Deanship of Graduate Studies

Al-Quds University

Genetic Screening of Xeroderma Pigmentosum Disorder in a Palestinian Family

Farouq Ziyad Rashad Nather

M.Sc. Thesis

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Thesis Approval

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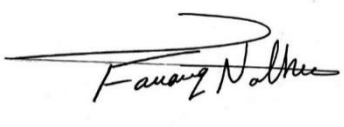
Declaration

I hereby verify that this master's thesis, submitted for the degree of Master, is the outcome of our research efforts. The content presented in the thesis is based on the work conducted since the approval of the research program. Throughout the preparation of this thesis, we have diligently adhered to all relevant ethics procedures and guidelines.

Signed: 

Farouq Ziyad Rashad Nather

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Abstract

Xeroderma pigmentosum (XP) is an uncommon autosomal recessive genetic disorder characterized by an extreme sensitivity of the skin to sunlight, particularly UV light, and an elevated risk of skin cancer. Some patients with XP also exhibit neurological symptoms. The majority of XP cases are attributed to mutations in eight specific genes (XPA through XPG and XPV). The XP-V subtype of the disease results from mutations in a gene called XPV, also known as POLH, responsible for encoding Pol eta, a member of the Y-DNA polymerase family. XP variant represents a milder form of XP caused by variants in the POLH gene. POLH encodes an error-prone DNA polymerase eta, which plays a crucial role in synthesizing past UV-induced photoproducts.

In the current study, we aimed to look for the underlying molecular cause of XP in a Palestinian family with multiple affected individuals suffering from the disease. WES analysis in the Proband led us to the identification of a homozygous frame-shift mutation c.106_118del p. (Val36Asnfs*8) in POLH which is responsible for the disease. Subsequently, clinical investigations and familial segregation analysis using Sanger sequencing were performed to check which family members are homozygous/ heterozygous (genotype) for the frameshift mutation. Interestingly, we discovered that a 60-year-old family member without any medical history is a homozygous for the mutation.

In conclusion, this study enabled us to establish the genetic diagnosis of XP in a Palestinian family by the identification of a new disease-causing mutation in the DNA polymerase eta (POLH gene). This Shows the significance of molecular diagnosis for accurate identification of the disease and provides valuable information for proper genetic counseling in families affected by XP.

Keywords: xeroderma pigmentosum (XP); autosomal recessive, polymerase eta, whole-exome sequencing, Y-DNA polymerase.

"الفحص الوراثي لمرض جفاف الجلد الصباغي في عائلة فلسطينية"

إعداد: فاروق زياد رشاد الناظر

إشراف: الدكتورة كفاية سليمان و الدكتورة فواز عواد

جفاف الجلد المصطبغ (XP) هو اضطراب وراثي جسمي متنحي غير شائع يتميز بحساسية شديدة للجلد لأشعة الشمس، وخاصة الأشعة فوق البنفسجية، وارتفاع خطر الإصابة بسرطان الجلد. بعض المرضى الذين يعانون من XP يظهرون أيضًا أعراضًا عصبية. تُعزى غالبية حالات XP إلى طفرات في ثمانية جينات محددة (XPA إلى XPG وXPV). ينتج النوع الفرعي XP-V من المرض عن طفرات في جين يسمى XPV، المعروف أيضًا باسم POLH، المسؤول عن ترميز Pol eta، وهو عضو في عائلة بوليميريز Y-DNA. يمثل متغير XP شكلاً أكثر اعتدالًا من XP الناجم عن المتغيرات في جين POLH. يقوم POLH بتشفير إيتا بوليميريز الحمض النووي المعرض للخطأ، والذي يلعب دورًا حاسمًا في تصنيع المنتجات الضوئية السابقة الناتجة عن الأشعة فوق البنفسجية.

في الدراسة الحالية، كنا نهدف إلى البحث عن السبب الجزيئي الكامن وراء مرض XP في عائلة فلسطينية تضم العديد من الأفراد المصابين الذين يعانون من المرض. قادنا تحليل WES في المريض إلى تحديد طفرة متماثلة في إطار التحول c.106_118del p. (Val36Asnfs*8) في POLH وهو المسؤول عن المرض. بعد ذلك، تم إجراء التحقيقات السريرية وتحليل الفصل العائلي باستخدام تسلسل سانجر للتحقق من أفراد الأسرة المتماثلين/غير المتجانسين (النمط الوراثي) لطفرة تغيير الإطارات. ومن المثير للاهتمام أننا اكتشفنا أن فردًا من العائلة يبلغ من العمر 60 عامًا وليس لديه أي تاريخ طبي هو متماثل الزيجوت بالنسبة للطفرة.

في الختام، مكنتنا هذه الدراسة من إنشاء التشخيص الوراثي لمرض XP في عائلة فلسطينية من خلال تحديد طفرة جديدة مسببة للمرض في DNA بوليميريز إيتا (جين POLH). يوضح هذا أهمية التشخيص الجزيئي لتحديد المرض بدقة ويوفر معلومات قيمة للاستشارات الوراثية المناسبة في العائلات المصابة بـ XP.

الكلمات المفتاحية: جفاف الجلد المصطبغ (XP)؛ جسمي متنحي، بوليميريز إيتا، تسلسل إكسوم كامل، بوليميريز Y-DNA.

Introduction

Xeroderma pigmentosum (XP) was first described by Moriz Kaposi which is a dermatologist in the year 1874 in Vienna dealing with four patients that consists of a dry thin skin viewing wrinkling, skin contraction, variable pigmentation, small dilatations of the vessels, and the development of skin-based tumors that are linked to a imperfect DNA repair that was reported by James Cleaver almost a century later (Fang, X., & Sun, Y.et al., 2019).

XP which is resulted due to the mutations to at least one of the 8 different genes (XPA, XPB, XPC, XPD, XPE, XPF, XPG and XPV) (Table1), which produces proteins that contribute in the nucleotide excision repair (NER) molecular cascade without the XP variant (XPV) type. The XPV is the only type of XP that is not caused by a mutation in the NER proteins instead it is resultant due to the mutations in the XPV (which is also known as POLH) gene. POLH gene encodes one of the DNA polymerase family proteins that's called DNA polymerase eta that helps in the repair of the DNA that is damaged by the UV (NA Bowden et al., 2015).

The classification of the XP is done by different complementation groups depending on the excat gene that's affected. The resultant of each complementation group depends on the presence of the causative mutation in the one of the XP family genes that are involved in the NER nor the POLH gene which encodes the XP-V which have an important role in the translation synthesis. Also the ERCC1 gene which also could be affected, which is an infrequent mutated one (Brambullo et al., 2022).

Table 1. Xeroderma pigmentosum (XP) groups: Table shows the frequency, ability to develop skin cancer, neurological abnormality, affected mutated gene, cellular repair capabilities and the sight of the chromosomal mutation (From: Lehmann, Seebode et al. 2018).

Complementation group	Frequency (%)	Skin cancer	Neurological involvement	Cellular repair capability	Defective gene	Chromosome
XP-A	30	++	+++	<10%	*XPA*	9q22.3
XP-B	0.5	+	+	3-7%	*XPB/ERCC3*	2q21
XP-C	27	++	+	10-20%	*XPC*	3p25
XP-D	15	++	+++	25-50%	*XPD/RECC2*	19q13.2-q13.3
XP-E	1	+	–	40-50%	*DDB1*	11q12-q13
					XPE-DDB2	11p12-p11
XP-F	2	+	-	10-20%	*XPF/ERCC4*	16p13.3
XP-G	1	+	++	<5%; 25%	*XPG/ERCC5*	13q33
XP Variant	23.5	+	–	100%	*Pol H*	6p21.1-p12

Oncogenic processes such apoptosis, differentiation, proliferation, and cell cycle disruption are carried out through the NER pathway with altered gene expression. Brain, head and neck, prostate, stomach, breast, bladder, colorectal, lung, endometrial, and melanoma, as well as non-melanoma skin cancers (NMSC), are one of the malignancies types that have been linked to XP syndrome (J. Zhao, S. Chen, H. Zhou et al., 2018).

1.1. Xeroderma pigmentosum

The rare genetic disorder XP which is autosomal recessive is considered to the patient's increased propensity and the development of skin malignancies in locations of sun-exposed.

XP is a genetic disease that is rarely autosomal recessive type that is characterized by the high susceptibility to progress skin cancers in sun exposed areas that patient’s deal with. One of the first scientists to define XP was Hebra and Kaposi in 1874, who described it as "a remarkable abnormality of pigmentation" and a "parchment-like drought, thinning, and wrinkling of the epidermis" (Hebra and Kaposi 1874). A little later, James Cleaver defined XP as a hereditary disorder, noting errors and defects in the cells of XP patients that had undergone UV radiation-induced DNA repair (Cleaver 1968).

Shortly after, De Weerd-Kastelein confirmed that the XP disease is a genetically heterogeneous disease. Ultimately, several groups of genetic problematic (XP-A to XP-G) and one variant (XP-V) were found and identified (Cleaver 1972, De Weerd-Kastelein, Keijzer et al. 1972, Keijzer, Jaspers et al. 1979). According to Kraemer and DiGiovanna (2015), the clinical and genetic characteristics of XP patients provide a model for the research of DNA repair and carcinogenic pathways.

1.1.1. Clinical characteristics and epidemiology

Geographical location affects the variation of estimated XP occurrences. As per the latest epidemiological investigation carried out in five Western European countries, autochthonous population incidence is estimated to be 1 per million births, but when immigrant population incidence is taken into account, this ratio doubles to 2,3 per million

births (Kleijer, Laugel et al. 2008). In areas where consanguinity has a long history, XP is more common. In Morocco and Tunisia, the prevalence of XP disease is estimated to be 1 in 80,000 and 1 in 10,000, respectively. Over 80% of cases in both nations, as well as the entire Mediterranean region, are caused almost only by XPC gene abnormalities. The main gene impacted in Japan, where the frequency of XP is around 1 in 20,000, is XPA (Hirai, Kodama et al. 2006).

Starting at age two, people with XP have a sensitivity to sunlight with the development of freckles and pigmentation lesions in sun-exposed areas. Early symptoms of photo-aging can be seen in the skin, which is typically dry, scaling, and atrophic (see Figure 2). According to Lehmann, McGibbon, and colleagues (2011), skin pigmentation is diverse and intermixes between zones that are hypo- and hyper-pigmented. These patients usually have issues with UV-exposed visual tissue. The prevailing abnormal symptoms frequently observed consist of conjunctivitis, corneal neovascularization, and dryness of the eyes. (Brooks, Thompson et al. 2013). According to Kraemer, Patronas et al. (2007), Bradford, and Goldstein et al. (2011), about one-fourth of XP patients also have and deal with neurological symptoms, including deterioration of speech function, progressive mental decline, hearing loss, peripheral neuropathy, ataxia, abnormal gait, and psychomotor changes that affect walking.

Skin cancer is the leading cause of death in XP sufferers. In comparison to the general population, they have approximately a 10,000 times increased chance of acquiring NMSC and a 2000 fold increased melanoma development before the age of twenty (Bradford, Goldstein et al. 2011). The earliest comprehensive case study of XP is dated 1987.

