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GENETICS OF HUMAN CARDIOVASCULAR ANOMALIES, CLEFT LIP PALATE AND CEREBRAL TUMORS

The aim of our research is to get insights into the molecular mechanisms underlying a variety of disorders of the cardiovascular and skeletal systems, as well as certain cancers. We are especially interested in evaluating the contribution of genetic variation to human disease. The precise cause of many disorders remains unknown, and current treatments are therefore aimed at alleviating symptoms. Identification of the primary causes as well as modulating factors would allow for the development of treatments that are more “curative” and specific. As this research is based on human DNA extracted from blood and tissue samples from patients, the group works closely 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; Consultation des Angiomes, Hôpital Lariboisière, Paris, and Centre labiopalatin, Cliniques Universitaires St-Luc).

VENOUS MALFORMATIONS AND GLOMUVENOUS MALFORMA- TIONS (“GLOMANGIOMAS”)

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Venous malformations (VM) are bluish-purple cutaneous and mucosal vascular lesions. They are often congenital, but can 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 clinico-genetic study allowed for their clinical differentiation.

We previously discovered that rare, hereditary venous malformations can be caused by an ac-

tivating mutation in the endothelial cell receptor tyrosine kinase TIE2/TEK (Fig1). The use of high-throughput technologies such as DH-PLC and High Resolution Melting, which allow for more efficient and sensitive genetic screens, was instrumental in the identification of several novel mutations amongst affected families [1]. Despite the ubiquitous presence of these germline mutations, the lesions they cause are localized. We therefore hypothesized that lesion-development may require a somatic second hit to locally disrupt the normal allele of the TIE2 gene. We obtained proof for this from one lesion, in which the ligand-binding region of the wild-type allele was deleted somatically, causing a local loss of its ability to function [2]. In addition, we discovered that at least 50% of the

be learnt as to precisely why this causes lesions. Towards this end, we have begun to carry out functional analyses of the role of TIE2 in VM-pathogenesis, using a variety of in vitro and in vivo methods. These include the generation of mouse models of the anomaly, by “knock-in” substitution of the normal TIE2 allele with the most frequently mutated forms associated with inherited VMCM and sporadic VMs respectively; Affymetrix expression profiling has also been used in order to compare the effects of the wild-type receptor with those of different mutant forms.

In contrast to VMs, glomuvenous malformations (GVM) are mostly, if not always, inherited. We discovered that GVM are caused by

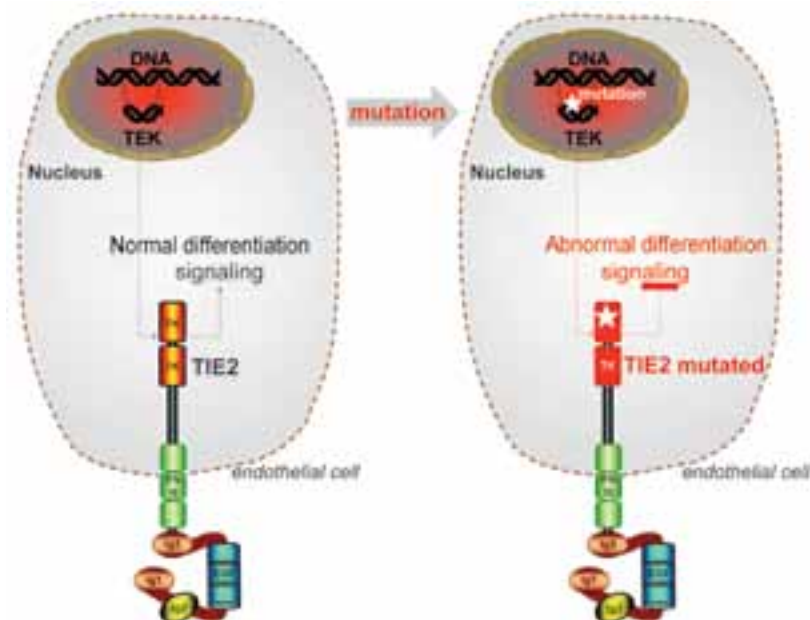


Fig. 1. Mutations in TIE2/TEK cause inherited and sporadic venous malformations. All of the mutations identified cause hyperphosphorylation of this tyrosine kinase receptor, resulting in abnormalities in signaling pathways known to be critical to endothelial cell function (Limaye et al, Nat Genet 2009).

