Pseudoxanthoma elasticum: Wide phenotypic variation in homozygotes and no signs in heterozygotes for the c.3775delT mutation in *ABCC6*

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Purpose: Pseudoxanthoma elasticum is an autosomal recessive disorder of elastic tissue in the skin, eyes, and cardiovascular system, caused by mutations in the *ABCC6* gene. The purpose of this study was to check variability in expression within one genotype and look for pseudoxanthoma elasticum signs in heterozygotes. **Methods:** We examined a relatively large, in comparison with the present literature, group of adult persons homozygous or heterozygous for the c.3775delT mutation in the *ABCC6* gene, from a genetically isolated population in the Netherlands. All participants filled out a questionnaire and underwent standardized dermatologic and ophthalmologic examinations with photography of skin and fundus abnormalities. Skin biopsies from affected skin or a predilection site and/or a scar were examined and compared with biopsies from controls. **Results:** Skin abnormalities, ophthalmologic signs, and cardiovascular problems varied greatly among the 15 homozygous participants. There was no correlation among severity of skin, eyes, or cardiovascular abnormalities. None of the 44 heterozygous participants had any sign of pseudoxanthoma elasticum on dermatologic, histopathologic, and/or ophthalmologic examination, but 32% had cardiovascular disease. **Conclusion:** Individuals homozygous for the c.3775delT mutation can have a highly variable phenotype. We did not find pseudoxanthoma elasticum eye or skin abnormalities in the heterozygous family members. ***Genet Med*** **2009:11(12):852–858.**

Key Words: *pseudoxanthoma elasticum, PXE, ABCC6, phenotypic variation, heterozygote*

Pseudoxanthoma elasticum (PXE) is a hereditary disorder of connective tissue. Elastic fibers of skin, eyes, and arteries become mineralized and degenerate. This leads to the formation of asymptomatic yellowish 2- to 5-mm papules, coalescing in larger plaques in the skin. The skin lesions usually begin symmetrically on the lateral side of the neck, followed by other flexural areas of the body. In patients with advanced PXE, reduced skin elasticity may lead to redundant thickened skin folds.[1,2] The first eye signs of PXE are mottling of the retinal pigment epithelium, which is called peau d'orange, and, subsequently, angioid streaks (AS). AS are cracks in Bruch membrane, an extracellular matrix between the retinal pigment epithelium and the choriocapillaris through which neovascular membranes may grow. This results in retinal hemorrhages and scarring, so-called disciform or wet macular degeneration (MD).[1,2] Additional eye signs of PXE are comets, white punched-out lesions, often with a slightly depigmented tail,[3] and paired hyperpigmented, symmetrical patches on either side of an AS,[1] such as the wings of a hovering bird of prey, which we call wing signs.

Because of mineralization of the mid-laminar layer of mid-sized arteries, patients with PXE have an increased risk of cardiovascular disease.[1,4] Gastric hemorrhage has been reported in 8–19% of patients.[1,2,5]

The inheritance of PXE is autosomal recessive.[6–9] The disease is caused by mutations in the *ABCC6* gene, which belongs to the ATP-binding cassette (ABC) family and encodes a transmembrane transport protein.[10–12] It is as yet unknown which molecules are transported by the protein and how its dysfunction causes PXE. It has also been suggested that heterozygous carriers of PXE can show PXE signs.[13–15] To date, more than 200 different mutations in *ABCC6* have been found in patients with PXE.[16,17] Most authors did not find a clear genotype-phenotype correlation.[7,16,18–21] The phenotype can be variable, also within families,[9,16,19,22–24] but because of the genetic heterogeneity and the autosomal recessive inheritance, extended pedigrees and larger series of patients with the same genotype are rare. We examined 15 patients with homozygous PXE and 44 heterozygous relatives from a genetic isolate in the Netherlands, all of them had the same mutation, a deletion of a T in exon 27 (c.3775delT). This mutation most likely leads to absence of functional protein, which is also the case for the majority of other mutations found in patients with PXE.[17] One aim of this study was to investigate the variability of the PXE phenotype within a relatively large group of patients with one single genotype, as a first step to unravel the cause(s) of phenotypic variability. Limited variability might point at an important role for the *ABCC6* genotype, whereas extensive variability would emphasize the importance of other genetic and nongenetic factors. The second aim was to look for signs and symptoms in heterozygous carriers.

