Spring 5-5-2016

Identification of XPA and XPC gene mutations in patients with xeroderma pigmentosum

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THE AMERICAN UNIVERSITY IN CAIRO
الجامعة الأمريكية بالقاهرة

School of Sciences and Engineering

Identification of *XPA* and *XPC* Gene Mutations in Patients with Xeroderma Pigmentosum

A Thesis Submitted to

The Biotechnology Program

In partial fulfillment of the requirements for the degree of Master of Science

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Thesis Committee Supervisor/Chair _____

Affiliation _____ Date _____

Thesis Committee Reader/Examiner _____

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Dept. Chair/Director Date Dean Date

Dedication

All Praise is to Allah for Everything.

To Prophet Muhammad (peace and mercy upon him); thanks for teaching me how to live.

To patients for their strength; to their parents for their care and awareness.

To my advisors; thanks for your time, effort, knowledge, guidance and encouragement.

To my mother; thanks for your love, passion, support, and guidance, you are my light and the reason I move forward.

To my father; thanks for your endless support, encouragement and for believing in me.

To my beautiful sisters; Omayma and Alaa, thanks for always being there for me; you are the magic beyond boundaries.

To my brothers; Ahmed and Waleed.

To our princess Dija; the blessing and happiness of our life.

Acknowledgments

My sincere appreciation and gratitude goes for my advisor **Prof. Suher Zada** for her instructive supervision, sincere encouragement and continuous guidance throughout the development of this work. I would like to express my deepest appreciation to **Prof. Khalda Amr,** for her sincere guidance, patience, great support, valuable advice, and dedication to finish this work. My genuine thanks and gratitude goes to **Prof. Ghada El-Kamah** for her support, encouragement, initiative enthusiasm to start this work for patients' interests and for always putting the patients first. It was a great honor to have three extraordinary helpful and enthusiastic professors as my advisors.

This work could not have been done without the cooperation of **Prof. Mohamad El Darouti**, Dermatology Department, Faculty of Medicine, Cairo University who was always acknowledged by patients and parents for his care and experience. I am grateful to young wonderful Egyptian physicians: **Heba Ahmed**, clinical geneticist from NRC, **Mona Radee**, dermatologist from Zakazig University and the enthusiastic ophthalmologist **Yosra Gamal** from Al-Demerdash, Ain Shams University hospital.

I would like to thank all members of **Medical Molecular Genetics Department,** for their kind encouragement especially **Mohamad Abdel Hamid, Hoda AbdAllah, Noha Hamdi, Aya Tarek, Nagham Elsayed, Wessam Elsayed, Miral Refaat and Asmaa Elkhouly.** Much appreciation to my AUC colleagues; **Aya Yusuf** and **Noha Ismail** for their support and guidance throughout graduate program and thesis execution.

I would like to express my gratitude to **Prof. Ahmed Moustafa** for his support and his Bioinformatics knowledge which reflected upon students of the Biotechnology program at AUC.

Finally, I would like to express much gratitude to the AUC for partially funding my academic coursework and to both NRC and AUC research grants for funding this research.

Abstract

The human body employs different DNA repair pathways to protect itself against cancers induced by DNA damage. The nucleotide excision repair (NER) pathway comprises different synchronously working DNA repair proteins; two of which are XPA and XPC. Mutations of any of the genes encoding the NER proteins cause an autosomal recessive genetic disorder called Xeroderma Pigmentosum **(XP)**. XP patients present with characteristic dry atrophic freckle-like pigmentation of the skin, photosensitivity and photophobia. Some patients develop neurodegenerative symptoms early in life, including mental retardation. Patients have a 10,000-fold increased risk for UV-induced skin cancers, moreover, a higher risk for ocular, oral and internal cancers. Death usually results from skin cancers, neurological deterioration, and internal cancers. XP is a rare disorder; it affects one per million individuals, however, higher incidences were observed in some geographical areas such as Mayotte islands (1:5000), Japan (1:22,000) and North Africa (1:10,000 in Tunisia and 1:80,504 in Morocco) due to geographical isolation, and high rate of intra-familial marriage. In North Africa, and in Egypt, *XPC* followed by *XPA* gene mutations are the most common. In Egypt, XP accounts for 15.9% of genetic skin disorders. Recently, *XPA* gene mutations were detected in four Egyptian XP patients only. In the current study, fourteen unrelated families having seventeen XP Egyptian patients were studied via direct sequencing for detection of both *XPA* and *XPC* gene mutations. This is the first study to identify mutations in both *XPA* and *XPC* genes in Egyptian XP patients with variable clinical features. Ten mutations were identified; four of which were recurrent mutations, three were novel mutations, and three mutations were reported previously in non-Egyptian XP patients. Carrier and prenatal screening were provided for the studied families. Spectra of *XPA* and *XPC* mutations in the Egyptian population were outlined with emphasis on clinical phenotypes. In conclusion, identification of pathogenic mutations provided a valuable tool for detection of recurring and private XP mutations in the Egyptian population. Mutation detection augments genetic counseling via carrier, prenatal and premarital screening, and provides a cornerstone for development of diagnostic strategies and future gene therapies.

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List of Abbreviations

µl: Microliter

1000G: 1000 Genome project

6-4PPs: 6-pyrimidine 4-pyrimidone lesions

BCC: Basal Cell Carcinoma

BLAST: Basic Local Alignment Search Tool

Bp: Base pair/s

C- terminal: Carboxylic group terminal

C7orf11: gene for chromosome 7 open reading frame 11 protein

CCDS: Consensus Coding DNA Sequence

cDNA: Coding Deoxyribonucleic Acid

COFS: Cerebrooculofacioskeletal syndrome

CPDs: Cyclobutane Pyrimidine Dimers

CS: Cockayne syndrome

CS-A and B: Cockayne syndrome type A and B

DDB2: damage-specific DNA binding protein 2

DDB2: gene for damage-specific DNA binding protein 2

ddNTP: 2',3'-dideoxynucleoside-5-triphosphate

DNA pol: DNA polymerase.

DNA: Deoxyribonucleic Acid

dNTP: deoxynucleoside-5-triphosphate

dsDNA: double stranded Deoxyribo Nucleic Acid

EC: Ethical Committee

EDTA: Ethylenediaminetetraacetic acid

ERCC: excision repair cross complementing genes

ExAC: Exome Aggregation Consortium

FI: Functional Impact

FIS: Functional Impact Score

GGR: Global Genome Repair

GTF2H5*:* general transcription factor 2H5 gene

HGMD: Human Genome Mutation database

hHR23B: human Homolog of the yeast RAD23B protein

Indel: Insertion-deletion

IRB: Institutional Review Board

IVS: Intervening Sequence

Kb: Kilo base

kDa: Kilo Dalton

m: month (in age context)

ml: mililiter

mRNA: messenger Ribonucleic Acid

MRI: Magnetic Resonance Imaging

MSA: Multiple Sequence Alignment

NCBI: National Center for Biotechnology Information

NER: Nucleotide Excision Repair

NLS: Nuclear Localization Signal

nm: nanometer

NMD: nonsense-mediated mRNA decay.

NRC: National Research Centre, Cairo, Egypt

N-terminal: Amine group terminal

OD260: Optical density at 260 nm

OD280: Optical density at 280 nm

OMIM: Online Mendelian Inheritance in Man

PCNA: proliferating cell nuclear antigen

PCR: Polymerase Chain Reaction

Pol H: The gene encoding polymerase η (eta), also known as *XPV*

RF-C: replication factor C

RNAPII: RNA polymerase II

RPA: Replication Protein A

SCC: Squamous Cell Carcinoma

SIFT: Sorting Tolerant From Intolerant

SNP: single nucleotide polymorphism

ssDNA: double stranded Deoxyribo Nucleic Acid

Taq: *Thermus aquticus*

TCR: Transcription Coupled Repair

TFIIH: transcription factor IIH

TTD: trichothiodystrophy

TTD-A: trichothiodystrophy type A

TTDN1: trichothiodystrophy non-phtosensitive type

UDS: Unscheduled DNA Synthesis

US: United States

UV: Ultraviolet radiation

UV-SENS syndrome: UV-sensitivity syndrome;

XP: Xeroderma Pigmentosum, as a disease

XPA to XPC and XPV (proteins are not italic): XPA to XPG and XPV repair proteins

XPA* to *XPG* and *XPV (genes are in italic): The genes encoding XPA to XPG and XPV repair proteins

XP-A to XP-G: XP complementation group A to G

XP-V: XP variant complementation group

Yr: year

***Examples of abbreviated sequence changes**:

c.1643_1644delTG: Deletion (del) of thymine and guanine bases (TG) at position 1643 and 1644 of coding DNA (c.).

ins: for insertion

c.1894C>T: Substitution of cytosine by thymine at position 1894 of coding DNA (c.).

p.Arg579X: Substitution of Arginine amino acid by stop codon (X) i.e. nonsense mutation at position 579 of the protein (p.).

p.Val548AlafsX25: Substitution of Valine amino acid by Alanine at position 548 of the protein (p.) and a frameshift which creates a stop codon at position 25 relative to substitution.

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Chapter (1): Literature review

1.1. Xeroderma Pigmentosum (XP)

Xeroderma pigmentosum (XP; OMIM: 278700-278780) is a rare autosomal recessive monogenic genodermatosis which is caused by a defect in DNA repair pathway, mainly the Nucleotide Excision Repair (NER). XP manifests as characteristic lesions in the skin, the eyes, specifically in areas exposed to the sunlight, and in some subtypes, symptoms of progressive damage of the nervous system (Kraemer *et al.*, 1987; Rapin *et al.*, 2000). Patients have increased risk of developing different types of skin cancers as well as cancers of other organs. Strict protection from sunlight is extremely important to reduce cancer risk (Kraemer *et al.*, 1987; Van Steeg & Kraemer, 1999). Patients usually die because of cancers or neurologic deterioration (Bradford *et al.*, 2011). XP has an overall incidence one per million, but in some regions such as North Africa, incidence is as high as 1 per 10:000. Mutations in different genes involved in NER pathway can cause XP phenotype. According to the mutated gene, XP can group into eight different XP groups (XP-A to XP-G and XP-V) which have overlapping clinical symptoms. Some groups are prevalent in a specific population; for example, in North African populations, XP-C then XP-A were found to be predominant (Kleijer *et al.*, 2008). The disease has no treatment which highlights the importance of management, prevention and early diagnosis. Molecular diagnosis is of special importance to predict disease course, detect carriers, provide prenatal diagnosis, and direct future corrective gene therapies (Kraemer & DiGiovanna, 2003/2014).

History

In 1874, XP was first reported and described in a textbook of dermatology. Kaposi and Hebra published in the book the first report of two cases diagnosed with XP. They described a characteristic dry, thin and wrinkled parchment-like skin or "*xeroderma*" and the freckle-like pigmentation or "*pigmentosum*". They have also reported involvement of the eye symptoms and the presence of cancers (Hebra & Kaposi, 1874; DiGiovanna & Kraemer, 2012). Later on in 1883, the discoverer of *Neisseria gonorrhea* bacteria; Albert Neisser described XP accompanied, for the first time, by neurological manifestations in two siblings in Germany (Kraemer *et al.*, 1987). In the meeting of the American Dermatological Association in 1888, 40 XP cases were

reported worldwide (Taylor, 1888; Kraemer *et al.*, 1987). In the 1900s, more reports had followed. In 1932, a syndrome named "De Sanctis-Cacchione syndrome" was reported in Italy by two physicians after whom the syndrome was named. The syndrome, which is very rare nowadays, has some special clinical features such as immature sexual development in addition to the XP skin manifestations (De Sanctis & Cacchione, 1932; Reed *et al.*, 1977; Kraemer *et al.*, 1987). In 1938 and 1940, the first two reports of XP in black individuals were published (Loewenthal & Trowell, 1938; King & Hamilton, 1940). In 1964, El-Hefnawi *et al.* reported 50 cases in Egypt, which were the highest reported number of cases in one country at that time. In Japan, where XP is prevalent, 50 patients were studied in 1977 (Takebe *et al.*, 1977). In a clinical review of 830 reported XP patients in 1987, Egypt presented 11% of cases worldwide (Kraemer *et al.*, 1987). It was known until 1968 that XP has a genetic basis but the underlying causative defect remained unclear. In 1968, a scientist named James Cleaver carried out an experiment that proved defective DNA repair in cell cultures of UV irradiated XP skin fibroblasts (Cleaver, 1968). UV induced damage, in the form of accumulation of thymidine dimers was found to be irreparable *in vitro* and *in vivo* (Reed *et al.*, 1969; Setlow *et al.*, 1969; Epstein *et al.*, 1970). There was one form of XP, named "XP Variant" or "XP-V" in which excision repair was efficient, but showed a defect in the repair mechanism post-replication (Burk *et al.*, 1971; Cleaver, 1972; Lehman *et al.*, 1975). Within mid to late 1970s, complementation tests employing cell fusion technique identified eight complementation groups; from A to G, and group V (Kraemer *et al.*, 1975a, 1975b; Lehman *et al.*, 1975; Arase *et al.*, 1979; Keijzer *et al.*, 1979). With advances in molecular biology, the genes harboring the defects responsible for each complementation group were identified across 1990s, correspondent proteins were isolated which facilitated the study and understanding of DNA repair pathways. Identification of the exact gene mutations in given populations through population and clinical studies continued up to the present time (Cleaver *et al.*, 1999; Soufir *et al.*, 2010; DiGiovanna & Kraemer, 2012; Ghafouri-Fard *et al.*, 2016; Jerbi *et al.*, 2016).

1.1.1. Clinical picture

Cutaneous symptoms

These symptoms appear at median age of one to two years, and are characteristically confined to sunlight exposed areas of the skin. Photosensitivity manifests as acute prolonged

reaction to sunlight in form of severe burns, blisters and erythema on minimal exposure (Kraemer *et al.*, 1987). Although severe photosensitivity after minimal sun exposure was recognized as a classical XP feature, about one third of XP patients do not burn but rather only tan (Bradford *et al.*, 2011; Sethi *et al.*, 2013). Hence, freckle-like hyperpigmented lesions or lentigines in sun exposed areas of the skin are characteristic to XP and are reported in all affected cases by the age of two (Sethi *et al.*, 2013). With continual sun exposure, photo-aging of the skin is observed; the skin shows dryness (xerosis), atrophy, parchment-like appearance, poikiloderma (hypo- and hyperpigmented lesions), cutaneous telangiectasia (spider-like veins), premalignant actinic keratoses, and eventually skin cancers (Kraemer *et al.*, 1987). Strict prevention of exposure to UV rays is a must and have to be started as early as possible, because the underlying DNA repair defect causes accumulation of photoproducts responsible for increased risk of skin cancers (Van Steeg & Kraemer, 1999). The association between UV exposure and increased skin cancer risk is consistent with and dependent on the nature of XP complementation groups. The majority of XP patients from complementation groups C, E and V do not show acute severe photosensitivity, and have no neurological symptoms. These patients are more prone to cutaneous cancers than patients from other complementation groups who either burn intensely on acute exposure or have neurological symptoms or both. This was explained by the early UV protection provided for patients who show severe photosensitivity; hence, reduced skin cancer risk. Neurological symptoms often hinder movement which explains less sunlight exposure and reduced skin cancer risk (Bradford *et al.*, 2011; Sethi *et al.*, 2013).

Ocular symptoms

The sunlight reaches eyelids, conjunctiva and cornea, where ocular symptoms of XP manifest (Kraemer *et al.*, 1987). At least one eye symptom appears in more than 90% of patients; closely as common as cutaneous symptoms, and appear in the first decade of life with median age of four years. Classic symptoms are photophobia, and severe keratitis. Eyelids show atrophy, freckling hyperpigmentation, ectropion (outward turning of lower eyelid); consequently, tearing abnormalities including dry eye. Lesions of inflammation in the conjunctiva may cause corneal opacity and vascularization (Kraemer *et al.*, 1987; Brooks *et al.*, 2013). The cornea shows scarring, and thinning with loss of endothelial lining due to UV damage, therefore, protection of the eyes is a necessity (Chaurasia *et al.*, 2014; Mohamed *et al.*, 2015). Less frequently,

hyperpigmentation affects the conjunctiva in form of conjunctival melanosis, which precedes ocular melanoma (Brooks *et al.*, 2013). Severity of ocular symptoms can increase to unilateral or bilateral blindness (Alfawaz & Al-Hussain, 2011). As in the case of skin manifestations, patients classified to complementation groups that show mild photosensitivity, develop ocular neoplasms more likely (Setlow *et al.*, 1969; Bradford *et al.*, 2011; Brooks *et al.*, 2013).

Neurological symptoms

Long term follow up of XP patients have shown that 24% of cases have progressive neurological abnormalities (Bradford *et al.*, 2011). Onset of these abnormalities can be as early as six months or as late as second decade of life with no relation to sunlight exposure (Kraemer *et al.*, 1987; Rapin *et al.*, 2000). The majority of patients show progressive course of cognitive and intellectual impairment, microcephaly, abnormal to absent tendon reflexes, sensorineural hearing loss, loss of ability to talk and walk, and ataxia. Since the course of neurological manifestations is progressive, hearing loss and decreased tendon reflexes provide diagnosis for the presence of XP with neurological abnormalities. Eventually, patients would manifest most of these neurodegenerative symptom (Rapin *et al.*, 2000). Combination of skin manifestations with intellectual disability, microcephaly, dwarfism and immature development of sex are features of "DeSanctis-Cacchione syndrome" (De Sanctis & Cacchione, 1932; Reed *et al.*, 1977). Early onset of neurodegenerative symptoms shows rapid deterioration, including DeSanctis-Cacchione syndrome. Intermediate (range between seven and 12 years) and late (between 12 to 21 years) onsets show slower deterioration with absence of retardation of sex development, and dwarfism (Rapin *et al.*, 2000). Both the peripheral and central nervous systems are involved; neurons show primary degeneration. DNA repair is concluded to be essential for nervous system integrity. This was evident in XP patients' autopsy findings that showed neuronal atrophy with lesions in cortex, cerebrum and cerebellum (Robbins, 1988, 1991; Rapin *et al.*, 2000). Neurological symptoms are confined to XPA, XPD, and to lesser extent XPG and XPF complementation groups (Rapin *et al.*, 2000; Kraemer & DiGiovanna, 2003/2014). Postmortem examination of an XPC patient has shown glioblastoma in the brain with absence of the above-mentioned neurodegenerative symptoms (Lai *et al.*, 2013). Case reports of XPC patients having neurodegenerative symptoms usually attributed these symptoms to other possible effects rather than that of XPC defect (Khan *et al.*, 2009; Soufir *et al.*, 2010).

Cancers

XP patients are highly susceptible to recurrent cancers of the skin (squamous and basal cell carcinoma, and melanoma), and the eyes. XP patients were compared to the general population of USA in a long term follow up study. Patients showed a 10,000-fold increased risk for basal cell and squamous cell carcinomas; the median age of onset of both carcinomas was nine years compared to 67 years for normal individuals. For melanoma, there was a 2000-fold increased risk. The median age for onset of melanoma was 22 years; 33 years earlier than normal individuals (Bradford *et al.*, 2011). Ocular neoplasms were assessed in another study which reported their onset in 11% of XP patients at median age of 16 years versus 60 years in general US population (Brooks *et al.*, 2013). Internal cancers have an overall 10-20 fold higher risk in XP patients; these include gliomas of the brain and the spinal cord, uterine, pancreatic, renal, and pulmonary cancers and leukemia (Kraemer *et al.*, 1987; Bradford *et al.*, 2011). Squamous cell carcinoma (SCC) of the oral cavity (as part of the skin) occurs much more frequently in XP individuals especially at the tip of the tongue. SCC of the tip of the tongue, being an unusual site for oral cancers in normal individuals, was suggestively attributed to its exposure to sunlight (Kraemer *et al.*, 1987; Bologna *et al.*, 2014).

Causes of death

Progressive neurological damage (31%) is the second cause of death after skin cancers (34%) while other cancer types cause 17% deaths in XP patients. The median age of death of patients with and without neurodegenerative manifestations were 29 years and 37 years, respectively (Bradford *et al.*, 2011).

1.1. 2. Etiology

XP is a photosensitivity disorder due to failure to repair DNA defects. Different mutations of genes encoding proteins involved in the nucleotide excision repair (NER) of DNA were identified in XP patients. NER is a highly conserved DNA repair mechanism in eukaryotes which repairs DNA damage lesions caused by UV radiation, chemicals, and oxidative stress (Rastogi *et al.*, 2010). XP has eight complementation groups; classical XP groups from XP-A to XP-G groups and XP variant (XP-V) according to the mutated gene (*XPA* to *XPG* and *XPV* (*Pol H*) genes) and its resultant defective protein product. Classical XP or "excision deficient XP"

encompasses groups where correspondent NER proteins fail to function normally and repair DNA resulting in accumulation of damage products and DNA structural distortions, eventually causing cancer (Van Steeg & Kraemer, 1999). XP variant group or "excision efficient XP" has functional NER but post-replication repair defect due to mutation in polymerase η (eta) gene (*Pol H*). Polymerase η is responsible for *de novo* synthesis of DNA at damaged sites after excision of induced erroneous lesions; thus, mutation of *Pol H* also predisposes to cancer (Masutani *et al.*, 1999).

DNA damage

DNA damage can be induced by UV, chemical agents or oxidative stress. Sunlight, to be specific, UV radiation of the sun induces DNA damage products i.e. photoproducts. These photoproducts are formed between adjacent pyrimidine (thymine and cytosine) bases. Two types of photoproducts are formed in a ratio of 3:1; cyclobutane pyrimidine dimers (CPDs) and 6-pyrimidine 4-pyrimidone lesions (6-4PPs), respectively. UV-A radiation (wavelength of 315–400 nm) represent majority of sunlight UV, almost 95% while UV-B rays have wavelength between 280 and 315 nm, and represent 5%. While UV-B produces more photoproducts than UV-A, the photoproducts induced by UV-A tend to be more mutagenic (Rünger *et al.*, 2012; Kraemer & DiGiovanna, 2014). UV-A can also produce oxidative lesions such as thymine glycol. DNA damage induced by light or chemicals or oxidative damage causes the formation of bulky lesions and DNA crosslinks. Photoproducts and other damage lesions are formed in different parts of human genome, either in the non-coding majority of human genome or the actively transcribed coding DNA regions. In all cases, NER is responsible for repairing these lesions together. Conversely, NER malfunction and continual exposure to mutagens causes the persistence of DNA damage lesions. This persistence ultimately distorts structure of the DNA molecule, interferes with replication and transcription, and hence affects cell cycle regulation and cellular function. Eventually, the cellular fate would be mutation, and tumor formation (Van Steeg & Kraemer, 1999; Rastogi *et al.*, 2010).

NER pathway

NER pathway represents sequential DNA repair steps. First, specific proteins (1) recognizes damage lesions, (2) unwind DNA at the recognized damage site, (3) marks damage

site by incision at either sides, (4) excises the damage lesion, (5) fills the resulting gap via *de novo* DNA synthesis, and finally, (6) ligates the newly synthesized DNA. NER includes two different pathways which differ in their machinery for recognizing DNA damage lesions. In actively transcribed DNA regions, the more rapid transcription coupled repair pathway (TCR-NER) is employed while the global genome repair (GGR-NER) is generally employed for all the genome, hence the name global. These two pathways use the same protein machinery for the remaining steps of NER (Kraemer & DiGiovanna, 2014). The roles and interactions of the proteins of NER pathway are illustrated in figure 1 (DiGiovanna & Kraemer, 2012).

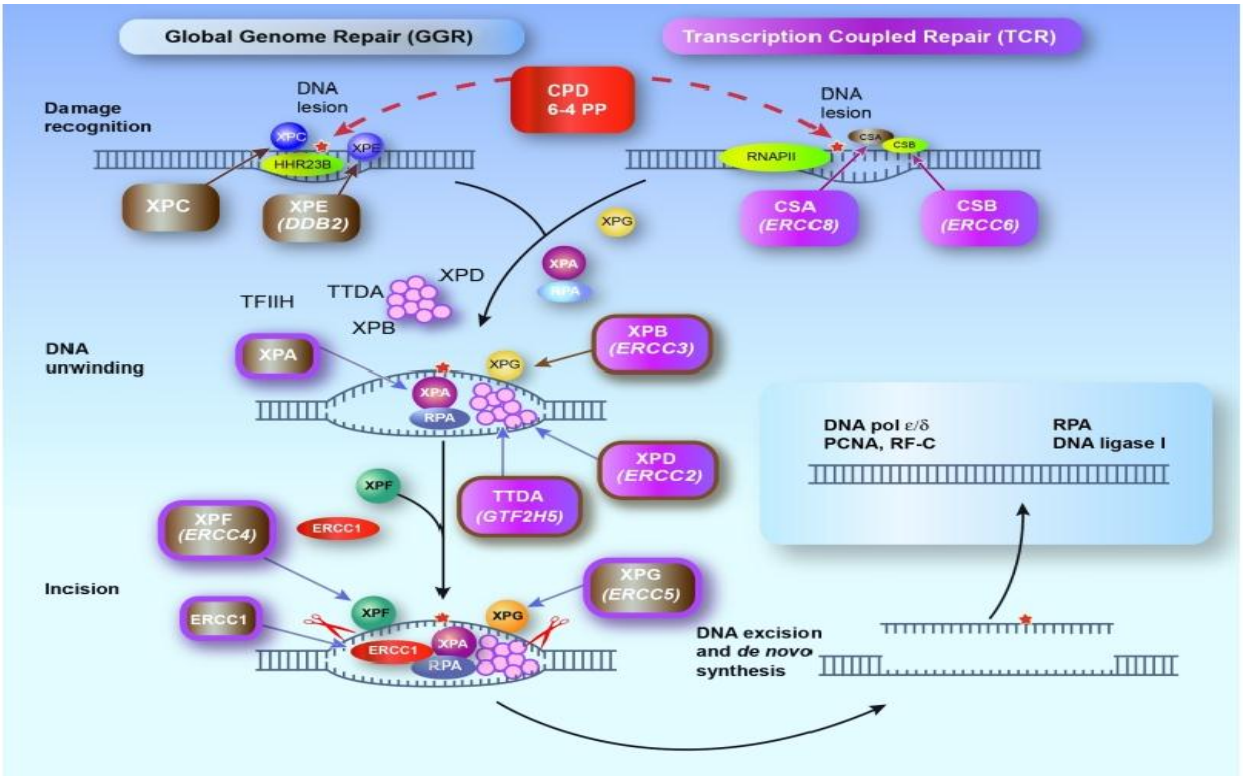


Figure 1: Nucleotide excision repair (NER) pathway (DiGiovanna & Kraemer, 2012). The figure shows GGR and TCR steps: damage recognition, DNA unwinding, incision, DNA excision, and *de novo* synthesis. Details are described in text. Rectangles are genes, and circles are gene products. CPD: cyclobutane pyrimidine dimers, 6-4 PP: 6-4 photoproducts, HHR23B: human homolog of the yeast RAD23B protein, RNAPII: RNA polymerase II, XP: xeroderma pigmentosum (A to G), CS: Cockayne syndrome (type A and B), *ERCC*: excision repair cross complementing (1 to 8), DDB2: damage-specific DNA binding protein 2 TFIIH: transcription factor IIH, TTDA or *GTF2H5*: general transcription factor 2H5, PCNA: proliferating cell nuclear antigen, RF-C: replication factor C and DNA pol: DNA polymerase. (Figure was obtained from DiGiovanna & Kraemer, 2012 with permission**)**

In the GGR, XPC protein in complex with the human homolog of the yeast RAD23B protein (hHR23B) recognizes DNA damage lesions. XPE (also named DDB2 i.e. damage-specific DNA

binding protein 2) binds to DNA damage lesions and interacts with XPC-hHR23B complex early in initial GGR recognition step (Sugasawa *et al.*, 2005). The XPC-hHR23B complex recruits a nine subunit transcription factor, TFIIH. TFIIH has two subunits, XPB and XPD, their DNA helicases functions to locally unwind DNA. The unwinding seems to signal the formation of a pre-incision complex of XPA and replication protein A (RPA), and the XPC-hHR23B complex is released (Rastogi *et al.*, 2010). This pre-incision complex anchors two endonucleases ERCC1-XPF and XPG which cut the damage lesions from both sides (ERCC1 is excision repair cross complementing 1) (Volker *et al.*, 2001; Fadda, 2016). After excision, the excised DNA leaves a gap which is filled by polymerase η with involvement of some facilitator proteins such as RPA, proliferating cell nuclear antigen (PCNA), and replication factor C (RF-C). Finally, DNA ligase I ligates the newly synthesized strand. The second NER pathway, TCR, involves all proteins from XPA to XPG with exception of XPC, and perhaps XPE. Instead of XPC, RNA polymerase II (RNAPII) recognizes DNA damage with two other proteins; CSA and CSB, their complex recruits XPG, TFIIH; consequently, the formation of pre-incision complex by XPA-RPA follows (Van Steeg & Kraemer, 1999). Mutations in *CSA* and *CSB* have been linked to complementation groups A and B of Cockayne syndrome (CS); a known photosensitivity disorder having defect in TCR only (Nance & Berry, 1992; Kraemer & DiGiovanna, 2014). In comparison between CS and XP, CS patients are not susceptible to cancer as in case of XP patients. CS patients have functional GGR but defective TCR while XP patients have defective GGR. Consequently, it was concluded that defective GGR is responsible for increased proneness to skin cancers. Furthermore, CS patients successfully repair 6-4 PPs but not CPDs lesions while both lesions cannot be repaired in XP patients, thus the role of 6-4 PPs in the increased cancer risk was suggested (Parris & Kraemer, 1993; Kraemer & DiGiovanna, 2014).

1.1.3. Epidemiology

XP is inherited in autosomal recessive manner with no sex bias. Asymptomatic carrier parents can give birth to affected cases, asymptomatic carriers and unaffected offsprings in a ratio 1:2:1; respectively. Incidence of XP in US and West-European countries is one per million. The frequency is higher in some populations, namely Mahori, Japanese, and North African populations, see table 1. The increase in frequency is due to high consanguinity rates which results from geographic or cultural factors. The Mayotte islands in the Indian Ocean, part of the

African Comorian islands, have the highest incidence of XP globally. Japan, being an island too, has a geographic isolation factor promoting consanguinity. North African countries have closely similar cultures and are surrounded by different geographical borders; the Mediterranean, the Sahara, the Red Sea and the Atlas mountains. Geographical isolation and cultures favorable of interfamilial marriages increase consanguinity rates. (Hirai *et al.*, 2006; Kleijer *et al.*, 2008; Soufir *et al.*, 2010; Doubaj *et al.*, 2012). Collectively, XP-A, C, D, and V groups represent 91% of total XP cases worldwide (Rivera-Begeman *et al.*, 2007). XP-C complementation group is the most prevalent worldwide. The second most prevalent group is XP-D in US and West-European countries, while in North African countries; it is XP-A group (Kleijer *et al.*, 2008; Tamura *et al.*, 2010; Bradford *et al.*, 2011). XP-C is exclusively prevalent in Mahori XP patients; a single founder splicing *XPC* gene mutation was found in all patients (Cartault *et al.*, 2011). A founder frameshift mutation due to two base pairs deletion in *XPC* gene was also identified in North Africa (Mahindra *et al.*, 2008; Ben Rekaya *et al.*, 2009; Soufir *et al.*, 2010; Tamura *et al.*, 2010). In Morocco, frequency of heterozygous carriers of this North African founder mutation was one per 250 newborns (Doubaj *et al.*, 2012). In Japanese population, XP-A followed by XP-V predominate (Moriwaki & Kraemer, 2001). It is estimated that one million (1%) of the Japanese population are heterozygous non symptomatic carriers of a founder *XPA* gene mutation (Hirai *et al.*, 2006).

Table 1: Incidences of XP in Mayotte islands, Japan and some North African countries.

Country	**Incidence**	**Reference**
Mayotte islands	1:5,000	**(Cartault *et al.*, 2011)**
Tunisia	1:10,000	**(Zghal *et al.*, 2005; Jerbi *et al.*, 2016)**
Japan	1:22,000	**(Hirai *et al.*, 2006)**
Libya	1: 50,000	**(Khatri *et al.*, 1999)**
Morocco	1:80,504	**(Doubaj *et al.*, 2012)**

In Egypt, the relatively increased incidence of XP compared to US and Europe was highlighted (German *et al.*, 1984). Egypt has high parental consanguinity rate which increases the prevalence of autosomal recessive genetic disorders. Studies estimated consanguineous marriages to be 33% to 35%; more than half of which are between first cousins (Temtamy *et al.*, 2010; Shawky *et al.*, 2011). High consanguinity has greater risk of mortalities among infants and children. The

consanguinity in families of history of recessive genetic disorders reached 78.8% (Shawky *et al.*, 2013). In 1964, XP was reported in 50 patients belonging to 34 families (El-Hefnawi *et al.*, 1964). A large study on the records of 660,280 Egyptian pediatric patients in the years between 1966 and 2009 reported the frequency of genetic disorders in the Egyptian population to be 43 per 1000 individuals. The frequency of genodermatosis among genetic disorders was approximately 2% of which nearly 16% (88 cases) were XP patients (Shawky *et al.*, 2012). Two earlier studies of XP in Egypt reported XP-A complementation group in 2 families, XP-C in 7 families, and only one family of XP-V via cell fusion complementation studies (Hashem *et al.*, 1980; Cleaver *et al.*, 1981). Only two molecular studies were performed 21 years later. One study was in 2005 on a banked XP-C Egyptian cell line where the North African two base pairs deletion mutation in *XPC* gene was identified (Ridley *et al.*, 2005). The other study followed after nine years at NRC identified three *XPA* gene mutations (one novel and two previously reported mutations) in four Egyptian families with severe XP form (Amr *et al.*, 2014).

1.1.4. Diagnosis

Diagnosis of XP depends on clinical picture of the patient, and family history with the aid of molecular diagnosis (Lambert & Lambert, 2015). Despite that XP subtypes vary in their clinical features in terms of the severity of cutaneous symptoms and presence/absence of neurological symptoms, overlapping does exist between XP subtypes themselves, and with other DNA repair disorders, such as Cockayne syndrome (CS) and trichiothiodystrophy (TTD), see figure 2 (DiGiovanna & Kraemer, 2012). Consequently, patients cannot be subtyped depending on their clinical features only e.g. XP with absence of neurologic abnormalities can be subtyped into three complementation groups, XP-C, E and V, which usually show milder photosensitivity but earlier cancer development. Still, reports of mutations in *XPA*, *XPD*, *XPC* and *XPF* genes causing this same phenotype do exist, see figure 2 (Sethi *et al.*, 2013). Furthermore, several researches reported mild XP-A forms with minor cutaneous and neurologic features where diagnosis could not be accomplished unless genetic analysis was carried out (Tanioka *et al.*, 2005; Sidwell *et al.*, 2006; Takahashi *et al.*, 2010).

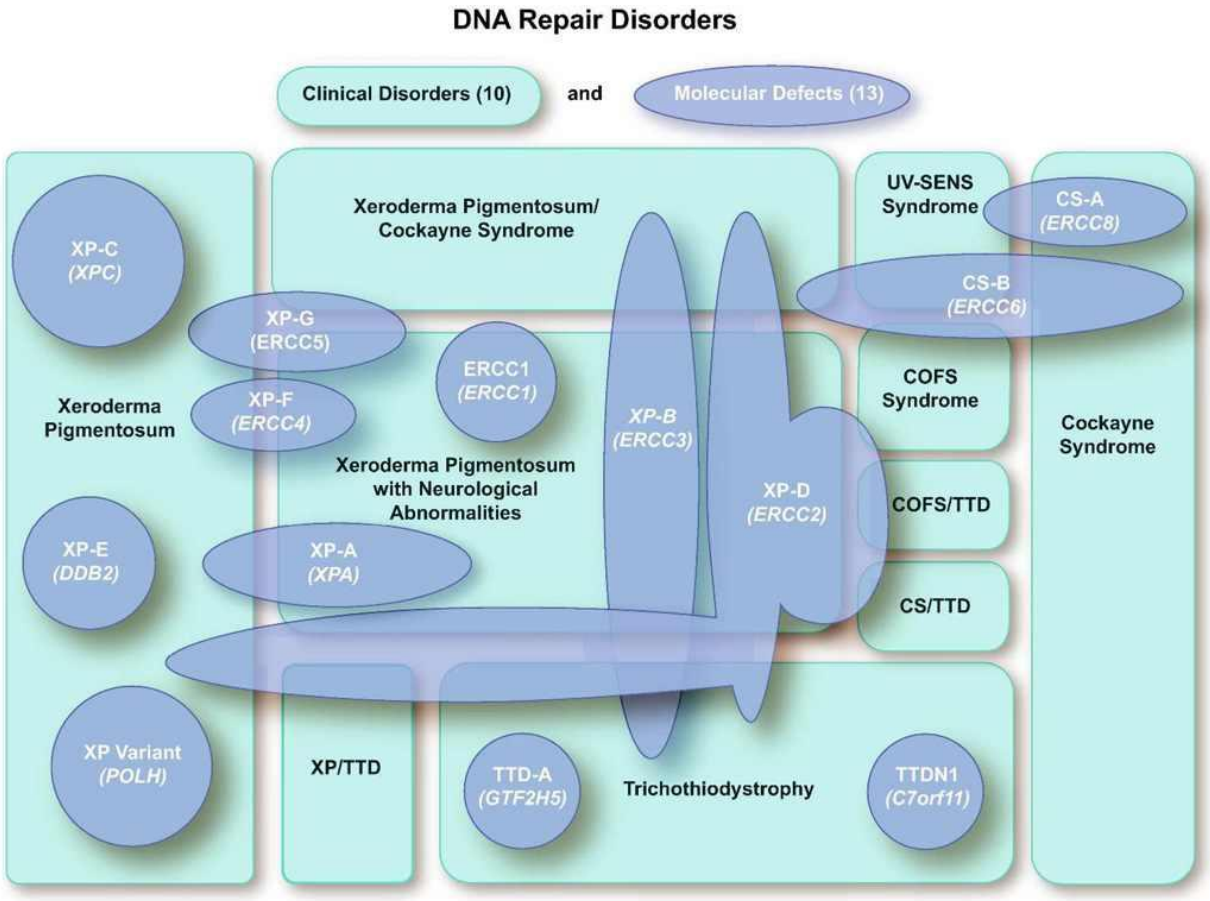


Figure 2: DNA repair disorders (DiGiovanna & Kraemer, 2012). The figure shows the relations between the clinical picture of ten DNA repair disorders and the defected genes underlying them. Ten clinical diseases in rectangles and 13 molecular defects are represented. Complementation groups of different disorders are in circles and their correspondent genes are in brackets. As illustrated, a single disorder can be caused by different mutations in more than one gene. On the other hand, diverse mutations in a single gene may lead to different clinical conditions. COFS: cerebrooculofacioskeletal syndrome; CS: Cockayne syndrome; TTD: trichothiodystrophy; UV-SENS syndrome: UV-sensitivity syndrome; and XP: xeroderma pigmentosum. Complex between two clinical conditions is denoted by slash "/" between them. For details on each clinical disorder, see reference by Kraemer & DiGiovanna, 2003/2014. CS-A and B: Cockayne syndrome type A and B; *C7orf11*: gene for chromosome 7 open reading frame 11 protein; *DDB2*: gene for damage-specific DNA binding protein 2; *ERCC*: excision repair cross complementing genes; *POLH*: polymerase η (eta) gene; TTD-A: trichothiodystrophy type A; TTDN1: trichothiodystrophy non-phtosensitive type; *GTF2H5:* general transcription factor 2H5 gene; XP-A to G: xeroderma pigmentosum type A to G; and *XPC*: xeroderma pigmentosum type C gene. (Figure was obtained from DiGiovanna & Kraemer, 2012 with permission)

With the help of functional and molecular tests on XP patients, the diagnosis can be confirmed, and the subtype (group A to G and V) can be identified. Subtyping is helpful in prediction of

disease course and degree of UV sensitivity (Sethi *et al.*, 2013; Lambert & Lambert, 2015). Molecular tests identifying the exact mutation causing the disease provide an important tool to subtype XP, detect carriers among at-risk family members, provide prenatal diagnosis, and direct future therapies under development. Functional analyses are carried out on skin biopsies from patients. Cell cultures from XP skin fibroblasts can be used to detect the survival of the cells post UV exposure; this is called UV sensitivity test. Cultures are also used for measuring unscheduled DNA synthesis (UDS) using radioactive thymidine; UDS is DNA synthesis taking place outside the S-phase. In both cases, XP-V group has results equivalent to normal cells. Complementation of DNA repair defect by fusion of XP cultured cells with previously subtyped XP cells is used for subtyping all XP groups, including XP-V. Unfortunately, functional tests are uncommon and commercially unavailable. Consequently, molecular tests for sequencing of the genes involved with XP phenotype are employed either by Sanger sequencing of each gene individually or by using multiple genes panel (Kraemer & DiGiovanna, 2003/2014; Warrick *et al.*, 2012; Ortega-Recalde *et al.*, 2013).

