

GENETICS OF HUMAN VASCULAR AND SKELETAL DISORDERS AND CEREBRAL TUMORS

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The basic aim of our research is to get insights into the molecular mechanisms behind human inherited diseases. We are especially interested in disorders affecting the cardiovascular and the skeletal system. In addition, we have initiated studies on cancerous tumors. As this research is based on human DNA extracted from blood and tissue samples obtained from patients, the group works tightly together with several clinicians and multidisciplinary centers worldwide (e.g. Centre des Malformations Vasculaires, Cliniques Universitaires St-Luc; Vascular Anomalies Center, Children's Hospital, Boston, USA and Centre labiopalatin, Cliniques Universitaires St-Luc).

Venous malformations and glomuvenous malformations ("glomangiomas")

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Venous malformations (VM) are bluish-purple cutaneous and mucosal lesions. They are often congenital, but can also appear later in life. They have a tendency to grow slowly with the growth of the child. Glomuvenous malformations (GVM, "glomangiomas") are a special subtype of venous anomalies. They are clinically similar to VMs, yet our recent studies have allowed clinical differentiation of these lesions (unpublished). In histologic examination, the pathognomonic sign of GVMs is the appearance of "glomus cells"

around the endothelium lining the convoluted channels. These cells are thought to be smooth muscle cells of origin.

We have previously identified that hereditary venous malformations can be caused by an activating mutation in the endothelial specific receptor tyrosine kinase *TIE2/TEK* (1). In contrast to inherited VMs, inherited glomuvenous malformations do not link to the *TIE2/TEK* gene. Instead, they link to *VMGLOM*, a locus on chromosome 1p21 (2). Analysis of the linked families allowed us to observe linkage disequilibrium between certain markers and the phenotype (3). Thus, the locus was narrowed to 1.48 Mbp, covered by a single YAC. Using this single YAC as template, we created a PAC (P1 bacterial artificial chromosome) contig for the reduced *VMGLOM* region and localized expressed sequences, and thus genes, into the locus. Characterization of these novel positional candidate genes recently led to the identification of the mutated gene, that we named "glomulin" (4). This novel factor does not have sequence identities to known proteins, nor does it contain known functional domains.

Thus, its molecular function is unknown. However, Northern-blot hybridizations showed that glomulin is widely expressed, as all analysed RNAs contained it (4). This is expected if the gene is important for the vasculature, as almost all tissues are invaded by blood vessels. However, we do not currently know, which cell types express glomulin.

As most of the identified mutations cause premature STOP codons in the coding sequence of glomulin (Fig. 1), loss-of-function is the most likely mechanism causing the disorder (4). Furthermore, we hypothesized that as the lesions are localized, a somatic second hit might be needed in the normal allele of the glomulin gene, for lesions to develop.

We have obtained proof for this from one lesion (4), but additional lesions need to be studied to confirm these promising results. To study glomulin function, we have cloned about 20kb of the murine glomulin gene (unpublished). This data has been used to create a construct for inactivating the glomulin gene by homologous recombination in murine embryonic stem cells. As the construct contains the lacZ marker gene within the glomulin sequence, homologous recombination would lead to an allele expressing β -galactosidase instead of glomulin. Thus, murine embryos containing such cells could be used to study the expression and the role of glomulin in development and angiogenesis.

