International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 4; October 2018; Page No. 33-39

Researching recent literature on pseudoxanthoma elasticum (PXE)

Sharika Akhter[1], Thomas Georgas[2], Georgios Giannoukos[3]
[1] University of Greenwich, United Kingdom
[2] Saint Francis Xavier College, London, United Kingdom
[3] Second Chance School, Greece

Abstract
Pseudoxanthoma Elasticum (PXE) is a rare genetic disorder, typically inherited in an autosomal recessive manner. It is also classed as a multisystem disorder as the disease involves the impairment of a number of organs and tissues. Literature is very limited in regards to the specific pathologically related histological/anatomical descriptions of certain affected tissues, especially cardiac tissues as well as the prevalence record of cardiovascular abnormalities in PXE patients. What this article aims to accomplish is to provide a detailed picture of the existing relevant literature on the field and discuss the prospects of discovering more about PXE. The article finishes with discussing how the human genome project is a perfect prospect to discovering more about PXE.

Keywords: pseudoxanthoma elasticum, multisystem disorder, cardiovascular abnormalities

Introduction

Pseudoxanthoma Elasticum (PXE) is a rare genetic disorder, typically inherited in an autosomal recessive manner. Prevalence of PXE is believed to be 1 in 25000. An autosomal dominant genotype can also be inherited and happens to be very rare compared to autosomal recessive inheritance (Nitschke *et al*., 2012) [49]. PXE can also be required through organ transplants from individuals with PXE, have an inherited condition of B-thalasamia (Fabbri *et al.*, 2009) [13] or due to reaction to penicillamine medication. It is also classed as a multisystem disorder as the disease involves the impairment of a number of organs and tissues. The tissues and organ typically affected are the eyes, flexor skin regions such as the neck and armpits, connective tissue such as the inside of the lower lip, tissue of the vascular walls (Finger *et al.,* 2009) [15]. Impairment of these organs and tissue manifest dermatological conditions around the flexor skin regions, vision disorders, and cardiovascular abnormalities. Ectopic mineralisation and fragmentation of elastic fibres in connective tissues, skin and vascular walls is the main cause behind the impairment of these organs (Nitschke *et al.*, 2012) [49]. The possible reason(s) behind such abnormal mineralisation in abnormal places still remain unclear (Nitschke *et al*., 2012) [49]. PXE is predominantly caused by mutations in the ATP-Binding Cassette subfamily C number 6 gene (ABCC6) gene. ABCC6 is a member of the ABC transporter protein family which play a number of essential transporting roles including transportation of endogenous substrates, drugs, lipids, proteins, sugars, metal ions and compounds alike (Vasiliou, Vasiliou and Nebert, 2008) [72]. What endogenous substrate is transported by the ABCC6 protein is still a mystery (Nitschke *et al.,* 2012) [49] which indicates that there is a need for more intensive investigations and researches to discover the underlying defect and what effect does it have on the substrate that is being transported.

The characteristic skin conditions were initially depicted by a French dermatologist Rigal in 1881 (Finger *et al.,* 2009) [15]. Soon after Rigal's description of the abnormal skin changes his colleague Balzer performed histological examination on the abnormal skin cells. However both could not comprehend the implications of their discoveries. Another French dermatologist Ferdinand-Jean Darier gave the condition the term Pseudaxonthoma Elasticum (PXE) in 1886 in order to distinguish PXE from common xanthoma that is characterized by yellow papules on the skin (Finger *et al.,* 2009) [15].

Angioid streaks were recognized as completely a separate entity of symptom by an English ophthalmologist Doyne in 1889 and two years later by a German ophthalmologist Plange in 1891 (Finger *et al*., 2009) [15]. Just a year after the discovery of German ophthalmologist Plange, an American-born German ophthalmologist, Hermann Knnap brought the clinical manifestation of angioid streaks to the attention of the scientific community in 1892. Hermann Knnap demonstrated an understanding that angioid streaks where the result of hemorrhages occurring in the posterior pole of the eye that led to pigmented streaks of dark brown or black (Finger *et al.*, 2009) [15].

Almost 40 years later, Ester Groenbald a Swedish dermatologist and James Strandberg an ophthalmologist, also of Swedish national were the first to recognize the connection between PXE and angioid streaks. They termed the syndrome after their surnames as being Groenbald-Strandberg syndrome, since which Pseudoxanthoma Elasticum and Groenbald-Strandberg are used alongside (Finger *et al.,* 2009) [15].

Typical Clinical Manifestations

Dermatological Abnormalities

Dermatological abnormalities start to manifest as the disease progresses. The dermatological condition is described as accruing yellow papules merging about the affected area of

large flexor skin surfaces such as the neck, axillary, groin and popliteal fossa [Figure 1] (Finger *et al*., 2009) [15]. Histological examination of these affected regions show fragmented basophilic elastic structures in both the upper and middle reticular dermis along with evident calcification of the elastic fibers (Nitschke *et al.*, 2012) [49].