1.1.5. Treatment and Management

XP has no treatment to date, thus early diagnosis is very important to initiate management and prophylactic measures. Physicians from different specialties should be visited regularly. Genetic counseling and patient education are crucial. As a rule of thumb, avoiding exposure to the sun and artificial UV sources should begin as early as possible. Sunscreens, protective sunglasses and surfaces that absorb UV are recommended. For the dryness, skin emollients, and artificial tears are recommended. For precancerous and cancerous lesions in the skin or the eyes or the oral cavity, approaches such as cryotherapy, electrodesiccation, surgical removal, and in severe cases corneal transplantation are employed (Kraemer & DiGiovanna, 2003/2014). Other unorthodox approaches were found beneficial such as: skin resurfacing, topical preparation containing DNA repair enzymes, and photodynamic therapy (Lambert & Lambert, 2015). Drugs such as retinoid derivatives can be used as a prophylactic agents against new multiple skin cancers; however, their toxicity and teratogenicity pose a major limitation (Kraemer *et al.*, 1988, 1990). Other agents for prophylaxis such as 5-fluorouracil and imiquimod creams increase immune responses against developing cancerous lesions (Hamouda *et al.*, 2001; Nagore *et al.*, 2003). Recently, gene therapy approaches have successfully corrected mutational

defect in XP-C patients' primary keratinocytes, which pave a road for skin grafting. Yet, these approaches are still under development and have not targeted except specific *XPC* gene mutations (Arnaudeau-Bégard *et al.*, 2003; Warrick *et al.*, 2012; Dupuy *et al.*, 2013; Kuschal *et al.*, 2013; Rouanet *et al.*, 2013).

1.2. *XPA* and *XPC* genes

XPC followed by *XPA* genes account for the majority of XP cases in North Africa (Tamura *et al.*, 2010). This finding agrees with the earlier reports of the identification of XP-A and XP-C complementation groups in XP cell lines from XP Egyptian patients (Hashem *et al.*, 1980; Cleaver *et al.*, 1981). In the next sections, *XPA* and *XPC* genes, their correspondent gene products and mutations are highlighted.

1.2.1. *XPA* gene and protein

Human *XPA* gene (NM_000380.3; OMIM: 611153) is located on the long arm of chromosome 9 at position 9q22.3. The gene has six exons and five introns transcribed into 1.4 kb mRNA. The gene encodes a 40 kDa protein of 273 amino acids. The XPA protein is involved in both types of NER pathway; particularly in verification of DNA damage occurrence via formation of a pre-incision complex. XPA was also found to interact with XPC-hHR23B complex in first DNA recognition step of GGR. When the two helicases subunits, XPB and XPD, of transcription factor TFIIH unwind DNA at the damage lesion, the XPA protein complexes with the replication protein A (RPA) creating a pre-incision complex at DNA damage lesion, see figure 1 (Van Steeg & Kraemer, 1999; Rastogi *et al.*, 2010). RPA is a trimer of three subunits; two of which, RPA34 and RPA70, bind to both ssDNA and XPA, and the third is vital for trimer formation. Aside from interaction with XPC-hHR23B complex, RPA and TFIIH, XPA also interacts directly with ERCC1-XPF complex to recruit its endonuclease function for incision. Indeed, XPA structure is very flexible, this fits and allows its interaction with all the core NER proteins, with exception of XPG that binds to RPA, in the bubble formed at DNA damage site. The XPA protein comprises three domains, see figure 3; a central DNA binding domain flanked by C- and N-terminal domains. The central domain, encoded by exons 3, 4, and 5 of *XPA*, is crucial for XPA functionality in DNA repair. As shown in figure 3, the central domain (from amino acid 98 to 219) has two subdomains; one has a zinc finger motif and the

other is rich in secondary loop structures; both domains participate in DNA binding. The central domain has the necessary structural conformation for binding the Y-shaped junction between ssDNA and dsDNA at the bubble formed at DNA damage site, as well as, for binding to RPA70 subunit. The N-terminal domain has 97 amino acid residues responsible for the binding to RPA34 subunit, and ERCC1 protein at two different sites. This domain, encoded by exons 1 and 2, has no contribution to DNA binding activity of XPA. Exon 1 harbors the sequence encoding the nuclear localization signal (NLS) of XPA. Disruption of N-terminal domain at sites corresponding to ERCC1 binding results in failure of XPA to recruit ERCC1-XPF endonuclease for the next DNA incision step of NER. The C-terminal domain, encoded by exon 6, is involved in TFIIH binding (Bartels & Lambert, 2007; Fadda, 2016).

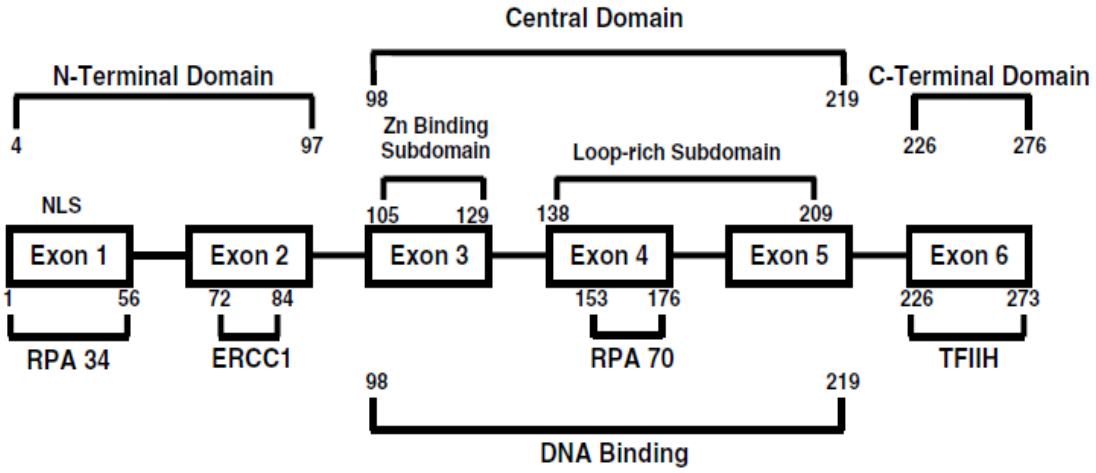


Figure 3: Schematic diagram of *XPA* gene and corresponding domains of XPA protein (Bartels & Lambert, 2007). The figure shows six exons and five introns of *XPA* gene. The three protein domains of XPA are indicated above the gene; N-terminal domain, the central DNA binding domain having 2 subdomains, and C-terminal domain. Below the gene are the binding sites for DNA and the proteins interacting with XPA; RPA 34 and 70, ERCC1 and TFIIH. The numbers refer to amino acid residues of XPA; XPA is 273 amino acids long. NLS is nuclear localization signal. (Figure was obtained from Bartels & Lambert, 2007 with permission)

1.2.2. *XPA* gene mutations

XP-A complementation group is considered the most severe XP group with the least residual DNA repair activity, less than 2% of normal DNA repair activity (Van Steeg & Kraemer, 1999). *XPA* has 36 mutations reported to cause XP-A phenotype to date. To the end of 2015, 35 mutations were reported according to human genome mutation database (HGMD;

www.hgmd.org) (Stenson *et al.*, 2014). From end of 2015 to date, one novel homozygous mutation was identified in exon 3 due to 5 bp deletion (c.349-353 delCTTAT) which resulted in severe neurologic deterioration (Ghafouri-Fard *et al.*, 2016). The identified *XPA* gene mutations fall into six classes: single base substitutions comprising five missense, seven nonsense mutations and nine splicing mutations, in addition to nine small deletions, four small insertions, and two indels (insertion and deletion), see figure 4. Neither were gross deletions, insertions or duplications identified nor complex rearrangements in *XPA*.

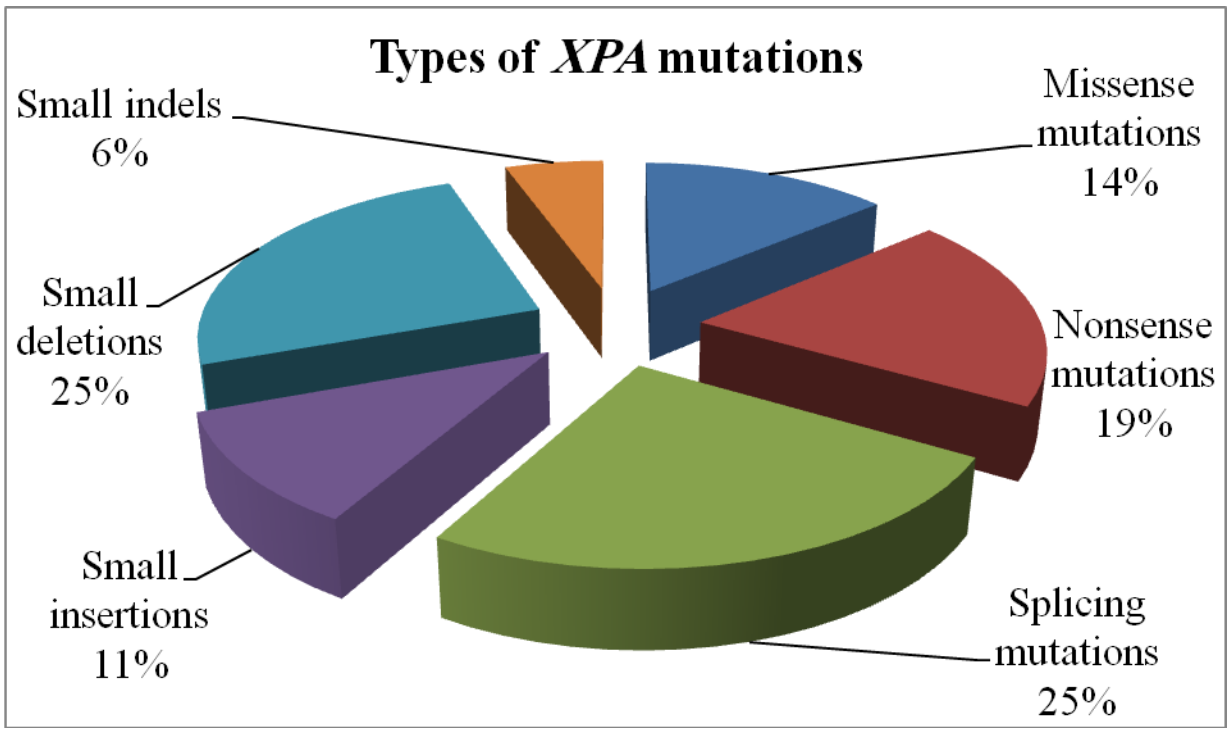


Figure 4: *XPA* gene mutations classified according to mutation types. The figure shows a pie chart of six different classes of the 36 mutations identified in *XPA* in percentage ratio. The figure was created based on data from literature search and human genome mutation database (HGMD; www.hgmd.org). Single base substitutions which result in change of one amino acid to another are termed missense mutations, while those which result in the formation of a premature stop codon are termed nonsense mutations. Splicing mutations are intronic mutations affecting mRNA sequence. Small insertions or deletions of 20 bp or less cause frameshift mutations. Indels refer to mutations due to both insertion and deletion of 20 bp or less.

The clinical phenotypes of the reported XPA mutations show variable severity of cutaneous and neurological symptoms owing to site and type of the mutation, and the resulting residual DNA repair activity (Nishigori *et al.*, 1994; Messaoud *et al.*, 2010b; Takahashi *et al.*, 2010). Phenotype-genotype correlation was apparent with respect to the mutation site. Patients with

mutations disturbing DNA binding domain encoded by exon 3, 4 and 5 of *XPA* showed severe clinical picture. On the other hand, *XPA* mutations in exon 6 which affect the regions close to *C*-terminus were found in patients having milder skin and neurologic presentation. This is consistent with the fact that truncated XPA proteins with intact DNA binding domains were shown to maintain some DNA repair activity in XPA patients, therefore, mild skin symptoms and slowly progressing neurologic deterioration (States *et al.*, 1998; Bartels & Lambert, 2007; Takahashi *et al.*, 2010). By looking into the sites of the reported XPA mutations, mutations in exons 3 to exon 6 represent the vast majority of XPA gene mutations, see figure 5.

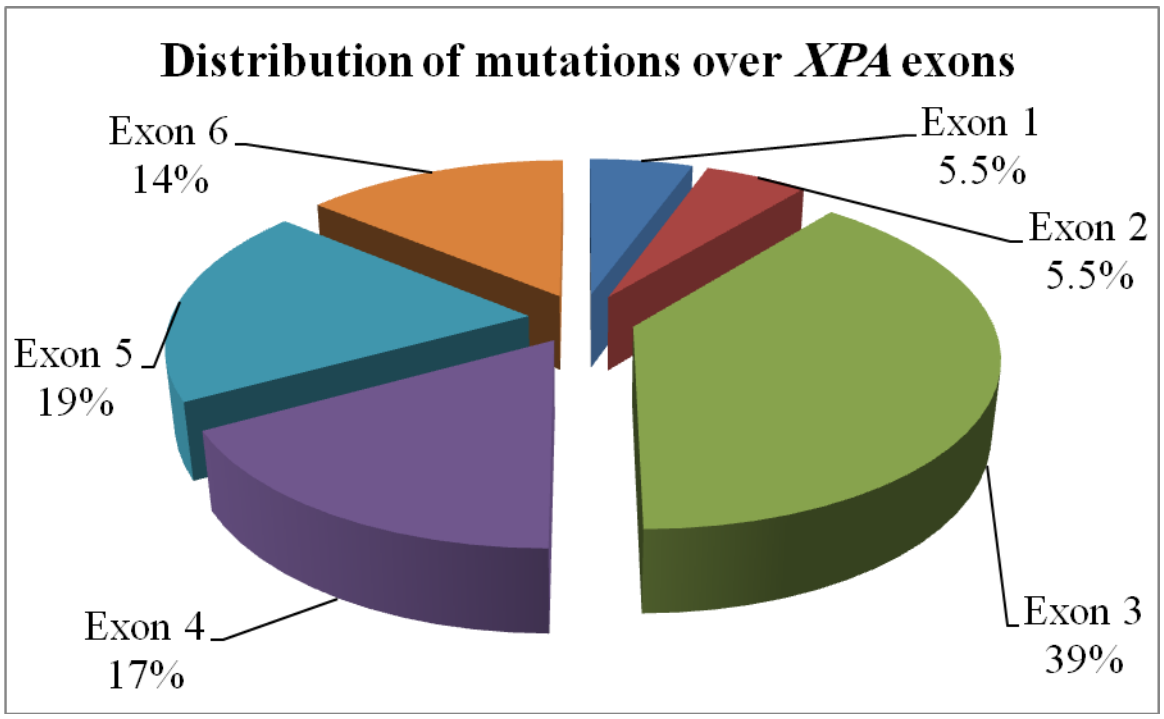


Figure 5: Distribution of previously identified mutations over *XPA* exons. The figure shows a pie chart for the distribution of different identified mutations on the six exons of *XPA* gene. The figure was created based on data from literature search and human genome mutation database (HGMD; www.hgmd.org). Exons number 3, 4, 5 and 6 harbor 89% of the identified *XPA* mutations. Splicing mutations in introns were added to the following exon percentage.

Founder mutations have been described in XPA. In Japan, where XPA is prevalent, a founder splicing mutation (c.3901G>C) in intron 3 was identified in 90% of Japanese XPA patients. The splicing mutation results in exon 3 skipping and premature stop codon in exon 4 producing negligible XPA protein, and severe phenotype (Satokata *et al.*, 1990). This Japanese founder mutation was estimated to be about 120 generations old (3,000 years ago) and one million people

of the Japanese population are estimated to be carriers of one mutated allele (Hirai *et al.*, 2006; Imoto *et al.*, 2013). Another founder mutation (c.682C>T p.R228X) was identified in Tunisian XPA patients; this mutation in exon 6 causes a premature termination of translation, at amino acid number 228, which results in relatively milder XPA phenotype (Nishigori *et al.*, 1993; Messaoud *et al.*, 2010a). Compound heterozygosity has been reported in XPA patients (Satokata *et al.*, 1992b; Tanioka *et al.*, 2005), this usually occurs in isolated populations where the frequent founder mutation is identified in one allele of a specific gene, and less frequent or rare mutation is found on another. (Zlotogora, 1994; Feingold, 1998). The two deficient alleles occasionally result in a phenotype milder than homozygous phenotype of any of the individual alleles (Negishi *et al.*, 2001; Takahashi *et al.*, 2010). In Egypt, three homozygous XPA mutations were identified; two of which were nonsense mutations, one novel mutation in exon 4 (c.553C>T, p.Q185X), and another mutation in exon 3 (c.331G>T, p.E111X). The nonsense mutation in exon 3 was identified in the Tunisian population before; hence, a common ancestor was suggested. The third mutation identified was a novel single nucleotide deletion (c.374delC) in exon 3 that causes 15 base pairs long frameshift and premature stop codon formation (p.Thr125IlefsX15). In compliance with the literature, these mutations in the exons 3 and 4 correspond to DNA binding domain; accordingly, their clinical phenotypes showed severe XP form in terms of neurologic manifestations (Messaoud *et al.*, 2011; Amr *et al.*, 2014).

1.2.3. *XPC* gene and protein

Human *XPC* gene (NM_004628.4; OMIM: 613208) is located on the short arm of chromosome 3 at position 3p25.1. The gene has 16 exons and 15 introns. It is transcribed into 3.5 Kb mRNA then translated into 940 amino acids (Khan *et al.*, 2002). XPC protein functions in the DNA recognition step of GGR only i.e. the NER concerned with the repair of the whole genome whether genes or non-transcribed regions. XPC functions in initial DNA damage recognition step via the formation of a trimer complex. XPC forms complex with two proteins hHR23B and centrin 2. Centrin 2 is required for XPC-hHR23B complex stability essential for DNA binding. The XPC-hHR23B protein complex induces conformational changes around DNA lesion. These changes facilitate TFIIH recruitment to unwind DNA via XPB and XPD helicases and assemble pre-incision complex (Masutani *et al.*, 1994; Sugasawa *et al.*, 1998; Araki *et al.*, 2001). Upon the assembly of pre-incision complex, XPC is released. Nevertheless, direct interaction between

XPC-hHR23B and XPA-RPA complexes was observed in the early DNA recognition step of some DNA crosslinked lesions(Thoma *et al.*, 2005). Similar to XPA, XPC protein has multiple domains. XPC can be divided into two regions: N-terminal region from amino acid number 1 to number 491, and C-terminal region from amino acid number 492 to 940. N-terminal region comprises the domain responsible for binding to XPA (amino acid 154 to 331). The *XPC* region from exon 1 to the middle of exon 9 encodes the N-terminal region. The C-terminal region comprises all the essential domains for binding to DNA, hHR23B, centrin 2 and TFIIH. The region between amino acid 606 and 766 is essential for mobility and recruitment of XPC at DNA damage lesions, see figure 6 (Bunick *et al.*, 2006; Camenisch *et al.*, 2009; Clement *et al.*, 2011; Feltes & Bonatto, 2015). The C-terminal region is encoded by XPC region from the middle of exon 9 to exon 16. Interestingly, XPC gene expression affects the type of DNA lesion to be recognized, hence repaired. It was reported that XPC, in normal level, repaired both CPDs and 6-4PPs. When the *in vivo* expression level decreased below normal or increased above normal, XPC selectively recognized CPDs only, however, negligible expression fails to repair neither CPDs nor 6-4PPs (Emmert *et al.*, 2000).

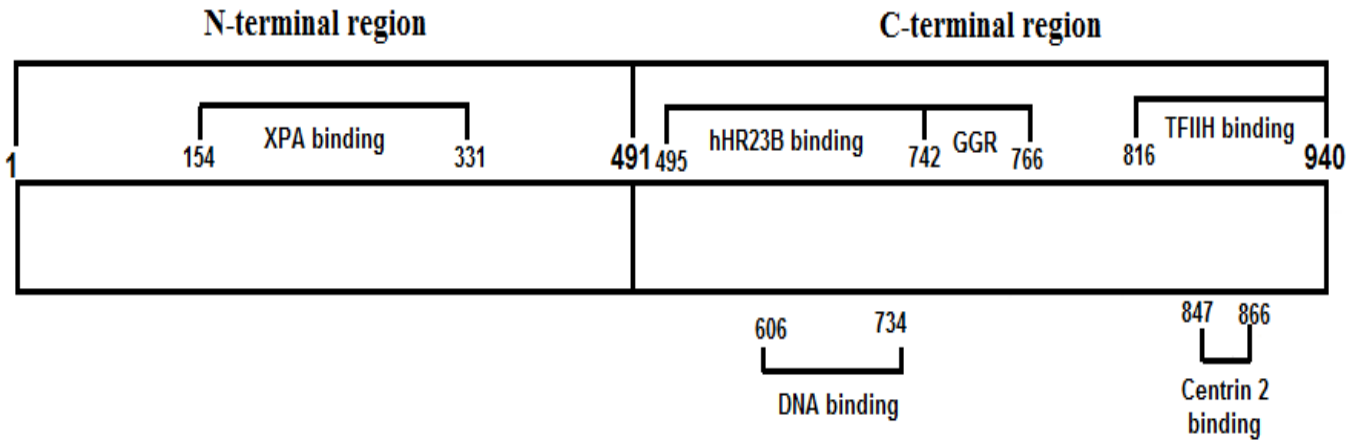


Figure 6: Schematic diagram of XPC protein. The figure shows the distribution of different domains over the 940 amino acid residues of XPC protein. The numbers refer to amino acid residues of XPC. XPC is divided into N- and C- terminal regions. The N-terminal region has the XPA binding domain. The C-terminal domain includes binding domains for DNA, hHR23B, centrin 2 and TFIIH. The DNA binding domain and the area denoted by GGR are essential for XPC recruitment into GGR pathway to perform its function. (Figure was modified from XPC figure by Feltes & Bonatto, 2015 with permission)

1.2.4. *XPC* gene mutations

Mutations in *XPC* gene are responsible for XP-C phenotype. Up till now, there are 60 identified mutations for XPC patients worldwide according to human genome mutation database (HGMD; www.hgmd.org) (Stenson *et al.*, 2014). According to the type of mutation, the majority (74%) of *XPC* gene mutations are small deletions of less than 7 bp, nonsense single base substitutions, and splicing mutations. Missense mutations, small insertions, and small insertion-deletion mutations are present at a lesser extent. Gross insertions, gross deletions and complex rearrangements of *XPC* are very minor, collectively, 5 of these mutations were identified, see figure 7.

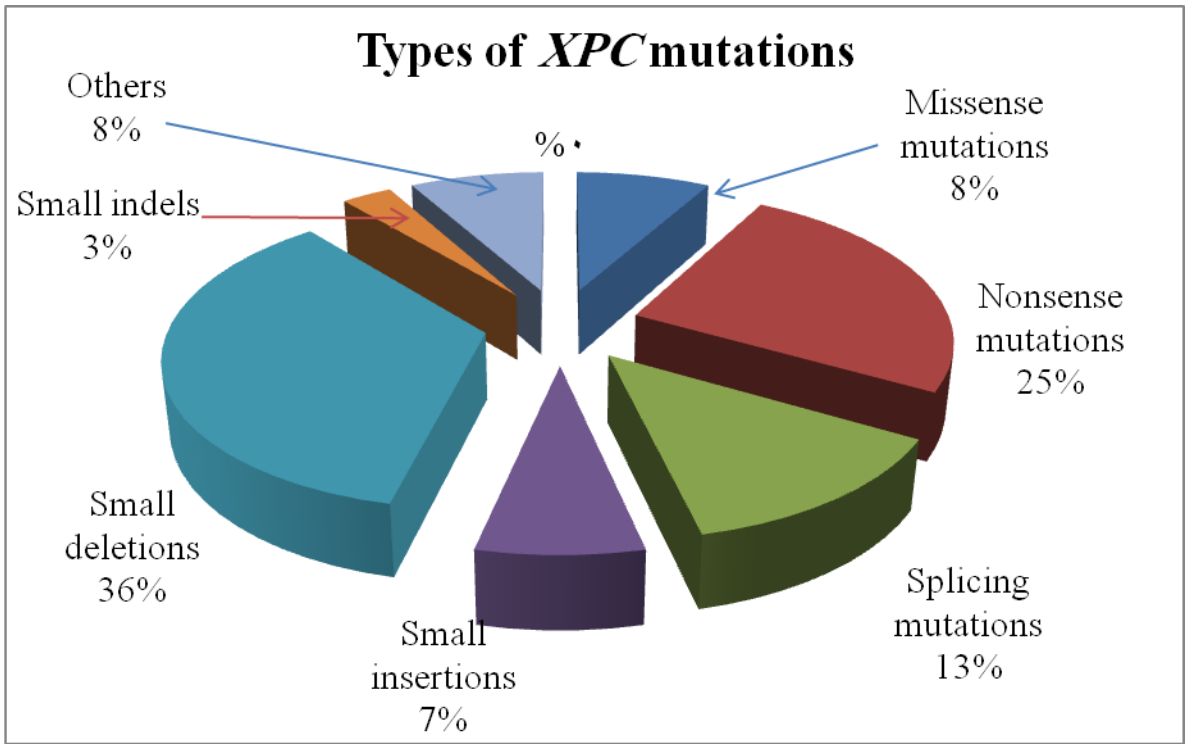


Figure 7: *XPC* gene mutations classified according to mutation types. The figure shows a pie chart of different classes of the 60 mutations identified in *XPC*. The figure was created based on information obtained from human genome mutation database (HGMD; www.hgmd.org). Small deletions and nonsense mutations have the biggest share, followed by splicing mutations. To a much lesser extent, missense mutations, small insertions and small indels were present. Other mutations such as large insertions, large deletions and complex rearrangements represent 8% (only 5 mutations) collectively.

According to the sites of the identified mutations, exon 9 evidently harbors more mutations than any other XPC exon; 28% of the identified *XPC* mutations see figure 8. Exon 9 encodes from

amino acid number 331 to number 624 of XPC protein, hence it encodes hHR23B and DNA binding domains of XPC, see figure 6. This might explain the importance of exon 9 integrity for XPC function. Nevertheless, increased number of mutations identified in exon 9 can be attributed to its large size; it is the largest exon in *XPC*. No hotspot exons were specifically reported. It is also noted that no mutations were reported in exons 7 and 16 of *XPC* gene.

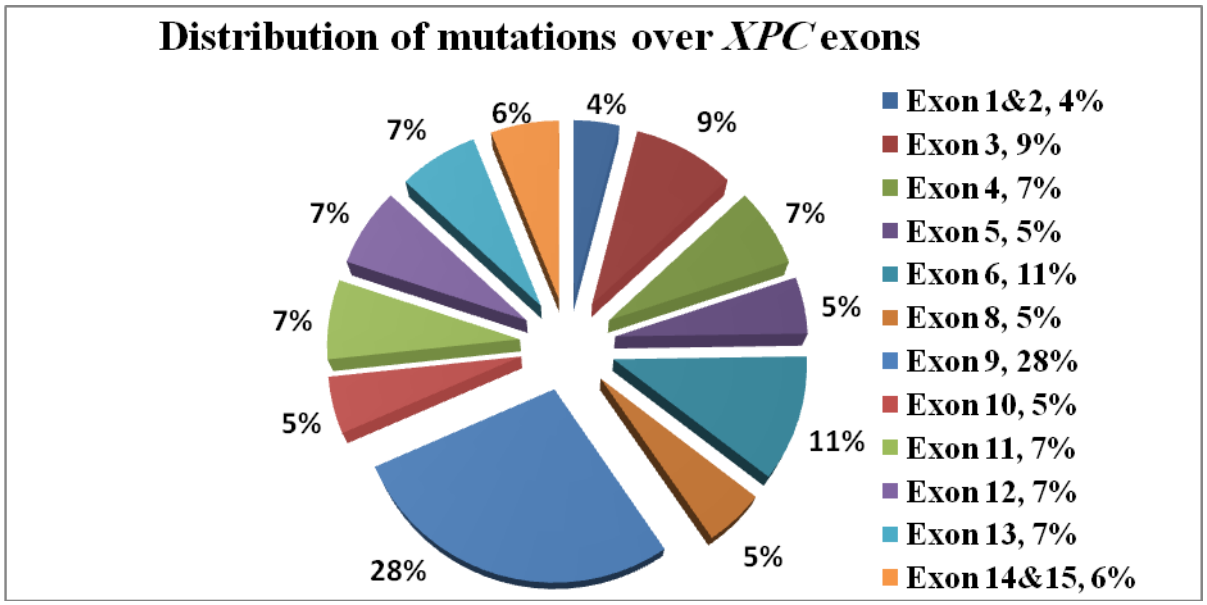


Figure 8: Distribution of previously identified mutations over *XPC* exons. The figure shows a pie chart for the distribution of different identified mutations on the exons of *XPC* gene. The figure was created based on data obtained from literature search and human genome mutation database (HGMD; www.hgmd.org). Splicing mutations in introns were added to the following exon percentage. No mutations were identified in exon 7 and 16. With exception to exon 9, each *XPC* exons seem to have small equal share of the identified mutations.

All the identified mutations were linked to XP clinical phenotypes that show photosensitivity of the skin and the eyes with early onset of skin cancers and no neurological abnormalities. Some exceptions were found to have neurologic manifestations in concurrence with XP-C genotype; this was explained by suspected involvement of homozygous mutations of other unknown genes due to consanguinity (Khan *et al.*, 2009; Soufir *et al.*, 2010; Schäfer *et al.*, 2013). *XPC* mutations usually result in more residual DNA repair (10-20% of normal DNA repair activity) and relatively less photosensitivity than *XPA* mutations which appears to be attributed to functional TCR in XPC patients. The relatively lower photosensitivity observed in XPC patients is thought to account for XPC patients receiving relatively late sun protection in their life, hence the early onset of UV-induced cancers (Sethi *et al.*, 2013). Sun exposure and environmental factors affect

the severity of clinical phenotype; nevertheless, in some *XPC* mutations the clinical severity was attributed to the effect of these mutations on normal *XPC* mRNA level or the functionality of the mutated protein, if any. As an example, patients who have a splicing mutation in intron 3 that results in 3% of normal *XPC* mRNA level manifested mild phenotype while patients with another splicing mutation in the same intron had severe phenotype due to negligible normal *XPC* mRNA level. Missense mutations result in unstable mutated proteins which might have some functionality to repair DNA at levels lower than normal. On the other hand, nonsense mutations could result in shorter than normal mRNA; these undersized mRNA are unstable and usually decay, consequently, the correspondent proteins, aside from being truncated, are undetectable (Khan *et al.*, 2004, 2009; Maquat, 2005; Schäfer *et al.*, 2013). A founder mutation has been identified in North Africa; specifically in 74% of 66 XP patients from Algeria, Morocco, and Tunisia. This founder mutation, dated back to 50 generations ago, is a two base pairs (TG) deletion in exon 9 (c.1643_1644delTG) which causes 25 codon long frameshift, and premature stop codon formation (p.Val548AlafsX25). The clinical picture of homozygous patients for this founder mutation is homogenous in terms of high severity, marked photosensitivity, multiple skin cancers, and manifestation of ocular symptoms (Mahindra *et al.*, 2008; Ben Rekaya *et al.*, 2009; Soufir *et al.*, 2010; Tamura *et al.*, 2010). A study was done on an Egyptian banked cell line previously identified as XP-C by complementation; this cell line showed the same homozygous TG deletion (Hashem *et al.*, 1980; Ridley *et al.*, 2005). The two base pairs TG deletion was also identified in an Italian patient (Chavanne *et al.*, 2000). Consequently, the North African founder mutation was suggested to be have spread in XP patients of the Mediterranean region as a whole (Tamura *et al.*, 2010). The migration of this mutation to the South of Europe can be further confirmed by a successive report of the North African founder in 25% of German XPC patients of Middle Eastern Ancestry (Schäfer *et al.*, 2013).

Study Objectives

Problem description

Egypt is characterized by high consanguinity rate (more than 33%) which fosters autosomal recessive disorders, and increases carrier frequencies and risk for child and infant mortalities. XP represents 16% of genetic skin disorders in Egypt. The disease has been described in Egyptian families long ago; however, the mutations underlying the disease were not well studied. Only three XPA mutations have been reported in four patients despite the evidence that XPC is the most common group in Egypt (Shawky *et al.*, 2012, 2013; Amr *et al.*, 2014).

Rationale

Identification of the disease causing mutations in a specific region or population, Egyptian patients herein, provides the advantage of (1) carrier detection, premarital counseling and prenatal diagnosis for at-risk family members, (2) diagnosis or confirmation of clinical examination for XP diagnosis, (3) correlation between certain prevalent mutations and their clinical picture is beneficial for disease management, genetic counseling, and decision making in prenatal diagnosis, and (4) identification of the prevalent mutations which permits the development of cost effective molecular diagnosis and targeted future treatments (Kraemer & DiGiovanna, 2003/2014; Ben Rekaya *et al.*, 2009; Warrick *et al.*, 2012; Dupuy *et al.*, 2013). The current study employed direct sequencing of *XPA* and *XPC* genes for identification of mutations in seventeen XP patients, their parents and available family members. This is the first study to be conducted to identify mutation spectrum of *XPC* gene mutations among Egyptian XP patients, and the second to identify *XPA* gene mutations.

Objectives

The current study aims at:

1- Identification of *XPA* and *XPC* gene mutations in XP patients.

2- Detection of carriers among at-risk family members.

3- Providing a preliminary mutation spectrum of XP in Egypt.

4- Correlation between clinical picture and underlying gene mutation.

Chapter (2): Patients and Methods

2.1. Patients

2.1.1. Patients Recruitment

The patients were recruited to the Genodermatoses clinic of the Medical Research Excellence Unit, National Research Center (NRC). Patients were referred from Cairo University hospital, Ain Shams University hospital and the National Cancer Institute.

2.1.2. Ethical approval

The study was approved by the Institutional review board (IRB) of the American University in Cairo (AUC) and the Ethical committee (EC) of the National Research Centre (NRC). Written informed consents were obtained from all participants, and guardians in case of children. Aims, methods, and possible results of molecular diagnosis were explained. The privacy and confidentiality of the obtained data were assured.

2.1.3. Selection criteria

The included patients were diagnosed with XP according to clinical examination, pedigree analysis and medical history. Patients having any other genetic disorder, even if combined with XP, were excluded.

2.2. Methods

2.2.1. Clinical Evaluation

Clinical evaluation included:

1. Full medical history taking including sex, age, initial complaint, progression of symptoms, the presence of any birth or pregnancy complications, and history of present illness especially previous history of cancers.
2. Pedigree analysis for at least 3 generations with emphasis on consanguinity, and family members having similar or different genetic abnormalities.
3. General clinical examination focusing on the skin, neurodegenerative symptoms, and the presence of ocular abnormalities.

2.2.2. Molecular investigation

A. Extraction of genomic DNA

Blood samples (3-5 ml) were withdrawn from patients, parents and available family members. The samples were sent to the Medical Molecular Genetics lab at NRC for molecular diagnosis. Genomic DNA was extracted from peripheral blood leucocytes of all patients and family members by salting out method (Miller *et al.*, 1988).

A.1. Reagents:

- Sucrose Triton (2X): pH 7.6

  0.64 M Sucrose (Q-BIO-gene, France)

  0.02 M Tris base (Q-BIO-gene, France)

  0.01 M Magnesium chloride, $MgCl_2$ (Q-BIO-gene, France)

  2% (v/v) Triton X-100 (Eastern Kodak Company, USA)

- Nuclei Lysis Buffer: pH 8.2

  10 mM Tris base (Q-BIO-gene, France)

  400 mM Sodium chloride, NaCl (Merck, Germany)

  2 mM Ethylenediaminetetraacetic acid, EDTA (Q-BIO-gene, France)

- 20% Sodium Dodecyl Sulfate (SDS) (Q-BIO-gene, France)
- Proteinase K (5 µg/µl) (Finzyme, Finland)
- Saturated NaCl (35%)

A.2. Procedure:

- Three to five ml of venous blood were withdrawn and transferred immediately to a polypropylene tube (purple cap) containing 0.5 M EDTA (pH 8.0) to prevent clotting.
- In a 50 ml falcon tube, 3 ml of blood sample was pipetted, followed by 25 ml of cold 2X sucrose triton, and the final volume was completed to 50 ml by distilled H_2O. The tube was allowed to stand on ice for 20 minutes during which mixing by inversion was done several times.

- The tube was then centrifuged at 5000 rpm for 20 minutes at 4 ºC using cooling centrifuge (SIGMA 3-16KL, Germany) and the supernatant was discarded leaving only the nuclear pellet.
- Onto the nuclear pellet, 3 ml of nuclei lysis buffer, 108 µl of 20% Sodium dodecyl sulphate (SDS) and 28 µl of proteinase K (5 µg/µl) were added. The mixture was vortexed to disrupt the pellet partially, followed by incubation at 54 ºC overnight for complete digestion.
- After incubation and digestion, 1 ml of saturated NaCl solution was added (for salting out DNA), the tube were vigorously shaken for 15 seconds by vortexing, and centrifuged at 5000 rpm for 15 minutes at 4 ºC.
- The supernatant was transferred to a clean 25 ml sterile falcon tube to which two volumes of cold absolute ethanol were added, and the tubes was inverted several times to precipitate the DNA.
- Precipitated DNA was collected on the tip of a glass Pasteur pipette. The tip holding DNA was washed in 70% ethanol for desalting, and DNA was left to dry on the tip.
- The DNA was then dissolved in 400-500 µl sterile H_2O and incubated overnight at room temperature (Miller *et al.*, 1988; Sambrook & Russell, 2001).

B. Fetal genomic DNA extraction

One family came back for prenatal diagnosis, after the specific pathogenic mutation has been identified in the proband. Extraction of fetal genomic DNA from amniotic fluid sample was done by QIAamp® DNA Mini Kit (Qiagen, Germany, www.qiagen.com; catalog number: 51304)**.** Amniotic fluid samples (10 ml) were withdrawn in the prenatal clinic, and sent to Medical Molecular Genetic lab, National Research Centre. Samples were placed into falcon tubes for centrifugation at 8,000 rpm for 30 min. After centrifugation, the supernatant was discarded, and the precipitated cells were used for extraction of fetal genomic DNA by QIAamp® DNA Mini Kit according to the manufacturer's protocol (Spin protocol). Briefly, 20 µl proteinase K and 200 µl AL (lysis buffer) are added to the precipitated cells, mixed by pipetting. The mixture was transferred to an eppendorf tube, and incubated at 56 ºC for 10 min. After incubation, 200 µls absolute ethanol were added to the mixture, mixed by vortexing and transferred to the spin column supplied. The column was centrifuged at 14,000 rpm at room temperature for 3 min, the flow through was discarded. Two washes were done with AW1 then

AW2 buffers with centrifugation at 14,000 rpm at room temperature for 3 min, and discarding flow through after each wash. After washes, the column was spun at 14,000 rpm at room temperature for 3 min for efficient drying. The column was transferred onto a new labeled eppendorf tube, 30 µl AE buffer (elution buffer) was applied onto the column and incubated for 10 min at room temperature. Finally, the column was centrifuged at 14,000 rpm at room temperature for 3 min, and the column was discarded leaving the extracted DNA in the eppendorf tube.

C. Checking the concentration and purity of the extracted genomic DNA

The purity and concentration of the extracted DNA were measured by applying 1µl of the extracted DNA on Nanodrop 2000c spectrophotometer connected to a computer (ThermoFisher Scientific inc., USA). The software of the nanodrop showed the purity and concentration of the applied DNA. The purity of DNA was estimated by the ratio between absorbance at 260 nm and 280 nm (OD260/OD280). The absorbance at 260 nm is the wavelength of maximum absorbance of DNA (OD260) while 280 nm is the wavelength of maximum absorbance of protein (OD280). A pure extracted DNA has an OD260/OD280 range from 1.8 and 2. Lower ratios indicate protein contamination and higher ratios indicate salts or RNA. The concentration of DNA was calculated in ng/µl by multiplying OD260 by 50 (an OD260 corresponds to 50 µg/ml of double stranded DNA in a quartz cuvette with 1-cm path length; Sambrook & Russell, 2001).

D. Polymerase chain reaction (PCR)

Extracted DNA samples of all patients underwent polymerase chain reaction (PCR) for amplification of coding regions and their flanking introns of *XPA* and *XPC* genes using sets of previously published primers (Rivera-Begeman *et al.*, 2007; Takahashi *et al.*, 2010; Ben Rekaya *et al.*, 2013). As mentioned before, patients with neurological abnormalities usually belong to XPA rather than XPC group. Consequently, the presence of neurological abnormalities prioritized screening for *XPA* mutations first while the absence of neurological abnormalities prioritized screening for *XPC* mutations first. In all cases, when the XP mutation was identified, parents and available family members were screened for carrying an allele of this specific mutation.

D.1. Principle:

Polymerase Chain Reaction (PCR) is an enzymatically catalyzed amplification reaction that synthesizes million copies of a targeted region of the DNA; hence the amplicon can be visualized on agarose gel. PCR is based upon the fact that any DNA region can be amplified provided that its flanking sequences are known. Components of the PCR are (1) the DNA template, (2) thermostable *Thermus aquaticus* polymerase enzyme, *Taq* polymerase, which adds nucleotides from 5' to 3' end complementing the template DNA, (3) forward and reverse oligonucleotide primers of approximately 20-30 bp length; these are designed specifically to complement either sides of the target DNA region, and four deoxyribonucleotides triphosphates (dNTPs) which are necessary for making copies of target DNA by the *Taq* polymerase. Magnesium ions are also added to function as cofactor for *Taq* polymerase. The reaction mixture enters multiple synthesis cycles that comprise three steps: denaturation, annealing and extension. Denaturation step at 95ºC separates the two strands of the DNA template by breakage of hydrogen bonds. Annealing step requires lesser temperatures for the primers to anneal to their complementary sequences on the separated single stranded DNA templates. Oligonucleotide primers are designed to be short and are added in relatively excess amount to anneal to the single stranded DNA templates before the two template strands anneal back together. The optimum annealing temperature depends on the GC base pairs content in the primer sequence. The extension step at 72ºC is required for *Taq* polymerase to synthesize complementary strands from 5' to 3' direction. Initial denaturation step is usually added before these multiple cycles to ensure all the DNA molecules are denatured. Similarly, a final extension step is usually added after the multiple cycles to ensure maximum product yield. Finally, the temperature is decreased to 4ºC to abolish the polymerase activity and conserve the amplified DNA product (Saiki *et al.*, 1988; Micklos *et al.*, 2003).

