Investigating the role of imprinted genes in pediatric sporadic brain arteriovenous malformations

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Arteriovenous malformation (AVM) is a vascular congenital defect affecting microvasculature of both brain and peripheral organs. Arteriovenous malformation of the brain (bAVM, OMIM #108010), in particular, affects up to 15 per 100,000 persons with no sex predominance. Almost 50% of the patients manifest intracerebral hemorrhage and epileptic seizures, as main clinical symptoms. Anatomically, lesions exhibit the direct shunt from arterioles to venules, lacking the normal capillary bed. Arterioles and venules are curled forming a tangle called nidus. At the nidus, pericytes are reduced. Feeding arteries and draining veins show impaired expression of vessel differentiation markers. These features result in loss of endothelial cells properties and increased permeability of the affected vessels. The high pressure of blood perfusing from arteries to the nidus increases risk of lesion rupture, resulting in intracerebral hemorrhage. Moreover, at the nidus, arterial and venous blood mixes, altering the normal oxygenation of the central nervous system (Barbosa Do Prado et al., 2019).

bAVM most often arises in adulthood. However, its severity is increased in children. In children, bAVM is more prone to rupture, anticipating the age of symptom onset. Pediatric bAVM represents about 3% of all bAVMs and usually occurs sporadically. Rarely bAVM is inherited as autosomal dominant character and only a few dozen of familial cases have been described. Most often, familial bAVM co-exists with another vascular syndrome known as hereditary hemorrhagic telangiectasia (HHT). HHT is an autosomal dominant disease arising due to perturbation of TGF-β/BMP9 signaling pathway, following germline mutations at the ENG, ACVR1, SMAD4 and GDF2 genes (Giraud et al., 2020).

Therefore, pediatric sporadic bAVM lesions are considered congenital and probably deriving from defects during embryonic vasculogenesis (Pezeshkpour et al., 2020). However, lesions show a severe remodeling rate, increasing their size over the years of the patient and have high recidivism risk. For these reasons, bAVM is considered a “dynamic” lesion resulting from continuous endogenous genetic stimuli driven by genetic factors. These stimuli include inherited germline and de novo genetic variants or epigenetic modifications occurring during embryo development. However, the low incidence of inherited cases makes it difficult to identify genetic factors involved in lesion development. To date, research is limited to the discovery of germline variants in genes related to the TGFβR2/SMAD pathway and of somatic mosaic-activating KRAS mutations that have been found in about 60% of adult patients (Scimone et al., 2020).

A recent study identified the reduced expression of METTL3 in bAVM specimens and this reduction was the greater the larger size of the nidus. In detail, METTL3 down-expression resulted in impaired RNA N6-methylation and continuously activation of Notch signaling pathway (Wang et al., 2020). However, these mechanisms are not sufficient to explain severity of pediatric bAVM. In this context, a new research performed on 112 trios proposed recessive and compound heterozygous mutations affecting angiogenesis-related genes as triggering bAVM development (Zhang et al., 2021) suggesting a different inheritance model for bAVM, usually described as a dominant condition.

In order to increase knowledge about signaling cascades perturbed in pediatric bAVM patients, we previously performed whole exome analysis on a small cohort of affected children. We focused on inherited germline mutations, and considered bAVM as the results of post-zygotic combination of parental alleles (Scimone et al., 2020a, b). However, even if it is useful, this mechanism might not be the only one or sufficient to explain lesion development. Therefore, we decided to focus our attention to another biological mechanism, not yet explored in the field of bAVM onset, that highlights the possible involvement of imprinted genes.

In zygote, genomic imprinting is an exceptional kind of allele-specific expression consisting in permanent hereditable germline-derived DNA methylation. This methylation leads to the selective inactivation of certain autosomal alleles, in relation to their parental inheritance pattern, conditioning the expressivity of the phenotype. Imprinting epigenetic modifications are refractory to embryonic gene expression reprogramming and, for this reason, are indicated as epigenetic heredity. Several genes involved in embryo development are imprinted in mammals.

An imprinting disease occurs when a mutation appears in the expressed allele of imprinted genes, being the wild-type one unexpressed (Chang and Bartolomei, 2020). Imprinting was observed for both coding and noncoding loci and, in the last few years, spectrum of imprinted genes looks set to grow. Currently, in human, more than 100 loci have been demonstrated to be selectively expressed, according to their parental origin. Most of these mutations have been predicted to be imprinted, following inferences from observation in other mammals (https://www.geneimprint.com/site/genes-by-species.Homo+sapiens.imprinted-All).