far more common sporadic VMs are caused by somatic mutations in TIE2 [2]. On this basis, we suggest somatic changes to be a general genetic mechanism in vascular malformations. All of the TIE2 mutations discovered thus far are intracellular and cause receptor hyperphosphorylation in vitro, although much remains to

loss-of-function mutations in a gene we named glomulin. So far, we have identified 36 different mutations in 140 families. As with VMs, we showed that GVMs appear locally because of the additional alteration of the second allele, likely in vascular smooth muscle cell precursors that populate the lesions. To better understand

the role of glomulin in normal and pathological conditions, we have created glomulin-deficient mice. While heterozygotes appear normal, homozygous knockouts are embryonic-lethal (Brouillard et al, unpublished). To enable studies beyond this time-point, we generated RNAi conditional knockdown mice, in which glomulin inactivation is cre-inducible (Brouillard et al, unpublished). When triggered during embryonic development, glomulin depletion is likewise lethal. To further study its role specifically in vascular development, we will instead knock it down in a cell-type specific manner. In vitro studies to identify the function as well as any interacting partners of glomulin, a molecule with no known domains or homologs, are also ongoing.

LYMPHEDEMA

A. Mendola, L.M. Boon, M. Vikkula in collaboration with K. Devriendt, KUL; D. Chitayat, Hospital for Sick Children, Toronto, Canada; I. Quere, Montpellier, France

Lymphedema is an external manifestation of lymphatic failure. It may be categorized as primary (idiopathic) or secondary (acquired) lymphedema. Primary hereditary lymphedema can occur at birth (Nonne-Milroy disease) or at puberty (Meige's disease). It is extremely difficult to treat lymphedema. Patients have a lifelong problem with progressive swelling of extremities. We use genetic approaches to unravel the pathophysiology with a view to ameliorating the condition. In some families with Nonne-Milroy disease, missense inactivating mutations in the VEGFR3 gene were identified. Moreover, some sporadic congenital primary lymphedemas are also explained by alterations in VEGFR3. We showed, for the first time, that recessive primary congenital lymphedema can be caused by a particular homozygous VEGFR3 mutation, which has a moderate effect on receptor function and can cause lymphedema only when both alleles are altered [3]. Mutations in the transcription factor gene SOX18 were identified in fami-

lies with autosomal recessive and dominant hypotrichosis-lymphedema-telangiectasia syndrome, and the forkhead transcription factor FOXC2 is mutated in some families with Meige disease, in association with distichiasis. We recently found that a proportion of sporadic fetal edema of unknown etiology is in fact attributable to mutations in the lymphedema-associated genes VEGFR3 and FOXC2 [4]. In collaboration with a group (RC Hennekam, Dept of Clinical Genetics, Academic Medical Center, The Netherlands) investigating Hennekam syndrome, characterized by lymphedema, lymphangiectasias, mental retardation and unusual facial characteristics, we identified CCBE1 as a gene that causes generalized dysplasia of lymphatic vessels in humans as well as animal models [5].

VASCULAR ANOMALIES AFFECTING CAPILLARIES

N. Revencu, N. Limaye, M. Amyere, L.M. Boon, 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; Virginia Sybert, Washington University, Seattle, USA

Capillaries, the smallest 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 of up to 12% in 1-year-old children, and typically undergo a period of rapid expansion, followed by spontaneous regression. We have an extensive collection of samples from sporadic as well as rare familial forms of hemangioma, and are using Affymetrix high-density whole genome SNP arrays in order to carry out linkage, loss of heterozygosity and copy number analyses on them in an effort to identify causative genomic variants. Work done with collaborators has demonstrated that per-

turbations of the vascular endothelial growth factor (VEGF) signaling pathway can cause hemangioma pathogenesis [6].

