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SHORT COMMUNICATION

High-Resolution Physical and Transcript Map of the Locus for Venous Malformations with Glomus Cells (*VMGLOM*) on Chromosome 1p21–p22

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Vascular anomalies are congenital lesions that usually occur sporadically, but can be inherited. Previously, we have described that venous malformations, localized bluish-purple skin lesions, are caused by an activating mutation in the TIE2/TEK receptor. Moreover, we mapped another locus to chromosome 1p21–p22, for venous malformations with glomus cells (*VMGLOM*). Here we report a physical map, based on 18 overlapping YAC clones, spanning this 5-Mb *VMGLOM* locus, from marker *GATA63C06* to *D1S2664.* In addition, we report a sequence-ready PAC map of 46 clones covering 1.48 Mb within the YAC contig, a region to which we have restricted *VMGLOM.* We describe 21 new STSs and nine novel CA repeats, seven of which are polymorphic. These data will enable positional cloning of genes for diseases mapped to this locus, including the *VMGLOM* gene, likely a currently unknown regulator of vasculogenesis and/or angiogenesis. © 2000 Academic Press

Venous malformations (VMs) are bluish-purple vascular lesions that can be single or multiple (16). They are most often localized on the skin and mucous membranes. In a family in which these lesions are inherited as an autosomal dominant trait, we identified a locus (*VMCM1*) on chromosome 9p21 that is linked to the phenotype (4). We found that the mutation in this locus is in the gene encoding the endothelial-specific receptor tyrosine kinase TIE2/TEK. The R849W mutation in the intracellular kinase domain leads to hyperactivation of the receptor in a ligand-independent manner (15). Another amino acid substitution, Y897S, identified in a separate family, seems to have a similar effect (5).

Since not all families with inherited venous malformations showed linkage to the *TIE2/TEK* gene, we used random linkage mapping to identify another locus on chromosome 1p21–p22 (3). Because of the presence of undifferentiated smooth muscle cells ("glomus" cells) in histologic slides of these venous malformations, we named this locus *VMGLOM.* The pathognomonic histologic finding suggests that, like *TIE2/TEK*, the *VMGLOM* gene regulates the development of smooth muscle layering in veins and thereby angiogenesis.

To identify the VMGLOM gene, we initiated positional cloning by creating a YAC-based physical map on the basis of information from the Whitehead database (17). We selected 18 overlapping YAC clones that cover the 5-Mb area between the polymorphic markers *AFMa205Xd5* and *D1S2775* that define the *VMGLOM* locus (3). The integrity of the clones was checked by amplifying markers 24 to 49 from the contig WC1.14 (17; Figs. 1A and 1B). These clones were used for the precise localization of additional STSs selected from various databanks (Figs. 1B and 1C) and created from our end-of-clone sequences (Table 1). We also identified the position of three polymorphic markers (*D1S188, D1S406,* and *D1S1170*) known to localize to this region (1). In contrast to Allikmets *et al.* (1), we were not able to localize marker *WI-7719* to our YAC map, and the order for markers *D1S2849* to *D1S286,* as well as for *D1S424* and *D1S406,* was inverted (Fig. 1). These results were later confirmed with our PAC map (Fig. 2). Based on the known sizes of the YAC clones, we estimate the size of the *VMGLOM* locus to be 5 Mb.

Having previously excluded three known genes as disease-causing in *VMGLOM* (3), we needed to identify new positional candidate genes. Thus, we selected more than 80 STSs from several databases on the basis of their localization to the vicinity of the *VMGLOM*