PATIENTS AND METHODS

Individuals registered at our institute as homozygous or heterozygous for the c.3775delT mutation in *ABCC6*, and their first degree family members, were invited to participate. All participants were from the same genetically isolated village of

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about 20,000 inhabitants in The Netherlands, where we also placed a call for participation in a local newspaper. New participants were included in this study if they carried one or two c.3775delT mutations and no other *ABCC6* mutations.

Permission for the study was given by the medical ethical committee of the Academic Medical Center in Amsterdam, and written informed consent was obtained from all participants.

Molecular analysis

DNA was isolated from peripheral blood by standard techniques. Polymerase chain reaction (PCR) primers, amplification conditions, and mutation analysis strategy were essentially carried out as described previously.[25] The *ABCC6* c.3775delT mutation was determined in all participants of this study by digestion of the exon 27 PCR fragment by the restriction enzyme BSTNI. The reactions were carried out according to the manufacturer's recommendations and digested PCR products were separated on 3% agarose gels.[25] The presence of this mutation, as indicated by the restriction fragment patterns, was confirmed by direct sequencing using standard procedures. The presence of the common deletion of exons 23 to 29 was excluded in the homozygous patients.

Clinical examination protocol

A questionnaire was sent to each individual and it inquired about the presence, age of onset and age of diagnosis of the various skin and eye signs and symptoms of PXE, cardiovascular problems (hypertension, angina pectoris, myocardial infarctions, cardiac valve abnormalities, arrhythmia, cerebrovascular incidents, and intermittent claudication), risk factors for cardiovascular disease (diabetes mellitus, serum cholesterol, smoking, height, and weight), gastrointestinal hemorrhages, general medical history, use of medication, and a family history of PXE. During their visit to the examination center, the geneticist went through this questionnaire with all participants. They next underwent a standardized dermatologic and ophthalmologic examination. The investigator (A.P.) who performed the dermatologic examination knew the genotype of the participants, but the ophthalmologist was blind to this information. Skin signs were scored as follows: papules one point, plaques two points, and redundant skin folds one point. Scores for every predilection site (chin, inner lower lip, neck, arm pit, inner elbow, wrist, navel, groin, and behind knee) were added. If plaques were present, papules were not scored. Because skin lesions were symmetrical in all the cases, left and right were not scored separately. Theoretically, the maximum score would be 27 points (three points at nine skin locations). Lesional skin was digitally photographed. Ophthalmologic examination was performed by an ophthalmologist (P.T.V.M.d.J.) with a great deal of experience in PXE and included biomicroscopy with a 90-diopter lens of the posterior pole of the eye fundus, indirect ophthalmoscopy of the peripheral retina and digital fundus photography were feasible. Because the participants were examined in a local study center with only a Snellen chart, slit lamp, and an ophthalmoscope, we noted "possible" for signs we might want to examine more closely later on. We disregarded these signs in the final evaluation.

Histopathology of skin

Permission was obtained from every participant to perform two 3-mm skin biopsies from skin with PXE signs (so-called lesional skin) and from a scar, if present. If no lesional skin was present, a biopsy was taken from a predilection site, preferably the lateral side of the neck. Similarly, skin biopsies were obtained from healthy volunteers and two deceased individuals, who had donated their tissues to research all from outside the genetic isolate. Biopsies were fixed in formalin and the slides were stained with hematoxylin and eosin, Verhoeff's stain, and von Kossa's stain. Slides of the homozygous, the heterozygous, and the control individuals were combined and randomly sorted, independently examined, and graded twice by two experienced dermatopathologists (J.T. and M.v.D.), who were blinded to identities and genotypes. Histopathology was considered typical for PXE if increase and fragmentation of elastin were combined with elastin clumping, with or without calcification.

