ONLINE MUTATION REPORT

Four common glomulin mutations cause two thirds of glomuvenous malformations (''familial glomangiomas''): evidence for a founder effect

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Background: Glomuvenous malformation (GVM) (''familial glomangioma'') is a localised cutaneous vascular lesion histologically characterised by abnormal smooth muscle-like ''glomus cells'' in the walls of distended endothelium lined channels. Inheritable GVM has been linked to chromosome *1p21-22* and is caused by truncating mutations in *glomulin*. A double hit mutation was identified in one lesion. This finding suggests that GVM results from complete localised loss of function and explains the paradominant mode of inheritance.

Objective: To report on the identification of a mutation in *glomulin* in 23 additional families with GVM.

Results: Three mutations are new; the others have been described previously. Among the 17 different inherited mutations in *glomulin* known up to now in 43 families, the *157delAAGAA* mutation is the most common and was present in 21 families (48.8%). Mutation *108C→A* was found in five families (11.8%), and the mutations *554delA+556delCCT* and *1179delCAA* were present together in two families (4.7% each). Polymorphic markers suggested a founder effect for all four mutations.

Conclusions: Screening for these mutations should lead to a genetic diagnosis in about 70% of patients with inherited GVM. So far, a mutation in *glomulin* has been found in all GVM families tested, thus demonstrating locus homogeneity.

In the past, glomuvenous malformations (GVM, MIM 138000) were improperly called ''glomangiomas'' or ''glomus tumours.'' GVM are vascular malformations and should be distinguished from paragangliomas (MIM 115310, 168000, and 605373) and from the (subungual) solitary glomus tumours.[1] Usually, GVM can be differentiated clinically from sporadic common venous malformations or inheritable cutaneomucosal venous malformation (VMCM, MIM 600195). GVM are often present at birth and slowly expand during childhood (fig 1). They are nodular and multifocal, rather than localised, frequently hyperkeratotic with a cobblestone-like appearance, and their colour varies from pink to purplish dark blue, as compared with the bluish hue of the typical venous malformations.[2] In addition, GVM are mainly located on the extremities, involve skin and subcutis, cannot be completely emptied by compression, and are often painful on palpation. In contrast, venous malformations commonly affect muscle and joints, can easily be emptied by compression, and are not usually painful on palpation.[2] Proper diagnosis is important as venous malformations are symptomatically improved by elastic stockings, whereas compression causes GVM to be painful. Resection and sclerotherapy are alternatives for both GVM and venous malformations.[2]

GVM is histologically characterised by distended vascular channels surrounded by variable numbers of mural ''glomus cells''.[3–5] Glomus cells stain positively for smooth muscle α-actin and vimentin, whereas they are negative for desmin, von Willebrand factor, and S-100 neuronal marker.[6] In addition, glomus cells have electronmicroscopic characteristics of smooth muscle[7] and thus are considered to be abnormally differentiated vascular smooth muscle cells.[6]

We previously reported that families with autosomal dominant inheritance of GVM link to chromosome *1p21-22*,[3] and we identified linkage disequilibrium for seven families in a region of 1.48 Mbp in the *VMGLOM* locus.[8] By positional cloning using YAC and PAC based physical maps,[9] we identified a novel gene, *glomulin (glmn)*, with unknown function, mutated in GVM.[5] Altogether, 14 different germline mutations were documented in affected individuals in 20 distinct families with high, but not complete, penetrance. Most mutations were thought to result in haploinsufficiency. Interestingly, we identified a somatic ''second hit'' mutation, also predicted to result in a truncated protein, in one patient, suggesting that the lesions arise because of complete localised loss of function of glomulin.[5] Thus GVM are inherited in a paradominant mode.

Our aim in this study was to identify additional mutations in *glomulin* in order to evaluate locus homogeneity and a possible founder effect. In addition, we hoped to uncover mutations that might increase our understanding of the function of glomulin. In all, we found seven different mutations in the 23 new families tested. Three mutations were novel and the four others, which had been documented previously, were found in 20 new families. Haplotypic analysis of the families with these common mutations revealed a strong founder effect. Finally, as all families with clinically unequivocal GVM proved to have a germline mutation, altered function of glomulin seems to be the only primary cause of the vascular lesions.

METHODS

Families and patients

Blood samples were collected from patients of 23 novel families with transmitted GVM (fig 2). All affected individuals had lesions resembling those described previously.[2 3 5] Informed consent was obtained for each participant, as