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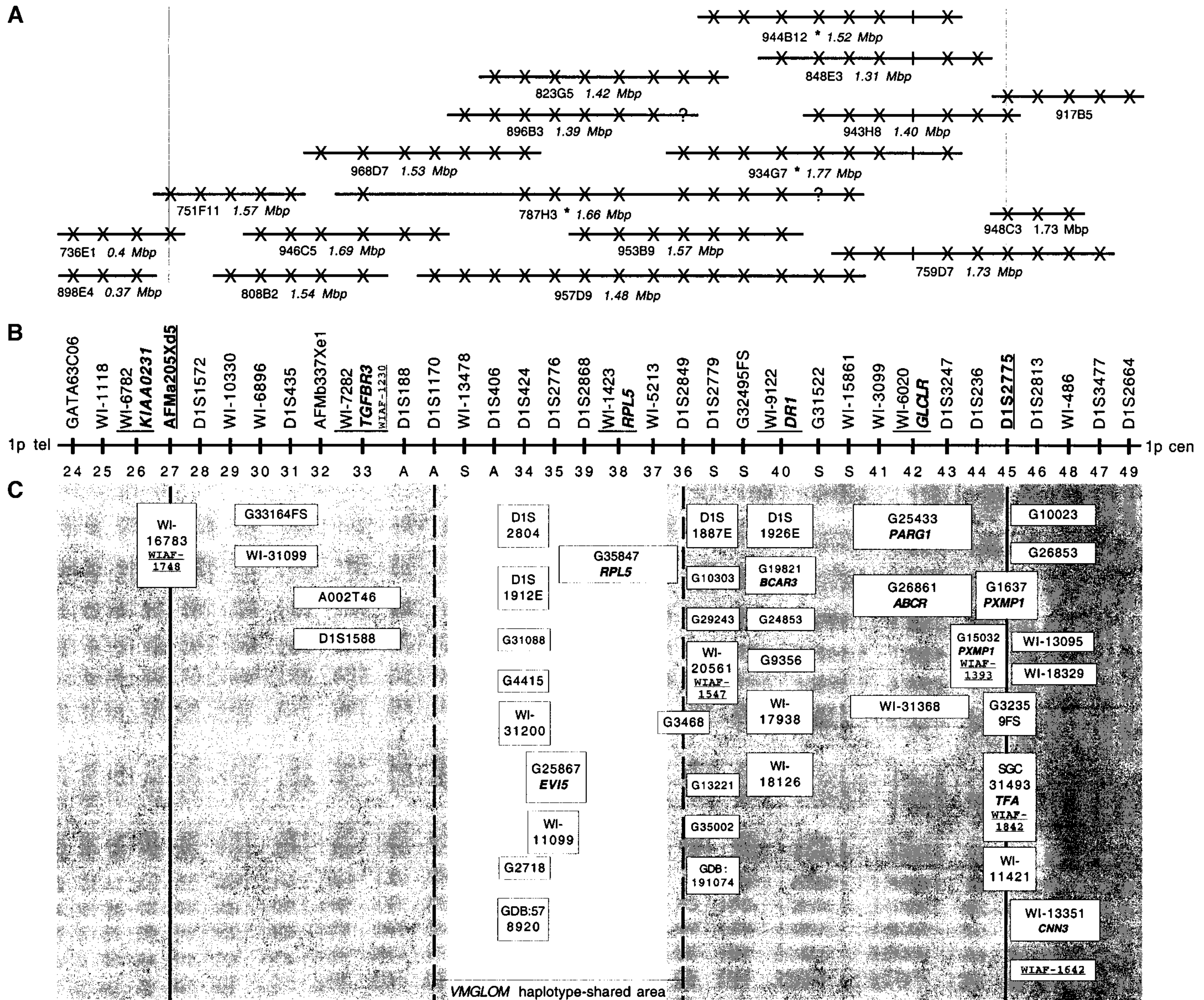

FIG. 1. Schematic representation of YAC map and STS localization. Genes, boldface italics; SNPs, small underlined capital letters (WIAF). (**A**) *YAC clone reported to be chimeric. Results for marker *WI-6020* marked with **I** to reflect database entries only. A question mark indicates unclear results. (**B**) Numbers under markers from the WC1.14 contig (17) indicate order in the map. A, from Ref. (1). S, placed during STS localization. Order for markers 36 to 39 is inverted to reflect the order in the PAC map (Fig. 2). (**C**) Boxes represent areas of localization of STSs. Vertical lines delimit the *VMGLOM* locus (unbroken lines) or a smaller, haplotype-shared area (dashed lines).