RESULTS

Fifteen patients (60% women) and 44 heterozygous relatives (75% women) from the genetic isolate participated in this study. The patients were aged between 30 and 74 years and the heterozygous relatives between 27 and 68 years. We obtained skin biopsies from 12 control individuals, aged 26–64 years. The clinical data are reported below in Tables 1–3. For privacy reasons, we left out the sex of the patients and noted their ages at 5-year intervals. We did not find marked differences between men and women.

Skin

The average age of onset of skin lesions was 16 years. In 12 (80%) of 15 patients, skin lesions were first noted on the neck and sometimes together with other locations. Three patients (20%) did not have obvious PXE skin lesions on the neck. Two of these (13% of total) did not have any papules or plaques (Fig. 1A). Skin lesions in the other patients varied from a few inconspicuous papules and prominent chin creases to extensive papules, plaques, and redundant skin folds at most of the flexural sites of the body (Fig. 1B and C). We could not detect an association between skin grade and age. No heterozygous family member had any skin signs of PXE.

Histopathology of skin

The number of biopsies to which each participant consented varied from zero to six. In 95% of 20 lesional skin biopsies from 13 patients, histopathology was typical for PXE (Table 1). None of the three scar biopsies showed the characteristic clumping. In two patients, we could not histopathologically confirm the PXE diagnosis. From one of these, four additional biopsies were taken from apparently thickened skin at the neck and from hyperlax skin at different locations, but none of these biopsies showed clumping or calcification of elastin.

We obtained 68 skin biopsies from 41 heterozygous participants and 21 biopsies from 12 control individuals. At first glance, the pathologists found increased and fragmented elastin as a sign of PXE in the heterozygous persons. After combining the slides with those of the controls and blinding the pathologists to the genotype, these signs seemed to be nonspecific for PXE. An increase in elastic fibers was found in 96% of the biopsies of the homozygous participants, in 63% of those of the heterozygous family members, and in 29% of the control biopsies. Elastin fragmentation occurred in 88% of the biopsies of the homozygous cases, in 57% of those of the carriers, and in 48% of the control biopsies. Statistical analysis using a χ^2 test showed that the differences in increase and fragmentation of elastin between patients and heterozygous persons and between heterozygous and control persons were not statistically significant ($\alpha = 0.01$). Therefore, it was impossible to differentiate between the three groups, based on these two signs. There was complete congruence between both dermatopathologists in scoring the elastin abnormalities.