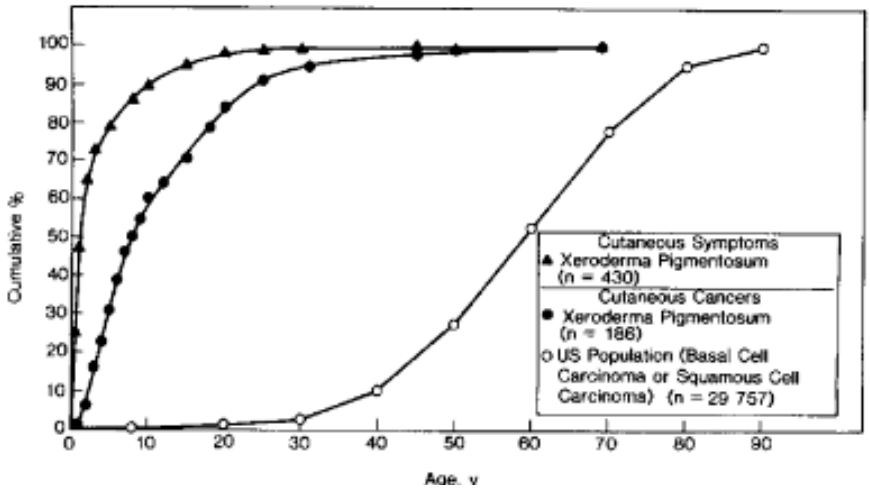


Figure 1. Age at which cancer and XP symptoms first appear (Kraemer, Lee et al. 1987).

A recent follow-up study which included a 106 XP patients, data on the median ages of the first melanoma and NMSC in the XP patients compared to population were given particular attention. The average age of the first melanomas and the first NMSCs in the general population is 67 years old and 55 years, respectively. The typical and average age of the appearance of the first melanoma diagnosis and finding in the XP patients is considered close to 22 years of age, while the median age of the first NMSC remains under 10 years old. These findings demonstrate that, in addition to XP patients developing skin malignancies earlier than the general population, there is also a change in the kind of initial cancer diagnosis. Melanomas normally tend to develop first in the normal population, whereas XP patients have a "preference" for the development of NMSC. This challenge shows that the two types of cancer have different methods of carcinogenesis (Bradford, Goldstein et al. 2010), A 10- to 20-fold rise in the prevalence of internal cancers in XP patients was also noted in a study from 1984 (Kraemer, Lee et al. 1985).

XP patients were found to have immune abnormalities. Studies conducted during the late 1980s and early 1990s revealed that NK from XP patients showed a diminished lytic activity, Furthermore, it has been suggested that this impaired function of NK cells could potentially contribute to XP's heightened vulnerability to cancer (Mariani, Facchini et al., 1992). Additionally, studies involving the activation of poly inosinic cytidylic acid demonstrated that XP peripheral blood lymphocytes (PBL) produced reduced levels of

IFN-γ, IFN-α (interferon-gamma and alpha), which are the NK cell activators (Gaspari, Fleisher et al. 1993), also According to Mariani, Facchini, et al. (1992), XP patients also exhibit reduced levels of circulating lymphocytes that are characterized by the presence of CD3+ CD4+.

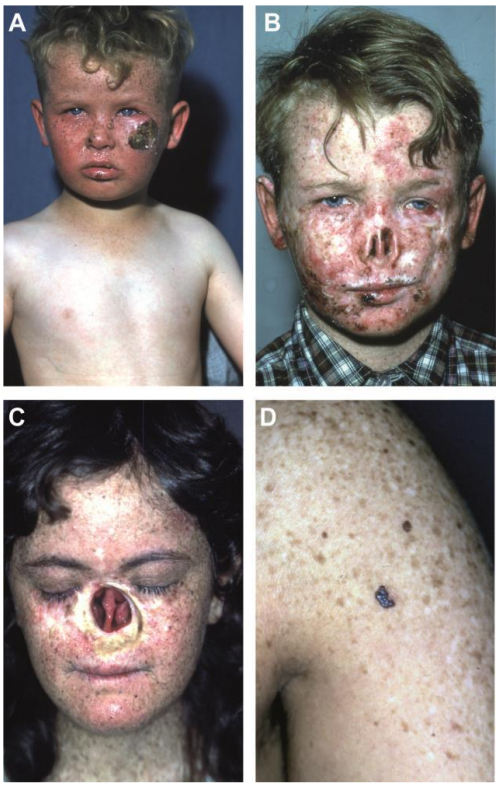


Figure 2. The illustration depicts two XP patients showcasing typical clinical characteristics. In Figure A, the patient exhibits regular freckling in sun-exposed regions, along with a severe sunburn on the face and multiple non-melanoma skin malignancies on specific facial areas (nose, lips, and cheek). (B) This photograph is of the same patient several years later. He has lost his nose due to the existence of a tumor, and he also has scars on his face after tumor excision, as well as further precancerous lesions. (C) This female patient, like the previous one, has lost her nose due to an invasive tumor, In Figure D, the female patient not only experienced the loss of her nose due to an invasive tumor but also presents with melanoma in sun-exposed areas, such as the arms.

1.1.2. Etiology and pathogenesis

The "classic" form of Xeroderma pigmentosum (XP) refers to the most common and well-known presentation of the genetic disease is caused by inherent functionality in the machinery that repairs DNA damages following UV exposure and which is the NER pathway. James Cleaver is the first scientist researcher to recognize the bond between the failure in DNA repair due to UV exposure and XP phenotype (Cleaver 1968). He conducted research that showed a significant proportion of XP cells exhibited a deficiency in unscheduled DNA synthesis (UDS) following UV irradiation, using a technique subsequently known as "unscheduled DNA synthesis" (UDS). The basic idea behind UDS is to include tagged nucleotides into freshly synthesized DNA patches during DNA repair. Previously, radio-nucleotides such as 3H-thymidine or 3H-bromodeoxyuridine were utilized; the level of incorporation was measured either through autoradiography or liquid scintillation counting. (Lehmann and Stevens 1980). Today, a strong criterion to validate XP diagnosis can now utilize a non-radioactive label, such as 5-Ethynyl-2'-deoxyuridine (EdU), to assess unscheduled DNA synthesis (UDS). This method provides a safer and more efficient alternative (Limsirichaikul, Niimi et al. 2009).

XP can be caused by mutations in any of eight genes (XPA through XPG and XPV). The genes XPA to XPG encode proteins which expression is changed during the DNA repair mechanism known as nucleotide excision repair (NER). Pol eta, a product of the POLH gene, belongs to the DNA polymerase family known as eta DNA polymerases. Its main role is to facilitate translesion DNA synthesis, a process that allows DNA replication to continue past damaged or distorted regions in the DNA template.

1.1.3. Genotype-phenotype correlation

The seven complementation genes' products play distinct functions in the NER, and their mutations in these genes are connected to a range of symptom severity, occurrence rate, and residual DNA repair levels, as assessed through unscheduled DNA synthesis (UDS) evaluation. (Table 1). For instance, XP-C and XP-E patients are less prone to experiencing acute sunburn, while individuals with XP-A (De Sanctis-Cacchione syndrome) are associated with severe neurological symptoms. (Moriwaki, Kanda et al. 2017).

Mutations in the NER pathway are unrelated to the variation (XP-V) disease. Therefore, NER impairment cannot be used as a diagnostic criterion. As an alternative, XP-V cells cause issues with the translesion synthesis (TS) technique used to replicate UV-damaged DNA strands. When there is no DNA damage, DNA polymerases (I) is in charge of replicating DNA; however, In the presence of DNA damage on the template, there is a switch from DNA polymerase I to other specialized DNA polymerases capable of carrying out DNA processing while the damage is being repaired. DNA strands with UV-related damages are replicated with the help of DNA polymerase eta (pol eta). Patients with XP-V have mutations in the POLH gene, which codes for pol eta. (Cordonnier, Lehmann et al. 1999, Gratchev, Strein et al. 2003).

Not all genetic diseases linked to NER pathway abnormalities are XP-related. Trichothiodystrophy (TTD) and Cockayne's syndrome (CS), as well as a number of other similar or overlapping diseases, are two other genodermatoses that have been identified (figure 3). As is the case with XP, one disease may result from mutations in several separate genes, but the contrary is also possible, This indicates that different mutations within the same gene can lead to diverse diseases or clinical conditions. (DiGiovanna and Kraemer 2012).

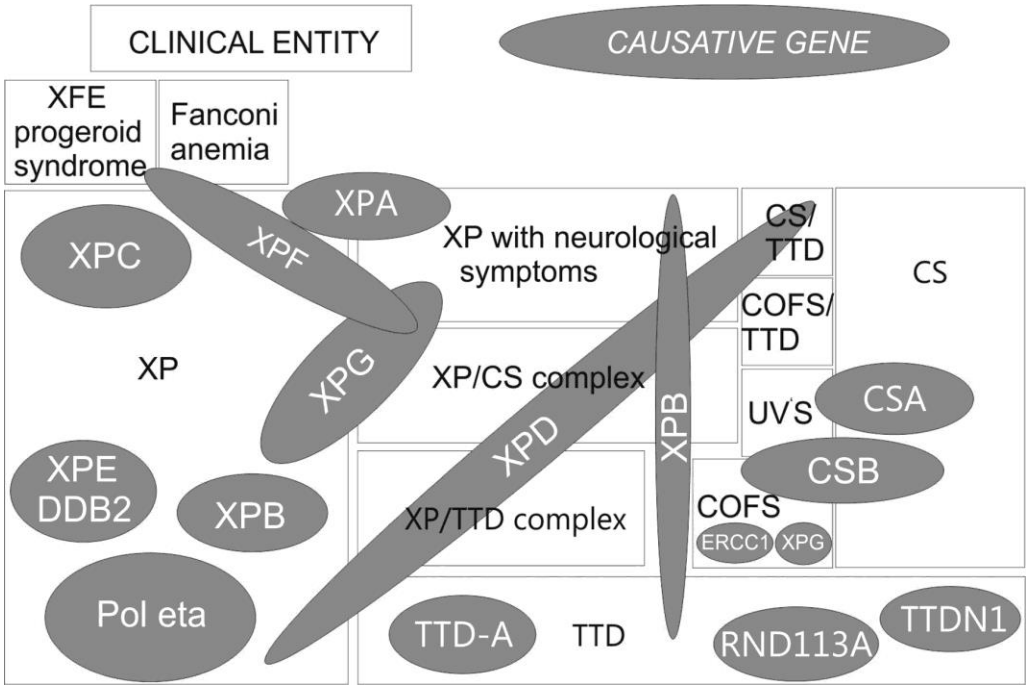


Figure 3. DNA Repair disorders (Adapted from (DiGiovanna and Kraemer 2012)