D.2. Primers:

For *XPA* gene, six reactions corresponding to its six exons and their flanking intronic regions were amplified for sequencing. For *XPC,* 14 fragments were amplified for sequencing; exon 9 is large, hence it was amplified by two reactions. A total of 20 primer pairs were purchased (HVD life sciences, Germany), see table 2 and 3.

Table 2: Primer pairs of six exons of *XPA* gene and their flanking intronic regions.

XPA exon	Forward primer / Reverse primer	Amplicon size (bp)	Temp. (°C)	Reference
1	5’CTCCGTGTCCGCGCATACCCAGACTC-3’	445	54	Takahashi *et al.*, 2010
	5’GGGAGAATCTGCACACATACGCCAGC-3’			
2	5’GTGATTGTGGACATCCTTGTGTTGTTTG-3’	343	56	
	5’TCACTGATTAAAGTAGTTATGGCATTAT-3’			
3	5’TCAGAAATATTTGTGGAATTGCTACGTT-3’	388	56	
	5’TTTGCCCTAAACCTACACATAAACATTA-3’			
4	5’TGCTAATTTAACCATGAGAGTTGCAGGA-3’	365	56	
	5’TAGTTTGTTATTAAGAATTTACCAGAGT-3’			
5	5’CTATAATATTTCGCAAGTCTGAATCACA-3’	274	56	
	5’ATTGGTGACATTAAACAGGAAGAATCTA-3’			
6	5’AGGCCGAACTAGTGAGGTAAGAAAGTAA-3’	470	55	
	5’TATACAAGGGTTTCATTCATCTAT-3’			

Table 3: Primer pairs of 13 exons of *XPC* gene and their flanking intronic regions.

XPC exon	Forward primer / Reverse primer	Amplicon size (bp)	Temp. ºC	Reference
4	5’ATGCCTCACTTCCTCCTTCC-3’	337	55	Ben Rekaya *et al.*, 2013
	5’CACTTTGATACTCAGTCCTGGTCCC-3’			
5	5’GATTCACTGTCATCCGAGGAGAAG-3’	309	55	
	5’CAAAGGCTCAGAGAGAGTAAGAAACTTG-3’			
6	5’TGAAAGACAAGACCAAAACAAAAACAG-3’	521	55	
	5’TGAAAGACAAGACCAAAACAAAAACAG-3’			
7	5’CTCCCTCTTTTTATTTTCTTGGCTG-3’	727	55	
	5’CTCCCTCTTTTTATTTTCTTGGCTG-3’			
8	5’TTGAACAAGCACCATAACAAACAAC-3’	335	55	
	5’TGCCCAAGTCTTCCCTAACACAG-3’			
9a	5’AATACATACATACAACCCTGAAGGATAGC-3’	641	59	Rivera-Begeman *et al.*, 2007
	5’CCTGGAGGCACTCTTGGACCC-3’			
9b	5’TGATGAGGATTCCGAACCTGG-3’	542	59	
	5’ACTGTGTCTTGGAGCCCCTGG-3’			
10	5’CCTTGGCTCCACCATCTGTTG-3’	496	58	Ben Rekaya *et al.*, 2013
	5’CCCTGTAACTGTTTTTCCCCTGC-3’			
11	5’AGATTAGGGTTTGTAAGTGGACACATC-3’	401	55	
	5’GGACTGGGAGGCTCATCATCAC-3’			
12	5’CTGGTAGGTGTGTTCTGAGGGTTC-3’	431	59	
	5’CGGTGTAGATTGGGCAGGTTC-3’			
13	5’GGCAGCATCAGAAGGGCTCAG-3’	358	60	
	5’AAATCCAGTGTAACATCCTGAAAATTG-3’			
14	5’AGGCTGGATAGGGGCTTTCAC-3’	492	58	
	5’CCTGCTGTATTCAGTGCTCGCTC-3’			
15	5’CCACTAAAGATTTTGGAGTCAGTAACG-3’	547	55	
	5’ACAGGGCTTGGGGCAGAAGAG-3’			
16	5’CCCTTGTCCTCCCAGAGTTACAC-3’	470	59	
	5’ATGCTGCCTCAGTTTGCCTTC-3’			

D.3. Reagents used in PCR:

- 5U/µl *Thermus aquaticus* (*Taq*) DNA Polymerase (ThermoFisher Scientific inc., USA)
- 10X *Taq* buffer with ammonium sulphate (ThermoFisher Scientific inc., USA)
- 2 mM deoxyribonucleotides triphosphates mix., dNTPs (ThermoFisher Scientific inc.)
- 25 mM $MgCl_2$ (ThermoFisher Scientific inc., USA)
- 25 pmol of oligonucleotide primers

D.4.Procedure:

A standard PCR reaction mix contained the following:

Material	**Final concentration**
10X *Taq* buffer	1X
25mM $MgCl_2$	1.5 mM
2 mM dNTP mix	0.2 mM
25 pmol forward primer	2.5 pmol
25 pmol reverse primer	2.5 pmol
Taq DNA polymerase (5U/µl)	0.1 unit
Genomic DNA	100-200 ng
Sterile distilled water	Up to 25 µl reaction

In each amplification reaction, the reaction mixture was made in a total volume of 25 µl containing 0.2 µg genomic DNA, 10X *Taq* buffer, 1.5 mM $MgCl_2$, 2 mM dNTPs mix, 2.5 pmol of each primer and 0.1 unit of *Taq* polymerase. PCR was carried out on Bio-Rad T100™ gradient thermal cycler (Bio-Rad Laboratories, USA). For *XPA*, the following conditions were used: initial denaturation at 95 ºC for 5 min, followed by 35 cycles of denaturation at 95ºC for 30 seconds, annealing at specified primers' temperatures for 30 seconds and elongation at 72 ºC for 30 seconds, followed by final extension of 7 min at 72 ºC. For *XPC*, the following conditions were used: initial denaturation at 95 ºC for 5 min, followed by 40 cycles of denaturation at 95ºC

for 30 seconds, annealing at specified primers' temperatures for 35 seconds and elongation at 72 ºC for 40 seconds, followed by final extension of 10 min at 72 ºC (Ben Rekaya *et al.*, 2009). The reactions were finally held at 4ºC.

E. Agarose gel electrophoresis of the amplified PCR product

The amplified PCR products were visualized on 2% agarose gel to ensure successful amplification reaction and correct amplicon size.

E.1. Principle:

Agarose gel electrophoresis is based upon migration of charged molecules in a porous agarose gel between two differently charged electrodes. The migration depends on the concentration of the gel, and the size and charge of the migrating molecules. Agarose gel is prepared in low concentration (1-2%) to enable the migration of the large sized chromosomal DNA. DNA samples are loaded into wells dug in the gel; samples are mixed with a loading dye to visualize sample loading and increase the sample viscosity. Negatively charged DNA can migrate from the negative to the positive electrode due to the negatively charged phosphate groups at the employed buffer pH. DNA samples of different sizes migrate as bands in the gel where larger sized DNA migrates more slowly than smaller sized DNA. The bands can be visualized on the gel by addition of ethidium bromide dye to the agarose gel preparation. Ethidium bromide dye intercalates into DNA double helix where the dye-DNA complex absorbs 300 nm UV light; reemits light in the visible range (590 nm); hence DNA bands can be visualized on the gel by using UV transilluminator (Sambrook & Russell, 2001; Micklos *et al.*, 2003).

E.2. Reagents used in agarose gel electrophoresis

- Agarose (molecular grade; Q.BIO-gene, France)
- 10X TBE (Tris-borate EDTA buffer) pH 8.3 (Q.BIO-gene, France):

  0.9 M Tris base
  0.89 M Boric acid

  100 mM EDTA (pH 8.0)
- 6X Loading dye solution (Fermentas, Germany):

0.09% Xylene cyanol FF
0.09% Bromophenol blue
60 mM EDTA (pH 8.0)
60% Glycerol

- 10 mg/ml Ethidium bromide (Q.BIO-gene, France)

E.3. Procedure:

The gel was prepared by adding 1.2 g of agarose to 60 ml of 0.5X TBE buffer. The agarose was dissolved by heating in a microwave oven and then cooled to 37 ºC. Ethidium bromide (3 µl) was added to the gel before pouring the gel into the gel chamber in presence of plastic combs to form wells for loading. The gel was allowed to solidify for 20 min at room temperature. An amount of the electrophoresis buffer (0.5X TBE) sufficient to fill the buffer tanks and cover the gel was added and the plastic combs were removed carefully.

One µl of the loading dye was mixed with 3 µl of each of the PCR products. The mixtures were pipetted into the wells of the gel. PCR fragments were related to a PhiX174 DNA/HaeIII molecular weight marker (Finzyme, Finland) which encompasses 11 digested fragments (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72-bp). The gel was electrophoresed for 30 min at 150 volt, then visualized and photographed using Bio-Rad photo-documentation system (Bio-Rad Laboratories, Inc).

F. Direct sequencing of PCR product

To detect the nucleotide sequence of each amplified reaction of *XPA* and *XPC*, Sanger sequencing or dideoxy chain termination was done. At first, PCR products are purified using the QIA purification kit (Qiagen, Germany), then the purified products enter a sequencing reaction by using Big Dye terminator kit (Applied Biosystems, USA) and one direction PCR primer (forward or reverse). The sequencing reaction product is purified before being injected to ABI prism 3130 DNA sequencer (Applied Biosystems, USA).

F.1.Principle:

DNA sequencing is defined as the determination of the precise sequence of nucleotides in a DNA sample. Sanger sequencing, also named dideoxy or chain termination method, is based on the use of specific terminators of DNA chain elongation; 2',3'-dideoxynucleoside-5-triphosphates (ddNTPs). When ddNTPs are incorporated by a DNA polymerase into a growing

DNA chain, the elongation reaction will be terminated because ddNTPs lack 3' hydroxyl group, therefore, they cannot form phosphodiester bond with the next incoming deoxynucleoside-5-triphosphate (dNTP). In Sanger sequencing, four reaction tubes are prepared; each contains dNTPs and one of ddNTPs (A, C, G, and T). The ratio of normal dNTPs to the ddNTP is high enough; therefore, DNA polymerase will succeed in adding several hundred dNTPs before the insertion of the ddNTP and chain termination. At the end, fragments terminated at different lengths of each tube were separated from longest to shortest on a gel to identify the sequence of the DNA sample. With technological advancement, automated DNA sequencing employs fluorescent tags of different colors specific for each ddNTP; thus, all four chain termination reactions can be performed in the same tube and their produced fragments are separated by capillary electrophoresis. A laser beam detects the wavelengths of these tags to generate a chromatogram of the DNA sequence with colored peaks corresponding to each nucleotide location on DNA sequence (Sanger *et al.*, 1977; Russell, 2002).

F.2. Kits used for direct sequencing:

- QIAquick PCR purification kit (Qiagen, Germany)
- BigDye® Terminator kit (Applied Biosystems, USA)
- CENTRI-SEP purification spin columns (ThermoFisher Scientific inc., USA**)**

F.3.Procedure of direct sequencing:

F.3.1. Purification of the PCR products to remove dNTPs and primers:

Purification of the PCR products was performed using QIAquick PCR purification kit (Qiagen, Germany; www.qiagen.com; catalog number: 28104). According to the kit protocol 5 volumes of Buffer PB were added to 1 volume of the PCR product and mixed well. The samples were then applied to QIAquick column and centrifuged for 1 min at 10,000 rpm. The washing step was carried out using 0.75 ml Buffer PE followed by centrifugation at 10,000 rpm for 1 min. PCR products were then eluted in 30 µl elution buffer followed by spinning the column at 10,000 rpm for 1 min.

F.3.2. Cycle sequencing of the purified PCR products:

Cycle sequence PCR was carried out using BigDye® Terminator kit (Applied Biosystems, USA; catalog number: 4336697). The BigDye® Terminator kit uses four different

fluorescent dyes to label ddNTPs, which are added to the primer through a cycle sequencing reaction. Reaction products were run in an automated sequencer to obtain the final sequence. To prepare the reaction mixtures the following components were mixed:

- 8.0 µl of the terminator ready reaction mix
- 10-30 ng of PCR products
- 3.2 pmol of primer of 10 µl /100ml conc. (forward or reverse primer)
- Sterile H_2O was added to adjust a 20 µl final reaction volume

Cycle sequencing PCR was carried out on Perkin Elmer thermal cycler (Applied Biosystems 2720, Singapore) using the following conditions: denaturation at 96 ºC for 1 min, followed by 25 cycles of denaturation at 96 ºC for 10 s, annealing at 60ºC for 5 s, and elongation at 60 ºC for 4 min. Finally, the reaction was held at 4 ºC,

F.3.3. Removal of dye terminators prior to sequencing:

CENTRI-SEP purification spin columns were used to remove dye terminators and other residuals before applying the sample to the sequencer according to manufacturer's protocol (ThermoFisher Scientific inc., USA; catalog number: 401762). First, the column was prepared by gentle tapping to ensure that the dry gel has settled in the bottom of the spin column. The powdered gel of the column was reconstituted by adding 800 µl of reagent grade water. The column cap was placed; the column was shaken briefly and left for 30 minutes at room temperature. Air bubbles were removed from the column gel by inverting the column and sharply tapping the column. Afterwards, the column was centrifuged at 3000 rpm for 2 minutes to remove any extra water. The 20 µl of completed terminator reaction mixture were transferred to the column at the top of the gel. The column was placed into 1.5 ml sample collection tube and was centrifuged at 3000 rpm for 2 minutes. The purified sample was collected in the bottom of the collection tube.

F.3.4. Preparation and loading the samples:

Twenty five µl of Hi-Di formamide (Applied Biosystems; catalog number: 4311320) were added to the tube containing the purified DNA sequencing reaction to suspend samples before injection. The mixture was mixed by vortexing, heated for 2 min at 95 ºC and it was held on ice until being ready to load the samples on the instrument. The samples were injected into

the automated 310 ABI Prism DNA sequencer. The output data was retrieved from the 310 ABI Prism DNA sequencing analysis software (Applied Biosystems, USA).

G. Mutation analysis software

The generated data of DNA sequences were analyzed using FinchTV 1.4.0 software, an application used to read DNA chromatogram files and display the DNA sequence graph (Geospiza, 2009). The obtained sequences were aligned using megablast of nucleotide BLAST (Basic Local Alignment Search Tool) for alignment of the submitted sequence query against highly similar sequences of human genomic reference database (Wheeler *et al.*, 2007, 2013) available from NCBI (National Center for Biotechnology Information; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Nucleotide changes were studied, and their effects on Consensus Coding DNA Sequence (CCDS) sequence of *XPA* (CCDS6729.1) and *XPC* (CCDS46763.1) were reported (Pruitt *et al.*, 2009; Farrell *et al.*, 2013). Three mutation prediction tools were used to predict functional effects of novel mutations MutationTaster, SIFT and mutation assessor. MutationTaster (www.mutationtaster.org) provides functional prediction for different nucleotide changes such as single base substitutions, insertions, deletions and short insertion deletions (indels). A nucleotide change can be classified as either a "disease causing" mutation or a harmless "polymorphism" with a probability score; the classification is based on a large data set of nucleotide changes from Human Genome Mutation database (HGMD) and 1000 Genome project (1000G) (Schwarz *et al.*, 2014). SIFT (Sorting Tolerant From Intolerant; http://sift.jcvi.org) tool predicts functional effects of single amino acid substitutions only based on evolutionary conservation of the amino acid position and its physical property. SIFT predicts either "damaging" or "tolerated" effect on protein function for a given amino acid substitution. SIFT predicts scores from 0 to 1, scores lower than 0.05 are considered "damaging" (Kumar *et al.*, 2009). Mutation assessor (http://mutationassessor.org) tool functions similar to SIFT depending upon evolutionary conservation via multiple sequence alignment to protein families and subfamilies homologs. Functional impact scores classify amino acid substitutions either as variations of "functional; medium or high" impact which have scores more than 1.9 or "non-functional; low or neutral" impact of scores less than 1.9 (Reva *et al.*, 2011).

Chapter (3): Results

The study included 14 unrelated families having 17 Egyptian patients clinically diagnosed with XP. Three of the studied families had two patients each. Molecular diagnosis identified *XPA* mutations in four patients and *XPC* mutations in 13 patients. One family came back for prenatal diagnosis. All available family members were screened for carrier detection.

3.1. Results of clinical investigation

The patients were 4 males and 13 females. Parental consanguinity was confirmed in 12 out of 14 families (85%) as shown in the following pedigree analysis.

3.1.1. Pedigree analysis

At least three generations pedigree analysis was drawn for each XP patient. Patients were numbered from XP1 to XP15. Square shapes refer to males and circle shapes refer to females. XP affected individuals were colored in black. Arrows point to probands while tilted slash refers to deceased individuals. Parental consanguinity (as line doubling) and other abnormalities in related family members were highlighted in the key of each pedigree, see figures 9 to 22.

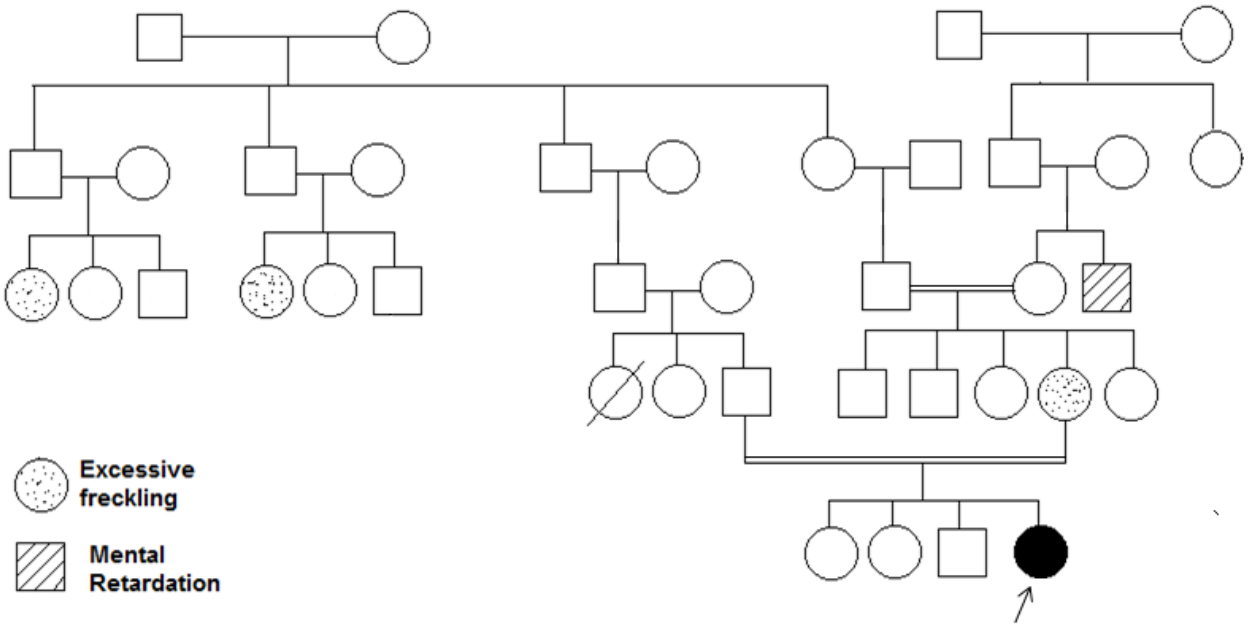


Figure 9: Pedigree analysis chart for XP family 1. The figure shows XP1 patient who was found to have a *XPA* mutation. Parents of XP1 are second cousins. Other abnormalities in related family members are shown.

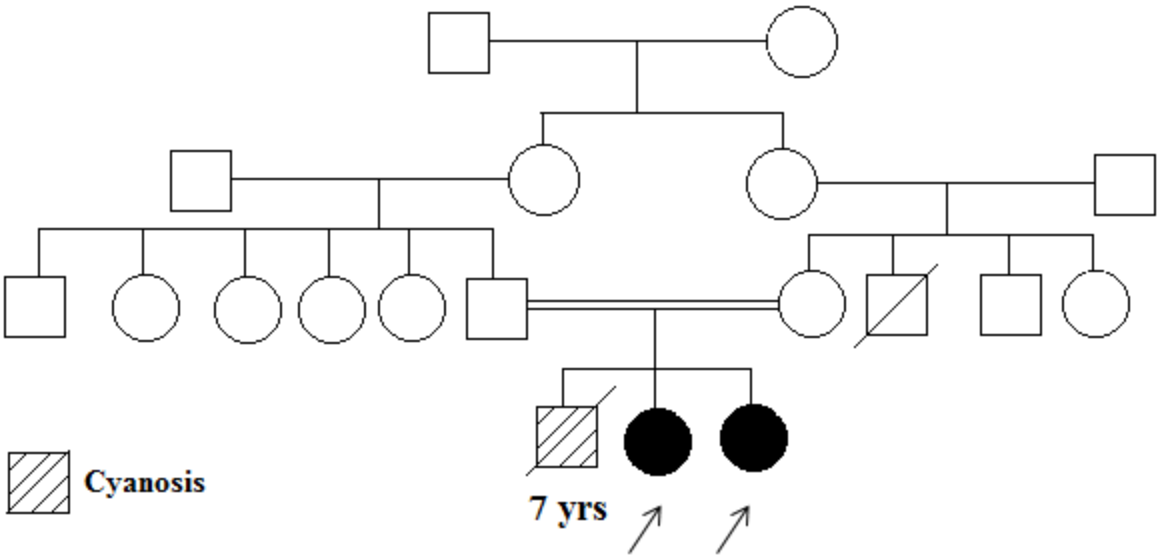


Figure 10: Pedigree analysis chart for XP family 2. The figure shows XP2 and XP3 patients who were found to have a *XPA* mutation. Parental consanguinity is evident in the pedigree; parents of XP2 and XP3 are first cousins.

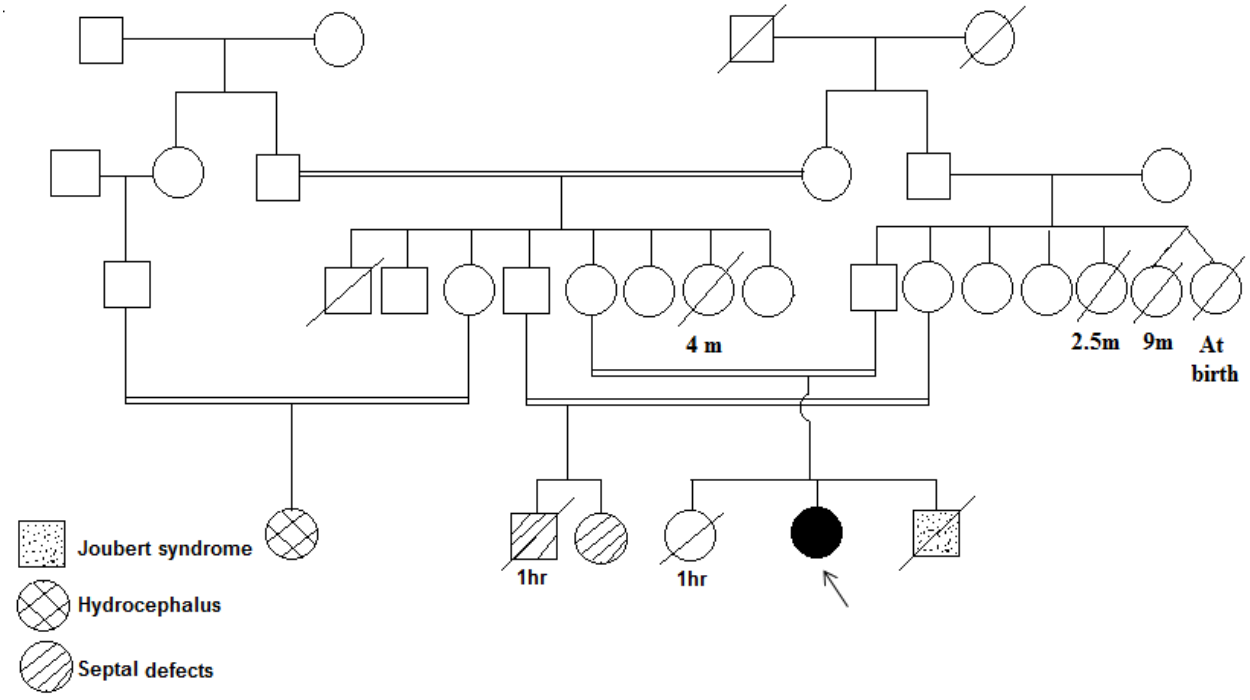


Figure 11: Pedigree analysis chart for XP family 3. The figure shows XP4 patient who was found to have a *XPA* mutation. Parents of XP4 are first cousins. Other abnormalities in related family members are shown.

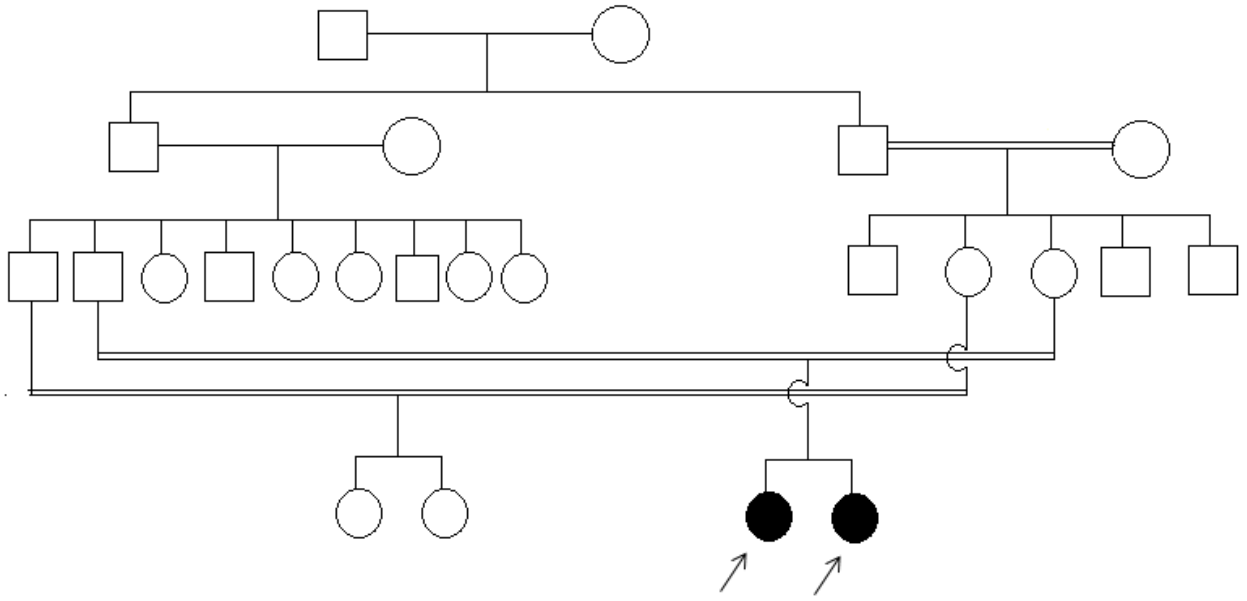


Figure 12: Pedigree analysis chart for XP family 4. The figure shows XP5 and XP6 patients who were found to have a *XPC* mutation. Parental consanguinity is evident in the pedigree; parents of XP5 and XP6 are first cousins.

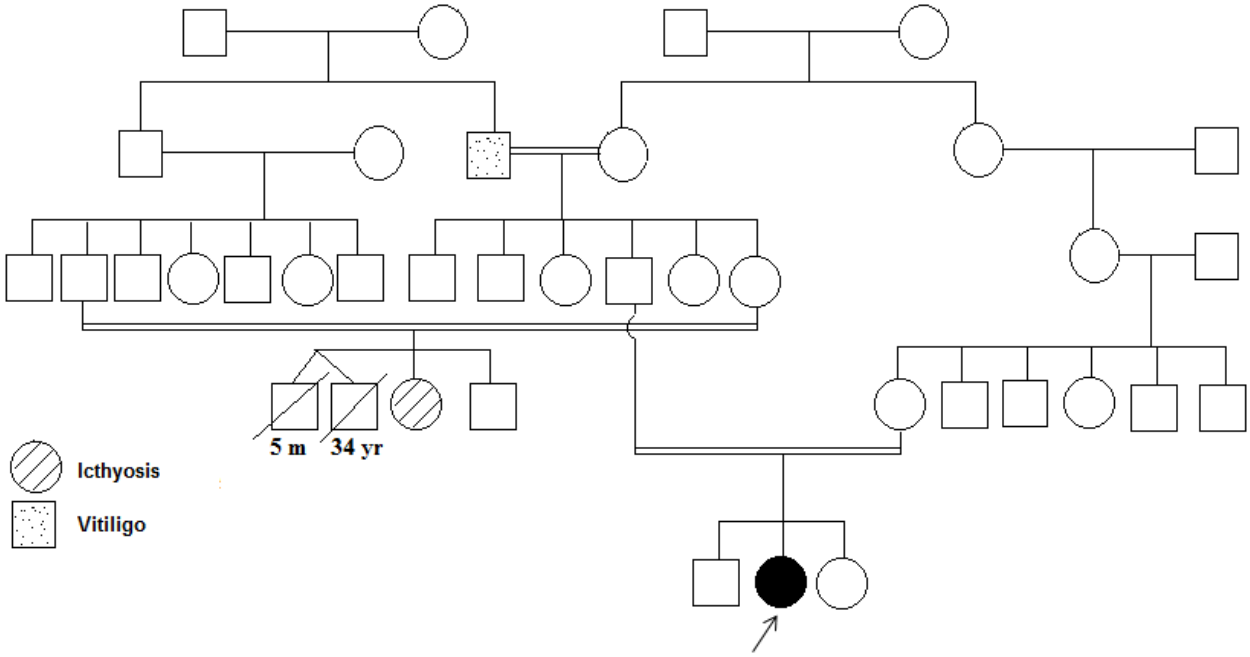


Figure 13: Pedigree analysis chart for XP family 5. The figure shows XP7 patient who was found to have a *XPC* mutation. Parents of XP7 are second cousins. Other abnormalities in related family members are shown.

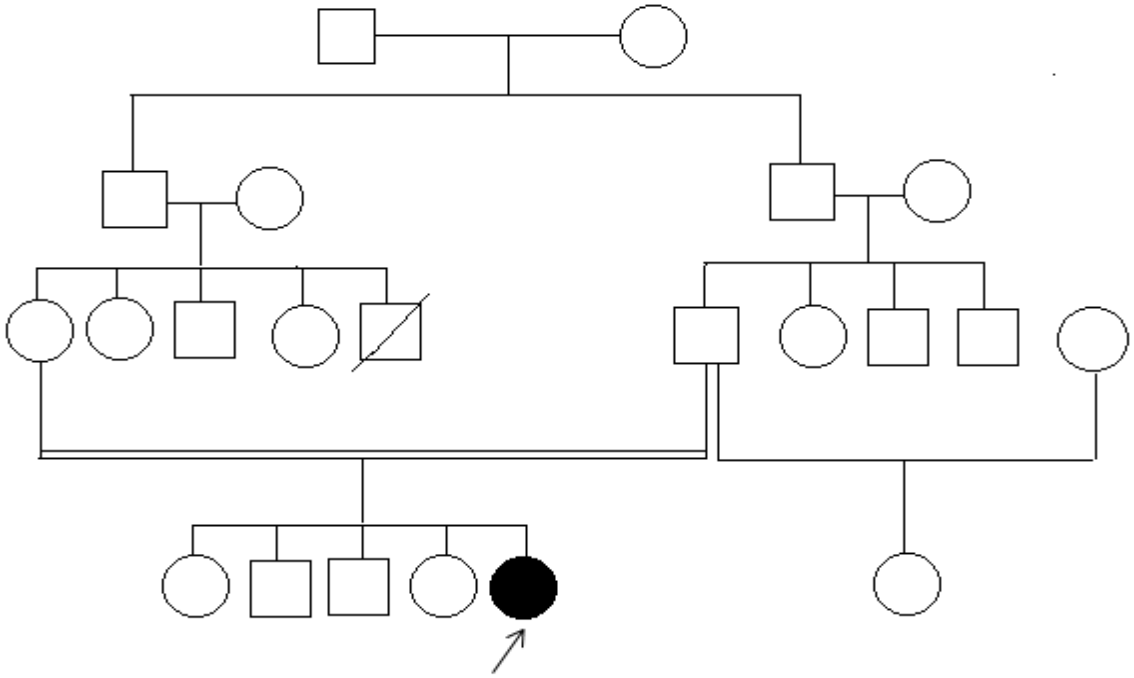


Figure 14: Pedigree analysis chart for XP family 6. The figure shows XP8 patient who was found to have a *XPC* mutation. Parental consanguinity is evident in the pedigree; parents of XP8 are first cousins.

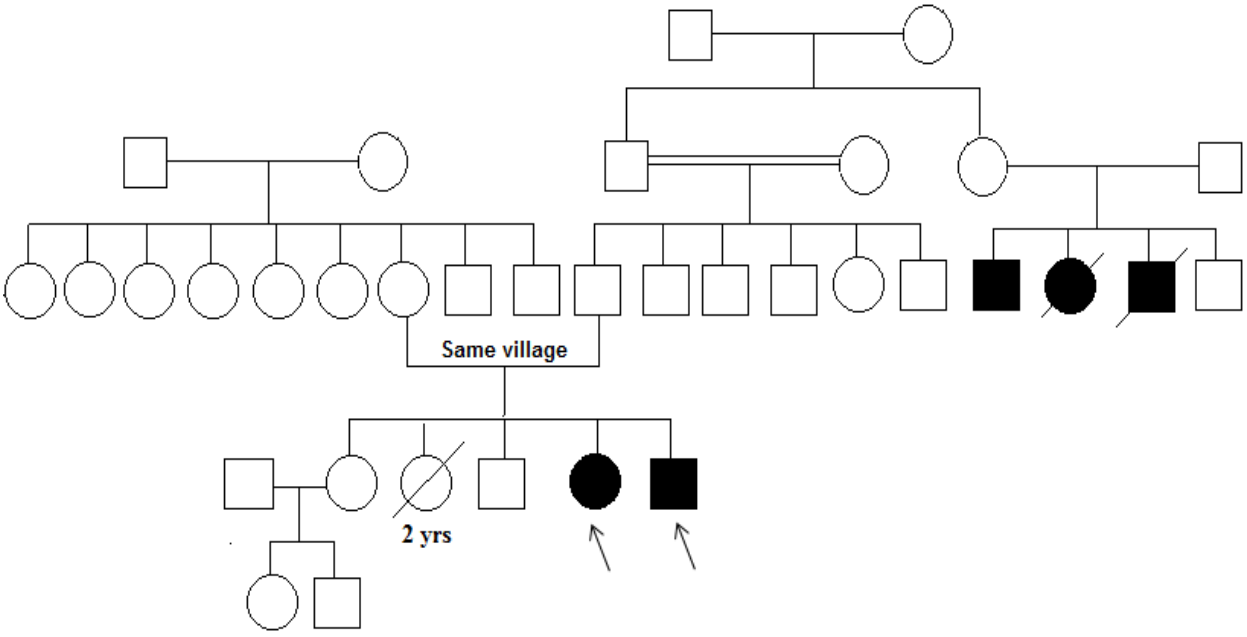


Figure 15: Pedigree analysis chart for XP family 7. The figure shows XP9 and 10 patients who were found to have a novel homozygous *XPC* mutation. Three other family members had history of XP diagnosis (shaded in black).

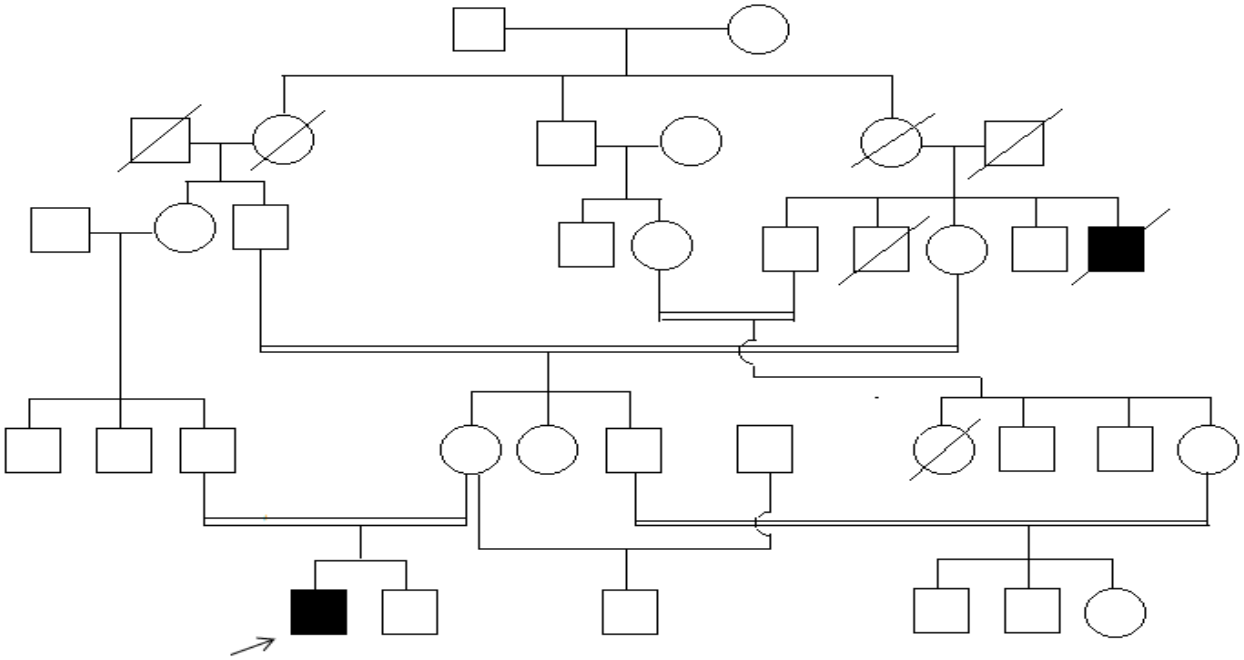


Figure 16: Pedigree analysis chart for XP family 8. The figure shows XP11 patient who was found to have a *XPC* novel mutation. Parental consanguinity is evident in the pedigree; parents of XP11 are first cousins. One family relative had XP.

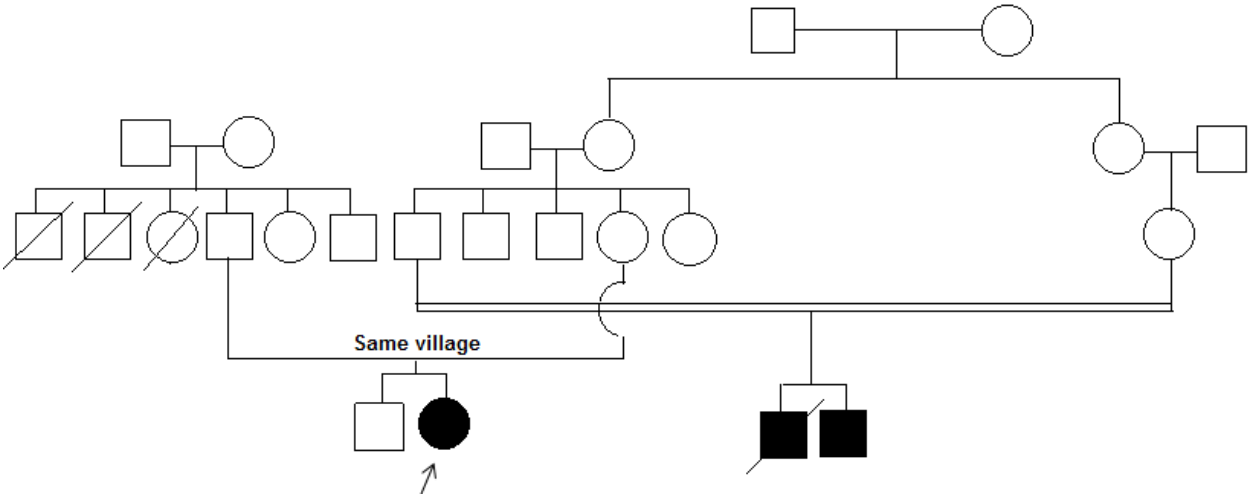


Figure 17: Pedigree analysis chart for XP family 9. The figure shows XP12 patient who was found to have a *XPC* mutation. XP was diagnosed in two first cousins of the patient.

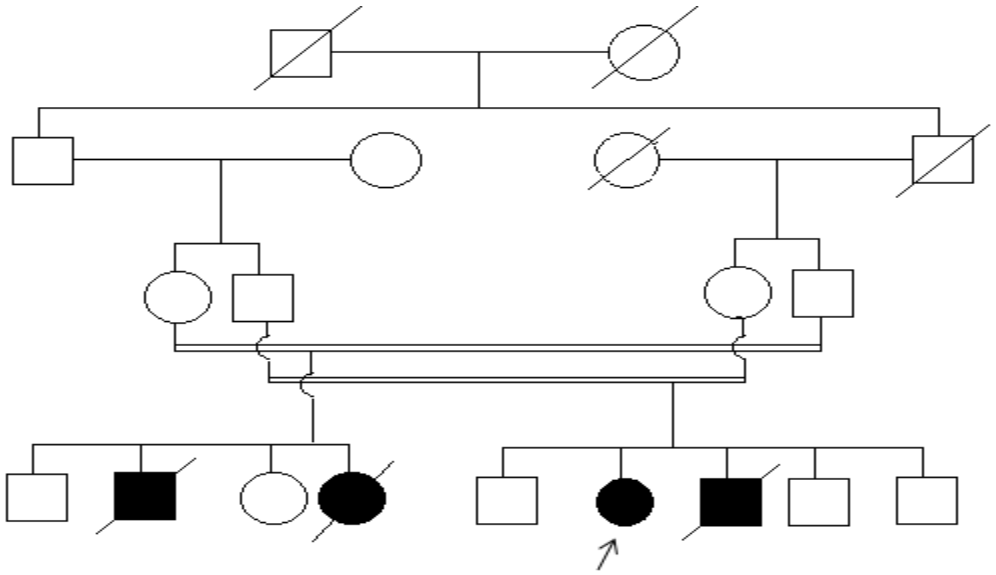


Figure 18: Pedigree analysis chart for XP family 10. The figure shows XP13 patient who was found to have a *XPC* mutation. Parental consanguinity is evident in the figure; parents of XP13 are first cousins. The patient had a sibling and two cousins who were XP patients.

