MINISTRY OF EDUCATION AND SCIENCE OF REPUBLIC OF KAZAKHSTAN

Kazakh National Research Technical University named after K.I. Satbayev

Institute of Chemical and Biological Technologies

Department of Chemical and Biochemical Engineering

Baitisheva Diana

Polymorphisms in APC gene in the personnel of atomic industry

DIPLOMA PROJECT

Major 5B070100 – Biotechnology

Almaty 2021

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Allowed to the Defence
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_____Raphikova Kh.S.
"18" May 2021

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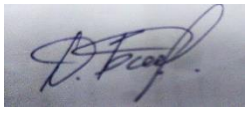
Completed by

Baitisheva Diana
Research mentor
Botbyev D.M.
Senior-lecturer



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_____Raphikova Kh.S.
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ASSIGMENT

On the completion of diploma project

Student: Baitisheva Diana
Topic: Polymorphisms in APC gene in the personnel of atomic industry
Approved by the order of University Rector №15 from "12" april 2021
Deadline for the completion of work "16" may 2021
Summary of the thesis:
a) Radiation
b) Low-dose radiation effects
c) Individual radiosensitivity
d) APC gene mutations
e) Methods for analyzing APC gene
The list of graphical material shown in: *13 slides*
Recommended main bibliography: *65 references*

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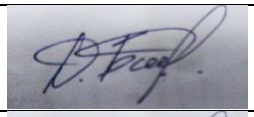
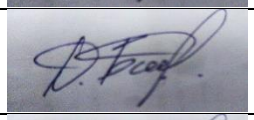
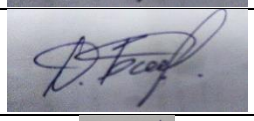
SCHEDULE

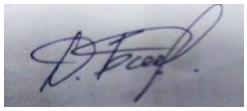
on preparation thesis

Name of sections, list of questions considered	Submission deadline	Note
Review of sources	25.01.2021	
Methods of analysis	31.03.2021	
Results of analysis	14.05.2021	

Signatures

of the consultants and the normative controller for the completed thesis project, indicating the sections of the project related to them

Name of sections	**Consultants**	**Date of signing**	**Signature**
Review of sources	D.M.Botbayev	26.01.2021	
Methods of analysis	D.M.Botbayev	01.04.2021	
Results of analysis	D.M.Botbayev	15.05.2021	
Normcontroller	M.E.Nursultanov	15.05.2021	

Research mentor _____  _____D.M.Botbayev

The students accomplished the task_____  _____ D.Baitisheva

Date “15” may 2021

АҢДАТПА

«Атомдық өндірістегі жұмысшылардың АРС геніндегі полиморфизмдер» дипломдық жоба 52 беттен, соның ішінде 22 кесте, 11 фигура және 14 суреттен тұрады.

Дипломдық жобаның мақсаты: аз мөлшерлі радиацияның АРС генінде пайда болатын мутацияларды зерттеу.

Бұл дипломдық жоба аз мөлшерлі радиацияның ДНҚ-ға әсері, жеке радиосезімталдылық және әр түрлі аз мөлшерлі радиацияның АРС геніне әсерін қарастырады. Осыған байланысты, әр түрлі елдерде өткен зерттеу жұмыстары, АРС геніне түрлі аз мөлшерлі радиация мөлшерлерінің әсері және жайлы мақалалар алынды.

АННОТАЦИЯ

Дипломный проект «Полиморфизмы в гене APC у работников атомной промышленности» состоит из 52 страниц, в том числе из 22 таблиц, 11 фигур и 14 рисунков.

Целью дипломной работы является изучения влияния малых доз радиации на ген APC.

Данный дипломный проект рассматривает влияние малых доз радиации на ДНК, индивидуальную радиочуствительность и мутации в гене APC при различных дозах малых доз радиации. Для этого были взяты исследования из разных стран, статьи о влиянии разных доз радиации на ген APC.

ANNOTATION

Thesis «Polymorphisms in APC gene in the personnel of atomic industry» consist of 52 pages, including 22 tables, 11 figures and 14 images.

Purpose of this project is to research about APC gene mutations caused by low-dose radiation.

This project reviews low-dose radiations' influence on DNA damage, individual radiosensitivity and APC gene mutations at different low doses radiation. In this case, studies from different countries, articles about different doses of low-dose radiation effects on APC gene.

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INTRODUCTION

Uranium industry is one of the big chapter of atomic industry. In 2019, 6 147 800 tons of uran were collected worldwide. In Australia it was more than 1,5 million tons, while in Kazakhstan it was slightly more than 900 thousand tones [1]. In addition, more than ten per cent of world reserves of uranium are located in Kazakhstan. In 2012, country had 12 per cent of world's uranium resources. So uranium mining has positive effects on economic, but due to wastes, nuclear reactors, nuclear weapons, it has a big amount of radiation and impact on uranium mining workers [2]. In 2018, there were 372 employees in uranium industry in USA [3].

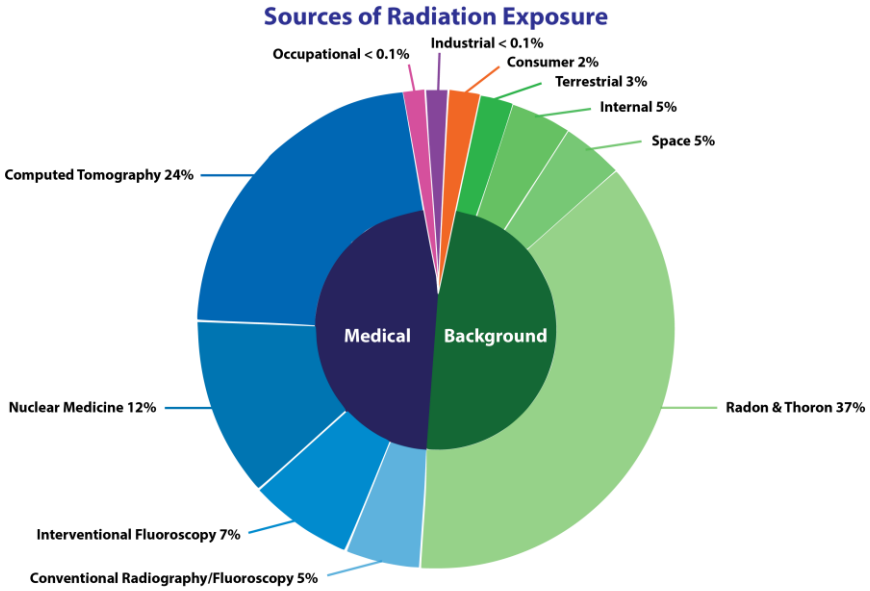
Radiation is particles or waves of energy that travels through the space. Particles transport energy into the atoms or molecules and they do not rip off electrons [4]. In everyday life, radiation surrounds us: light, heat, the microwaves, radio waves. Depends on the sources of radiation, it can be divided into three big groups: medical, background and other [5]. Medical radiation includes tomography, nuclear medicine, fluoroscopy, radiography, whereas background radiation includes internal, space, and radon and thoron radiation [6]. Little amount radiation, less than one per cent, comes from industry and occupation (Figure 1).

Due to different doses of radiation can damage tissue and/or organs. It depends, also, different sensitivity of organs and tissue. When organism is affecting by large dose of radiation during the short amount of time, it is feeling immediately. Chronic effects are cause of changes in behavior and motor function comes from central nervous system diseases and nausea. During the decades, radiation accumulated and leads to cancer, cataracts and vision impairment, degenerative cardiac disease. In the nuclear power industry and other activities that use ionizing radiation such as medical diagnostics and therapy, limits are set to ensure doses are at a safe level. Radiation dosage depends on natural background, local geology, radionuclides in the human body and some dosage of radiation comes from diagnostic examinations [7].

Radiation is one of the cause of mutations in genes that can lead to cancer. Familial adenomatous polyposis is autosomal dominant disease is appears due to mutations in APC genes.

The aim of this work is to find out different types of mutation in APC gene and recognize how low-dose radiation can effect on this gene.

The relevance of this project is Nuclear power production is still developing and increasing in counties and low-dose radiations' effects is not still studied well, so APC gene can be used to define low-dose radiation effects during time and in different doses. Also, colorectal cancer is the second leading cause of cancer mordability and mortality worldwide, which is caused by mutations in APC gene.



Average Annual Radiation Dose

Sources	Radon & Thoron	Computed Tomography	Nuclear Medicine	Interventional Fluoroscopy	Space	Conventional Radiography/ Fluoroscopy	Internal	Terrestrial	Consumer	Occupational	Industrial
Units											
mrem (United States)	228 mrem	147 mrem	77 mrem	43 mrem	33 mrem	33 mrem	29 mrem	21 mrem	13 mrem	0.5 mrem	0.3 mrem
mSv (International)	2.28 mSv	1.47 mSv	0.77 mSv	0.43 mSv	0.33 mSv	0.33mSv	0.29 mSv	0.21 mSv	0.13 mSv	0.005 mSv	0.003 mSv

(Source: National Council on Radiation Protection & Measurements, Report No. 160)

Figure 1. Radiation from different sources

1 Radiation

1.1 Effects of radiation on humans

Radiation can damage directly or indirectly in cellular level. High dose radiation can lead to death, but it is not only effects of radiation, it can effect on skin, hair, eyes. On skin appears reddening like sunburn and skin starts to dry out when there are gamma, x-ray and beta radiation. Hair loss is one of the skin effects because of radiation. Men can have sterility which could be temporary or permanent, while women have permanent sterility, but in the higher doses. On some high-doses radiation appears cataracts, neutrons effectively produce cataracts, because water stops neutrons. Damages of organs and tissues can be cause of a rapid body response that called “Acute Radiation Syndrome” [8].

First symptoms of Acute Radiation Syndromes are nausea; vomiting, fatigue and appetite lose. In addition, there can be appeared hematopoietic, gastrointestinal and central nervous system syndromes. When hematopoietic syndrome takes place, blood-forming organs are affected which are the most sensitive. During gastrointestinal syndrome, very sensitive gastrointestinal system are affected, while brain and muscles affected by central nervous system [8].

Nowadays, modern identity of radiation-related cancer risks are based on Hiroshima and Nagasaki atomic bombs’ effects and patients who had therapy and diagnostic radiation. In addition, radiation leads to cancer, but cancer risks from radiation are smaller than from chemical compounds and bad habits. Hemoblastosis is related with radiation damage of bone marrow hematopoietic system that appears during the high-dose radiation are most researched radiation effects, and it is using like an indicator of radiation effects [9].

1.1.1 Direct damage

In this type of damage, radiation interacts with the atoms of the DNA or with some cell component. Such damages destroy cell ability to reproduce and survive and leads to DNA mutations. When large amount of molecules are destroying, then chromosome do not replicate normally, this is changing information which is given to offspring. Even DNA has repair mechanism there can be errors. Most damages are repaired, but some of them are badly in repairing, so it also has effects to cell and offspring [8].

1.1.2 Indirect damage

Indirect damage also can be called “the carcinogenic effect”. Electron from radiation goes through tissues and ionize atoms and molecules, so there are take place chemical reactions which is the starting of biological effects. Large amount of adsorbsion occurs in the water: ionizing particles goes through water and make it

ionized (H_2O^+). This ionized water react with normal molecule of water and produce hydroxyl radical and hydronium. Hydroxyl radical can make back reactions, because this molecule is highly reactive because of unpaired electron. All of these reactions called “radiolysis”:

$$H_2O \xrightarrow{radiation} e, H^+, H_2O_2, H_2.$$

All of these products attacks molecules in cells that leads to biological damages. The most damaging effects come from hydroxyl radical, because it is oxidizing agent and takes a hydrogen atoms from deoxyribose of DNA [8].

1.2 Low-dose and high-dose radiations

A unit called “Sievert” (Sv) is using for measure the dose of radiation. Background radiation has 0,01 mSv per day, but in some places it can be higher and in reach 0,1 mSv per day. The higher dose of radiation which can reach 150 mSv is called high-dose radiation which has immediate effect on people. Between background radiation and high-dose radiation is low-dose radiation. Effects of low-dose radiation is not be immediately noticeable, so it has long-term biological effects. Estimation of this radiation is not complete [10].

Atomic industry has low-dose radiation, because radiation accumulates during the years and its average annual dose less than 100 mSv. In 2009, analysis of radiation workers in 2009, observed 174541 workers. That study show, that more half of workers have less than 10 mSv lifetime dose and only six per sent of workers have radiation lifetime dose more than 100 mSv (Table 1) [11]. In Canada, annual dose limit for nuclear energy workers is 50 mSv and typical annual dose received by workers in a uranium mine or nuclear power plants is 1 mSv, while five-year dose limit is 100 mSv (Image 1) [12]. In USA, from 1983, 100 000 nuclear plants workers are under the control of the US Nuclear Regulatory each year, so there is no worker who has annual radiation more than 50 mSv. A chest x-ray send 0,1 mSv to lung tissue, mammography examination dose is 3 mSv, and astronauts get about 2,4 mSv during three days in the space [13]. Moreover, National registry for radiation workers analyzed radiation lifetime dose by birth year of workers (Table 2). The bigger amount of older people work at atomic industry, than younger workers, so there are less workers who born after 1975 and affected by different doses of radiation. Nevertheless, workers born between 1960 and 1964 were more affected by radiation less than 10 mSv. Total number of workers were similar, except people younger than 1970 year. The most number of workers who had more than 100 mSv radiation dose born between 1930 and 1934 [11].