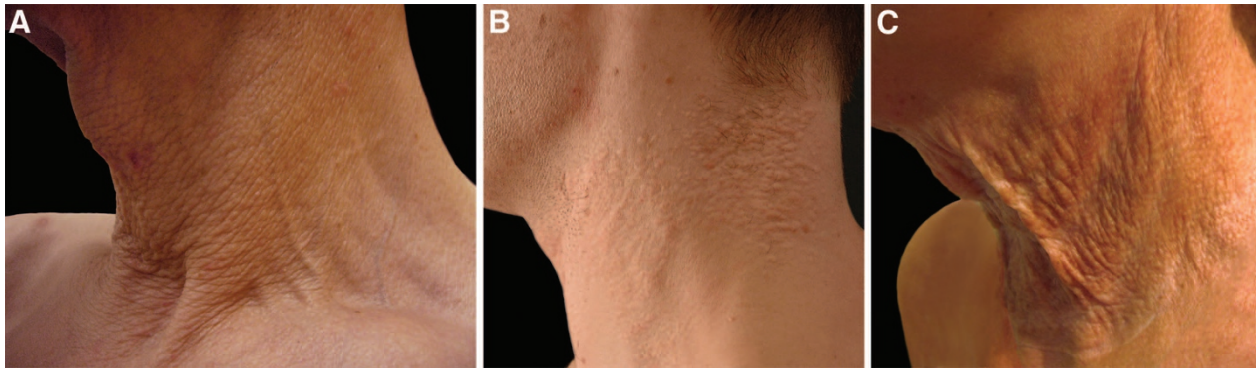

Fig. 1. A, No characteristic skin lesions, but hyperlaxity and excess of creases at the left side of the neck of Patient 9. B, Characteristic papules and plaques at the left side of the neck of Patient 4. C, Papules, plaques, and loss of elasticity at the left side of the neck of Patient 12.

Table 1 Clinical and histological skin signs of 15 patients with PXE homozygous for the *ABCC6* c.3775delT mutation

						Biopsy		
No.	Age[a]	Age at onset of skin signs	Age at diagnosis	Affected skin sites	Skin score	loc.	Lesional skin	Typical for PXE
1	30–34	8	10	ne,ax,el,na,gr	14	Neck	+	+
						Neck	+	+
2	30–34	9	10	ne,ax,el,na	9	Neck	+	+
3	30–34	8	9	ne,ax,gr	8	Neck	+	+
						Neck	+	+
4	35–39	22	22	ne,ax,el,gr	10	Neck	+	+
						Neck	+	+
5	45–49	16	31	ne,ax,el,na,gr	11	Axilla	+	+
						Abdomen	+	+
6	50–54	43	50	ne,ax,ch	6	Neck	+	+
7	50–54	?	41	ne,ax,el,gr	6	Groin	+	+
8	55–59	36	36	ne,ax,el,na,gr,ch	10	Neck	+	+
						Elbow	+	+
9	55–59	?	44	ch	1	Neck	−	−
						Thorax	scar	−
10	55–59	NA	37	NA	0	Elbow	−	−
						Thorax	scar	−
11	55–59	12	43	ne,ax,el,na,gr,kn,ch	18	Elbow	+	+
						Elbow	+	+
12	60–64	12	12	ne,ax,el,na,gr,kn,ch	18	Neck	+	+
13	60–64	10	47	ne,ax,gr,ch	4	Neck	+	−
						Axilla	+	+
14	65–69	?	47	ax	3	Axilla	+	+
						Leg	scar	−
15	70–74	0	50	ne,ax,el,gr,kn,ch	14	?	+	+

[a]For privacy reasons in this small community, age is given in 5-year intervals.
ne, neck; ax, axilla; el, inner elbow; na, navel; gr, groin; kn, knee; ch, chin; loc., localization; NA, not applicable; ?, unknown.

Table 2 Ophthalmic signs and symptoms in the homozygous patients

No.	Age (yrs)[a] at exam.	Age (yrs) at start visual loss	Visual acuity		Eye signs						
			OD	OS	AS	co	MD wet	MD dry	pa	pdo	wi
1	30–34	NA	0.8	0.8	+	+				+	
2	30–34	NA	1.6	1.0	+	+			+	+	
3	30–34	21	1.0	1.6	+	+	+				
4	35–39	16	0.8	1.0	+		+		+		+
5	45–49	NA	0.7	0.9	+	+				+	+
6	50–54	33	0.5/60	0.5	+	+	+	+	+	+	
7	50–54	NA	1.2	1.0	+				+	+	+
8	55–59	48	0.6	2/300	+	+	+	+	+	+	
9	55–59	NA	0.8	0.8	?	+			+	+	
10	55–59	37	1/60	1/60	?		+	+	+		
11	55–59	43	2/300	0.5	+		+		+		
12	60–64	40	1/∞	1/∞	?		+	+	+		
13	60–64	47	0.1	1/60	+	+	+	+	+	+	+
14	65–69	47	0.125	1/60	+	+	+	+	+	?	
15	70–74	50	1/60	1/60	?		+	+	+		

[a]For privacy reasons in this small community, age is given in 5-year intervals.
OD, oculus dexter (right eye); OS, oculus sinister (left eye); AS, angioid streaks; co, comets; exam., examination; MD, macular degeneration; NA, not applicable; pa, peripapillary atrophy; pdo, peau d'orange; wi, wings; ?, possible.