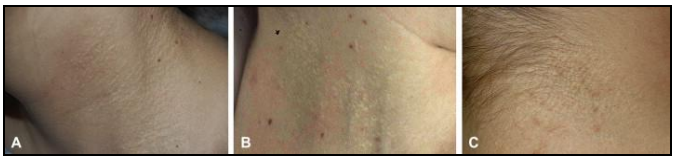

Fig 1: Photos of the neck of patient 1 (A), patient 2 (B) & patient 3 (C) showing yellow papules around the side of the neck (from Bercovitch *et al.,* 2011)

Ophthalmological Abnormalities

Ophthalmoscopy/fundoscopy of the eye reveals pigment irregularities, which is defined by a term called peau d'orange. Peau d' orange is described to appear as small dark spots against a background of whitish or opaque fundus (Gliem *et al*., 2013) [18]. The spots can be observed initially at the posterior pole of the eye which gradually precedes towards the peripheral pole as the disease progresses (Gliem *et al*., 2013) [18]. Possible pathophysiology behind the occurring of Peau d' orange is due to calcification of the Bruch's membrane (BM) also known as the retinal pigment epithelium (RPE) of the eye (Charbel Issa *et al.,* 2010). Peau d' orange is understood to progress into Angioid streaks within a time frame of 1 to 8 years [Figure 2] (Finger *et al.,* 2009) [15]. Angioid streaks are more prominently witnessed on ophthalmoscopy/fundoscopy however sometimes they are hardly seen on some eyes, leading to an inaccurate diagnosis (Finger *et al.,* 2009) [15]. It was suggested by Kofler (1917) that angioid streaks occur due to ruptures in the (BM). The advancement of Angioid streaks and Peau d' orange does lead to impaired vision and is reported to have a greater impact on the quality of life of PXE patients than the impact cardiovascular abnormalities. Angioid streaks can further progress to subretinal chorodial neovascularisation (CNV) which is defined as debris accumulation, atrophy and scarring of the RPE (Gliem *et al.,* 2013) [18].

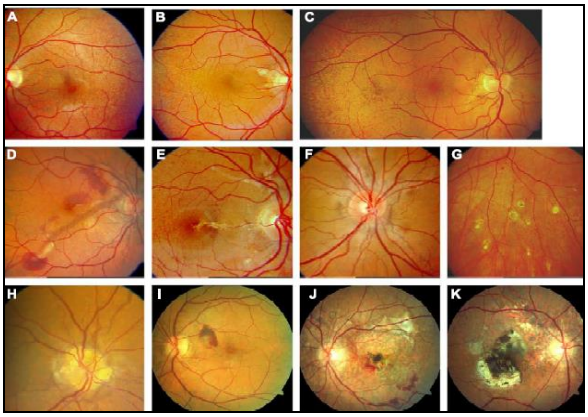

Fig 2: Funduscopy demonstrating the initial appearance of Peau d' orange (A), growing into angioid streak (E) which is further progressing into CNV (K) (adapted from Gliem *et al.,* 2013) [18].

Cardiovascular Abnormalities Concomitant with GIH and RH

Since the condition also affects the vascular walls particularly the arteries, patients suffer from cardiovascular abnormalities. The cardiac abnormality occurs alsoowing to the calcification of the connective tissues of the vessel wall. It is observed that in arteries of small to medium-sized arteries such as the coronary artery, calcification initiates from the internal and external elastic laminae which progresses into the media and intima (Lever, Elder and Elenitsas, 1997) [36]. The nature of such calcification resembles that of atherosclerosis (Mendelsohn, Bulkley and Hutchins, 1978) [43]. This causes the thickening of the endocardium which contributes to arterial hypertension and decreased peripheral pulses (Nitschke *et al*., 2012; Hu *et al.,* 2003) [49]. On a more rare and severe cases, thickening of the endocardium can also lead to restrictive cardiomyopathy, mitral valve prolapse or stenosis, angina pectoris and even sudden cardiac failure(Finger *et al.,* 2009; Nitschke *et al.,* 2012) [49].

Literature is very limited in regards to the specific pathologically related histological/anatomical descriptions of the affected cardiac tissues as well as the prevalence record of cardiovascular abnormalities in PXE patients (Finger *et al*., 2009) [15]. Most patients with PXE have been reported to not experience cardiovascular abnormalities until they reach the third or fourth decade of life (Hacker *et al*., 1993; Schachner and Young, 1974). However instances of early onset has been observed to occur as early as at 9 years of age, of which early onset of atherosclerosis is regarded as a serious impediment (Hacker *et al.,;* Schachner and Young, 1974; Nitschke *et al*., 2012). Also cardiomyopathy is described as a rare occurance (Hu *et al.,* 2003; Lever, Elder and Elenitsas, 1997) [24, 36]. In a study by Farber *et al*., (1964), 200 PXE patients were employed to investigate the prevalence of each of their individual cardiovascular problems. The prevalence of decreased peripheral pulses showed the most prevalence (25%), followed by arterial hypertension (22.5%), angina pectoris (19%) and intermittent claudication (18%). Thus, this particular study implies that the subsequent decreased peripheral pulse is more common than the later types and the rare and severe types.

Often gastro-intestinal haemorrhage (GIH) is noticed with the cardiovascular problems and is reported to have been frequently present. GIH were found in 13% of the patients employed in the study by Farber *et al.,* (1964). The cause of GIH still remains implicit however it is thought that GIH does not ensue due to a spontaneous rupture in a GI vessel as it is seen in a disease called Ehlers-Danlos disease (Chassaing *et al.,* 2005; cross referenced) [6]. Instead the haemorrhage is believed to exhibit due to calcification of the thin-walled arteries situated right under the gastric mucosa layer (Lever, Elder and Elenitsas, 1997) [36].