Abbreviations: GVM, glomuvenous malformation

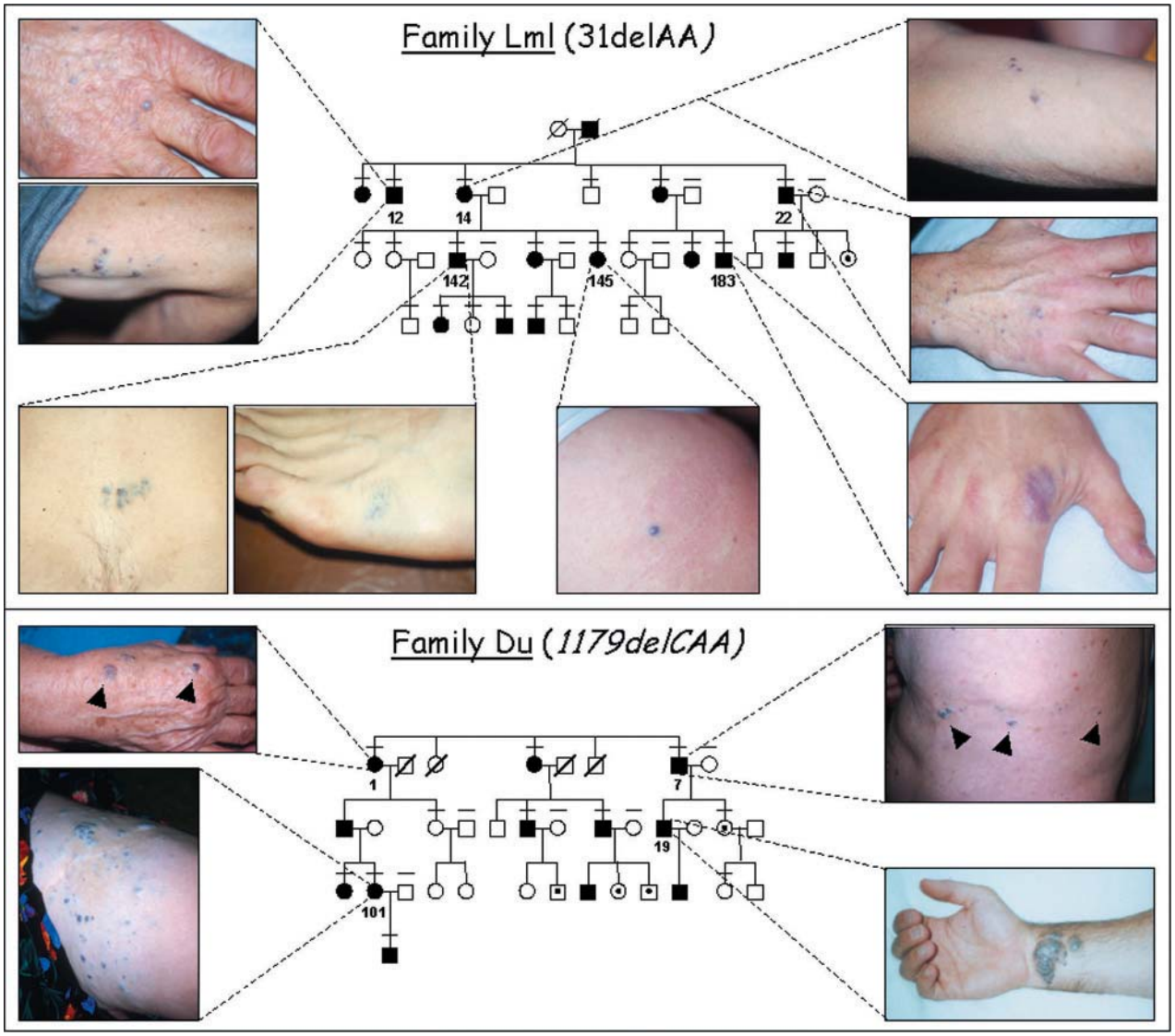

Figure 1 Phenotypic variation in glomuvenous malformations. Most lesions are small and localised. Individual Du-19 has a large raised lesion on wrist. Lml-183 has a plaque-like purple lesion. Du-101 has a broad cobblestone-like lesion. Small horizontal bars indicate individuals examined clinically. Reproduced with permission.

approved by the ethics committee of the medical faculty of the Université catholique de Louvain, Brussels, Belgium.

Screening on genomic DNA

Screening for mutations was done on genomic DNA from one or two patients per family. Venous blood samples were drawn from all participants and DNA was extracted from the buffy coats (QIAamp DNA blood mini kit; Qiagen Inc, Valencia, California, USA) or from whole blood (DNA purification kit; Gentra Systems Inc, Minneapolis, Minnesota, USA). Mutational screening was by SSCP, heteroduplex, and size difference analyses, as described.[5] Fragments showing abnormal migration were reamplified and sequenced on a CEQ2000 capillary sequencer (Beckman Coulter).

Segregation of mutations

Segregation of the three novel mutations (*738insT*, *1150delAG* and *1293delA+1296delAAA*) was assessed by sequencing. Mutations *108C→A*, *157delAAGAA* and *1179delCAA* were tested by allele-specific PCR (table 1) and mutation *554delA+556delCCT*, which creates a *Spe*I restriction enzyme cutting site, was checked by restriction enzymatic digestion (table 1).