1.1.4. Xeroderma pigmentosum Prevalence.

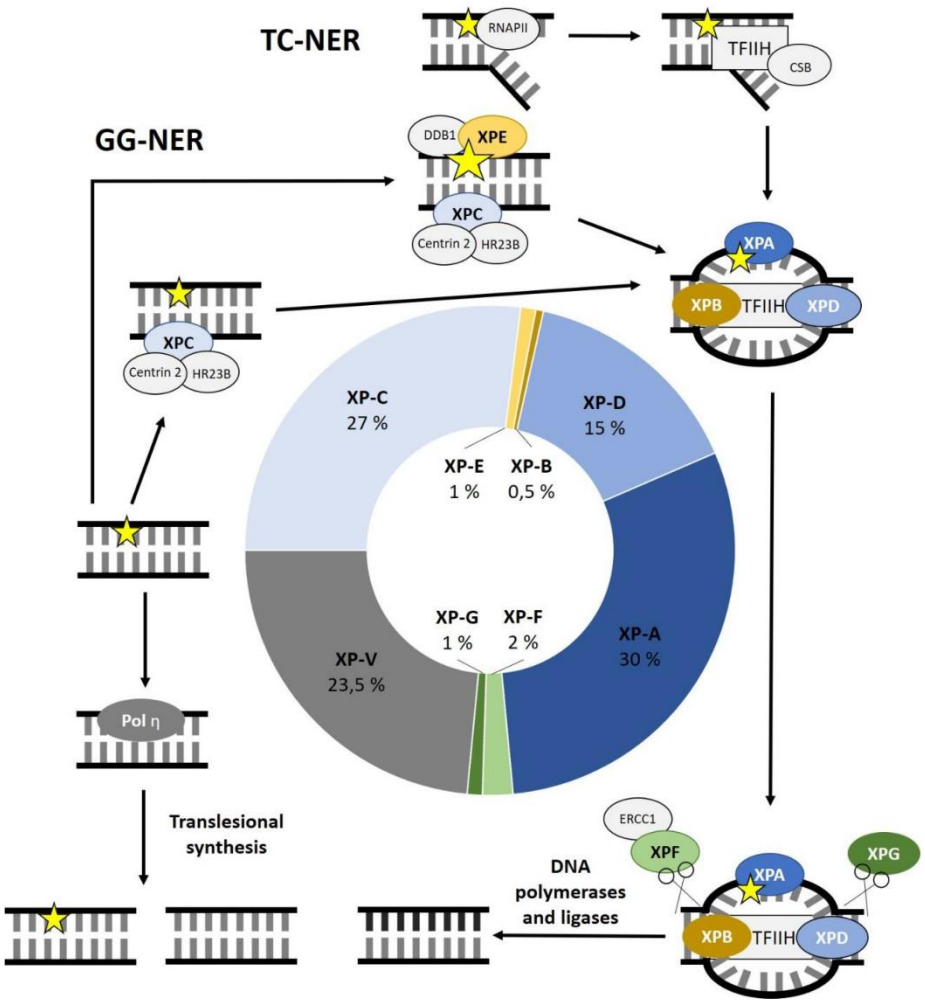
XP is an uncommon genetic disorder that occurs at a global rate of 1 in 1 million individuals. It can be classified into seven complementation groups, namely XP-A to XP-G and XPV. Despite the variant form's impact on translesional DNA polymerase eta, all seven XP groups are involved in the NER process. Within these groups, specific genes are more commonly affected than others. Notably, XP-A is the most frequently encountered subtype. XP-A is the most frequent subtype, accounting for approximately 30% of all XP patients, while XP-C follows closely at 27%, and XP-D constitutes nearly 15% of the affected individuals. On the other hand, XP-F, XP-E, and XP-G are less commonly affected, with incidences of 2%, 1%, and 1%, respectively. XP-B is the least frequently impacted XP complementation group, representing only 0.5% of XP cases. XPV defects constitute 23.5% of all cases (Figure 4).

The prevalence of XP can vary in different regions, which may be attributed to factors such as isolation, cultural practices, or limited mobility. For instance, in Japan, the prevalence of XP is 1 in 22,000 individuals, and approximately 1% of the Japanese population carries a founder mutation in XPA. Similarly, Among the Northern African population, founder mutations in XPC have been documented, and in Iraqi families of Jewish descent, founder mutations have been reported in XPD. (Martens et al., 2021).

The prevalence of XP is highest in regions with increased rates of consanguinity, such as North Africa and the Middle East. In the USA and Europe, the prevalence is estimated to be around 1 in 1 million. However, in specific areas like Morocco and Saudi Arabia, where consanguinity rates are higher, the frequency is likely to be approximately 15–20 cases per 1,000,000 individuals, similar to Libya. The prevalence of XP varies significantly within complementation groups due to differences in individual UV radiation exposure and protective measures.

Notably, XPC and XPA are the most common types of XP in the USA, Europe, North Africa, and Japan, respectively, as reported by AlWatban & Binamer (2017) and Black (2016).

It is crucial to underscore that there exists significant diversity in the regional incidence of XP. Although the disease is expected to be relatively common in Africa and the Middle East, there is a lack of studies focusing on the prevalence of XP in specific areas, such as Palestine (West Bank, Gaza). As of now, no papers or studies have covered XP cases in this region. Therefore, conducting epidemiological studies is essential to determine the actual prevalence of XP, which will aid in devising policy planning and developing healthcare systems to improve the care and survival of affected patients, as emphasized by Tembo et al. (2022).



Martens et al., 2021

Figure 4. In this section, a comprehensive examination of the Nucleotide Excision Repair (NER) process and the prevalence of gene mutations associated with Xeroderma pigmentosum (XP) is presented. The Global Genome (GG)-NER pathway comprises the XPC complex, which is responsible for recognizing and detecting DNA modifications induced by damage. HR23B and centrin 1 play roles in increasing the activity of XPC and stabilizing the XPC complex, respectively. The UV-DDB complex, containing DDB1 and XPE, aids in recognizing minor distortions in the DNA by inducing a more noticeable kink. The Transcription-coupled (TC)-NER process initiates when DNA damage stalls RNA polymerase II (RNAPII), leading to the recruitment of CSA, CSB, and UVSSA. These proteins initiate the activation of the transcription factor II H (TFIIH) complex, comprising ten subunits, including the DNA helicases XPB and XPD. XPB and XPD work together to

unwind the double-stranded DNA around the lesion. Subsequently, the endonucleases XPG and XPF-ERCC1 participate in the removal of the damaged single-stranded DNA resulting in a gap that is subsequently repaired by DNA polymerases and DNA ligases. XPA plays a pivotal role in orchestrating incisions and identifying single-stranded DNA damage within the Nucleotide Excision Repair (NER) pathway. Patients with XP variant (XPV) have a mutation in the translesional DNA polymerase. The diagram in the center depicts the distribution of affected complementation groups in Xeroderma pigmentosum (XP), showing the frequency of involvement of each group. XP-V patients exhibit a relatively milder phenotype, characterized by a later onset of symptoms and a slower disease progression compared to other XP subtypes. XP-V patients do not have any ocular or neurological problems. According to several studies, this type of XP is underdiagnosed. As a result, XP-V patients account for just 20 to 30% of all XP cases. Due to a lack of DNA polymerase eta (), cells from XP-V patients are extremely hypermutable after UV exposure. Under normal circumstances, DNA polymerase eta (η) is responsible for catalyzing translesion synthesis (TLS) by incorporating dAMP opposite thymine residues of a cyclobutane thymine dimer (CPD).

When DNA polymerase eta is absent, the role of bypassing UV-induced lesions falls upon the highly error-prone DNA polymerase iota. As a consequence, there is an accumulation of UV-induced mutations, leading to an elevated susceptibility to skin cancer. The genetic code for Pol eta is carried by the POLH gene, which is the human equivalent of the yeast Rad30 gene. Pol eta plays a crucial role in safeguarding genome stability by mitigating the effects of DNA damage induced by UV or cisplatin exposure. Over 60 mutations in the POLH gene have been identified in cell lines derived from XP-V patients hailing from various geographic regions, including Russia-Armenia, Germany, Korea, Algeria, Lebanon, Iran, USA, Europe, Asia, and turkey.

1.2. UV irradiation and the skin

UV light, an electromagnetic radiation spanning the 100-400nm range, is widely acknowledged as a potent mutagen with established mutagenic effects. UV light can be classified into three main categories: UVA, UVB, and UVC, as reviewed by Seebode et al. in 2016.

1.2.1. Penetration

UVC irradiation, with wavelengths ranging from 100 to 280nm, possesses the shortest wavelength yet the highest energy, aligning with DNA's absorption maximum at 245nm. However, the ozone-containing stratosphere efficiently blocks the majority of UVC radiation, and any residual UVC that manages to reach the Earth's surface is absorbed by the skin's corneal barrier (stratum corneum). On the other hand, UVB (280-315 nm) and UVA (315-400 nm) radiation have the ability to penetrate the atmosphere, resulting in DNA damage upon exposure (Rastogi et al. in 2010).

UVA irradiation is renowned for its influence on skin aging, primarily due to its ability to penetrate deeper skin layers, particularly the dermis, where elastin and collagen fibers are located. In contrast, UVB radiation can only penetrate into the deepest layers of the epidermis, specifically the stratum basale. (see Figure 5).

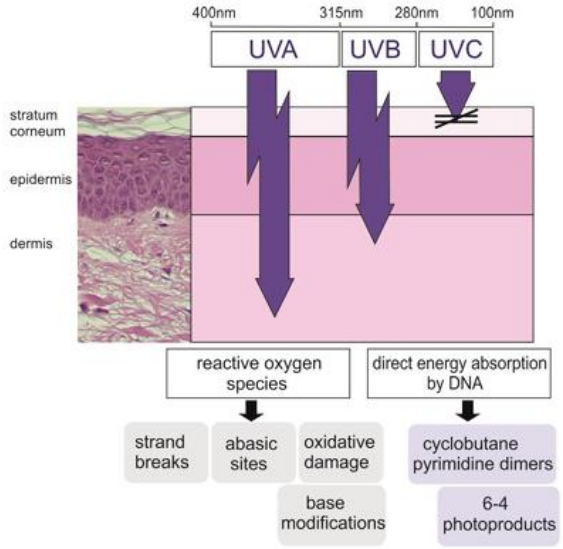


Figure 5. The impact of UV irradiation on human skin and its penetration depth are illustrated in the left panel, displaying a histological section of human skin (hematoxylin-eosin staining, x40). The right panel provides a schematic representation of how UV and visible light penetrate the skin. UVC irradiation (100-280 nm) is effectively blocked by the stratosphere, preventing it from reaching the earth's surface. On the other hand, UVB radiation (280-315 nm) is capable of penetrating the stratum basale, directly impacting DNA and giving rise to the formation of bulky DNA lesions, including 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs). UVA irradiation (315-400 nm) primarily reaches the dermis, leading to the generation of reactive oxygen species. These reactive oxygen species are responsible for causing strand breaks, abasic sites, oxidative damage, or base modifications within the skin.
The illustration credits go to Dr. rer. nat. Christina Seebode (From: Lehmann, Seebode et al. 2018).

