Genetic Aspects of Cerebrovascular Malformations

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Summary

In the last decade there have been fundamental advances in our understanding of the pathogenesis of vascular malformations. These advances have resulted from the application of molecular methods to identify disease genes, rather than from immunohistochemical or physiological studies. This presentation reviews the genetic basis of a variety of cerebral vascular malformations which occur as part of well-characterised diseases inherited in an autosomal dominant manner. These highlight the diversity of mechanisms which can perturb vascular development, and should have significant implications for the development of new therapies.

Introduction¹

To familiarise the general reader with terminology and methods, it is helpful to review briefly the principles involved in finding disease genes. Details of the double-stranded structure of DNA need to be considered in order to explain why base-pairing is so strict: adenine (A) with thymine (T); guanine (G) with cytosine (C). The base pairing of DNA explains the generation of the mobile messenger molecule mRNA which carries the genetic code from the DNA in the nucleus, out into the cyto-

plasm where the protein-making machinery of the ribosomes decodes the base sequence in triplets to generate a protein. The base-pairing of DNA also explains how DNA can replicate faithfully, enabling genes to be passed on from parent to child. Differences between DNA molecules exist and account for genetic variation between individuals. The majority of DNA variations (polymorphisms) remain silent. Only a tiny fraction alter a gene product, and only a proportion of these are mutations which lead to disease.

Some of the silent polymorphisms can be used to locate mutated disease genes. Due to the efforts of the Human Genome Project, the chromosomal locations and relative genetic positions of numerous, otherwise insignificant polymorphisms are now known. The inheritance of these polymorphisms can be tracked through generations of a particular family, enabling the transmission of regions of the chromosome to be followed. In families transmitting a genetic disease, these linkage analyses can indicate that a particular region of the chromosome contains the gene that when mutated, causes the disease. This allows molecular analyses to focus on genes in the region, before identifying differences in sequences in the particular gene that is responsible for the disease.

Figure 1 Genes resulting in vascular malformations.

Specific Diseases

A spectrum of abnormal vessels spanning venous, capillary venous, arteriovenous malformations and arterial dysplasia will be discussed. Each type of malformation can occur as part of an inherited disease resulting from a single mutation in a gene on one of chromosomes 1-22 (the autosomes). There will be a second normal copy of the gene on the other chromosome, as all of the autosomes are paired. In such autosomal dominant diseases, males and females are equally affected, and each affected individual will have one affected parent. This makes the genetic basis of the disease relatively easy to recognise, in contrast to autosomal recessive diseases in which mutations are needed in both copies of the gene, and therefore affected individuals have parents who are usually silent carriers.

Cerebral cavernous angiomas, Bean syndrome, Hereditary haemorrhagic telangiectasia (HHT, Rendu-Osler-Weber syndrome) and

neurofibromatosis are four well-recognised autosomal dominant diseases which result in vascular malformations and dysplasia in different parts of the circulation. A key question is whether the abnormal vessels in these diseases result from similar processes occurring in cells of different type, location or maturity, or whether the diseases reflect very different pathological processes converging on the overall process of vascular development.

Hereditary Haemorrhagic Telangiectasia (HHT)

Arteriovenous malformations in HHT are thought to result from dysregulated signalling by TGF- β family members due to mutations in endoglin, ALK-1, and at least one further, as yet unidentified gene.

Hereditary Haemorrhagic Telangiectasia (HHT, Rendu-Osler-Weber syndrome) is most commonly recognised due to the presence of

Figure 2 Cartoon illustrating pathways implicated in the development of cerebrovascular malformations.

nasal, mucocutaneous and gastrointestinal telangiectasia, and/or pulmonary arteriovenous malformations². A significant proportion of patients also develop cerebral arteriovenous malformations³. In a particular family, HHT results from a mutation in one of at least three different genes. Two of these genes have been identified - *endoglin* on chromosome 9⁴, and *ALK-1* on chromosome 12⁵. The location of the third locus is unknown^{6,7}. A large number of mutations have been identified in *endoglin* and *ALK-1* in different families. It appears that the mutated *endoglin* and *ALK-1* genes produce no protein, resulting in an individual with half normal levels of the respective protein. There is continued debate as to whether mutations in *ALK-1* can result in as severe a spectrum of HHT disease as mutations in *endoglin*: cerebral AVMs may be more common in *endoglin* families although formal data is awaited.

The proteins encoded by *endoglin* and *ALK-1* are expressed on the surface of vascular en-

dothelial cell and involved in signalling by growth factors of the transforming growth factor- β (TGF- β) family^{8,9}. It is not clear exactly how the genetic mutations result in the vascular malformations, although from our knowledge of the normal function of particularly the *endoglin* protein, we can speculate that HHT results from the abnormal regulation of processes such as cell proliferation, migration and/or adhesion to the extracellular matrix¹⁰. The majority of abnormal vascular lesions in HHT develop later in life - cerebral AVMs are probably the first to develop, around the time of birth. This indicates that vascular development must begin normally to generate capillary-like endothelial cell tubes, but become abnormal at a later stage. This is supported by data on timing of expression of the normal *endoglin* protein, and most importantly by data from the *endoglin* null mouse which dies at 10.5 days gestation after the development of a rudimentary vascular network¹¹.