Haplotypic analysis

Haplotypes were determined by genotyping several affected individuals in each family in order to identify the alleles segregating with the mutation, as previously described.[8] We used all the short tandem repeat markers we had mapped to the *VMGLOM* locus.[9]

Multiple alignment of glomulin proteins

Human and murine glomulin amino acid sequences were retrieved from GenBank database (accession numbers Q92990 and CAD92739, respectively). Protein sequences for chimpanzee, dog, rat, xenopus, and zebrafish were deduced from sequences extracted in silico from EST and genomic sequences, using ''Blast'' at NCBI (http://www.ncbi.nlm.nih.gov/) and ''Blat'' at UCSC (http://genome.ucsc.edu/cgi-bin/hgGateway). These fragmented nucleotidic sequences were first assembled using Sequencher software 4.1.2 (http://www.genecodes.com/) in order to maximise coverage of the

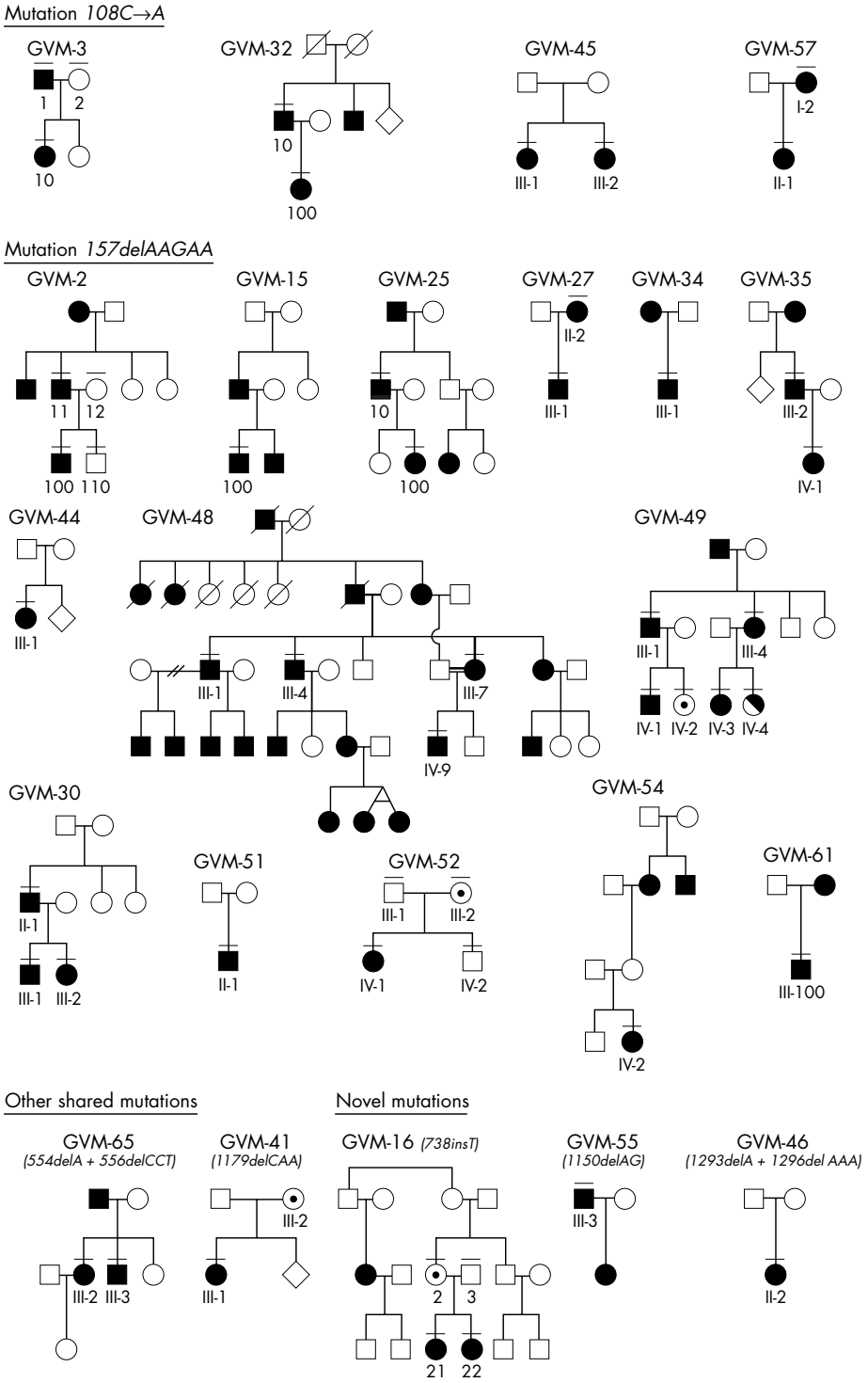

Figure 2 Twenty three new families with inherited glomuvenous malformations (GVM) presented in order of occurrence of mutations in *glomulin*; shared mutations first. Individuals indicated by numbers were tested. Black symbols, affected individuals; dotted symbols, carriers; half black symbols, phenocopies; small horizontal bars indicate individuals examined clinically.

open reading frame. The consensus sequences were then translated into amino acid sequences using DNA Strider 1.0 (http://www.cellbiol.com/DNAStrider1_1_sit.bin). The proteins were aligned using ClustalW (http://www.ebi.ac.uk/clustalw/) and identical or similar residues were highlighted using Boxshade (http://bioweb.pasteur.fr/seqanal/interfaces/boxshade.html).

