Comprehensive re-assessment of causality of *ABCC6* missense variants associated with pseudoxanthoma elasticum

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Introduction

Pseudoxanthoma elasticum (PXE) is an ectopic mineralization disorder affecting the elastic fibers of the skin, the eye and the cardiovascular system. It is caused by biallelic mutations leading to loss-of-function of the ***ABCC6*** gene. To date, over 400 genetic variants have been reported in *ABCC6.* However, high phenotypic variability, a lack of genotype-phenotype correlations and contemporary population genomics raise the question whether all these variants are pathogenic. Especially for missense substitutions it is challenging to predict the impact on protein function (Fig 1b). We therefore re-evaluated *ABCC6* missense variants and classified them according to pathogenicity.

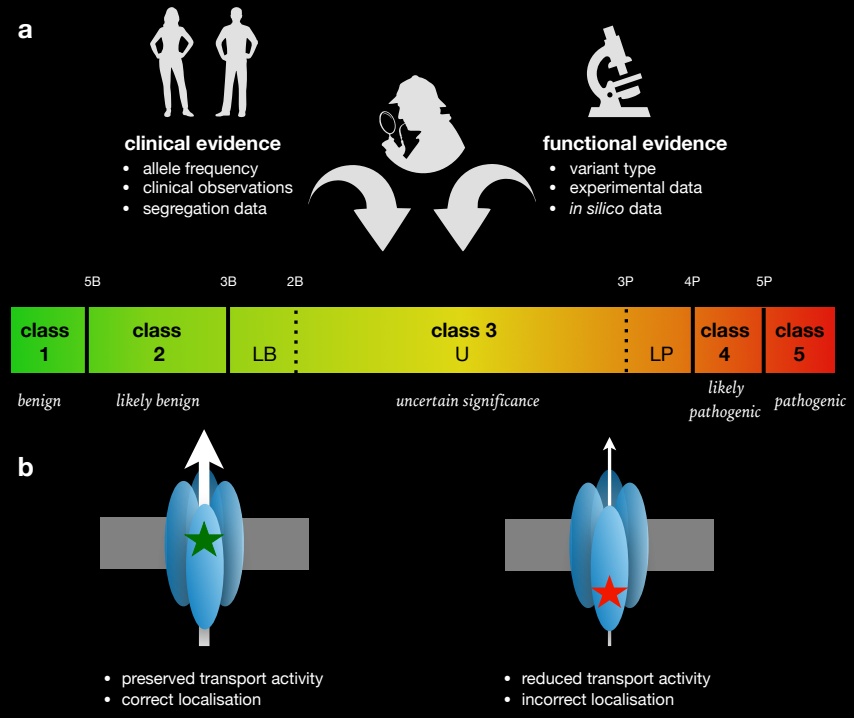

Fig 1*: (a) Evaluation and scoring of clinical and functional evidence leads to classification of the variant based on its total score. (b) Effect of genetic variation on the protein function. The green star represents a benign variant, the red star a pathogenic variant.*

Findings

Comprehensive classification revealed that 74% of *ABCC6* missense variants are class 3 (Fig 2). (Likely) pathogenic variants account for 12% (class 4) and 9% (class 5) respectively, almost all of which are located in the protein's functional domains. (Likely) benign variants are the least represented (1% and 3% respectively). VUS classification was further refined into those that lean towards likely benign (1% of class 3), those that are truly uncertain (69% of class 3) and those that lean towards likely pathogenic (30% of class 3) based on their total score (inset Fig 2).

Taking the latter also into account, **43% of *ABCC6* variants is (likely) pathogenic** when considering all available population, clinical, experimental and *in silico* data (Fig 3). This is significantly different from the variant database ClinVar, where 87% of *ABCC6* missense variants are said to be pathogenic and only 11% are VUS.

Conclusions

Evaluation of *ABCC6* missense variants using the most recent and comprehensive criteria reveals an **overestimation of (likely) pathogenic variants in literature and databases**. Data that are missing for many variants are experimental validation and - surprisingly - segregation data. Our results underline that variant classification should be done systematically and with caution, as variant interpretation has **important consequences for patients and carriers identified via familial or expanded carrier screening.** The high number of VUS confirms the need for functional testing to prove or refute their causality before returning them to patients.

Methodology

We evaluated all *ABCC6* missense variants from ClinVar, from literature and novel variants from in-house patient screenings. In total, 234 variants were analysed using the numerical score-based variant classification system **Sherloc** (Nykamp *et al.* 2017). Clinical and functional evidence were scored with benign (B) or pathogenic (P) points using hierarchical decision trees. Classification was based on the variant's total score: benign (class 1), likely benign (class 2), variants of uncertain significance (VUS) (class 3), likely pathogenic (class 4) and pathogenic (class 5). To distinguish between different types of VUS we created additional subclasses: those that are truly uncertain (3 U) and those leaning towards likely benign (3 LB) or likely pathogenic (3 LP) (Fig 1a).

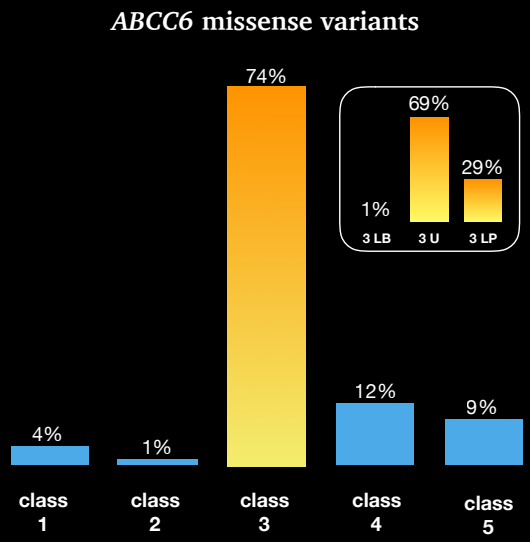

Fig 2*: ABCC6 missense variants classified according to pathogenicity (total variants = 234). Inset: subclassification of class 3 variants into class 3 with likely benign (LB), likely pathogenic (LP) or unclear (U) tendency (in percentage of total class 3 variants).*

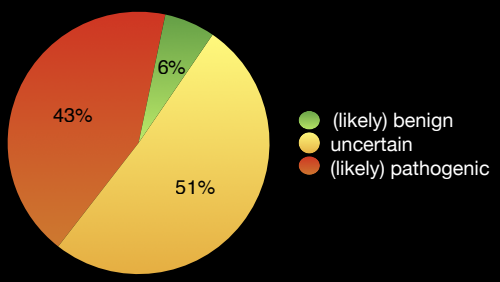

Fig 3: Distribution of (likely) benign (class 1 + 2 + 3 LB), uncertain (class 3 U) and (likely) pathogenic (class 3 LP + 4 + 5) ABCC6 missense variants.

References

Nykamp K et al. *Genetics in Medicine*. **2017,**19(10):1105-1117.

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