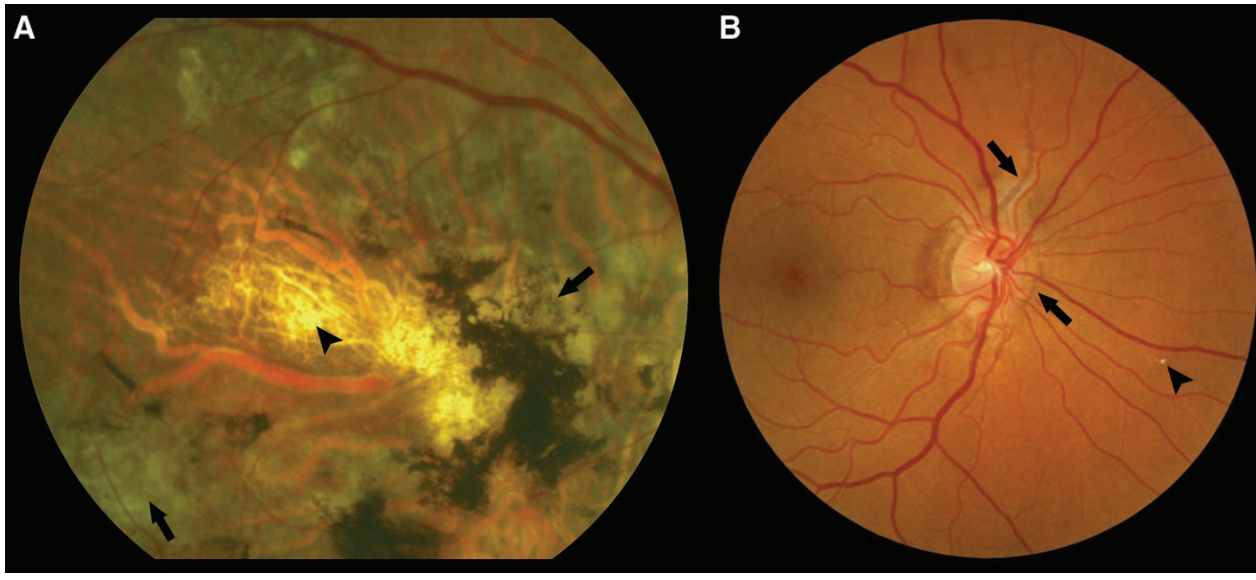

Fig. 2. A, Wet (arrows) and dry (arrow head) macular degeneration in the right eye of Patient 12, probably obscuring previously present angioid streaks. B, Angioid streaks (arrows) and one comet (arrow head) in the right retina of Patient 1.

Eyes

The ophthalmologic signs and symptoms of the patients are summarized in Table 2. Patients 3 and 4 experienced loss of visual acuity at a relatively young age because of a trauma, but they still had normal vision. All five patients younger than 50 years still had visual acuity of at least 0.8 in the best eye, and all but one of the six patients older than 56 years were legally blind because of MD (Fig. 2A). Of all patients, 60% showed peau d'orange and 73% AS (Fig. 2B). The remaining 27% possibly had AS. These latter patients had peripapillary atrophy and/or MD, which could be the reason why the AS was not clearly visible anymore. Comets (Fig. 2B) were found in 60%, wings in

Table 3 Cardiovascular signs and symptoms in the homozygous patients and heterozygous family members