RESULTS

Identified mutations

Screening for *glomulin* mutations on genomic DNA identified the previously described 5 bp deletion *157delAAGAA* in 14 new GVM families (figs 2 and 3). Co-segregation analysis showed the deletion in all affected individuals whose DNA was available for testing. Altogether two unaffected carriers

Table 1 Primers and conditions for detection of the common *glomulin* mutations by allele specific polymerase chain reaction or restriction enzyme digestion

	108C→A	*157delAAGAA*	*554delA+556delCCT*	*1179delCAA*
Forward primer	GATACGTGTGTTATTTACGTTAC	GATACGTGTGTTATTTACGTTAC	TTGATGAGCGACAACTTGATC	GTGATGAAGTCTGGGTAAGC
Reverse primer	ATGTGATTATTCTCTTCCCAAG	ATGTGATTATTCTCTTCCCAAG*	TAAGTCCACTGTGAGATGTTC	AACAATTACATGGCATTAACATG
Wt product size	324 bp	324 bp	304 bp	207 bp
Mutation specific (=3rd) primer	F: AGTTAGCTGGGCAAAGATGA	R: GTACGCACCTTA|TTCATTTTG	–	R: TTGCCTTGTGAATCCAAC|TTAA
Restriction enzyme	–	–	*Spe* I	–
Mutant product(s)	130 bp	219 bp	111 and 193 bp	117 bp
Annealing temp	55°C	55°C	62°C	58°C

*Only 50% of this primer should be used in the allele specific polymerase chain reaction.
| = site of deletion; underlined nucleotide = substitution.
bp, base pair; temp, temperature.

(individuals with a mutation but no lesion) and one presumed phenocopy, with a single lesion of 2 cm in diameter on the foot, were detected in families GVM-49 and GVM-52 (fig 2). In four other families, we found the previously reported nonsense mutation *108C→A* (figs 2 and 3). Co-segregation analysis showed this mutation in all affected individuals (fig 2). Two other known mutations were identified: *554delA+556delCCT* segregating with the disease in family GVM-65 and *1179delCAA* in family GVM-41 (figs 2 and 3). In the latter, one obligate carrier was detected (fig 2).

In addition, three novel mutations were discovered, each in one family (fig 2 bottom and fig 3): *738insT*, an insertion of a thymidine in exon 8, was detected in family GVM-16 with one unaffected carrier (fig 2); *1150delAG* was found in one member of family GVM-55; and one member of family GVM-46 harboured the mutation *1293delA+1296delAAA* (fig 2). All three mutations cause reading frameshifts that predicted a premature STOP codon in the sequence.

To date, we have identified *glomulin* mutations in all GVM families tested. *157delAAGAA* represents 48.8% of all the inherited mutations; *108C→A* represents 11.8%, and mutations *554delA+556delCCT* and *1179delCAA* represent 4.7% each (fig 4). Thus these four mutations account for 70% of all GVM families; the remaining 30% being represented by a mutation unique to each family.

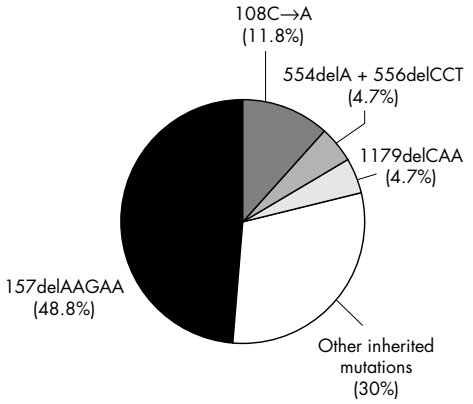

Figure 4 Diagram showing frequency of inherited *glomulin* mutations. "Other inherited mutations" comprises 13 mutations identified in a single family each.

Haplotype analysis

Haplotype analysis for mutation *157delAAGAA* showed that all 21 families with this alteration share a common haplotype for various markers in the *VMGLOM* locus (fig 5). Based on the alleles, families can be grouped by genetic proximity with regard to the *VMGLOM* locus—for example, families GVM-25, GVM-27 and F, or GVM-2 and GVM-48. The smallest shared area for *157delAAGAA* was between polymorphic markers *33CA1* and *75CA1*, an area of 825 kbp. The distance of marker *33CA1* from the mutation is about 80 kbp. Similar haplotype sharing was observed for the five families with *108C→A* mutation, in a region shared between markers *D1S188* and *D1S236* (fig 5). Families with mutations *554delA+556delCCT* and *1179delCAA* also showed clear evidence of haplotype sharing (fig 5).

