COMMENTARY

Commentary on Variants in the ABCC6 Gene Implicated in Pseudoxanthoma Elasticum, a Heritable Ectopic Mineralization Disorder

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Significant progress has been made in understanding pseudoxanthoma elasticum (PXE), which results from mutations in ABCC6. The low prevalence of PXE and its heterotypic presentation confound genotype-phenotype correlations and the characterization of many identified variants. Clinical and laboratory studies have recently correlated ABCC6 function with circulating pyrophosphate, which serves as a potent anti-mineralization factor (Jansen et al., 2014, 2013). The loss of plasma membrane-resident, functional ABCC6 underlies the pathophysiology of PXE. While a growing number of ABCC6 sequence variants have been identified, the specific function of ABCC6 in normo- and patho-physiology and its substrate are not well established. Variants have been shown to alter gene expression, protein biosynthesis and trafficking, and protein function. Ongoing international genetic studies are expanding the list of variants, but genotype-phenotype correlations are not well established (Bartstra et al., 2021). Understanding the impact of ABCC6 variants on transporter structure and function and their association with disease pathophysiology is a central focus of the study by Kowal et al. (2021).

These physiological studies inform our understanding of the pathophysiology and development of PXE and have the potential to refine our understanding of normo-physiological maintenance by ABCC6.

Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder characterized by ectopic mineralization in multiple tissues. PXE was first associated with mutations in ABCC6 in 2000 (Bergen et al., 2000; Ringpfeil et al., 2000). ABCC6, a basolateral ATP-Binding Cassette (ABC) -transporter, is most highly expressed in the liver and kidney and expressed at low levels in multiple disease-affected tissues. Clinical and laboratory studies have recently correlated ABCC6 function with circulating pyrophosphate, which serves as a potent anti-mineralization factor (Jansen et al., 2014, 2013). The loss of plasma membrane-resident, functional ABCC6 underlies the pathophysiology of PXE. While a growing number of ABCC6 sequence variants have been identified, the specific function of ABCC6 in normo- and patho-physiology and its substrate are not well established. Variants have been shown to alter gene expression, protein biosynthesis and trafficking, and protein function. Ongoing international genetic studies are expanding the list of variants, but genotype-phenotype correlations are not well established (Bartstra et al., 2021). Understanding the impact of ABCC6 variants on transporter structure and function and their association with disease pathophysiology is a central focus of the study by Kowal et al. (2021).

These studies use heterologous adenovirus-mediated expression of human ABCC6 in an Abcc6⁻/⁻/Rag1⁻/⁻ mouse to evaluate protein expression and plasma pyrophosphate concentration associated with wildtype or variant ABCC6 gene sequences. Prior work has demonstrated that adenoviral delivery and expression of ABCC6 was sufficient to rescue the calcification phenotype measured in the Abcc6 knockout animals (Li et al., 2019). Further, these studies established that changes in measured plasma pyrophosphate levels correlate with ectopic calcification, establishing the use of plasma pyrophosphate as a surrogate biomarker (Dedinszki et al., 2017; Li et al., 2019; Pomozi et al., 2017). Building upon these in vivo studies, Kowal et al. (2021) characterize three gene variants (S400F, L420V, and R1064W) whose bioinformatic predictions lack agreement across multiple computational platforms. This experimental analysis suggests that one, S400F, is pathogenic, whereas the other two, L420V and R1064W, are likely benign. More broadly, this study defines a platform that can be used for the in vivo evaluation of ABCC6 alleles of interest.

In PXE, experimental gene variant analyses are of particular interest. PXE is both rare and heterotypic in its presentation (Bartstra et al., 2021). Many variants have been identified in a limited number of individuals and multiple alleles are reported in single families or single individuals. In addition, genotype-phenotype correlations are not well established in this population. PXE presentation in affected individuals may differentially present with circulatory, gastrointestinal, cardiac, and retinal symptoms. The variability in disease presentation suggests that environmental, behavioral and/or other genetic modifiers play key roles in disease development and progression. This combination of heterotypic disease presentation in a small population confounds de novo predictions for allele pathogenicity. As a result, experimental evaluation will likely be required to provide clinical interpretation and guidance for many individuals with ABCC6 sequence variants.

Biochemical, cell biological, and functional analyses are powerful tools to examine the molecular basis of disease. Previous efforts to characterize gene variants, specifically missense mutations, in ABCC6 have relied

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largely on in vitro cell culture and biochemical approaches (Ran et al., 2018). These studies can readily evaluate the impact of missense and small indel mutations on protein biosynthesis, structure, and subcellular localization. However, human ABCC6 has been largely refractory to functional analysis in these in vitro systems (Jansen et al., 2013). The lack of robust functional measurements confounds the characterization of variants that otherwise traffic and localize to the plasma membrane. Given the diversity in reported ABCC6 variants and their locations within the structure of the transporter, it is likely that many will impact protein function. The development of adequate functional assays is critical for variant characterization and underlies the need for the in vivo murine system described here.

The development of this model system follows in vivo allele studies of Abcc6 in zebrafish (Danio rerio) (Li and Uitto, 2018). Morpholino knockdown of Abcc6 homologs resulted in developmental defects (pericardial edema and curled tails) that could be readily scored approximately one week after fertilization. Coinjection of morpholino and human ABCC6 mRNA partially rescued this knockdown phenotype in these animals. Gene complementation studies of PXE-associated gene variants provided early in vivo characterization of PXE-associated alleles. The rapid development and phenotypic presentation in zebrafish supported its utility. However, the differences in environmental conditions, development, and observed calcification raised concerns over the physiological relevance of this system. Though the phenocopy from human to mouse is not complete, the Abcc6 mouse knockout develops ectopic calcification and is a more robust, physiologically-relevant model for these allelic studies.

There are several limitations that should be considered when evaluating this in vivo murine system. Expression of ABCC6 is not under physiological regulation and is limited to cells infected by the adenoviral vector. The pyrophosphate measurement in this study does not distinguish between ABCC6 expressed in native and non-native cells in the transduced tissues. It is possible that cell-specific changes in protein trafficking, protein-protein association, and transporter regulation influence ABCC6 activity. The expression of ABCC6 in cells that would otherwise not produce ABCC6 may alter its regulation and function and some consideration for the granularity of the adenoviral vector tropism is warranted. Similarly, ABCC6 expression levels may confound the analysis of mutations with intermediate functional activity. Mutations that modestly decrease transporter activity could be mischaracterized if transporter expression is elevated in this system. Moderate changes in transport activity might not be fully captured with the heterologous expression provided by the viral vector if protein expression is elevated. Thus, the pyrophosphate measurement may not fully capture the impact of ABCC6 mutations with mild to moderate molecular phenotypes and may require a more granular analysis of expression and activity. Despite these considerations, the murine system represents a significant step forward in the physiological analysis of ABCC6 and reported variants.

This system also opens the door to address important, unanswered physiological questions in PXE. The ability to engineer and introduce specific ABCC6 sequences for in vivo analysis allows for the annotation of many PXE-associated alleles that are incompletely characterized by existing in vitro analyses. In addition, the potential to utilize tropic vectors for ABCC6 expression may facilitate more granular studies of its tissue- and cell-specific function in the development of PXE. These physiological studies inform our understanding of the pathophysiology and development of PXE and have the potential to refine our understanding of normophysiologic maintenance by ABCC6.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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