Original Article

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Pulmonary affection of patients with Pseudoxanthoma elasticum: Long-term development and genotype-phenotype-correlation

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SUMMARY Pseudoxanthoma elasticum (PXE) is a rare, heritable disease caused by various, mainly recessively transmitted mutations in the *ABCC6* gene. Due to calcification of soft connective tissue phenotypic hallmarks are progressive loss of vision, alternation of the skin and early onset atherosclerosis. Beside these main features patients also suffer from impaired alveolar diffusion. The present study focused on impaired lung functioning based on a large cohort of patients with PXE, its long-term development, and genotype-phenotype correlation. Retrospectively, 98 patients and 45 controls were enrolled. All patients underwent body plethysmography and carbon monoxide diffusion testing. Of 35 patients three or more body plethysmographic records were available for long-term analysis. For genotype-phenotype analysis *ABCC6* genotypes were grouped as two missense, mixed, or two nonsense mutations. Patients with PXE showed significantly reduced vital capacity ($p < 0.05$), diffusion capacity ($p < 0.01$), and diffusion transfer coefficient ($p < 0.05$). Over a mean period of 38 months diffusion capacity ($p < 0.05$) and diffusion transfer coefficient ($p < 0.01$) dropped significantly whereas lung volumes remained unchanged. Genotype-phenotype correlation revealed no connection between gene variants and lung functioning. In conclusion, PXE is accompanied by progressive reduction of alveolar diffusion indicating progressive alterations of lung tissue. Genotype-phenotype correlation with genotypes sorted as missense and nonsense mutations do not explain impaired lung functioning.

Keywords Pseudoxanthoma elasticum, lung functioning, restrictive lung disease, alveolar diffusion, genotype-phenotype-correlation

1. Introduction

Pseudoxanthoma elasticum (PXE) is a rare, genetic, metabolic disease caused by autosomal recessive mutations of *ABCC6* gene (*1-3*) with an estimated prevalence between 1:25.000 and 1:56.000 (*4-6*). The human ATP-binding cassette family C member 6 (*ABCC6*) gene encodes an ABC transporter protein, which is mainly expressed in liver and kidneys. *ABCC6* deficiency is associated with low plasma pyrophosphate levels (*7*). Pyrophosphate is one main inhibitor of systemic calcification (*8*). Mutations of *ABCC6*, therefore, would result in decreased blood levels of pyrophosphate and, subsequently, systemic calcification. Resulting characteristic PXE phenotype consists of progressive loss of vision (*9,10*), formation of yellowish papules and coalescing plaques in the skin (*1,11*), and early-onset atherosclerosis (*12,13*). Still, the main substrate of *ABCC6*-encoded transporter protein remains unknown and pathology, subsequently, is yet to be illuminated.

Since PXE is a rare disease, current research mostly focuses on these main features of the disease while other less obvious characteristics remain unexplored until now. Nonetheless, they are essential to fully understand PXE and the medical condition of afflicted persons.

One of these less obvious characteristics is the affection of the lung in patients with PXE. The first report of impaired lung functioning in patients with PXE was published by our department in 2016 by Pingel *et al.* (*14*). Herein, the authors reported reduced carbon monoxide (CO) diffusion capacity in a group of 35 patients with PXE, which was interpreted as a preclinical state of interstitial lung disease. This assumption was supported by several postmortem

examinations of patients with PXE: for example, Jackson and Loh (1980) reported one case with a substantial amount of calcified deposits in alveolar septa with fibrous thickening and perivascular fibrosis (*15*). Puvaneswary (1986) observed bilateral radiographical opacities due to pulmonary calcification induced by elastic tissue damage (*16*). Yamamoto *et al.* presented a case of a woman with calcified nodules scattered in alveolar septa (*17*). Lately, Vos *et al.* (2018) found pleural lesions in a patient with PXE (*18*). Recently, our department characterized pathological nailfold capillaries in patients with PXE (*19*). Herein, body plethysmography revealed reduced vital capacity and Tiffeneau Index in patients with PXE compared to a control group. However, no impairment of CO diffusion capacity occurred.