Low-dose radiation estimated which based on epidemiological studies of population which is affecting by medical x-rays, mixed gamma and neutron radiation from the Hiroshima and Nagasaki atomic bomb. Actually, there is no

evidence of cancer risks from the neutron radiation, so the biological effects of low dosage radiation should be estimated experimentally. However, there is no cellular enough sensitive tests. These tests should also identify the DNA double stand break. This type of radiation damage or change cells, whereas high-dose radiation kill these cells. There are three categories of effects [14]:

1. Genetic – effect when changes from radiation appear in offspring;
2. Somatic – effect when damage suffer individual. There can be cancer like primary result;
3. In-utero – effect when embryo or fetus are suffered by radiation.

People who are having diagnostic radiotherapy (patients with tuberculosis), radiation therapy high-dose radiation and people who are working on uranium deposits and other nuclear power factories have high-dose radiation. This can kill large amount of cells that will lead to damage organs and tissues. This radiation studies are making epidemiologic basis of estimation of radiation-related risks [14].

Table 1 - Radiation lifetime doses in workers

Lifetime dose (mSv)	**Number of individuals**	
Less than 10	118 776	68 %
10 – 49.9	35 402	20 %
50 – 99.9	9 869	6 %
More than 100	10 504	6 %
TOTAL	174 541	100%

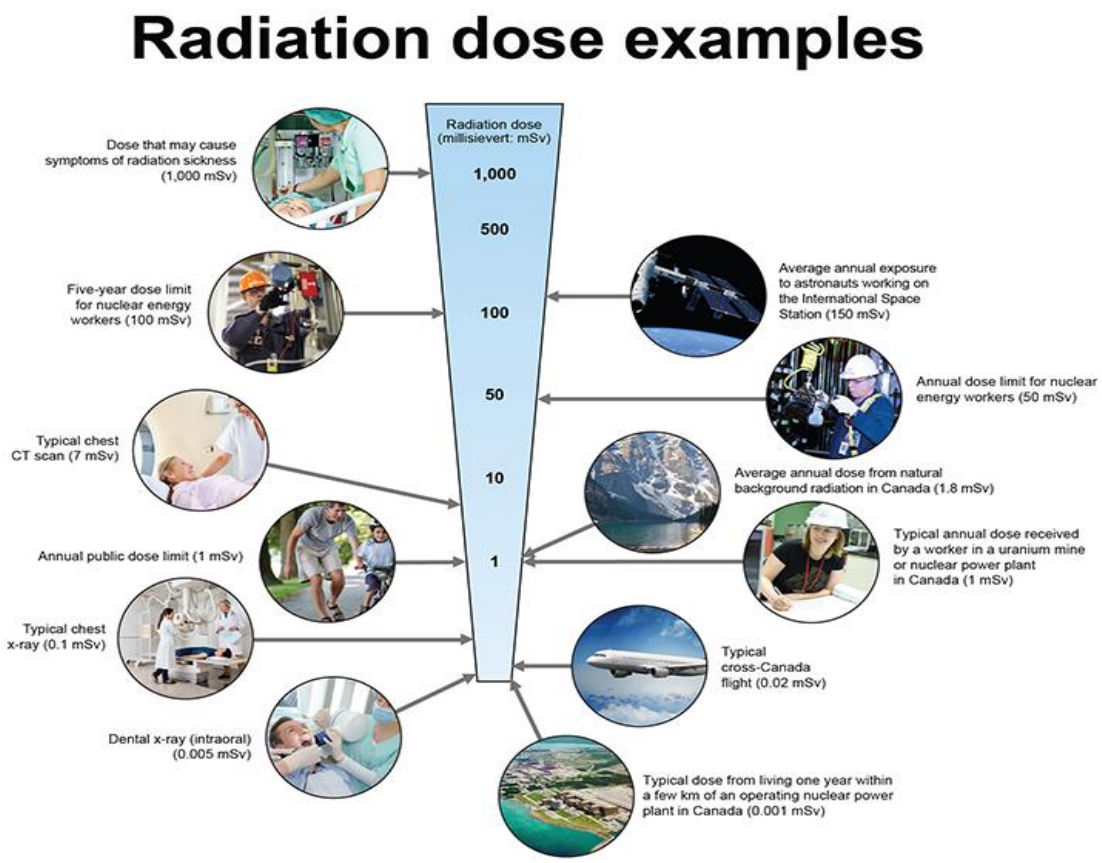


Image 1 - Radiation doses from different sources in Canada

Table 2 - Number of workers who had different doses during the work related to their birth year

Period	Lifetime dose				Total number of workers	
	Less than 10	10-49.9	50-99.9	More than 100		
1940-1944	10 843	3 356	903	894	15 996	14.2%
1945-1949	14 231	3 656	1 007	899	19 793	17.6%
1950-1954	12 236	3 290	812	784	17 212	15.4%
1955-1959	13 903	3 198	815	577	18 493	16.5%
1960-1964	14 161	2 576	525	229	17 491	15.6%
1965-1969	11 548	1 453	222	32	13 255	11.8%
1970-1974	7 113	485	37	1	7 636	6.8%
After 1975	2 211	74	0	0	2 285	2.1%
TOTAL	**86 246**	**18 088**	**4 321**	**3 416**	**112 161**	**100 %**

1.3 Influence of low-dose radiation

Effects of low-dose radiation have not been concluded, and there are many researches that are studying. There was a linear no-threshold hypothesis, in which relationships between radiation dose and adverse effects at high levels also applies to low-dose radiation and provides a basis for occupational health and other radiation protection standards [9]. At low exposure levels, the body's natural machinery repairs radiation-caused damages and other cellular damage soon after it occurs. Moreover, some adaptive response is stimulated that protects cells and tissues, and other external agents' influence at their low levels [13].

Mutations from radiation are different from spontaneous mutations and from ultraviolet and chemical mutagens-caused mutations. Ionizing radiation leads to appearing of point mutations in one gene and leads to disappearing of other genes. The primary event of mutagen effects is losing genes during DNA deletions. To analyze effect of radiation on DNA, alpha-particles are more important, it is estimated that an average in uranium miner, 96% of the bronchial cells go through one alpha-particle. However, 10^{-7} alpha-particle go through one bronchial cell [15].

In 2017, there was a study about connection between low-dose X-ray radiations and somatic mutations. In study, Fe ion beam was moved through cells with LDR. As a result, increasing of number of mutation started at 0.05 Gy, but these mutations was not stable, because at 0,1 Gy mutations decrease. Stable rising of mutation started from 0,15 Gy and at 1 Gy number of mutations was more than 17 mutants per 104 survivors (Figure 2) [16].

Some studies show that DNA mutations can be decreased by radiation, because radiation stimulates repair mechanism in cells. That effect was shown on human lymphocytes and mice cells. Adaptive response of cell can be transient or individual. In vitro adaptive lymphocyte response occurs approximately four to six hours after exposure to a conditioning dose in a dose range of approximately from five to 200 mSv and remains effective for three cell cycles. After the control dose,

the repair is manifested in a decrease to below the expected level of chromosomal aberrations. Moreover, bone marrow cells and mice spermatocytes exposed to a control dose that followed the conditioning dose and showed a decrease in the number of chromosomatic breaks compared to cells exposed to the control dose alone [8].

In the USA, in 2012, Massachusetts Institute of Technology studied low-dose radiation effects on mice. Any impact of radiation on the DNA was not leading to damages, although, control group had some damages. Also, there are other studies concluded that people who have less than 1000 mSv per year at low-dose radiation will not suffer adverse health effects [7].

Futhermore, some studies showed that low-dose radiation, which is approximately under 10 mSv, has positive effects on health, it is called hormesis. Hormesis appear in the result of adaptive cell reaction of organism. Ionizing radiation's positive effect is showing by decreasing of cancer and by a resistance to the higher dose of radiation. Nevertheless, hormesis did not proved conclusively. Therefore, from 2015 the USA has the Low-dose Radiation Research Act that calls for an assessment of low-dose radiation in USA and worldwide [17].

In China, connection between immune system and low-dose radiation was analyzed. LDR hormesis was observed in population who exposed to high-background radiation at a low-dose rate which is 1,96 mSv per year in Yangjiang, Guangdong Province. DNA repair ability of T cells were higher than in people of low-background radiation areas. Moreover, this is observed in thymocytes, T helper differentiation and cytokine secretion and pro-apoptosis, pro-activity proteins and interaction with APC occur. Furthermore, Chinese scientists concluded that low-dose radiation has different biological effects on germ cell. When low-dose radiation effects on germ cells, there can be apoptotic cell death or genomic and inheritable effects. During apoptotic cell death, cells' apoptotic response increases, p53 protein expression in spermatogonia and spermatocytes and mitochondrial pathway occur. During genomic and inheritable effects, chromatid aberrations reduces, chromosomes translocation reduces, also DNA repair, antioxidant activity appear [18].

In addition, today, some studies show that LDR has no negative effects on the articular cartilage, but that kind of studies observed only apoptosis effects. Apoptosis effect of radiation is one of the main object of studies, because main purpose of radiotherapy is to enhance the efficacy of radiation in tumors. Four types of low-dose radiation, which is used in clinical setting, has different effects (Table 3) [19]. Negative effects of beta radiation was observed in 1961. During study, tibia's epiphyseal cartilage and osteoblasts were observing and they had x-radiation intensities from 400 to 1800 administeted from one to ten days. As a result, osteoblastic activity was shown and proliferation with excessive matrix production was found [20]. The study of low-dose radiation and its anti-tumor effects showed

that combination of high-dose radiation and low-dose radiation's gamma radiation delayed primary and secondary tumor growth. It is clear that the inflammatory response does indeed occur in response to radiation and may be the molecular mechanisms that induce the early activation of ostoclasmediated bone resorption. Bone strength is reduced and people under the risk of fractures with concomitant mortality as a late complication. Nevertheless, these studies cannot show exact doses of LDR and its types that will have positive, negative effects on organisms [21].

In 1980s, in USA, nuclear power workers were observed, they had high-dose (5 mSv) and low-dose (less than 5 mSv). As a result, authors concluded that there were any negative effects from low-dose gamma radiation. In addition, there was no connection between mortality from cancer or leukemia and cumulative low-dose radiation, where average dose was less than 50 mSv in Hanford Site, Rocky Flat Nuclear Weapons Plant and Oak Ridge National Laboratory. In Canada, the risks of cancer was declined between 1 and 49 mSv, and when radiation dose was raised to 100 mSv, cancer risks were increased [13]. A Canadian Nuclear safety Commission reported that cancer mortality among the nuclear workers, who worked after 1965, was lower than among all country population. That kind of conclusion was made in the United Kingdom: mortality from myeloma and leukemia was lower than it was found in the general population . Also, in 1999, there were analyses, which showed the general mortality was lower than it was expected by comparison to the general country population [12]. In Japan, 120 000 nuclear power workers were observed during 4,5 years. There was not any connection between radiation dose and general factors, so connection between cancer risks and radiation doses do not depends to each other, it also depends general factors (lifestyle) [12].

In Russia, long-term effects of radiation on nuclear power plant workers in Ozersk city were analyzed. Workers had 200 mSv average radiation per year and maybe it was higher till 1953, but then, during next twenty year, radiation doses declined to 5 mSv. Despite that, the accumulated radiation was high, mortality from cancer was less than in the other population in the city [12].

In uranium miners, correlation between radon exposure and lung cancer was shown many times. For example, French uranium miner workers' mortality from lung cancer was 34% between 1946 and 2007, and this risk was raised with cumulative radon exposure. That kind of correlation was in Germany, Canada and in the USA [12].

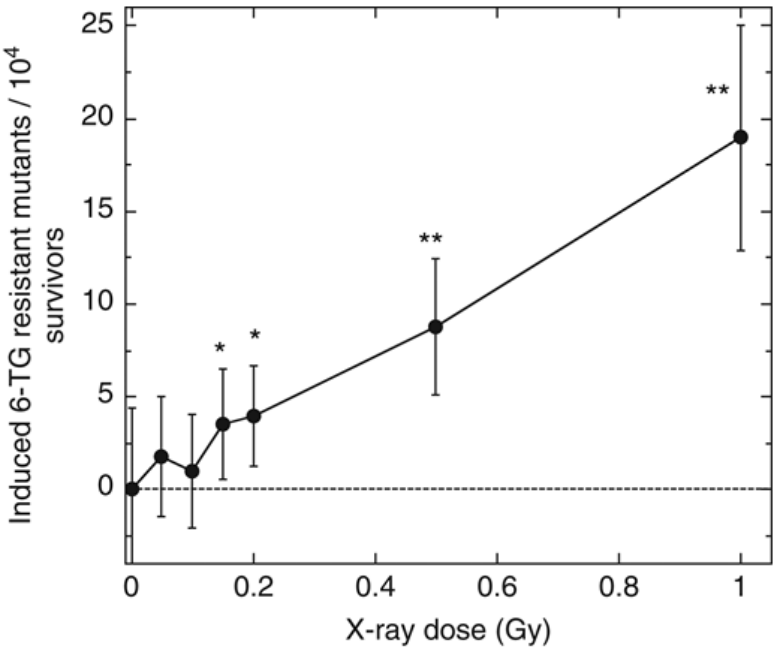


Figure 2 - Connection between x-dose rate and induced mutations number

Table 3 - The effects of four of low-dose radiation on articular carliage

Type of radiation	Positive effect	Negative effect	No effect
Beta		+	
Gamma	+	+	
X-ray	+	+	+

1.4 Individual radio sensitivity

Radiation sensitivity describes effect of ionizing radiation to individual organism or the organism reply to ionizing radiation. Radio sensitivity depends on species. For example, bacteria that found near nuclear reactor could live and multiply. Farther, in each tissue and cell in one organism has different level of sensitivity. Bone marrow, lymphoid tissue, epithelium of the mucous membrane of the small intestine are very sensitive and muscle, nervous, bone tissues are relatively stable. In addition, the hematopoietic, immune and reproductive systems are the most sensitive in human organism. So, it is known from the radiotherapy in the past that every person have different respond to radiation [22].