CMs occur in 0.3% of newborns. Unlike hemangiomas, they persist throughout life if not treated. Certain capillary malformations affect other organs, such as the brain in the case of cerebral capillary malformations or CCMs. We discovered that inherited hyperkeratotic cutaneous capillary-venous malformations (HCCVM) associated with CCM are caused by a mutation in the KRIT1 (Krev interaction trapped 1) gene, suggesting it is important not only for cerebral but also for cutaneous vasculature. In addition, genome-wide linkage mapping on families with inherited capillary malformations led us to identify a linked locus CMC1. Screening of positional functional candidate genes resulted in the identification of mutations in the RASA1 gene, a modifier of the Ras signaling pathway. This implies that RAS pathway modulators may serve as a novel therapy for these patients in the future. Ongoing studies have led to the identification of RASA1 mutations in 90 index patients. This has allowed for a more precise clinical description of the clinical signs and symptoms associated with this newly recognized disorder that we have named Capillary malformation-arteriovenous malformation (CM-AVM) [7]. Importantly, capillary lesions can be associated with deeper, more dangerous anomalies about 20% of the time; these include arteriovenous malformations and fistulas (AVM/AVF), Parkes Weber, and Vein-of-Galen aneurysmal malformations, which warrant careful clinical management

CLEFT LIP AND PALATE

M. Ghassibé, L. Desmyter, M. Quentric, N. Revencu, M. Vikkula, in collaboration with Y. Gillerot, B. Bayet, R. Vannijck, Ch. Verellen-Dumoulin, N. Deggouj, St-Luc, UCL

Cleft lip and palate (CLP) is a congenital anomaly of complex etiology. Predisposition is

governed by numerous genetic loci, in combination with environmental factors. Clefts have an incidence of 1/700 births.

We collected DNA samples from a large number of patients affected with popliteal pterygium syndrome, as well as van der Woude syndrome, the most common cleft syndrome. We showed that IRF6 is the major causative gene in our Belgian cohort. This study in turn led to several collaborations that allowed us to carry out a genotype-phenotype correlation on hundreds of patients from different ethnic backgrounds. Results showed that IRF6 is mutated in 69% of VWS patients and 97% of PPS patients. Interestingly, mutation-distribution is non-random: 80% are localized in IRF6 exons 3, 4, 7 and 9 for VWS, and 72% in exon 4 for PPS patients. These findings are of great importance for clinical diagnosis, mutational screens and genetic counseling. We also demonstrated that IRF6 predisposes to non syndromic clefts in Europe and that it is mutated in familial clefts with minor lip anomalies. In parallel, we identified a new gene, FAF1, responsible for cleft palate only and Pierre Robin sequence [8]. This gene is associated with clefts across populations. Zebrafish studies confirmed its role during embryonic development and jaw formation (Fig 2). In parallel, we generated a mouse model where we knocked-out the gene and we are currently phenotyping the mouse in order to understand the mechanism behind craniofacial development and cleft occurrence.

CEREBRAL TUMORS

T. Palm 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. We are especially interested in two types of cerebral tumors: oligodendrogliomas and ependymomas. To better understand the molecular alterations