locus (6, 12–14). Every marker was amplified by PCR on six overlapping YACs covering the whole region (736E1, 751F11, 946C5, 957D9, 944B12, and 759D7; Fig. 1A), with genomic DNA as positive control. Forty-eight positive markers were identified (a complete list of tested STSs is available via http://www.icp.ucl.ac.be/vikkula). Finer localization of these 48 markers was performed by testing all the YAC clones in the vicinity of the positive one(s). Using this strategy, 5 markers, *WI-13478, D1S2779, G32495FS, G31522,* and *WI-15861,* were precisely localized between existing markers of the YAC map (Fig. 1B), whereas the 43 other STSs were only roughly localized (Fig. 1C). A Blast homology search (2) was performed for each marker, and several genes were retrieved: *acidic calponin (CNN3), breast cancer anti-estrogen resistance 3 (BCAR3), Evi-5 homologue (EVI5), peroxisomal 70-kDa membrane protein (PXMP1), PTPL1-associated RhoGAP (PARG1),* and *KIAA0231* (Figs. 1B and 1C).

Concurrently with these studies, we identified haplotype sharing in *VMGLOM* among 12 families, resulting in the reduction of the candidate region from *AFMa205Xd5–D1S2775* to *D1S1170–D1S2849* (9). Based on the size of the YAC 957D9, containing the whole region showing haplotype sharing, the *VMGLOM* locus should be less than 1.48 Mb (Fig. 1). Thus, we undertook the creation of a more precise physical map of this area (Fig. 2). In the Sanger Centre database (12), we found 20 PAC clones containing STSs of the *VMGLOM* haplotype-shared area. Each clone was