This sensitivity will help firstly in medical purposes, because many people need radiotherapy. Furthermore, predicting radiation sensitive individuals would allow the radiation protection industry to tailor dose limits to an individual, reducing risk to the worker.

In 1989, Little and his colleagues described problem of predicting radio sensitivity, and they showed variability of radiation in cells. Due to their work, there was shown that radio sensitivity cannot be used to predict cancer risks and these changes in individual radio sensitivity cannot measure cells are normal or sensitive. It is, also, known that person with high radio sensitivity has more chance to have

cancer. Thus, radio sensitivity can be result of defects of repair DNA damage or defects in the processing and signaling of this damage [23].

1.4.1 Cell division and radiosensitivity

There is connection between cell division and sensitivity level. If organism has no cell division, sensitivity is low. When organism has active cell division, radiation sensitivity is high (Figure 3).

Actively dividing cells that are less differentiated tend to show higher radio sensitivity. For example, hematopoietic stem cells in bone marrow are differentiated into various blood cells, while dividing actively. Immature (undifferentiated) hematopoietic cells that have divided (proliferated) from stem cells are highly sensitive to radiation and die due to a small amount of radiation more easily than differentiated cells. As a result, the supply of blood cells is suspended and the number of various type of cells in blood decreases. In addition, the epithelium of the digestive tract is constantly metabolized and is highly sensitive to radiation. On the other hand, nerve tissues and muscle tissues, which no longer undergo cell division at the adult stage, are known to be resistant to radiation.

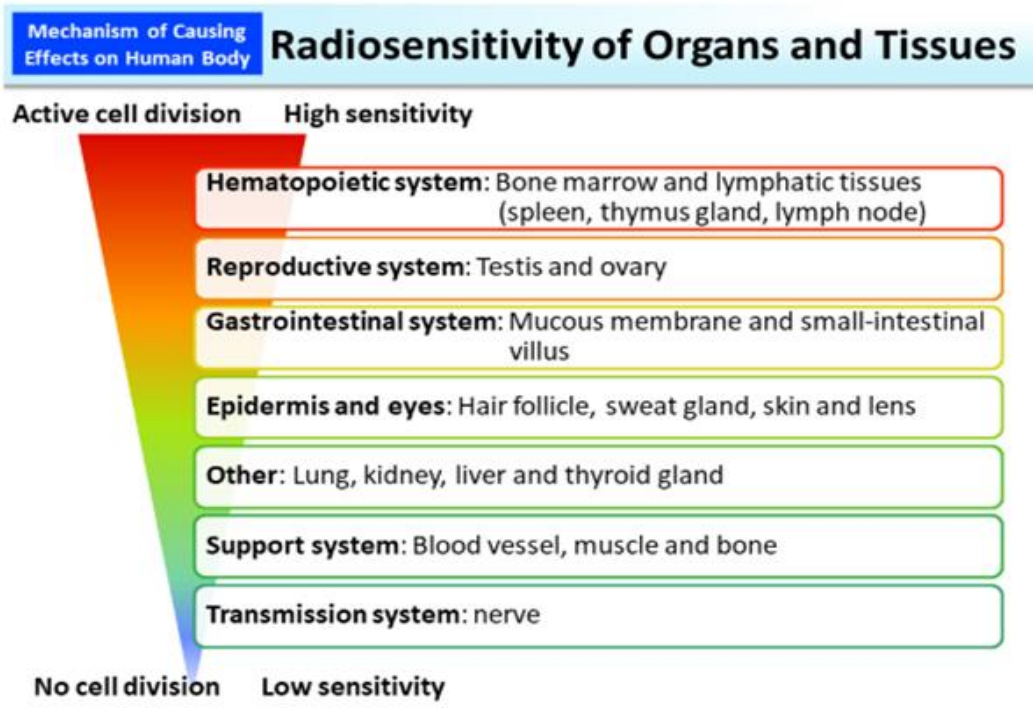


Figure 3 - Correlation between cell division and radio sensitivity of these cells

1.4.2 Radiation sensitivity assays

There are three tests, which are used to assess radiosensitivity and in test, fibroblasts or lymphocytes are using:

- Survival assay – cells are effected with different dosage of radiation and these doses are compared;

- Micronucleus assay – micronucleus are observed by using X-rays. The amount of X-ray when micronucleus yields are higher is correlate with radio sensitivity;
- G2 chromosomal assay – chromatid-type are observing in the G2 phase of cell cycle after X-ray affects. All phases of cell cycle has different radio sensitivity, so it is important to do correlation exactly in G2 phase. The amount of chromosomal damage shows radio sensitivity.

2 Polymorphism

Polymorphism is a different variation in the same section of the base pair sequence for the DNA strands. There can be enzyme and DNA polymorphisms.

DNA polymorphism has four different types [24]:

1. Single nucleotide polymorphism is a sequence of DNA where only one nucleotide is differ. Due to one nucleotide differences, there can be found millions of SNPs throughout the human genome (Image 2).

2. Tandem repeat polymorphism described is a repetition of a series of nucleotides. The polymorphism consists of the number of repeats (Image 3).

3. Structural variants involves deletions or intersetions of nucleotide sequence, inversions and translocations. When an allele is missing one nucleotide or few nucleotides, it is called deletion (Image 4). When the allele is adding one or few nucleotides that are differ from original, it is called insertion (Image 5). An inversion polymorphism occurs when one allele has a nucleotide sequence that is reversed in another allele. Inversions usually occur when a chromosome breaks in two places and DNA repair mechanisms mistakenly splice the middle fragment back but in reverse order (Image 6). Typically, they involve many thousands of base pairs. A translocation occurs when a section of DNA is deleted from one chromosome and then inserted into another chromosome (Image 7).

4. Sequence polymorphisms - the ultimate polymorphism is to actually have the whole sequence of nucleotides for a region for a large number of DNA strands and then examine all of the differences among the strands. Here, the DNA differences could be a SNP, a tandem repeat or a structural change. There is no accepted term for this phenomenon, so we call them sequence polymorphisms. In effect, sequence polymorphisms subsume all known DNA polymorphisms [24].

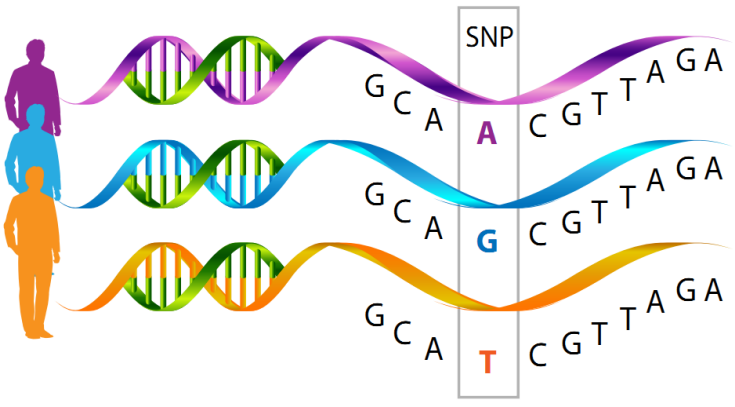


Image 2 - Single nucleotide polymorphism

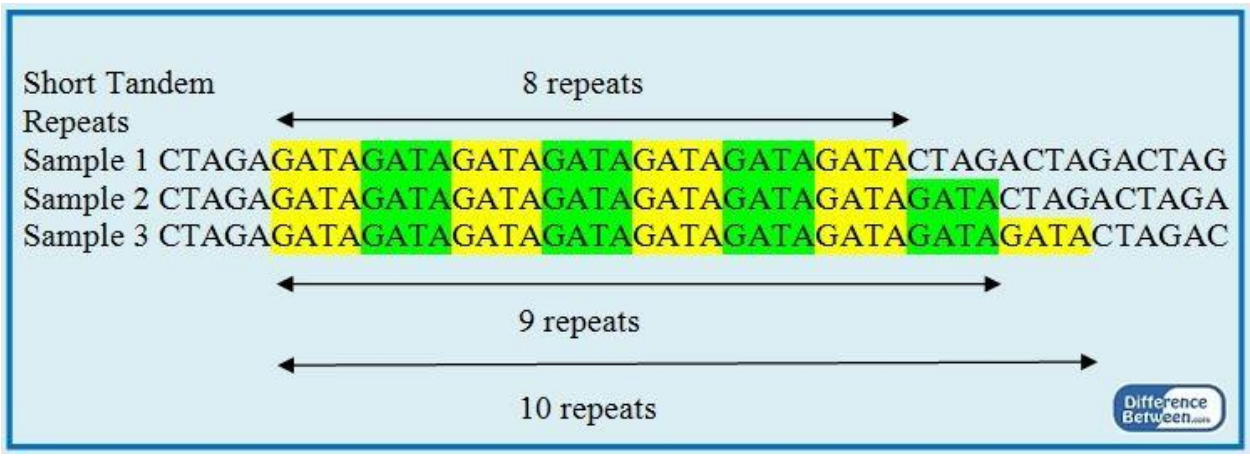


Image 3 - Tandem repeat polymorphism

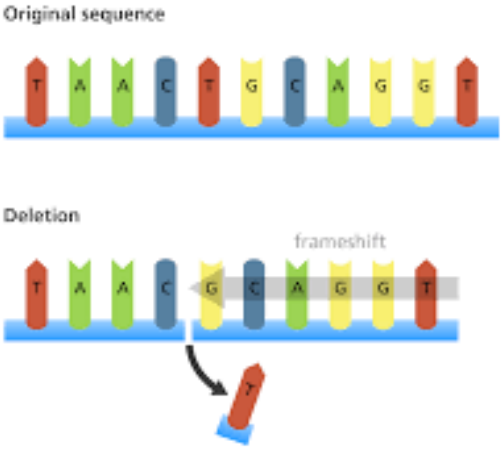


Image 4 – Deletion

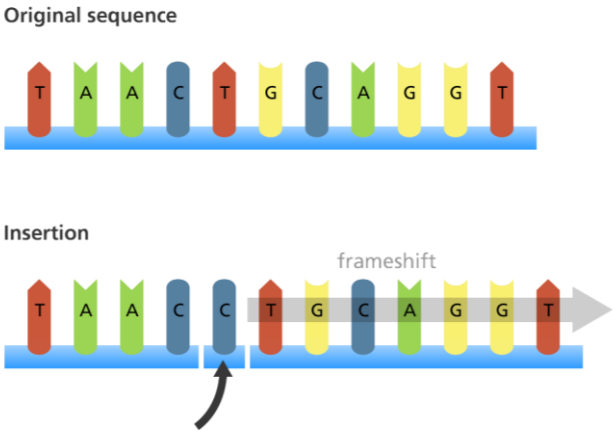


Image 5 - Insertion

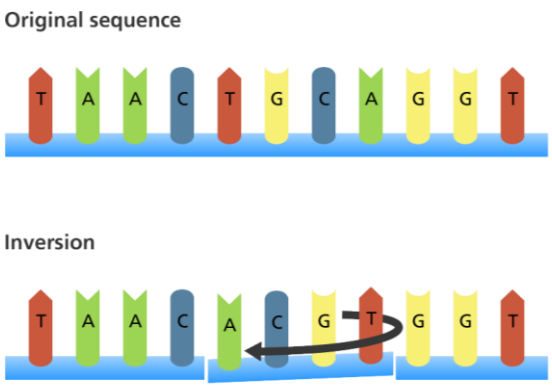


Image 6. Inversion

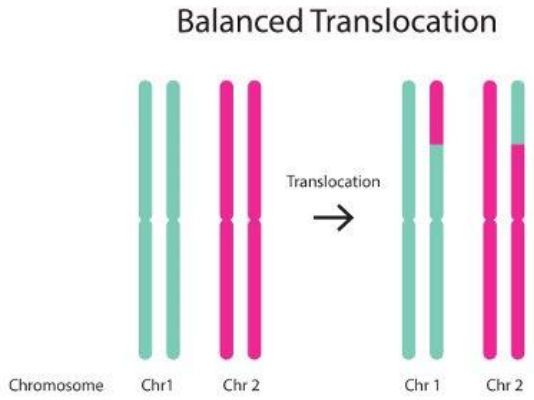


Image 7. Translocation

3 APC gene

Adenomatous Polyposis Coli gene is a tumor suppressor gene, which located in the fifth chromosome, in 5q21 site (Image 8) [25] and product is a 312 kDa protein that has multiple domains, through which it binds to various proteins, including β-catenin, CtBP, Asef, IQGAP1, EB1 and microtubules. This gene has some transcripts and they are tissue-specific. The APC gene is expressed in many tissues and consists of 8 535 nucleotides pairs and spanning 21 exons [26].