There is a wide interindividual variance of characteristic features in patients with PXE. Therefore, many attempts have been made to describe genotype-phenotype correlations to better predict individual risk of a severe course of the disease (*20-23*). However, this is difficult regarding the variety of pathogenic *ABCC6* mutations (*22-24*) and possible moderating cofactors such as mutations the *ENPP1* and *GGCX* genes (*25,26*). Additionally, often small sample sizes hinder informative value of these studies. Due to the complicacy of reasonable grouping *ABCC6* mutations, classification according to functionality of the translated protein has been established (*22,23*). Therefore, mutations are classified *via* their resulting protein as missense and nonsense mutations or truncating and non-truncating variants, respectively. Recently, Legrand *et al.* (*22*) and Bartstra *et al.* (*23*) showed that patients with nonsense, or truncating variants respectively, were more severely affected from eye lesions and arterial calcification. Genotype-phenotype correlation regarding lung functioning has never been attempted.

This retrospective study intended to clarify the severity of impaired lung functioning by means of a large cohort of patients with PXE. It further aimed for enlightening the development of lung functioning parameters in long-term follow up, and, in a final step, for specific genotype-phenotype-correlations in relation to impaired lung function.

2. Patients and Methods

This study surveyed body plethysmographic data of patients with PXE assessed between August 2014 and December 2020. It was conducted according to the principles of the Declaration of Helsinki for Human research and has been approved by the local ethics committee of the University of Bonn (no. 126/21). Written informed consent has been obtained from all patients and controls. Diagnosis of PXE was confirmed either genetically or by the results of fundoscopy combined with the results of skin biopsy.

2.1. Patients and controls

Inclusion criteria were sufficient information concerning baseline characteristics and conduction of body plethysmography at baseline. If patients showed three or more records of body plethysmography, records of one-year-follow-up as well as the latest record were included to illustrate long-term development of lung functioning parameters.

In total, 103 patients with PXE were surveyed. 5 Patients were excluded due to missing body plethysmographic data. Therefore, 98 patients were included. Of those, 35 patients presented with three or more records of body plethysmography (baseline, FU-1, FU-2) and entered subgroup analysis of long-term development. Of 69 patients *ABCC6* genotype was available.

Body plethysmographic data of 45 patients without PXE were assessed during clinical routine serving as control group. Baseline characteristics are presented in Table 1, no intergroup differences between baseline and control occurred.

2.2. Body plethysmography

All patients and controls underwent body plethysmography and CO-diffusion testing. Examinations were performed by qualified personnel using Body plethismograph Jaeger® respectively Alveo-Diffusionstest Jaeger® in single breath mode according to current guidelines (*27*). All assessed values were recorded as standard value and percentage of predicted value. The latter were calculated by integrated software during body plethysmography referring to reference values provided by the Global Lung Initiative (*28*). Abbreviations corresponding to percentage of predicted values are labeled with "%". The following parameters entered statistical analysis: total lung capacity (TLC, TLC%), vital capacity (VC, VC%), residual volume (RV%), forced expiratory volume (FEV1, FEV1%), Tiffeneau Index (FEV1/FVC), and Hb-adjusted diffusion parameters (DLCO/SB%, DLCO/

Table 1. Baseline characteristics

Variables	PXE Baseline (n = 98)	Control (n = 45)	p
Gender [female] (%)	63 (64.3)	21 (46.7)	0.067
Age [years]	49.6 ± 14.2	54.1 ± 13.8	0.078
BMI [kg/m^2]	27.33 ± 6.02	26.50 ± 4.23	n.s.
Nicotine abuse* (%)	46 (46.9)	26 (57.8)	n.s.
Packyears	5.8 ± 10.5	9.1 ± 20.0	n.s.
Diabetes (%)	3 (3.1)	3 (6.7)	n.s.
Hypertension (%)	38 (38.8)	17 (37.8)	n.s.
Renal dysfunction (%)	0 (0.0)	0 (0.0)	n.s.
Dyslipidemia (%)	36 (36.7)	10 (22.2)	n.s.
COPD (%)	2 (2.0)	3 (6.7)	n.s.
Asthma (%)	0 (0.0)	0 (0.0)	n.s.