Venous malformations

Venous malformations result from mutations in at least two genes, one of which, TIE2, encodes an endothelial cell surface receptor for a different series of extracellular signalling molecules, the angiopoietins.

The relationship of cerebral venous malformations to venous malformations elsewhere in the circulation is not clearly defined. In a proportion of VM patients, the disease is inherited as an autosomal dominant trait, including at least one condition (Bean syndrome), in which cutaneous, gastrointestinal and cerebral VMs occur. In contrast to HHT in which the earliest studies indicated that mutations in at least two genes were likely to cause disease, for several years, only a single gene (*TIE2* on chromosome 9¹²) was known to result in venous malformations. Furthermore, the small number of families presented had an identical mutation which had arisen on different occasions. It is now known that another mutation in *TIE2*¹³, and more importantly, mutations in at least one further gene on chromosome 1¹⁴ can result in venous malformations in different families. The VMs in chromosome 1 families appear to be morphologically different to those in *TIE2* families.

TIE2 encodes another protein expressed on the surface of vascular endothelial cells but unlike endoglin and ALK-1, the *TIE2* protein does not appear to be involved in signalling by the TGF- β family. Instead, it is involved in signalling by another series of ligands, the angiopoietins. These are not (as their name suggests) involved in the earliest stages of blood vessel formation, but again appear to influence how a simple endothelial cell-lined tube develops into a mature vessel, albeit by an apparently different mechanism to endoglin and ALK-1^{15,16}.

Arterial malformations in Neurofibromatosis type I

Neurofibromatosis type I results from mutations in a gene which encodes an intracellular signalling molecule implicated in vascular development.

Neurofibromatosis type 1 (in which a high proportion of individuals have abnormal arteries^{17,18}) results from mutations in *neurofibromin* on chromosome 17¹⁹. Unlike the HHT and venous malformation genes, *neurofibromin* en-

codes an intracellular protein. This interacts with the GTPase activating proteins (GAPs) which deactivate members of the Ras family²⁰. Ras proteins cycle between a GTP-bearing (active) and GDP-bearing (inactive) states, and are involved in numerous aspects of cell signalling, particularly mediating the proliferation response to growth factors. Two of the mammalian Ras proteins, *neurofibromin* and p120-rasGAP, act synergistically in embryonic vascular development, at a stage of reorganisation of the early yolk sac vascular plexus, and embryonic dorsal aorta²¹. It has been proposed that some of the numerous and complex intracellular roles of Ras proteins may be to operate downstream of endothelial cell specific receptors including *TIE2*, mutated in venous malformations²¹.

Cerebral cavernous malformations cavernous angiomas

The first of the three known CCM genes has been recently identified as KRIT1 which encodes a probable intracellular signalling protein.

Autosomal dominant cerebral cavernous malformations are well recognised, and can result from a mutation in one of three different genes on chromosomes 3 and 7 (*CCMs 1-3*^{22,23}). The first of these genes (*CCM1* on the long arm of chromosome 7) has been identified recently as *KRIT1*²⁴. The *KRIT1* protein resides in the cellular cytoplasm, probably attached to fibres of the cell cytoskeleton. Although not a great deal is known about *KRIT1*, it is suspected its known interaction with another protein Krev, that like neurofibromin, *KRIT1* is involved in intracellular signal transduction²⁵. Furthermore, *KRIT1* may also influence intracellular switches between activated and inactivated members of the Ras family, although involvement in other signalling pathways relevant to endothelial cells is also predicted²⁴.

Conclusions and Perspectives

This discussion has focussed on four diseases and nine different genes. There is still much to be understood about the way in which the genetic mutations lead to the development of abnormal vascular structures, and particularly the susceptibility of endothelial cells in different parts of the circulation. It is possible that *TIE2*

(mutated in venous malformation families) signals in part through the downstream signalling pathways perturbed in patients with neurofibromatosis and cerebral cavernous malformations¹⁶, but there is no evidence to suggest this is true of the proteins mutated in HHT. Thus distinct pathogenic pathways appear to be highlighted by these genetic disorders.

We should recognise that these diseases represent just the first of the cerebral vascular malformation diseases to be defined at a molecular level. These conditions, due to the autosomal dominant pattern of inheritance, were rela-

tively easy to recognise as genetic conditions amenable to molecular analyses. In contrast the inherited nature of disease in patients with vascular malformations due to autosomal recessive and polygenic diseases has probably been overlooked. The first stages of the recognition process are awaited from the neurovascular clinics.

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