The APC gene provides instructions for making the APC protein, which plays a critical role in several cellular processes. The APC protein acts as a tumor suppressor, which means that it keeps cells from growing and dividing too fast or in an uncontrolled way. It helps control how often a cell divides, helps control how it attaches to other cells within a tissue, and whether a cell moves within or away from a tissue. This protein also helps ensure that the number of chromosomes in a cell is correct following cell division. The APC protein accomplishes these tasks mainly through association with other proteins, especially those that are involved in cell attachment and signaling [27].

One protein with APC associates is beta-catenin. Beta-catenin is main parner of APC in its tumor suppression function and it helps control the activity (expression) of particular genes and promotes the growth and division (proliferation) of cells and the process by which cells mature to carry out specific functions (differentiation). Beta-catenin also helps cells attach to one another and is important for tissue formation. Association of APC with beta-catenin signals for beta-catenin to be broken down when it is no longer needed [28].

The protein APC gene plays important role in the epithelium of the colon:

1. In control of the cell cycle (Wnt biochemical pathway) – gene play role in apoptosis, differentiation of neurons, degradation of β-catenin in cytoplasm, segregation of chromosomes, it participates in the assembly of the fission spindle. Wnt or beta catenin pathway is a highly conserved pathway and is involved in several cellular processes starting with embryogenesis to organismal development to homeostasis and tissue development in adult life [28].
2. In intercellular adhesion and regulation of cell migration – gene interacts with intercellular beta-catenin, which is a component of adherens junctions, so it links E-cadherin with a-catenin and actin cytoskeleton. In colorectal cancer, expressions of APC with truncated APC leads to increasing of E-cadherin level on cell membrane and leads to translocation of beta-catenin from nucleus and cytoplasm to the cell periphery that increase cell adhesion. Mutants of APC leads to decreasing of cell adhesion [28].

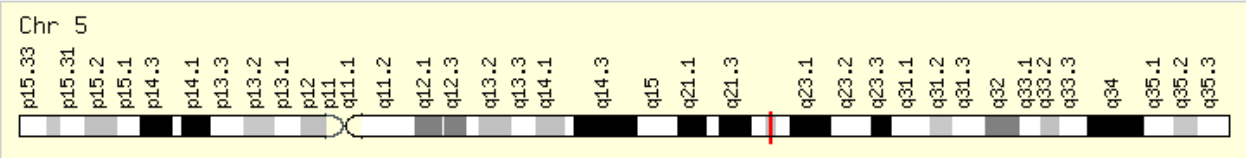


Image 8 - Location of APC gene in chromosome 5

3.1 Diseases related to APC mutation

Familial Adenomatous Polyposis is one of the hereditary disease that can lead to colon cancer. For the first time it was described as a Gardner's syndrome in 1925, and the clinical diagnosis depends on the detection of adenomatous polyps. Polyps are small non-cancerous growth. One or two polyps in adult organisms is normal as they get older. These polyps are at increased risk for malignancy [29].

Over time, it was known that FAP has different syndromes and Gardner's syndrome is just one of them. In 1882, W.Cripps described FAP in one family's brother and sister and concluded that FAP has family accumulating, so it has genetic conditioning. In addition, some families can have Attenuated FAP (AFAP) that is a mild form of FAP. The developing of AFAP is slower than developing of FAP and have fewer amount of polyps [29]. Nevertheless, cancer risk is still high. The incidence of FAP in the population is approximately one in 8000, polyps occurs at teen ages, and they are malignancy near 40 years. In FAP APC gene is missing or alerted, so it do not work correctly. If one parent have FAP, it mean he has alerted gene and normal gene, so offspring has 50 per cent change to have alerted APC gene (Image 9) [29]. Due to amount of polyps, FAP classified as:

- Classical or typical form – 100-500 polyps;
- Diffusion or total polyposis – over 2000 polyps;
- Attenuated form or multiple polyposis – 10-100 polyps.

Turcot syndrome is a genetic disease comparised of an association of colorectal cancer with a cancerous brain tumor which is called medulloblastoma and this syndrome is connected with FAP. The first time, when FAP was connected with medulloblastoma, was described in 1949. In 1959, Turcot described in brother and sister who had FAP that developed to central neural system tumor, adenocarcinoma, medulloblastoma in the spinal cord [30].

In addition to causing large numbers of colonic adenomas, which have a 100% lifetime risk of progressing to CRC, FAP is associated with extracolonic conditions including osteomas, epidermoid cyst, congenital hypertrophy of the retinal pigment epithelium (CHRPE), upper gastrointestinal (UGI) polyps or adenomas, desmoid tumors, and other malignancies at sites including brain, thyroid, and biliary tract [28].

Desmoid tumor is appears from connective tissues, which provide strength and flexibility of bones, ligaments, muscles. Due to APC gene mutations desmoid tumors take place in abdominal cavity, but sometimes it could be in another place of

body. Some APC gene mutations lead to truncating of APC protein, which cannot interact with beta catenin. Therefore, large amount of beta-catenin leads to out of control of growing up and division of cells and at this place, desmoid tumors appear [31].

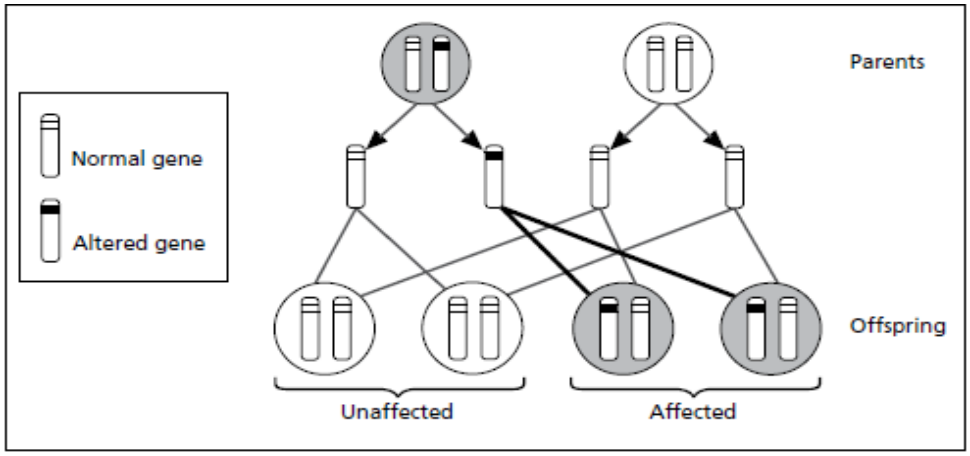


Image 9 - FAP running through family

3.2 Mutations in the APC gene

Now, more than 700 mutations of APC are registered, and their analysis show some features. To make databases, information from patients with Familial Adenomas Polyposis and Colorectal Cancer were taken. Most of mutations lead to truncation of the APC protein or to nonsense mutations in 30%, also 68 per cent deletions and insertions present. 1061 and 1309 codons are hot spots of mutations (Table 4). In addition, dinucleotide CpG frequently methylated in the genome of vertebrates. Deamination of the 5-methylcytozine lead to the replacing Cytosine (C) to Thymine (T) after the DNA replication. The replacing Cytosine to Thymine or Guanine (G) to Adenine (A) have same rates and these kind of mutations take 13 per cent of all APC mutations. In germline and in somatic mutations, the most often occurring mutation is the replacing C to T [32].

Mutations in APC gene lead to violation work of degradation complex and lead to increasing of beta-catenin, which goes to nucleus and activate transcription. Mutations appear with damaging in regulation of S and G phases' cell division of colon's epithetic cells and in 95% situations, mutations lead to cancer.

75 per cent of patient can have malignant tumors of the stomach, uterus, breasts, and central nervous system. Gardner's syndrome and colon cancer are associated with mutations in an APC gene, so during mutations Family Adenomatous Polyposis (FAP), an autosomal dominant disorder characterized by the development of multiple colorectal polyps, which can develop in colon [28]. The most common mutations that associated with the synthesis of a truncated APC protein: frame shift mutations (68%), nonsense mutations (30%) [33], large deletions of one-five pairs of nitrogen bases (two per cent) [34].

Mutation points are located in the 1309 and in the 1061 codons, and their frequency are 17 and 8.6 per cent. Somatic APC mutations occur in exon 15, more than 50% occur between 1286-1513 codons that known as the mutations cluster region [35]. Mutation in the 1309 codons are appeared with thousands of polyps. Polyps demonstrates appearing of FAP which usually appears in the youth or in the third decade of life. Moreover, APC mutations find out in six per cent of patients in Central and Eastern Europe. This mutation replaces isoleucine to lysine at 1309 codon of APC protein. In the beginning, this mutation was not dangerous, but then it was find out that increasing risk of colon cancer to 10 or 20 per cent. Also, somatic mutations of APC can lead to stomach cancer [36].

APC gene has two promotors: 1A and 1B. Promotor 1A is more active then promotor 1B. Hypermythylation of promotor can be one of the mechanism in colorectal tumors where only one mutation of APC is located. Researches indicated that hypermethylation of promotor 1A can be in colorectal adenomas and carcinomas, but they cannot be in normal colonic mucosa and it described in gastrointestinal tumors and it is found in normal gastric mucosa. Hypermethylated tumors are not able to express APC transcripts. Study showed that deletions in the gene decreased transcriptions in promotor 1B to 39-45 per cent [37].

Full length of APC promotes apoptosis, whereas truncated APC have anti-apoptosis functions. Truncated APC are unable to accelerate apoptosis in vitro and in vivo, so these APC promote cell survival with other mechanisms. Some studies show that truncated APC in SW480 sells compromises DNA replication and there occurring down regulation of cells by cell cycle components. In normal epithelium, wild type (WT) of APC appears and colorectal cancer's (CRC) cells depends on truncated APC for growth and survival (Image 10). In the early stage of CRC, truncated APC can promote tumorigenic properties. After different genetic alterations and in late stages of CRC cell with altered signaling network start to be addicted to truncated APC for cell survival. This leads to late adenoma or carcinoma where polyps appears, so there cell death occurs [38].

Moreover, de novo mutations can be in APC gene. De novo mutation is a genetic changes that occurs for the first time in one family member as a result of a mutation of the germline cells in parent or a variant that occurs in the fertilized egg itself during late embryogenesis. All de novo mutation can be called de novo difference or new mutation. That kind of mutations are still studying so there is no enough information about its effects [32] And described mutations in codon 1309 and 1061 are de novo mutations which appears frequently than other mutations.

Table 4 - Mutations in APC gene

	Total	Germline	Somatic
Total	737	332	402
Frameshifts			
deletion	432 (58%)	220 (66%)	210 (52%)
insertion	73 (10%)	21 (6%)	52 (13%)
Point mutation			
missense	16 (2%)	6 (2%)	12 (3%)
nonsense	216 (30%)	85 (25%)	129 (32%)
G → A	7	5	2
G → A at CpG	1	0	1
C → T	44	17	26
C → T at CpG	94	38	56
A → T	8	5	3
A → G	5	0	5
A → C	0	0	0
T → G	5	2	3
T → C	2	0	2
T → A	3	2	1
C → A	19	9	10
C → G	15	10	5
G → T	28	3	24
G → C	1	0	1

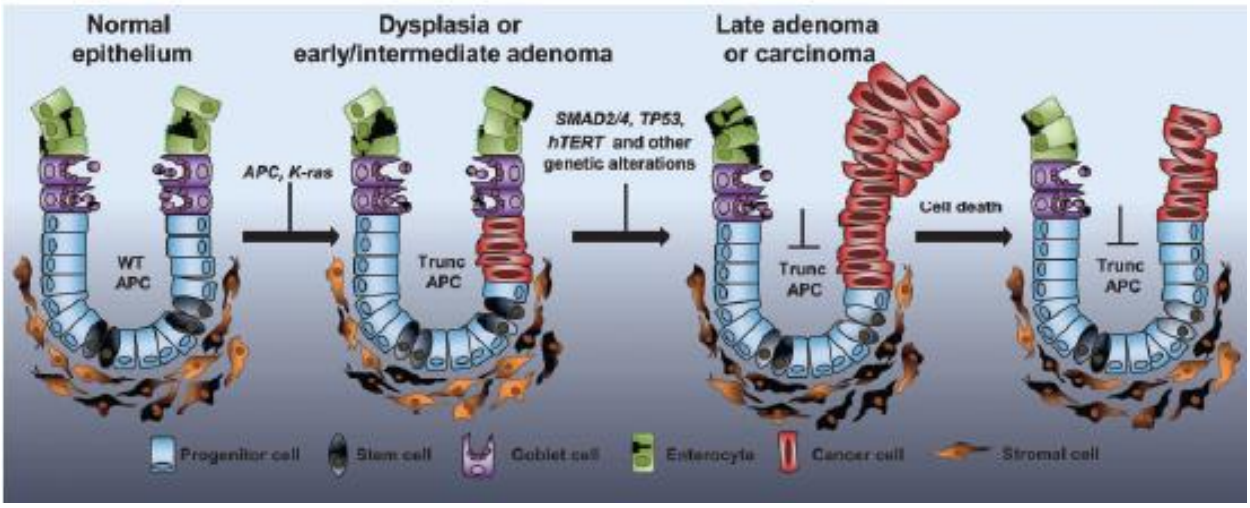


Image 10 - Dependency of colorectal cancer (CRC) cells on truncated APC

4 Polymorphisms of mutations in APC gene

4.1 Methods

To analyze mutations in laboratory conditions various methods are used: Polymerase Chain Reaction, RNA protection analysis, The Protein Truncation Test, Single Stranded Conformation Polymorphism, DNA sequencing, Denaturing Gradient Gel Electrophoresis.

