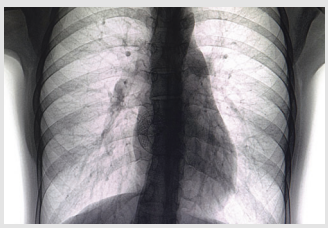
Genetics in Medicine | NEWS

NEWS

SORBS2 variants promote CHD pathogenesis in 4q deletion syndrome



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Chromosome 4q deletion syndrome is a genetic condition that manifests variably with intellectual disability and craniofacial, cardiovascular, and gastrointestinal abnormalities. About half of 4q deletion patients have congenital heart disease (CHD). The critical cardiovascular deletion region, 4q32.2-q34.3, contains several genes, including *HAND2*, which is thought to be mainly responsible for CHD. However, 4q deletions in some patients with CHD do not cover *HAND2*. Liang and colleagues recently reported in *eLife* (https://elifesciences.org/articles/67481) that *SORBS2*, a gene encoding an adapter protein for signaling complexes, within 4q35.1, regulates cardiac development and that variants in the gene contribute to CHD pathogenesis. The researchers knocked down *SORBS2* expression to about 40% of wild type with short hairpin RNAs in human embryonic stem cell lines. In vitro cardiac differentiation showed that *SORBS2*-deficient cardiomyocytes displayed a higher percentage of disorganized sarcomeres than wild-type cells and that cardiomyocyte contraction was much weaker. *SORBS2* knockdown also decreased expression of the secondary heart field (SHF) cardiac progenitor markers *TBX1*, *ISL1*, and *MEF2C*, which give rise to cardiac structures that are commonly defective and lead to CHD in 4q deletion patients. RNA sequencing analysis revealed that *SHH* and SHH signaling targets were reduced in *SORBS2* knockdown cells but exogenous SHH upregulated *ISL1* and *MEF2C* and rescued cardiomyocyte differentiation defects in these cells. Next the researchers investigated a role for SORBS2 in cardiac development in knockout mice. They found that 40% of $SORBS2^{-/-}$ hearts (12/30) exhibited atrial septal defect (ASD). ASD penetrance was similar to the ratio of postnatal lethality, indicating that ASD may contribute to early postnatal death. A third of animals showed dorsal mesenchyme protrusion, which originates from SHF, further supporting the idea that *SORBS2* haploinsufficiency in 4q deletion contributes to CHD pathogenesis via SHF development. Finally, the researchers performed targeted sequencing in 300 complex CHD cases. *SORBS2* and *KMT2D*, a well-established CHD gene, showed statistically significant mutation burden after correction for multiple testing, indicating that rare *SORBS2* variants have levels of enrichment in CHD similar to those in known CHD genes. Missense variants identified in the cohort produced protein aggregates in cells. Most ASD cases (85%) were in patients harboring *SORBS2* variants, and patients with ASD showed an enrichment of *SORBS2* rare damaging variants compared with non-ASD patients. The authors conclude that *SORBS2* variants contribute to CHD pathogenesis in 4q deletion patients. —*V. L. Dengler, News Editor*

Three-hit mechanism behind aggressive cerebral cavernous malformations

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Cerebral cavernous malformations (CCMs), vascular lesions in the brain or spinal cord, are typically slow-growing and clinically silent. Classic genetic studies support a monogenic basis for the disease via loss-of-function variants in *KRIT1*, *CCM2*, or *PDCD10*, genes encoding components of the heterotrimeric CCM protein complex. However, some CCMs are fast-growing and prone to bleeding and can lead to hemorrhagic stroke, seizures, and neurologic deficits. In a study recently published in *Nature* (https://doi.org/10.1038/s41586-021-03562-8), Ren and colleagues found that clinically symptomatic and aggressive CCMs arise through a cancer-like three-hit paradigm. The researchers first knocked out *Krit1* in endothelial cells of mature mice and observed that loss of this gene produced vascular lesions in the testis but not brain. To determine whether CCM formation requires proliferative signals, they next used an inducible transgene to drive expression of a gain-of-function *Pik3ca* variant that is known to accelerate cell proliferation in cancers in brain endothelial cells. These mice developed small lesions in the hind- and forebrain. However, double-mutant $Krit1^{KO}$;$Pik3ca^{GOF}$ mice revealed a synergistic effect on lesion formation. Direct injection of adeno-associated virus (AAV) vector encoding Cre recombinase into $Krit1^{fl/fl}$ or $Krit1^{fl/+}$;$Pik3ca^{GOF}$ adult mice via cranial windows did not induce lesions. In contrast, AAV-Cre injection into $Krit1^{fl/fl}$;$Pik3ca^{GOF}$ mice resulted in large, mulberry-like cavernomas. To learn whether human CCMs harbor variants that promote cellular proliferation, the researchers next sequenced nearly 80 surgically resected CCM lesions across a panel of 66 genes. They identified gain-of-function *PIK3CA* variants in 71% of CCMs. Sequencing also uncovered three distinct genetic hits—in two CCM alleles and one *PIK3CA* allele—in nine familial CCM cases and six presumed sporadic CCM cases. Single-nucleus DNA sequencing revealed that variants in *PIK3CA* and CCM genes arose in the same cells. Follow-up experiments showed that CCM loss of function and *PIK3CA* gain of function enhanced mammalian target of rapamycin (mTOR) signaling in endothelial cells. Treatment with rapamycin, an FDA-approved therapy for venous and lymphatic malformations, nearly ablated CCM growth in adult mice with compound variants in *PIK3CA* and CCM genes. The findings indicate that aggressive CCMs arise via a three-hit mechanism in which loss of vascular "suppressor genes" and gain of vascular "oncogenes" result in excessive vessel growth. The authors conclude that rapamycin or another mTORC1 inhibitor may slow or block this growth. —*V. L. Dengler, News Editor*

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