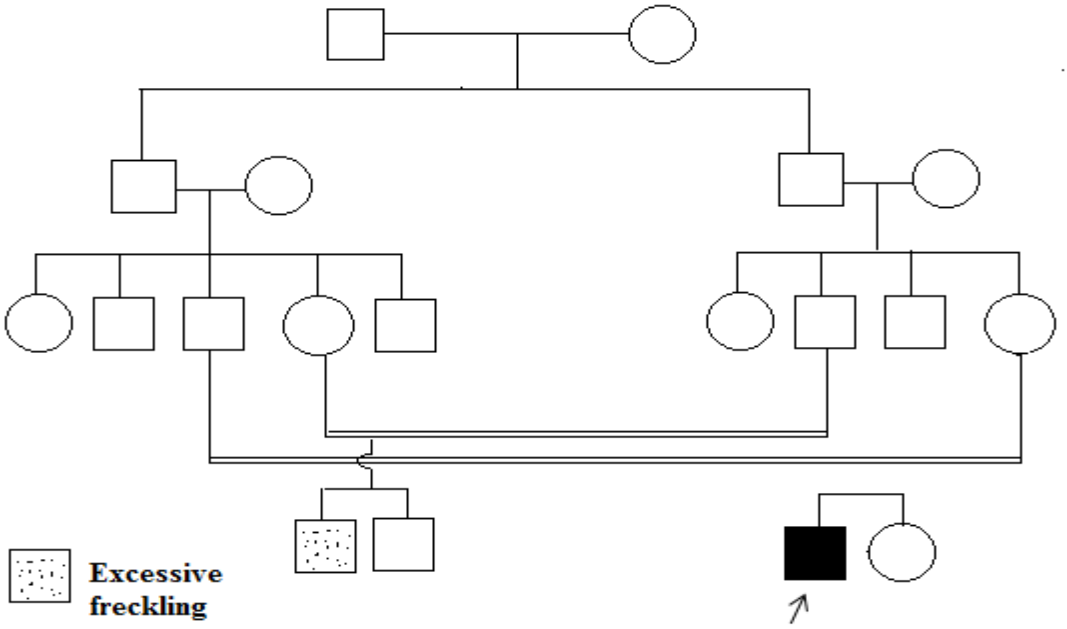


Figure 19: Pedigree analysis chart for XP family 11. The figure shows XP14 patient who was found to have a *XPC* mutation. Parental consanguinity is evident in the figure; parents of XP14 are first cousins. The patient had no other affected relatives; only one cousin had excessive freckling.

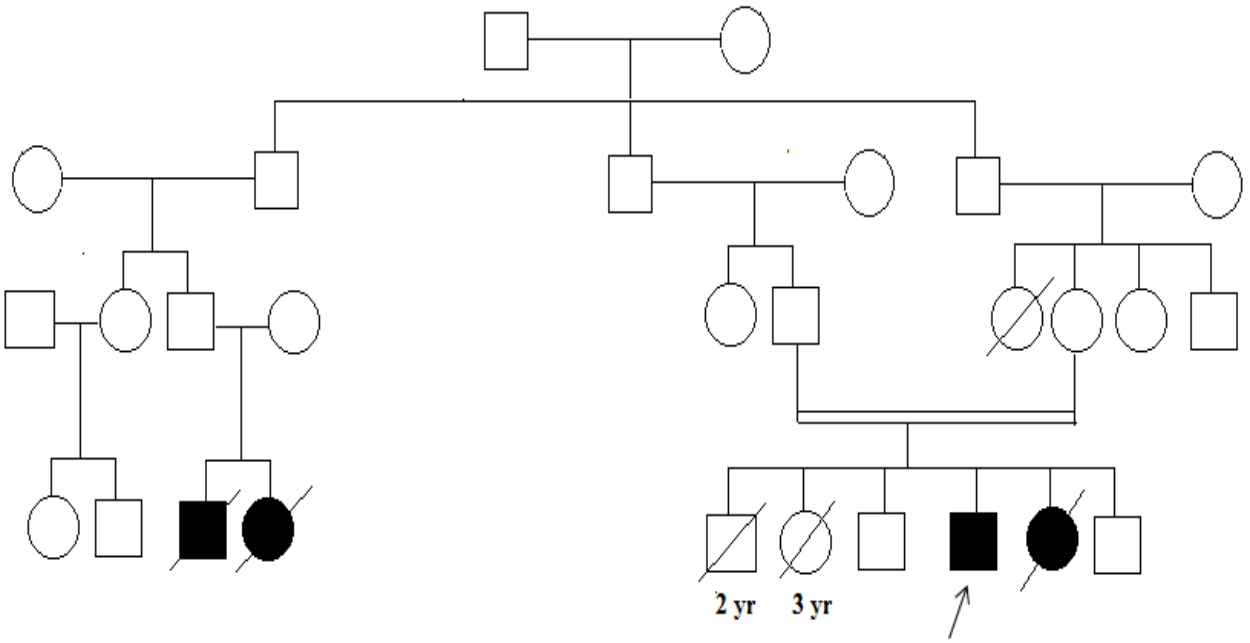


Figure 20: Pedigree analysis chart for XP family 12. The figure shows XP15 patient who was found to have a *XPC* mutation. Parental consanguinity is evident; parents of XP15 are first cousins. The patient had a sibling and two second cousins who died because of XP complications.

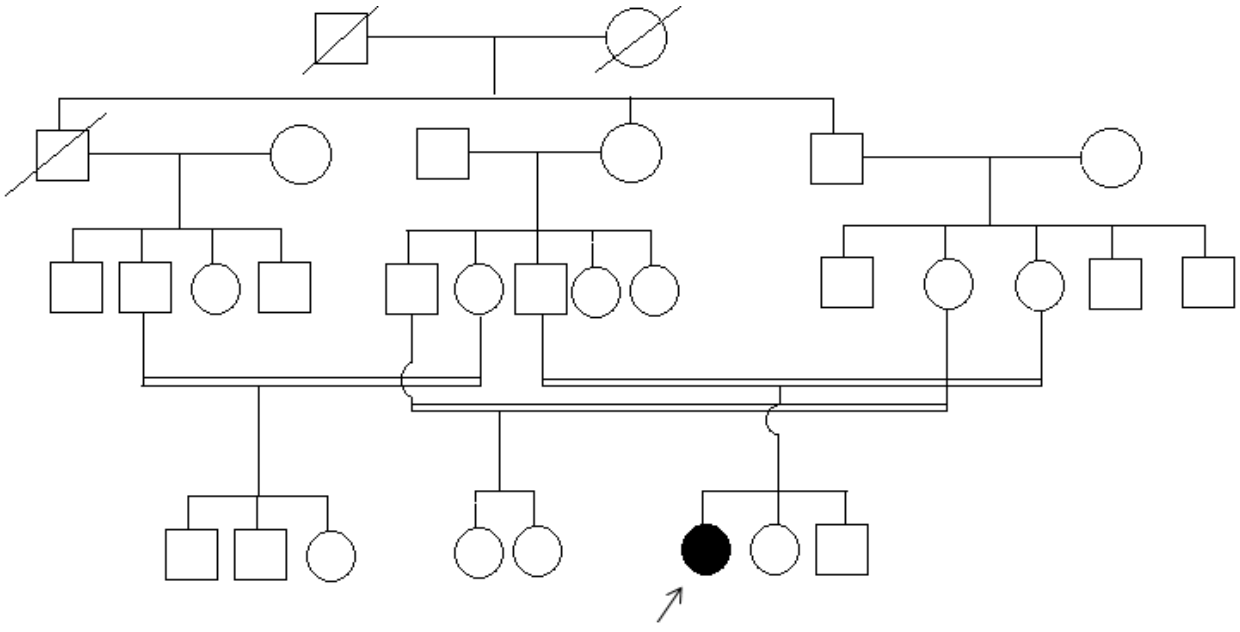


Figure 21: Pedigree analysis chart for XP family 13. The figure shows XP16 patient who was found to have a *XPC* mutation. Parental consanguinity is evident in the pedigree; parents of XP16 are first cousins.

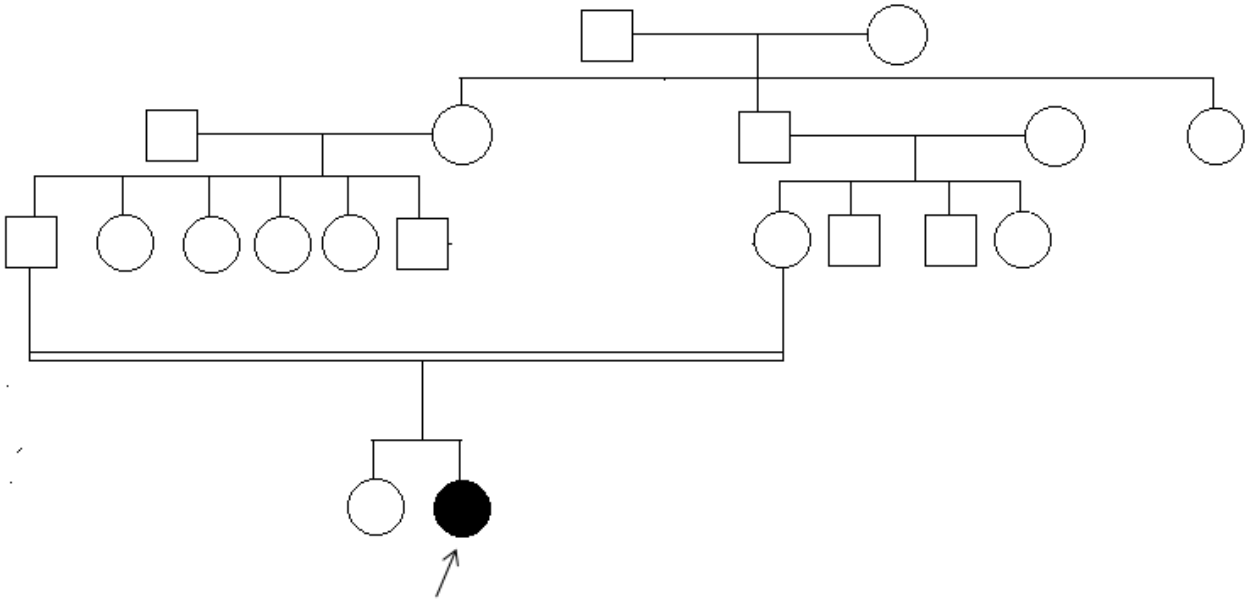


Figure 22: Pedigree analysis chart for XP family 14. The figure shows XP17 patient who was found to have a *XPC* mutation. Parental consanguinity is evident in the figure; parents of XP17 are first cousins.

3.1.2. Clinical description

In the studied families, patients (13 females and 4 males) were equally distributed between Upper and Lower Egypt. The age of the four XP-A patients ranged from 2-7 years (median age = 4 years). For 13 XP-C patients, their age ranged from 4-16 years with exception of two patients having 25 and 32 years (median age = 10.5 years). Age of onset of the first symptom ranged from 4 months to 3 years (median age for XPA = 5 months, for XPC = 8 months). Basic clinical features were summarized in tables 4, 5, 6 and 7. In 16 out of 17 cases, the first symptoms were cutaneous in form of photosensitivity and/or freckle-like pigmentation/lentigines on the face or on sun exposed areas of the skin in general. With exception of XP3 in table 4, all patients had the classic XP skin abnormalities including xerosis, skin atrophy**,** lentigines and poikiloderma (hyper-& hypo-pigmented patches) on face and extremities. Some patients developed telangiectasia or actinic keratosis. Based on clinical history, XP3 was protected since birth from sunlight; therefore she had no obvious XP skin abnormalities. Table 4 shows the four XPA patients exhibiting neurological manifestations unlike XPC patients (table 5, 6 and 7). All of XPA patients showed microcephaly, mental retardation, cerebellar affection and delayed speech. Limb anomalies were observed in XP2. All XPA patients had high photosensitivity; only XP2 developed multiple recurrent cancers. Ocular symptoms, namely, photophobia, conjunctivitis and keratitis, were found in the majority of

patients (16/17). Eyelid abnormalities were also observed (5/17), and to a lesser extent corneal opacities (3/17). Oral examination of some patients showed limited mouth opening, dry mouth, scarring of lips and tongue ulcers. Cutaneous, ocular and oral lesions usually develop to BCC (10/17) and SCC tumors (8/17) of the skin (9/17), the eyes (9/17), and nose (4/17). SCC of the tongue is infrequent (1/17). Only one XPC patient developed melanoma (XP15; table 6).

Table 4: Clinical data of the four XPA patients (XP1 to XP4)

Patient	**XP 1**	**XP2**	**XP3**	**XP4**
Sex	F	F	F	F
Age	2 yr 3m	7yr	4yr 3m	4yr
Consanguinity	+	+		+
Governorate	Kafr el-Sheikh	Dakahlia (Mansoura)		Sohag
Nature of 1st Symptom	Cutaneous: freckle-like pigmentation on face and arms	Cutaneous: severe sunburn	Cutaneous: dry skin Neurologic: delayed development	Cutaneous: dry skin, freckle-like pigmentation on face, legs and arms
Age of onset	6 m	4 m	4 m	6m
Skin symptoms	-Photosensitivity -Xerosis -Lentigines and poikiloderma on face and arms	-Photosensitivity -Lentigines on face and hands -Actinic keratosis	No obvious skin lesions	- Photosensitivity -Xerosis -Lentigines on face, hands and few on forearms - Poikiloderma on face
Ocular symptoms	Conjunctivitis	-Photophobia -Keratitis -Multiple conjunctival projections	-	Conjunctivitis
Neurological manifestations	+	+	+	+

-Mental retardation -Delayed speech -Hearing loss -Others	+ + + (moderate to severe) -MRI: periventricular leucomalacia	+ + - -Axonal neuropathy - Difficulty in movement	+ + - -	+ + - -
Oral symptoms	-	Open bite	-	-Dry lips -Mandibular macrognathia -Bifid tip of tongue -Long philtrum
History of cancers	-	Skin: Multiple recurrent BCC and SCC Ocular: SCC in both eyes	-	-
Others	-Microcephaly -Cerebellar affection	-Microcephaly -Cerebellar affection - Limb anomalies	-Microcephaly - Cerebellar affection	-Microcephaly -Cerebellar affection

Table 5: Clinical data of four XPC patients (XP5 to XP8) having the same genetic mutation

Patient	**XP5**	**XP6**	**XP7**	**XP8**
Sex	F	F	F	F
Age	9yr	5 yr 6m	4yr	25yr
Consanguinity	+		+	+

Governorate	Faiyum		Dakahlia (Mansoura)	Ismailia
Nature of 1st Symptom	Cutaneous: freckle-like pigmentation on nose spread to the whole face	Cutaneous: dry skin, freckle-like pigmentation on face, legs and arms	Cutaneous: freckle-like pigmentation on nose spread to the whole face	Cutaneous: dry skin, hyperpigmented spots on face, arms and legs
Age of onset	6 m	6 m	6 m	3 yr
Skin symptoms	-Photosensitivity -Xerosis -Lentigines and poikiloderma on face and extremities.			
Others	Hypopigmented patches on arms and legs	Minute papule on left cheek	Erythema and hyperpigmented papules on forehead, nose and upper lip	Few Lentigines and poikiloderma on on back and trunk
Ocular symptoms	Photophobia, keratitis and conjunctivitis			
Others	Ectropion of the lower left eye lid	-	Severe blepharitis (eyelid inflammation)	Progressive corneal opacity
Neurological Symptoms	-	-	-	-
Oral symptoms	-	-	-Papules above the lower lip	-Dry mouth -Limited mouth opening -Crowded lower anterior teeth -Moderate periodontitis with areas of gingival recession -Severe lip scarring

				- Short philtrum -Tongue ulcers with reddish areas at tip
History of cancers	Multiple recurrent SCC of skin, both eyes, face, nose and ear	-	-	Multiple recurrent BCC and SCC of skin and face
Others	-Xanthoma -Secondary infections in skin and eye SCC lesions	-	-	-

Table 6: Clinical data of XPC patients (XP9 to XP13) having different genetic mutations

Patient	**XP9**	**XP10**	**XP11**	**XP12**	**XP13**
Sex	F	M	M	F	F
Age	16yr	12yr	12yr 4m	6yr	10yr 5m
Consanguinity	- (Same village)		+	- (Same village)	+
Governorate	Beni Suef		Dakahlia (Mansoura)	Alexandria	Giza
Nature of 1st Symptom	Cutaneous and ocular: photophobia and sunburn	Cutaneous and ocular	Cutaneous: few freckle-like pigmentation on the face	Cutaneous: few freckle-like pigmentation on the face	Cutaneous: freckle-like pigmentation on face and hands
Age of onset	2 yrs	8 m	9 m	8 m	8 m
Skin symptoms	-Photosensitivity -Xerosis				

Others	-Lentigines and poikiloderma on face and hands (all over the body in XP9 and XP10)				
	Telangiectasia	-	Atrophic skin Actinic keratosis	-	-
Ocular Symptoms	Photophobia Conjunctivitis Keratitis Right eye is excised	Severe ocular symptoms; both eyes were excised	Photophobia Conjunctivitis Keratitis Corneal opacity	Photophobia Conjunctivitis	Photophobia Conjunctivitis
Neurological Symptoms	-	-	-	-	-
Oral symptoms	-Dry mouth -Limited mouth opening	Severe symptoms progressed to tumor in lips and mandible	-	-	-
History of cancers	-BCC of conjunctiva and tongue -SCC of upper and lower eyelids, eye globe and the right eye orbit - Skin tumor at the side of the right philtrum	-Recurrent BCC and SCC on face, eyes, neck and maxilla -Both eyes, nose and mandible were excised	-BCC on anterior of the chest - Recurrent BCC of the skin.	Small submandibular tumor	Multiple recurrent BCC and SCC of skin and eyes

Table 7: Clinical data of XPC patients (XP14 to XP17) having the same genetic mutation

Patient	XP14	XP15	XP16	XP17
Sex	M	M	F	F
Age	8yr	32yr	10yr 6m	10yr 6m
Consanguinity	+	+	+	+

Governorate	Minya	Giza	Gharbiyah (Tanta)	Giza
Nature of 1st Symptom	Cutaneous: freckle-like pigmentation that spread to the whole face	Cutaneous: freckle-like pigmentation on face, legs and arms	Cutaneous: freckle-like pigmentation on face, legs and arms	Cutaneous: freckle-like pigmentation on face, legs and arms
Age of onset	6m	2 yrs	8 m	8 m
Skin symptoms	-Photosensitivity -Lentigines and poikiloderma on face and extremities			
Ocular symptoms Others	-Photophobia -Keratitis -Conjunctivitis			
	Corneal opacity	-	-	-
Neurological Symptoms	-	-	-	-
Oral symptoms	-	-Limited mouth opening -Crusted lips -Ulcer on anterior of tongue -Very deep overbite -Loss of upper anterior vestibule	-Dry mouth and lips -Tongue has ulcers at tip	-
History of cancers	BCC on nose, scalp and in the eyes	Multiple BCC, SCC of the skin, nose and lower left eyelid and melanoma of the skin	BCC on nose, scalp and in the eyes	Multiple recurrent BCC and SCC of skin and eyes

3.2. Results of molecular investigation

3.2.1. PCR amplification of *XPA* and *XPC*

Polymerase chain reaction (PCR) was carried out for the six exons and their flanking introns of *XPA* gene, and the 13 exons and their flanking introns of *XPC* gene using specific primers which resulted in amplification of these fragments with specific amplicon sizes, see sizes in table 2 and 3. Using 2% agarose gel electrophoresis, the amplified PCR products were run to visualize successful amplification reaction and correct amplicon size, see figures 23-27. Successfully amplified PCR products were directly sequenced to detect mutations in *XPA* and *XPC* genes.

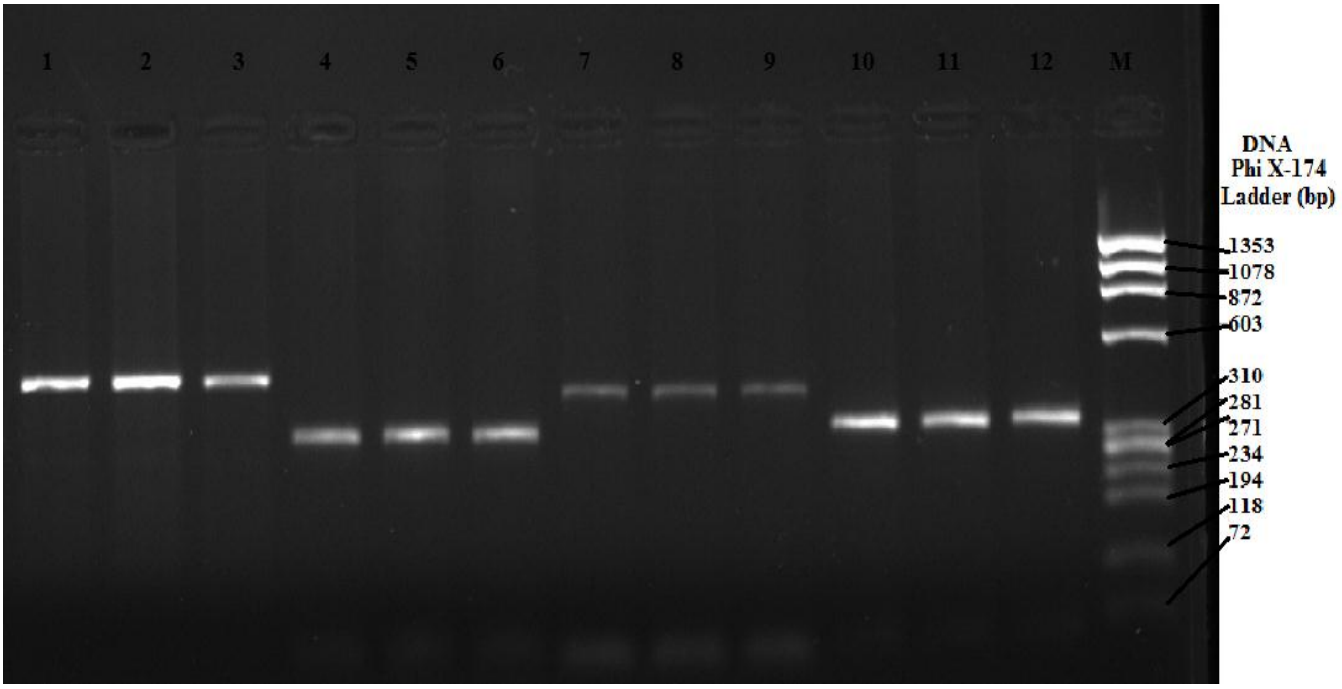


Figure 23: Gel electrophoresis of PCR amplicons of *XPA* exon 1 to exon 4. The figure shows PCR products of XP1, XP2 and XP4 of *XPA* exon 1 (lanes 1, 2 and 3) of size 445 bp, exon 2 (lane 4, 5 and 6) of size 343 bp, exon 3 (lanes 7, 8 and 9) of size 388 bp and exon 4 (lanes 10, 11 and 12) of size 365 bp. The "M" lane refers to PhiX174 DNA/HaeIII molecular weight marker (Finzyme, Finland) which encompasses 11 digested fragments; their sizes are illustrated on the right.

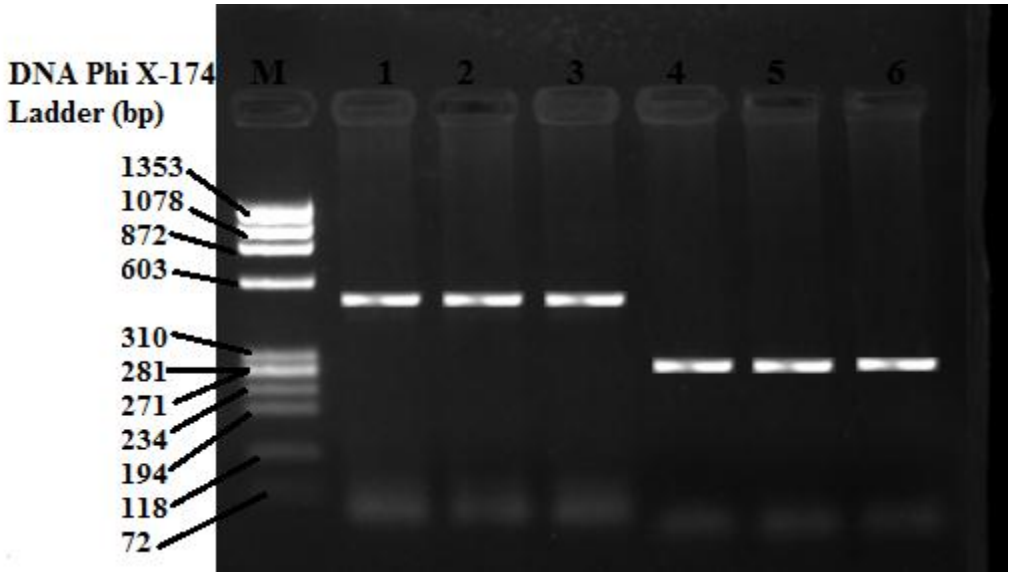


Figure 24: Gel electrophoresis of PCR amplicons of *XPA* exon 5 and 6. The figure shows from the left PhiX174 DNA/HaeIII molecular weight marker in "M" lane which encompasses 11 digested fragments; their sizes are illustrated on the left. Lane 1, 2 and 3 shows PCR products of exon 6 fragments of XP1, XP2 and XP4 of size 470 bp while lane 4, 5 and 6 shows PCR products of exon 5 fragments of size 274 bp.

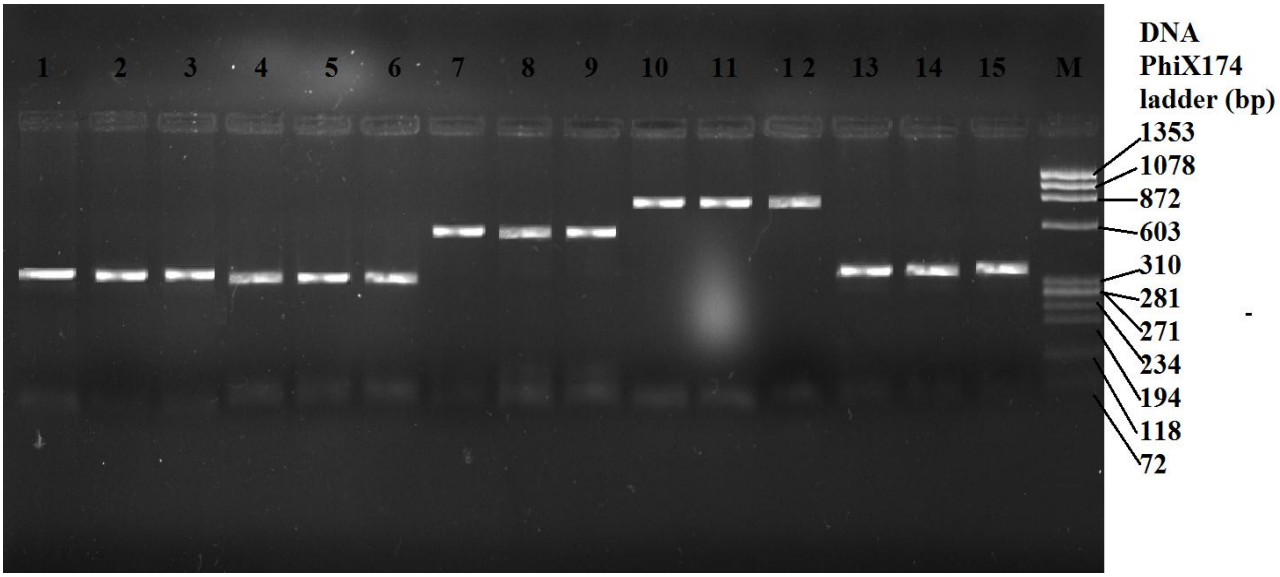


Figure 25: Gel electrophoresis of PCR amplicons of *XPC* exon 4 to exon 8. The figure shows PCR products of three XPC patients' samples of *XPC* exon 4 (lane 1, 2 and 3) of size 337 bp, exon 5 (lane 4, 5 and 6) of size 309 bp, exon 6 (lane 7, 8 and 9) of size 521 bp, exon 7 (lane 10, 11 and 12) of size 727 bp, and exon 8 (lane 13, 14 and 15) of size 335 bp. "M" lane refers to PhiX174 DNA/HaeIII molecular weight marker which has 11 digested fragments; there sizes are illustrated on the right.

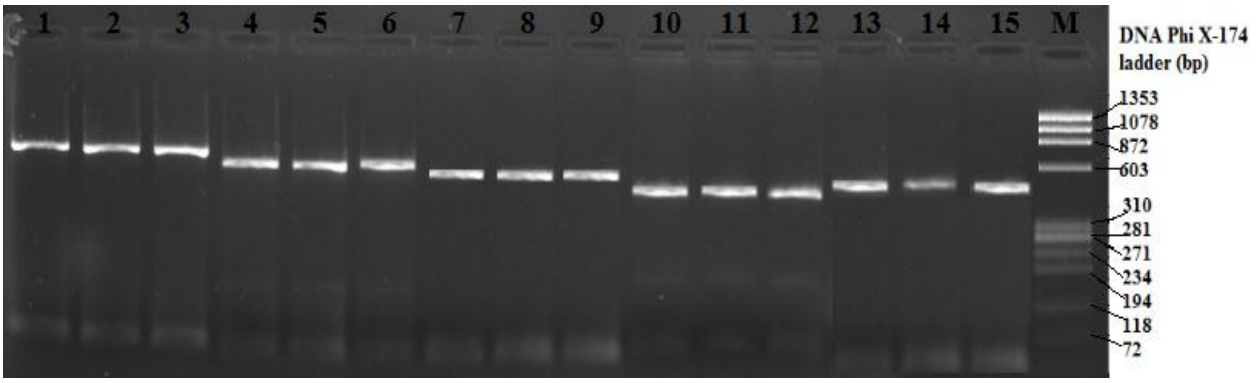


Figure 26: Gel electrophoresis of PCR amplicons of *XPC* exon 9 to exon 12. The figure shows PCR products of three XPC patients' samples of *XPC* exon 9a (lane 1, 2 and 3) of size 641 bp, exon 9b (lane 4, 5 and 6) of size 542 bp, exon 10 (lane 7, 8 and 9) of size 496 bp, exon 11 (lane 10, 11 and 12) of size 401 bp, and exon 12 (lane 13, 14 and 15) of size 431 bp. The "M" lane refers to PhiX174 DNA/HaeIII molecular weight marker which has 11 digested fragments; their sizes are illustrated on the right.

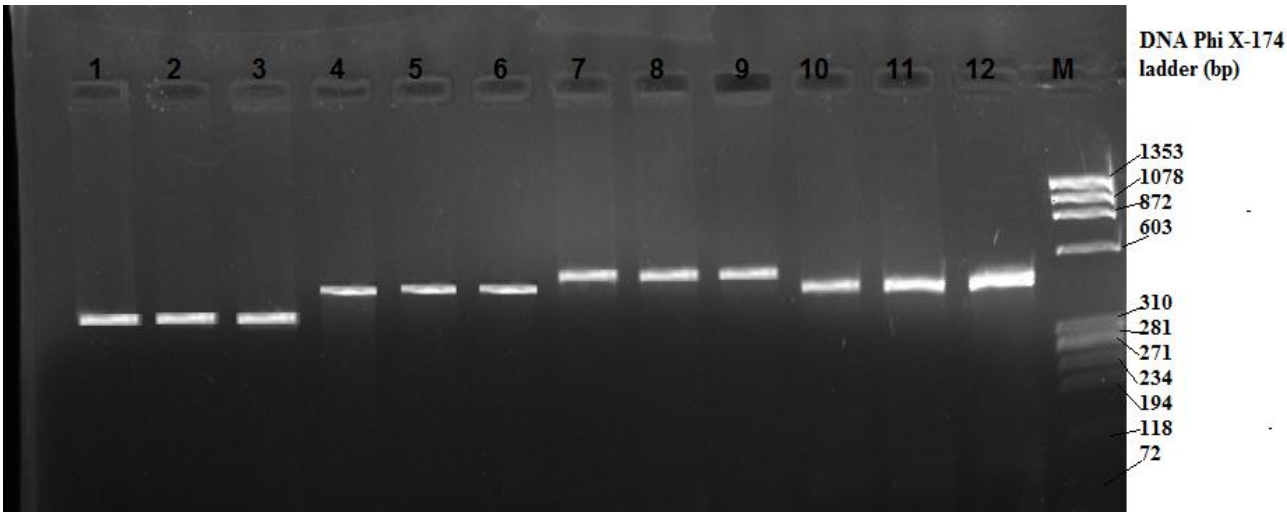


Figure 27: Gel electrophoresis of PCR amplicons of *XPC* exon 13 to exon 16. The figure shows PCR products of three XPC patients' samples of *XPC* exon 13 (lane 1, 2 and 3) of size 358 bp, exon 14 (lane 4, 5 and 6) of size 492 bp, exon 15 (lane 7, 8 and 9) of size 547 bp, and exon 16 (lane 10, 11 and 12) of size 470 bp. "M" lane refers to PhiX174 DNA/HaeIII molecular weight marker which has 11 digested fragments; their sizes are illustrated on the right.

3.2.2. Sequencing results

When successfully amplified PCR products were sequenced, the generated sequencing data were obtained, visualized by FinchTV 1.4.0 software, and aligned against human genomic reference database using nucleotide BLAST (Basic Local Alignment Search Tool). Mutation analysis revealed the presence of ten different mutations in all of the 17 studied XP patients; these mutations are compiled in table 8. Nucleotide sequence changes are expressed in their complementary DNA (cDNA) positions and their consequent protein changes are described based on CCDS sequence of *XPA* (CCDS6729.1) and *XPC* (CCDS46763.1). Nomenclature of the mutations is based on Human Genome Variation Society (HGVS) recommendations (Den Dunnen & Antonarakis, 2000). For confirmation of the detected, all parents were screened for carrying the same mutation of their affected offspring which validates autosomal recessive inheritance. In addition to 34 heterozygous parents, 10 family members were screened for carrying their correspondent proband mutation; seven of them were found to be heterozygous carriers. Fetal genomic DNA from family 2 showed an affected fetus having the same homozygous mutation as XP2 and XP3.

Table 8: Compilation of the XP molecular results

Patient	Gene	Exon (E) or Intron (IVS)	Mutation		Type of mutation	Genotype	Reference	Number of carriers among screened family members
			Nucleotide Change	Protein or mRNA Changes				
XP1	*XPA*	E4	c.553C>T	p.Gln185X	Nonsense	Homozygous	Amr *et al*, 2014	4/5
XP2 & XP3	*XPA*	E3	c.374delC	p.Thr125IlefsX15	Small deletion	Homozygous	Satokata *et al*, 1992b	2/2 + An affected fetus
XP4	*XPA*	E5	c.619C>T	p.Arg207X	Nonsense	Homozygous	Satokata *et al*, 1992a	2/4
XP5 & XP6	*XPC*	IVS12	c.2251-1G>C	Three abnormally spliced mRNA: exon 13 skipping, intron 12 retention, and 44 bp deletion in exon 13	Splicing	Homozygous	Cartault *et al*, 2011	2/2
XP7								2/2
XP8								2/2
XP9 & XP10	*XPC*	E6	c.668_669delTC	p.Ile223MetfsX45	Small deletion	Homozygous	**Current study**	3/3
XP11	*XPC*	E10	c.1894C>T	p.Gln632X	Nonsense	Homozygous	**Current study**	3/3
XP12	*XPC*	E4	c.526_527delAGinsCA	p.Arg176Gln	Small indel	Compound heterozygous	**Current study**	2/2
		E9	c.1103_1104delAA	p.Gln368ArgfsX6	Small deletion		Chavanne *et al*, 2000	
XP13	*XPC*	E9	c.1735C>T	p.Arg579X	Nonsense	Homozygous	Chavanne *et al*, 2000	4/4
XP14	*XPC*	E9	c.1643_1644delTG	p.Val548AlafsX25	Small deletion	Homozygous	Li *et al*, 1993	3/3
XP15								2/2
XP16								2/2
XP17								2/2

A. *XPA* gene mutations

All XPA (XP1 to XP4) patients have neurological abnormalities and were screened for *XPA* gene mutations first. Three previously identified *XPA* gene mutations were detected in a homozygous state in the four patients descending from three unrelated pedigrees. Two of the *XPA* gene mutations are C to T substitutions (c.553C>T and c.619C>T) at different exon positions which create premature stop codons i.e. nonsense mutations, and produce truncated proteins. The c.553C>T mutation changes the CAG codon number 185 of glutamine to TAG i.e. a premature stop codon (p.Gln185X; see figures 28 to 30). The c.619C>T mutation changes the CGA codon number 207 of arginine to TGA i.e. a premature stop codon (p.Arg207X; see figures 34 to 36). The third *XPA* gene mutation was detected in two siblings and fetal DNA from the same family. This third *XPA* mutation is a small one base pair homozygous deletion of a cytosine base at position number 374 (c.374delC, see figures 31 to 33). The deletion of C changes ACT codon number 125 of threonine into ATT codon of isoleucine, results in a frameshift mutation and creates of a premature stop codon 14 codons downstream (p.Thr125IlefsX15), hence a truncated XPA protein is produced.

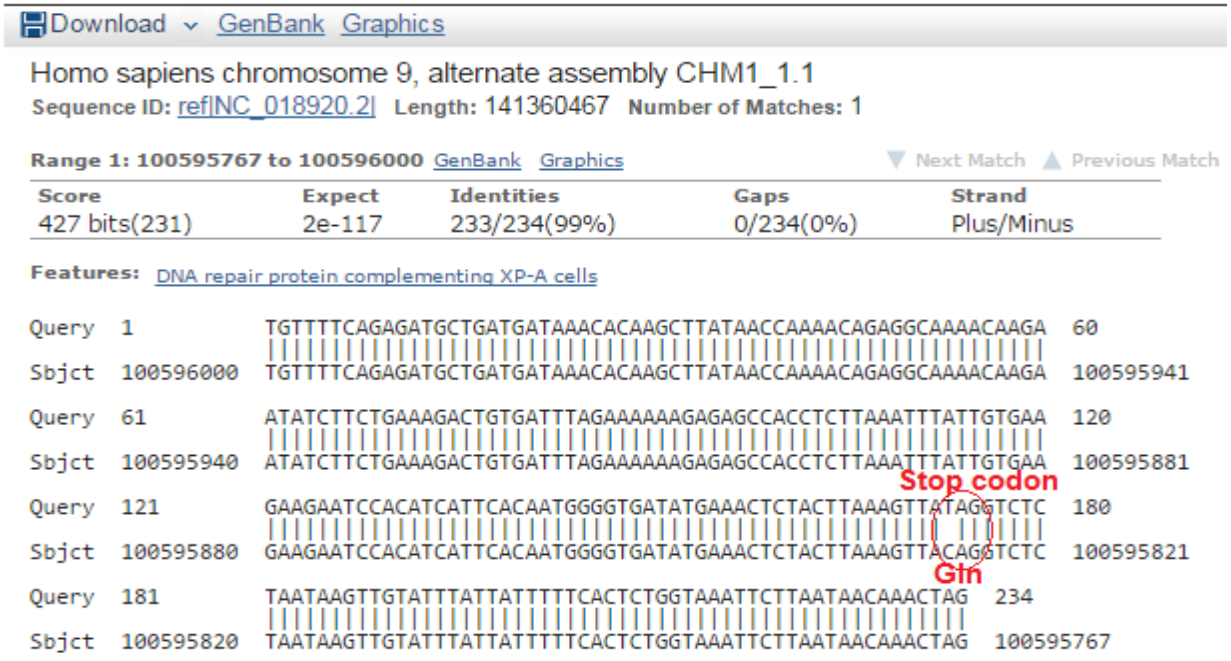
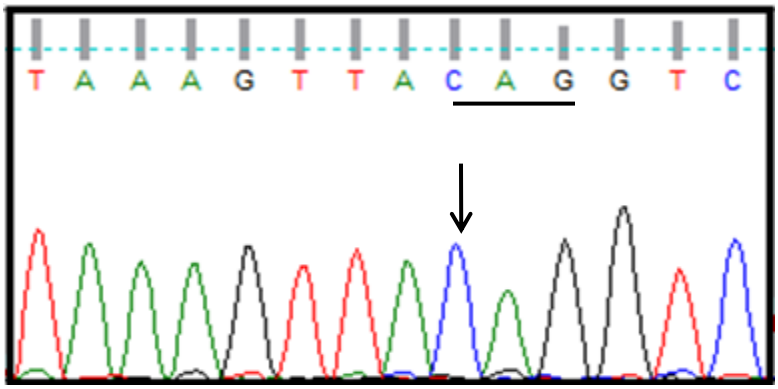


Figure 28: BLAST preview of query sequence of *XPA* exon 4 of XP1 patient. The figure shows alignment of exon 4 of XP1 against a reference sequence from human genome database of NCBI. In the red circle, C to T substitution is shown where the CAG codon of glutamine is changed into a premature stop codon (TAG).