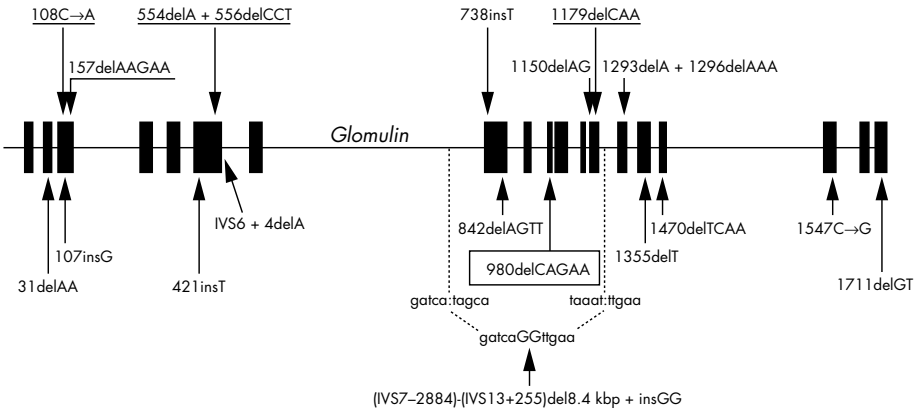

Figure 3 Schematic representation of *glomulin* summarising all known mutations (adapted from Brouillard *et al*[5]). Mutations reported here are marked above the gene. Mutations found in more than one family are underlined. Somatic second hit is boxed.

DISCUSSION

Four previously known mutations were identified in 20 new families. We also found three novel mutations that would be predicted to cause reading frameshifts and premature truncations.

Founder effect for glomulin mutations

Mutation *157delAAGAA*, previously documented in seven families,[5] was detected in 14 additional kindreds. The prevalence of this mutation among patients with GVM is thus 48.8% (fig 4). As in the seven previously reported families,[8] strong haplotype sharing was observed among all 21 white families with this mutation, independent of their country of origin (fig 5). Thus this mutation is a common ancestral alteration. Mutation *108C→A*, found in family Ba,[5] was detected in four additional families that all share a common haplotype (fig 5). The prevalence was 11.8% in the GVM families (fig 4). Mutations *554delA+556delCCT* and *1179delCAA* were also detected in one new family, giving a prevalence of 4.7% for each (fig 4). Again, haplotype sharing was detected for both mutations (fig 5). Altogether, these four mutations in *glomulin* were identified in 70% of the GVM families (fig 4). Although the alterations in the gene cause abnormal cutaneous vascular development, they do not appear to affect reproduction—that is, there is normal endometrial and placental angiogenesis. The high prevalence of these common mutations makes cost-effective genetic testing possible in 70% of patients (table 1).

1179delCAA is an atypical glomulin mutation

The *1179delCAA* mutation, which corresponds to removal of one asparagine at position 394, was identified for the second time. It contrasts with all other mutations that result in premature truncation of glomulin. It could be that the *1179delCAA* allele is unstable or destroyed either at the RNA or at the protein level. Alternatively, the mutation could produce a cryptic splice site and result in premature truncation, as in the case of the ''missense'' mutations in *KRIT-1*.[10 11] However, RT-PCR (reverse transcriptase polymerase chain reaction) on RNA extracted from EBV transformed lymphoblasts (illegitimate transcripts) did not reveal aberrant splicing, and the wild type and mutant alleles were present in equivalent amounts (data not shown). Although the asparagine is not conserved between human and other species (in which it is often an aspartate), most of *glomulin* is highly conserved and a deletion at this position is not present in known *glomulin* sequences (fig 6). As individuals with the asparagine 394 deletion have cutaneous lesions indistinguishable from those caused by truncation at whatever site in the protein, it appears that the residue 394 is important for the structure or function of glomulin.

Locus homogeneity for GVM

The findings in this report underscore our earlier report that premature truncation is most likely to cause loss of function of glomulin. This is the predominant cause of inheritable GVM. Interestingly, mutations were found in members of all families affected by GVM. The four other GVM families that have been studied genetically and reported to date were also shown to link to the *VMGLOM* locus.[12] Thus the likelihood of locus heterogeneity for GVM is very small, yet rare variants cannot be excluded.

Double hit explains phenotypic variation

After the identification of *glomulin* mutations as the cause of GVM, the questions remained as to why these anomalies are