*Current and former nicotine abuse. BMI: Body Mass Index, COPD: Chronic obstructive pulmonary disease.

VA%). DLCO/SB (diffusion capacity) describes the amount of CO diffusing from alveoli into the blood in 10 ± 2 seconds. DCLO/VA (diffusion transfer coefficient) describes CO diffusion in relation to alveolar volume. Isolated reduced values of DLCO/SB indicate impaired gas distribution (*e.g.*, emphysema) whereas concomitant reduction of DLCO/VA implicates impaired diffusion (*27*).

Obstructive and restrictive body plethysmographic pattern was defined according to Pellegrino *et al.* (2005). Thereby, an obstructed pattern was assumed in patients with reduced FEV1/FVC (< 70%) and normal VC or, respectively, reduced VC and normal TLC. Restrictive pattern was diagnosed in patients presenting with normal FEV1/FVC and reduced VC and TLC (*29*).

2.3. Genotype-phenotype analysis

All 69 patients with available mutational analysis were included in genotype-phenotype analysis. Patients without or incomplete *ABCC6*-sequencing were excluded as well as those without a detected mutation on the second allele. Grouping of the remaining 58 patients was performed according to Legrand *et al.* (2017) (*22*). Therefore, mutations were sorted by mutation type as missense and nonsense mutations. As a result, included patients were assigned to three groups according to mutation combination of their alleles (missense/missense, missense/nonsense, nonsense/nonsense). Intergroup differences were calculated by means of baseline TLC%, VC%, RV%, FEV1%, and CO-diffusion parameters.

Patients with complete *ABCC6* sequencing and long-term body plethysmography data were analyzed as a subgroup according to the development of diffusion parameters in relation to genotype.

2.4. Statistical analysis

Statistical analysis was performed using IMB® SPSS® Statistics, Version 26. To calculate intergroup differences of nominal and ordinal scaled variables Cramer's V and χ^2-test, respectively, were applied. Continuously scaled parameters were compared *via* independent-sample *t*-test respectively ANOVA for calculating intergroup differences in long-term follow up subgroup analysis. Two-tailed *p*-value was defined significant at 0.05-level. Continuously scaled variables are presented as mean ± standard deviation.

3. Results

No significant differences regarding baseline characteristics occurred (Table 1). Of note, the control group was insignificantly older, reported a higher amount of pack years, and contained more men compared to PXE.

3.1. Body plethysmography at baseline

Results of body plethysmography are presented in Table 2. Four patients presented with restrictive pattern, two showed obstructive body plethysmographic pattern. Patients with PXE showed significantly reduced values of TLC, VC, VC%, and FEV1 compared to control. No differences occurred regarding TLC%, FEV1%, and RV%.

Regarding diffusion parameters both DLCO/SB ($p < 0.01$) and DLCO/VA ($p < 0.05$) were significantly lower in patients with PXE. 40% of patients with PXE showed reduced DLCO/SB% corresponding to a Z-score ≤ -1 (decreased DLCO/SB% ≥ one standard deviation) compared to control ($p < 0.001$).

3.2. Long-term development of body plethysmographic parameters

A total number of 35 patients merged into subgroup analysis for long-term development (Table 3). First