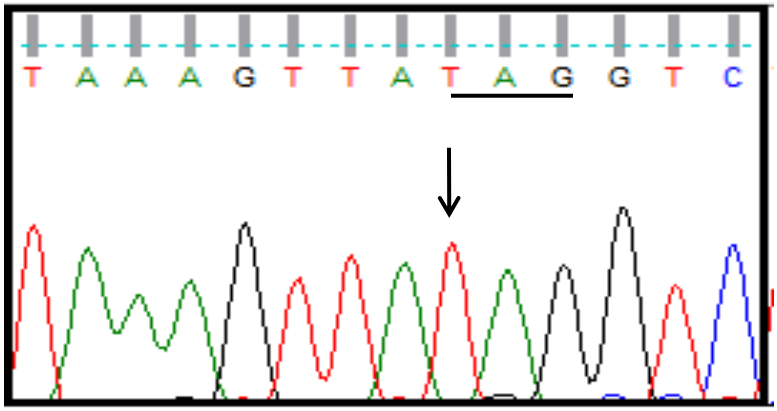
```
Exon 4 -
  aaaataactaatttatataatggacttaatctgttttcagA GAT GCT GAT GAT AAA 135

136 CAC AAG CTT ATA ACC AAA ACA GAG GCA AAA CAA GAA TAT CTT CTG 150

151 AAA GAC TGT GAT TTA GAA AAA AGA GAG CCA CCT CTT AAA TTT ATT 165

166 GTG AAG AAG AAT CCA CAT CAT TCA CAA TGG GGT GAT ATG AAA CTC 180

                    185
181 TAC TTA AAG TTA CAGgtctctaataagttgtatttattatttttcactctggtaa
                    Gln
                    TAG
                    Stop codon
```

Figure 29: cDNA sequence of *XPA* exon 4 with c.553C>T (p.Gln185X) mutation of XP1. The figure shows exon 4 of *XPA* gene where 185th CAG codon of glutamine (highlighted in yellow) changes into TAG; a premature stop codon (p.Gln185X) due to c.553C>T mutation.

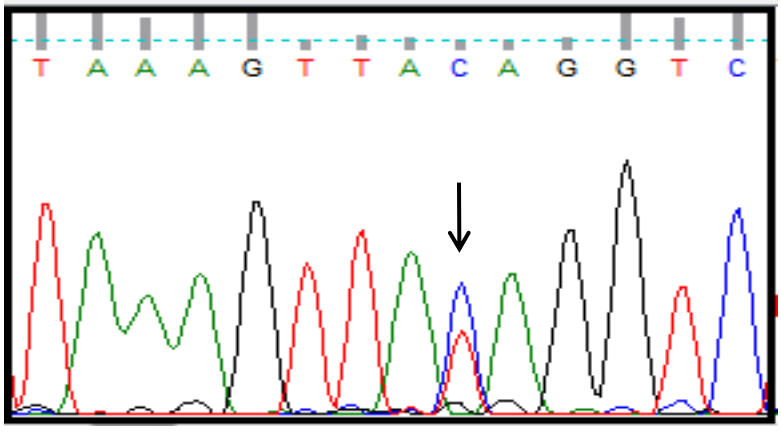
Codons are shown in capital letters, numbers refer to codon number in cDNA sequence, and small letters refer to flanking intronic sequences.



(A) Wild type sequence



(B) Mutant sequence



(C) Heterozygous carrier sequence

Figure 30: Sequencing analysis of exon 4 of *XPA* gene in XP1 patient with c.553C>T (p.Gln185X) mutation. The figure shows a portion of the sequencing chromatogram of (A) wild

type sequence, (B) mutant sequence detected in XP1 which has a homozygous C to T substitution that changes the CAG 185th codon of glutamine to a premature stop codon (TAG), and (C) heterozygous carrier sequence detected in both parents and two out of three siblings.

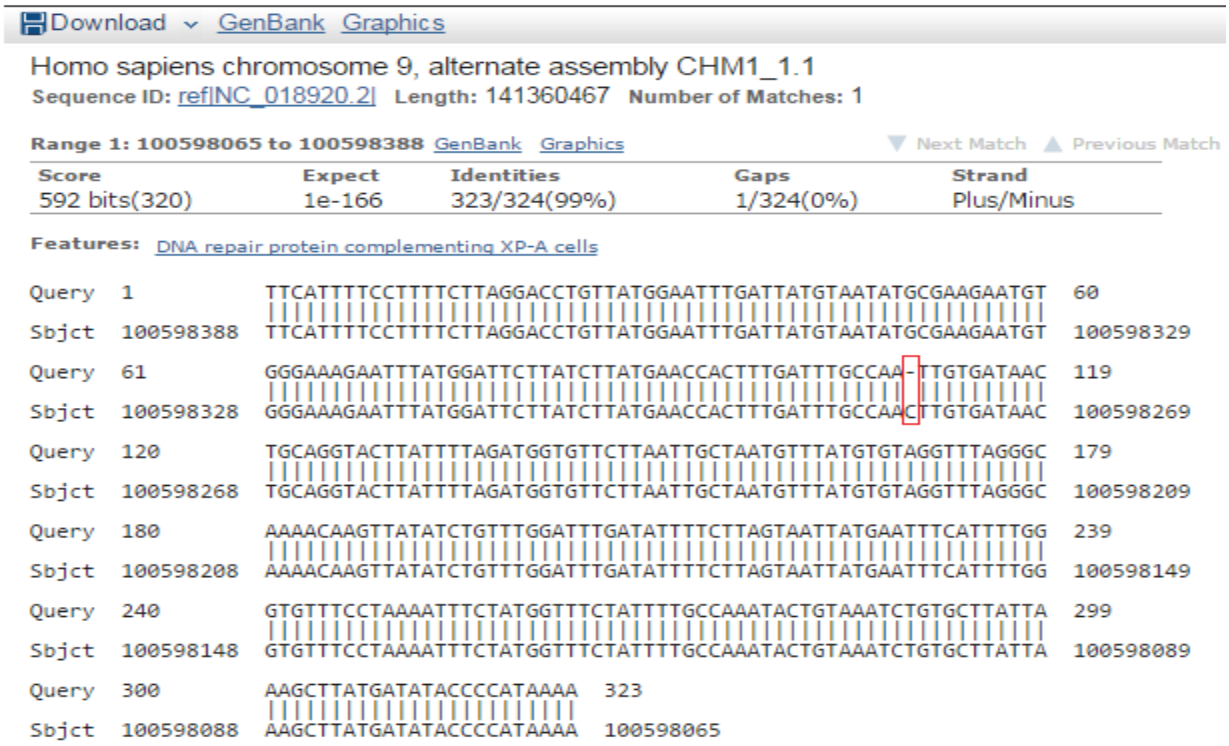


Figure 31: BLAST preview of query sequence of *XPA* exon 3 of XP2 patient. The figure shows alignment of exon 3 of XP2 against a reference sequence from human genome database of NCBI. In the red box, the single C base deletion is shown.

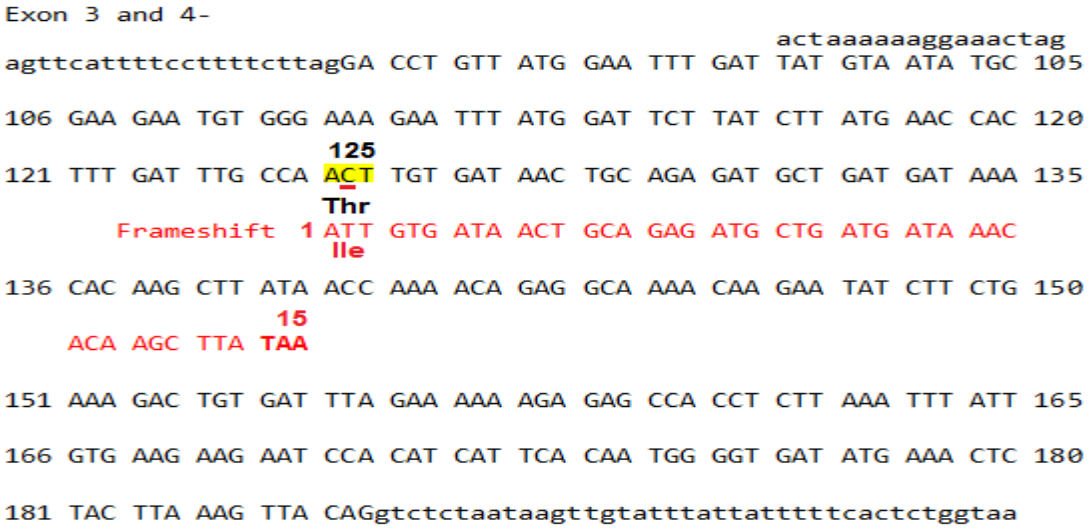
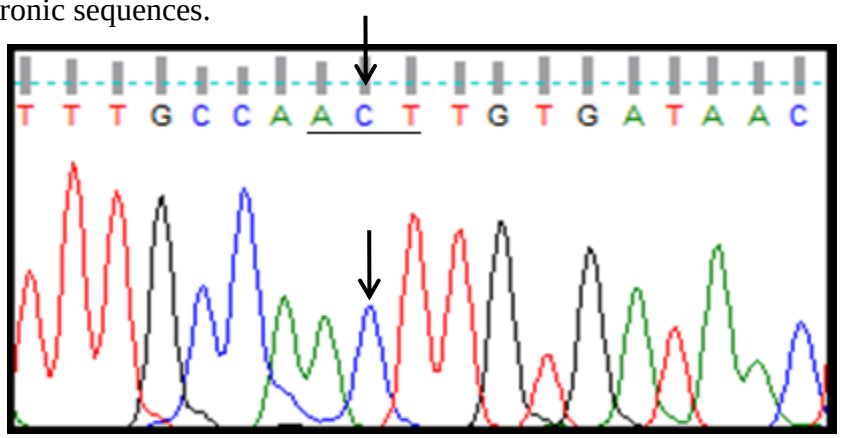
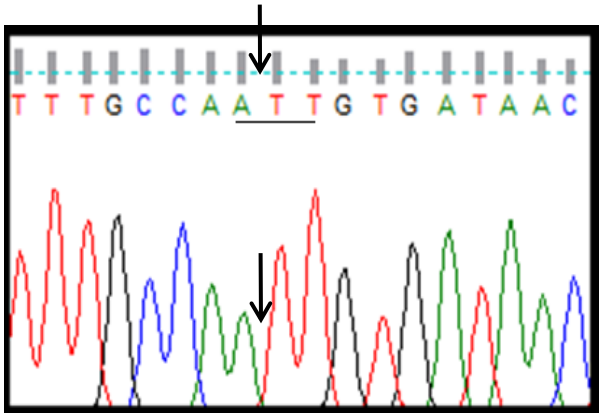


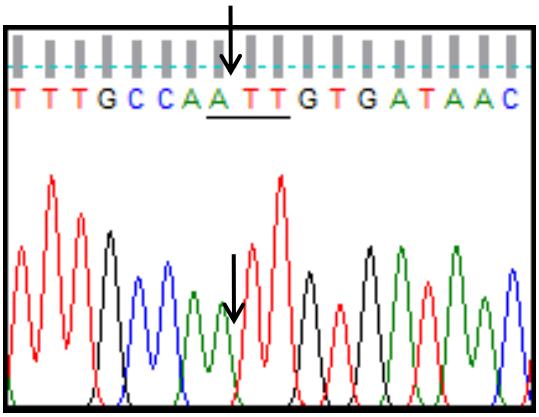
Figure 32: cDNA sequence of *XPA* exon 3 and 4 with c.374delC (p.Thr125IlefsX15) mutation of XP2, XP3 and fetal DNA of family 2. The figure shows exon 3 and 4 of *XPA* gene where 125th codon of threonine (highlighted in yellow) changes into ATT codon of isoleucine due to c.374delC deletion (underlined red). The resultant frameshift mutation

(p.Thr125IlefsX15) forms a premature stop codon (TAA) at position 15 of frameshift. Codons are shown in capital letters, numbers refer to codon number in cDNA sequence, and small letters refer to flanking intronic sequences.



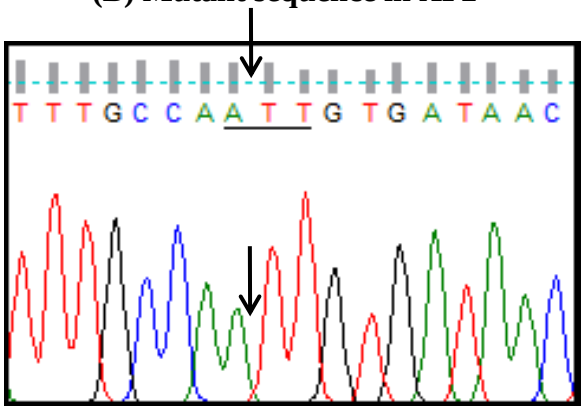
(A) Wild type sequence

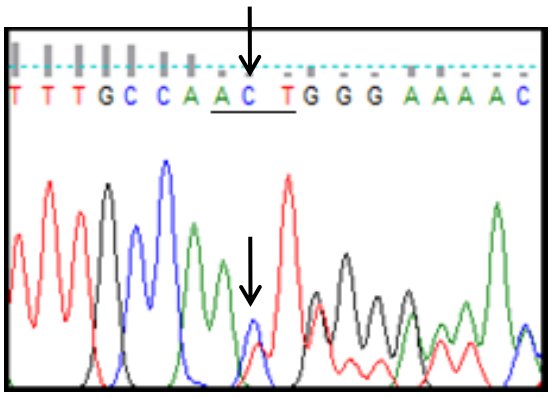




(B) Mutant sequence in XP2

(C) Mutant sequence in XP3





(D) Mutant sequence in fetal DNA

(E) Heterozygous carrier sequence

Figure 33: Sequencing analysis of exon 3 of *XPA* gene in XP2 and XP3 patients with c.374delC mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP2 where there is a homozygous deletion of C base at position 374 of cDNA which changes 125th ACT codon of threonine into ATT codon of

isoleucine, and results in frameshift mutation (p.Thr125IlefsX15), (D) the same mutant sequence was detected in the sibling XP3 and in fetus (D). (E) Heterozygous carrier sequence detected in both parents.

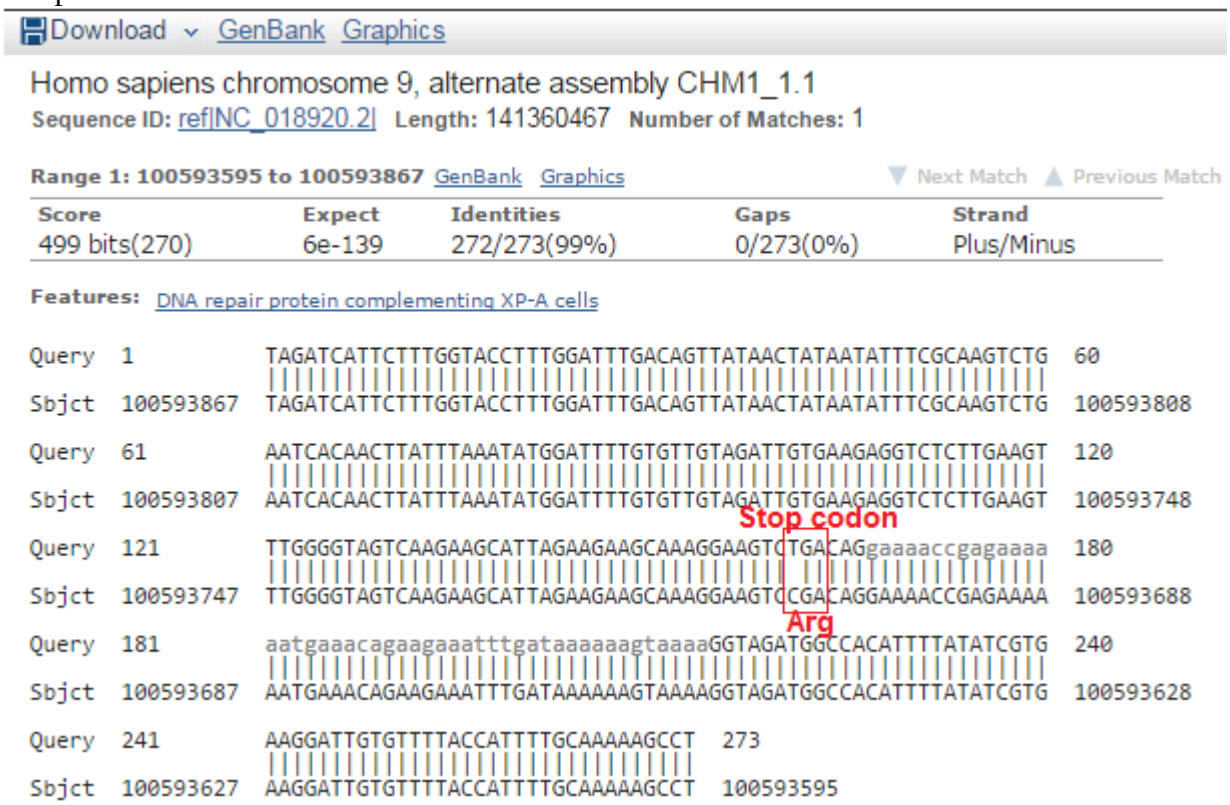


Figure 34: BLAST preview of query sequence of *XPA* exon 5 of XP4 patient. The figure shows alignment of exon 5 of XP4 against a reference sequence from human genome database of NCBI. In the red box, C to T substitution is shown where the CGA codon of arginine is changed into a premature stop codon (TGA).

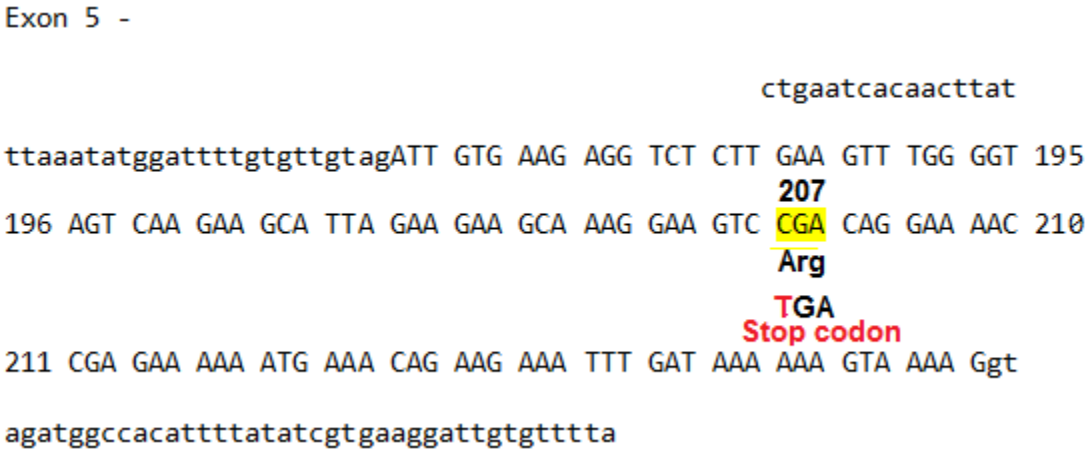
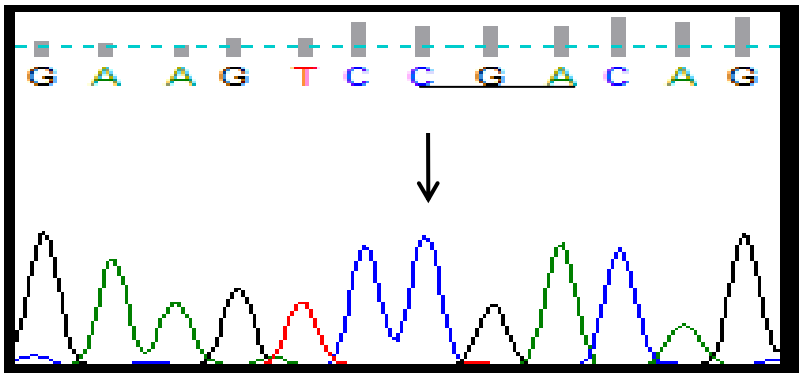
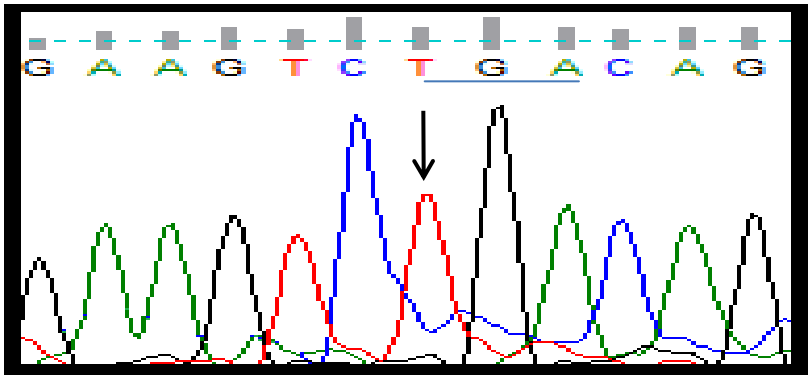


Figure 35: cDNA sequence of *XPA* exon 5 with c.619C>T (p.Arg207X) mutation of XP4. The figure shows exon 5 of *XPA* gene where 207th CGA codon of arginine (highlighted in yellow) changes into TGA; a premature stop codon (p.Arg207X) due to c.619C>T mutation.

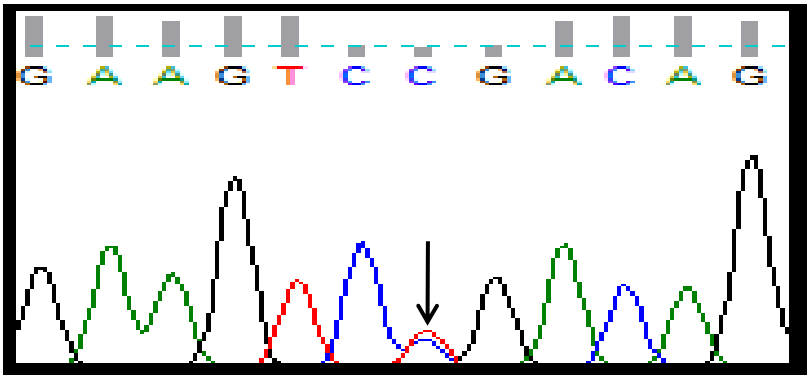
Codons are shown in capital letters, numbers refer to codon number in cDNA sequence, and small letters refer to flanking intronic sequences.



(A) Wild type sequence



(B) Mutant sequence



(C) Heterozygous carrier sequence

Figure 36: Sequencing analysis of exon 5 of *XPA* gene in XP4 patient with c.619C>T (p.Arg207X) mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP4 which has a homozygous C to T

substitution that changes the 207th CGA codon of arginine to a premature stop codon (TGA), and (C) heterozygous carrier sequence detected in both parents.

B. *XPC* gene mutations

Seven different *XPC* gene mutations were detected. Two *XPC* mutations were the most frequent. The first was an acceptor splicing site mutation due to G to C substitution at 3' end of intron 12 i.e. one base pair upstream exon 13 (c.2251-1G>C); this mutation was detected in four patients (XP5 to XP8) descending from three unrelated pedigrees, see figures 37 to 40. A cytosine-adenine single nucleotide polymorphism (C/A SNP, rs2279017) was observed 6 bases upstream exon 13 (i.e. IVS12-6). The A/A genotype of the SNP was found in the wild type sequence, C/C genotype in the mutant sequences of XP5 to XP8 and C/A genotype in both parents' carrier sequences, see figure 38 to 40, with exception to father of XP5 and XP6 who had C/C genotype, see figure 38. The second frequent *XPC* mutation was detected in four patients (XP14 to XP17) descending from four unrelated pedigrees. It is a small two base pairs deletion in exon 9 (c.1643_1644delTG, see figures 56 to 61) which creates a premature stop codon. Three *XPC* novel mutations were identified in XP9 to XP12 which have not been reported before. A small homozygous two base pair deletion in exon 6 of *XPC* (c.668_669delTC, see figures 41 to 43) was identified in two siblings, XP9 and XP10; this novel TC deletion from ATC codon of isoleucine amino acid would result in a frameshift mutation where ATG codon of methionine is created at this position with consequent premature stop codon formation 45 codons later (p.Ile223MetfsX45). Another *XPC* novel mutation was a nonsense small homozygous C to T substitution in exon 10 (c.1894C>T, see figures 44 to 46) identified in XP11; this mutation changes CAG codon of glutamine into a premature TAG stop codon (p.Gln632X). The third identified novel nucleotid change in *XPC* was a small insertion deletion (indel) in exon 4 of XP12 where two bases (AG) were deleted and two bases (CA) were inserted at the same position (c.526_527delAGinsCA, see figures 47 to 49); hence no frameshift. This indel in exon 4 results in replacement of AGA codon of arginine to CAA codon of glutamine (p.Arg176Gln) i.e. single amino acid substitution. XP12 was found to be compound heterozygote, i.e. the patient has two different mutated alleles; one for the novel indel of exon 4 and the other for a previously identified two base pairs deletion (c.1103_1104delAA; p.Gln368ArgfsX6, see figures 50 to 52) in exon 9. A previously identified mutation in exon 9 was detected in XP13; the patient had a

homozygous nonsense mutation due to C to T substitution which creates a premature stop codon (c.1735C>T; p.Arg579X, see figures 53 to 55). In all patients, parents were found to be carriers for one mutant allele; no *de novo* mutations were identified.

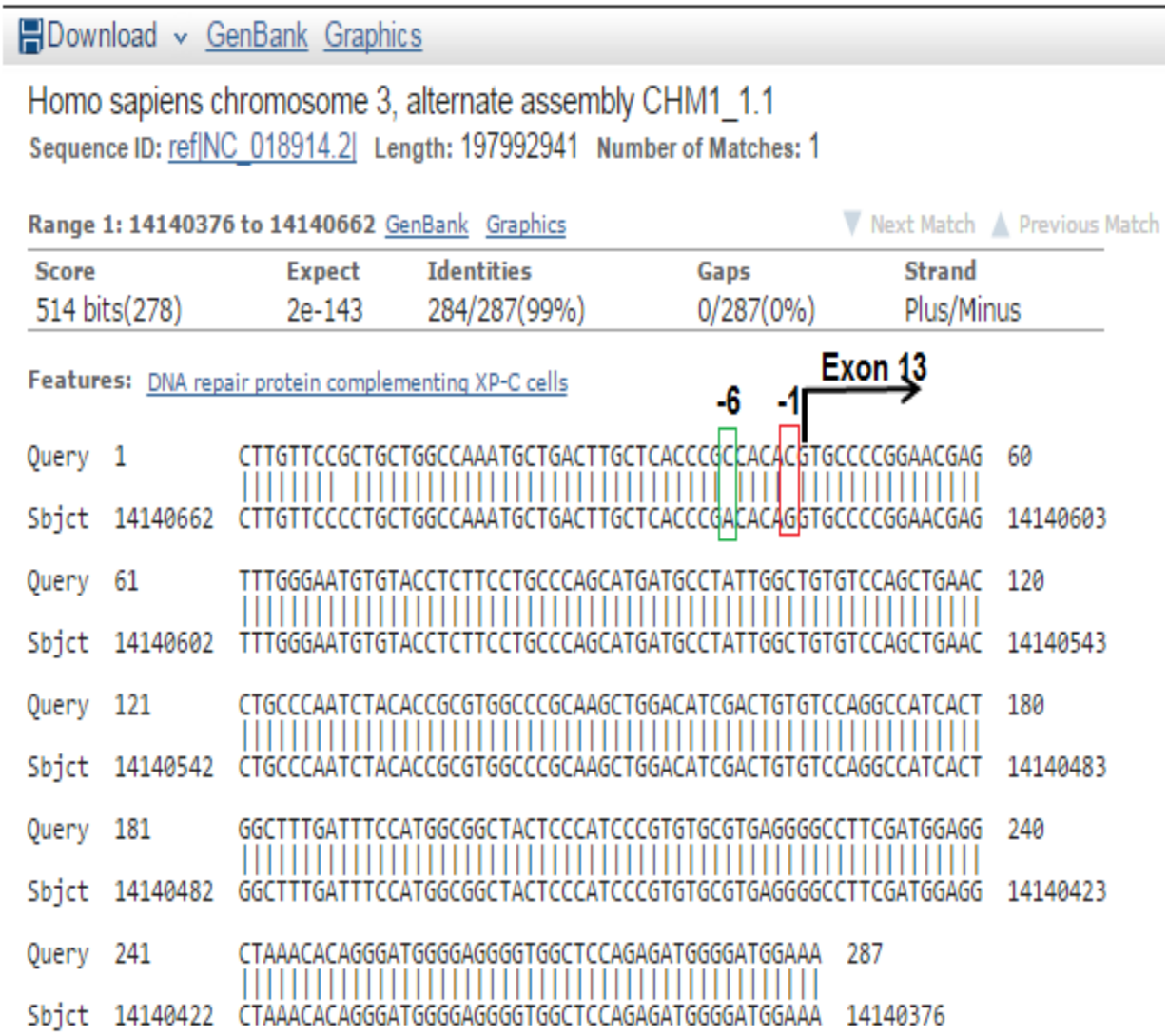
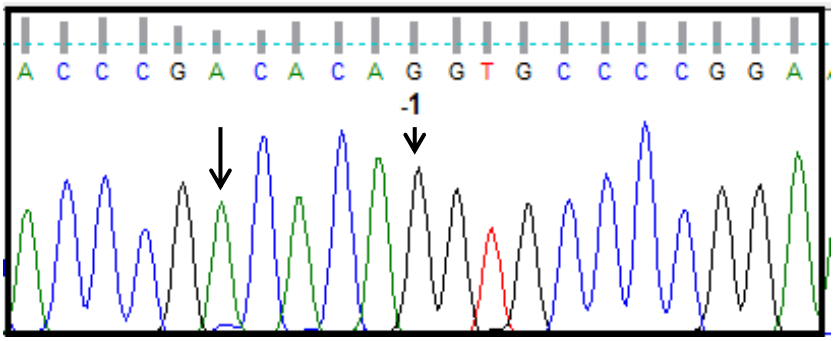
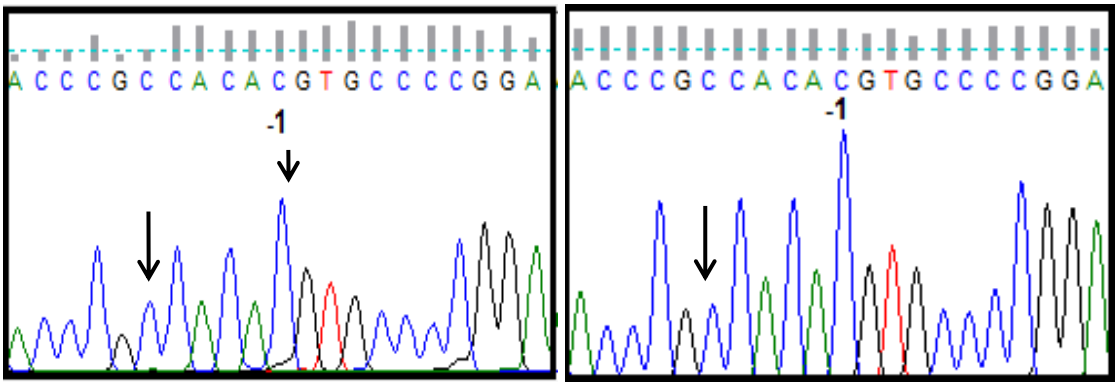


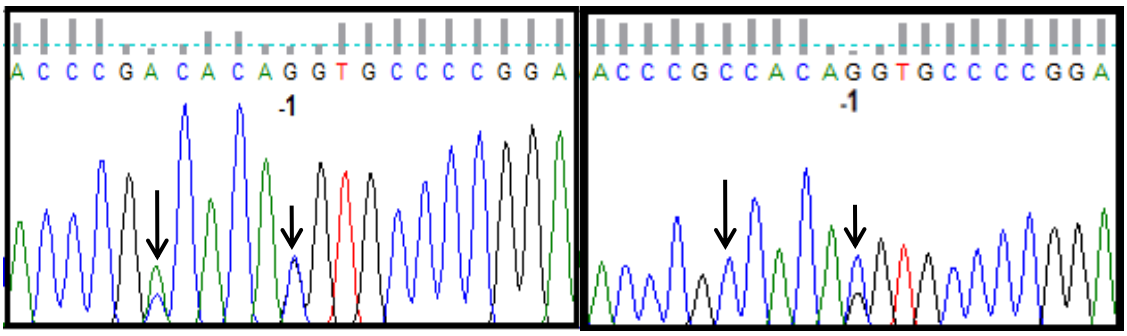
Figure 37: BLAST preview of query sequence of *XPC* exon 13 of XP5 patient. The figure shows alignment of XP5 exon 13 against a reference sequence from human genome database of NCBI. In the red box, G to C substitution is shown in intron12/exon 13 junction (IVS12-1) i.e. c.2251-1G>C. In the green box, A to C substitution at (IVS12-6) is shown; it is a previously identified single nucleotide polymorphism (SNP) where the C allele of the SNP is known to be associated with the c.2251-1G>C mutant allele. Similar BLAST results were obtained in case of XP6, XP7, and XP8 patients.



(A) Wild type sequence

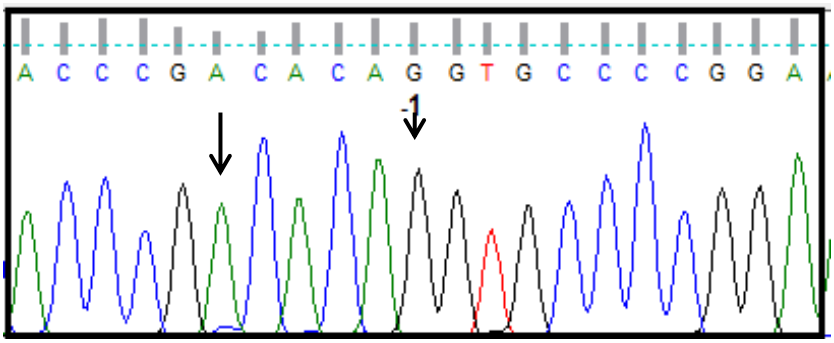


(B) Mutant sequence in XP5 (on the left) and XP6 (on the right)

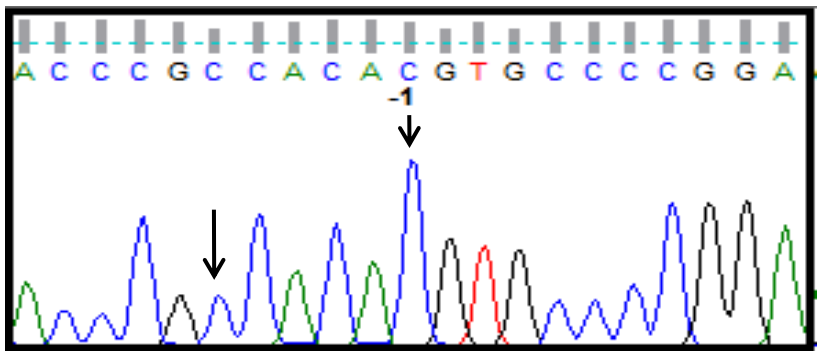


(C) Heterozygous carrier sequence in mother (on the left) and father (on the right)

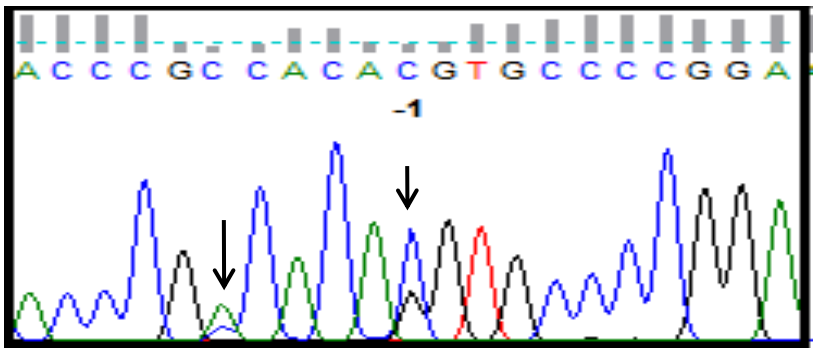
Figure 38: Sequencing analysis of intron 12 and exon 13 of *XPC* gene in XP5 and XP6 patients with c.2251-1G>C mutation. The figure shows end of intron 12 and beginning of exon 13 in the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP5 and her sibling XP6; the mutation is a homozygous G to C substitution at -1 bp of splice site and (C) heterozygous carrier sequence detected in both parents. A single nucleotide polymorphism (C/A; IVS12-6) is indicated by the arrow upstream of mutation: A/A genotype in the wild type (A), C/C genotype in the mutant sequences (B), C/A genotype in the sequence of the mother (C, on the left) and C/C genotype in the father (C, on the right).



(A) Wild type sequence

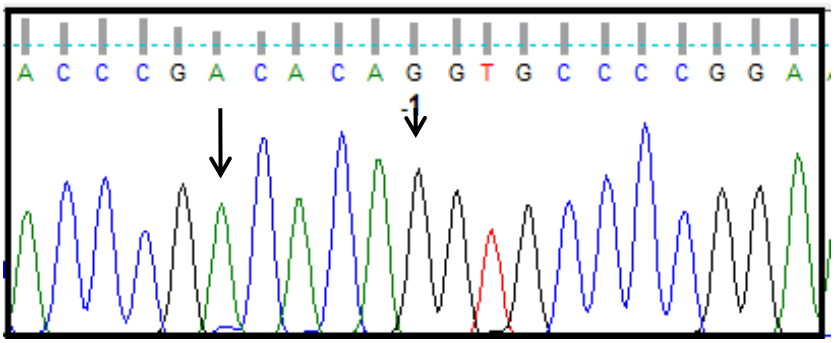


(B) Mutant sequence

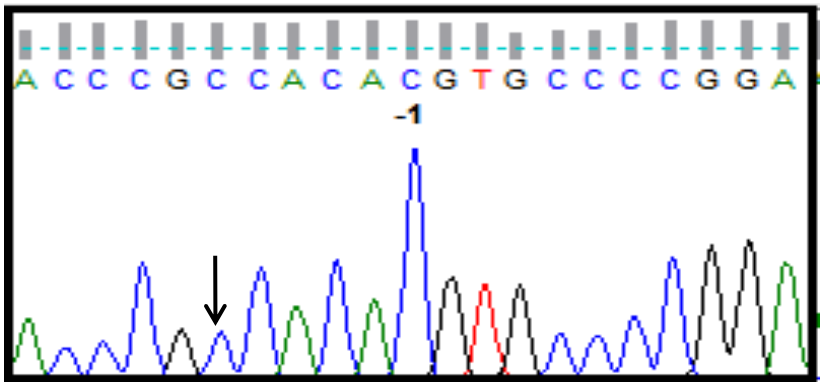


(C) Heterozygous carrier sequence

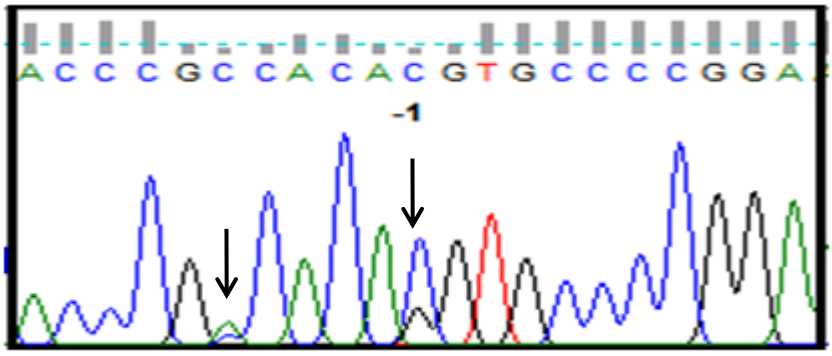
Figure 39: Sequencing analysis of intron 12 and exon 13 of *XPC* gene in XP7 patient with c.2251-1G>C mutation. The figure shows end of intron 12 and beginning of exon 13 in the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP7; the mutation is a homozygous G to C substitution at -1 bp of splice site and (C) heterozygous carrier sequence detected in both parents. The SNP (C/A; IVS12-6) is indicated by the arrow upstream of mutation: A/A genotype in the wild type (A), C/C genotype in the mutant sequence (B), and C/A genotype in the sequence of both parents (C).



(A) Wild type sequence



(B) Mutant sequence



(C) Heterozygous carrier sequence

Figure 40: Sequencing analysis of intron 12 and exon 13 of *XPC* gene in XP8 patient with c.2251-1G>C mutation. The figure shows end of intron 12 and beginning of exon 13 in the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP8; the mutation is a homozygous G to C substitution at -1 bp of splice site and (C) heterozygous carrier sequence detected in both parents. The SNP (C/A; IVS12-6) is indicated by the arrow upstream

of mutation: A/A genotype in the wild type (A), C/C genotype in the mutant sequence (B), and C/A genotype in the sequence of both parents (C).