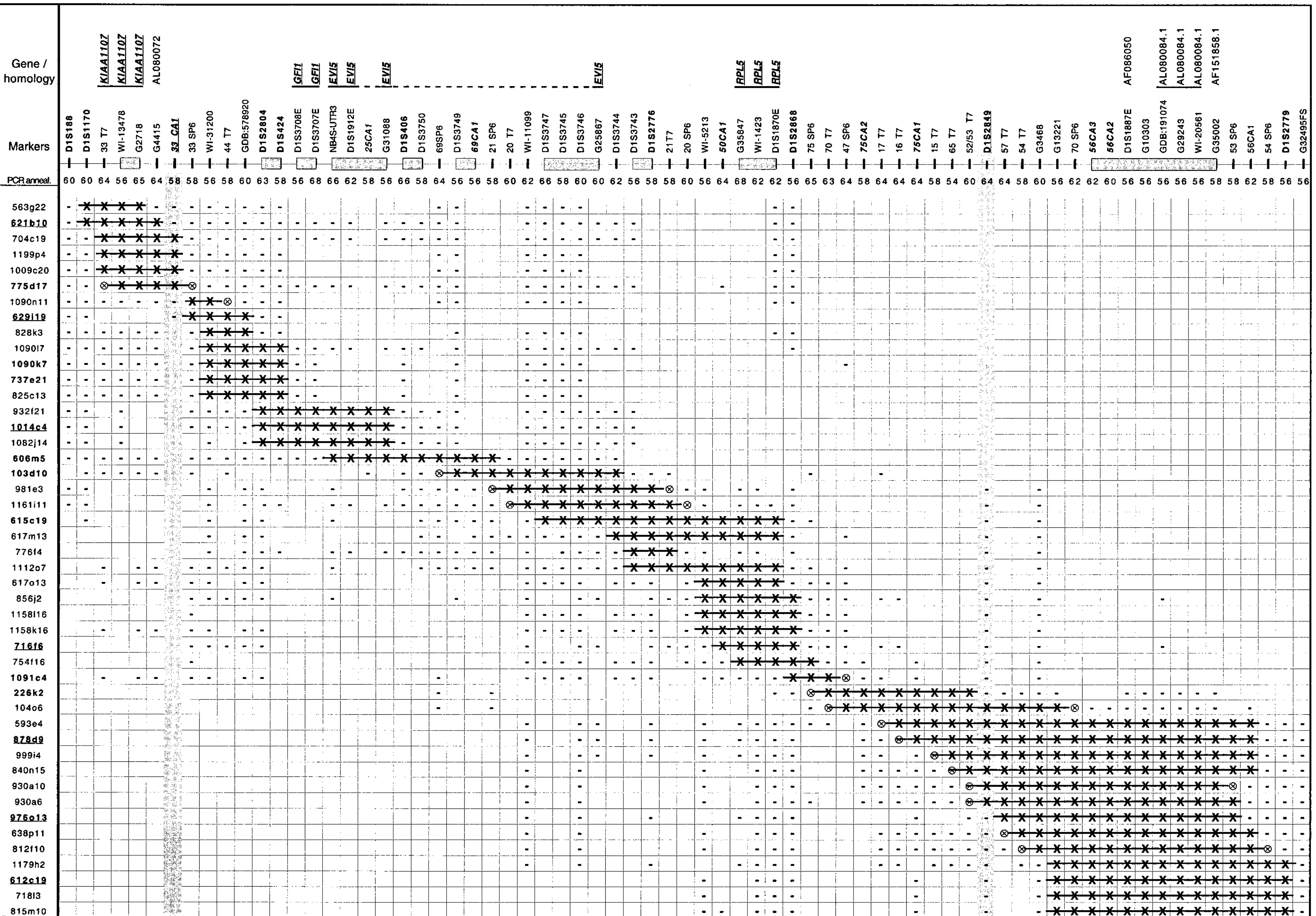

FIG. 2. Schematic representation of PAC-based STS and transcript map of VMGLOM. Gene names, boldface italics, underlined; polymorphic markers, boldface type; novel CA repeats, boldface italics; markers with unknown order, gray boxes. Fingerprinted PAC clones, boldface type; underlined clones were selected for sequencing (12). **X,** positive PCR result; −, negative PCR result; ⊗, new end-of-clone STS. Best annealing temperature for PCR is given for each STS.

TABLE 1

PCR Primer Sequences for the 22 New End-of-Clone STSs

Name	PAC	Primer 1	Primer 2	Size (bp)	Annealing temperature (°C)
15 T7	999i4	AGC AAA CTT ACT GGC AGT GC	GCT ACT TGG AGC TGA GCA G	217	58
16 T7	878d9	AGG AGA ATG GCG TGA ACC C	TCA AAG ATT CTT CCT TCC TGC	146	64
17 T7[a]	593e4	ACT CAG GAA TGG AGT CAT GG	CAT GCT CAC AGG GTA GAT AC	191	64
20 SP6	1161i11	TCT AAA GTC TTG TCA CAG TGC	GTC TAT ATG GCA TGT TTC TCC	196	60
20 T7	1161i11	AGC AGG TAT GTC ACA CAG TG	TAT GGC TTG GAT CTC CCT TC	218	60
21 SP6[a]	981e3	TAT CTT CAA TGA AAT CCC AAT AC	GTT TTG ACA AAG TAT CAG ATT GC	168	58
21 T7	981e3	ACT GCC CTC ATA CTA CCA TG	CTG AAC CAA CCA TGC ATC AC	238	58
33 SP6	775d17	GCC ACT TGT ATG TAG GAG AG	TGG CTA CTA ACA GAC ATC AAC	320	58
33 T7	775d17	GAC GTC CTA TCC AGT AGA AG	TTT CAC TGG ACC TTC CTG AG	246	64
44 T7	1090n11	CTC TAG AGA GTA TGC GTC TC	ACA CTG ACT ACT ATG GAA CTG	231	58
47 SP6	1091c4	ATG GAG AAC TCC AGT GAG AG	TAA GTT CTG AAT GCA TGG GTC	205	64
52/53 T7	930a6/930a10	AGA CTG ATA ATT CTG AGC TAT C	ATG CAT GCA GGC CAC ATA TG	266	60
53 SP6	930a10	TAC TCT GGA TCT CTC ACA GG	TAG CTT GTC CTC TCT TGC TG	258	58
54 SP6	812f10	AGC AGT TAT TTC TGG TGG TAG	TGG AGA TTT AGA CAG TTT ATA AC	168	58
54 T7	812f10	CCA GGG TGG TCT CAA ACT C	TAA GTC TAT GAT CCA TTT CGA G	166	58
57 T7	638p11	GAG AGT GAG ACC CTG TCT G	AAA GGA CAG AGA ATC AAC CTG	167	64
65 T7	840n15	GTG ACA GGA GCC AAT GAA TG	GGG ATG TCT AGA CAG AAG TG	240	54
69 SP6	103d10	GGG TTA GGG GTA AAG GGT GG	CGA AGA ACC TGG TAT GCA GG	122	58
69SP6 bis[a]	103d10	TCC TGC ATA CCA GGT TCT TC	TGT TCT GCT GGT AGT AGT CC	265	64
70 SP6	104o6	TGA GCC ACC ACG CCC AAC	TGC GCA CGT TCT TGT GCT G	165	62
70 T7	104o6	ATA TCT GAC ATC TCA GAG TGG	CTG GCC TGA ATT TCA GAG TC	258	63
75 SP6	226k2	CTC TGC ATA GAG TCA GCA AG	CCA CCA TGC CCA GTT TCT C	188	65