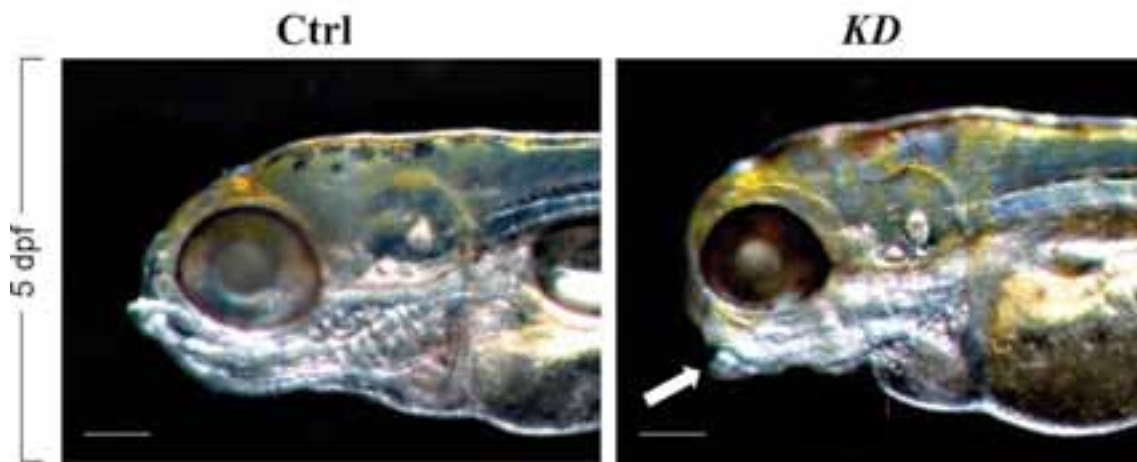


Figure 2 – FAF1 mutations cause abnormalities in jaw development across species. Morpholino-induced knockdown of the protein in zebrafish causes an open-mouthed appearance due to retrognathia, similar to the phenotype in human lacking FAF1. (Ghassibé-Sabbagh et al, Am J Hum Genet 2011)

leading to ependymomal oncogenesis, we performed microarray-based expression profiling on a series of 34 frozen ependymomas. Results of our profiling study are in concordance with the “oncology recapitulates ontology” hypothesis, in which genes implicated in stem cell fate decisions may be important for supporting cancer stem cells as well. Pathways activated in high grade ependymomas were consistent with the histological appearance of a more aggressive tumor phenotype. Using array-CGH, we recognized a subgroup of supratentorial ependymomas affecting young adults, which are characterized by trisomy of chromosome 19.

Within the posterior fossa compartment, ependymomas cluster into three sub-groups. The first corresponds with ependymomas that are histologically of WHO grade II, the second with those of WHO grade III, and the third with a group of ependymomas of a bi-phasic appearance, combining regions of both grades. This sub-group shares gene-sets with tumors of both other groups, and in addition has a glycogen metabolism signature of its own. Whether these groups correspond to three distinct tumoral entities, or demonstrate multifocal tumor progression remains to be investigated.

NEUROENDOCRINE TUMORS

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Vikkula, in collaboration with P. Rustin, INSERM
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Pheochromocytomas and head and neck paragangliomas are neuroendocrine tumours derived from the neural crest. Paragangliomas are associated with parasympathetic ganglia and are usually non-secreting. By contrast, pheochromocytomas are derived from paraganglia associated with the orthosympathetic system and are characterized by increased secretion of catecholamines and paroxysmic hypertension.

The current project aims to look at the nature and frequency of mutations in the known predisposing genes in pheochromocytoma and paraganglioma in Belgium and to detect possible genotype-phenotype correlations. A multicentric collaboration including the main academic centers from Belgium has therefore been established.

The SDH genes code for the subunits of succinate dehydrogenase, at the crossroad of the mitochondrial respiratory chain and Krebs

cycle. Three of the four subunits of succinate dehydrogenase, i.e. SDHD, SDHB, and more rarely SDHC, have been associated with paraganglioma and pheochromocytoma. Furthermore, SDHB mutations have been associated with an increased risk of recurrence and malignancy in several European series. In our study, the prevalence of SDHB was unexpectedly high in head and neck paraganglioma. Surprisingly, tumours associated with such mutations are mainly late-onset unilateral tumours without evidence of recurrence or malignancy. We also described a family with a very rare presentation of severe head and neck paraganglioma with liver and spine localization. No evidence of mutations was found in the known predisposing genes by dHPLC and/or SSCP. An in depth search for the genetic abnormality underlying this unusual form of paraganglioma is currently under way. Last, we are currently involved in an international collaborative effort to look for the clinical spectrum associated with mutations of TMEM127, a recently described pheochromocytoma susceptibility gene

ESSENTIAL HYPERTENSION

A. Persu, Division of Cardiology, Saint-Luc, UCL; N. Limaye, and M. Vikkula.

High blood pressure - commonly called hypertension - is found in almost 20 % of the adult population worldwide and affects 2 million Belgians. Despite the wide range of anti-hypertensive therapies available, blood pressure is adequately controlled in only 30-40% of hypertensive patients. In a large majority of cases, no specific cause is found ("essential hypertension") and high blood pressure is thought to reflect the interplay of lifestyle (obesity, high salt intake) and genetic factors; 30-50 % of blood pressure level is thought to be genetically determined. Despite this, conventional linkage and association studies have failed to establish the role of genetic variants in susceptibility.