Our idea that mutations in expressed alleles of imprinted genes can lead to early bAVM development comes from the observation that lesions arise in children despite the healthy parents. This hypothesis is also supported by data collected during our previous studies.

In detail, we examined a small cohort of four affected children (mean age 5.5, male/female ratio 1:1). The group was heterogeneous for both age of onset and clinical manifestation, comprising a child already symptomatic at birth. Following neuroradiological diagnosis, they were investigated by whole exome sequencing in order to detect inherited mutations most likely linked to bAVM development (Scimone et al., 2020a, b). Among the wide spectrum of genes carrying variants in each patient, we found that at least 20 coding loci under a reduced imprint expression due to imprinting mechanism. Therefore, these loci were selected and variants were filtered according to the minor allele frequency (< 0.01) reported in the GnomAD database (https://gnomad.broadinstitute.org/) and the functional classification. In detail, 1 frameshift and 18 missense mutations affecting imprinted coding loci were identified. Of these, three were novel mutations not yet reported in the public variant annotation databases such as Ensembl (http://www.ensembl.org/index.html), dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and GnomAD (https://gnomad.broadinstitute.org/). Disrupting effect of these mutations on the protein structure was predicted by the SIFT prediction tool (Vaser et al., 2016). Then, mutated genes were functionally enriched and clustered according to the biological pathways in which they participate. Most of these genes were annotated to pathways related to transport and homeostasis of cations within the cell (Figure 1). Cation imbalance in non-excitable cells was shown to cause hyperpolarization of the membrane potential. Moreover, impairment of cation concentration was shown to enhance proliferation rate in brain capillary endothelial cells (Yamamura et al., 2016) and we also previously focused on the cation imbalance as possible bAVM stimulating factor (Scimone et al., 2020b).

However, network analysis revealed that 6 imprinted genes (MKRN3, NDN, SLCO2A3, KCNQ1, OSBP1L, LRPL1) may be involved in the regulation of angiogenic process and, in detail, with the TGFβ/SMAD and VEGF signaling (Figure 1). Clearly, this knowledge is it is not yet very thorough and needs further investigation in order to confirm involvement of these loci in brain vasculature development, at the embryo stage. About allele-specific expression, MKRN3 and NDN are maternally imprinted, while SLCO2A3, KCNQ1 and OSBP1L show paternal imprinting. LRPL1 expression pattern are, instead, not yet well characterized. Therefore, we are now going on the study by genotyping the parents of the patients in order to decipher the inheritance pattern.
of the mutations. If they are carried by the expressed alleles, our hypothesis will be strongly encouraged.

Although imprinting diseases usually comprise complex phenotypes, recent results highlighted the impairment of single imprinted loci in congenital vascular defects. Alveolar air-blood barrier dysfunction and, in particular, the alveolar capillary dysplasia with misalignment of pulmonary veins, were shown to arise following FOXF1 mutations or due to aberrant epigenetic modifications at its imprinting control regions that are targets of long noncoding RNAs (Szafranski et al., 2016). Likewise, H19/IGF2 shares the imprinting control region. However, H19 and IGF2 undergo to paternal and maternal imprinting, respectively. Recently, it was demonstrated that common single nucleotide polymorphisms within the imprinting control region of H19/IGF2 promote infantile hemangioma onset. Moreover, the effects of polymorphisms on phenotype severity changed according to their inheritance pattern, suggesting a specific parent of origin effect (Schultz et al., 2015).

Taken together, these findings even more point out the complexity of the imprinting mechanisms as well as the variability of the phenotype spectra. In our study, we are firstly focusing on likely gene-disrupting mutations. However, several variants at regulatory sequences, as 5′-Untranslated and 3′-Untranslated regions were detected and they will be further considered. Pediatric bAVM is classified as congenital defect arising during embryo development (Pezeshkpour et al., 2020). Based on these observations, we hypothesize the possibility that pediatric bAVM can arise due impairment of imprinted genes. Inherited mutations in expressed alleles of imprinted genes, indeed, might explain the autosomal dominant-like pattern of the disease, showed by the affected children despite their healthy parents.

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