[a] Used as probe; 20-bp overlap between 69SP6 and 69SP6 bis, as primer 1 of 69SP6 bis is complementary to primer 2 of 69SP6.

tested by PCR for all the markers localized between *D1S1170* and *D1S2779* (Fig. 1). A manual analysis of the results allowed clustering of the PACs in four contigs. With a second search in the Sanger Centre database, we picked 23 additional PACs. None closed the gaps between the PAC clusters.

To join the different PAC islands, we generated 21 new STSs by sequencing the ends of the protruding PAC clones (identified as *T7* or *SP6;* Table 1). Marker *33SP6,* from the centromeric end of clone 775d17, closed the first gap (Fig. 2). Similarly, marker *21SP6* enabled us to bridge clone 981e3 with clone 606m5. However, the novel markers *47SP6* and *17T7* inside the last gap did not reach any clone from the other cluster (Fig. 2). Thus, a screening of the Human RPCI-1 PAC Library filters (7, 8), using the amplified *17T7* as a probe, was performed. This resulted in the identification of two new PACs, 104o6 and 226k2, which bridged the two contigs (Fig. 2). To obtain double coverage for the single-linked point in the map around marker *21SP6,* additional new clones were screened from the PAC library with *21SP6.* Clone 103d10, which overlaps clones 606m5 and 981e3, was identified. This overlap was confirmed with the novel STS *69SP6* (Fig. 2).

To identify new polymorphic markers, nine PAC clones were selected for CA-repeat screening. Seven of these were not positive for a known CA repeat and two contained one CA repeat (Fig. 2). These PACs were subcloned, and the libraries were screened by hybridization with a radiolabeled $(GT)_{16}$ probe. More than 40 positive subclones were sequenced. This enabled us to

TABLE 2

PCR Primer Sequences for Novel CA Repeats

Name	PAC	Forward	Reverse	Approximate size (bp)	T° for mapping	Heterozygosity in controls	Alleles identified
25CA1[a]	606m5	GAG GTC AGG AGT TCG AGA C	GTA GGA GTG CAT CAC CAT GC	139	PCR Not specific	PCR Not specific	—
33CA1	775d17	TCT GAC TTT GAC GTT GTA ACC	CAG TCA CTT CTC TTT CAT CAG	157	55	12/16	7
50CA1	617o13	TGT AAA GAG CTG CTG CAC TC	AGC AAA GTG ACA TCT GAC TTC	129	64	15/16	6
56CA1[b]	612c19	CTT GAA GCC AGG AGT TTG AG	CAG CCT CCC AAG TAG CTA G	141	55	0/16	1
56CA2	612c19	ATG TTG TTC AGC GCA ACC TC	AGC CTG GAT GAC AGA ATG AG	181	55	7/16	4
56CA3	612c19	GGT ATG GTG AAT GAA GCA TTC	TGA GCT CCT GAA TTA CAC ATC	148	58	6/16	2
69CA1	103d10	TTT TTA AAT CCC ATA ACT TGC C	GCA GTG GTG AGA GAG TGG	175	58	11/16	6
75CA1	226k2	CAA CAG GTT GAG AAG GCA AG	GGG TGA CAG AGT GAG ACT G	169	58	11/16	5
75CA2	226k2	CAA CAT AGA TCC TGG GAA CG	GAG AAT CTG ACC TTG AAG GC	195	58	10/16	5