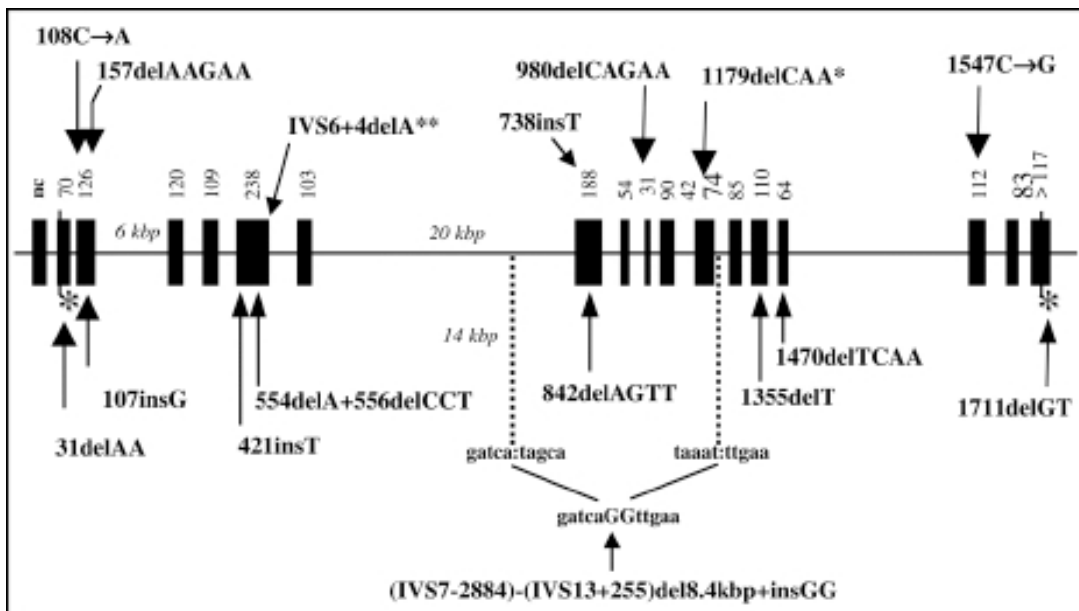


Fig. 1. Glomulin gene – structure and mutations. The size of exons and of the three largest introns are given; other introns are to scale. Exon 1 is noncoding (nc), exon 2 contains the translation start site, and exon 19 contains the TGA stop codon. Above the sequence line, the white arrowheads indicate 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 asterisk (**) indicate a split-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.

Lymphedema

A. Irrthum, L. Boon and M. Vikkula in collaboration with K. Devriendt, KUL

Primary lymphedema can occur at birth (Meige's disease) or at puberty (Milroy disease). We identified a family in which primary lymphedema was present at birth in several family members. Genetic studies confirmed linkage to 5q33-34 and led to the identification of a mutation in the VEGFR3 gene (5). *In vitro* expression studies demonstrated that the mutated receptor has lost its autophosphorylation capacity (5). The continued studies have recently led to the identification of linked candidate regions in a family with supposedly recessively inherited congenital lymphedema (unpublished). The identification of the mutated gene should shed further light into factors that are important for lymphatic development

Vascular anomalies affecting capillaries

I. Eerola, LM. Boon and M. Vikkula in collaboration with J.B. Mulliken, Children's Hospital, Boston, USA, S. Watanabe, Showa University School of Medicine, Tokyo, Japan, A. Domp Martin, CHU de Caen, France and Virginia Sybert, Washington University, Seattle, USA

Capillaries, the small blood vessels that connect arterioles to venules, can give rise to various anomalies, two of which are very common: 1) hemangioma, a benign, localized overgrowth of capillary-like vessels, and 2) capillary malformation (CM; commonly known as portwine stain), a localized maldevelopment of capillary like vessels. Hemangiomas have a frequency up to 12 % in 1-year-old children, and CMs occur in 0,3% of newborns. Whereas hemangiomas usually disappear spontaneously, capillary malformations stay throughout life, if not treated. Other types of cutaneous capillary anomalies also exist, in addition, some can affect other organs, like CCMs, cerebral capillary malformations.

As the molecular mechanisms leading to these localized capillary lesions are unknown, we have started to collect clinical information

and samples from families in which more than two individuals are affected. These studies, led to the discovery that inherited hyperkeratotic cutaneous capillary-venous malformations (HCCVM) associated with cerebral capillary malformations are caused by a mutation in the *KRIT1* (Krev interaction trapped 1) gene (6). This suggests that *KRIT1*, a possible intracellular signaling molecule, is important not only for cerebral but also for cutaneous vasculature. As our Northern-hybridisation results showed that the *KRIT1* transcript is bigger than expected, we verified the length of the *KRIT1* cDNA. Using *in silico* cloning we identified 8 novel exons, four of which are translated (7).

In addition to these studies, we have performed a genome-wide linkage mapping on families with inherited capillary malformations. Large parts of the genome were excluded, finally leading to the identification of a linked locus (8). Identification of the causative gene is an ongoing important project.

Cardiopathies

I. Gutierrez-Roelens, A. Irrthum and M. Vikkula, in collaboration with T. Sluysmans, St-Luc, UCL and M. Gewillig and K. Devriendt, KUL

The cardiovascular system may encounter developmental problems affecting the heart. These cardiac defects, cardiopathies, vary from physiological septal defects to life-threatening complex malformations. To get insight into the molecular mechanisms behind these phenotypes, we have started to collect samples from families with possibly hereditary cardiopathies. In two families, in which atrial septal defect is associated with progressive atrioventricular conduction defect, we identified two novel mutations in the *CSX/Nkx2.5* gene (9) (Fig. 2), an important transcription factor for cardiac development. Identification of mutation carriers is crucial, as in the few studied families the first "symptom" has sometimes been sudden death. Identification of mutations allows genetic testing in the respective families, enabling tight follow-up and preventive pacemaker implantation.