Age at onset CVD (yrs)	CVD	Other risk factors for CVD
Homozygous		
?	Hypertension	Diabetes mellitus
52	Ischemic stroke, MI	Smoking
53	AP	Hypercholesterolemia, smoking
53	Aortic valve calcification and stenosis	No
51	IC, hypertension, TIA	Hypercholesterolemia
Heterozygous		
13	Hypertension	No
28	Hypertension	No
?	Hypertension	Hypercholesterolemia, smoking
42	Hypertension	No
26	Hypertension	Hypercholesterolemia
51	MI	Smoking
49	Cardiac valve insufficiency, arrhythmia	No
?	Hypertension	Hypercholesterolemia, smoking
54	Hypertension, mitral valve insufficiency	Hypercholesterolemia
43	IC, MI	Smoking
57	Hypertension	Smoking
?	Hypertension	No
40	IC, TIA, hypertension	Hypercholesterolemia
59	AP, arrhythmia, IC, hypertension	Hypercholesterolemia

AP, angina pectoris; CVD, cardiovascular disease; IC, intermittent claudication; MI, myocardial infarction; TIA, transient ischemic attack.

27%, and peripapillary atrophy in 80%. There were no heterozygous family members with eye signs of PXE.

Cardiovascular signs

Five (33%) of 15 patients and 14 (32%) of 44 heterozygous family members had a history of cardiovascular problems. Their data are summarized in Table 3.

As far as the participants were aware, they had never had a gastrointestinal hemorrhage.

DISCUSSION

Phenotype in the patients with PXE

Our results demonstrate that the phenotype within the group of 15 patients, homozygous for the same mutation (c.3775delT) in *ABCC6*, is variable. Skin abnormalities varied from severe PXE lesions at age of 30 years to no PXE skin signs around the age of 60 years (Table 1). The number of affected locations varied from zero to seven, with an average number of four. Axilla (87% of patients) and neck (80%) were most frequently affected, followed by groin (67%), inner elbow (60%), chin (47%), navel area (40%), and popliteal space (20%). The variability of skin signs could not be attributed to an age effect. Also eye signs and symptoms were highly variable (Table 2). In accordance with the literature, all six patients younger than 50 years still had close to normal visual acuity in both eyes and the six patients older than 56 years had severe visual loss in at least one eye. The other eye signs were not confined to certain age strata. The most consistent sign was AS, which was present in 73% of the patients, taking into account that peripapillary atrophy is common in the general population. We did not find a clear correlation between the skin score and the eye abnormalities.

Of the 15 patients, 33% had a history of cardiovascular disease. In this small group, we could not demonstrate any association between severity of skin or eye abnormalities and cardiovascular problems. Their number was too small to draw conclusions about the relative risk of cardiovascular disease for patients with PXE.

Marked intrafamilial phenotypic variability was known from small[9,22–24] and large[16,19] studies. Christen-Zäch et al.[26] examined 25 haplotypic homozygous patients (with unknown mutations) and 67 heterozygous carriers from genetic isolates in Switzerland. Considerable intrafamilial phenotypic variation was seen in the patients and no correlation was found between the severity of skin, eyes, or cardiovascular lesions within one patient.[26]

Different *ABCC6* mutations could explain variations between and sometimes even within families. However, so far clear genotype-phenotype correlations could not be demonstrated in large groups of nonrelated patients with PXE.[7,16,18–21,27] In two of these studies, a significantly lower age at diagnosis and/or a higher number of affected organs were found in case of mutations leading to the absence of (functional) ABCC6 protein.[19,27] The deletion of a T in exon 27 (c.3775delT) in our cases leads to a frameshift and a premature chain termination, which probably results in the absence of a functional protein. Apparently, this can also cause a variable phenotype. It is to be expected that this also holds for other mutations, leading to absence of a functional protein. Most patients with PXE have such mutations.[17]