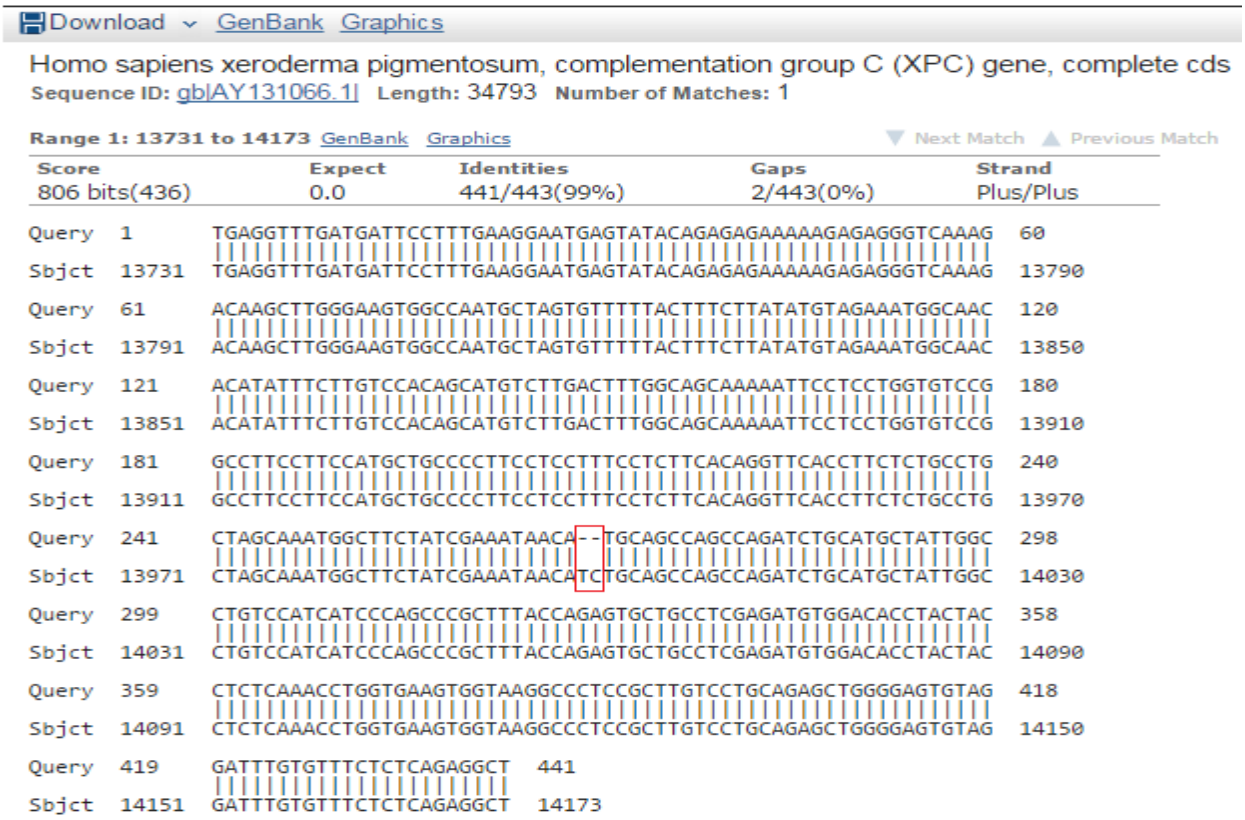


Figure 41: BLAST preview of query sequence of *XPC* exon 6 of XP9 patient. The figure shows alignment of exon 6 of XP9 against a reference sequence from human genome database of NCBI. In the red box, the deletion of two base pairs (TC) is shown. Similar alignment results were obtained for XP10.

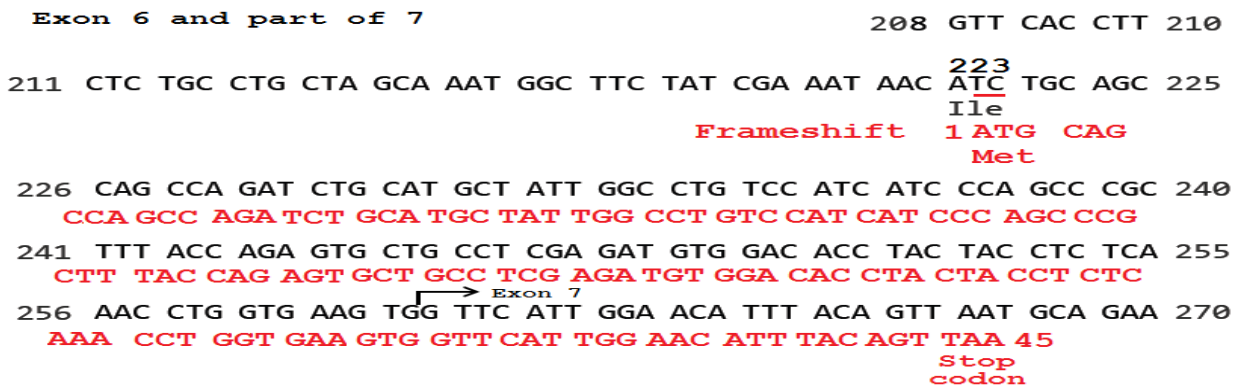
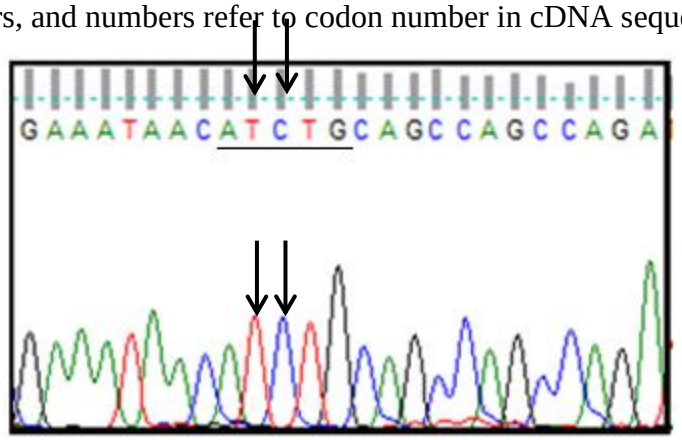
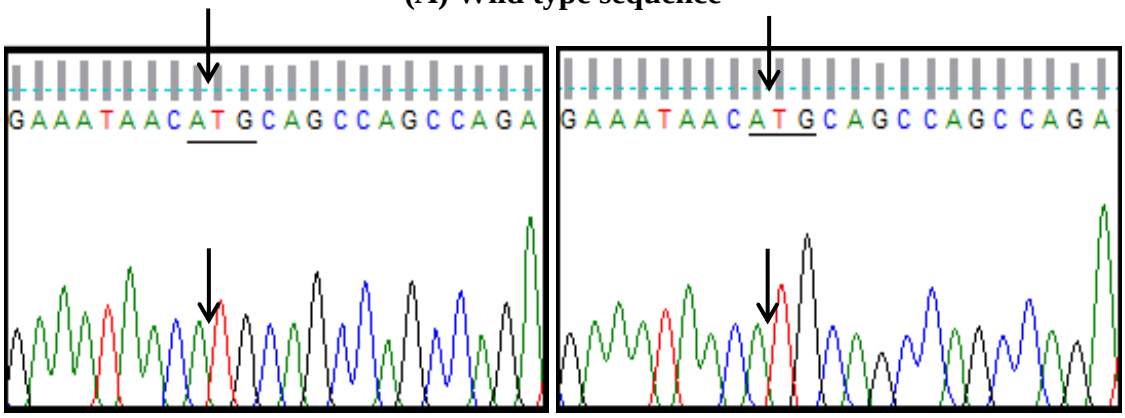


Figure 42: cDNA sequence of *XPC* exon 6 and part of exon 7 with novel c.668_669delTC mutation of XP9 and XP10 siblings. The figure shows exon 6 and part of exon 7 of *XPC* gene

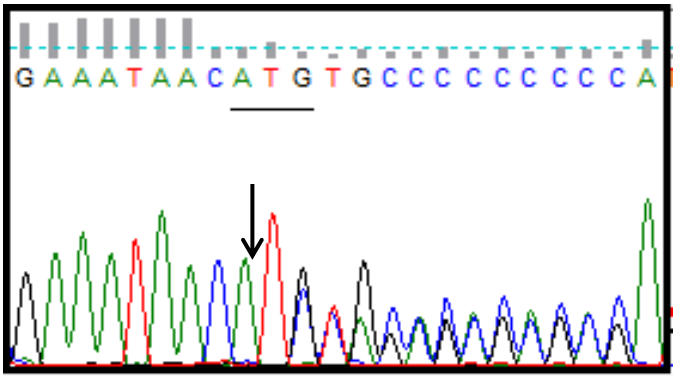
where 223rd ATC codon of isoleucine changes into ATG codon of methionine due to c.668_669delTC deletion (underlined red). The resultant frameshift mutation (p.Ile223MetfsX45) forms a premature stop codon (TAA) at position 45 of frameshift. Codons are shown in capital letters, and numbers refer to codon number in cDNA sequence.



(A) Wild type sequence



(B) Mutant sequence in XP9 (on the left) and XP10 (on the right)



(C) Heterozygous carrier sequence

Figure 43: Sequencing analysis of exon 6 of *XPC* gene in XP9 and XP10 patients with novel c.668_669delTC mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence having TC bases, (B) mutant sequence detected in XP9 and her sibling XP10;

the mutation is a homozygous 2 bp (TC) deletion at position 668 and 669 of cDNA which changes ATC codon of isoleucine into ATG codon of methionine and causes a frameshift mutation (p.Ile223MetfsX45), and (C) heterozygous carrier sequence detected in both parents, and a sibling.

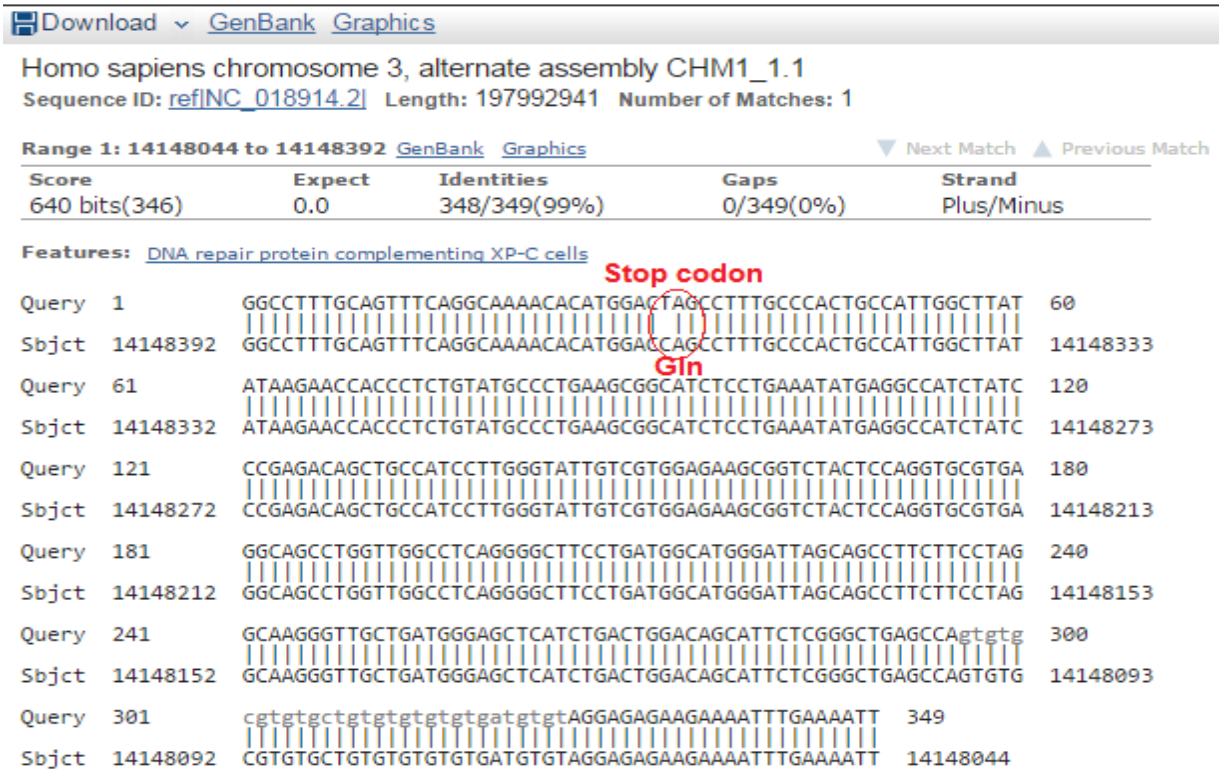


Figure 44: BLAST preview of query sequence of *XPC* exon 10 of XP11 patient. The figure shows alignment of exon 10 of XP11 against a reference sequence from human genome database of NCBI. In the red circle, C to T substitution is shown where the CAG codon of glutamine is changed into a premature stop codon (TAG).

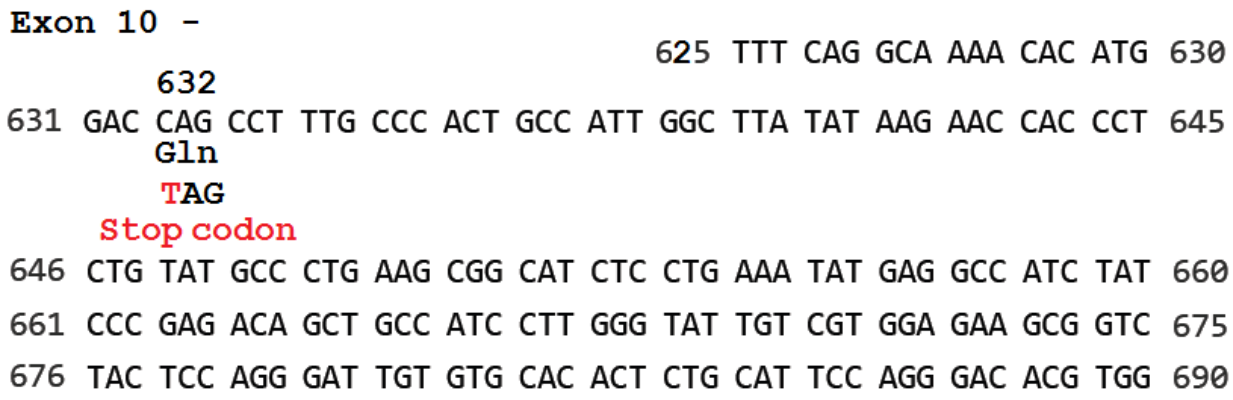
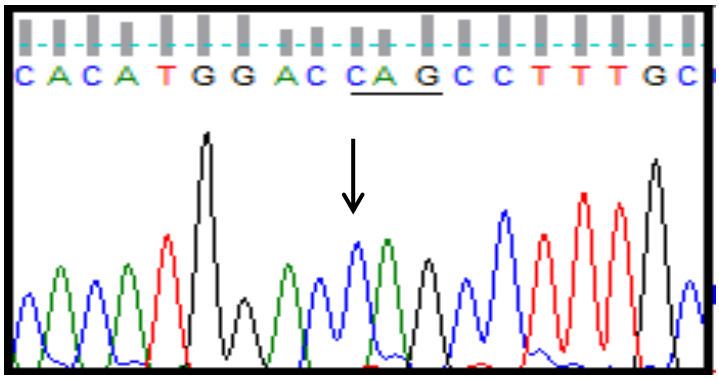
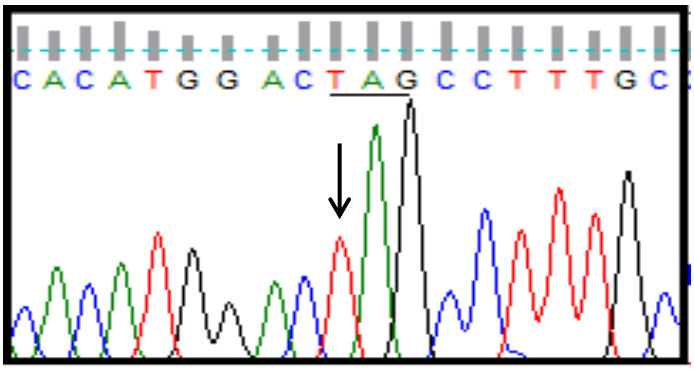


Figure 45: cDNA sequence of *XPC* exon 10 with novel c.1894C>T (p.Gln632X) mutation of XP11. The figure shows exon 10 of *XPC* gene where 632nd codon of glutamine (CAG) changes

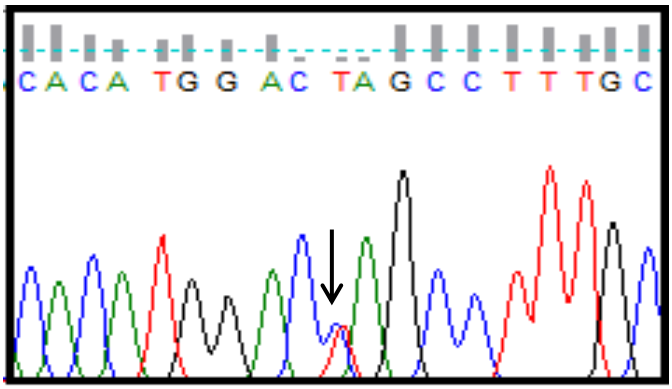
into TAG; a premature stop codon (p.Gln632X) due to c.1894C>T mutation. Codons are shown in capital letters, and numbers refer to codon number in cDNA sequence.



(A) Wild type sequence



(B) Mutant sequence



(C) Heterozygous carrier sequence

Figure 46: Sequencing analysis of exon 10 of *XPC* gene in XP11 patient with novel c.1894C>T (p.Gln632X) mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP11 which has a

homozygous C to T substitution that changes the 632th CAG codon of glutamine to a premature stop codon (TAG), and (C) heterozygous carrier sequence detected in both parents.

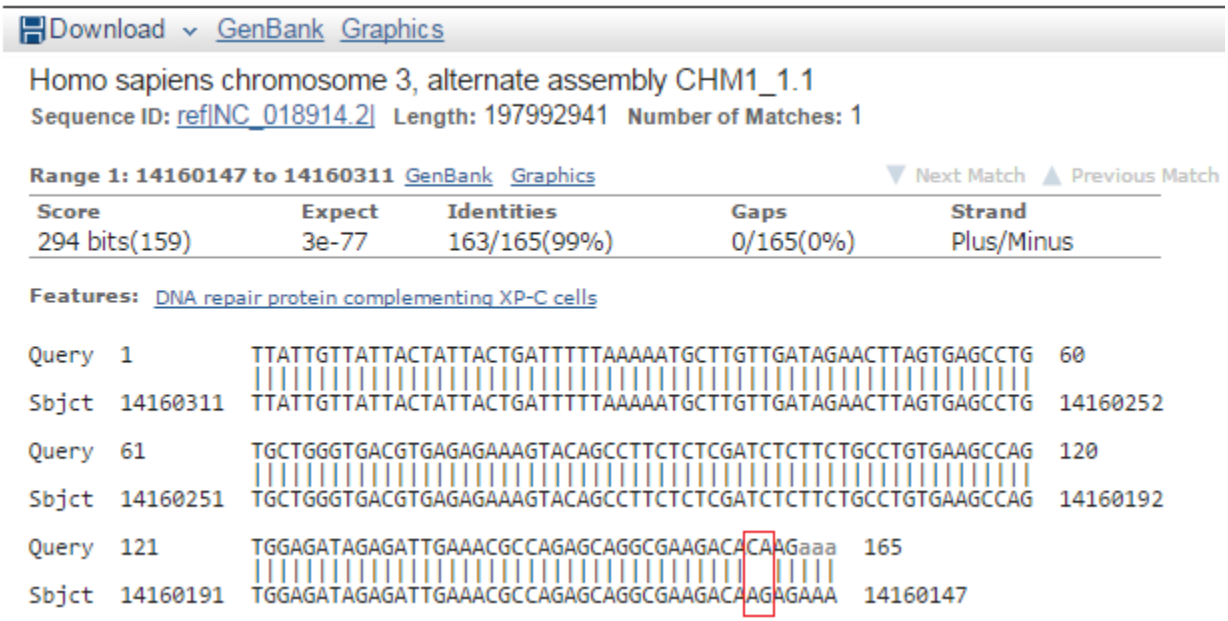


Figure 47: BLAST preview of query sequence of *XPC* exon 4 of XP12 patient. The figure shows alignment of exon 4 of XP12 against a reference sequence from human genome database of NCBI. In the red box, the displacement of AG by CA (delAGinsCA) is shown**.**

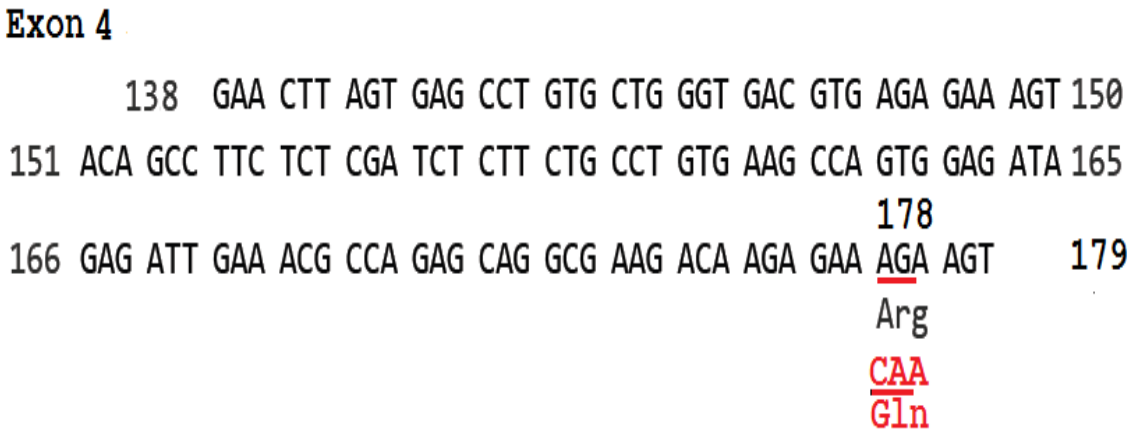
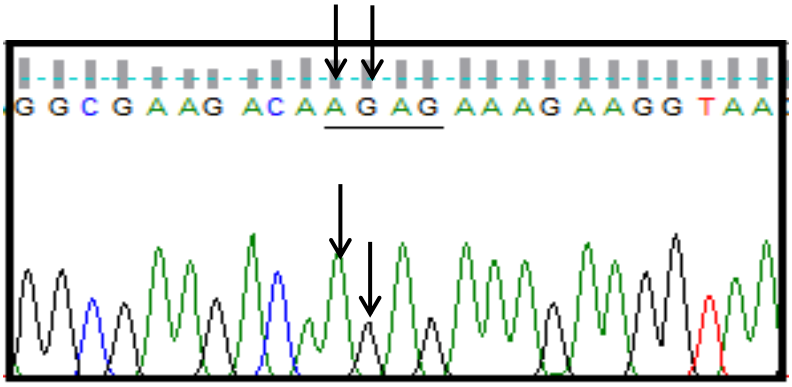
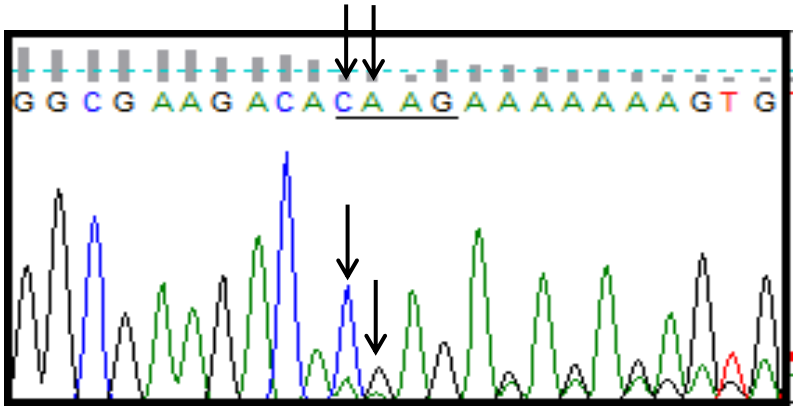


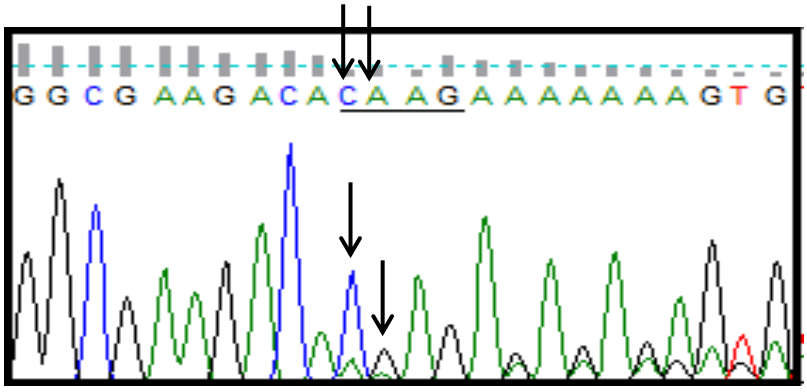
Figure 48: cDNA sequence of *XPC* exon 4 with novel insertion deletion c.526_527delAGinsCA of XP12. The figure shows exon 4 of *XPC* gene where 178th AGA codon of arginine changes into CAA codon of glutamine i.e. single amino acid substitution (p.Arg176Gln) due to CA insertion instead of AG (underlined red). Codons are shown in capital letters, and numbers refer to codon number in cDNA sequence.



(A) Wild type sequence in father



(B) Heterozygous mutant sequence in XP12



(C) Heterozygous mutant sequence in mother

Figure 49: Sequencing analysis of exon 4 of *XPC* gene in XP12 patient with a novel heterozygous insertion deletion c.526_527delAGinsCA. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence detected in one parent, (B) heterozygous mutant sequence detected in XP12 which shows deletion of 2 bp (AG) and the insertion of another 2 bp (CA) instead, and (C) heterozygote mutant sequence which was also detected in the other parent.

Figure 50: BLAST preview of query sequence of *XPC* exon 9 of XP12 patient. The figure shows alignment of fragment 9a of exon 9 of XP13 against a reference sequence from human genome database of NCBI. Underlined in red is the observed heterozygous pattern which begins at the red circle of AA deletion.

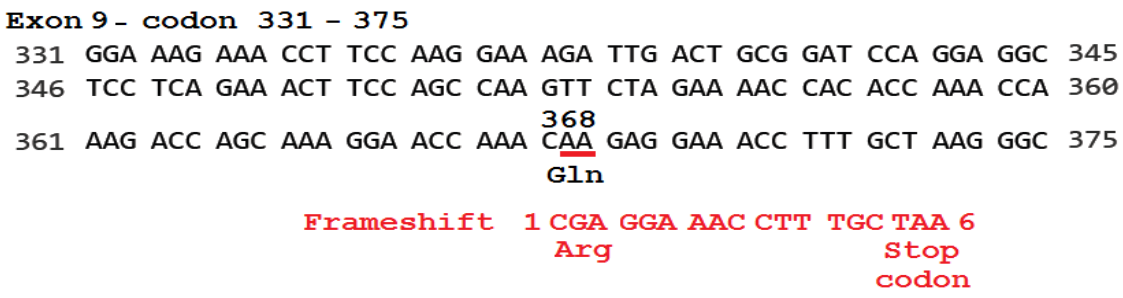
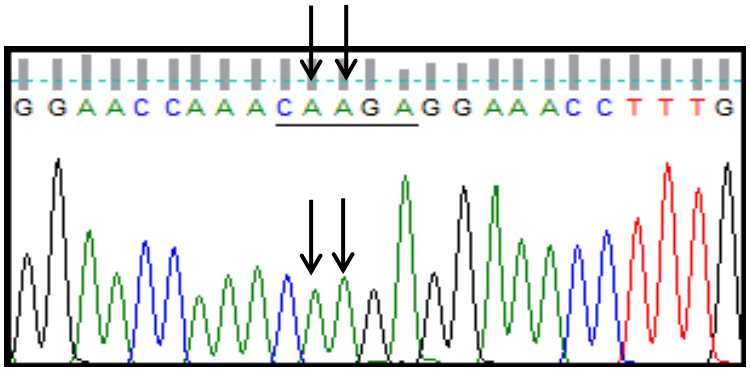
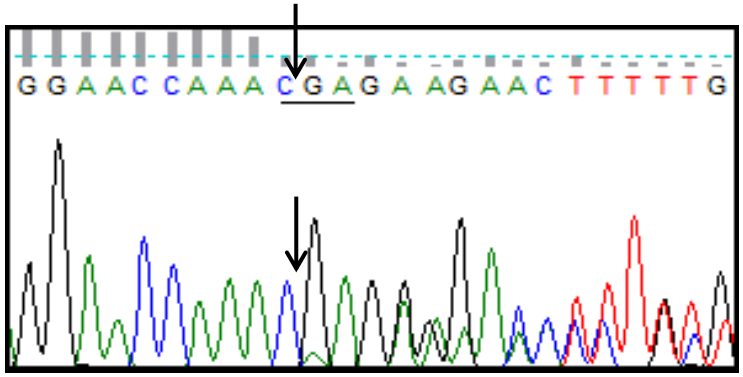


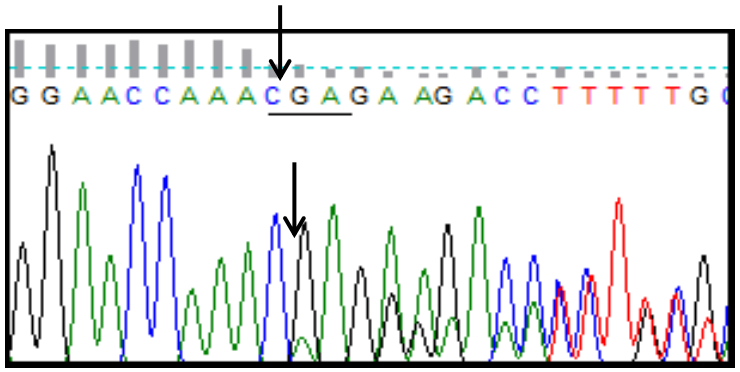
Figure 51: cDNA sequence of *XPC* exon 9 with c.1103_1104delAA (p.Gln368ArgfsX6) mutation of XP12. The figure shows from codon 331 to 630 of exon 9 of *XPC* gene where two bp (AA, underlined in red) of 368th CAA codon of glutamine are deleted, therefore, CAA codon changes into CGA codon of arginine (p.Gln368Arg), and a frameshift mutation results (p.Gln368ArgfsX6**)** and a premature stop codon (TAA) is created at position 6 of frameshift. Codons are shown in capital letters, and numbers refer to codon number in cDNA sequence.



(A) Wild type sequence in mother



(B) Heterozygous mutant sequence in XP12



(C) Heterozygous mutant sequence in father

Figure 52: Sequencing analysis of exon 9 of *XPC* gene in XP12 patient with heterozygous c.1103_1104delAA mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence detected in one parent, (B) heterozygous mutant sequence detected in XP12 which shows a small deletion of 2 bp (AA) which changes 368th CAA codon of glutamine into CGA codon of arginine, and results in frameshift (p.Gln368ArgfsX6), and (C) heterozygote mutant sequence which was also detected in the other parent.

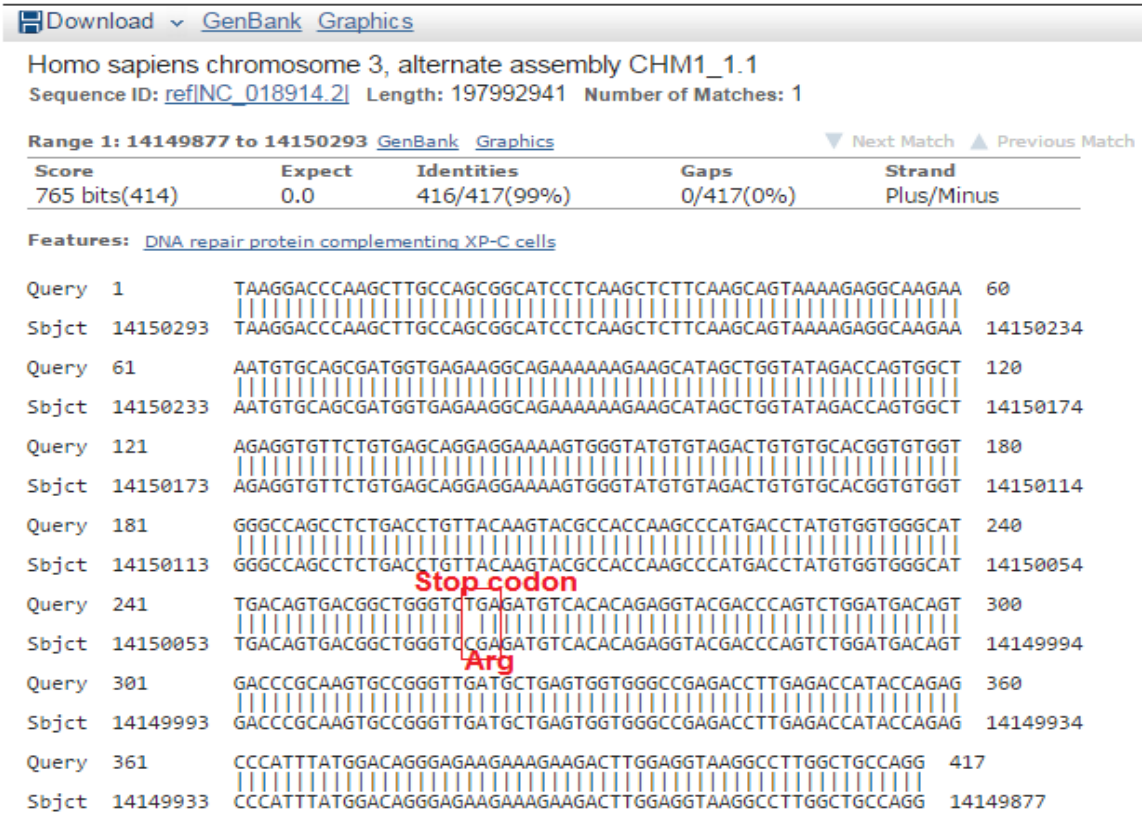
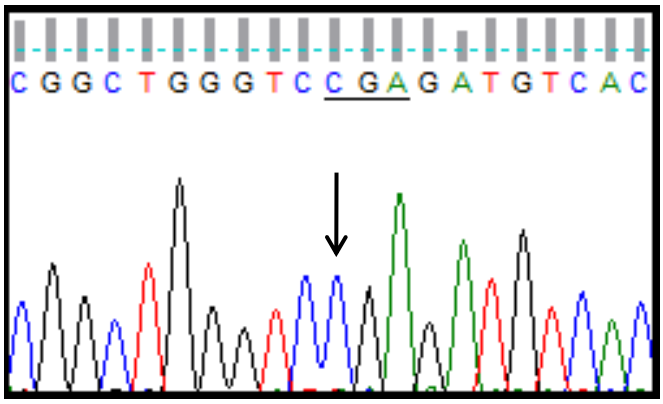


Figure 53: BLAST preview of query sequence of *XPC* exon 9 of XP13 patient. The figure shows alignment of fragment 9b of exon 9 of XP13 against a reference sequence from human genome database of NCBI. In the red box, C to T substitution is shown where the CGA codon of arginine is changed into a premature stop codon (TGA).

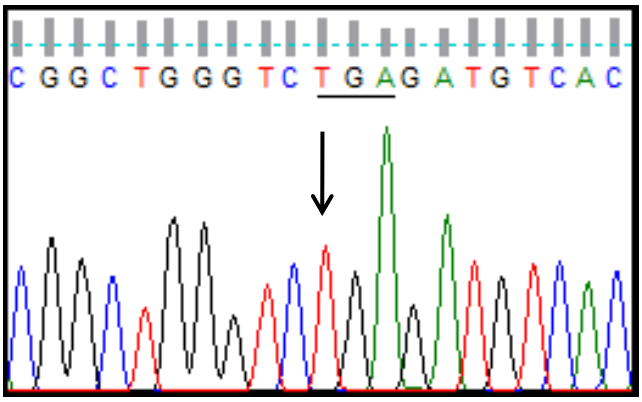
Exon 9 – codon 571-630

```
                                        179
571  GGC ATT GAC AGT GAC GGC TGG GTC CGA GAT GTC ACA CAG AGG TAC  585
                                        Arg
                                        TGA
                                    Stop codon
586  GAC CCA GTC TGG ATG ACA GTG ACC CGC AAG TGC CGG GTT GAT GCT  600

601  GAG TGG TGG GCC GAG ACC TTG AGA CCA TAC CAG AGC CCA TTT ATG  615

616  GAC AGG GAG AAG AAA GAA GAC TTG GAG TTT CAG GCA AAA CAC ATG  630
```

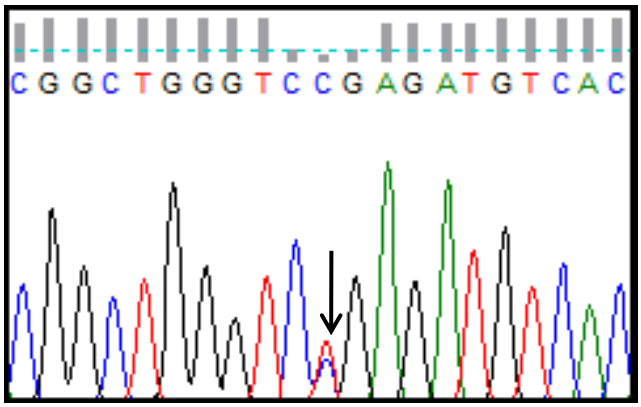
Figure 54: cDNA sequence of *XPC* exon 9 with c.1735C>T (p.Arg579X) mutation of XP13. The figure shows from codon 571 to 630 of exon 9 of *XPC* gene where 579th CGA codon of arginine changes into TGA; a premature stop codon (p.Arg579X) due to c.1735C >T mutation. Codons are shown in capital letters, and numbers refer to codon number in cDNA sequence.



(A) Wild type sequence



(B) Mutant sequence



(C) Heterozygous carrier sequence

Figure 55: Sequencing analysis of exon 9 of *XPC* gene in XP13 patient with c.1735C>T (p.Arg579X) mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP13 which has a homozygous C to T substitution that changes the 579th CGA codon of arginine to a premature stop codon (TGA), and (C) heterozygous carrier sequence detected in both parents.

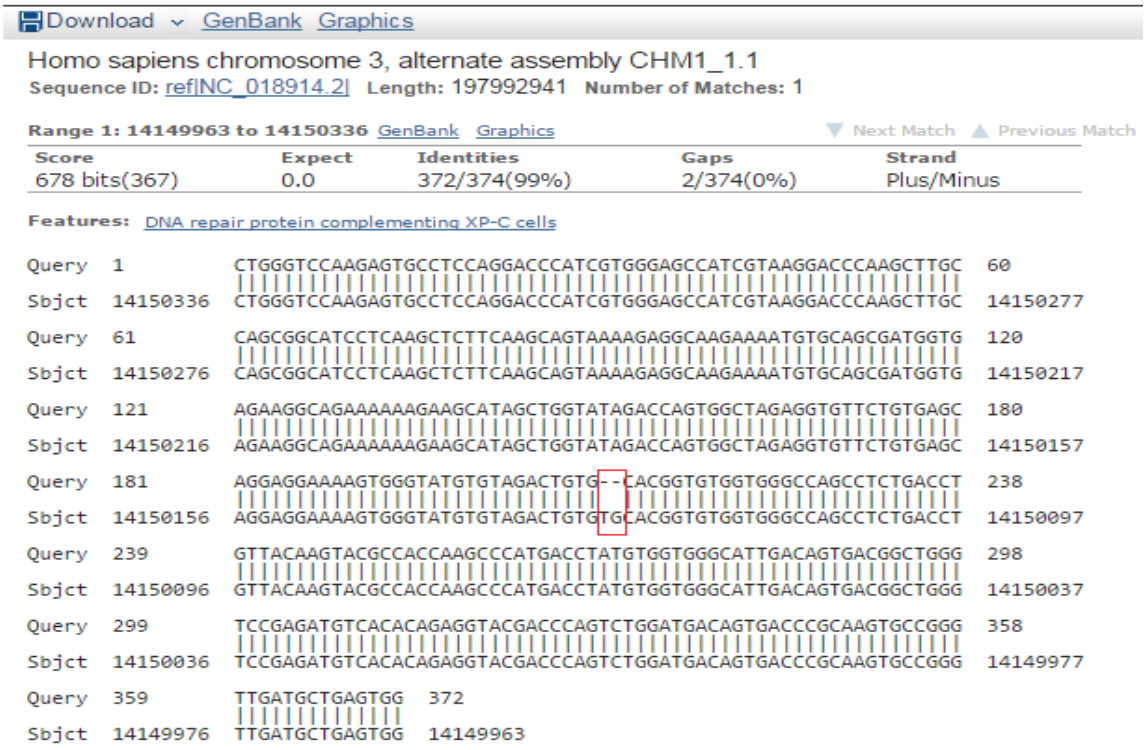


Figure 56: BLAST preview of query sequence of *XPC* exon 9 of XP14 patient. The figure shows alignment of fragment 9b of exon 9 of XP14 against a reference sequence from human genome database of NCBI. In the red box, the deletion of two base pairs (TG) is shown. Similar alignment results were obtained for XP15, XP16 and XP17 patients.