1.2.2. UV induced DNA lesions

UVA causes indirect DNA damage by producing free radicals, but UVB and UVC cause direct DNA damage by crosslinking neighboring pyrimidine bases and forming bulky adducts. There are two forms of lesions: (CPDs) and (6-4 PPs) which refers to cyclobutane pyrimidine dimers and 6-4 pyrimidine photoproducts. (Lippke et al., 1981; Mitchell & Nairn, 1989) (see Figure 6). CPDs make up 75% of the lesions and entail the formation of a four-membered ring through the coupling of the C=C double bonds of pyrimidines. On the other hand, 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs) account for 25% of the lesions and involve a crosslink between C6 of one pyrimidine and C4 of the other, causing a more pronounced distortion of the DNA backbone (Vink & Roza, 2001; Yokoyama et al., 2012).

Inside the cellular environment, 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs) undergo rapid repair within a six-hour timeframe, while cyclobutane pyrimidine

dimers (CPDs) remain unresolved for up to 12 hours (50%). (Kobayashi et al., 2001). Consequently, CPDs are the main culprits behind the long-lasting UV-induced mutations. (You et al., 2001).

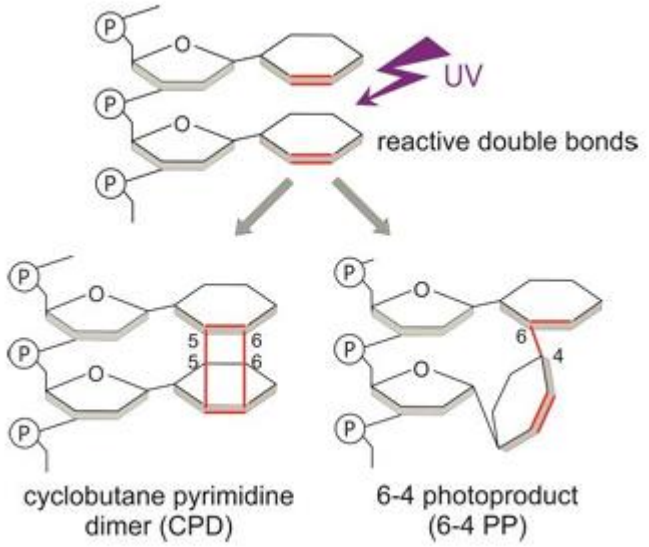


Figure 6. UVB irradiation promotes direct DNA absorption, which results in the creation of DNA photoproducts. Adjacent pyrimidine bases (thymidines (T) and cytosines (C)) crosslink, resulting in the formation of CPDs and 6-4 PPs in a 3:1 ratio, as reported by Seebode et al. in 2016.

During DNA replication, cells have a mechanism to deal with DNA damages through translesion polymerases if the damage remains unrepaired by the time the cell enters the S-phase of the cell cycle. A common mutation observed in squamous cell carcinomas (SCCs) is the C to T or CC to TT transition in TP53 genes, which represents a typical UV signature mutation (Brash et al., 1991). These signature mutations adhere to the A-rule, wherein the translesion polymerase η, lacking proofreading capability, inserts two adenines opposite the crosslinked pyrimidine bases (as reviewed in Matsumura & Ananthaswamy, 2002). Consequently, alternative pathways have evolved for DNA damage repair.

XP patients, who exhibit a significant predisposition to skin cancer development, present a distinctive disease model that allows for the investigation of the consequences of unrepaired DNA lesions in skin carcinogenesis and the progression of skin cancer. (as reviewed in DayaGrosjean, 2008).

1.3. DNA repair

As mentioned earlier, UV light has played a crucial role in the early phases of evolution, owing to its capacity to be absorbed by DNA, resulting in the emergence of long-chain RNA molecules and the evolution of more complex organisms. (Mulkidjanian et al., 2012). Consequently, UV-induced mutations may be perceived as a pivotal force driving evolution and promoting the diversification of life forms. However, under the influence of evolutionary pressures, all living organisms have ultimately evolved an intricate system of DNA repair pathways to counteract the adverse consequences of DNA damage and ensure the preservation of genome integrity. This complex system is known as the DNA damage response (DDR), as reviewed by Giglia-Mari et al. in 2011.

1.3.1. DNA repair mechanisms

Cells have developed multiple repair mechanisms to safeguard genome integrity and effectively manage diverse types of DNA damage, as examined comprehensively by Lombard et al. in 2005. While several types of DNA repair mechanisms exist, my focus will be on discussing Nucleotide Excision Repair (NER) due to its crucial role in the development of most types of Xeroderma Pigmentosum (XP).

1.3.1.1. The Nucleotide excision repair pathway (NER)

Fortunately, cells have evolved a specialized mechanism to detect and repair UV lesions and other bulky adducts, known as the nucleotide excision repair (NER) pathway. The NER pathway composed four main steps: damage recognition, damage verification, dual incision, and gap filling (see Figure 7).

NER encompasses two sub-pathways that differ in their recognition step. Transcription-coupled NER (TC-NER) operates during transcription, where UV lesions cause RNA polymerase to stall at the damage site. Subsequently, RNA polymerase backtracks to create space on the DNA for damage verification (Lans et al., 2019). On the other hand, in the global genome NER (GG-NER) sub-pathway, recognition occurs simultaneously throughout the genome as it is scanned for damage (Marteijn et al., 2014). In this context, XPC serves as the central damage recognition factor (Sugasawa et al., 1998), binding to short unpaired regions that form around UV lesions. While XPC directly recognizes 6-4PPs, which cause extensive distortion in the DNA double helix resulting in single-stranded DNA, CPDs create three distortions that only slightly weaken base pairing between nucleotides. To recognize CPDs, XPC depends on the UV-DDB complex, comprising DDB1 and the damaged DNA-binding factor DDB2 (Fig. 7).

Once XPC and CETN2 are bound to the lesion, the transcription factor TF2H binds subsequently. TF2H contains helicases, XPB, and XPD, which unwind the DNA and validate the damage (Fig. 7). If the verification process fails to detect any damage, the entire process is aborted (Mathieu et al., 2013).

After unwinding, the strand containing the lesion is excised and removed. The endonucleases XPF-ERCC1 and XPG cut the strand on both sides of the lesion, creating a gap of 24-32 nucleotides (Fagbemi et al., 2011). Finally, DNA polymerase fills this gap, and DNA ligase seals the nicks (Ogi et al., 2010) (Fig. 7).

The NER pathway effectively prevents the accumulation of mutations by proficiently recognizing, verifying, and removing UV-induced DNA lesions. Consequently, the NER pathway plays a critical role in preventing skin cancer. In individuals with xeroderma pigmentosum (XP), harmful mutations in NER pathway components render the NER machinery non-functional. As a result,

UV lesions remain unrepaired, leading to the accumulation of mutations and the eventual development of skin cancer. Strikingly, by the age of 20, XP patients face a 10,000-fold increased risk of developing skin cancer compared to healthy individuals (DiGiovanna et al., 2012).

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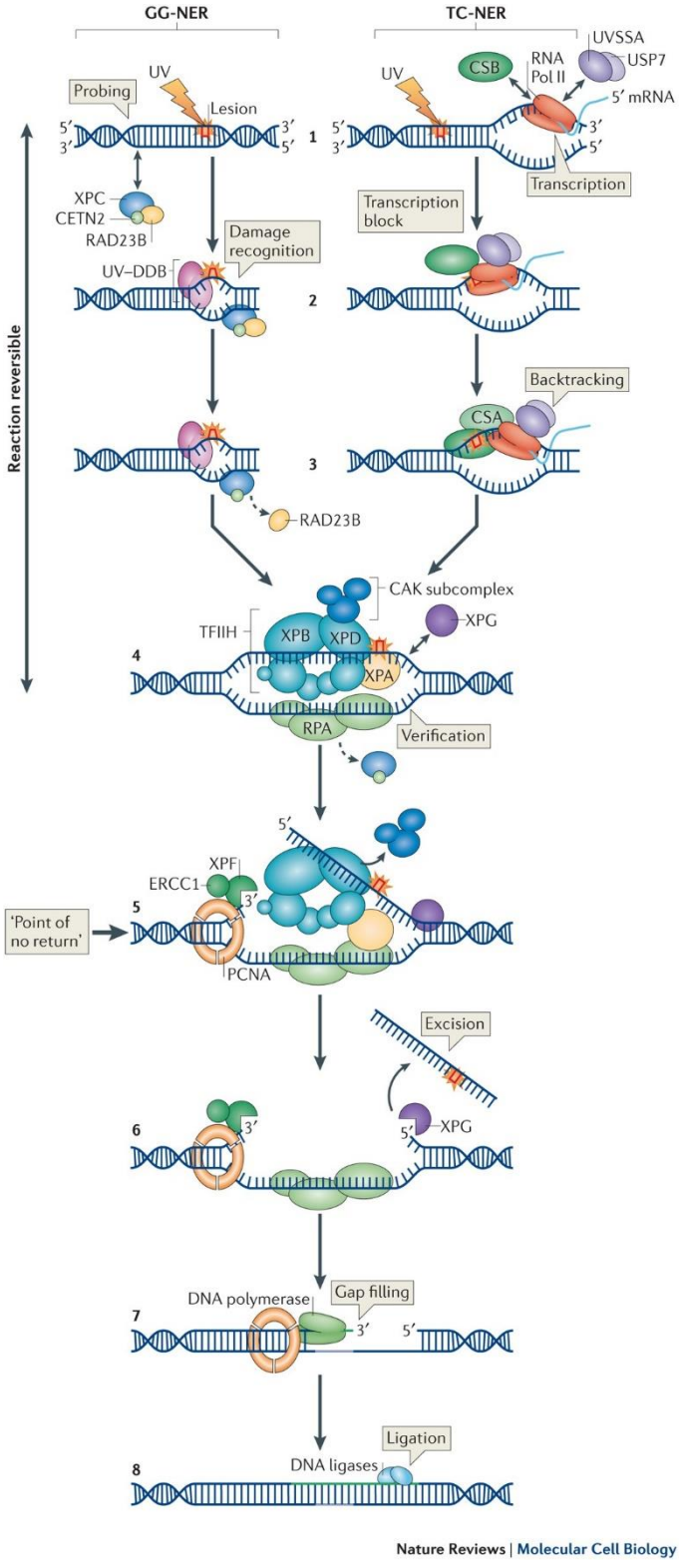


Figure 7. The nucleotide excision repair (NER) pathway involves the recognition of UV lesions either by an RNA polymerase in transcribed regions (TC-NER) or by XPC throughout the genome (GG-NER). After verification by TF2H, the damaged DNA is excised. Subsequently, the resulting gap is filled by a DNA polymerase, and ligases seal the nicks to restore DNA integrity (Figure adapted from Marteijn et al., 2014).

2. Aim of the study

Xeroderma pigmentosum (XP) is a rare autosomal recessive hereditary disease characterized by heightened photosensitivity and DNA repair deficiencies. Among the different types of XP, XPV is one of the most frequently occurring worldwide.

The prevalence of XP exhibits significant regional variation, with higher expected rates in Africa and the Middle East. However, there is a lack of studies focusing on the mutation spectrum of XPV in the Middle East, particularly in Palestine. Notably, clinical features of XPV can range from severe to mild, and they are closely associated with the specific mutation site in the XPV gene.

To address this, the objective of this study is to conduct mutational analysis and identify the molecular defect in a Palestinian patient with XP-V. Additionally, the study aims to screen the patient's family using whole exome sequencing (WES) as a demonstration of how next-generation sequencing can be a powerful and rapid approach for determining XP cases.