Renal hypertension (RH) owing to calcification of renal arteries in kidney, occasionally concomitant with calcification / mineralisation of other more profound and inner organs such as the spleen, liver and breast tissue has also been observed in PXE patients, with RH affecting one-quarter of PXE patients (Crespi, Derchi and Saffioti, 1991 cross referenced only abstract available; Nitschke *et al.,* 2012) [9, 49]. It is believed that RH along with GIH further precedes to haematemesis

(vomitting of the blood) and melena (egestion of black tarry faeces) in 15% of PXE patients (Fah, 1991; Wilson, 1990) [14, 73].

Additional Clinical Manifestations of PXE

Besides gastro-intestinal bleeding and haemorrhage, other types of haemorrhage and bleeding from organs such as in bladder, renal, nose, subarachnoid, pulmonary and joint bleedings have also been documented but is said to happen very rarely. In women, menometrorrhagias appears to be frequent event (Fah, 1991; Uitto, Pulkkinen and Ringpfeil, 2001) [14, 58].

Ectopic mineralisation/calcification of spleen and hepatic malformations as discussed earlier in the earlier section also occurs, however a different nature of splenic and hepatic vascular malformations have been recognised where the pathogenesis of the vascular malformation was not fully understood (Korn et al., 1987) [30].

PXE can also have implications on pregnancy in women with PXE. The placenta is mainly the most affected and subjected to structural deformities due to excessive abnormal mineral precipitation and formation of matrix like fibrinoidin the placenta (Gheduzzi et al., 2001) [17]. The abnormal mineralisation affects the septa of the placenta, stroma of villi, stem of tertiary chorionic villus and the decidua. Two small infarcts in the placenta with heavy fibrin deposition in the stem of tertiary chorionic villus have been documented in the case of mother with PXE, investigated by Elejalde et al. (1984). 20 women with PXE were employed in a study conducted by Viljoen et al. (1987) in whom 54 pregnancies where monitored for possible deformities in the placenta. Atleast 12 pregnancies resulted in first trimester miscarriage. Only three women out of the 20 women had successful deliveries. Thus this indicates that the abnormal mineralisation in the placenta has a high potential in interfering with the foetus(s)' metabolism, hence disrupting their development. Not only structural deformities of placenta account for miscarriages but hypertension which is a pretty common attribute of PXE, also leads to complications during pregnancy(Viljoen *et al*. 1987). Hypertension had a significant impact on atleast 7 pregnancies in the study by Viljoen *et al*. (1987). Although some literatures do classify such events as rare occurrence (Gheduzzi *et al*., 2001; Hu *et al*., 2003) [17, 14], PXE still has the potential to affect pregnancy and at times very severely. i.e. increasing the risk of miscarriage in first trimester which is evident from the number of unsuccessful pregnancies, atleast 14 out of 54 pregnancies have been affected in the study (Viljoen *et al.* 1987) due to PXE implications.

Conclusion

Human Genome Project (HGP) is piloted by an international collaboration of scientists all around the world and is capable of providing a great platform for investigating the aetiology of various disease at the genetic level. HGP was approved and incited by the Department of Energy (DOE) in 1985, USA (Saraswathy *et al.*, 2014). The National Institute of Health (NIH) and DOE together took the assignment to fund HGP which also involved 25 laboratories in 5 countries. The project officially commenced in 1990 for a time frame of 15 years with an estimated cost of US $ 200 million. Within this time frame, the objective of HGP was to determine the order of 3 billion DNA nucleotides through DNA sequencing. Both governments' and private sectors' arising social enthusiasm, strong support and curiosity to investigate the root cause of rare life-threatening conditions started to drive the progression of HGP. The ambition was achieved and completed in April 2003 (Genome.gov, 2015); Saraswathy *et al.*, 2014).

HGP not only aids in discovering the genomic cause but also helps in early detection of rare inherited genetic disorders through newborn screening. It provides the opportunity to manufacture personalised medicines to suit specific treatment-related needs of individual patients (Hefti and Beck, 2014) [21]. Also it assists in understanding genomic profile expression of certain patients as a response to certain medicines (Marchionni, 2008) [41]. With emerging advancement in technology and genomic understanding, several gene based therapy treatments such as Talens have been established and is implemented in patients with genetic conditions such as sickle cell anaemia, duchene muscular dystrophy (Ousterout *et al.*, 2013; Sun *et al.*, 2012) [51, 65]. Thus HGP offers a great opportunity for investigating a rare genetic disorder such as Pseudoxanthoma Elasticum (PXE).