[a] Specific on the PAC but not on genomic DNA.
[b] Not polymorphic.

identify nine different CA repeats (Table 2). The sublibrary from clone 828k3 did not show any clone containing a putative repeat, and the eight CA repeats identified from clone 981e3 revealed only the known *D1S2776.* Three of 12 subclones from PAC 612c19 were identical to *D1S2779.* The nine novel markers were tested by PCR for their specificity on genomic DNA. All except *25CA1* gave a unique signal. Genotyping of 16 unrelated control individuals for the eight specific STSs showed that seven of them were polymorphic (Table 2).

To integrate additional published information into our PAC map, 10 recently reported markers (10) were localized (Fig. 2). We noted that 2 of them, *D1S2868* and *D1S1870E,* had an inverted localization. The whole map is now covered by 46 clones and 69 STSs, 4 of which correspond to known genes: *Ribosomal protein L5 (RPL5), KIAA0231, EVI5* (10), and *GFI1,* a growth factor independence gene (11). In addition, STSs *G4415, D1S1887E, G35002, GDB:191074, G29243,* and *WI-20561* represent 4 putative genes as they correspond to an uncharacterized cDNA or to an EST cluster (14). On average, the *VMGLOM* area should correspond to roughly 47 genes. However, as the gene encoding EVI5 was shown to cover 270–300 kb (10) (around *NB4S-UTR3* and *G25867;* Fig. 2), and the gene *GFI1* covers 50 kb (11), less than 30 genes would be expected to reside in the remaining maximum of 1.1 Mb. In addition, as 1p21–p22 is considered a low-density gene area on the basis of the number of EST hits (6), the number of genes should be even lower. Thus the tags we have for 8 genes may represent the majority of the genes in the area. Only 3 of these, *GFI1, EVI5,* and *RPL5,* localize to the minimal *VMGLOM* locus and represent positional candidates for venous malformations with glomus cells (Fig. 2). *EVI5* is involved in a translocation breakpoint in stage 4S of neuroblastoma that creates the *NB4S* fusion gene between *EVI5* and the *TRNG10* gene from chromosome 10q21 (10), and *GFI1* and *RPL5* do not seem obvious candidates. Thus, we may still lack a tag for the *VMGLOM* gene.

To sequence and identify genes in the area covered by the PAC map, one should select clones presenting a minimum of overlap. Seven of the 46 clones have already been selected for sequencing (Fig. 2 and Ref. (12)). To cover the whole region, clones 775d17, 1090k7 or 737e21, 606m5, 103d10, 615c19, 1091c4, and 226k2 should also be selected. Fingerprinting of these 15 clones by *Hin*dIII restriction digestion confirmed the overlaps (data not shown). To date, clone 976o13 is completely sequenced, and clones 621b10 and 1014c4 are unfinished (12). No additional gene can be retrieved with these sequences by Blast.

In summary, we report a 5-Mb YAC map and a PAC map of maximally 1.48 Mb of chromosome 1p21–p22, with a high density of STS markers. This gives the necessary tools for sequencing this part of the human genome and for the identification of genes in this locus. In addition, we report seven novel, highly polymorphic dinucleotide repeat markers, which will be useful for linkage, haplotypic, and association analyses involving this chromosomal area. We conclude that these data will enable the identification of the gene responsible for venous malformations with glomus cells, likely a novel gene important for vascular development.

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