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CLINICAL UTILITY GENE CARD

Clinical utility gene card: for pseudoxanthoma elasticum

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1. Disease characteristics

1.1 Name of the disease (synonyms)

Pseudoxanthoma elasticum (PXE), Grönblad-Strandberg syndrome.

1.2. OMIM# of the disease

264800

1.3. Name of the analyzed genes or DNA/chromosome segments

ABCC6 located in 16p13.11 chromosomal segment. The corresponding protein is also named Multidrug Resistance-Associated Protein 6; MRP6.

1.4. OMIM# of the gene(s)

603234

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1.5. Mutational spectrum

More than 300 distinct pathogenic variants have been reported in the *ABCC6* gene [1]. Most of these variants are gathered in the public *ABCC6* variants database (https://databases.lovd.nl/shared/genes/ABCC6). These include missense, nonsense, frameshift, splice-site single-nucleotide variants (SNVs), and small deletions/insertions. In addition, large deletions or duplications of all or part of the gene are also frequently identified. Pathogenic variants are responsible for a partial or complete loss-of-function.

Most variants are private, although two recurrent variants have been described. The SNV c.3421 C > T, p.(Arg1141-Ter) is found with a prevalence of 25% in various ethnic backgrounds, and a large deletion of >16 kb encompassing exons 23–29 (c.2996–1724_4209–478del, also called "del23–29") has a prevalence of 28% in Americans of European descent [2] and 11% in Caucasians [1]. Few other variants are found with a low level of recurrence.

Causative variants are distributed throughout the gene, but two intracellular domains are significantly enriched with missense variants: the eighth intracellular loop and the nucleotide binding fold [1].

Polymorphic variants are listed in the dbSNP Database (http://www.ncbi.nlm.nih.gov/snp/), the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), and in the Genome Aggregation Database (http://gnomad.broadinstitute.org). Please note that the abovementioned databases include pathogenic variants.

1.6. Analytical methods

The analysis should at least combine Sanger sequencing and an analysis of the recurrent deletion del23–29.

Sanger sequencing targets the 31 coding exons and flanking intronic sequences of the *ABCC6* gene (NCBI reference sequence: NG_007558.2, NM_001171.5). Specific primers are needed to avoid amplification of the very similar *ABCC6* pseudogenes Ψ1 and Ψ2 sequences [3].

The del23–29 can be analyzed with a published method using multiplex long-range PCR [4] or by multiplex ligation-dependent probe amplification (MLPA).

MLPA or any other technique targeting genetic imbalances of *ABCC6* exons should be systematically performed in cases with single heterozygous point variants or suspected hemizygosity based on sequencing results. However, MLPA lacks coverage for several *ABCC6* exons (exons 1, 3, 6, 16, 19, 20, 29, and 31).

Combining Sanger sequencing with multiplex PCR and MLPA identifies variants in ~90% of patients. Indeed, deep intronic variants, large deletions, and duplications including exons not explored by MLPA would not be detected using this strategy.

1.7. Analytical validation

Sequencing of both DNA strands for SNVs. Disease-causing variants should be confirmed using genomic DNA from an independent sample.

When an unknown variant (UV) is identified, analytical validation should systematically integrate interspecies conservation analysis of the mutant nucleotide(s) and amino-acid(s), as well as the surrounding sequence(s). The identified variant(s) should systematically be searched in polymorphisms and disease variants databases.

1.8. Estimated frequency of the disease (incidence at birth ("birth prevalence") or population prevalence)

Estimated population prevalence ranges between 1/25,000 and 1/50,000 [5].

Incidence of *de novo* pathogenic variants is very low [1, 6]. All ethnic groups are involved. There is a sex bias toward women (sex ratio 1:2 [1]).

If known to be variable between ethnic groups, please report:

The frequency does not seem to vary between different ethnical backgrounds. However, in consanguinous populations occurrence of new cases could theoretically increase.

1.9. Diagnostic setting

	Yes.	No.
A. (Differential) diagnosis	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☐	☒

Comment: A first comment must be made concerning differential diagnoses. One first differential diagnosis has to be considered in case of severe and early-onset arterial calcifications. This recessive disorder is named generalized arterial calcification of infancy (GACI) and is caused by pathogenic variants in the *ENPP1* gene encoding ecto-nucleotide pyrophosphatase/phosphodiesterase 1. It is characterized by hydroxyapatite deposits in the internal elastic lamina of large and medium-sized arteries associated with intimal proliferation, leading to severe arterial stenoses, and organs' ischemia. Causal variants in *ENPP1* are found in approximatively 75% of cases [7, 8]. Among the remaining 25%, some cases harbor mono- or biallelic *ABCC6* pathogenic variants. Hence an important overlap of genotypes and phenotypes shows that GACI and PXE belong to the same clinical spectrum of ectopic calcification [9]. To date, no digenism has been described between these two genes.