References

1. Eacute Cuwe C, Dalle S, Ronger-Savl&eacute S, Skowron F, Balme B, Kanitakis J, Thomas L. Elastosis perforans serpiginosa Associated with Pseudo-Pseudoxanthoma elasticum during Treatment of Wilson & rsquo; s Disease with Penicillamine. Dermatology. 2005; 210(1):60-63. NCBI [Online]. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15604549 [Accessed on 7 April 2018]
2. Belinsky M, Kruh G. MOAT-E (ARA) is a full-length MRP/cMOAT subfamily transporter expressed in kidney and liver. Br J Cancer, [online] 80, pp.1342–1349. Available, 1999, at: http://www.scopus. com/record/display. url?eid=2-s2.0-00330 20785& origin= inward&txGid=03484B30321553F86AB12142C3E475EE.iqs8TDG0Wy6BURhzD3nFA%3a1 [Accessed 10 Apr. 2018].
3. Belinsky M, Chen Z, Shchaveleva I, Zeng H, Kruh G. Characterization of the Drug Resistance and Transport Properties of Multidrug Resistance Protein 6 (MRP6, ABCC6). Cancer Research, [online]. 2002; 62:6172-6177. Available at: http://cancerres.aacrjournals.org/ content /62/21/6172.full.pdf+html [Accessed 21 Mar. 2018].
4. Bercovitch L, Martin L, Chassaing N, Hefferon T, Bessis, D, Vanakker O, Terry S. Acquired pseudoxanthoma elasticum presenting after liver transplantation. Journal of the American Academy of Dermatology. 2011; 64(5):873-878. Science Direct [Online]. Available at: http://ac.els-cdn.com/S0190962210003841/1-s2.0-S0190962210003841-main.pdf?_tid=c56317a0-e4e0-11e4-83f2-00000aacb361& acdnat=1429261656_ 0ee77ef0aa3b3307919a507cf9ff5830 [Accessed on 3 April 2018]
5. Borst P, Evers R, Kool M, Wijnholds J. A Family of Drug Transporters: the Multidrug Resistance-Associated