Polymerase chain reaction – is one of the genetic engineering method, which used to make millions or billions of specific DNA samples' copies. During the process, Taq polymerase, small DNA sample, buffer solution and PCR primers are needed. DNA polymerase is using for DNA replication which is making new DNA stands. Taq polymerase is an enzyme which isolated from Thermus aquaticus bacterium and it used because of its heat-stable. PCR primers are short pieces of single-stand DNA, which have approximately 20 nucleotides. In one PCR reaction two primers are used, they are complementary to given single-stand DNA. Primers can be extended by the Taq polymerase when they are bound with the DNA sample.

For PCR, all components of reaction are assembled in a tube and next steps occur (Image 11) [39]:

1. Denaturation is a reaction of separating double DNA stand by heat, which is 96C.
2. Annealing is the cooling of reaction between 55 and 65 C, by that primers can complementary bound with single-stand DNA.
3. Extension is the process of heating in the higher temperature (72C) by that Taq polymerase can extends primers and it is synthesis of new DNA stands.
4. All steps are repeating 25-35 times and take 2-4 hours [39].

RNAse Protection analysis is high sensitive method which identify and calculate the number of specific mRNAs in total RNA molecule. The assay uses in vitro transcribed 32P-labeled antisense RNA probes that hybridize in solution to their complementary cellular mRNAs. After that, single-standed RNAs are degested with RNAse. RNAse is deleted with K protease. Since it is possible to synthesize "sense" mRNAs having the same sequence as the mRNA of the target cell, it is possible to construct appropriate standard curves and use them to quantify changes in mRNA levels in tissues. Since the assay requires perfect sequence complementarity for complete protection, it not only serves as a quantitative tool, but also provides compelling evidence for the existence of a particular mRNA in a given tissue [40].

The protein truncation test (PTT) is a method which detects mutations at the protein level. This method is simple and fast way to identify biologically relevant mutations. It is based on the analysis of size of transcription and translation products in vitro. Proteins with lower mass, than it was expected by full-length protein. These

proteins are taking as a products of translation which presented as a result of truncating frame shift or stop mutations in the analyzed gene. However, sample PTT method has low sensitivity, so it can be used to analyze only in samples which have large amount copies of mutations. To solve that problem, PTT had modified and now there are presented digital PTT and gene capturing. Firstly, DNA or mRNA source PCR amplification goes with direct primers which contains T7-promoters and translation initiation signals, so these products can be used in in vitro transcription and translation to identification of small truncated proteins' products. Usually mRNA is using, but it has induced nonsense mutations [41].

Single stranded conformation polymorphism (SSCP) is an analysis which is widely used to identify different genomic variants in a large number of samples and in large range of organisms [42]. Analysis detects sequence through electrophoretic mobility differences. DNA with mutations has differences in mobility, in comparison with DNA without any mutation sequences. To analyze, DNA should be extracted and that first step of this method. DNA extraction are depending on sample's characteristics. Next step is PCR, so analyzing DNA region is copied. After that, copied DNA samples are mixed with Hi-Di Formamide mix, and after heat denaturation, electrophoresis is going. Electrophoresis is started with adding PCR products with electro kinetics method in capillary. DNA fragments are separated by size by fluorescent which is a result of high voltage. Separated DNA fragments are detected by a laser-camera system (Image 12) [43].

DNA sequencing is a process of detecting the nucleic acid sequence. This methods help in medical and biological researches and discoveries. In Switzerland's study used direct DNA sequence. This method needs radioactive labeling at one of the 5' end of the DNA and purification of DNA fragment. Chemical treatment leads to breaking of nitrogen bases (A, T, G, C).

Denaturing gradient gel electrophoresis (DGGE) is a method which is used to separate short and medium-size DNA fragments based on temperature gradient to denature the sample during it is moving by acrylamide gel. It helps in identification one nucleotide polymorphisms without DNA sequencing [44]. DNA samples are amplified by PCR and then electrophoresed in gel. Samples are denatured on gel by their melting in different places of gel. In this gel matrix, melting domains create single stranded nucleotide branches. Denatured samples are shown as patterns and by these patterns can be compared with known DNA samples to identify the species present in the sample (Image 13) [45].

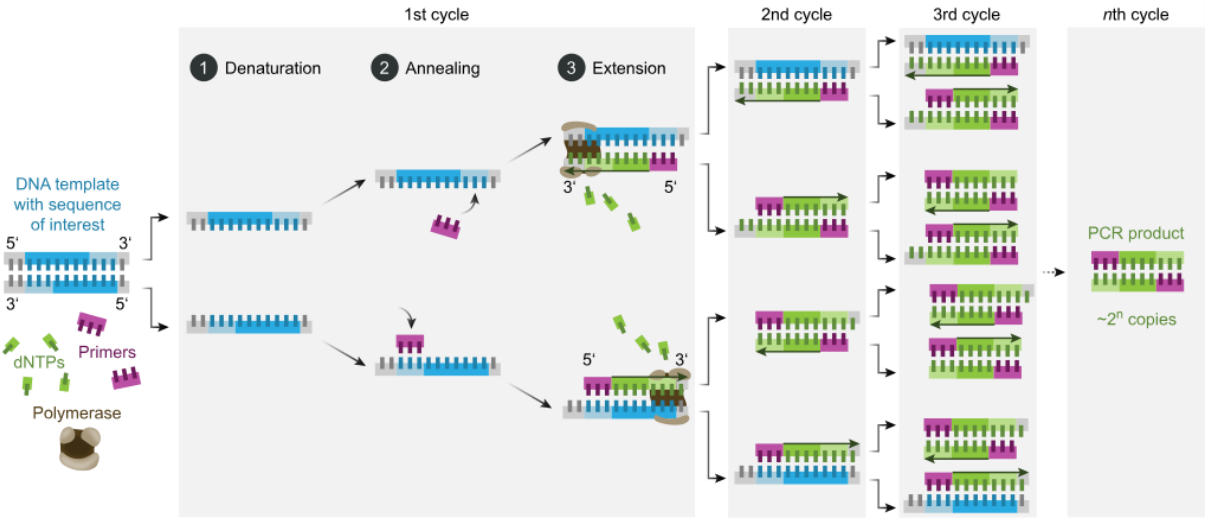


Image 11 - PCR steps

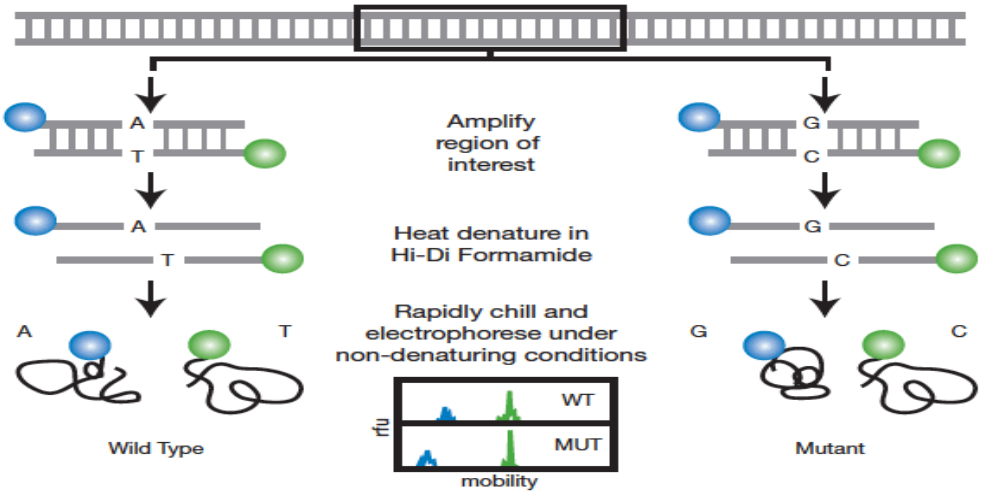


Image 12 - Single-stand Conformation Polymorphism

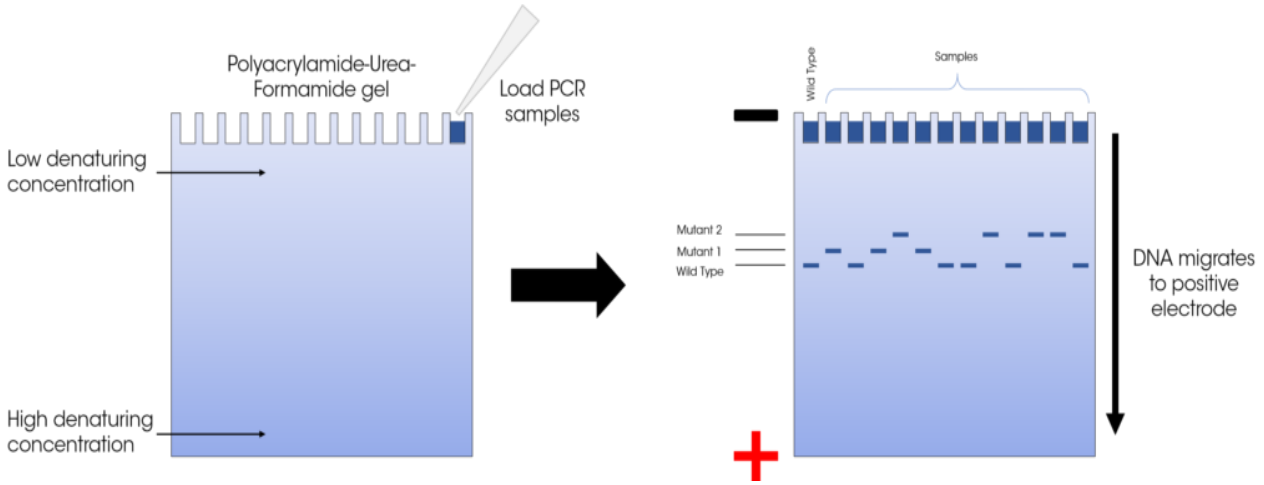


Image 13 - Denatured gradient gel electrophoresis method

4.2 Mutations in APC gene (researches)

In 1992, APC gene mutations in tumors were analyzed. For that PCR, RNase protection analysis and cloning and sequence analysis methods were used. 95 per cent of somatic mutations lead to truncated APC protein. Also two mutations effect on APC protein activity. In the study, 43 mutations were found and 21 of them are point mutations and 22 mutations are frameshift mutations. Deletions were from one to 31 base pairs (bp) in codons, 31 bp deletion in codon 1293 and 8 bp deletion in codon 1353, 11 point mutations were in Cytosine on CpG or CpA (Table 5) [46].

In addition, during studying gastric cancer, there is possibility gastric cancers are considered to originate from the FAP. For this study, DNA of gastric cancer was analyzed by RNAse by PCR test. In the APC gene in 44 of tumors were found somatic mutations: nonsense, missense and delition. To analyze, firstly, normal APC from healthy gastric was taken and then its expression lhe APC in several normal tissue of mice with reverse transcription-PCR. The APC gene was expressed in a wide variety of organs, including the stomach. DNA sequences of the APC gene were amplified by PCR and these products were analyzed by RNAse protection analysis. From 279 to 1666 codons were found mutations in patients with FAP. All of these mutations were somatic mutations and they were found in three types of tumors (Table 6) [47].

Table 5 - Number and types of mutations in tumors

Mutation type	Number of mutation
Point Mutation	21
Nonsense	16
Missense	2
Splice	3
Frameshift Mutations	22
Deletion	19
Insertion	2
TOTAL	43

Table 6 - Mutations in APC gene in tumors

Tumor	Codon	Nucleotide change	Mutation
C23	1068	tcaaGGA	Deletion
C20	1286	GAA – TAA	
C11, C15	1114	CGA – TGA	
C27	1293	AcacaggaagcagattctgctaataccctgcAAA	Deletion
C7, C21	1309	GAaaagaT	Deletion
C21, C42	1315	TCA – TAA	
C22	1353	gaatttcTTC	Deletion
C16	1420	CCcA	Deletion
C40, A52	1429	GAA - TAA	
C3	1513	GAC - TGAG	Insertion

4.3 Mutations and de novo mutations in APC gene through countries

In Russia, to analyze mutations in APC where used 65 blood samples and 12 samples did not have FAP. In this group, 37 mutations were found, which is 57% of group. Only to types of mutations were repeated: deletion in 1061 codon and deletion in 1309 codon (Table 7). In general, each sample has its own mutation in APC, so there are some deletion of nucleotides, changing of nucleotides, which leads to appearing stop-codons. Mutations in APC gene occur from 1061 – 1309 codons more than in other codons (Figure 4). In addition, 90 per cent of mutations lead to synthesis of truncated APC and other 10 per cent are missense mutations [34].