Table 2. Results of body plethysmography

Variables	PXE (*n* = 98)	Control (*n* = 45)	*p*
Restrictive pattern [*n* (%)]	4 (4.1)	2 (4.4)	n.s.
Obstructive pattern [*n* (%)]	2 (2.0)	3 (6.7)	n.s.
TLC [l]	5.86 ± 1.23	6.58 ± 1.40	< 0.01
TLC% [%]	102 ± 14	104 ± 16	n.s.
VC [l]	3.57 ± .84	4.10 ± 1.07	< 0.01
VC% [%]	95 ± 15	101 ± 17	< 0.05
RV% [%]	119 ± 34	121 ± 31	n.s.
FEV1 [l]	3.04 ± .71	3.35 ± .87	< 0.05
FEV1% [%]	99 ± 16	103 ± 14	n.s.
FEV1/FVC [%]	85 ± 10	83 ± 6	n.s.
DLCO/SB% [%]	78 ± 13	85 ± 10	< 0.01
DLCO/VA% [%]	87 ± 13	93 ± 12	< 0.05
DLCO/SB% (Z-score ≤ -1) [*n* (%)]	39 (40.0)	4 (8.9)	< 0.001
DLCO/VA% (Z-score ≤ -1) [*n* (%)]	12 (12.2)	1 (2.2)	0.062

Abbreviations amended with % represent percentage of predicted value; TLC: total lung capacity; VC: vital capacity; RV: residual volume; FEV1: forced expiratory volume; FEV1/FVC: Tiffeneau Index; DLCO/SB: CO-diffusion capacity; DLCO/VA: diffusion transfer coefficient.

Table 3. Long-term development of body plethysmographic parameters

Variables	Baseline (n = 35)	FU-1 (n = 35)	FU-2 (n = 35)	p
Period to baseline [months]		12 ± 4	38 ± 12	
Restrictive pattern [n (%)]	1 (2.9)	2 (5.7)	1 (2.9)	n.s.
Obstructive pattern [n (%)]	0 (0.0)	0 (0.0)	0 (0.0)	n.s.
TLC% [%]	100 ± 12	103 ± 13	108 ± 13	< 0.05
VC% [%]	97 ± 12	98 ± 12	97 ± 12	n.s.
RV% [%]	113 ± 24	119 ± 30	128 ± 27	n.s.
FEV1% [%]	103 ± 15	102 ± 14	99 ± 14	n.s.
FEV1/FVC [%]	87 ± 6	85 ± 6	85 ± 6	n.s.
DLCO/SB% [%]	78 ± 11	77 ± 14	70 ± 10	< 0.05
DLCO/VA% [%]	87 ± 12	86 ± 13	77 ± 12	< 0.01
DLCO/SB% (Z-score ≤ -1) [n (%)]	14 (40)	18 (51)	29 (83)	< 0.001
DLCO/VA% (Z-score ≤ -1) [n (%)]	4 (11)	6 (17)	15 (43)	< 0.01

Abbreviations amended with % represent percentage of predicted value; TLC: total lung capacity; VC: vital capacity; RV: residual volume; FEV1: forced expiratory volume; FEV1/FVC: Tiffeneau Index; DLCO/SB: diffusion capacity; DLCO/VA: diffusion transfer coefficient.