Proteins. JNCI Journal of the National Cancer Institute, 92(16), pp.1295-1302. NCBI [Online]. Abstract available at, 2000, http://www.ncbi.nlm.nih.gov/pubmed/10944550 [Accessed on 4 April 2018]
6. Chassaing N, Martin L, Calvas P, et al. Pseudoxanthoma elasticum: a clinical, pathophysiological and genetic update including 11 novel ABCC6 mutations. Journal of Medical Genetics. 2005; 42(12):881-892. NCBI [Online]. Abstract available at: http:// www.ncbi.nlm.nih. gov/pubmed/15894595 [Accessed on 4 April 2018]
7. Clinicaltrials.gov. Magnesium Supplements In The Treatment Of Pseudoxanthoma Elasticum (PXE) - Full Text View - ClinicalTrials.gov. [online], 2015, Available at: http://clinicalt ria ls .gov /show /NCT 0152 5875 [Accessed 13 Apr. 2018].
8. Coatesworth A, Darnton S, Green R, Cayton R, Antonakopoulos G. A case of systemic pseudo-pseudoxanthoma elasticum with diverse symptomatology caused by long-term penicillamine use. Journal of Clinical Pathology. 1998; 51(2):169-171. Abstract available at: http://www.ncbi.nlm.nih. gov/ pubmed/ 9602696 [Accessed on 8 April 2018]
9. Crespi G, Derchi L, Saffioti S. Sonographic detection of renal changes in pseudoxanthoma elasticum. Urol Radiol. 1991; 13(1):223-225. NCBI [Online]. Abstract available at : http://www. ncbi. Nlm .nih. gov/ pubmed/ 1598746 [Accessed on 3 April 2018]
10. Dabisch-Ruthe M, Kuzaj P, Götting C, Knabbe C, Hendig D. Pyrophosphates as a major inhibitor of matrix calcification in Pseudoxanthoma elasticum. Journal of Dermatological Science. 2014; 75(2):109-120. Science Direct [Online]. Available at:http://ac.els-cdn.com/ S0923181 114001121 /1-s2.0-S09231 81 114001121-main.pdf?_tid=4dfcbbb4-e4e3-11e4-a8c00 0000aa b0f 02 &acdnat=1429262744_c9ac4598b2263306330782ab217b 8143 [Accessed on 5 April 2018]
11. Dean M, Rzhetsky A, Allikmets R. The Human ATP-Binding Cassette (ABC) Transporter Superfamily. *Genome Research*. 2001; 11(7):1156-1166. NCBI [Online]. Available at: http://w ww. Ncbi .nlm. nih.gov/ pmc/articles/PMC2752038/ [Accessed on 28 March 2018]
12. Deeley R, Cole S. Function, evolution and structure of multidrug resistance protein (MRP). *Seminars in Cancer Biology*. 1997; 8(3):193-204. NCBI [Online]. Avaialable at:http://www.ncbi.nlm.nih.gov/pubmed/9441948 [Accessed on 7 April 2018]
13. Fabbri E, Forni G, Guerrini G, Borgna-Pignatti C. Pseudoxanthoma elasticum-like syndrome and thalassemia: An update. Dermatology Online Journal, [online]. 2009; 15(7). Available at: https:// escholarship. org/uc/item/1cw031gj [Accessed on 11 April 2018].
14. Fah L. Pseudoxanthoma elasticum—a visual diagnosis. *Schweiz Med Wochenschr*. 1991; 121:660-663. NCBI [Online]. Abstract available at: http:// www.ncbi. nlm.nih. gov/pubmed/2047826 [Accessed on 12 April 2018]
15. Finger R, Issa P, Ladewig M, Götting C, Szliska C, Scholl H, Holz F. Pseudoxanthoma Elasticum: Genetics, Clinical Manifestations and Therapeutic Approaches. Survey of Ophthalmology. 2009; 54(2):272-285. Science Direct [Online]. Available at: http://ac.els-cdn.c o m/ S00 39625708002580/1-s2.0-S0039625708002580-main.pdf?_tid=d6b89dd2-e4e4-11e4-bbbb-00000aacb361&acdnat=1429263403_8bfb795af3189ca8d 841399241e301b9 [Accessed on 11 April 2018]
16. Genome go. all about The Human Genome Project (HGP). [online] Available at, 2014, http://www.genome. gov/10001772 [Accessed 10 Apr. 2018].
17. Gheduzzi D, Taparelli F, Quaglino D, Di Rico C, Bercovitch L, Terry S, *et al*. The Placenta in Pseudoxanthoma Elasticum: Clinical, Structural and Immunochemical Study. Placenta. 2001; 22(6):580-590. Science direct [Online]. Available at: http://ac.els-cdn. com/S0143400401906873/1-s2.0-S0143400401906873-main.pdf?_tid=1bed283c-e4e5-11e4-be04-00000a ab0 f2 7&acdnat=1429263519_ae1a88fe919f6283863bb2b5342 63e12 [Accessed on 13 April 2018]
18. Gliem M, Zaeytijd J, Finger R, Holz F, Leroy B, Issa P. An update on the ocular phenotype in patients with pseudoxanthoma elasticum. *Front. Genet.*, 4. NCBI [Online]. Available at, 2013, http://www. ncbi.nlm. nih.gov/ pmc/articles/PMC3617449/ [Accesed on 13 April 2018]
19. Hacker S, Ramos-Caro F, Beers B, Flowers F. Juvenile Pseudoxanthoma Elasticum: Recognition and Management. Pediatr Dermatol. 1993; 10(1):19-25. NCBI [Online]. Abstract available at: http://www. ncbi.nlm. nih.gov/pubmed/8493160 [Accessed on 7 April 2018]
20. Hamlin N, Beck K, Bacchelli B, Cianciulli P, PasqualiRonchett, I, Le Saux O. Acquired Pseudoxanthoma elasticum-like syndrome in beta-thalassaemia patients. British Journal of Haematology. 2003; 122(5):852-854. NCBI [Online]. Abstract available at: http:// www.ncbi.nlm. nih.gov/pubmed/12930400 [Accessed on 13 April 2018]
21. Hefti M, Beck A. The Human Genome Project and Personalized Medicine. Reference Module in Biomedical Sciences, [online], 2014, 3418-3422. Abstract available at: http://www.sciencedirect.com/ science/article/ pii/B9 780123864567066041 [Accessed 10 January 2018].
22. Higgins C. ABC Transporters: From Microorganisms to Man. Annual Review of Cell Biology. 1992; 8(1):67-113.
23. Hoffbrand A, Moss P, Pettit J. Essential haematology. Malden, Mass.: Blackwell Pub, 2006.
24. Hu X, Plomp A, van Soest S, Wijnholds J, de Jong P, Bergen A. Pseudoxanthoma elasticum: a clinical, histopathological, and molecular update. Survey of Ophthalmology. 2003; 48(4):424-438. Avaiable at : http://ac.els-cdn.com/S0039625703000535/1-s2.0-S00 3 9625703000535-main.pdf?_tid=4d380a36-e4e7-11e4-a5b 1-00000 a acb360& acdnat= 1429264461_ 138c94a 9c 905b4ec9e00cad069508a06[Accessed 15 April 2018]
25. Ilias A, Urban Z, Seidl T, Le Saux O, Sinko E, Boyd C, Sarkadi B, Varadi, A. Loss of ATP-dependent Transport Activity in Pseudoxanthoma Elasticum-associated Mutants of Human ABCC6 (MRP6). Journal of Biological Chemistry, [online]. 2002; 277(19):16860-16867. Available at: http://www. scopus. com/record/display. url?eid=2- s2.0-00370533 55& origin=inward&txGid=D940AEC51A79DAB5AA15533