In Europe, 680 families were analyzed. Lots of mutations in APC gene were because of deletion some nucleotides and only seven mutation were repeated: families have insertion in 1859-1860, 3799, 4022, 4025 codons, others have deletion in 509, 1863, 3467 codons. In general, more mutations occur between 1000 and 1500 codons (Figure 5). Deletion of a few nucleotides usually appears because of mistakes in replication, which occurs in specific DNA region [48].

In Finland, clinical implications and the prevalence of APC gene mutations were analyzed into patients with FAP and with desmoids. Blood from the Finnish Familial Polyposis Registry were taken and they were used for DNA and RNA extraction. Also information about diagnosis, colorectal cancer, number of polyps were retrieved from hospital records. In the study, 38 different mutations were found, but only 14 mutations were registered in the database of human genome mutations. In general, there were small deletions, small insertions, splicing and nonsense mutations (Figure 6). The most mutations were in codons 1060, 1309 and 1407 (Table 8) [49].

Study from University of Bonn in Germany from 2004, where 917 patients from 1990 to 2002 with FAP were observed to mutations in germline in APC gene. Analysis are made by SSCP and PTT methods. 410 patients exactly had FAP with 4100 colorectal adenomas, 171 patients had an attenuated or suspected FAP with from five to 100 adenomas and the number of adenomas of other patients were not identified. In 448 patients, there are 49 per cent, patients a pathogenic germline mutation was identified. So 563 patients had the familial cases, which is 90% of mutations, and 58 patients had de novo mutations, which is 10%. Suspected de novo mutations were identified in 52 of 58 patients which is 90 per cent (Figure 7). More de novo mutations were in codon 1309, there was deletion of AAAGA nucleotides [50]. In 2005, 1166 non-FAP patients' blood samples from 1991 were taken for study. 634 mutations were identified and 94% (597 mutations) of them were point mutations, and other mutations were large deletions (37 mutations). In some codons were more than one mutations, so in codon 141 were seven mutations. In codon 1309 were found deletion of 5 bp, deletion of GAAA and substitution of G to T. In codon 1061 was only one mutation (deletion of CAAA) [51].

In the UK, 190 FAP and 15 non-FAP patients were analyzed by PCR method. In these cases, twenty seven different mutations were identified in 47 patients which leaded to truncated APC protein. Single nucleotide changes were found in two patients with FAP and one without FAP, but changes of C to T in codon 1171 leaded to Cisteine replacing arginine which not considered to be responsible for the FAP phenotype. 5 bp deletion was the most frequently mutation in this study, and they were "ACAAA" deletion in codon 1061 and "AAAGA" in codon 1309 (Table 9) [52].

In 1998, in Switzerland, phenotypic differences in FAP was analyzed, 161 parents from 50 FAP families were taken. To analyze mutations were used PTT, SSCP and DNA sequencing methods. In 36 families, APC germline mutations were found in different codons, including in codon 1309 which were in two families. However, in codon 1987 mutations were found in ten families. The FAP patients' diagnosis of colonic polyposis according to age were also analyzed. Diagnosis was found in about 25% of 20-29 age, but the most APC-negative patients was at age 40-49, while the most number of APC-positive patients was at age 20-29 (Figure 8) [53] (Table 10).

In 2004, Polish patients with FAP were analyzed: 79 from the central west, 17 from the northwest, 7 from the south east, six from the southwest, two from the north and nine from the central east of Poland. 140 patients had typical FAP and eight patients had atypical FAP. In the analysis of 120 patients and 35% of them had 22 different APC gene mutation (Table 11). Deletion of A in codon 636 is a de novo mutation, mutations 2413C-T and 4393-4394InsGA, 4387insGA were also de novo mutations. Mutation in codon 1309 were also found, and it was in 15 families, mutation in codon 1061 was in six families. Moreover, there were found mutation in codon 1068 (3202-3205delTCAA). By ages, mutations presented at any age from four to 47, but some patients ages were not noun. Four years old patient had mutation 3921-3924delAAAA and it was duodenal polyps, while 1492delT leaded to desmoid tumor and cerebral flax tumor in one patient, and in another patient, it leaded to brain fibromatosis. In one patient mutation in codon 1309 leaded to liver and lung cancer. Stomach polyps and desmoid were presented by mutations 4266-4276delTCTTCCAGATA and 4667insA [54].

In study of Belgian patients in 2002, patients with FAP were analysed. In 40 patients, APC gene's exon 15 was analyzed and for that, PTT method was used. In this study, known mutations and de novo mutations were found. Moreover, mutations mostly were in exon 15, while mutations in codon 357 and 491 were in exon 9 and 11 respectively. Six families had deletions of five base pairs in codon 1309, while other mutations were found only in one patients. Mutations were presented from 10 to 27 years (Table 12) [55].

In Taiwan, medical examination were in the Hualien Tzu Chi medical center for 74 patients. All patients were analyzed with colonoscopy and no one has CRC

family history. Therefore, they were taken with 80 patients with CRC, groups had 40 males and 40 females, so all mutations were in APC gene. Mutations were in exons 9, 11 and 15, so most mutations were in exon 15 (Table 13). Mutations in codons 1061 and 1039, like in other studies, have not been reported. Therefore, APC gene mutations can be depend on population differences. In this case, there were three new mutations in Taiwan's population. Mutation in 460 codon leaded to expression of a truncated APC protein. In codons 1125 and 1126, missense mutations occurred, and they are leading to damage of APC signal function [56].

In Japan, in 1997, 75 FAP tumor were collected and there were 29 gastric adenomas and 11 gastric fundic gland polyps, for that PCR and SSCP methods used. 47 APC somatic mutations were detected in 21 FAP patients. All detected mutations leaded to stop-codons and then to truncated APC protein. Eleven mutations were 2-4 base pair deletion and 25 mutations were 1 or 2 bp insertion, while 25 mutations were nonsense point mutations. Gastric mutations in APC gene were located in 1450, 1462-1465, 1554-1556 codons (Table 14). Nevertheless, 26 tumors did not have any detections of mutations in gene, but A insertion was more frequented mutation in tumors (20 of all tumor had that type of mutation). In comparison of diseases and mutations, founded mutations leaded to carcinoma (insertion in codon 1554), severe adenoma (substitution in codon 1450, deletions in 1462-1464) and moderate adenoma (insertion in codon 1506, substitutions in codon 1564 and 1539) (Table 14) [57].

In 2001, study from Australia was published. In this study, SSCP, DGGE and PTT methods were used. To analysis, 53 patients were taken and 27 mutations in APC gene were found. There were deletions, substitutions and insertions. More frequent mutation was T substitution, and in codon 5773 was the biggest deletion of base pairs (Table 15). Nevertheless, de novo mutation in codon 1309 was not found. Also study related to age showed that the most mutations were present in age group from 10 to 19, but the biggest amount of people with FAP was at age 40-49 (Figure 8). Also there were group of patients with a mixed adenomatous hyperplastic polyposis, which was the biggest at age above 60 [58].

In 2020, study from New Zealand was published. 25 patients' DNA were analyzed and there were 14 female and 11 male under the age 50, PCR method was used. Most diagnosis were between 41 and 50 years (72%) and only one patient had diagnosis when he was under 30 (Table 16). Most mutations were in exon 15 between 1200 and 1800 codons (Figure 10). There were lots of substitutions and the most frequent was C changing to T (Table 17). Also the most of mutations were nonsense mutations, while three of all mutations were missense and three were frameshifts. Moreover, four patients had two types of mutations in the same time (Table 17) [59].

Study from University of Cape Town, South Africa, in 2000, analyzed hereditary non-polyposis colorectal cancer (HNPCC). Patients' blood analyzed by

PCR and PTT methods. In 20 patients were found nine mutations and more frequent mutation was in codon 1309 (seven families), then in codon 564 (five families) in exon 15. Also, in this study, insertion of A in codon 1426 was found (Table 18). The most frequent mutation types is substitution C to T in codons 302, 564, 901 [60].

In South Korea, 83 FAP families were studied in 2005. For gene analyzing PCR, PTT were used. 71 per cent of patients had 45 different germline mutations, while 34 patients had 24 different frameshift mutations. In this study, 17 mutations were new, and there was big deletion of nucleotides, in codon 1247, 31 bp delition (Table 19). Also, de novo mutation in codon 1309 was found and it was the most frequent mutation in this study, six patients had that mutation, while mutation in codon 1289 was in five patients. Mutations were found from 16 to 58 years old. Moreover, most of patients had colorectal cancer and it was not depend on number of polyps and mutation type [61].

In 2004, study from Argentina was published where 51 FAP unrelated patient were analyzed by SSCP, DNA sequencing and PCR methods. The most mutations were found in exon 15, while 12 patient did not have any mutations. In codon 1309 (10 patients or near 20 %) was the most number of patients with mutations, then in codon 1061 (two repetition). Mutations were not depended on age and CRC having (Table 20) [62].

To sum up, APC gene mutations studies were done in different countries in different years: from 1997 to 2020. Mutations from APCE gene mutations database were found in these studies, but there, also, were de novo mutations. To found that mutations PCR, SSCP, DNA sequencing, PTT methods were used. As de novo mutations were mutations in codon 1309, 1061, which were presented in many countries, but in Australia, in Japan and in Taiwan these mutations were not presented. In other countries that mutations were the most frequent, but in Switzerland, the most frequent was mutation in codon 1987. In total, mutation in 1309 is 6 per cent and in codon 1061 is near 4 per cent (Table 21). In Australia, in codon 5773 was large duplication of nucleotides (17 bp). These mutations were different from mutations in patients in different studies. Therefore, it can be concluded, that APC gene mutations depends on the nationality or living place. By age, mutations was found in four years old patient, so mutation can be found from four to older than 70.

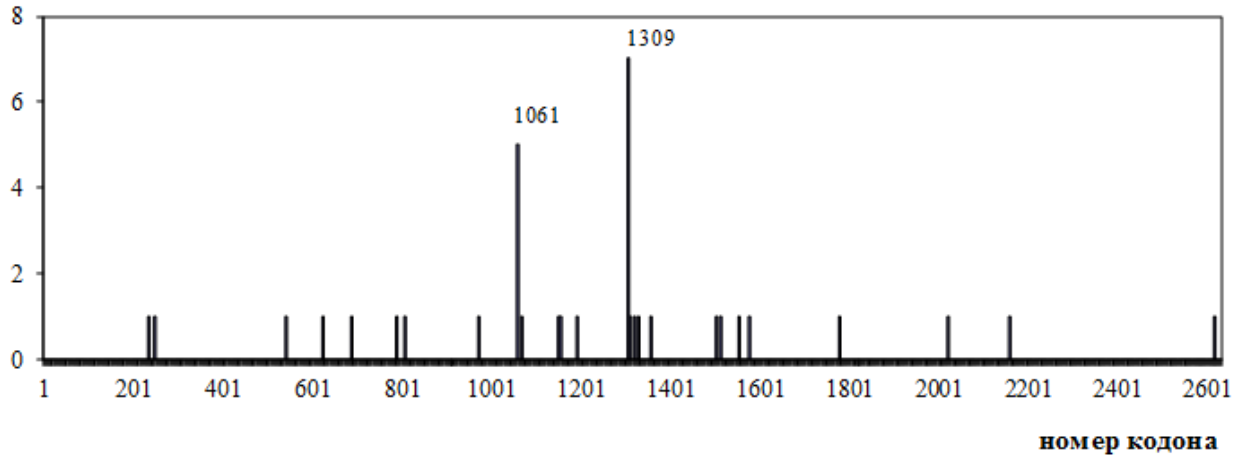


Figure 4 - Distribution of mutations in APC gene in Russia

Table 7 - Mutation in APC gene in Russia

Codon	Mutation frequency	Mutations
541	1	Q541X
1556	1	T1556S
1580	1	1580del4
1319	1	1319delCC
1331	1	1331delG
1061	5	1061del5
2160	1	T2160A
1192	1	1192delA
1155	1	1155del5
1515	1	1515insT
232	1	R232X
974	1	974delGT
1156	1	1156delAA
1309	7	1309del5
805	1	R805X
790	1	790delAG
1152	1	1152delA
247	1	Q247X
1310	1	1310insA
2023	1	2023delG
1503	1	S1503X
688	1	688delA
1068	1	S1068X
1780	1	P1780S
2621	1	S2621C
621	1	621-622del4
1356	1	S1356X