Mutation		157delAAGAA																			
Family	Position	25	27	F	35	61	Sch	Sh	34	44	54	Bln	Bt	49	52	51	2	48	50	Bl	15	T
Origin	(Mbp)	Aus	USA	Sco	UK	Spa	Ger	USA	Fra	USA	Fra	Fra	Be	Fra	UK	USA	Be	Fra	Fra	USA	Aus	Ita
A205XD5	89.640	241/**245**	241/**245**	**245**/R	241	241/245	245	245	241/245	**241**/245	**241**	**241**	239	241	241	241/245	239	241	241	241	**241**/245	**241**
D1S435	91.026	**175**	**175**	**175**	159	159/161	157	161	161/177	**157**/177	**157**	**157**	**157**	177	161	161/175	**161**	**161**/R	175/R	175	157/**175**	**175**
B337XE1	91.435	**281**	**281**	**281**	**281**	259/263	267	267	263/267	**263**	**263**	**263**	**263**	**263**/267	**263**	259/281	**263**	**263**	259	263	**259**/267	**259**
D1S188	91.968	**166**	**166**	**166**	152/**166**	154/**166**	**166**	**166**	**166**	**166**/168	152/**166**	**168**	**168**	**168**	**168**	156/162	**152**	**152**	152	164	**166**/168	**166**
D1S1170	92.014	**118**	**118**	**118**	**118**	**118**	**118**	**118**	**118**/126	**118**/122	114/**118**	**118**	**118**	**118**	**118**	**118**/122	**118**	**118**/122	**118**	**118**	**122**	**122**
33CA1	92.150	**156**	**156**	**156**	**156**	**156**	**156**	**156**	142/**156**	**156**	152/**156**	**156**	**156**	**156**	**156**	136/**156**	**156**	**156**	142/**156**	**156**	142/**152**	**152**
Glomulin	92.183-92.236																					
D1S424	92.352	**223**	**223**	**223**	**223**	**223**/225	**223**	**223**	225/227	209/**223**	209/**223**	225	225	225	**223**	**223**	**223**	**223**	**223**	**223**	**223**/227	**223**
D1S2804	92.353	**179**	**179**	**179**	**179**	**179**	**179**	**179**	**179**/191	**179**/185	**179**/185	**179**	**179**	**179**	**179**	**179**	**179**	**179**	**179**	**179**	-	**179**
D1S406	92.499	**200**	**200**	204	**200**	**200**/212	**200**	**200**	**200**/208	**200**/208	196/**200**	196	**200**	**200**	204	**200**/208	**200**	**200**/204	**200**	**200**	204/208	**200**
69CA1	92.544	**171**	**171**	**171**	**171**	**171**	**171**	**171**	169/**171**	169/**171**	169/**171**	**171**	169/**171**	169/**171**	**171**/175	**171**	**171**	**171**	**171**	**171**	169/**171**	**171**
D1S2776	92.682	**206**	**206**	**206**	**206**	**206**/210	**206**	**206**	194/**206**	196/**206**	194/**206**	**206**	**206**	**206**	**206**	**206**/210	**206**	**206**	**206**	**206**	194/**206**	**206**
50CA1	92.735	**127**	**127**	**127**	**127**	121/**127**	**127**	129	125/**127**	125/**127**	**127**	**127**	125/**127**	125/**127**	**127**	119/**127**	**127**	**127**	**127**	**127**	121/**127**	**127**
D1S2868	92.807	**146**	**146**	**146**	**146**	**146**/150	**146**	**146**	144/**146**	**146**/148	144/**146**	**146**	**146**	**146**	**146**	**146**/150	**146**	**146**	**146**	**146**	**146**/148	**146**
75CA1	92.975	171/**173**	171/**173**	**173**	171/**173**	171/**173**	**173**	**173**	**173**	169/**173**	171/**173**	**173**	171/**173**	**173**	**173**	**173**/176	**173**	**173**	**173**	171/**173**	-	171
D1S2849b	92.991	**179**	**179**	**179**	**179**	175/**179**	**179**	**179**	177/**179**	**179**	**179**/181	**179**	**179**	**179**	**179**	177/**179**	**179**	**179**	**179**	**179**	**179**/181	179
D1S2779	93.172	**231**	227/**231**	**231**	**231**	229/**231**	**231**	233	**231**/243	229/**231**	**231**	**231**	**231**	**231**	**231**	**231**/237	**231**	**231**	229	229	**231**/241	241
D1S236	94.282	**190**	**190**	**190**	214	**190**/214	212	194	212	194/218	194/212	**190**	210	212	194	**190**	212	214	214	194	194	212
D1S2775	94.717	**201**	**201**/205	**201**	201/205	199/203	201	201	201/205	201	197/199	199/**201**	201/205	203	197/199	197/199	199/201	199/201	199/201	199/201	-	201

Mutation		108C>A				
Family	Position	45	57	3	Ba	32
Origin	(Mbp)	USA	Aus	USA	Yug	Aus
A205XD5	89.640	**241**	**241**	**241**	241	239
D1S435	91.026	**157**	**157**/161	**157**	**157**	**157**
B337XE1	91.435	263/**267**	**267**	**267**	**259**/267	**259**
D1S188	91.968	**158**	154	**158**	**156**/162	152/**156**
D1S1170	92.014	**118**	**118**	**118**	**118**/122	**118**
33CA1	92.150	**156**	**156**	**156**	**156**	**156**
Glomulin	92.183-92.236					
D1S424	92.352	**209**	**209**	**209**	**209**	**209**/223
D1S2804	92.353	**185**	**185**	**185**	**185**	**185**
D1S406	92.499	**208**	**208**	**208**	204/**208**	**208**
69CA1	92.544	**169**	**169**	**169**	**169**	**169**
D1S2776	92.682	**196**	194/**196**	**196**	**196***	**196**/210
50CA1	92.735	**125**	**125**	**125**	**125**	**125**
D1S2868	92.807	**148**	**148**	**148**	**148**	**148**
75CA1	92.975	**171**	**171**	**171**	**171**	**171**
D1S2849b	92.991	**179**	**179**	**179**	**179**	**179**
D1S2779	93.172	**233**	**233**	**233**	**233**	**233**
D1S236	94.282	**190**	**190**	216	**194**/212	**194**
D1S2775	94.717	**199**	**199**	201	197/201	199