In an effort to find genetic variations that account for a significant proportion of blood

pressure heritability, and to study the interactions between known variants with mild to moderate effects, we set up a multicentric national genome wide association study (HYPERGEN) with the support of the Belgian Hypertension Committee. We aim to recruit at least 1000 hypertensive patients and 1000 normotensive subjects. Detailed phenotyping including renin and aldosterone dosages are obtained in all patients, and genotyping will be performed using SNP chip technology.

HEMATOLOGICAL MALIGNANCIES AND TUMORS OF SOFT TISSUE AND BONE

H. Antoine-Poirel, V. Havelange, F. Duboux, J. Bodart, G. Ameye, Human Genetics Center, St.Luc, UCL; with M. Vikkula

The explosion in the identification of genetic biomarkers of hematological malignancies and tumors of soft tissue and bone over the past 20 years has had significant impact on diagnosis, prognosis and treatment, as well as our understanding of the genetic and epigenetic processes that lead to tumorigenesis. Our aim is to characterize genomic alterations in both types of malignancy because their oncogenic mechanisms exhibit significant similarities. Towards this end, we use a variety of techniques including conventional and molecular cytogenetics or FISH, molecular biology, and microarrays to identify partner genes in reciprocal translocations and study their functional consequences. We characterized new partner genes of known multipartner genes MLL/11q23, ABL/9q34, PDGFR β /5q33, and USP6/17p13, as well as unknown partner genes. We demonstrated that the functional consequences may be more complex than transcriptional dysregulation by promoter-swap of fusion genes.

In an international therapeutic trial of children with mature B-cell lymphomas, we showed the adverse prognostic impact of chromosomal alterations of 13q and 7q, detected by cytogenetics. Using genome-wide SNP array

technology, we found that most 13q alterations lead to an amplification of the microRNA 17-92 cluster, known to interact with the MYC oncogene, a finding confirmed with mature miRNA expression profiling. In addition, we detected numerous cryptic genomic alterations including partial uniparental disomies. Their prognostic value is currently under study in collaborations within different therapeutic trials across Europe

SNP-CHIP PLATFORM AND NEXT GENERATION SEQUENCING PLATFORMS

M. Amyere and M. Vikkula

We host the UCL oligonucleotide microarray platform (Affymetrix), currently used by several groups in the de Duve Institute and UCL for expression profiling as well as genotyping. We also collaborate with several groups from around the world on whole genome mapping studies. In work done with Dr Jüppner from Harvard Medical School, Boston, we genotyped a large family with a new form of hypophosphatemia and mapped this autosomal recessive form (designated ARHP) to chromosome 4q21. This allowed for the identification of causative homozygous mutations in DMP1 (dentin matrix protein 1), which encodes a non-collagenous bone matrix protein expressed in osteoblasts and osteocytes [9]. In collaboration with G. Matthijs from KULeuven, we have used autozygosity mapping along with expression profile analysis to identify a new gene for congenital glycosylation disorder. In collaboration with Dr Vermeesch, also from KULeuven, we genotyped a large family with autosomal-dominant microtia. Copy number analysis led to the identification of five tandem copies of a copy number-variable region at chromosome 4p16, linked to the disease. With the same group, we established that chromosome instability is common during early human embryogenesis in study of 23 pre-implantation embryos from 9 fertile couples. Additionally, rearrangements such as segmental imbalances

were observed in 70% of the 23 embryos tested. This explains low human fecundity and identifies post-zygotic chromosome instability as a leading cause of constitutional chromosomal disorders [10]. In an exciting development, this platform will be complemented by a High Throughput Sequencing platform, funded by the Fondation Contre le Cancer. This will further enhance our ability to detect mutations and identify genes that participate in various pathogenic processes.

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