Ongoing studies focus on various forms of atrio-ventricular septal defects and heterotaxia.

Osteoarthritis and osteochondrodysplasias

M. Ghassibé, V. Wouters, M. Vikkula, in collaboration with D. Manicourt, B. Bayet, R. Vanwijck, N. Revencu and Ch. Verellen-Dumoulin, St-Luc, UCL)

Our main project is in collaboration with Centre labio-palatin, St Luc, to unravel the molecular background of syndromic and non-syndromic cleft lip and/or palate. Numerous

blood samples of affected individuals, and their parents and siblings have been collected. This will be continued so as to allow us to do association studies in the future. In addition, collaboration with the cleft lip and palate center of the CHRU Lille has been initiated. These studies have recently led to the identification of mutation causing Van der Woude syndrome (unpublished).

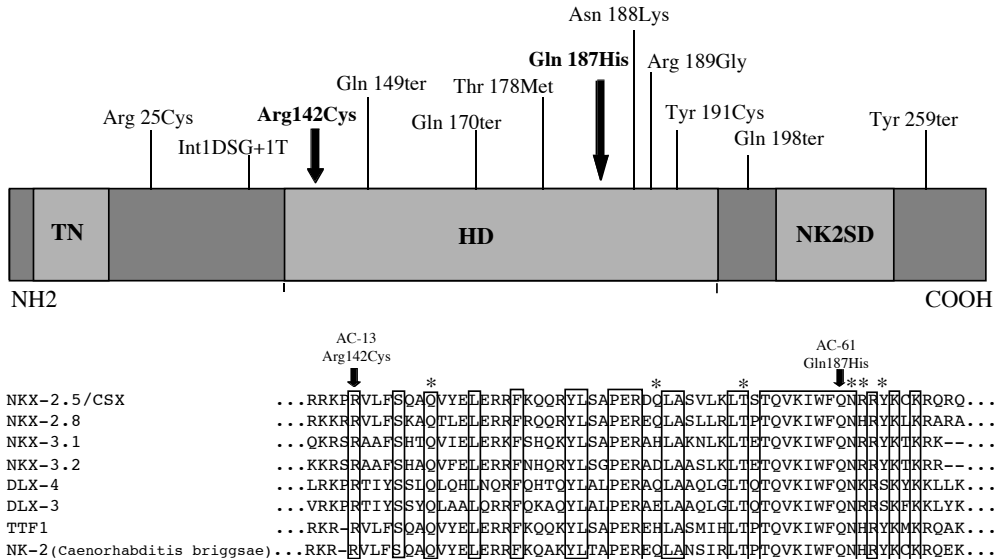


Fig. 2. Schematic representation of CSX/NKX2.5 with tinman domain (TN), homeodomain (HD) and NK2 specific domain (NK2SD). Ten published mutations and two novel missense mutations (bold) from families AC-13 and AC-61 marked above. Top, phenotypes other than ASD with AV block associated with each mutation. For two most 5' mutations, no individual with "ASD with AV-block" reported. Bottom, multiple alignment of amino acid sequences of homeodomains of different human NK-2 proteins and an NK-2 orthologue. *, previously described mutation, □, AC-13 and AC-61 mutations (9). Conserved amino acid boxed.

Cerebral tumors

E. Rousseau and M. Vikkula, in collaboration with C. Godfraind, Laboratory of Neuropathology, St-Luc, UCL)

Morphological characterization and classification of tumors is not always clear. Thus, better (molecular) criteria are needed. In addition, the causative genes are often unknown. We are especially interested in two types of cerebral tumors: oligodendrogliomas and ependymal tumours. Using DNA, extracted from formalin-fixed and paraffin-embedded tissues, we have performed loss-of-

heterozygosity testing. A restricted screening was performed in a number of oligodendroglial tumours as well as in a large series of ependymal tumours. For oligodendrogliomas, this allowed us to identify and define specific histological characteristics for tumors that have lost chromosome 1p and 19q and that are known to have a preferable response to chemotherapy (10). In addition, we identified methylation difference in ependymomas (unpublished).

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