940D90ABA.FZg2ODcJC9ArCe8WOZPvA%3a41 [Accessed 10 Apr. 2018].
26. Issa P, Finger R, Götting C, Hendig D, Holz F, Scholl H. Centrifugal Fundus Abnormalities in Pseudoxanthoma Elasticum. Ophthalmology. 2010; 117(7):1406-1414. [Online]. Abstract available at: http://www.aaojournal. org/article/S0161-6420(09)01296-2/abstract [Accessed 11 April 2018]
27. Jiang Q, Takahagi S, Uitto J. Administration of Bone Marrow Derived Mesenchymal Stem Cells into the Liver: Potential to Rescue Pseudoxanthoma Elasticum in a Mouse Model (Abcc6−/−). Journal of Biomedicine and Biotechnology, 2012,.1-11. [Online]. Available at: http://www.hindawi.com/journals/bmri/2012/818937/ [Accessed on 4 April 2018]
28. Kool M, van der Linden A, de Haas M, et al. Expression of human MRP6, a homologue of the multidrug resistance protein gene MRP1, in tissues and cancer cells. Cancer Res, [online]. 1999; 59:175-182. Available at: http://www. scopus.com/record/display.url?eid=2-s2.0-0032893268&origin=inward&txGid=03484B30321553F86AB12142C3E475EE.iqs8TDG0Wy6BURhzD3nFA%3a11 [Accessed 10 Apr. 2018].
29. Kool M, van der Linden M, De Haas M, Baas F, Borst P. Expression of Human MRP6, a Homologue of the Multidrug Resistance Protein Gene MRP1, in Tissues and Cancer Cells. Cancer Research, [online]. 1999; 59:175-182. Available at: http://cancerres. aacrjournals. org/content/59/1/175.full.pdf+html [Accessed on 21 March 2018].
30. Korn S, Selinacht J, Huth C, Feller A. Cardiovascular manifestation of pseudoxanthoma elasticum (Gronblad-Strandberg-syndrome). Thorac Cardiovasc Surg. 1987; 35:191-194. Europe PubMed Central [Online]. Abstract available at: http://europepmc.org/abstract/MED/2442842 [Accessed on 1st April 2018]
31. Le Saux O, Bunda S, VanWart C, Douet V, Got L, Martin L, Hinek A. Serum Factors from Pseudoxanthoma Elasticum Patients Alter Elastic Fiber Formation In Vitro. J Investig Dermatol. 2006; 126(7):1497-1505. Nature [Online]. Available at: http://www. nature.com/ jid/ journal/v126/n7/full/5700201a.html [Accessed on 30 March 2018]
32. Le Saux O, Martin L, Aherrahrou Z, Leftheriotis G, Váradi A, Brampton C. The molecular and physiological roles of ABCC6: more than meets the eye. Frontiers in Genetics, 3. NCBI [Online]. Available at, 2012, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3520154/ [Accessed on 27 March 2018]
33. Le Saux, O, Urban Z, Tschuch C, Csiszar K, Bacchelli B, et al. Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. *Nature Genetics*, [online]. 2015; 25:223-227. Available at: http:// www. nature.com/ng/journal/v25/n2/full/ng0600_223.html#a2 [Accessed on 18 February 2018].
34. Le Saux O, Urban Z, Tschuch C, Csiszar K, Bacchelli B, Quaglino D, et al. Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. Nat Genet. 2000; 25(2):223-227. Abstract available at:http://www. ncbi.nlm.nih.gov/pubmed/10835642 [Accessed on 23 March 2018]
35. Lee P, Costumbrado J, Hsu C, Kim Y. Agarose Gel Electrophoresis for the Separation of DNA Fragments. Journal of Visualized Experiments. 2012; (62). NCBI [Online]. Abstract available at: http://www. ncbi. nlm.nih.gov/pubmed/22546956 [Accessed on 10 April 2018]
36. Lever W, Elder D, Elenitsas R. Congenital diseases (Genodermatoses). Histopathology of the Skin, chap 6J.B.Lippincott Company, Philadelphia, 1997, 117-151.
37. Li Q, Uitto J. Mineralization/Anti-Mineralization Networks in the Skin and Vascular Connective Tissues. The American Journal of Pathology. 2013; 183(1):10-18. Science Direct [Online]. Available at: http://ac.els-cdn.com/S0002944013002575/1-s2.0-S0002 94 4013002575-main.pdf?_tid=c56541f0-e4eb-11e4-9f47-00000aab0f27&acdnat=1429266380_ead74ec2396a03ec9f04d6fc729d8677 [Accessed on 15 March 2018]
38. Li Q, Jiang Q, Uitto J. Ectopic mineralization disorders of the extracellular matrix of connective tissue: Molecular genetics and pathomechanisms of aberrant calcification. Matrix Biology. 2014; 33:23-28. Science Direct [Online]. Available at: http://ac.els-cdn.com/S 0945053X1 3001 005/1-s2.0-S0945053X13001005-main.pdf?_tid=0dde 206e-e4ec-11e4-b09f-00000aacb35f&acdnat=142926 6502_77f663409872bac6544a06c4f4ee9456 [Accessed on 16 March 2018]
39. Liang S, Quirk D, Zhou A. RNase L: Its biological roles and regulation. IUBMB Life (International Union of Biochemistry and Molecular Biology: Life), [online]. 2006; 58(9):508-514. Available at: http://onlinelibrary. wiley.com/doi/10.1080/ 15216540600838232/pdf [Accessed on 12 April 2018].
40. Lorenz-Depiereux B, Schnabel, D, Tiosano D, Häusler G, Strom T. Loss-of-Function ENPP1 Mutations Cause Both Generalized Arterial Calcification of Infancy and Autosomal-Recessive Hypophosphatemic Rickets. The American Journal of Human Genetics. 2010; 86(2):267-272. NCBI [Online]. Abstract available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2820166/ [Accessed on 15 April 2018]
41. Marchionni L. Impact of gene expression profiling tests on breast cancer outcomes. [Rockville, Md.: Agency for Healthcare Research and Quality. NCBI [Online . Available, 2008, at: http://www. ncbi. nlm. nih.gov/books/NBK38451/ [Accessed on 18 March 2018]
42. Martin L, Douet V, VanWart C, Heller M, Le Saux O. A Mouse Model of β-Thalassemia Shows a Liver-Specific Down-Regulation of Abcc6 Expression. The American Journal of Pathology. 2011; 178(2):774-783. NCBI [Online]. Available at: http://www. ncbi.nlm. nih.gov/ pmc/articles/PMC3069908/ [Accessed on 12 March 2018]
43. Mendelsohn G, Bulkley B, Hutchins G. Cardiovascular manifestations of Pseudoxanthoma elasticum. Arch Pathol Lab Med. 1978; 102(1978):298-302. NCBI [Online]. Abstract available at: http://www. ncbi. nlm.nih.gov/pubmed/580722 [Accessed on 6 February 2018]
44. Morita M, Imanaka T. Peroxisomal ABC transporters:

Structure, function and role in disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2012; 1822(9):1387-1396. Science Direct [Online]. Available at: http://www.sciencedirect. com/science/ article/pii/S0925443912000373 [Accessed on 20 February 2018]
45. Na S, Choi M, Kim M, Lee J, Cho S. Penicillamine-induced Elastosis Perforans Serpiginosa and Cutis Laxa in a Patient with Wilson's Disease. Annals of Dermatology. 2010; 22(4):468. NCBI [Online]. Available at:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2991731/ [Accesed on 7 February 2018]
46. Nature.com. Scientists Can Make Copies of a Gene through PCR | Learn Science at Scitable. [online], 2014, Available at: http://www.nature.com/ scitable/ topicpage/ scientists-can-make-copies-of-a-gene-6525968 [Accessed on 8 April 2018].
47. Ncbi.nlm.nih.gov. Polymerase Chain Reaction (PCR). [online] Available at, 2014, http://www. ncbi.nlm. nih.gov/probe/docs/techpcr/ [Accessed on 8 April 2018].
48. Nebert D, Zhang G, Vesell E. From Human Genetics and Genomics to Pharmacogenetics and Pharmacogenomics: Past Lessons, Future Directions. Drug Metabolism Reviews. 2008; 40(2):187-224. NCBI [Online]. Available at : http://www.ncbi.nlm.nih.gov/pmc/a rticles/PM C2 75 26 27/ [Accessed on 21 February 2018]
49. Nitschke Y, Baujat G, Botschen U, Wittkampf T, du Moulin M, Stella J, et al. Generalized Arterial Calcification of Infancy and Pseudoxanthoma Elasticum Can Be Caused by Mutations in Either ENPP1 or ABCC6. The American Journal of Human Genetics. 2012; 90(1):25-39. Science Direct [Online]. Available at: http://ac.els-cdn.com/S0002929711004988/1-s2.0-S00 02929711004988-main.pdf?_tid=8e063982-e4ee-11e4-b907-00000aacb3 5f&acdnat=1429 267576_c2017 aeed 47593723d1d3e66ad2f3bdd [Accessed on 29 January 2018]
50. Ogt.co.uk. Understanding and measuring variations in DNA sample quality. [online] Available at, 2015, http://www.ogt.co.uk/resources/literature/483_understanding_and_measuring_variations_in_dna_sample_quality [Accessed on 8 April 2018].
51. Ousterout D, Perez-Pinera P, Thakore P, Kabadi A, Brown M, Qin X, *et al*. Reading Frame Correction by Targeted Genome Editing Restores Dystrophin Expression in Cells From Duchenne Muscular Dystrophy Patients. *Mol Ther*, [online]. 2013; 21(9):1718-1726. Available at: http://www.ncb i.nlm.nih.go v/pubmed/23732986 [Accessed on 9 April 2018].
52. Paulusma C, Bosma P, Zaman G, Bakker C, Otter M, Scheffer G, *et al*. Congenital Jaundice in Rats with a Mutation in a Multidrug Resistance-Associated Protein Gene. *Science*, [online]. 2002; 271(5252:1126-1128. Available, 1996, at: http://www.scopus. com/record/display.url?eid=2-s2.0-0029981430&origin= inward & txGid=D940AEC51A79DAB5AA15533940D90ABA.FZg2ODcJC9ArCe8WOZPvA%3a11 [Accessed on 10 April 2018].
53. Pelt-Verkuil E, Belkum A, Hays J. Principles and technical aspects of PCR amplification. [Dordrecht]: Springer. [Online]. Available at:, 2008, http://link. springer.com/book/10.1007%2F978-1-4020-6241-4 [Accessed on 12 April 2018]
54. Poon E, Mason G, Oh C. Clinical and histological spectrum of elastotic changes induced by penicillamine. Australasian Journal of Dermatology. 2002; 43(2):147-150. NCBI [Online]. Abstract avialble at: http://www.ncbi.nlm.nih.gov/pubmed/11982575 [Accessed on 22 February 2018]
55. Pope F. Autosomal dominant pseudoxanthoma elasticum. Journal of Medical Genetics. 1974; 11(2):152-157.
56. Pope F. Two Types of Autosomal Recessive Pseudoxanthoma Elasticum. Arch Dermatol. 1974; 110(2):209.
57. Ringpfeil F, Lebwohl M, Christiano A, Uitto J. Pseudoxanthoma elasticum: Mutations in the MRP6 gene encoding a transmembrane ATP-binding cassette (ABC) transporter. Proceedings of the National Academy of Sciences. 2000; 97(11):6001-6006. NCBI [Online]. Available at: http://www.ncbi.nlm.nih. gov/pmc/articles/PMC18548/ [Accessed on 2 February 2018]
58. Ringpfeil F, Pulkkinen L, Uitto J. Molecular genetics of pseudoxanthoma. *Experimental Dermatology*. 2001; 10:221-228. Wiley online Library [Online]. Available at: http://onlinelibrary.wiley.com/doi/10.1034/j.1600-06 25. 2001.100401.x/full [Accesed on 2 February 2018]
59. Sakamoto N, Akasaka K, Yamamoto T, Shimada H. A Triplex DMA Structure of the Polypyrimidine: Polypurine Stretch in the 5′ Flanking Region of the Sea Urchin Arylsulfatase Gene. Zoological Science. 1996; 13(1):105-109. NCBI [Online]. Abstract available at: http://www.ncbi.nlm.nih.gov/pubmed/8688804 [Accessed on 9 April 2018]
60. Schachner L, Young D. Pseudoxanthoma Elasticum with Severe Cardiovascular Disease in a Child. Archives of Pediatrics & Adolescent Medicine. 1974; 127(4), p.571. NCBI [Online]. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4544975 [Accessed on 23 March 2018]
61. Scheffer G, Hu X, Pijnenborg A, Wijnholds J, Bergen A, Scheper R. MRP6 (ABCC6) Detection in Normal Human Tissues and Tumors. *Lab Invest*, [online]. 2002; 82(4):515-518. Available at: http://www.r eadcube.com/articles/10.1038/labinvest.3780444 [Accessed on 12 April 2018].
62. Schneider E, Hunke S. ATP-binding-cassette (ABC) transport systems: Functional and structural aspects of the ATP-hydrolyzing subunits/domains. FEMS Microbiology Reviews. 1998; 22(1):1-20. Oxford Journals [Online]. Available at: http://femsre. Oxford journals. org/content/22/1/1.long [Accessed on 25 March 2018]
63. Shampo M, Kyle R, Kary B. Mullis—Nobel Laureate for Procedure to Replicate DNA. Mayo Clinic Proceedings. 2002; 77(7):606. NCBI [Online]. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12108595 [Accessed on 10 February 2018]
64. Sherer D, Bercovitch L, Lebwohl M. Pseudoxanthoma elasticum: Significance of limited phenotypic expression in parents of affected offspring. Journal of the American Academy of Dermatology. 2001; 44(3):534-537. NCBI