The second differential diagnosis is a recessive condition named PXE-like disorder with multiple coagulation factor deficiency (PXE-like) and is caused by pathogenic variants in *GGCX* encoding γ-glutamyl carboxylase [10]. PXE-like shares some manifestations with PXE: yellowish papules or leathery plaques associated with fragmentation and calcification of elastic fibers in the dermis, ocular peau d'orange, and/or angiod streaks (AS), and accelerated arteriosclerosis. Differential diagnosis relies on more widespread and severe skin lesions and inconstant association with retinopathy, cardiac malformations, and deficiency of the vitamin-K dependent factors resulting in abnormal bleeding tendency [11]. Cases with PXE-like skin phenotype and no clotting factor deficiency were found to harbor pathogenic variants in both genes, one variant in *GGCX* and one variant in *ABCC6*, revealing digenic inheritance [12].

Another differential diagnosis is about angiod streaks, which are not pathognomic of PXE. Indeed hemoglobinopathies, and in particular sickle-cell disease and β-thalassemia, should be considered.

A second comment is needed concerning the family screening. Identifying two pathogenic variants in a patient enables to test the siblings. Asymptomatic parents are expected to have transmitted one pathogenic variant each, and their screening is not relevant. Few cases of pseudo-dominant transmission have been described and were proven to occur in families where three mutations co-segregate [1, 13]. In these latter cases, the parents are tested to properly define inheritance of the variants.

A third comment relates to prenatal diagnosis. A prenatal test is technically feasible. However, in most cases PXE is not a life-threatening condition and is neither responsible for major disability. Thus pregnancy termination is not required.

2. Test characteristics

	Genotype or disease		A:true positives	C: false negative
	Present	Absent	B:false positives	D:true negative
Test				
Pos.	A	B	Sensitivity: specificity:	A/(A+C) D/(D+B)
Neg.	C	D	Pos. predict. value: Neg. predict. value:	A/(A+B) D/(C+D)

2.1. Analytical sensitivity

(proportion of positive tests if the genotype is present)

100%. If only one mutation is identified by the combination of Sanger sequencing and multiplex PCR targeting the recurrent deletion del23–29, the analysis should be completed by MLPA to detect other large rearrangements. Unfortunately only 23 of the 31 exons of *ABCC6* are investigated by MLPA. In case of obvious clinical diagnosis and a negative MLPA result, a high-density array-CGH or another semi-quantitative technique covering the 31 exons should also be performed.

2.2. Analytical specificity

(proportion of negative tests if the genotype is not present)

Analytical specificity reaches nearly 100%. False positive results are rare and could be explained by misinterpreting known or unknown variants: some SNV were historically classified as pathogenic, and should be reclassified as polymorphism due to their frequency in the general population (for example, p.(Arg391Gly) minor allele frequency is 0.8% in Caucasians but was initially reported pathogenic [3]). ABCC6 was found to have a role in hepatic ATP release and then its conversion in inorganic pyrophosphate (PPi), a key inhibitor of ectopic mineralization [14]. However, no functional test (e.g., measurement of plasma PPi level) is available to confirm or deny the pathogenic effect of UV.

2.3. Clinical sensitivity

(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

Clinical sensitivity theoretically reaches 100%. A series published in 2017 raises clinical sensitivity to 82% for all clinical PXE cases and points out that only one pathogenic variant is identified in some cases with uncomplete or atypical phenotype (harboring only skin or ocular manifestations) [1].

Two hypotheses are proposed to explain these results. Heterozygous carriers might display slight skin or eye manifestations [15], which could correspond to a milder phenotype in carriers. Alternatively, the second causal variant could be a non-coding variant not detected by current analysis methods [1].

Inherited disorders close to PXE, like GACI, related to *ENPP1* deficiency, or PXE-like, related to *GGCX* deficiency, could also explain incomplete *ABCC6* genotypes. Taking into account the clinical overlap between these conditions and PXE, molecular screening of these two genes should be performed in patients with no or one *ABCC6* variant.

2.4. Clinical specificity

(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors, such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

Clinical specificity is nearly 100% in adults. Indeed, prevalence of skin and eye symptoms increases with age [16].

2.5. Positive clinical predictive value

(life-time risk to develop the disease if the test is positive)

Non-penetrance in adulthood is rare and positive predictive value is close to 100%. The clinical severity increases with age and is unpredictable due to intra-familial and inter-familial variability.

2.6. Negative clinical predictive value

(probability not to develop the disease if the test is negative)

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested and two causative variants identified:

100%. If the non-affected relative does not carry the two causative variants or carries only one variant, he or she has no risk to develop the disease.

Index case in that family had not been tested:

The theoretical recurrence risk is of 25% for the siblings due to recessive inheritance.