The specific objectives were as follows:

1. To identify the causal mutation and its type, whole exome sequencing was performed for the proband XP patient.

2. To screen for the XP disease-causing mutation identified by exome, Sanger sequencing has been conducted for the other members of the XP family.

3. Material and Methods

3.1.Participants

The research study received approval from the ethics committee of Al-Quds University. All study participants provided written informed consent, including permission to publish any relevant case details in future publications (Appendix A1).Members of the XP patient's maternal side of the family (mostly males) were recruited in the current study with a number of 24 samples.

3.2.Data Collection

Clinical history data, clinical findings and laboratory results were retrospectively collected for the proband patient who was diagnosed with XP at the age of 16 after his first tumor appearance on his nose. The proband XP patient and the other family members were given a survey which represents the aim of the study with details of what XP disorder and asking if they were dealing with any of the main symptoms of the XP in general with the check-up of the presence of any lesions, patient and family members were also provided with Sample collection

3.3.DNA Extraction

5ml of peripheral blood samples were obtained with the assistance of an authorized nurse and collected using a peripheral anticoagulation blood tube. Genomic DNA was extracted from the collected blood samples using the Genomic DNA Mini Kit (Blood/Cultured Cell) Fresh Blood Protocol. To ensure the quality and quantity of the DNA, A260⁄A280 measurements were performed using a Nanodrop spectrophotometer (Nanodrop; Fisher Scientific, Wilmington, DE, USA).

3.4.Whole Exome Sequencing (WES) including CNVs and Mitochondrial genome

The WES test was conducted at the Molecular and genetics lab in Istishari Arab Hospital. The process involved taking a blood sample from the patient, followed by Whole Exome Sequencing (WES) that included the examination of CNVs and the mitochondrial genome. The quantification of DNA was performed using Qubit v.3, and its quality was verified through gel electrophoresis.

For library preparation, the TruSeq Capture Exome Kit (Illumina) was used, which allows for coverage of 45 Mb of exonic content. The kit's probe set was designed to enrich 214,405 exons. Afterward, the data were subjected to sequencing on NextSeq 500, and the reads were mapped to the reference human genome (h19) using BWA aligner. Before variant calling through GATK (Genome Analysis Toolkit), the BAM-formatted mapped reads underwent several preprocessing steps, which included eliminating PCR duplicates, realigning around indels, and recalibrating base quality.

The final list of variants was annotated using ANNOVAR with information from various databases, including minor allele frequency (MAF) data like PopFreqMax, as well as variant effect predictors such as SIFT, PolyPhen-2, and REVEL.

To filter out less reliable variants, those with low coverage, synonymous changes, predicted to be benign by SIFT, PolyPhen-2, and REVEL, MAF > 1% on gnomAD, PopFreqMax, and our Palestinian in-house database were excluded.

3.5.Primers Design

Primer design for the segregation analysis and Sanger sequencing of the POLH gene was performed using Primer3 software. **Amplification of the gene of POLH was done by using the primer pair: XP-F135: TGGCTACTGGACAGGATCGA** and **XP-R135: CACCACCCTTCCATGATTTGT** that amplifies 135 bp for the POLH gene. (Table 2) shows the sequencing of the forward and reverse primer.

Table 2. Forward and reverse primers for the 135 base pair POLH gene.

XP-F135			5’TGGCTACTGGACAGGATCGA3’
XP-R135			CACCACCCTTCCATGATTTGT

3.6.Polymerase Chain Reaction (PCR) of POLH gene

PCR was conducted in a 25 ul total reaction volume using the PCR-Biosystems Ready mix (2X PCRBIO HS Taq Mix Red, Jerusalem, Israel) and the Gene Amp PCR-system 9700 thermal cycler (Applied Biosystems, CA, USA). For the PCR, three microliters of eluted DNA from the blood sample were utilized. The reaction conditions consisted of an initial predenaturation step at 95°C for 15 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 60 seconds, all repeated for 34 amplification cycles. To serve as a PCR-negative control, the reaction buffer without human DNA was also included.

3.7.Agarose gel electrophoresis

The amplicon for the characterized gene was subjected to electrophoresis on 2% agarose gels (SeaKem® LE agarose gel) running at 120 V in 1x Tris-Acetate-EDTA buffer (TAE) with ethidium bromide (0.5 g/mL) for visualization under UV light using a gel documentation system (the Bio-Imaging Systems MiniLumi transilluminator). To determine the molecular weight of the amplicon, either a 100 bp or 50 bp molecular weight standard ladder (ThermoScientific GeneRuler) was used as a reference on each gel.

3.8.DNA sequencing:

The PCR products were sequenced utilizing an ABI 3730xl DNA Analyzer (Hylab Co., sequencing service). Sequencing was conducted in both directions with the same primers employed in the PCR. The ensuing chromatograms were meticulously examined, and the sequences were assembled using Bio-edit software-version 6.0.7.

To align the sequences of the POLH gene, the Multalin Multiple sequence alignment tool (http://multalin.toulouse.inra.fr/multalin/) was employed.

4. Results

4.1.Proband XP patient

A 29-year-old male presented with an unusual increase in freckle-like pigmentation on sun-exposed areas, which first appeared when he was 8 years old. The patient, a Biologist, did not exhibit photophobia or ocular lesions, and there were no neurological abnormalities (Fig. 8). A review of the family history revealed two other individuals with similar symptoms. Notably, the patient's parents were second cousins, indicating consanguinity.

The pigmentation changes were mainly concentrated in UV-exposed regions such as the face, upper thorax, upper limbs, and dorsal hands (Fig. 8). These symptoms progressively worsened until the age of 18, after which the condition stabilized. Sunlight exposure exacerbated the disease throughout the entire period. However, the palms, planta pedis, mucous membranes, and nervous system were not affected.

At the age of 16, the patient developed a large basal cell carcinoma (BCC) on his nose that required surgical removal. It was during this time that he was diagnosed with XP, although the specific type of disease was not yet identified. Since then, the patient has undergone multiple surgeries to address tumors that appear in sun-affected areas due to the disease. Over the past 12 years, various tumor types, including BCC, SCC, and melanoma, have been detected in the patient's ongoing struggle with this condition.

In January of 2023 a melanoma in situ was detected in the patient’s scalp which was 4mm in size, and afte which he was diagnosed with stage II melanoma, and started his treatment with PD-1 Immunotherapy on the 22th of May 2023, after he underwent wide excision of the tumor with sentinel lymph node biopsy which showed that there was no metastasis of melanoma (figure 9). Abnormal renal function was also detected, with small elevated levels of creatinine and uric acid at the age of 26.

Figure 8. The XP patient displayed hyper-pigmented lesions on both the facial region and the dorsal areas of the hands and back.

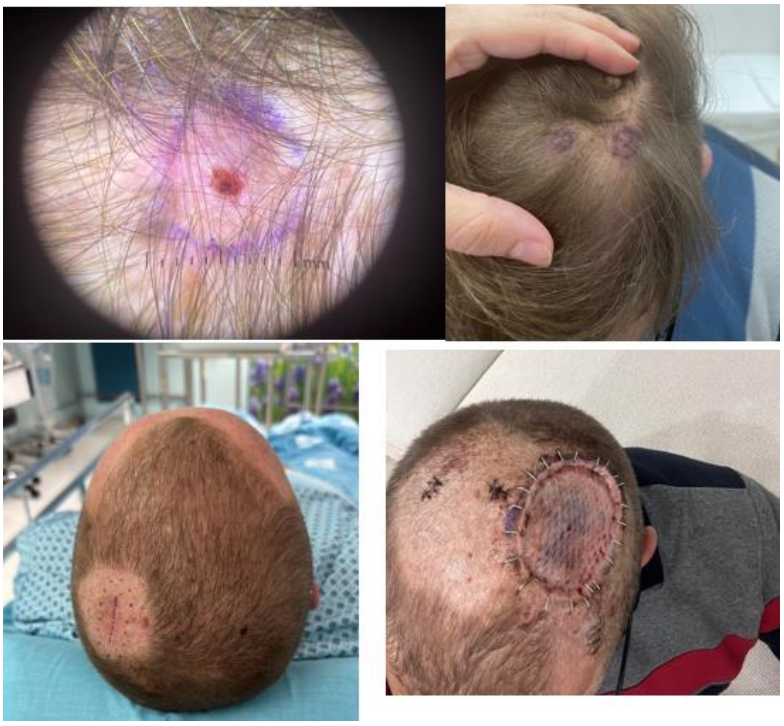
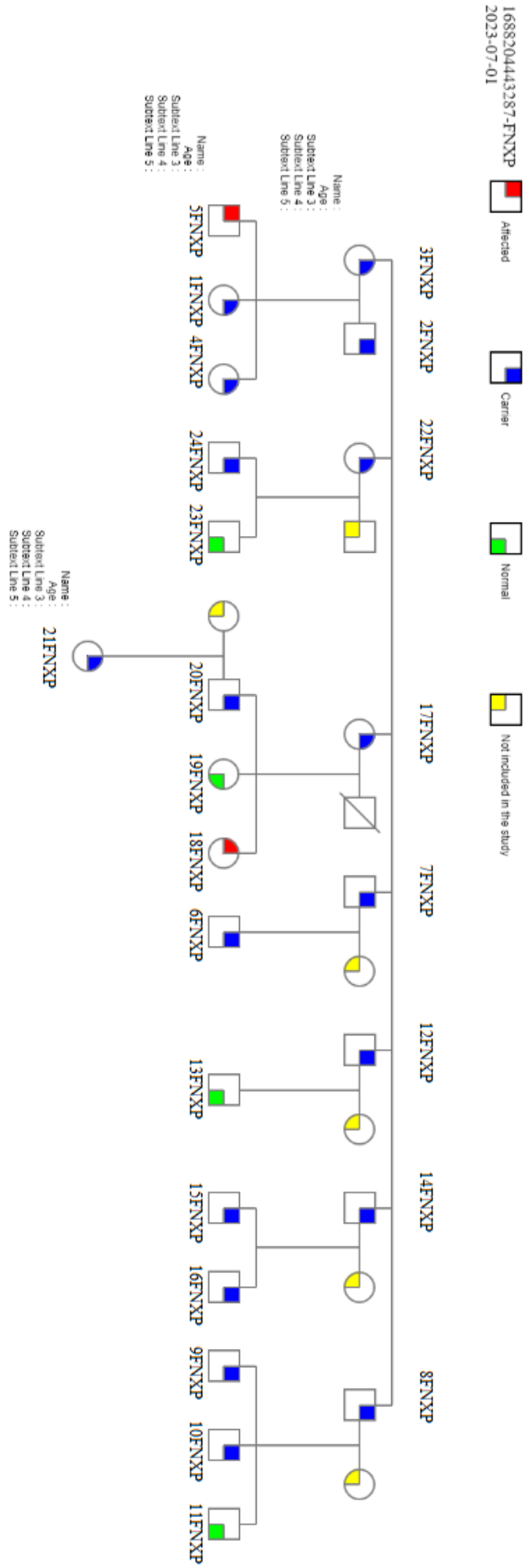


Figure 9. Melanoma before and after surgery : Melanoma in situ discovered by the dermatologist, Hadassah Ein Kerem hospital (upper left and right), where wide excision of the tumor and a sentinel lymph node biopsy were performed (lower left),followed by skin grafting (lower right).