[Online]. Abstract available at: http://www. ncbi. nlm. nih.gov/pubmed/11209132 [Accesed on 7 February 2018]
65. Sun N, Liang J, Abil Z, Zhao H. Optimized TAL effector nucleases (TALENs) for use in treatment of sickle cell disease. *Molecular BioSystems*, [online]. 2012; 8(4):1255. Available at: http://www.ncbi. nlm.nih. gov/ pubmed/ 22301904 [Accessed on 9 April 2018].
66. Terry S. Disease advocacy organizations catalyze translational research. *Front. Genet.*, 4. Frontier [Online]. Available at, 2013, http://journal. frontiersin. org/ article/10.3389/fgene.2013.00101/full [Accessed on 28 January 2018]
67. Thomas P, Cote G, Wohllk N, Haddad B, Mathew P, Rabl W, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science*, [online]. 1995; 268(5209):426-429. Available at: http://www. scopus. com/record/ display.url?eid=2-s2.0-0029021696&origin= inward & txGid=D940AEC51A79DAB5AA15533940D90ABA.FZ g2ODcJC9ArCe8WOZPvA%3a1 [Accessed on 10 April 2018].
68. Thornton B, Basu C. Real-time PCR (qPCR) primer design using free online software. Biochemistry and Molecular Biology Education. 2011; 39(2):145-154. Springer [Online]. Available at:v http:// online library. wiley.com/doi/10.1002/bmb.20461/abstract
69. Uitto J, Boyd C, Lebwohl M, Moshell A, Rosenbloom J, Terry S. International Centennial Meeting on Pseudoxanthoma Elasticum: Progress in PXE Research. J Investig Dermatol. 1998; 110(5):840-842.
70. Uitto J, Pulkkinen L, Ringpfeil F. Molecular genetics of pseudoxanthoma elasticum: a metabolic disorder at the environment–genome interface?. Trends in Molecular Medicine. 2001; 7(1):13-17.
71. Uitto J, Váradi A, Bercovitch L, Terry P, Terry S. Pseudoxanthoma Elasticum: Progress in Research toward Treatment: Summary of the 2012 PXE International Research Meeting. J Investig Dermatol. 2013; 133(6):1444-1449.
72. Vasiliou V, Vasiliou K, Nebert D. Human ATP-binding cassette (ABC) transporter family. Hum Genomics. 2008; 3(3):281. NCBI [Online]. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2752038/ [Accessed on 9 February 2018]
73. Wilson D. Clinical Methods: The History, Physical and Laboratory examinations. 3rd ed. Boston: Emory University School of Medicine, Atlanta, Georgia, 1990, 439-442.
74. Yakovchuk P, Protozanova E, Frank-Kamenetskii M. Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. Nucleic Acids Research, [online]. 2006; 34(2):564-574. Available at: http://nar.oxfordjournals.org/content/34/2/564.full.pdf+html [Accessed on 8 April 2018].
75. Zebardast N, Adelman R. Intravitreal Ranibizumab for Treatment of Choroidal Neovascularization Secondary to Angioid Streaks in Pseudoxanthoma Elasticum: Five-year Follow-up. Seminars in Ophthalmology. 2012; 27(3-4):61-64.