Mutation	554delA+ 556delCCT	
Family	BG	65
Origin	USA	USA
A205XD5	241	245
D1S435	157/177	159
B337XE1	**263**/267	**263**
D1S188	156/**166**	**166**
D1S1170	**122**	**122**
33CA1	142/**156**	**156**
Glomulin		
D1S424	**223**/227	**223**
D1S2804	**179**/191	**179**
D1S406	**208**/212	**208**
69CA1	**169/171**	**169/171**
D1S2776	194/**206**	196/**206**
50CA1	**127**	**127**
D1S2868	**146/148**	**146/148**
75CA1	171/**173**	**173**
D1S2849b	**177**/181	**177**
D1S2779	**231**	**231**
D1S236	214	190
D1S2775	201	197

Mutation	1179delCAA	
Family	41	Du
Origin	Arg	USA
A205XD5	**241**	**241**
D1S435	**157**	**157**
B337XE1	**259**	**259**
D1S188	**166**	**166**
D1S1170	118/**122**	**122**
33CA1	**142**/146	**142**
Glomulin		
D1S424	**225**	**225**
D1S2804	185/**195**	**195**
D1S406	200/204	208
69CA1	169	171
D1S2776	194	206
50CA1	129/133	121
D1S2868	150	144
75CA1	176	171
D1S2849b	179	181
D1S2779	235	231
D1S236	190/192	218
D1S2775	197/199	197

Figure 5 Haplotypes showing strong founder effect for the four common *glomulin* mutations. Numbers indicate size of the allele(s) segregating with the disorder. Alleles shared between families are indicated by grey shading. Families were grouped by maximising proximity. Location of *glomulin* is marked in black. Physical position of markers is given according to the July 2003 genome assembly of UCSC. Note that order of markers *D1S424* and *D1S2804* was inverted compared with previous reports. The origin of the families is shown at the top: Arg, Argentina; Aus, Australia; Be, Belgium; Fra, France; Ger, Germany; Ita, Italy; Sco, Scotland; Spa, Spain; UK, United Kingdom; USA, United States of America; Yug, Yugoslavia. Note that sizes of shared alleles for marker *D1S188* are 2 bp larger than previously reported for families Ba and Bl.[8]