Figure 10. Pedigree analysis for the XPV family



4.2.XPA Specific mutation Sequencing

The patient genetics counselor approved to do as a first step working on the situation a screening of one of the genetic mutation that are found in the Palestinians population for XPA c.619C>T (p.Arg207Ter) that was covered by the insurance in Hadassah Ein Kerem Hospital and it showed that the patient has a normal XPA gene (figure 11)

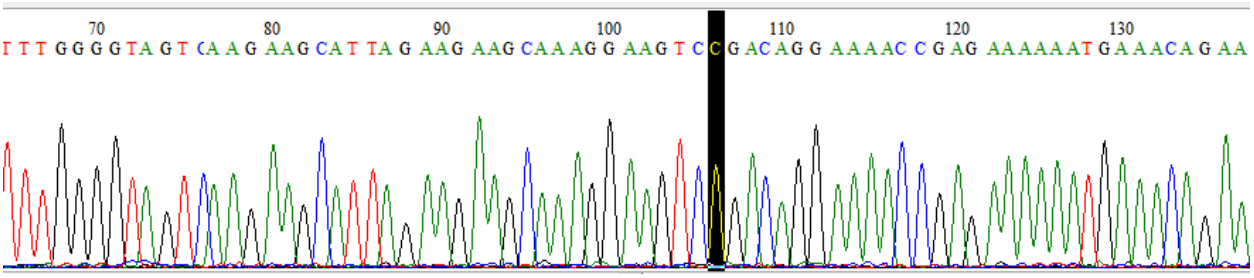


Figure 11. Electropherogram displaying the genomic DNA sequence for the XP patient which shows the normal sequencing of the XPA c.619C>T (p.Arg207Ter).

4.3.Whole Exome Sequencing (WES) including CNVs and Mitochondrial genome

Through the analysis of Whole Exome Sequencing (WES) data, which also included CNVs and the Mitochondrial genome, we successfully identified a pathogenic homozygous variant in the POLH gene: c.106_118del p. (Val36Asnfs*8). This confirms the genetic diagnosis of an autosomal recessive variant form of XP. No other clinically significant variants associated with the XP phenotype were found (Table 3).

Table 3 presents the results of the POLH gene mutation analysis after the WES test, providing details such as variant coordinates, zygosity, in silico parameters, allele frequencies, and the type and classification of the gene.

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS	ALEELE FREQUENCIES	TYPE AND CLASSIFICATION
POLH	NM_006502.2: c.106_118del	Homozygous	Align-GVDG:N/A SIFT:N/A MutationTaster:N/A Conservation_nt:N/A Conservation_aa:N/A	gnomAD:- ESP:- 1000G:-	Frameshift Pathogenic (class 1)

*IAH VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

Class 1 – Pathogenic
Class 2 – Likely pathogenic
Class 3 – Variant of uncertain significance (VUS)
Class 4 – Likely benign
Class 5 – Benign
Additionally, other types of clinical relevant variants can be identified (e.g. risk factors, modifiers).

4.3.1. Interpretation of the POLH variant

The POLH variant c.106_118del p. (Val36Asnfs*8) results in a change in the reading frame, beginning at codon 36. The altered reading frame leads to a stop codon 7 positions downstream. This particular variant has been previously reported as disease-causing for XP, variant, according to HGMD Professional 2022.1, with a reference by Johnson et al., 1999. As per the ACMG recommendations, it is classified as pathogenic (class 1).

4.4.PCR for the patient with the XPV and the family members

After the mutation was known and the primers were designed,the patient and his family member samples were taken to be checked using PCR to see which are homozygous/ heterozygous or normal for the mutation. (Figure 12, 13, 14).

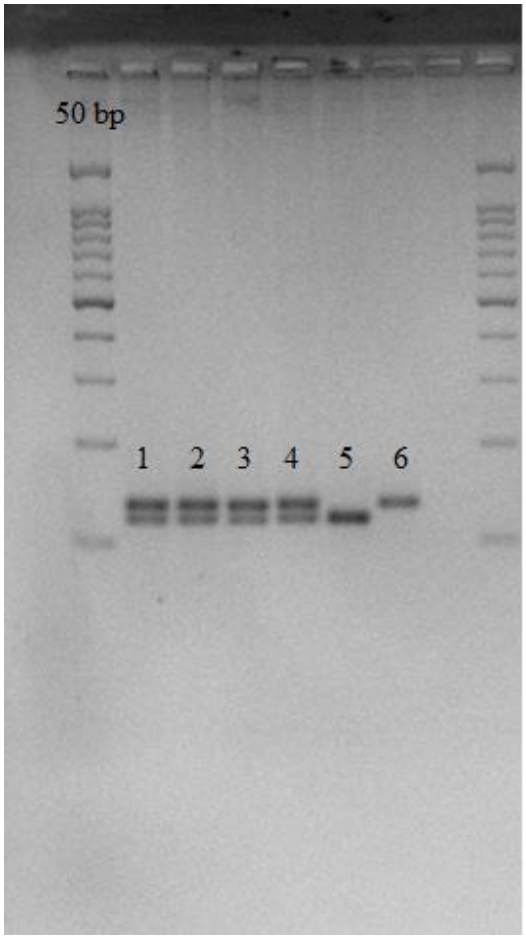


Figure 12. Upon conducting agarose gel electrophoresis analysis of the PCR products for the POLH gDNA variant c.106_118del p. (Val36Asnfs*8), distinct differences in size were observed. The homozygous affected individual #5 (5FNXP) displayed a contrasting size compared to the heterozygous individuals #1, 2, 3, and 4 (1FNXP, 2FNXP, 3FNXP, 4FNXp), as well as the healthy control. A molecular size marker (GeneRuler) in the form of a 50 kb DNA ladder was used for reference.

Table 4. Numbering of the PCR which show the XPV patient and his family PCR codes.

#	PCR code
1	**1FNXP (XPV Patient's sister)**
2	**2 FNXP (XPV Patient's father)**
3	**3 FNXP (XPV Patient's mother)**
4	**4 FNXP (XPV Patient's sister)**
5	**5 FNXP (XPV Patient)**
6	**NC**

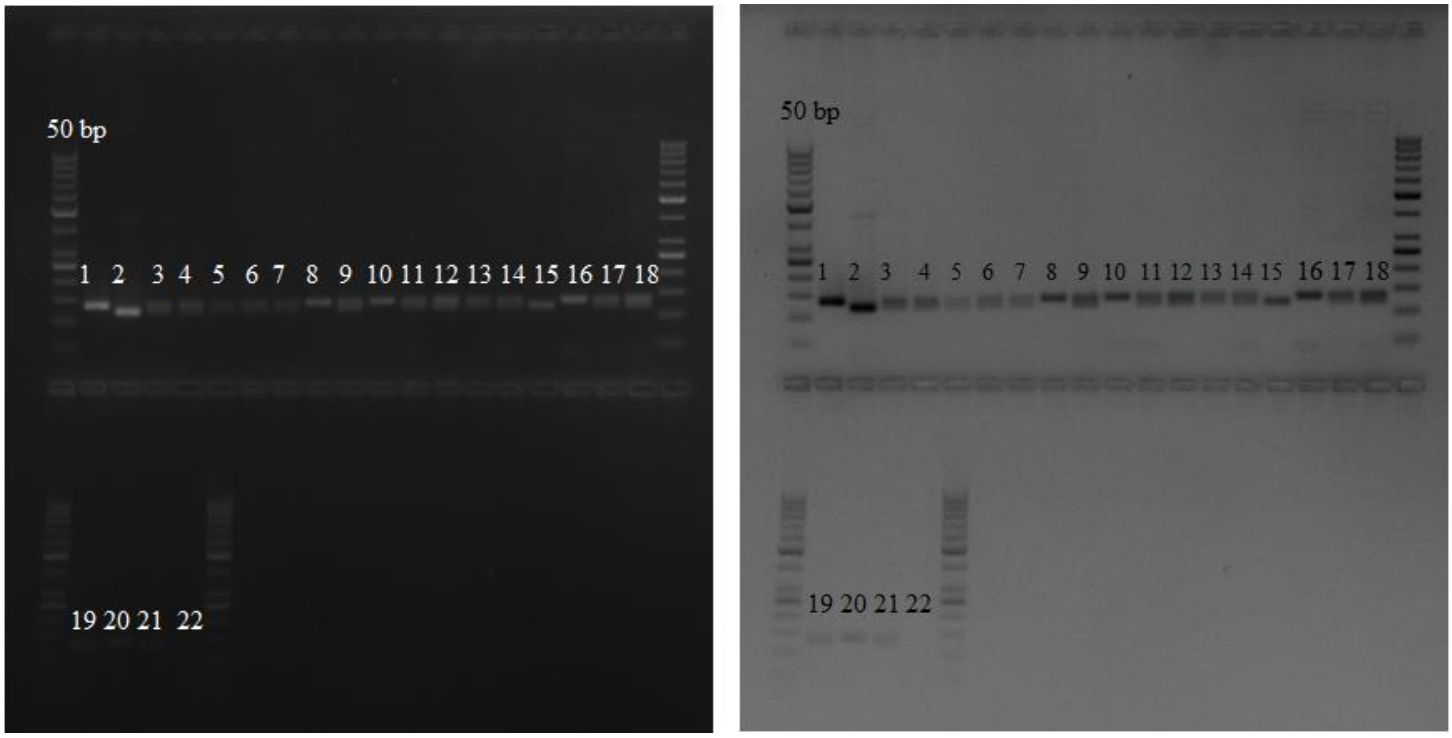


Figure 13.

Agarose gel electrophoresis analysis of the PCR product for the POLH gDNA variant c.106_118del p. (Val36Asnfs*8) reveals a distinct difference in size for the homozygous affected individual #2 and #15 (5FNXP, 18FNXP) compared to normal individuals #8, 10, 16 and 20 (11FNXP, 13FNXP, 19FNXP, 23FNXP) and a heterozygous individuals #3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 17, 18, 19 and 20 (6FNXP, 7FNXP, 8FNXP, 9FNXP, 10FNXP, 12FNXP, 14FNXP, 15FNXP, 16FNXP, 17FNXP, 19FNXP, 20FNXP, 21FNXP and 23FNXP). (Marker: 50 kb DNA ladder Molecular size marker (Gene Ruler).

Table 5. Numbering of the PCR which show the XPV patient and his maternal relatives samples PCR codes.