Within most families, especially within sibships, and within our study population, variation cannot be explained by different genotypes at the *ABCC6* gene, so other genes and/or environmental factors must play a role. Previously, three variations in the gene for xylosyltransferase II were found, which were associated with a more severe phenotype in patients with PXE.[28] Xylosyltransferase II plays a role in proteoglycan metabolism. In another study, promoter polymorphisms of the *SPP1* gene were significantly more often present in patients with PXE than in controls, so that it was suggested that *SPP1* is a modifier gene for PXE.[29] Furthermore, a correlation was reported between polymorphisms in three genes encoding for antioxidant enzymes (CAT, SOD2, and GPX1) and age of onset of PXE.[30] Higher serum concentrations of the calcification inhibitor matrix Gla protein (MGP) were correlated with later onset of PXE. Also, a certain MGP haplotype, formed by two *MGP* polymorphisms, seemed to be protective.[31] As an environmental factor, high-calcium intake could perhaps influence disease severity.[32] Three of the six patients treated with the phosphate-binder aluminum hydroxide showed improvement in skin lesions.[33] The results of all these studies have not yet been replicated in subsequent studies. To our knowledge, no other genetic or environmental factors have been reported to influence

the phenotype. Only the cardiovascular problems of patients with PXE are well known to be influenced by many other factors, such as smoking, serum lipids, hypertension, body mass index, and diabetes mellitus, as they are in the general population.

Phenotype in carriers of PXE

In the 44 heterozygous family members, we did not find any PXE skin or eye signs, not even in 68 skin biopsies when compared with control biopsies. In the literature, several signs and symptoms of PXE have been reported in individuals, who were (probably) heterozygous for an *ABCC6* mutation. Mild skin and ocular abnormalities were reported in three of the six parents of patients, but mutational analysis of *ABCC6* had not been performed.[14] Histopathologic abnormalities were found in skin biopsies from some probably heterozygous first-degree relatives of patients with PXE.[13,34,35] Most frequent abnormalities were increase and fragmentation of elastin, which were found less frequently in controls, but which we consider aspecific for PXE. In some biopsies, calcification of elastic fibers was present, but no clumping was mentioned.[13,34]

Christen-Zäch et al. did not find any skin or eye signs of PXE in 67 heterozygous persons. From four of them (6%) skin biopsies were examined, which were also normal.[26] A study of 17 heterozygous persons revealed comets at fundoscopy in two, and no other eye and/or macroscopic skin lesions.[16] Martin et al.[15] reported on four heterozygous carriers with varying skin and/or eye manifestations of PXE. In summary, some authors found (mostly mild) skin, eyes, and/or histopathological abnormalities in heterozygous family members of patients with PXE, but others did not. Several explanations for the different findings in these studies are conceivable: (1) Most studies contained only small numbers of patients. There could be selection bias. Heterozygotes with positive findings will be reported more likely than those with negative findings. (2) Expression in heterozygotes might be different for different genotypes. (3) Most observers were not blinded to the genotype and not all studies included control persons. In our experience, these two conditions are important for reliable results. (4) Putative heterozygous persons with clinical expression could be homozygous or compound heterozygous. DNA studies were not always performed and, if one mutation has been found, a second as yet undetectable mutation cannot be excluded.

In the literature, the risk for cardiovascular disease seemed to be increased in heterozygous persons,[36,37] whereas they did not have any skin or eye signs.[37,38] Vanakker et al.[16,39] found peripheral atherosclerosis in 7 of 17 heterozygous persons (and calcifications in several organs in 4 of 17). In our study, the prevalence of a positive history for cardiovascular disease was equal between homozygous (33%) and heterozygous (32%) family members. The absence of an exactly matched control population makes it hard to draw conclusions from our study about a possibly increased risk of cardiovascular problems for heterozygous persons.

In conclusion, homozygosity for the c.3775delT mutation in *ABCC6* can cause a highly variable phenotype, especially concerning skin and cardiovascular abnormalities, comparable with the phenotypic variation seen between different genotypes. Future research should elucidate potential genetic and environmental factors that contribute to this variation. Persons heterozygous for the c.3775delT mutation did not have skin or eye signs of PXE.

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