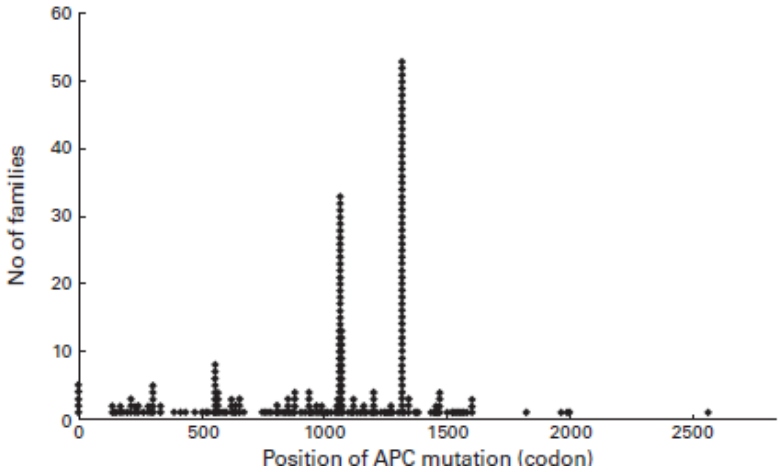


Figure 5 - Distribution of mutations in APC gene in 680 families in Europe

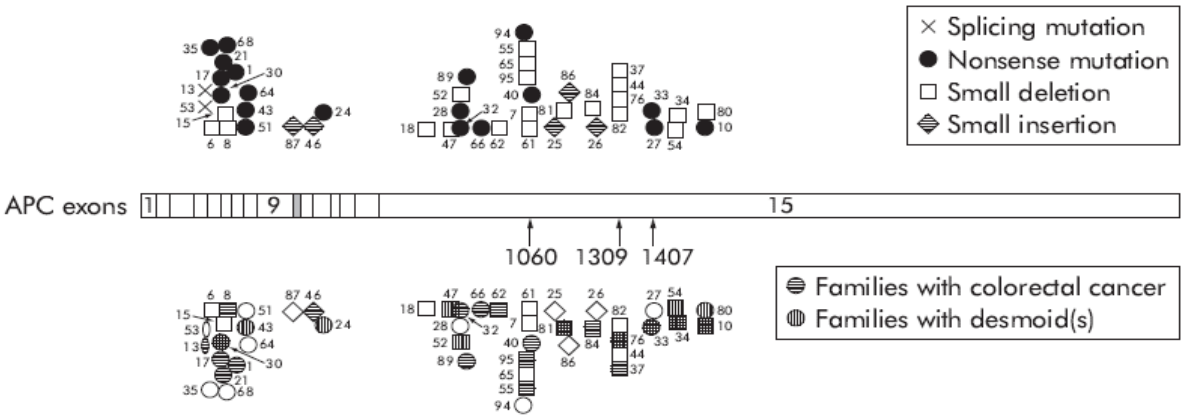


Figure 6 - Distribution of APC mutations in Finland

Table 8 - APC gene codons with the most number of mutations in Finland

Codon	**Mutation type**	**Nucleotide changes**	**Frequency**
1309	Deletion	AATAAAAGA***acaaaa***GT	
1060	Deletion	GATG***aaat***AAACAA	
1407	Nonsense	CAG - TAG	

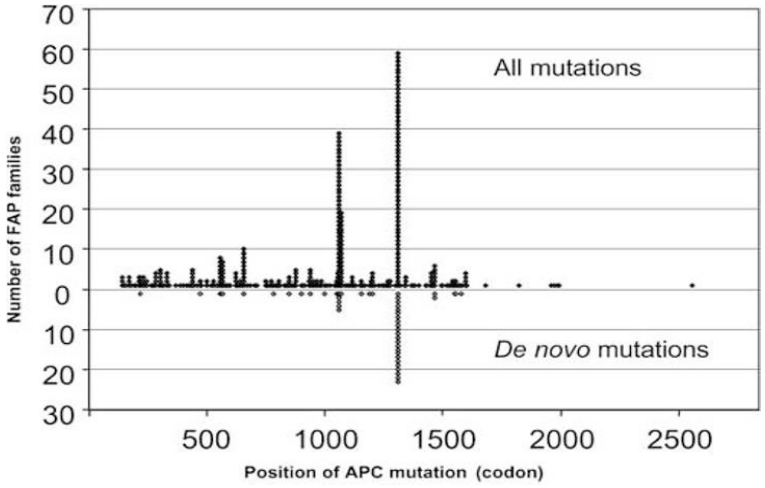


Figure 7 - Germline mutations in the APC gene including de novo mutations in Germany

Table 9 - APC gene mutations in the UK

Codon	Exon	Mutation	Mutation frequency
177	4	Deletion T	1
232	6	Substitution C to T	3
233	6	Substitution C to T	1
283	8	Substitution C to T	9
452	10	Insertion TG	1
456	10	Deletion TTTCA	1
457	10	Substitution C to A	1
495	11	Insertion A	1
486	11	Substitution C to G	1
500	11	Substitution C to T	1
527	12	Deletion AGCACTTGTG	1
541	12	Substitution C to T	2
564	13	Substitution C to T	1
585	14	Deletion C	1
609	14	Deletion G	2
625	14	Substitution C to T	1
640	14	Deletion G	1
648	14	Insertion A	1
657	15	Deletion A	1
764	15	Substitution T to A	1
770	15	Insertion A	1
931	15	Deletion TCAA	1
934	15	Deletion TTAC	1
935	15	Insertion A	1
1023	15	Insertion T	1
1045	15	Substitution C to T	2
1061	15	Deletion A	11
1068	15	Deletion TCAA	2
1122	15	Deletion AATC	1
1309	15	Deletion AAAGA	10
1395	15	Deletions A and T	1
1449	15	Substitution A to T	1
1450	15	Substitution T to C	1
1465	15	Deletion G	1
1493	15	Deletion A	1
1822	15	Substitution A to T	3
2738	15	Substitution t to C	1

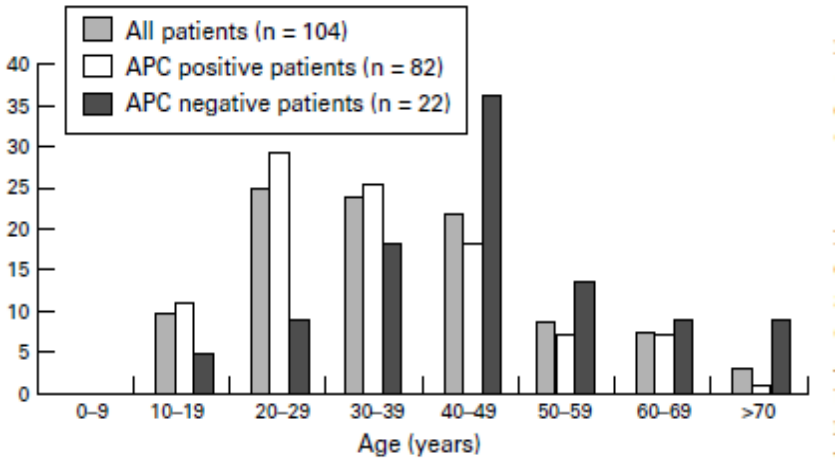


Figure 8 - Age at diagnosis of colonic polyposis in FAP patients in Switzerland

Table 10 - Mutations frequency in Switzerland

Codon with mutation	151	169	232	498	793	805	849	935	1061	1062	1309	1987
Frequency of mutation	1	1	1	1	1	1	1	1	2	1	3	10

Table 11 - Mutation frequency in APC gene in Polish families

Codon	**Mutation**	**Mutation frequency**	**Ages**
601	delG	1	-
636	delA	1	26
892	delCA	1	-
1491	insT	2	12, 10, 36, 16, 35,37, 47
2413	subC-T	1	-
2509	delC	1	16
2626	subC-T	2	16, 30
2922	insG	1	-
3119	insCTCTGGAA	1	-
3165	delTAATA	1	-
3183	delACAAA	6	-
1068	delTCAA	2	26
3371	delA	1	13
1158	delGA	1	19
3515	delA	1	36
3578	delCAGT	1	-
1199	delA	1	-
3921	delAAAA	1	4
1309	delAAAGA	15	20,26, 13, 36, 12, 18
4266	delTCTTCCAGATA	1	31
1465	insGA	1	-
4667	insA	1	45

Table 12 - APC mutations in Belgium families

Codon	Exon	Mutation	Mutation frequency	Age
357	9	1087insA	1	-
491	11	1494delC	1	-
704	15	2128delG	1	18
966	15	2911delAATA	1	-
1028	15	3104delTT	1	10
1061	15	3201delACAAA	1	-
1199	15	3614insA	1	23
1309	15	3945delAAAGA	6	-
1347	15	4057CG – A	1	13
1465	15	4411delAG	1	-
1474	15	4439delC	1	27
1538	15	4630delGA	1	-

Table 13 - APC mutations in Taiwan

Exon	9	10	11	11	13	15	15	15	15	15	15	15	15
Muta-tion	1050 T to G	1378 delG	1458 T to C	1488 A to T	1635 G to A	3374 T to C	3378 C to G	4479 G to A	4725 A to G	5034 G to A	5268 T to G	5465 T to G	5880 G to A

Table 14 - Somatic mutations in APC gene in gastric tumors in Japan

Mutation frequency	20	9	8	1	1	2	1
Codon	1554-1556	1450	1462-1465	1506	1462-1465	1564	1539
Mutation	A insertion	C to T	AG deletion	TA insertion	AGAG deletion	T to G	C to G
Diagnosis	Carcinoma	Severe adenoma	Severe adenoma	Moderate adenoma	Severe adenoma	Moderate adenoma	Moderate adenoma

Table 15 - Mutations in APC gene in Australia

Codon	Mutation type	Nucleotide change
301	T substitution	GGGAA**G**GATC to GGGAATGATC
487	T substitution	AACTT**G**AGAA to AACTTTAGAA
451	4 bp deletion	AAG**AAGA**AAA to AAGAAA
996	C deletion	GTCG**C**GAACT to GTCGTGAACT
996	T substitution	TGTCG**C**GAACT to TGTCGTGAACT
1005	14 bp deletion	TTGCT**AGCTATGTCTAGCT**CCAAGAC to TTGCTCCAAGAC
1495	T substitution	TAAGA**C**GATAT toTAAGATGATAT
1619	A insertion	CTTACAGCAG to CTTA**A**CAGCAG
1907	G insertion	GAGGTGGGAT to GAGG**G**TGGGAT
2096	A substitution	GCATTATGGGA to GCATTA**A**GGGA
2225	TT deletion	CAATA**TT**ATGT to CAATAATGT
2537	C deletion	TCGTT**C**TGAAA to TCTCGTTTGAA
2799	ACTT deletion	AAACACTTACAAT to AAACACAAT

Table continuation

2803	TACA deletion	CACTTACAATTT to CACTATTT
2907	T deletion	GTAGTAGTGA to GTAGAGTGA
3183	ACAAA deletion	AATAAA**ACAAA**GTG to ATAAAGTG
3231	TTATA deletion	CCTGTTTATACTG to CCTGTCTG
3459	TGAA deletion	CAGCATGAAGAAG to CAGCAGAAG
3283	T substitution	TTGGACAGCA to TTGGATAGCA
3620	A deletion	AGTAAAACCG to AGTAAACCG
4330	A insertion	TCCTCAAAC to TCCTCAA**A**ACA
4424	C deletion	TGCTG**C**AGTT to TGCTGAGTT
4647	A insertion	AACCAAGAGA to AACCAA**A**GAGA
5773	17 bp duplication	AACCCATACTTCAGAAACAATCCAC to AAACC**CATACTTCAGAAACAACCATAC TTCAGAAACAA**TCCAC

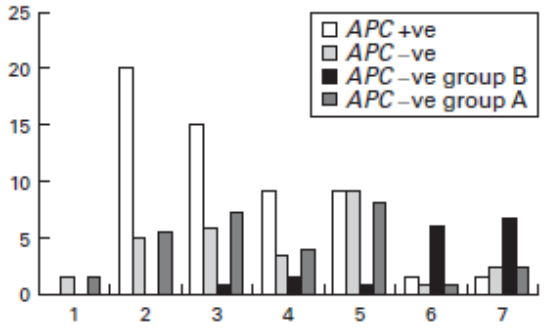


Figure 9 - Studying APC mutations related to patients' age in Australia.