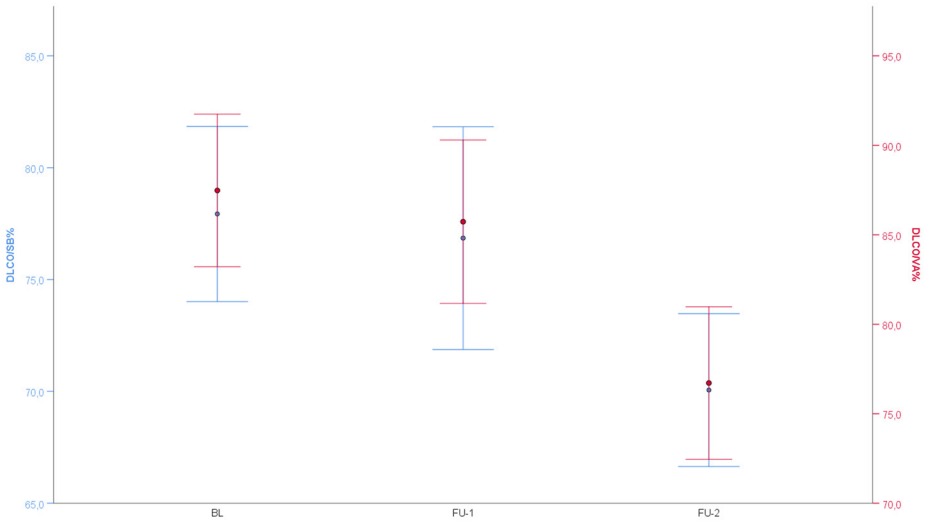

Figure 1. Mean values of diffusion capacity (DLCO/SB%) and diffusion transfer coefficient (DLCO/VA%) at baseline (BL), after 12 months (FU-1), and after 38 months (FU-2).

follow up examination (FU-1) after baseline was conducted after 12 ± 2 months. The second follow up examination (FU-2) was performed after 38 ± 12 months. None of the patients observed in long-term development showed an obstructive ventilatory pattern. Further, no increase of patients with restrictive pattern was observed. No relevant intergroup differences regarding body plethysmography occurred. Diffusion parameters decreased over time (Figure 1). Decrease of DLCO/VA% was even more distinct ($p < 0.01$) compared to DLCO/SB% ($p < 0.05$). Also, the number of patients with relevant reduced DLCO/SB% ($p < 0.001$) and DLCO/VA% ($p < 0.01$) (Z-score ≤ -1) grew significantly.

3.3. Genotype-phenotype-correlation

ABCC6 mutation analysis was available for 69 patients with PXE. Within those, 46 different mutations occurred. Mutations and their incidence are presented in Supplemental Table S1(*http://www.irdrjournal.com/action/getSupplementalData.php?ID=89*). The c.3421C>T (p.Arg1141*) mutation was detected in 49 alleles and, therefore, occurred most frequently by far. Interestingly, in 11 patients with complete *ABCC6* mutation analysis no mutation on the second allele was found. Further, to the best of our knowledge, the seven mutations c.3179C>G (p.Pro1060Arg), c.2399G>A (p.Gly800Arg), c.2230A>C (p.Thr744Pro), c.2090C>T (p.Pro697Leu), c.1589T>C (p.Leu530Pro), c.3679_3770insC, and c.2071-1G>A on *ABCC6* gene have not been reported previously.

No intergroup differences between patients with missense/missense (m/m; n = 11), missense/nonsense (m/n; n = 18), and nonsense/nonsense (n/n, n = 29) occurred (Table 4A). Of those, 25 patients had three or more body plethysmographic records and entered subgroup analysis (Table 4B, Figure 2). The decrease of diffusion parameters seen in long term analysis was mirrored by all three groups (m/m; m/n; n/n).

Although diffusion values were lower in patients with n/n mutation pattern, no level of significance has been reached.

4. Discussion

Clinical data on impaired lung functioning of patients with PXE is scarce. A comprehensive PubMed search using the search item "*Pseudoxanthoma elasticum AND lung*" yielded two results (*14,19*). Therefore, this study is the largest clinical investigation of lung functioning in patients with PXE up to now. It was demonstrated that PXE is frequently accompanied by reduced diffusion parameters. Moreover, patients with PXE presented with significantly reduced TLC, VC, VC% and FEV1. The combination of reduced total lung capacity, vital capacity, and diffusion parameters can be interpreted as restrictive lung disease. This conclusion, however, cannot be drawn unconditionally from present data. That is, on the one hand, due to VC% values within reference and, on the other hand, due to stable or even increasing values of TLC% and VC% in long-term development. Moreover, there was no relevant number of patients with a restrictive ventilatory pattern. With 2% of patients with an obstructive ventilatory pattern in PXE, chronic obstructive pulmonary disease (COPD) is underrepresented in this sample compared to the literature (*30*). This may be due to relatively young age and a low mean number of pack years in this sample. Also, patients with PXE, being aware of their diagnosis, often live a healthy lifestyle.

Therefore, these results mainly indicate an isolated impairment of alveolar diffusion in PXE and, subsequently, confirm the assumptions of Pingel *et al.* (2016) (*14*).