#	PCR code
1	**23con (PC)**

2	**5 FNXP (XPV Patient)**
3	**6 FNXP (XPV Patient's male cousin)**
4	**7 FNXP (XPV Patient's maternal uncle)**
5	**8 FNXP(XPV Patient's maternal uncle)**
6	**9 FNXP (XPV Patient's male cousin)**
7	**10 FNXP (XPV Patient's male cousin)**
8	**11 FNXP (XPV Patient's male cousin)**
9	**12 FNXP (XPV Patient's maternal uncle)**
10	**13 FNXP (XPV Patient's male cousin)**
11	**14 FNXP (XPV Patient's maternal uncle)**
12	**15 FNXP(XPV Patient's male cousin)**
13	**16 FNXP (XPV Patient's male cousin)**
14	**17 FNXP (XPV Patient's maternal aunt)**
15	**18 FNXP (XPV Patient's female cousin)**
16	**19 FNXP (XPV Patient's female cousin)**
17	**20 FNXP (XPV Patient's male cousin)**
18	**21 FNXP (XPV Patient's female cousin) (20 FNXP daughter)**
19	**22 FNXP (XPV Patient's maternal aunt)**
20	**23 FNXP (XPV Patient's male cousin)**
21	**24 FNXP (XPV Patient's male cousin)**
22	**N.C**

Table 6. A table shows the affected, carrier and the normal individuals.

Sample #	Health situation
1FNXP	**Carrier**
2FNXP	**Carrier**
3FNXP	**Carrier**
4FNXP	**Carrier**
5FNXP	**Affected**
6FNXP	**Carrier**
7FNXP	**Carrier**
8FNXP	**Carrier**
9FNXP	**Carrier**
10FNXP	**Carrier**
11FNXP	**Normal**
12FNXP	**Carrier**
13FNXP	**Normal**
14FNXP	**Carrier**
15FNXP	**Carrier**
16FNXP	**Carrier**
17FNXP	**Carrier**
18FNXP	**Affected**

19FNXP	**Normal**
20FNXP	**Carrier**
21FNXP	**Carrier**
22FNXP	**Carrier**
23FNXP	**Normal**
24FNXP	**Carrier**

Some of the samples were not detected precisely and it needed more space to run the gel, so another PCR was done for the samples to try to make it as clear as possible, figure 13 shows that.

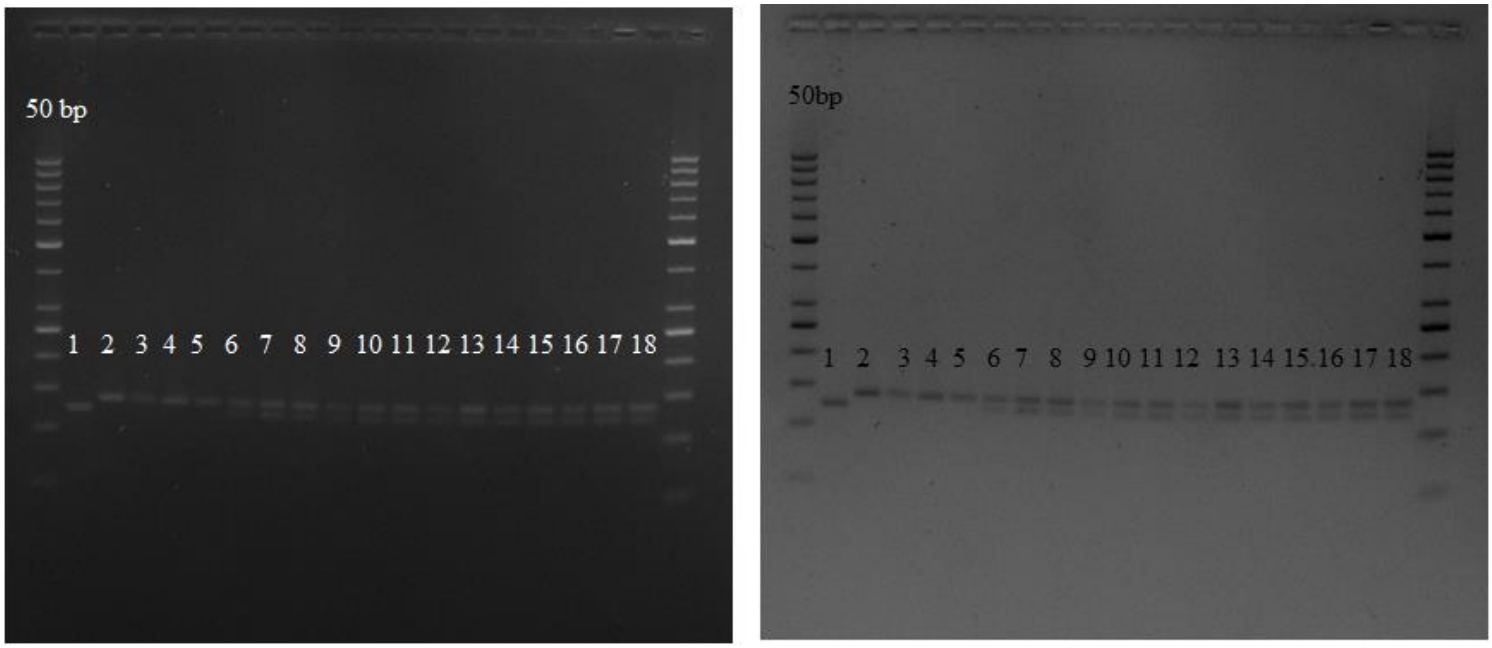


Figure 14. Second Agar gel electrophoretic analysis of the PCR POLH gDNA variant c. 106_118del p. (Val36Asnfs*8) shows difference in the size between homozygous affected individual #1 and #20 (5FNXP, 18FNXP) compared to normal individuals #2, 3, 4 and 5 (11FNXP, 13FNXP, 19FNXP, 23FNXP) and a heterozygous individuals #6, 7, 8, 9,10, 11, 12, 13, 14, 15,

16, 17 and 18 (22FNXP, 24FNXP, 6FNXP, 7FNXP, 8FNXP 9FNXP, 10FNXP, 12FNXP, 14FNXP, 15FNXP, 16FNXP, 17FNXP, 20FNXP, 21FNXP and 18FNXP). (Marker: 50 kb DNA ladder Molecular size marker (Gene Ruler).

Table 7. Numbering of the PCR which show the XPV patient and his maternal relatives samples PCR codes.

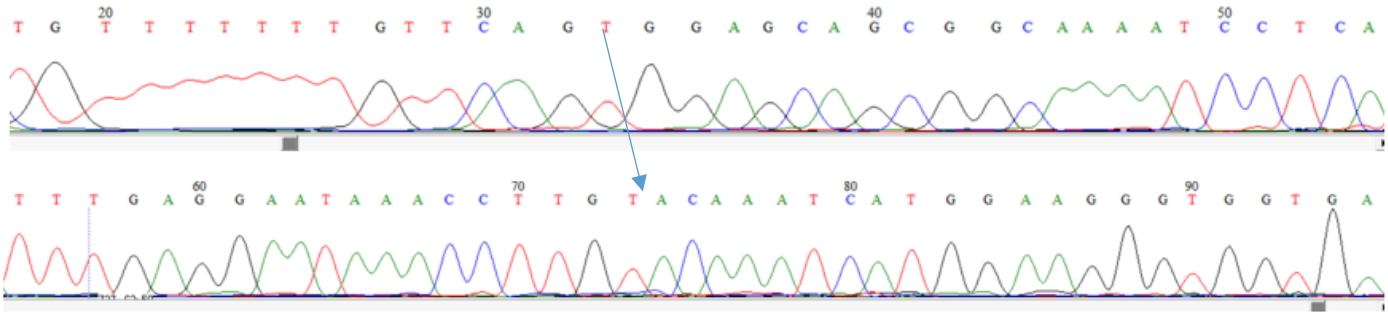
#	PCR code
1	**5 FNXP (XPV Patient)**
2	**11 FNXP (XPV Patient's male cousin)**
3	**13 FNXP (XPV Patient's male cousin)**
4	**19 FNXP (XPV Patient's female cousin)**
5	**23 FNXP (XPV Patient's male cousin)**
6	**22 FNXP (XPV Patient's maternal aunt)**
7	**24 FNXP (XPV Patient's male cousin)**
8	**6 FNXP (XPV Patient's male cousin)**
9	**7 FNXP (XPV Patient's maternal uncle)**
10	**8 FNXP(XPV Patient's maternal uncle)**
11	**9 FNXP (XPV Patient's male cousin)**
12	**10 FNXP (XPV Patient's male cousin)**
13	**12 FNXP (XPV Patient's maternal uncle)**
14	**14 FNXP (XPV Patient's maternal uncle)**
15	**15 FNXP(XPV Patient's male cousin)**

16	**16 FNXP (XPV Patient's male cousin)**
17	**17 FNXP (XPV Patient's maternal aunt)**
18	**20 FNXP (XPV Patient's male cousin)**
19	**21 FNXP (XPV Patient's female cousin) (20 FNXP daughter)**
20	**18 FNXP (XPV Patient's female cousin)**
21	**N.C**

4.5.Sanger Sequencing

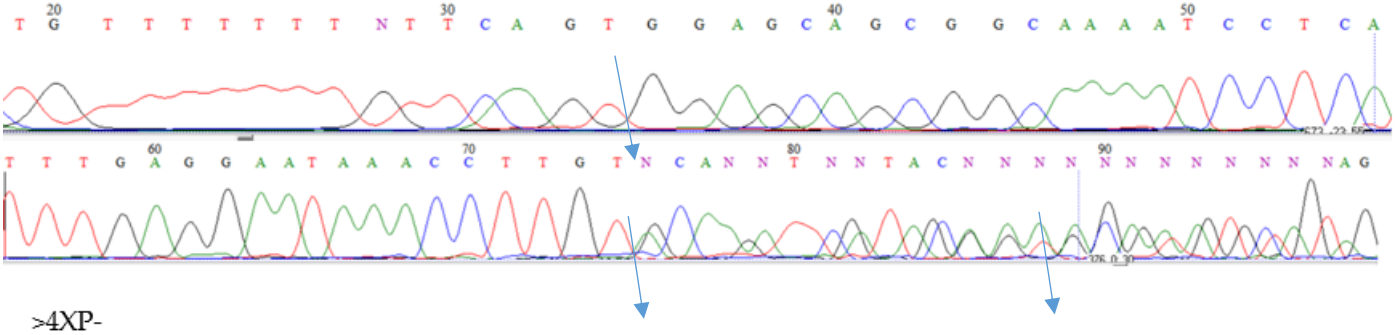
To validate the existence of pathogenic mutations, a series of samples (including infected, carrier, and normal samples) underwent direct Sanger sequencing using an ABI3500 sequencer (Applied Biosystems, Foster City, CA, United States), as illustrated in Figure 15.