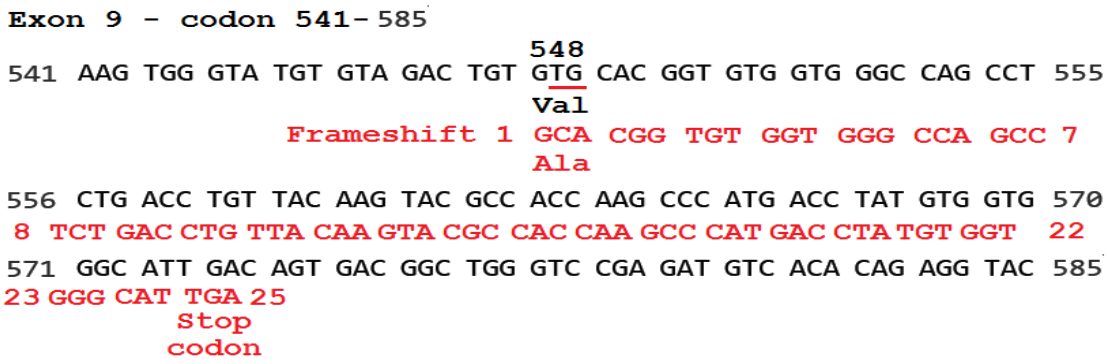
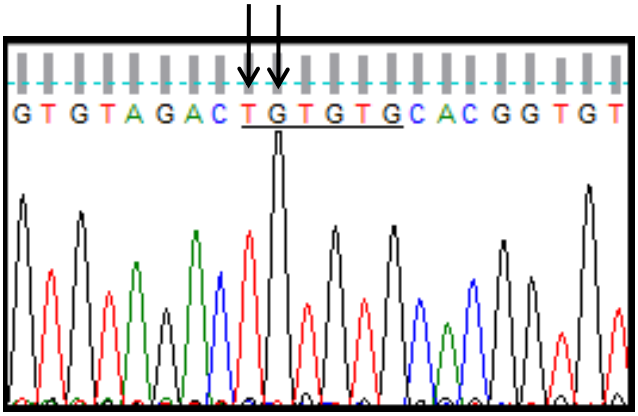
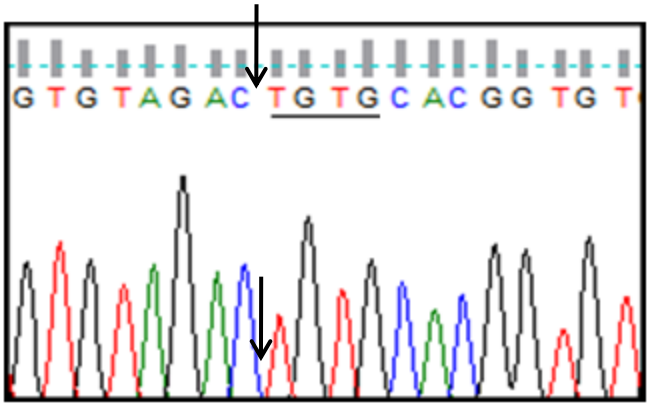


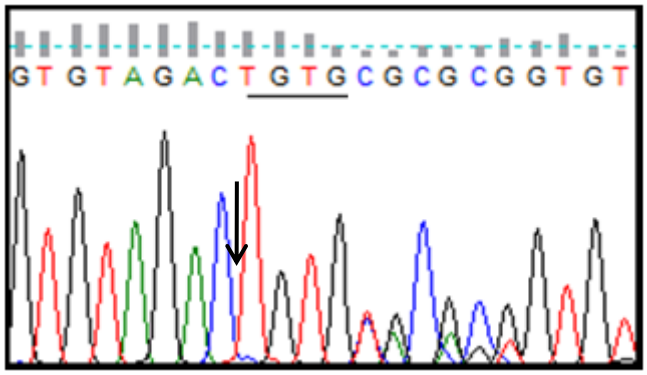
Figure 57: cDNA sequence of *XPC* exon 9 with c.1643_1644delTG (p.Val548AlafsX25) mutation of XP14, XP15, XP16 and XP17. The figure shows codon 541 to 585 of *XPC* gene where 548th GTG codon of valine changes into GCA codon of alanine due to c.1643_1644delTG deletion (underlined red). The resultant frameshift mutation (p.Val548AlafsX25) forms a premature stop codon (TGA) at position 25 of frameshift. Codons are shown in capital letters, and numbers refer to codon number in cDNA sequence.



(A) Wild type sequence

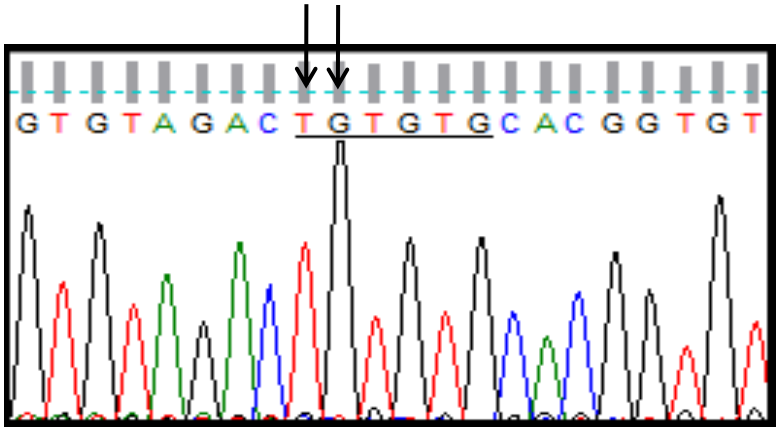


(B) Mutant sequence

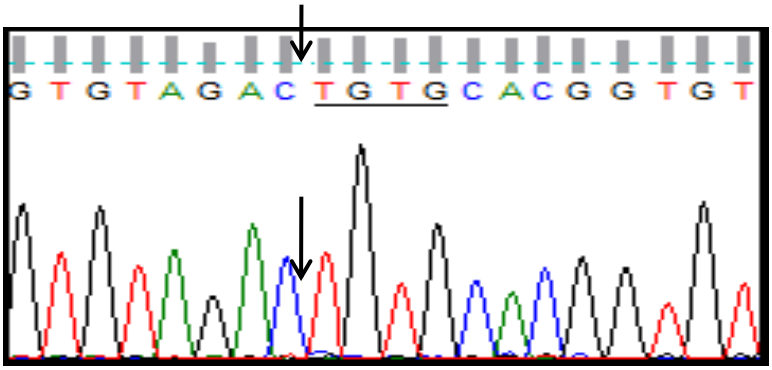


(C) Heterozygous carrier sequence

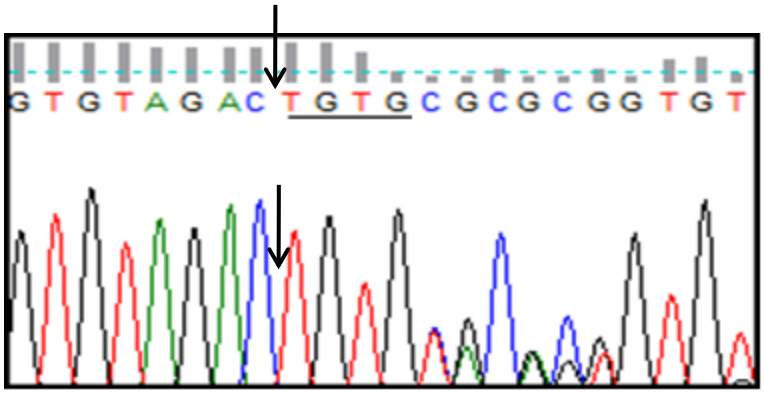
Figure 58: Sequencing analysis of exon 9 of *XPC* gene in XP14 patient with c.1643_1644delTG mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP14; the mutation is a homozygous 2 bp (TG) deletion at position 1643 and 1644of cDNA which changes GTG codon of valine into GCA codon of alanine, and causes a frameshift (p.Val548AlafsX25**)**, and (C) heterozygous carrier sequence detected in both parents.



(A) Wild type sequence

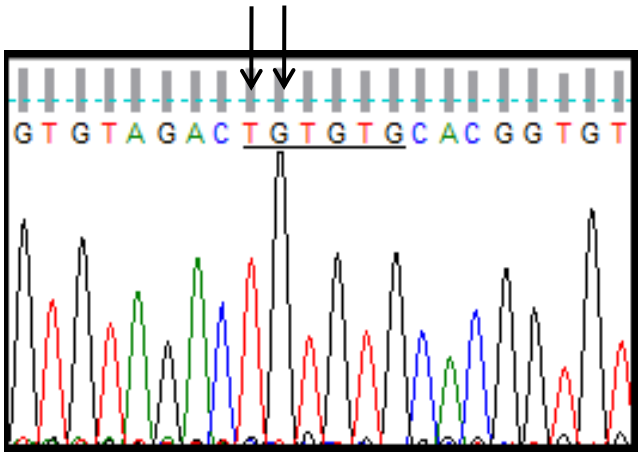


(B) Mutant sequence

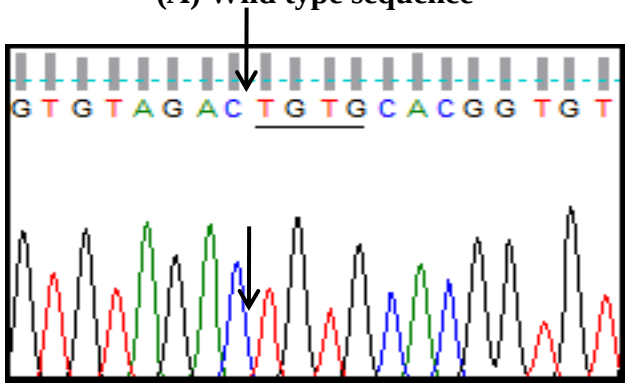


(C) Heterozygous carrier sequence

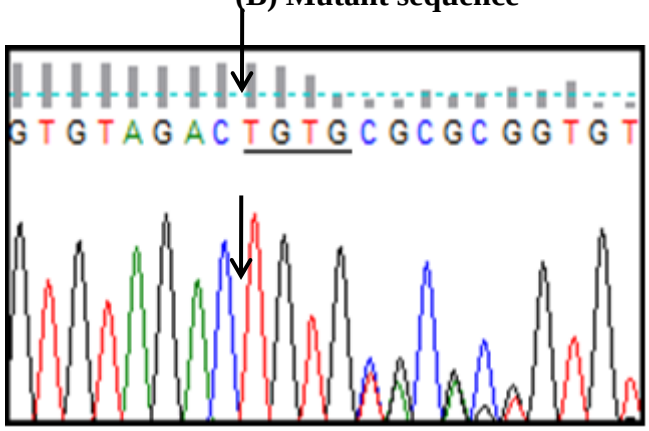
Figure 59: Sequencing analysis of exon 9 of *XPC* gene in XP15 patient with c.1643_1644delTG mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP15; the mutation is the same homozygous 2 bp (TG) deletion at position 1643 and 1644of cDNA which changes GTG codon of valine into GCA codon of alanine, and causes a frameshift (p.Val548AlafsX25**)**, and (C) heterozygous carrier sequence detected in both parents.



(A) Wild type sequence

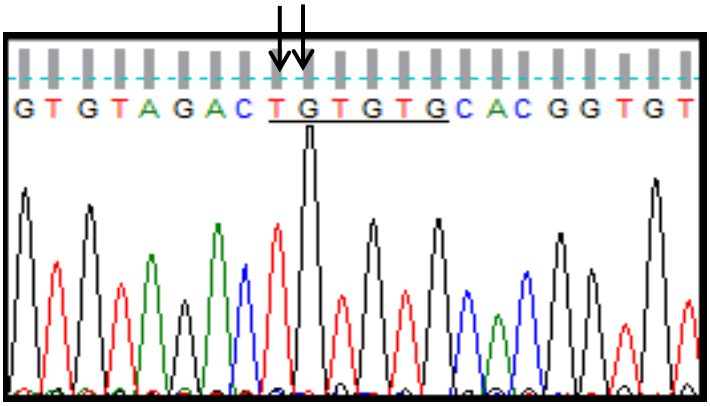


(B) Mutant sequence

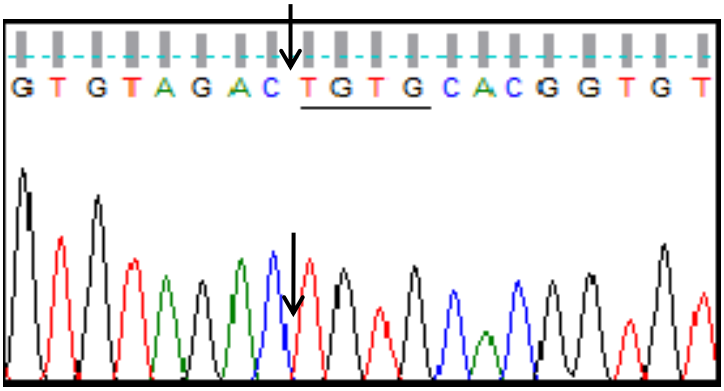


(C) Heterozygous carrier sequence

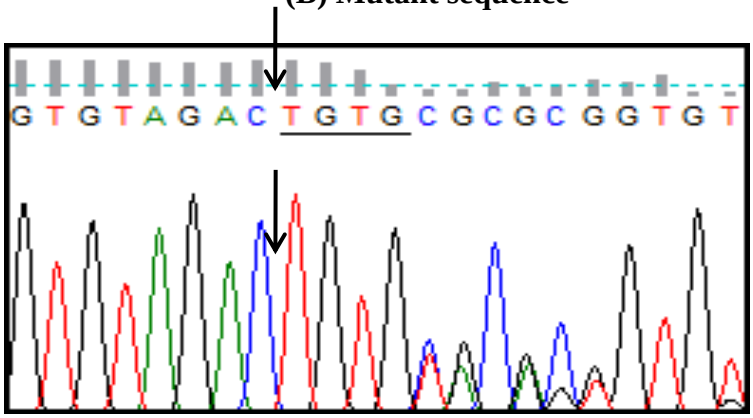
Figure 60: Sequencing analysis of exon 9 of *XPC* gene in XP16 patient with c.1643_1644delTG mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP16; the mutation is the same homozygous 2 bp (TG) deletion at position 1643 and 1644of cDNA which changes GTG codon of valine into GCA codon of alanine, and causes a frameshift (p.Val548AlafsX25**)**, and (C) heterozygous carrier sequence detected in both parents.



(A) Wild type sequence



(B) Mutant sequence



(C) Heterozygous carrier sequence

Figure 61: Sequencing analysis of exon 9 of *XPC* gene in XP17 patient with c.1643_1644delTG mutation. The figure shows a portion of the sequencing chromatogram of (A) wild type sequence, (B) mutant sequence detected in XP17; the mutation is the same homozygous 2 bp (TG) deletion at position 1643 and 1644of cDNA which changes GTG codon of valine into GCA codon of alanine, and causes a frameshift (p.Val548AlafsX25**)**, and (C) heterozygous carrier sequence detected in both parents.

3.2.3. Analysis of novel mutations

Three novel mutations in *XPC* gene were identified; a frameshift mutation (c.668_669delTC, p.Ile223MetfsX45), a nonsense mutation (c.1894C>T, p.Gln632X), and a single amino acid substitution due to insertion deletion (c.526_527delAGinsCA, p.Arg176Gln). According to literature search and human genome mutation database (HGMD; www.hgmd.org), these mutations have not been previously identified. In order to predict functional effect of these newly identified mutations, MutationTaster was used for the three novel mutations while SIFT and mutation assessor were used for single amino acid substitution (p.Arg176Gln). MutationTaster predicted the three mutations to be "disease causing". The calculated probability scores were 1 for both the frameshift c.668_669delTC and nonsense c.1894C>T mutations, and 0.99 for insertion deletion mutation (c.526_527delAGinsCA), see figures 62 to 64. For the substitution of arginine (R) amino acid by glutamine (Q) at position 176 (p.Arg176Gln or R176Q) caused by the insertion deletion c.526_527delAGinsCA, SIFT predicted "damaging" effect of score of 0.025; lower than the cutoff of 0.05, see figure 65. Mutation assessor predicted "medium functional" impact of score of 2.35; higher than threshold score of 1.9, see table 9.

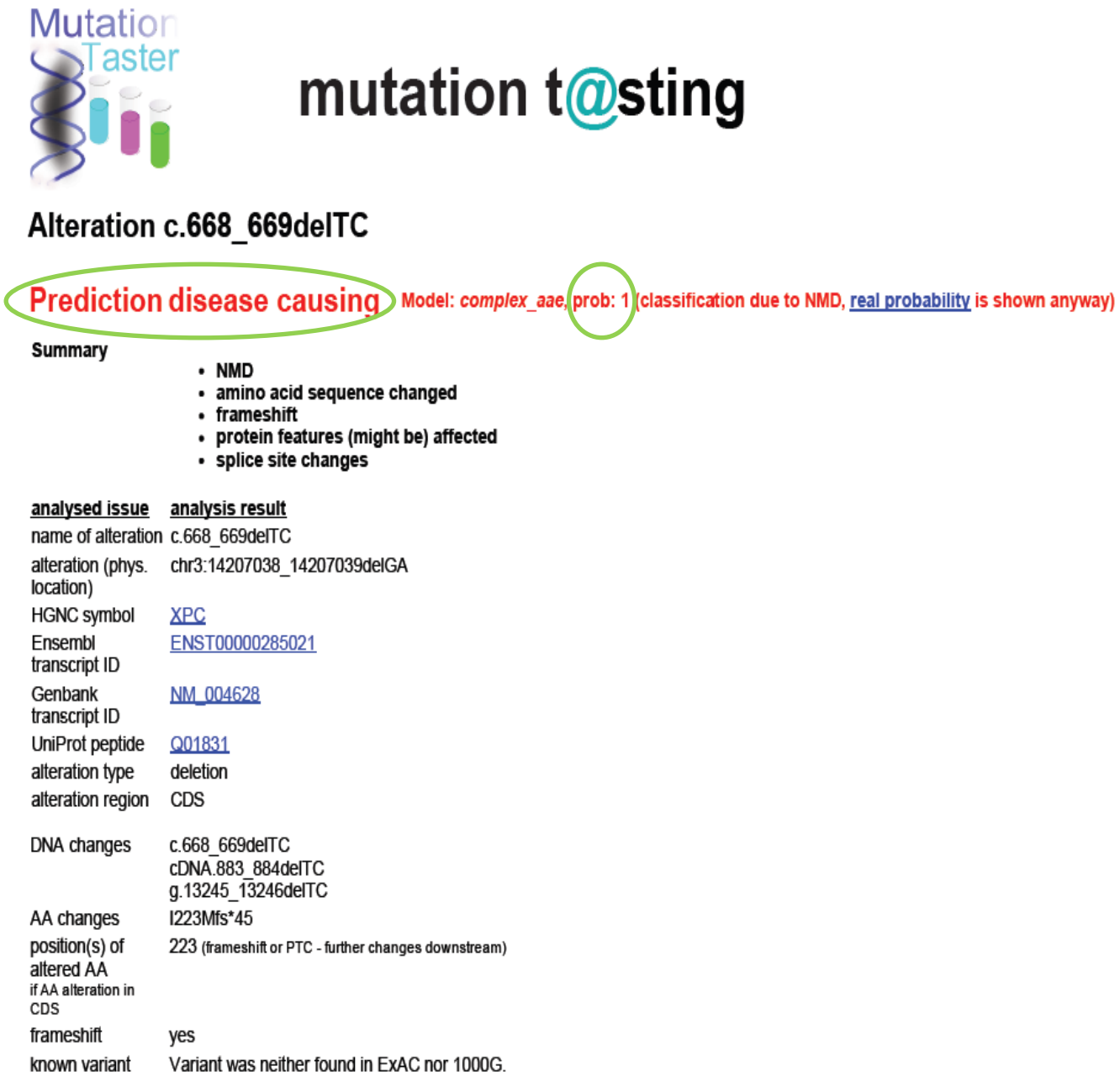


Figure 62: Preview of MutationTaster prediction tool result of the novel frameshift mutation (c.668_669delTC, p.Ile223MetfsX45). The figure shows in the two green circles the "disease causing" prediction result of probability 1. NMD refers to nonsense-mediated mRNA decay.

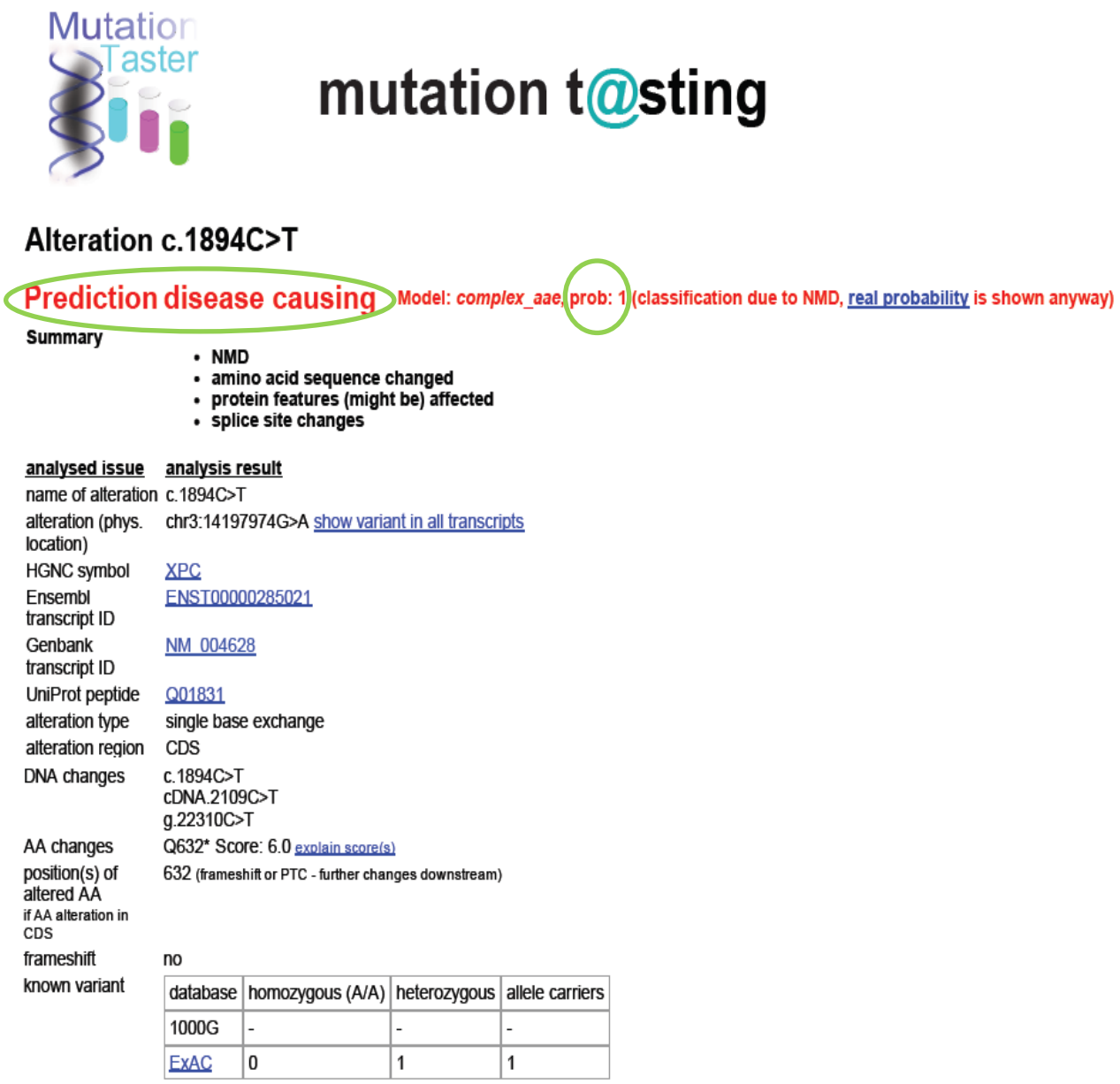


Figure 63: Preview of MutationTaster prediction tool result of the novel nonsense mutation (c.1894C>T, p.Gln632X). The figure shows in the two green circles the "disease causing" prediction result of probability 1. The table refers to the presence of a polymorphism at the same position in the Exome Aggregation Consortium (ExAC) database. NMD refers to nonsense-mediated mRNA decay.

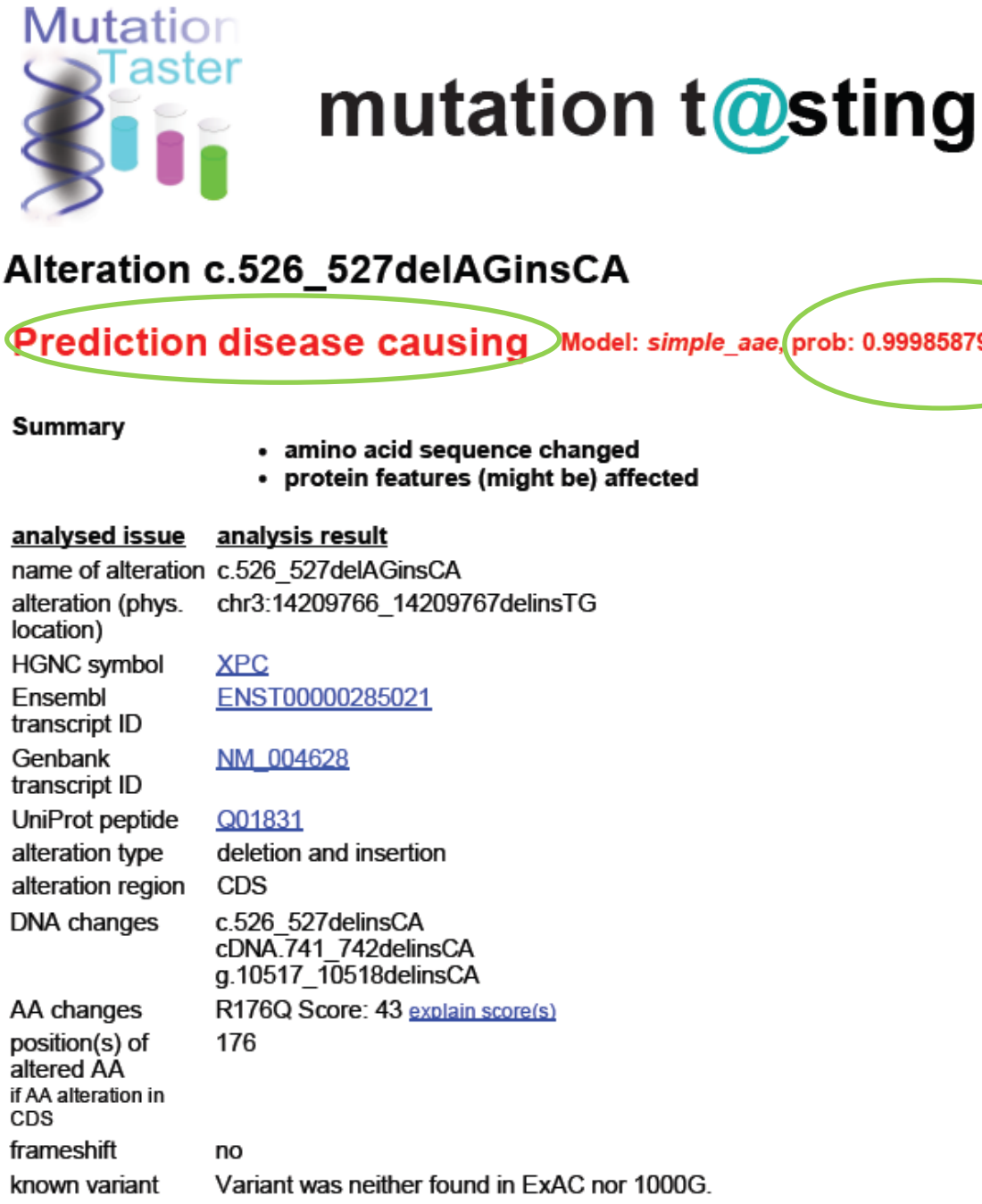


Figure 64: Preview of MutationTaster prediction tool result of the novel insertion deletion (c.526_527delAGinsCA, p.Arg176Gln). The figure shows in the two green circles the "disease causing" prediction result of probability 0.99.

VARIATION		PROTEIN SEQUENCE CHANGE				SIFT PREDICTION			
ROW_NO.	INPUT	PROTEIN_ID	POSITION	RESIDUE_REF	RESIDUE_ALT	SCORE	PREDICTION (cutoff=0.05)	MEDIAN_INFO	#SEQ
1	Q01831,176,R,Q	Q01831	176	R	Q	0.025	Damaging	2.87	59

Figure 65: Preview of SIFT prediction tool result of the novel single amino acid substitution p.Arg176Gln or R176Q. The figure shows SIFT score of 0.025 which is lower than cutoff of 0.05. The mutation is predicted to be "damaging". The prediction was based upon multiple sequence alignment of 59 sequences of 2.87 MEDIAN_INFO (median conservation score, its ideal value is between 2.75 and 3.5)

.

Table 9: Mutation assessor result table for the novel single amino acid substitution p.Arg176Gln or R176Q

Mutation	XPC_HUMAN R176Q
Amino acid variant	R176Q
Gene	XPC
Codon start position	chr3:14184769
FI (Functional Impact)	**medium**
FIS (Functional Impact Score)	**2.35**
MSA height (Multiple Sequence Alignment)	**31**
Uniprot	XPC_HUMAN
Uniprot position	176
Uniprot residue	R
Refseq	NP_004619
Refseq position	176
Refseq residue	R

In bold is the predicted "medium" functional impact of p.Arg176Gln which has a score of 2.35 which is higher than mutation assessor threshold of 1.9 based on alignment with 31 sequences.

Chapter (4): Discussion

Xeroderma Pigmentosum (XP) is one of the rare genetic diseases affecting the skin, the eyes and the nervous system. It is an autosomal recessive disorder caused by defective DNA nucleotide excision repair pathway (NER). The defect accumulates DNA damage products, which eventually cause cancers (Van Steeg & Kraemer, 1999). Clinical symptoms start early in life and are related to exposure to UV of sunlight which causes severe photosensitivity reactions and different types of cutaneous and ocular cancers (Kraemer *et al.*, 1987; Kraemer & DiGiovanna, 2003/2014; Bradford *et al.*, 2011). In some XP patients, neurological symptoms manifest (Rapin *et al.*, 2000). XP has eight different complementation groups which are attributed to mutations in *XPA* to *XPG* and *XPV* (*Pol H*) genes; these genes encode proteins involved in NER pathways (Kraemer *et al.*, 1975a, 1975b; Lehman *et al.*, 1975; Arase *et al.*, 1979; Keijzer *et al.*, 1979). The current study focuses on identifying mutations of *XPA* and *XPC* genes; XP-A and XP-C complementation groups have been reported in Egyptian XP patients (Hashem *et al.*, 1980; Cleaver *et al.*, 1981). Identifying common and rare mutations of *XPA* and *XPC* provides information necessary for preventive measures such as carrier detection, premarital and prenatal diagnosis and counseling as well as for development of potential gene therapies (Ben Rekaya *et al.*, 2009, 2013; Warrick *et al.*, 2012; Dupuy *et al.*, 2013).

4.1. Inheritance and Consanguinity

In autosomal recessive disorders, unaffected carrier parents i.e. heterozygotes, have 25% risk of having affected offspring. Patients can be (1) compound heterozygous harboring two different mutated alleles of the same gene or (2) homozygous who have two identically mutated alleles of the same gene when parents are related i.e. consanguineous or when parents come from limited gene pool with high mutation rate (Mueller & Young, 1995). In a worldwide study of 830 XP cases, parents were reported to be first cousins in 21% of cases, and 67% of cases reported a previously affected XP relative (Kraemer *et al.*, 1987). The consanguinity rates were even higher in populations were XP is prevalent such as Mayotte islands, Japan, and Tunisia (Hirai *et al.*, 2006; Tamura *et al.*, 2010; Cartault *et al.*, 2011). In Egypt, consanguineous marriages represent 33% to 35% which was attributed to cultural, social and economic reasons. The frequency of consanguinity increases in rural more than urban areas. Moreover, consanguinity was reported in 78.8% of the Egyptian families which have history of autosomal recessive diseases (Temtamy *et*

al., 2010; Shawky *et al.*, 2011, 2013). Among the first clinical studies on XP in Egypt, a study reported 100% consanguinity rate in 34 families having 50 XP patients (El-Hefnawi *et al.*, 1964). In a cell complementation study of six Egyptian families, only one family was reported to have no parental consanguinity (Hashem *et al.*, 1980). In the current study, parental consanguinity was confirmed in all families but two (85%). The studied XP patients were equally distributed between Upper and Lower Egypt, mostly from rural regions of their governorates, with no segregation of common mutations in a specific area, see figure 66.

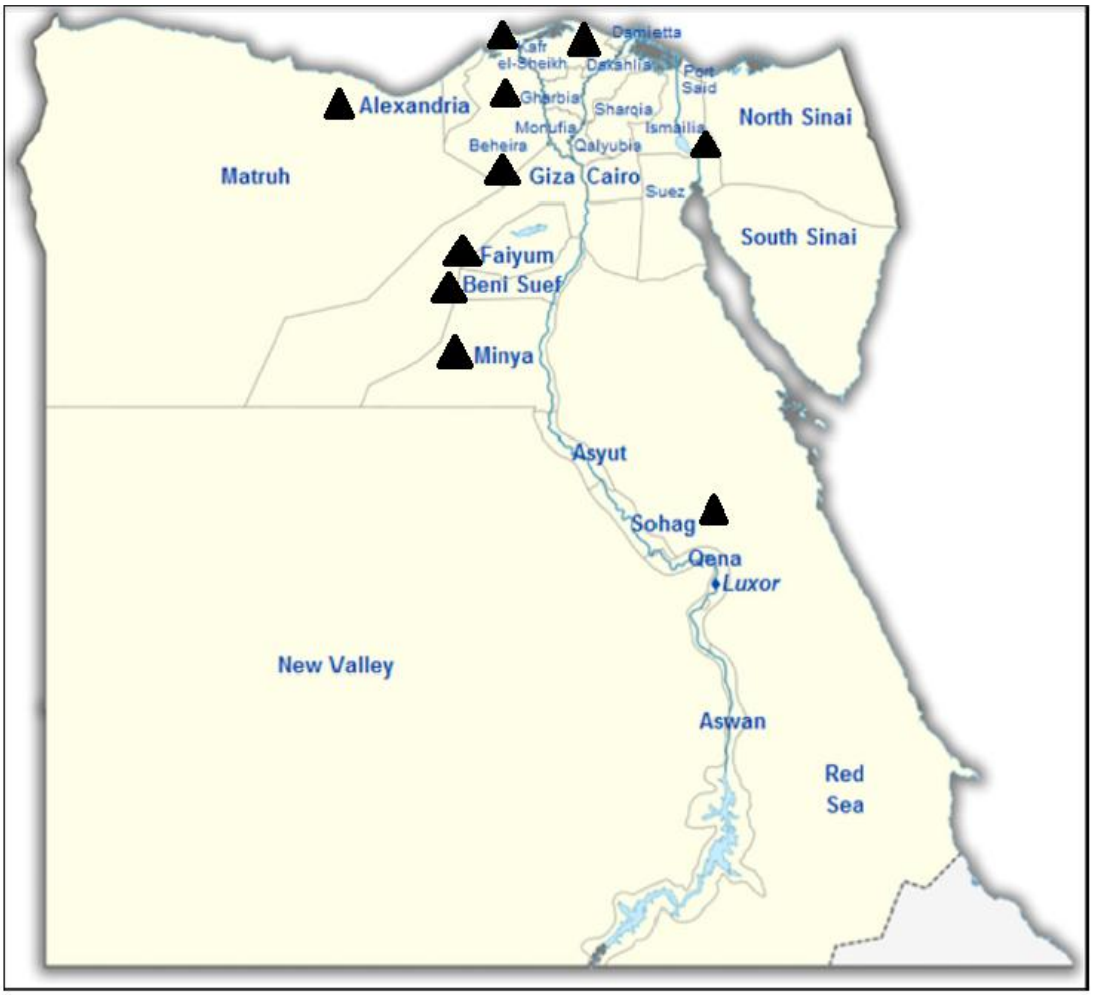


Figure 66: Geographical distribution of the studied XP families on Egypt's map. Black triangles refer to the governorates which represent the origins of the studied families. The map shows equal distribution between Upper and Lower Egypt.

All 17 patients had parents who were first cousins or to a lesser extent second cousins with exception of two families. In family 7, parents of XP9 and XP10, who originated from the same village, denied consanguinity (see figure 15), however, the identified homozygous *XPC* mutation

in XP9 and XP10 (see figure 43) suggests that their parents might have a common ancestor. Parents of XP12 also denied consanguinity and they originate from the same village (see figure 17); this is consistent with the identified compound heterozygous *XPC* mutation see figure 49 and 52, where two different heterozygous mutant alleles were detected.

4.2. Xeroderma Pigmentosum group A

Xeroderma Pigmentosum group A (XP-A) is attributed to mutations in *XPA* gene which has 6 exons and is located on chromosome 9. This group has the least residual DNA repair activity and is considered the most severe among XP groups (Van Steeg & Kraemer, 1999). Phenotype of group A is heterogeneous. An established phenotype-genotype correlation is that XP-A involves early onset of photosensitivity and neurological symptoms of different onsets which range from mild to severe (Rapin *et al.*, 2000; Bradford *et al.*, 2011). Consequently, patients diagnosed clinically as XP with neurological abnormalities were screened first for *XPA* gene mutations. Indeed, neurological abnormalities were observed only in 4 studied XPA patients among 17 Egyptian XP patients herein. Clinical variability depends upon the mutation site within *XPA* gene, hence the residual DNA repair activity. Exons 3, 4 and 5 of *XPA* encode the DNA binding domain of XPA protein; mutations in these exons are reported to account for severe clinical pictures (States *et al.*, 1998; Bartels & Lambert, 2007; Takahashi *et al.*, 2010). As a result, exons 3, 4 and 5 of *XPA* gene were targeted first for mutation detection. In the current study and the study by Amr *et al*, all the detected *XPA* mutations among XP-A Egyptian patients were confined to exons 3, 4 and 5 (Amr *et al.*, 2014). Direct gene sequencing was employed in both studies for mutation detection because all the previously identified 36 *XPA* gene mutations were classified as single base substitutions and small deletions or insertions or indel (insertion-deletion; see figure 4) which can be easily explored by direct gene sequencing (Stenson *et al.*, 2014).

4.2.1. Clinical characterization of XP-A patients

Cell complementation studies on Egyptian XP patients have identified four XP-A patients descending from three unrelated families; patients had median age of 7 years and their symptoms started at median age of 4 months (Hashem *et al.*, 1980; Cleaver *et al.*, 1981). Amr *et al.* studied four XP-A patients descending from four unrelated Egyptian families where three *XPA* mutations were identified; median age of patients was 8 years and their symptoms started at median age of

5.5 months (Amr *et al.*, 2014). The current study involved four patients (XP1 to XP4) descending from three unrelated consanguineous families diagnosed clinically as XP with neurological abnormalities; their median age was 4 years and symptoms started at median age of 4 months. Collectively, XP started as early as the first 6 months of life in Egyptian XP-A patients. All XP-A Egyptian patients have presented with profound photosensitivity, microcephaly and mental retardation with only one exception in the study by Cleaver *et al.* where a patient had 35 years with no neurological symptoms (Cleaver *et al.*, 1981).

In the current study, XP1 to XP4 have shown early onset mental retardation, microcephaly, cerebellar affection and delayed speech with neither dwarfism nor hypogonadism, see table 4. Other neurological abnormalities were reported such as hearing loss in XP1, and axonal neuropathy in XP2. Limb anomalies were observed in XP2. Similarly, Amr *et al.* reported severe phenotype of Egyptian XPA. In agreement with phenotype-genotype correlation, severe clinical picture of XP1 to XP4 and of the patients studied by Amr *et al.* can be attributed to mutations in exons 3, 4 and 5 (Amr *et al.*, 2014).

In concordance with the earlier Egyptian studies and recent studies on different populations, XP1 to XP4 patients had early onset photosensitivity (Cleaver *et al.*, 1981; Bradford *et al.*, 2011; Sethi *et al.*, 2013). Early photosensitivity increases parental awareness of the importance of patients' protection from sunlight; thus protection was early implemented in XP1, XP3 and XP4 and decreased their ocular symptoms and proneness to cancers. To illustrate, XP2 and XP3 are two siblings with the same *XPA* mutation but different clinical severity and picture. XP2 was 7 years old, the first case in her family, and she was protected from sunlight later in her life, thus she developed multiple recurrent skin and ocular cancers. On the other hand, XP3, who was 4 years, is the second case in the family, and she was protected from sunlight since birth, thus she had no obvious classical XP abnormalities. In addition to parental awareness of sunlight protection, it was suggested by Amr *et al.* that age difference may play a role in the development of cancers i.e. XP patients who are older in age have more chance for developing cancers. On the contrary, Amr *et al.* studied XP1GE patient who was 8 years old i.e. one year older than XP2, however, XP1GE had not developed any malignancies as in case of the 4 years old XP3 herein. Neurological manifestations were reported in both XP2 and XP3 confirming the independence of neurodegeneration from UV protection and supports the hypothesis that accumulation of

endogenous DNA damage lesions and oxidative stress could cause brain atrophy and other accompanying neurological symptoms in XPA patients (Rapin *et al.*, 2000).

4.2.2. Mutational spectrum of *XPA* in the Egyptian Population

Mutation analysis in the current study revealed three previously identified homozygous *XPA* mutations in exons 3, 4, and 5 which encode the central DNA binding domain of XPA protein, see table 8 and figure 3. The first is the homozygous single nucleotide substitution of cytosine by thymine in the last codon of exon 4 which changes CAG of glutamine to TAG i.e. a premature termination codon (c.553C>T, p.Gln185X; see figures 28 to 30). Nonsense mutations, as well as mutations which create premature termination codons, produce unstable short mRNAs that are susceptible to NMD (nonsense-mediated mRNA decay). NMD is a protective pathway in mammals which downregulates unstable mRNAs to decrease expression of potentially harmful truncated proteins (Maquat, 2005). Homozygous p.Gln185X mutation was detected in XP1 of family 1 herein; XP1 had two out of three siblings who were heterozygous for p.Gln185X allele. The same homozygous p.Gln185X mutation was first reported by Amr *et al.* in two patients descending from two unrelated families (Amr *et al.*, 2014). Of interest is that p.Gln185X has not been reported before except in the Egyptian population, hence the current study suggests the presence of a founder effect for this private mutation which needs to be confirmed by haplotype analysis. In brief, a haplotype is a series of single nucleotide polymorphisms (SNPs) surrounding a specific mutation. The presence of the same haplotype in different patients carrying the same mutation indicates "a founder effect" i.e. a common ancestor. On the other hand, the recurrence of the same mutation in different patients carrying different haplotypes indicates a hot spot region (Tamura *et al.*, 2010).