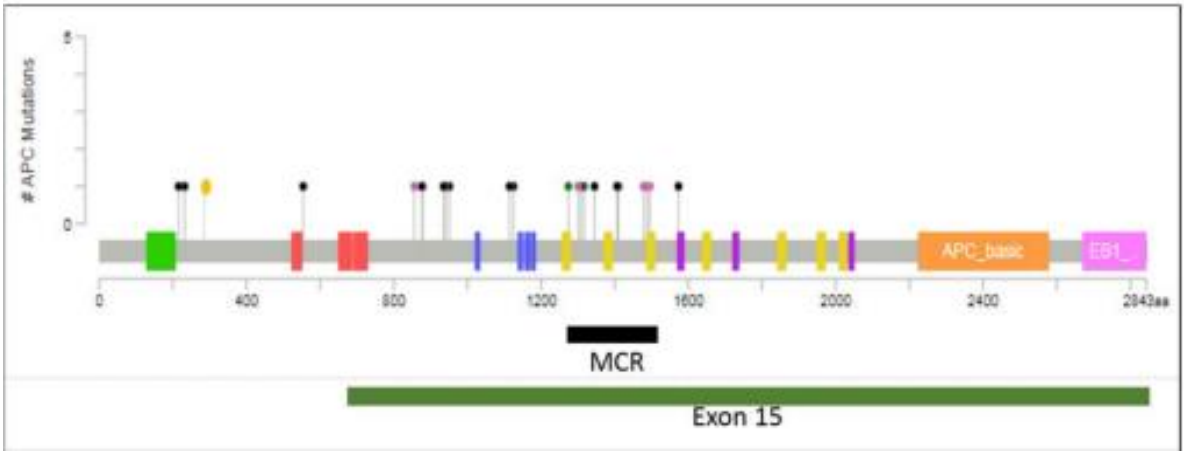


Figure 10 - Distribution of mutations in APC gene in New Zealand (MCR – mutation cluster region)

Table 16 - Age of diagnosis CRC in New Zealand

Age	**Number of patients**	**Percentage**
Under 30	1	4
32-40	6	24
42-50	18	72

Table 17 - Mutations in the APC gene in New Zealand

Mutation	Mutation type
2626 C to T	Nonsense
1660 C to T	Nonsense
2853 T to G	Nonsense
4488 delT 2805 C to A	Nonsense
2636 delA	Frameshift
4033 G to T	Nonsense
4463 delT 3826 delT	Frameshift Frameshift
4616 C to T 3949 G to C	Nonsense Missense
4717 G to T 2821 G to T	Nonsense
4485 delT 2626 C to T	Frameshift Nonsense
3925 G to T	Nonsense
3740 C to T 3340 C to T	Missense Nonsense
4230 C to A	Nonsense
3374 T to C	Missense
2563 dupGA	Frameshift

Table 18 - APC gene mutations in South Africa

Exon	Codon	Nucleotide change	Mutation frequency
8	302	906 C to T	1
10	441	1323 del T	1
13	564	1692 C to T	5
15	901	2071 C to T	1
15	1061	3182 del AGAAA	2
15	1095	3285Ins A	1
15	1309	3936 del AGAAA	7
15	1426	4276 Ins A	1

Table 19 - APC gene mutations in South Korea
(From 480 to 1291 were found in this study, from 1061 to 2040 were found in studies before)

Codon	Mutation	Mutation frequency	Age	CRC
480	*1438 C to T*	*1*	*24*	*No*
745	*2232-2233dupCT*	*1*	*16*	*No*
1142	*3426delT*	*1*	*47*	*No*
1247	*3739-3769del31*	*1*	*-*	*No*
1311	*3931-3935delATTGG*	*1*	*19*	*Yes*
1444	*4332dupA*	*1*	*38*	*No*
1574	*4722-4725delACTA*	*1*	*35*	*No*
216	*646 C to T*	*2*	*31, 32*	*No*
695	*2083 C to T*	*1*	*33*	*No*
805	*2413 C to T*	*1*	*31*	*No*

Table continuation

831	*2493dupA*	*1*	*36*	*No*
935	*2895 C to G*	*3*	*28, 53, 38*	*Yes*
943	*2828 C to G*	*1*	*38*	*No*
976	*2927-2928delGA*	*1*	*24*	*No*
1168	*3502-3506delGAAGA*	*1*	*58*	*Yes*
1291	*3871 C to T*	*1*	*39*	*Yes*
1464	*4391-4394delAGAG*	*2*	*31*	*No*
1061	3183-3187delACAAA	3	43, 29, -	Yes, no, yes
1075	3225 T to A	2	37, 46	Yes
1098	3294-3295delTG	2	31, 61	Yes, no
1289	3867 T to A	5	52	Yes
1309	3927-3931delAAAGA	6	35, 20, 21, 38, 18, 39	Yes, yes, no, yes, yes, no
1370	4108 A to T	1	23	No
2040	6118 C to T	1	48	Yes

Table 20 - APC gene mutations in Argentina

Exon	**Codon**	**Mutation**	**Mutation frequency**	**Age**	**CRC**
6	346	sub C to T	1	17	Yes
11	480	sub C to T	1	36	No
12	1621	sub C to T	1	26	No
15	2522	delT	1	41	Yes
15	2626	Sub C to T	1	24	Yes
15	2893	delACTT	1	20	No
15	2802	delT	1	17	No
15	831	delC	1	33	Yes
15	3081	delTA	1	32	No
15	1061	delACAAA	2	34, 39	Yes, no
15	1062	delAA	1	49	No
15	1063	delTCAA	1	24	No
15	3367	Sub C to T	1	42	Yes
15	3510	delA	1	34	Yes
15	1193	sub C to T	1	42	No
15	1193	dupG	1	36	Yes
15	1234	delAA	1	47	Yes
15	1468	dupA	1	19	No
15	1327	delTT	1	23	No
15	1233	dupG	1	25	No
15	1272	3815 C to G	1	14	No
15	1309	delAAAGA	10	13, 13, 21, 26, 28, 28, 28,31, 34, 31	No, no, no, no, no, no, yes, no, no, no
15	1373	4084-4085delTC	1	2	no
15	1389	4280delC	1	44	Yes
15	1453	4348 C to T	1	19	No
15	1465	4393-4394delAG	1	35	Yes

Table continuation

15	1445	4526-4533dupTGAGCCTC	1	19	No
15	1556	4669-4670delAT	2	16, 46	No, yes
15	1578	4888-4891delGTTA	1	13	No
-	-	No mutation	12	21, 25, 30, 33, 36, 41, 43, 45, 47, 57, 30	No, no, no, no, yes, no, no, no, yes, no, no

Table 21. de novo mutations in different countries

Country | Codons	**Number of patients**	**1309**	**1061**
South Africa	20	7 (35%)	2 (10%)
Germany	917	53 (5.8%)	32 (3.4%)
United Kingdom	205	10 (4.9%)	11 (5.4%)
Switzerland	161	3 (1.9%)	2 (1.3%)
Belgium	40	6 (15%)	1 (2.5%)
Australia	53	0	0
Russia	77	7 (9%)	5 (6.5%)
Finland	47	4 (8.5%)	7 (14.9%)
Taiwan	74	0	0
Poland	120	15 (12.5%)	6 (5%)
South Korea	83	6 (7.2%)	-
TOTAL	1 797	111 (6.2%)	66 (3.7%)

4.4 Radiation therapy and APC gene

Radiation therapy used as a type of cancer treatment. Most often x-rays and protons are used to kill cancer cells [62].

Proton radiotherapy is the most used method because protons is effective to make point DNA damage in the place of tumors without negative effects on the nearby cells. Nevertheless, proton radiation therapy was not researched properly as a photon therapy [63].

In 2016, effects of proton radiation was analyzed on APC mice. These mice were affected by two Sievert of x-ray, acute high dose-rate protons and low dose-rate protons. Median survival of non-affected mice was 763 day, while survival of APC mice, which was not affected by low dose-rate protons and was affected, were 351 days and 327 days. To analyze effect of radiotherapy, mice were observed after 50 days proton radiation and at this moment in tumor was not any significant changes. However, after 1000 days medium number of polyps in control group were 3,2, in mice after x-ray therapy was 4,4 and in mice after low dose-rate was 7,2. To reduce that effect, mice had diet with CDDO-EA and after that number of polyps were reduced (Figure 10). As a result, protons' exposure on mice induced persistent of DNA damage responses, which leaded to senescence-inflammatory response and CDDO-EA is reducing DNA damages [64].

In the USA, CRC risks were observed in people who survived after atomic bombs and radiation workers. Moreover, analyzing patients, who had prostate cancer and radiation therapy, showed that risks of a second malignant neoplasm of the colon cancer was high, and it is supporting the opinion that radiation therapy is a factor for CRC. On the molecular level, radiation has carcinogenic effects by chromosomal instability, which leads to loosing of reparation genes functions and suppressor genes like APC gene. Therefore, there is prediction that cosmic radiation leads to damages in molecular level and then to cancer [9].

To analyze radiation therapy effects on patient with FAP, 36 patients from The Hereditary Gastrointestinal Cancer Registry (HGCR) with familial gastrointestinal cancers were taken. These patients were treated by radiotherapy and the record gave all demographic and clinical information about patients. 18 of these patients had genetic diagnosis of FAP, so 15 patients of them had 18 course of treatment with radiation. Doses range was from 18 to 162.9 Sv. Five patients had desmoid tumors, three had colon cancer and two patients had prostate cancer, and breast cancer, melanoma, medullablastoma, gastric cancer, glioma of undetermined type was in one of each other patients (Table 22). As a result, 14 patients had effects as nausea, dermatitis and diarrhea [30].

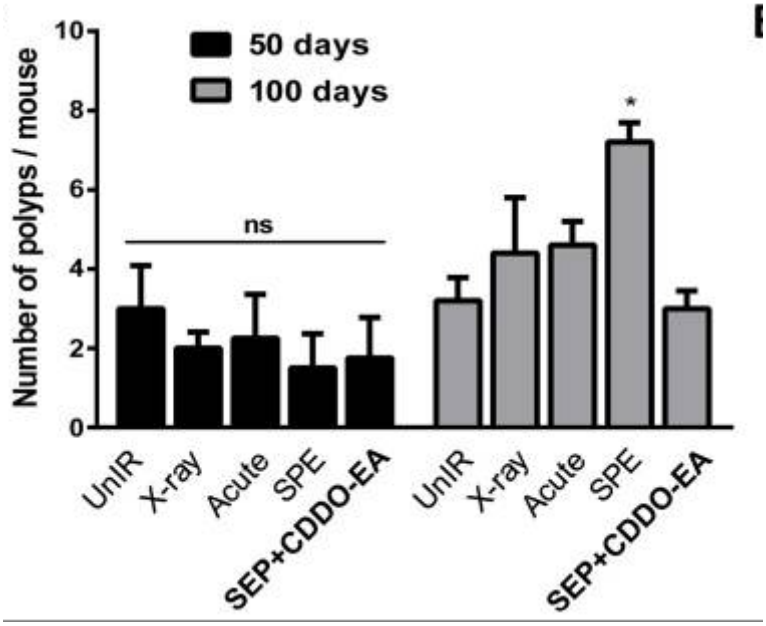


Figure 11 - Number of polyps in APC mice after 50 and 100 days after radiotherapy (UnIR – ; SPE – low dose-rate radiation; SEP+CDDO-EA – low dose-rate radiation with CDDO-EA)

Table 22 - Effects after radiotherapy at different diagnosis

Patient's age	RT dose	Diagnosis	Effects
67	60 Sv/1 fx	Melanoma	Dermatitis
68	18 Sv/1 fx	Melanoma	-
68	20 Sv/4 fx	Melanoma	-
11	54 Sv/30 fx	Medulloblastoma	Hypothyroid

44	162,9 Sv	Prostate	-
47	45 Sv/25 fx	Gastric	Nausea
42	50,4 Sv/28 fx	Desmoid	Nausea, fatigue
44	48,6 Sv/27 fx	Desmoid	Dermatitis, fatigue
51	50,4 Sv/28 fx	Rectal	Diarrhea
70	60 Sv/30 fx	Prostate	-
20	50.4 Sv/28 fx	Desmoid	Nausea, dermatitis
35	50.4 Sv/28 fx	Glioma	Fatigue, dermatitis, nausea, neuropathy
26	50.4 Sv/28 fx	Rectal	Small bowel, pruritus, dermatitis
25	45 Sv/25 fx	Desmoid	Dermatitis, nausea
29	50.4 Sv/28 fx	Desmoid	Colon perforation, fatigue

CONCLUSION

Radiation effects on people everywhere and it is significant to estimate effects of different doses, especially at atomic industry where workers faced with low-doses radiation every day. However, LDR is cumulated in organisms and has long-term effects, so its influence is not fully researched. In that case, personal radiation sensitivity can played important role in studying, because each organism have different respond to radiation as tumor formation due to DNA damage. During radiation therapy, there could be new tumors.

At uranium manners, workers are effected by LRD from 10 Sv to more than 100 Sv, so during five year they have medium LRD dose – 50 Sv. Any doses of LDR lead to appearing of DNA mutations. The more radiation dose, the more mutations in DNA, which researched in APC mice. APC mutations were found in persons' who did not have any disease of gene mutations, so there are many mutations which do not lead to diseases. In the future, APC gene can be used to analyze LDR effects on human organisms. APC gene mutations can be in different organisms without any symptoms. Nevertheless, mutations can lead to appearing polyps and then to colon cancer or gastric cancer. Although, there is database of APC gene mutations, APC gene should be analyzed in specific area (country), not in nationality, because this gene's mutations are different in various countries. Moreover, gene has de novo mutations and these mutations could be at any age from four year. In the personnel of atomic industry, radiation dose also does not depend on age and mostly these workers have less than 10 mSv the work. Although, than older workers, than more radiation they have cumulated. Therefore, all types of mutations and their relation to age should be studied before using it in LRD research.

LIST OF SYMBOLS AND ABBRAVIATIONS

LRD – Low dose radiation
APC – Adenomatous Polyposis Coli
DNA – Deoxyribose Nucleic Acid
FAP – Familial Adenomatous Cancer
CRC – Colorectal Cancer
AFAP – Attenuated Familial Adenomatous Cancer
PCR – Polymerase Chain Reaction
A – Adenine
T – Thymine
C – Cytosine
G – Guanine
bp – base pair
PTT – Protein Truncated Test
SSCP – Single Stranded Conformation Polymorphism
DGGE – Denaturing Gradient Gel Electrophoresis

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