(A)



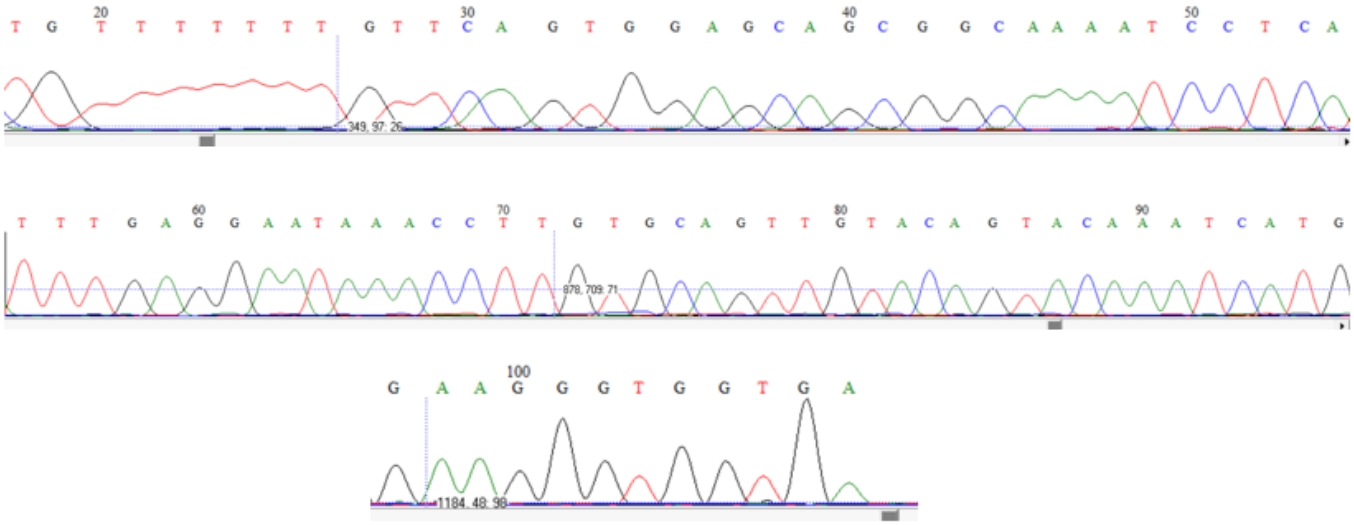
>5xp-
TGTTTTTTTGTTCAGTGGAGCAGCGGCAAAATCCTCATTTGAGGAATAAACCTTGTACAAATCATG
GAAGGGTGGTGA

(B)



>4XP-
TGTTTTTTTNTTCAGTGGAGCAGCGGCAAAATCCTCATTTGAGGAATAAACCTTGTNCANNT
NNTACNNNNNNNNNNNNAGGAAGGGTGGTGA

(C)



>11XP-
TGTTTTTTTGTTCAGTGGAGCAGCGGCAAAATCCTCATTTGAGGAATAAACCTTGTGCAGTTG
TACAGTACAAATCATGGAAGGGTGGTGA

Figure 15. PCR products amplified from exon 2 of the POLH gene variant c.106_118del p. (Val36Asnfs*8) were subjected to direct sequencing. A distinct blue-red coloration indicated the position of a 13-base pair deletion. (A) The XPV patient displayed a homozygous frame-shift mutation in the coding region at Nucleotide position 105-119 in the proband's sequence. (B) Patient's sister (4FNXP) exhibited a heterozygous frame-shift mutation. (C) The unaffected cousin's sequence served as a reference homozygote and did not show any mutations.

5. Discussion

XPV patients comprise approximately 20-25% of all XP cases. In contrast to XP groups A-G, XPV is a milder form of the condition, characterized by less severe skin lesions and a lower incidence of tumors. Moreover, symptoms typically emerge at a later stage in life, and patients generally exhibit an extended life expectancy with a reduced occurrence of neurological complications. (Gratchev et al., 2003). Similar pigmented diseases with sun sensitivity, like XPV, often go undiagnosed until a later age since patients do not display noticeable symptoms such as skin damage or cancerous changes in sun-exposed areas.
XPV patients often exhibit a wide range of severity in their skin lesions (Inui et al., 2008), which can pose considerable challenges in molecular diagnosis (Ben Rekaya et al., 2018).

XPV is characterized by a predisposition to cancer and results in photosensitivity, as well as skin and ocular damage in certain patients. Additionally, sun exposure can lead to the development of precancerous skin lesions in affected individuals. (Broughton et al., 2002). XPV is associated with a diverse array of tumor types and neurological. The research subject (5FNXP), a 29-year-old male with XP, presented with a variety of tumor types, including BCC, SCC, and melanoma, along with minor renal dysfunction, as observed in this study. However, the precise relationship between the primary disease and the associated renal dysfunction symptoms remains uncertain. Previous cases of XP have demonstrated instances of end-stage renal disease as well, suggesting a potential link between XP and renal complications. (Radwan et al., 2015).

To understand the genetic basis of skin disorders in humans, a family was studied, including a patient (5FNXP) with XP, whose parents were second cousins. The patient developed hyper pigmented macules in early childhood, primarily in UV-exposed areas like the face, upper thorax, upper limbs, and dorsal hands. These symptoms worsened until the age of 18, after which the condition stabilized, though sunlight continued to exacerbate the disease. Notably, the palms, soles of the feet, mucous membranes, and nervous system were unaffected. At 16 years old, the patient developed a large basal cell carcinoma on the nose, leading to a diagnosis of XP, though the specific type was not determined. Since then, the patient has undergone

numerous surgeries to address tumors that appear on sun-exposed areas, with BCC, SCC, and melanoma being the types of tumors detected over the last 12 years.

The patient's genetics counselor conducted a screening for the XPA c.619C>T (p.Arg207Ter) genetic mutation, commonly found in the Palestinian population, which showed negative results, indicating that the patient did not have XPA-type XP.

Whole-exome sequencing is a swift and effective approach for studying genetic information, especially in the context of complex and monogenic genetic diseases, such as skin disorders. (Choi et al., 2009).

In the context of rare disorders, employing whole exome sequencing can minimize errors in detecting mutations within hotspot regions. Particularly for Mendelian disorders, Next-Generation Sequencing (NGS) can prove valuable in comprehending pathogenesis and identifying novel gene mutations associated with the condition. (Yang et al., 2013). Furthermore, because it allows for the rapid detection of homozygous harmful mutations by comparing coding sequences between affected and unaffected people, this technique is very successful in researching genetic illnesses with recessive inheritance patterns (Bamshad et al., 2011).

Given that the initial sequencing results for the XPA c.619C>T (p.Arg207Ter) gene were negative, high-throughput sequencing was employed to determine the genetic basis of the disease. Whole-exome and Sanger sequencing revealed a pathogenic POLH mutation in the patient and his maternal cousin. Taking into account the patient's medical history, laboratory findings, and existing reports (Inui et al., 2008), the diagnosis of XPV caused by the POLH variant c.106_118del p. (Val36Asnfs*8) was confirmed, but If we notice that the mutation starts with CA and the next nucleotides just after the deletion is also CA meaning that we can consider the deletion starts with CA at position 104 as named by (Johnson et al) or consider it starts with GT at position 106 and ends with CA. In all cases, at the amino acid level, the deletion should start at Val36 position which is wrongly considered at Ala35 in Johnson et al paper The POLH gene encodes specific DNA polymerases responsible for repairing DNA damage and preventing the mutagenicity of UV-induced DNA modifications. The DNA polymerase eta is a 713-amino acid-long protein encoded by POLH, with a highly

conserved amino terminus seen in Y-family polymerases, as well as the active site responsible for polymerase activity. After UV exposure, the C-terminal region, which contains around 120 amino acids, plays an important function in nuclear localization regulation and assembly into replication forks. The core 240 amino acids' function is still unknown. To date, 92 XPV-related variants in the POLH gene have been documented, including missense, nonsense, indels, and splicing site changes. (http://www.hgmd.cf.ac.uk; accessed 01 October 2021).

Additionally, POLH has been implicated in hypermutation during immunoglobulin class change recombination. A hundred POLH mutations have been associated to XPV pathogenesis Most of these mutations result in premature termination codons in mRNA, which causes degradation via the nonsense-mediated decay (NMD) mechanism. (Inui et al., 2008). Opletalova et al. (Opletalova et al., 2014) the phenotype/genotype correlation in 23 XPV patients was summarized, indicating that the type of missense mutation was substantially linked with clinical severity. Cumulative UV exposure is a major predictor of cancer development in patients with truncating mutations, underscoring the significance of sun protection. The detected alteration in this case was a frameshift mutation. The proband displayed an unstable general condition with a low tumor burden, consistent with another report (Ben Rekaya et al., 2011).

6. Conclusion

NGS technology presents both advantages and challenges. The complexity of known genes with multiple exons makes first-generation sequencing of all exons time-consuming and problematic, leading to imprecise disease diagnosis (Ben Rekaya et al., 2018). However, for diseases with unidentified mutations, NGS proves more effective than first-generation methods, offering comparable costs (Kleijer et al., 2008). Nonetheless, downstream bioinformatics analysis of NGS data remains a bottleneck, hindering the seamless integration of NGS into clinical practice (Ortega-Recalde et al., 2014; Smith et al., 2014). Although various bioinformatics tools have been applied, establishing a reliable NGS platform demands considerable time and resources. Nevertheless, our experience demonstrates that whole-exome sequencing is an efficient approach for XP diagnosis and may hold promise for future etiology classification. Advancements in diverse technologies will progressively deepened our understanding of XP, fostering continuous developments in treating this disease.

In our study, we identified a frameshift mutation in POLH associated with XPV in an affected family. Notably, this mutation had not been studied since its initial detection in 1999 by Robert E. Johnson (Johnson et al., 1999). During genetic screening of the XPV patient's family, we further discovered the presence of this mutation in a 60-year-old female with no previous medical records related to the condition.

In conclusion, our results highlight the potential of whole-exome sequencing as a powerful strategy for determining the molecular etiology of XP, and its implementation in the near future may lead to significant advancements in understanding and managing XPV. Furthermore, our findings contribute valuable clinical and molecular insights into XPV patients.

7. References

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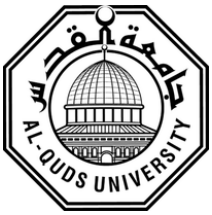
8. Appendix

A1. Participant's survey



Al-Quds University **Sample #:**

Faculty of Graduate Studies

Department of Biochemistry and Molecular Biology

Participant Name: **Date of birth**: **Survey Age**:

Gender: Male Female

Xeroderma pigmentosum (XP) is a hereditary condition characterized by an extreme sensitivity to sunlight, which poses a significantly elevated risk of developing skin cancer and other medical complications. Individuals with XP exhibit an extraordinary sensitivity to both ultraviolet (UV) type A and UV type B radiation from the sun. Even minimal exposure to UV radiation can lead to severe sunburn and blistering, starting at a very young age. This heightened sensitivity to UV radiation results in increased freckling and areas of lighter skin pigmentation. People with XP may also experience dry skin. The condition carries a substantial risk of developing squamous cell and basal cell skin cancers, as well as melanoma. Due to the severity of sun sensitivity and the associated risks, individuals with XP must take stringent measures to protect their skin from sunlight and minimize potential health complications.
Significance of the study: The main purpose of this research study is to detect the mutation of an XP patient with screening the patient's family.

Symptoms	Yes	No

Sensitive to sun		
Presence of freckles		
Dry skin		
Irregular dark spots		
Eye sensitivity		
Corneal ulcerations		
Spider veins		
Abnormal presence of lesions		
Neurological abnormalities		

I, the undersigned, give my consent for the publication of identifiable details, which can include photograph(s) and/or videos and/or case history and/or details within the text ("Material") to be published in future publications. And accept taking a blood sample from me for the purpose of this study.

Participant's signature _____.

Researcher's Signature_____