The second *XPA* gene mutation is a frameshift mutation detected in XP2, XP3 and a fetal DNA of the same family due to homozygous deletion of one cytosine nucleotide in exon 3 (c.374delC, p.Thr125IlefsX15, see figures 31 to 33) which results in a premature termination codon 14 codons downstream. Premature termination codon causes instability of XPA mRNA via NMD, production of truncated XPA protein, and nullification of the residual DNA repair activity. The c.374delC mutation was first reported in a Caucasian patient from Europe, and then in one Egyptian patient (Satokata *et al.*, 1992b; Amr *et al.*, 2014). Detection of c.374delC mutation in the current cohort suggests a founder effect which requires haplotype analysis for confirmation. Migration flow between Europe and North Africa could explain the occurrence of the same

mutation in two different continents; the same elucidation observed in other genetic disorders, including XP-C (Ben Rekaya *et al.*, 2009).

The third mutation is another nonsense mutation in exon 5 of XP4 due to single nucleotide substitution of cytosine by thymine which converts CGA of arginine to TGA i.e. a premature termination codon (c.619C>T, p.Arg207X, see figures 34 to 36), hence a truncated protein would be produced. The p.Arg207X nonsense mutation was identified for the first time in a Palestinian XP patient who had severe skin symptoms and De Sanctis-Cacchione syndrome; the nonsense mutation destabilizes mRNA and produces insignificant residual DNA repair (Satokata *et al.*, 1992a). The same p.Arg207X mutation was reported in a Brazilian XP patient who had severe photosensitivity but intermediate onset of neurological symptoms although the patient had complete absence of XPA protein. XPA mutations are very rare in Brazil (Santiago *et al.*, 2015). It can be suggested that the migration flow of Arabs from Ottoman Empire to Brazil in the late 19^{th} century could play a role in the detection of Arabian genetic alleles in Brazil in some genetic disorders provided that a common ancestor is proposed (Alberto *et al.*, 1999).

To outline the *XPA* mutational spectrum in Egypt, the current study complements the earlier molecular study by Amr *et al.* (Amr *et al.*, 2014). Collectively, four *XPA* mutations have been identified in the Egyptian population: (1) the private p.Gln185X which was confined to Egyptians only, (2) the c.374delC which was identified also in a Caucasian patient from Europe, (3) the p.Arg207X which was identified in one Palestinian and one Brazilian XP patients, and (4) a p.Glu111X mutation which was identified by Amr *et al.* in an Egyptian patient and identified previously in three patients from Tunisia, hence a common ancestor was proposed (Messaoud *et al.*, 2012), see figure 67. It is evident that all four *XPA* mutations are truncating mutations due to premature termination codons which disrupt the central domain of XPA protein (from amino acid 98 to 219) and interferes directly with the ability of XPA protein to bind to damaged DNA lesions (Bartels & Lambert, 2007). Consequently, all patients had severe phenotype of XP-A with marked early onset of skin photosensitivity and devastating neurological symptoms. The effect of genetic counseling and parental awareness of the importance of protection from exposure to sunlight and other UV sources has direct impact on development of malignancies, but not on the neurological affection.

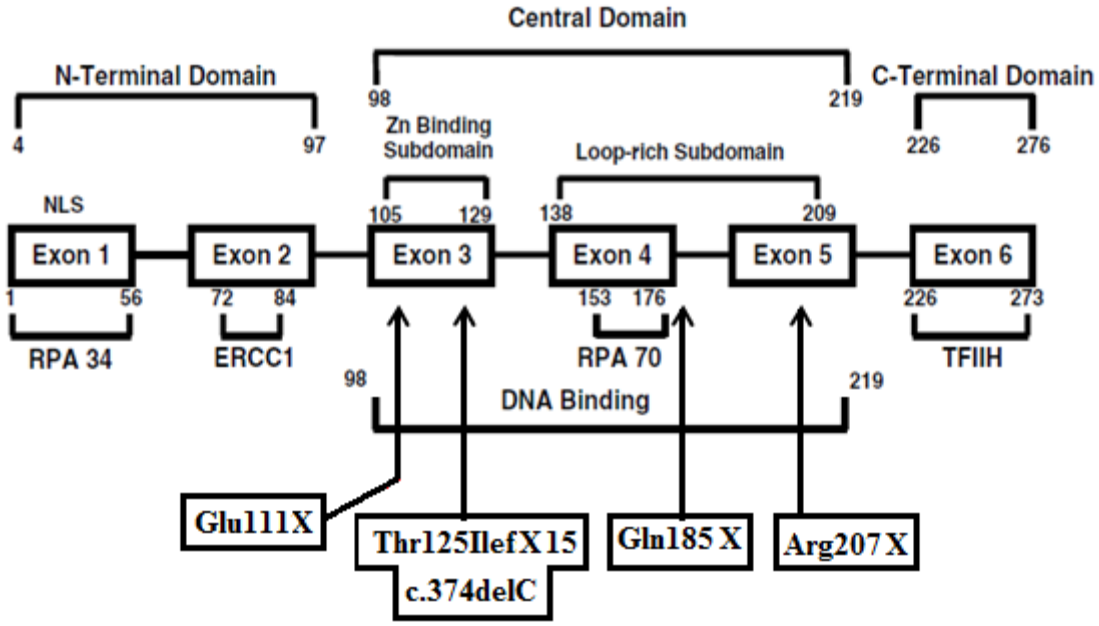


Figure 67: Mutational Spectrum *XPA* gene in the Egyptian population. The figure illustrates the four XPA mutations which were identified in Egyptian XPA patients. Mutations are described in text; see figure 3 above for full description (original figure was obtained from Bartels & Lambert, 2007 with permission).

4.3. Xeroderma Pigmentosum group C

Xeroderma Pigmentosum group C is attributed to mutations in *XPC* gene on chromosome 3. Residual DNA repair activity of XP-C ranges from 10-20% of normal which is larger than that of XP-A (Van Steeg & Kraemer, 1999). XP-C phenotype usually shows XP without neurological abnormalities but with early onset recurrent skin cancers due to comparatively lower UV exposure resulting from lower photosensitivity than XP-A (Bradford *et al.*, 2011; Sethi *et al.*, 2013). Accordingly, the 13 XP patients in the current study who showed no evidence of neurological abnormalities were screened for *XPC* mutations first. This is the first study to identify *XPC* mutations in Egyptian XP patients, and their related family members. Few Egyptian XP patients with XP have been reported to be among XP-C complementation group via complementation test. Nevertheless, XP-C was relatively more frequent than XP-A in the studied XP patients. (Cleaver *et al.*, 1981). .*XPC* gene has 16 exons. To date, 60 mutations have been reported in XPC patients; the majority of these mutations were detectable by direct sequencing, see figure 7 (Stenson *et al.*, 2014). Being the largest *XPC* exon, exon 9 harbors the largest number of mutations, see figure 8. Moreover, a North African founder mutation in exon 9 was

identified in large number of XPC patients in the Maghreb region; the same mutation was reported in a sole study on an Egyptian banked cell line (Li *et al.*, 1993; Ridley *et al.*, 2005; Soufir *et al.*, 2010). Accordingly, the current study targeted exon 9 first by direct gene sequencing which successfully identified pathogenic mutations in six patients.

4.3.1. Clinical characterization of XP-C patients

Eight Egyptian patients were previously assigned to XP-C group via cell complementation test in the study by Cleaver *et al.*; their median age was 7 years and their symptoms started at 4 months to 3 years (median age = 11 months) (Cleaver *et al.*, 1981). In the current study, 13 patients were found to be XP-C via direct sequencing of *XPC* gene; their median age was 10.5 years and their first symptoms appeared also from 6 months to 3 years (median age = 8 months). Overall, it is observed that XP-C (median age of onset = 8-11 months) has slightly later onset than XP-A (median age of onset = 4-5.5 months). It is possible that the sunny weather in Egypt around the year is linked to the cutaneous nature of first symptom among the 17 studied patients and the ocular symptoms observed in all XP-C patients, and to lesser extent XP-A patients.

No neurological symptoms were reported in XPC Egyptian patients in the cell complementation study by Cleaver *et al*. or XPC patients herein. Previous studies have suggested that the exception of occurrence of neurological affection among XP-C patients could be explained by the factor of consanguinity which could result in homozygous mutations in other unidentified genes (Khan *et al.*, 2009; Soufir *et al.*, 2010; Schäfer *et al.*, 2013). The former suggestion might explain the occurrence of genetic abnormalities, other than XP, among some of the studied families, see figure 9, 11 and 13.

The clinical data of the current cohort, displayed in table 5, 6 and 7, show cutaneous and ocular symptoms of variable severity and recurrence of different types of cancers which is the typical description of XP-C in other populations (Soufir *et al.*, 2010; Cartault *et al.*, 2011). Ocular symptoms such as conjunctivitis and keratitis were as common as skin symptoms in XP-C. Skin cancers were detected in 69% of XP-C patients, mainly BCC and SCC with only one case of skin melanoma (XP15; see table 6). Melanomas are generally rare in XP patients and usually appear in XP patients of relatively old age which was the case for XP15 who was 32 years old (Kraemer *et al.*, 1987; Bradford *et al.*, 2011). Ocular malignancies occurred in 53% of the studied XP

cohort herein, 61% in XP-C alone, which is close to a study by Mortada who reported 40% ocular malignancies in 55 unclassified Egyptian XP patients (Mortada, 1967).

Age difference may play a role in terms of the development of cancers. The youngest XP patients were XP6 and XP7 of age 5.5 and 4 years, respectively; they had no history of cancers' development and did not present any tumors at examination. XP12 who was 6 years had no history of cancers too, but at examination a small submandibular tumor was observed of unknown nature. XP12 was found to have compound heterozygosity for two different mutations which could contribute to decreased severity of tumor development; this was also observed in compound heterozygotes of both *XPC* and *XPA* genes' mutations (Negishi *et al.*, 2001; Yasuda *et al.*, 2007; Jacobelli *et al.*, 2008; Meneses *et al.*, 2015). Conclusively, the decreased rate of tumor development in XP6, XP7, and XP12 might be attributed to their young age and the previously recognized late onset of recurrent tumors in XP-C (Bradford *et al.*, 2011; Sethi *et al.*, 2013). On the other hand, the three oldest XP patients were XP8, XP9, and XP15 of age 25, 16 and 32 years, respectively; they had the latest onset of XP symptoms; between 2 and 3 years which is in agreement with the study by Cleaver *et al.* where XP-C patients of late onset (2-4 years) were the oldest in age among their studied cohort (Cleaver *et al.*, 1981); thus molecular diagnosis provides an effective tool to pre-symptomatically identify these cases as early as possible (Jerbi *et al.*, 2016). Late onset of symptoms implies late diagnosis and consequent late protection from sunlight which accounts for recurrent tumors. Nevertheless, it was the poikiloderma (hyper-& hypopigmented patches) on sun exposed areas of the skin that was observed the first symptom not sunburns; hence, parental awareness of protection from sunlight might be hindered by the relatively lower photosensitivity of XP-C, consequently the higher tumor occurrence and recurrence (Bradford *et al.*, 2011; Sethi *et al.*, 2013).

When the clinical data were segregated according to the detected *XPC* mutation, see table 5 and 7, no significant clinical differences could be detected between patients having the same genetic mutations and patients with other mutations. The c.2251-1G>C genetic mutation of XP5 to XP8 was reported previously as a founder mutation in black Mahori patients while XP14 to XP17 patients had the well-established c.1643_1644delTG North African founder mutation. Comparison between the Egyptian Caucasian XP5 to XP8 patients and the black Mahori patients can provide an insight of the effect of different ethnicities on the clinical presentation of XP because they all share a common mutation. The majority of Black Mahori XPC patients had first

symptoms of ocular nature unlike the cutaneous nature in case of Egyptian XPC patients bearing the same mutation (Cleaver *et al.*, 1981; Cartault *et al.*, 2011). On the other hand, the clinical presentation of XP14 to XP17 patients is similar to that observed in North African patients who had the same North African founder mutation in terms of low to moderate photosensitivity, multiple recurrent skin cancers and the occurrence of ocular symptoms (Soufir *et al.*, 2010; Jerbi *et al.*, 2016).

4.3.2. Mutational spectrum of *XPC* in the Egyptian Population

Thirteen patients descending from 11 unrelated Egyptian families were found to have *XPC* mutations. Seven different mutations are reported herein; interestingly, two mutations were more prevalent; one was a North African founder mutation, and the other was a founder mutation in Mayotte of the Comorian islands. Four patients (XP14 to XP17) were homozygous for the North African small deletion of two base pairs from exon 9 (c.1643_1644delTG, see figures 56 to 61). Parents of XP14 to XP17 and an unaffected sibling of XP14 were found to be heterozygous carriers of c.1643_1644delTG. The deletion of thymine and guanine bases (TG) at positions 1643 and 1644, respectively, of *XPC* coding region changes GTG codon of the amino acid valine at position 548 to GCA codon of alanine, a frameshift results, and a premature termination codon (TGA) at position 25 of frameshift (p.Val548AlafsX25), see figure 57. The premature termination codon renders an unstable *XPC* mRNA which is prone to nonsense-mediated mRNA decay (NMD), hence the low level of *XPC* mRNA. *XPC* mRNA levels of XPC patients which have c.1643_1644delTG mutation decayed to less than 25% of normal levels (Schäfer *et al.*, 2013). Chavanne *et al.* reported the existence of another abnormal mRNA probably due to aberrant splicing at a cryptic splice site (c.1627) rather than the authentic one at the beginning of intron 9; they explained that the two nucleotide deletion (TG) in mRNA might cause changes in mRNA secondary structure favoring the aberrant splicing (Chavanne *et al.*, 2000). The c.1643_1644delTG mutation would produce a protein of 571 amino acids instead of 940 amino acids which resulted in the truncation of the majority of C-terminal region of XPC, see figure 6, where the majority of XPC functional domains exist, including DNA binding domain. Normal XPC protein was completely absent in keratinocytes and fibroblasts of XPC patients with homozygous c.1643_1644delTG mutations, therefore, it was concluded that XPC is not indispensible for cellular proliferation or cellular viability (Chavanne *et al.*, 2000; Ridley *et al.*, 2005; Rezvani *et al.*, 2008). Nevertheless, homozygous c.1643_1644delTG mutations were

reported to reduce residual DNA repair to less than 10% of normal which explains severity of clinical picture (Soufir *et al.*, 2010).

The c.1643_1644delTG mutation was first identified by Li *et al.* in an XP cell line (Li *et al.*, 1993). Many XPC patients, mostly from North Africa, were reported to have c.1643_1644delTG mutation in homozygous state, see table 10; the largest cohort from Tunisia and Morocco (Ben Rekaya *et al.*, 2013; Senhaji *et al.*, 2013; Jerbi *et al.*, 2016). Soufir *et al.* identified c.1643_1644delTG in 87% of North African XPC patients. Haplotype analysis has proven the presence of common ancestor of the c.1643_1644delTG mutation in Maghreb countries, namely Tunisia, Algeria and Morocco. The common ancestry was estimated to have occurred 1,250-1,500 years ago; this dates with the Saracens' i.e. Arab-Muslims' conquest of South Europe. As a result, Soufir *et al.* proposed that founder effect included the Mediterranean region because of previous reports in Egypt, Spain and Italy (Soufir *et al.*, 2010). The current study highlights the recurrence of c.1643_1644delTG mutation in Egypt, the studied XP patients herein which hold the same mutation show similar clinical picture of North African cohort. Egypt and North African countries, especially Tunisia, hold the same historical rule of Abbasids (641-969) and Fatimid dynasties (969-1171) which extended to the Middle East(Amr *et al.*, 2014). Unsurprisingly, c.1643_1644delTG mutation was also reported in Sudanese patients in the Middle East and German patients of Arabian and Libyan ancestries, see table 10 (Mahindra *et al.*, 2008; El-Harith *et al.*, 2012; Schäfer *et al.*, 2013).

Table 10: Previous reports on c.1643_1644delTG (p.Val548AlafsX25) mutation

Origin	Cohort	Genotype	Reference
Italian	One patient	Homozygous	Chavanne *et al*., 2000
Egypt	One cell line	Homozygous	Ridley *et al.*, 2005
African American	One cell line	Heterozygous	Khan *et al*., 2006
Spanish	One cell line	Heterozygous	
Moroccan	One cell line	Homozygous	
Algerian	One cell line	Homozygous	
Sudanese	Two brothers	Homozygous	Mahindra *et al*., 2008
Tunisian	20 patients from 14 families	Homozygous	Ben Rekaya et al., 2009
North African from Maghreb countries	56 patients from 49 families	Homozygous	Soufir *et al.*, 2010
Sudanese	Two siblings	Homozygous	El-Harith *et al*., 2012
Moroccan	17 patients from 16 families	Homozygous	Senhaji *et al.*, 2013
German of Arabic ancestry	Three patients	Homozygous	Schäfer *et al.*, 2013
German of Libyan ancestry	One patient	Homozygous	
Tunisian	60 patients from 43 families	Homozygous	Jerbi *et al.*, 2016

The c.1643_1644delTG (p.Val548AlafsX25) mutation represents 36% of the 13 Egyptian XPC patients herein which comparable to 76% of 24 Moroccan XPC patients and 93% of 64 Tunisian

XPC patients (Senhaji *et al.*, 2013; Jerbi *et al.*, 2016). It is debatable that a different common ancestor might exist, therefore, haplotype analysis can provide further insight on common ancestry with consequent implication on cost effective molecular diagnosis and screening.

The second *XPC* mutation recurred in four patients (XP5 to XP8) descending from three unrelated pedigrees, see figures 37 to 40. The splicing mutation was found in homozygous state where guanine base was substituted by cytosine at the last base of 3' end of intron 12 (c.2251-1G>C) i.e. one base upstream to exon 13, therefore, the AG splice acceptor site of intron 12 changed into AC. Cartault *et al* reported this mutation previously in 22 black XP patients. Molecular characterization of c.2251-1G>C has shown that the disruption of the AG splice acceptor site of intron 12, via change into AC, results in aberrant splicing. Three abnormal mRNAs were detected in XPC patients with c.2251-1G>C mutation: one where intron 12 was retained, the second where exon 13 was skipped and the third were 44 base pairs were deleted from exon 13. Residual DNA repair was as low as 15% of normal (Cartault *et al.*, 2011). Similar molecular consequences and abnormal splicing products were reported in a black XP patient who had deletion of the two AG base pairs of the splice acceptor site of intron 12 with the insertion of two base pairs (CC) instead at 3 base pairs upstream of the deleted splice acceptor site (Khan *et al.*, 2006).

The c.2251-1G>C splicing mutation was reported only in 22 black Mahori XP patients from 17 families where all patients were homozygous; thus a founder effect was proposed and proven. The Mahori population is a small private population of the Comorian islands with high consanguinity, geographical isolation, and unified life style of its people which led to exceptionally high prevalence of XP (one per 5,000 individuals). The age of c.2251-1G>C splicing mutation was to be approximately 770 years old coinciding with Mahori migration from Africa. The recurrence of this mutation in our Caucasian Egyptian cohort suggests a common ancestor due to migration flow inside Africa, possibly by trade routes between Egypt and the Mayotte islands of the Indian Ocean. As mentioned before, clinical features of Caucasian XP5 to XP8 are homogenous with those of the black Mahori XP patients who have the same c.2251-1G>C splicing mutation with exception of ocular symptoms that tend to start in black XP patients earlier than in Caucasians; this exception is probably ethnic rather than mutation related given the same observation in black XP patients when compared to XP Caucasians with different *XPC* mutation (Cartault *et al.*, 2011). Consequently, no specific phenotype correlates with

c.2251-1G>C splicing mutation genotype. With reference to molecular diagnosis, it is recommended that screening for c.2251-1G>C should not be exclusive to black African XP families but rather to African XP families in general.

A single nucleotide polymorphism (SNP; rs2279017; c.2251-6C>A) in intron 12 was identified 6 bases upstream to the start of exon 13 of *XPC* i.e. 6 bases upstream to the c.2251-1G>C mutation. The C allele of this SNP was found to be associated with c.2251-1G>C mutation in Mahori population (Cartault *et al.*, 2011). In the current cohort, XP5 to XP8 who were homozygous for c.2251-1G>C mutation were also homozygous for C allele of c.2251-6C>A SNP, i.e. C/C genotype, while their parents were heterozygous for c.2251-6C>A SNP i.e. A/C genotype, see figure 38 to 40, with exception to father of XP5 and XP6 who had C/C genotype, see figure 38. Interestingly, one patient with a different *XPC* mutation, XP9, also had C/C genotype while three patients (XP12, XP16 and XP17) had A/A genotype of c.2251-6C>A SNP. It can be concluded that C/C genotype of c.2251-6C>A SNP might not be exclusively associated with *XPC* c.2251-1G>C mutation. The association between c.2251-6C>A SNP and cancer risk was addressed in some studies. Previously Khan *et al.* identified the c.2251-6C>A SNP and genotyped it together with another two *XPC* SNPs in a small cohort of human fibroblast cell lines. They explained that A/A genotype of c.2251-6C>A SNP increased the level of a mRNA isoform that skipped exon 13 in normal fibroblast cells; hence, DNA repair was decreased which might contribute to increased cancer-risk (Khan *et al.*, 2002). Recently, a large case-control study on melanoma identified a series of 5 SNPs within *XPC* gene, including one C allele of c.2251-6C>A; these SNPs form a distinctive haplotype which was associated with increased risk for melanoma (Torres *et al.*, 2013). Another case-control study associated C/A genotype with sporadic colorectal cancer risk (Gil *et al.*, 2012). Further studies are required to assess association of c.2251-6C>A SNP with melanoma or generally carcinoma risks in the Egyptian population especially in family members of *XPC* patients to benefit genetic counseling for internal cancer investigation.

Two previously reported mutations were reported in XP12 and XP13. XP12 was found to be heterozygous for c.1103_1104delAA where two adenine base pairs (AA) were deleted from exon 9. Similarly, father of XP12 was heterozygote c.1103_1104delAA mutant allele, see figures 50 to 52. The AA deletion changes CAA codon of glutamine amino acid at position 368 of XPC protein to CGA codon of arginine, and results in a frameshift where TAA stop codon is created

at position 373 of XPC (p.Gln368ArgfsX6). With premature termination of translation, a truncated protein of 372 amino acid might be produced which lacks the entire C-terminal domain and a part of N-binding domain, see figure 6. The sole previous report of c.1103_1104delAA was in an Italian XP patient, also in a heterozygote status, where *XPC* mRNA expression was reduced, DNA repair activity fell to 20% of normal and Western blot failed to detect normal XPC protein. The Italian XP patient was compound heterozygote i.e. heterozygote of 2 mutant alleles: the c.1103_1104delAA in exon 9 and c.128delC (Pro43HisfsX36) in exon 2 (Chavanne *et al.*, 2000). Besides heterozygote c.1103_1104delAA mutation, XP12 was found to carry maternal allele with novel indel (insertion deletion) to be discussed later. Similar to XP12, the Italian XP patient with the same heterozygote mutation was among the three youngest Italian XP patients and had not shown any tumors. XP12 had her first XP symptoms at 8 months but she has not developed any tumors except a small submandibular tumor at age of 5.5 years. It is questionable whether this would be attributed to compound heterozygosity or the relatively young age of both the Italian patient (4 years) and XP12 (6 years).

In XP13, a previously identified nonsense mutation was detected due to substitution of cytosine by thymine nucleotide close to 3' end of exon 9 at position 1734 of *XPC* coding region (c.1735C>T), as a result, CGA codon of arginine becomes a premature stop codon TGA at 579th amino acid position (p.Arg579X), see figures 53 to 55. The resultant *XPC* transcript was of normal size but comprised the C to T substitution. Another *XPC* transcript was detected by in an Italian XP patient and three Turkish XP patients who were homozygous for the same c.1735C>T mutation. Surprisingly, the abnormal transcript was the same transcript caused by the c.1643_1644delTG mutation. Both mutations are located at 3' end of exon 9 (882 base pairs) cause aberrant splicing indirectly. By computational analysis, the cryptic splice donor site at 1627th nucleotide of exon 9 was predicted to be activated by both mutations via changes in secondary structures of their correspondent mRNAs; therefore, the abnormal transcript that lacks 246 nucleotides from exon 9 is produced. Overall, the mRNA level was reduced, most probably via nonsense-mediated mRNA decay of the short length transcripts, and DNA repair level fell to 14-20% of normal. As expected, the truncated protein (p.Arg579X) was not recruited to the DNA damage sites, thus it failed to excise DNA damage lesions (De Jesus *et al.*, 2008). The homozygous c.1735C>T mutation was notably associated with severe phenotype and reports of premature death. XP13 had a sibling and two cousins who were XP patients and died at young

age; one cousin died at age of 7 years, see figure 18. When one healthy cousin and his mother were screened for c.1735C>T mutation, they were found to be heterozygous carriers. The one Italian patient died at 15 years age, and two of the three Turkish patients from same family died at 10 and 16 years. Similar to these four patients, XP13 had shown recurrent cutaneous and ocular malignancies of early onset. The occurrence of the homozygous c.1735C>T mutation in both Italy and Turkey was attributed to common ancestry which dates back to about 300-540 years ago. It is possible that XP13 has common ancestry with the reported Italian and Turkish families. Egypt is historically and geographically linked to both Italy and Turkey, particularly, during Roman and Ottoman age and through Mediterranean Sea (Chavanne *et al.*, 2000; Gozukara *et al.*, 2001).

Three XPC novel mutations are reported herein. XP9 and XP10, two siblings, were found to be homozygous for c.668_669delTC mutation in exon 6. Heterozygous sequence of c.668_669delTC mutation was detected in their parents, but not in their healthy sibling, see figure 43. No mutations were identified in this location before according to Human Genome Mutation Database (HGMD). By analysis of *XPC* CCDS (CCDS46763.1), the novel TC deletion causes a frameshift mutation. The ATC codon of isoleucine at position 223 of XPC protein becomes ATG codon of methionine and a premature termination codon (TAA) is created 44 codons downstream (p.Ile223MetfsX45) i.e. at position 267. The resultant truncated protein is predicted to be 267 amino acids long instead of the complete 940 amino acids long XPC protein which corresponds to the absence of both half of N-terminal domain and C-terminal domain where all the known functional domains of XPC exist, see figure 6. MutationTaster was used to predict functional effect of this novel deletion, see figure 62. The c.668_669delTC mutation (p.Ile223MetfsX45) was predicted to be disease causing (probability = 1) due to its susceptibility to nonsense-mediated mRNA decay (NMD). A splice donor site might be gained at c.663 which results in abnormally spliced mRNA which loses 116 nucleotides from 3' end of exon 6. Characteristically, both XP9 and XP10 have shown poikiloderma all over the body rather than being confined to sun exposed areas of the body. XP9 who was 16 years started tumor development at 6 years old and XP10 who was 12 years old started at 3 years old. Interestingly, both XP9 and XP10 had aggressive ocular and facial malignancies at young age which progressed to the excision of one eye in XP9 and both eyes, mandible and nose in XP10.

The second novel mutation was a nonsense mutation that was identified in XP11 who had a homozygous C to T substitution (c.1894C>T) at exon 10 which changed in CAG codon of glutamine into a premature termination codon (TAG; p.Gln632X; figures 44 to 46). Both parents and a sibling were heterozygote for the c.1894C>T mutation. Expectantly, it was predicted by MutationTaster that (c.1894C>T; p.Gln632X) is disease causing mutation (probability = 1). The resultant short mRNA which encodes 631 amino acids would be susceptible to NMD, see figure 63. No abnormally spliced mRNA was predicted to be produced but rather the scores of the original splicing acceptor and donor sites were increased. The resultant truncated protein would have an interrupted DNA binding and hHR23B domains with complete loss of centrin2 and TFIIH binding domains, and the region necessary for XPC recruitment to GGR pathway, see figure 6. XP11 who was 12 years old had developed first symptoms of XP at 9 months. Characteristically, XP11 had actinic keratosis, corneal opacity and developed small BCC at anterior chest wall at first, then recurrent BCC of the skin.

As mentioned before, XP12 was found to be heterozygous for c.1103_1104delAA which was inherited paternally. In consistency with non-consanguineous parents, another heterozygous allele was inherited maternally. The maternal allele was a novel heterozygous deletion of AG and concurrent insertion of CA at the same position (c.526_527delAGinsCA) of exon 4 of *XPC*, see figures 47 to 49. Since the two deleted bases were replaced by another two bases, no frameshift occurs. The insertion deletion (indel) was predicted to cause single amino acid substitution where AGA codon of arginine would be replaced by CAA codon of glutamine (p.Arg176Gln) in XPA binding domain of XPC protein, see figure 6.

To validate the damaging effect of p.Arg176Gln, three different computational prediction tools were used: MutastionTaster, SIFT and mutation assessor. MutationTaster predicted that c.526_527delAGinsCA (p.Arg176Gln) to be "disease causing" mutation (probability = 0.99) when compared to functionally characterized pathogenic mutations from HGMD and 1000 Genome project (1000G), see figure 64 (Schwarz *et al.*, 2014). SIFT tool predicted that the single amino acid substitution (p.Arg176Gln) has "damaging" effect with a score of 0.025 (cutoff = 0.05) compared to 59 closely similar protein sequences, see figure 65 (Kumar *et al.*, 2009). Mutation assessor tool assigned p.Arg176Gln to "medium functional" impact on XPC protein functions with score of 2.35 (cutoff = 1.9) where the mutated XPC protein was compared to 31 related protein sequences, see table 9 (Reva *et al.*, 2011). In the three prediction tools, no

SNPs were identified at the same nucleotide position. Conclusively, it can be predicted that the p.Arg176Gln single amino acid substitution affects the XPC protein structure, hence it is possibly pathogenic and further functional characterization is required. This is the first report of a single amino acid substitution due to indel in *XPC*. All reported single amino acid substitutions in XPC were due to missense mutations which do not alter the length of the protein, unlike frameshift mutations. Nonsense and frameshift mutations which result in protein truncations tend to have more drastic cellular effects (Yasuda *et al.*, 2007; De Jesus *et al.*, 2008). Only two small indels were previously reported in *XPC*, none of them resulted in a single amino acid substitution (Khan *et al.*, 2006; Sun *et al.*, 2015; Vaz-Drago *et al.*, 2015). Five pathogenic missense mutations have been reported in *XPC* before; all of them were due to single nucleotide substitution. Interestingly, three of these pathogenic missense mutations were identified in one allele of compound heterozygous XPC patients while the other mutant allele showed a frameshift mutation. In these three reports, patients were reported to have long survival and delayed onset of either tumor development or of XP symptoms. On the cellular level, both mutated *XPC* mRNA and protein were reduced to low levels. According to the mutated amino acid residue, the mutated XPC protein might have partial activity or might destabilize the protein product (Yasuda *et al.*, 2007; Jacobelli *et al.*, 2008; Meneses *et al.*, 2015). XP12 had two mutant alleles, one for the frameshift c.1103_1104delAA mutation which produces a truncated XPC protein and the other for the p.Arg176Gln due to c.526_527delAGinsCA insertion deletion which presumably produces a full length mutated protein. Apparently, the p.Arg176Gln does not affect DNA binding domain of XPC. Consequently, it is predictable that the full length Arg176Gln mutated protein retains some functionality which results in relatively delayed cancer onset in XP12 which was the case for the three reported compound heterozygous XP patients.

The studied XP patients herein provide a preliminary *XPC* gene mutational spectrum in Egyptians. In the seven *XPC* mutations reported, see figure 68, two were recurrent, two were previously reported and three were novel mutations. With reference to geographical distribution, the recurrent c.1643_1644delTG mutation, the c.1103_1104delAA deletion and c.1735C>T nonsense mutation were notably reported in XP patients of the Mediterranean region which reflects the effect of geographical location rather than ethnicity in XP genetics while the recurrent c.2251-1G>C splicing mutation asserts the African component of Egyptian population. Nevertheless, other *XPC* mutations, such as the novel c.668_669delTC, c.1894C>T, and

c.526_527delAGinsCA mutations, were neither reported in the cohorts in North Africa and the Mediterranean nor in the few reported cases in sub Saharan Africa. With respect to *XPC* mutation types, the reported *XPC* mutations were deleterious mutations, either nonsense or splicing or frameshift mutations which deplete XPC protein levels. A novel heterozygous indel mutation was reported and predicted computationally to alter the protein function without affecting its length. Nevertheless, further functional characterization is required to assess cellular effects of this novel indel mutation.

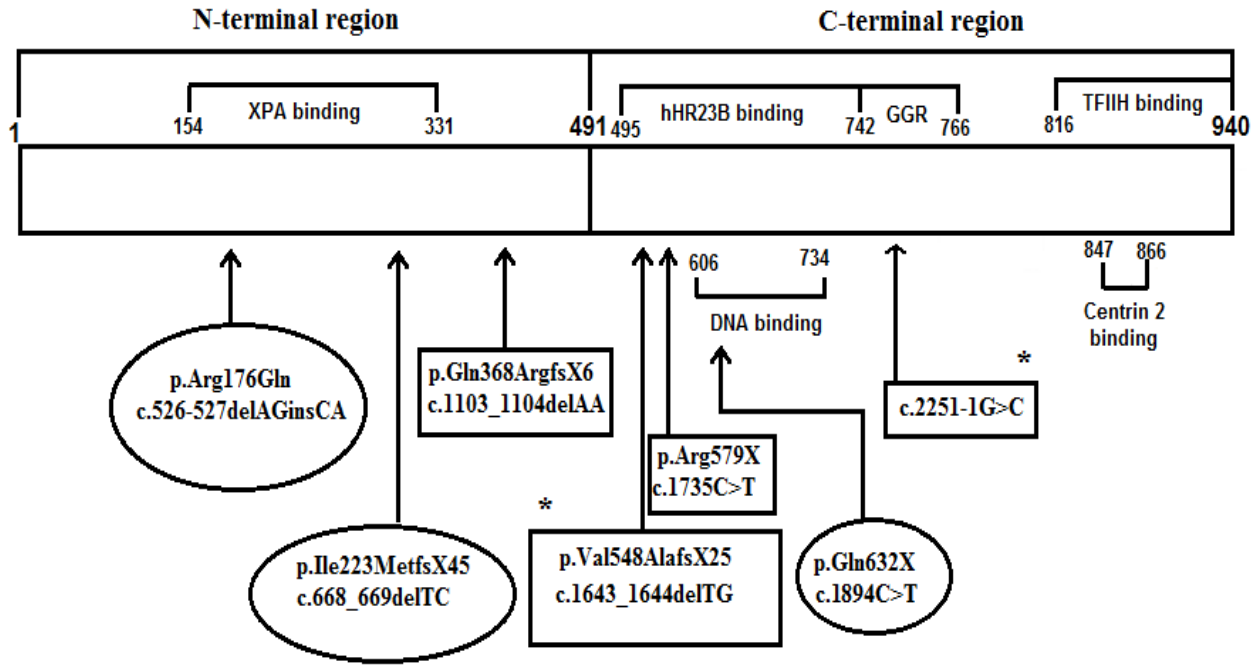


Figure 68: Mutational Spectrum *XPC* gene in the Egyptian population. The figure illustrates the seven XPC mutations which were identified in Egyptian XPC patients. Novel mutations are in oval shapes while previously reported mutations are in rectangular boxes. The two recurrent mutations have (*) sign. Note that c.2251-1G>C is a splicing mutation which occurs one base pair before exon 13; exon 13 starts at amino acid number 750.Mutations are described in text; see figure 6 above for full description (figure was modified from XPC figure by Feltes & Bonatto, 2015 with permission).

Conclusion

This is the first study to detect XP gene mutations among Egyptian patients from different XP complementation groups with variable clinical features. High consanguinity rates, recurrence of XP carriers and the occurrence of more than one patient in the same family were disclosed among the studied families. Clinically, XPA showed severe neurological abnormalities in Egyptian XPA patients, such as mental retardation, developmental delay, cerebellar and neuropathic affection, with early onset photosensitivity and relatively delayed onset of tumor development. Three *XPA* mutations were identified in four XP patients and a fetus. The mutation spectrum of *XPA* in the Egyptian population was expanded. *XPA* mutations were confined to exons 3, 4, and 5 which encode DNA binding domain of XPA protein. Among 13 XPC patients, seven mutations were reported and *XPC* mutation spectrum was identified. Recurrent *XPC* mutations were evident of Mediterranean and African components of Egyptian population. Three *XPC* novel mutations were identified. In some cases, phenotype genotype correlations were deciphered. One homozygous mutation was evident of previously reported severe phenotype and premature death while a compound heterozygous mutation showed delayed onset of tumor development. Population based mutation spectrum provides a base for simple and efficient diagnostic as well as therapeutic approaches. Clinically, XPC has slightly later onset than XPA with relatively late photosensitivity but increased risk for recurrent tumors. Still, the extent of protection from sunlight, age of the patient and late diagnosis are important factors for interpretation of consequent phenotype. Provided with early diagnosis and consequent stringent protection from sunlight, XPC patients could have better quality of life. Characterization of XP patients clinically and molecularly serves as a foundation for effective genetic counseling and follow up to provide efficient carrier screening, prenatal diagnosis and premarital counseling.

Future recommendations

1. The outlined XPA and XPC mutation spectrum should serve as a guide for future molecular diagnosis of XP in Egyptian patients.
2. Estimation of prevalence of XP in Egyptians via expansion of molecularly diagnosed XP Egyptian patients.
3. Carrier and prenatal screening should be mandated for at risk family members.
4. Estimating the carrier rate of the recurrent c.1643_1644delTG and c.2251-1G>C splicing mutation mutations among Egyptians.
5. Haplotype analysis of the identified XP mutations to provide an insight on common ancestry and migration flows.
6. Establishment of primary fibroblast cell lines to characterize novel mutations and their consequent effects on protein products.
7. Use of primary fibroblast cell lines of Egyptian XP patients for application of recent corrective gene and skin graft therapies.

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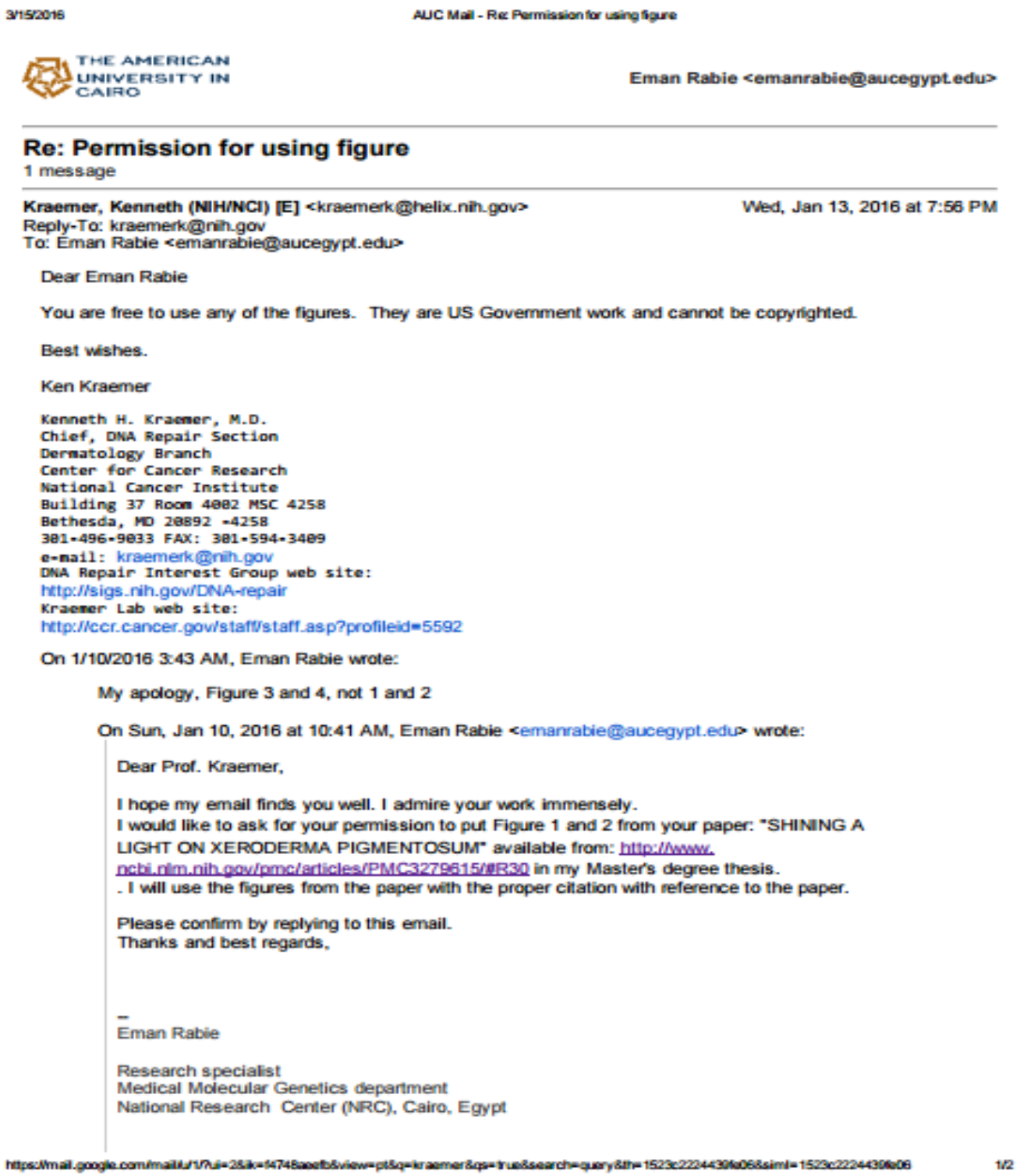

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