In general, reports of long-term development in patients with PXE are rare. This investigation surveyed body plethysmographic data over a mean period of 38 months. During this time, diffusion parameters dropped significantly whereas mobilizable lung volume remained unchanged. This indicates a progressive impediment of diffusion through the alveolar-capillary membrane. A rationale based on pathology, however, is not easy to find since there still is a lack of knowledge regarding high-resolution CT-Imaging of the lungs and

Table 4A. Diffusion parameters in relation to genotype

Variables	m/m (n = 11)	m/n (n = 18)	n/n (n = 29)	p
TLC% [%]	102 ± 14	102 ± 12	98 ± 13	n.s.
VC% [%]	99 ± 20	98 ± 11	94 ± 15	n.s.
RV% [%]	116 ± 20	117 ± 24	108 ± 27	n.s.
FEV1% [%]	101 ± 25	103 ± 11	102 ± 13	n.s.
DLCO/SB% [%]	76 ± 11	79 ± 8	82 ± 15	n.s.
DLCO/VA% [%]	88 ± 12	88 ± 8	92 ± 15	n.s.

Abbreviations amended with % represent percentage of predicted value; TLC: total lung capacity; VC: vital capacity; RV: residual volume; FEV1: forced expiratory volume; DLCO/SB: CO-diffusion capacity; DLCO/VA: diffusion transfer coefficient; m: missense mutation; n: nonsense mutation.

Table 4B. Long-term development of diffusion parameters in relation to genotype

Variables	m/m (n = 6)	m/n (n = 9)	n/n (n = 10)	p
Baseline				
DLCO/SB% [%]	81 ± 8	77 ± 6	77 ± 13	n.s.
DLCO/VA% [%]	90 ± 9	90 ± 8	85 ± 16	n.s.
FU-1				
Period to baseline [months]			12 ± 4	
DLCO/SB% [%]	77 ± 5	77 ± 10	74 ± 12	n.s.
DLCO/VA% [%]	86 ± 12	88 ± 12	84 ± 14	n.s.
FU-2				
Period to baseline [months]			38 ± 12	
DLCO/SB% [%]	73 ± 9	71 ± 11	68 ± 8	n.s.
DLCO/VA% [%]	80 ± 16	81 ± 16	73 ± 12	n.s.

Abbreviations amended with % represent percentage of predicted value; DLCO/SB: CO-diffusion capacity; DLCO/VA: diffusion transfer coefficient; m: missense mutation; n: nonsense mutation.

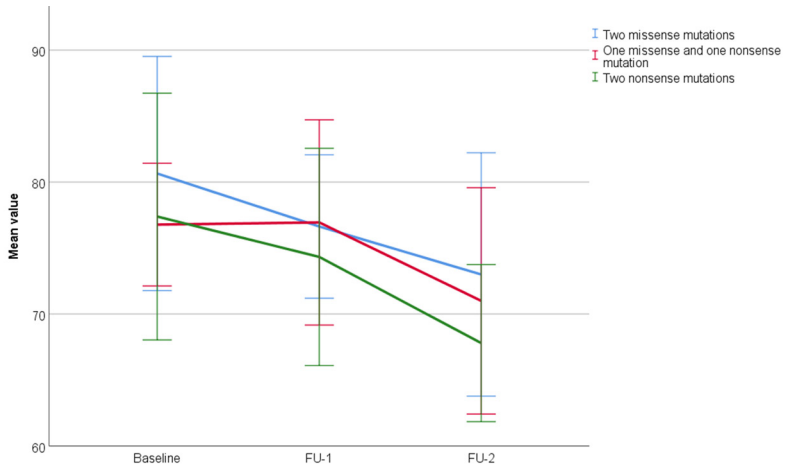

Figure 2. Mean values of diffusion capacity (DLCO/SB%) and diffusion transfer coefficient (DLCO/VA%) at baseline, after 12 months (FU-1), and after 38 months (FU-2) according to mutation type.