3. Clinical utility

3.1. (Differential) diagnostics: The tested person is clinically affected

(To be answered if in 1.9 "A" was marked)

3.1.1. Can a diagnosis be made other than through a genetic test?

No.	☐ (continue with 3.1.4)	
Yes,	☐	
	clinically.	☒
	imaging.	☐
	endoscopy.	☐
	biochemistry.	☐
	electrophysiology.	☐
	other (please describe):	Histopathology.

3.1.2. Describe the burden of alternative diagnostic methods to the patient

Clinical diagnosis (combining family history and physical examination) associated with histopathological analysis of a skin biopsy is the only alternative method.

As the combination of skin and eye typical lesions is present in most cases with one or two variants [1], both organs must be evaluated by expert clinicians in dermatology and ophtalmology. Skin examination can reveal yellowish papules in the flexural locations or coalescent plaques, which rarely affect non-flexural sites. Histopathological skin analysis confirms the diagnosis and shows an increase and fragmentation of elastin fibers with typical clumping and calcification on light microscopy and von Kossa staining.

PXE ocular signs, peau d'orange, and angioid streaks in the retina are detected by fundus. They are sometimes associated with complications: retinal hemorrhages and neovascularization.

Cardiovascular and renal complications are rare (10% of cases). Their evaluation is generally used for severity assessment but not for clinical diagnosis.

3.1.3. How is the cost-effectiveness of alternative diagnostic methods to be judged?

Physical examination, skin biopsy, and fundus are cost-effective, compared to genetic screening. However, clinical diagnosis does not help for screening the at-risk relatives, particularly in young adults and children.

3.1.4. Will disease management be influenced by the result of a genetic test?

No. ☐

Yes. ☒

Therapy (please describe)	There is no specific treatment for PXE. The treatment depends on clinical manifestations (*cf* Management).
Prognosis (please describe)	Genotype–phenotype correlation shows a more severe eye and vascular phenotype in patients with total loss-of-function variants. This implies an enhanced follow-up based on fundoscopy and vascular imaging.
Management (please describe)	The current management of PXE is multidisciplinary (dermatologist, ophthalmologist, cardiologist, vascular and plastic surgeon, geneticist, nutritionist). The management and treatment depend on clinical manifestations and include: -Eye management: the patients should learn to use the Amsler grid to control for central visual disturbances. Eye protection is needed for high-risk activities. Intravitreal injections of vascular endothelial growth factor (VEGF) antagonists are used to prevent neovascularization in cases of retinal hemorrhages. -Lipid levels should be monitored periodically and treated if necessary (disease-causing lipid alterations are not linked to PXE but they should be avoided to prevent atherosclerosis and aggravation of existing vascular lesions due to PXE). Endovascular and surgical intervention are required for severe peripheral vascular disease. -Surgical intervention can be necessary for gastrointestinal bleeding. -Plastic surgery is sometimes used for redundant skin reduction. -Other treatments are currently being assessed: magnesium intake, phosphate binders, and bisphosphonates such as etidronate (bisphosphonates are pyrophosphate analogs, they are used to treat ectopic mineralization thanks to their antimineralization and antiosteoclastic activities) [17].

3.2. Predictive setting: The tested person is clinically unaffected but carries an increased risk based on family history

(To be answered if in 1.9 "B" was marked)

3.2.1. Will the result of a genetic test influence lifestyle and prevention?

If the test result is **positive** (please describe):

Dietary and lifestyle modifications are needed to delay or prevent complications. Cardiovascular risk factor (particularly overweight, hypertension, lipid disorders, and smoking) prevention and treatment is essential: smoking cessation, weight loss, daily walking, moderate physical exercise, etc. Aspirin and other non-steroidal anti-inflammatory medications should be avoided because of gastrointestinal hemorrhage risk. Contact sports without appropriate eye and head protection should also be avoided to prevent eye trauma that could cause retinal hemorrhage. Magnesium supplementation could be also proposed to PXE patients to prevent connective tissue mineralization (the benefits observed in mice have to be proven in humans) [18].

If the test result is **negative** (please describe):

No.

3.2.2. Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

Lifestyle and prevention should be exactly the same as above if the clinical diagnosis is certain.

3.3. Genetic risk assessment in family members of a diseased person

(To be answered if in 1.9 "C" was marked)

3.3.1. Does the result of a genetic test resolve the genetic situation in that family?

Genetic testing is necessary for genetic risk assessment in siblings.

3.3.2. Can a genetic test in the index patient save genetic or other tests in family members?

Not applicable

3.3.3. Does a positive genetic test result in the index patient enable a predictive test in a family member?

Family screening can be performed after identification of two causal variants in the index case.

3.4. Prenatal diagnosis

(To be answered if in 1.9 "D" was marked)

3.4.1. Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Not applicable.

4. If applicable, further consequences of testing

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

The genetic test confirms the clinical diagnosis of PXE and allows genetic counselling, family screening, and prevention of complications. For example, prevention of eye trauma with adapted protection in high-risk activities can be proposed to the concerned relatives.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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