NT5E Mutations and Arterial Calcifications

Hilaire et al
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Recessive mutations in the NT5E gene were recently described in lower-extremity vascular calcification. ENPP1 mutations were previously described in a severe disorder of vascular calcification in infancy. Together these data support that extracellular adenosine and pyrophosphate are essential components of the pathways in vascular disease.

Arterial calcification by impairing blood flow and reducing vessel compliance increases the risk for vascular disease in aging, diabetes, and renal disease. Whether or not arterial calcification is an active participant in vascular disease, it is readily identifiable, providing the rationale for less invasive means of coronary artery screening. The molecular mechanisms governing vascular calcification overlap substantially with the mechanisms of bone formation. The recent work of St Hilaire et al1 demonstrates that mutations in the gene encoding CD73, an ectonucleotidase, cause peripheral arterial calcification.

The NT5E gene encodes the cell surface protein CD73, which catalyzes the conversion of AMP to adenosine and inorganic phosphate (Figure). The genetic defect was identified using a single family with 5 affected individuals, ages 44 to 54 years, each of whom had significant vascular calcification of the lower extremities. Because their parents were third cousins, it was reasonable to assume that a homozygous recessive gene was responsible. Using a genotyping array containing a million single nucleotide polymorphisms, the authors identified a single region of homozygosity shared among the affected siblings. This region spanned 22 MB and contained 92 genes, including the NT5E gene. NT5E encodes the second enzyme in the pathway that converts extracellular ATP to adenosine. The first enzyme in this pathway, ENPP1 (also known as NPP1), was previously implicated in a more severe calcification disorder known as idiopathic infantile arterial calcification.2 Idiopathic infantile arterial calcification is characterized by calcification of the internal elastic lamina. Also a recessive disorder, the most severe mutations are those that associate with loss of enzymatic function. ENPP1 encodes an ectonucleotide pyrophosphatase that utilizes extracellular ATP as its substrate, generating AMP and pyrophosphate. Patients with idiopathic infantile arterial calcification often die in infancy, with more extensive vascular calcification afflicting nearly all arterial beds, including the coronary arteries.

The genetic findings in the 3 families described by St Hilaire are compelling in that a truncating mutation was found in the index family (p.S221X).1 A second unrelated family was homozygous for a missense change p.C358Y, and a single affected individual in a third family was a compound heterozygote with the same nonsense mutation from family 1 and a novel insertion that led to a frameshift and stop codon. None of the heterozygous parents displayed features of calcification, suggesting that a threshold of enzymatic activity is sufficient to prevent vascular calcification.

Vascular Pathology and Implications for Smooth Muscle

Vascular calcification from NT5E mutations was largely restricted to the iliac, femoral, and tibial arteries, producing claudication. Joint calcification was also present. Joint calcification also produced pain, leading to the speculation that nerve tissue may be involved in this pathway leading to enhanced pain perception.3 Detailed histopathology on a single patient showed vascular calcification was primarily in the media.4 Disrupted elastic fibers were reminiscent of pseudoxanthoma elasticum. ABCC6 gene mutations have been described in pseudoxanthoma elasticum, and it was
speculated that the unknown ligand for the ABCC6 protein could be adenosine or that adenosine concentration may be critical to the formation of these disrupted elastic fibers. This model fits well with the data supporting smooth muscle as a key cellular target for calcification.5,6 Smooth muscle may undergo transdifferentiation to an osteogenic cell type on stimulation unless blocked by pyrophosphate. The absence of coronary artery involvement in NTSE mutants is intriguing and may suggest that distinct arterial beds are more or less susceptible to the adenosine and pyrophosphate levels.

Animal Models Exhibit Extravascular Calcification

Nt5e null mice have reduced basal coronary flow and reduced time to carotid artery occlusion in an experimental model.7 Increased plaque formation was seen in Nt5e null mice after carotid wire injury, and treatment with the adenosine agonist was able to prevent abnormal neointima formation after injury, supporting that adenosine was the mediator.8 The more severe human disorder from ENNP1 mutations also has murine equivalent referred to as ttw mice.9 Ttw mice are so named for “tip-toe walking,” which develops from ossification of the specific spinal ligament, indicating that these pathways are important for calcification of extravascular tissues. Aortic media calcification can be seen in mice with Ennp1 mutations, and studies using bone marrow stromal cells support that chondrogenesis can be inhibited by exogenous pyrophosphate.10,11

Lessons From Rare Diseases

The index family used to identify NTSE gene mutations was evaluated and studied through the National Institutes of Health Undiagnosed Diseases Program. With careful phenotyping and the ability to rapidly assess the genome, identification of rare diseases is more feasible than ever. Currently, more than 4000 rare diseases have been genetically solved and many have pointed to pathways highly relevant for complex disorders, and the findings of St Hilaire et al are an example of a successful outcome of such approaches. The discovery that ENNP1 and NTSE together play a role in calcification emphasizes the role of adenosine and pyrophosphate in the genesis of vascular disease. The other components of this pathway are also likely candidate genes for mediating or modifying vascular disease. Together, these data point to agents that modify this pathway as potential therapies for treating both rare and potentially more common forms of vascular disease.

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Disclosures

None.

References

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