histological characteristics of lung tissue. Nonetheless, at least three different explanatory approaches are needed to discuss this context. First, decreasing CO-diffusion capacity can be explained by emphysema. However, emphysema does not explain increasing CO-diffusion transfer coefficient which relates CO diffusion to lung surface participating in gas exchange (*27*). Moreover, chronic pulmonary obstructive disease as representative of pulmonary disease with emphysema is not frequently accompanied by reduction of both, CO-diffusion capacity and coefficient (*31*). Second, impaired diffusion parameters may be due to structural alterations of the alveolar-capillary membrane as indicated by cited case studies (*15-18*). Reduced diffusion parameters would, therefore, be induced by progressive calcification of lung tissue and fragmentation of elastic fibers. This results in thickening of alveolar-capillary membrane and hindered diffusion. Third, impaired gas exchange can be caused by alterations of pulmonary capillaries. Especially, capillary dilatations have been associated with reduced alveolar diffusion (*32*). Since there is no data on morphology of pulmonary capillaries in PXE it remains a matter of speculation weather or not pulmonary capillaries in PXE are altered. However, alterations of nailfold capillaries correlate with capillary alteration in different sites of the body (*33*). Nailfold capillaries in PXE show a highly pathological pattern with ramification, dilatations, and perivascular edema (*19*). If this pattern is mirrored by pulmonary capillaries, this might also explain reduced diffusion parameters as well as unimpaired vital and total lung capacities. Autopsy studies and high-resolution CT-scans are necessary to shed light on pathology of pulmonary involvement in PXE.

According to our clinical experience patients with PXE do not frequently suffer from dyspnea. However, physical fitness is often limited by intermittent claudication due to early onset atherosclerosis and vascular occlusion (*12*) as well as visual impairment (*9*). Therefore, dyspnea may not occur due to a lack of physical activity. Reduced diffusion parameters should be taken into account regarding, for example, medical consultations in questions of physical resilience in patients with PXE.

Genotype-phenotype correlation showed no significant association between impaired diffusion parameters and mutational pattern in patients with PXE. Patients with nonsense mutations on both alleles, however, showed insignificant lower values of DLCO/SB and DLCO/VA in long-term follow up. This would be in line with the finding of Legrand *et al.* and Bartstra *et al.* (*22,23*) who found patients with two *ABCC6* nonsense mutations to present with a more severe PXE phenotype. However, the present study is most likely underpowered to elaborate significant differences in this matter.

Nonetheless, grouping according to missense and nonsense mutations is arbitrary. It cannot be applied on every symptom of PXE. For example, severity of skin alterations neither correlates with truncated proteins nor nonsense mutations (*22,23*). Therefore, phenotypic impairment of diffusion in the lungs may not be explainable by mutation locus on the *ABCC6* gene. Larger cohorts as well as analysis of other PXE causing genes such as *ENPP1* or *GGCX* or genetic co-factors are needed to resolve the open questions of genotype-phenotype correlations in PXE.

This study has several limitations. Although this study included approximately between seven and ten percent of all patients with PXE in Germany, the first and foremost limitation is the sample size which restrains validity of the results. Also, retrospective and single-center study design without the possibility of blinding examining personnel regarding diagnosis of PXE lessens the validity. Larger studies are needed regarding genotype-phenotype correlation including analysis of other PXE causing genetic factors such as *ENPP1* or modifier genes.

5. Conclusions

This study encompasses the largest evaluation of body plethysmographic data in PXE up to now. Patients with PXE presented with significantly reduced vital capacity as well as impaired diffusion capacity and diffusion transfer coefficient. Beyond that, it revealed relevant progression of impaired alveolar diffusion over a mean period of 38 months. This indicates progressive alterations of lung tissue in PXE or pathologies of pulmonary capillaries without influencing mobilizable lung volumes. Well-established grouping of *ABCC6*-mutations according to missense and nonsense mutations did not reveal any association with impaired alveolar diffusion. More research is needed in this matter.

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