP48 (a truncated form of glomulin), it is interesting to note that, in vitro and in vivo, in a yeast-two-hybrid system, FAP48 interacts with FKBP59 and FKBP12 (Chambraud et al. 1996). FKBP12 is known to bind to—and, thereby, inhibit signaling through—the TGFβ type I receptor (TβRI) (Chen et al. 1997), and TGFβ signaling is important for both SMC maturation and expression of SMC cytoskeletal markers. Indeed, TβRI-deficient murine embryos lack SMCs around endothelial-lined yolk-sac vessels (Larsson et al. 2001), and, when endothelial cells and SMCs are cocultured with an antibody to TGFβ, SMC differentiation does not occur (Hirschi et al. 1998). Glomulin, like FAP48, may function to repress this inhibition. Accordingly, complete loss of glomulin function would result in increased binding of FKBP12 to TGFβ receptors, inhibition of TGFβ signaling, altered expression of SMC proteins such as desmin, and altered differentiation of VSMCs.

In summary, the data that have been presented here demonstrate that a novel gene, *glomulin,* plays a role in normal VSMC differentiation, since mutations in this gene are responsible for the presence of SMC-like glomus cells in inherited GVMs ("glomangiomas"). Developmental studies of normal and glomulin-deficient mice, as well as functional studies of resected GVM tissues and of in vitro expression systems are needed to get further insights into the role and function of glomulin in both vascular morphogenesis and vascular pathologies.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

BLAST, http://www.ncbi.nlm.nih.gov/BLAST/(for blastn, blastx, and tblastn searches)

GenBank Overview, http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html (for *FAP48* [accession number U73704], *glomulin* mRNA [accession number AJ302735], and glomulin genomic sequences [accession numbers AJ302727–AJ302734])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/OMIM/ (for VMCM [MIM 600195] and GVM [MIM 138000])

PredictProtein, http://www.embl-heidelberg.de/predictprotein/

SMART, http://smart.embl-heidelberg.de/

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