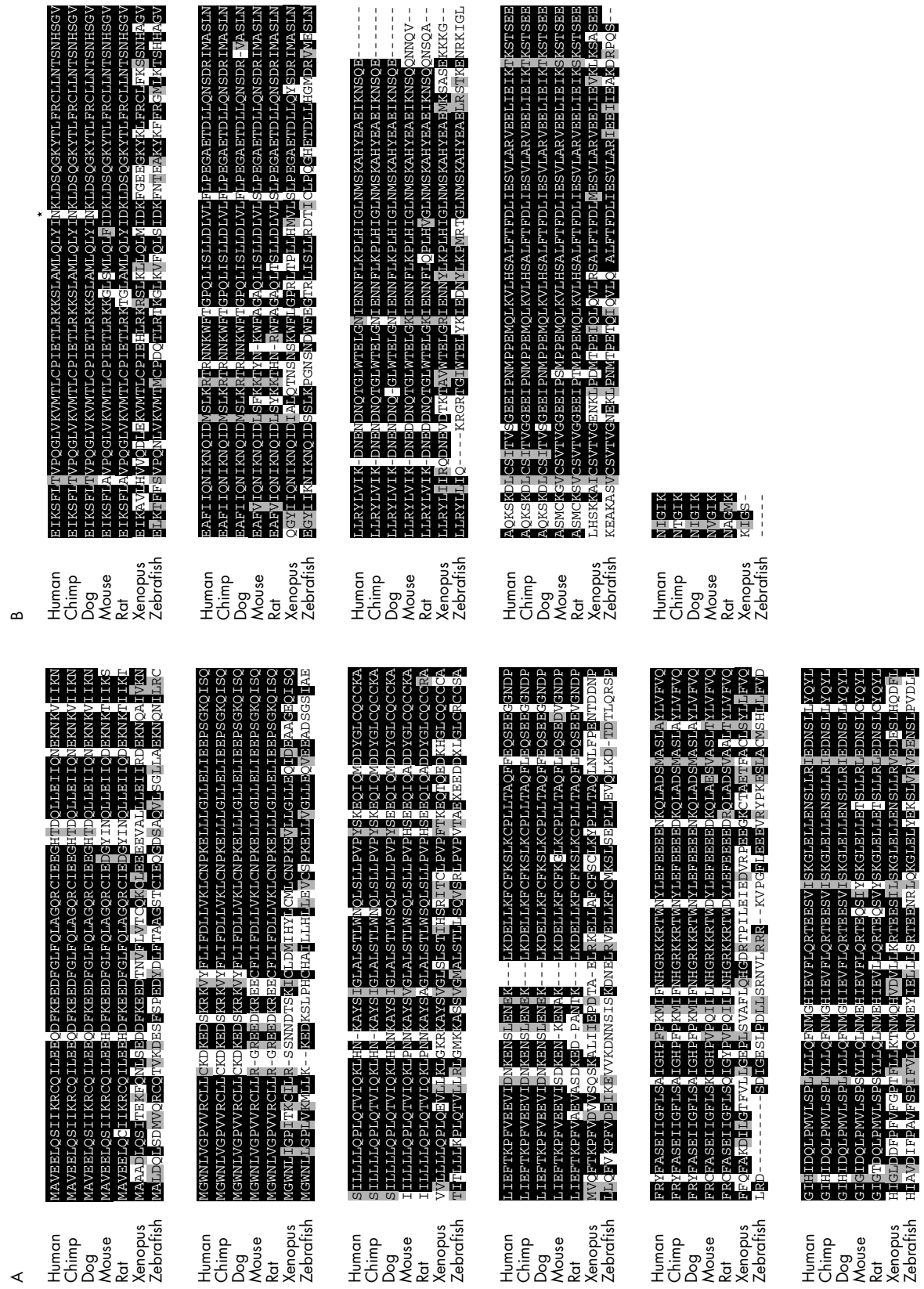

Figure 6 Multiple alignment of glomulin proteins of different species (known or deduced from fragments of nucleotidic sequences found in databases) showing high degree of homology. Identical residues are boxed in black and similar ones in grey. The asterisk indicates the asparagine (N) 394 lost because of mutation 1179delCAA.

multifocal instead of arising in all veins, and why only in the skin? According to our data, there is no phenotype–genotype correlation—that is, the position of a mutation in *glomulin* does not explain the clinical findings, such as localisation, extent, or number of lesions. Moreover, for the same germline mutation, the expressivity is variable from family to family and from patient to patient (fig 1). Frequently, a single individual in a family is more severely affected and is brought to medical attention, whereas most of the other affected individuals have small lesions and have never considered treatment.[2] We have proposed that Knudson's double hit model could explain this variation.[5 13] Support for this hypothesis was the discovery of a somatic mutation—a second hit affecting the ''normal'' allele—in one resected GVM lesion, resulting in a complete localised loss of function of glomulin.[5] This paradominant mode of inheritance would explain the presence of the 22 unaffected carriers in our series (fig 2 and Brouillard *et al*[5]), and is underscored by the age dependent variation in penetrance, which reaches its maximum (92.7%) by 20 years.[5] If no second hit occurs in a cell in which the function of glomulin is important (likely to be the vascular smooth muscle cells), no lesion would develop. Thus the localisation, size, and number of lesions are defined by the random occurrence of post-zygotic mutations. This also suggests that somatic mutations early in development would result in larger, segmental lesions, whereas mutations occurring later in development would only cause small punctate lesions. The low frequency of extensive lesions (>5 cm) and the high frequency of localised lesions (<5 cm) observed in GVM patients fit this model.[2] The double hit proposal would also explain the multifocality of the lesions as the result of several independent somatic mutations. This is supported by the fact that 17% of affected individuals develop new lesions in time.[2] However, these all stay small. What is not clear is whether non-hereditary GVM exists and why only cutaneous and subcutaneous veins are affected.

The two hit scenario may also apply to other inheritable vascular anomalies for which loss of function is predicted,[13] such as cutaneous and cerebral capillary-venous malformation (CCM) caused by mutations in the *KRIT1* or *malcavernin* gene,[14–17] capillary malformation-arteriovenous malformation (CM-AVM) caused by mutations in *RASA1*,[18] and even congenital lymphoedema caused by loss of function mutations in the VEGFR3 receptor.[19 20] For CCM, two different somatic mutations have been reported in *KRIT-1* in a tissue sample.[21]

GVM: a primary vascular smooth muscle cell defect?

Mural ''glomus cells'' are pathognomonic for GVM. It seems that the localised complete loss of function of glomulin alters recruitment and differentiation of vascular smooth muscle cells. Our developmental in situ hybridisation studies on murine embryos reinforce this idea.[22] *Glomulin* expression was mainly identified in vascular smooth muscle cells during development.[22] The expression was particularly obvious in large arteries and veins, and yet patients with GVM do not have structural abnormalities of the major blood vessels.

The signalling pathways involving glomulin remain unknown. The best candidates are the transforming growth factor β (TGFβ) pathway, through a possible interaction of glomulin with the immunophilin FKBP12,[23–25] and the hepatocyte growth factor (HGF) pathway, by direct interaction with the HGF receptor c-MET and activation of the p70S6 kinase.[25] In both instances, loss of function of glomulin may result in alteration of the signalling pathway, and could provide targets for therapy. Glomulin was also reported to interact with Cul7, knockout of which presents with cutaneous haemorrhages.[26] Interestingly, for inherited mucocutaneous venous malformations which are caused by activating mutations in the angiopoietin receptor TIE2/TEK, there are diminished numbers of vascular smooth muscle cells.[27] Thus in both disorders venous dilatation may simply be secondary to decreased or altered mural cellular support.

Conclusions

All *glomulin* mutations identified up to now indicate that familial GVM lesions are caused by loss of function in a paradominant fashion. Seventy per cent of families with inherited GVM share one of the four common mutations, whereas the other 30% have unique mutations. Thus screening of these four common mutations should be the first step in molecular diagnosis of a patient thought to have GVM.

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