EYE COLOUR INHERITANCE IN HUMANS

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Abstract. Around 8000 years ago, in Europe appeared the first people with blue eyes. For a long time, scientists thought that the gene, responsible for the eye colour, had a dominant allele for brown eyes and recessive one for light eyes. With the development of molecular biology and genetics, however, it was discovered that the eye colour inheritance pattern is not that simple. Nowadays, scientists know that eye colour is a polygenic trait – many genes are responsible for it, which explains the enormous eye colour variation in humans.

This project examines eye colour inheritance in people. It focuses on the most important genes, responsible for eye colour – HERC2 and OCA2. The mentioned genes and their manifestation were investigated within a family by construction of a genealogical pedigree and using molecular-biological methods. The results from the experiment led to confirmation of the theoretical knowledge.

The aim of this study is to present the basic principles that underlie eye colour genetics and to explore the essential methods used by scientists daily to research complex biological topics as eye colour heredity.

Keywords: eye colour; genetics; polygenic trait; HERC2; OCA2

Theoretical analysis of eye colour heredity

What is eye colour?

The eye is our sensory organ, responsible for our light perception of the surrounding world. **The role of eye colour** is to limit the amount of light entering the eye. The structure responsible for the eye colour is the **iris**. It has six layers of cells – anterior limiting layer, stroma, iris sphincter muscle, iris dilator muscle, anterior pigment epithelium and posterior pigment epithelium. The pigmentation of the eye depends on the two pigmented epithelial layers and the minuscule amount of pigment in the stroma. The pigment in the iris is **melanin**, which is also responsible for hair and skin colour. Higher concentration of melanin leads to brown eyes, as melanin is brown, while lower concentration leads to lighter colours like blue, green or hazel. The latter colours, however, form not because of another pigment but because of the structure and density of the iris which scatters incoming light. Such colour is called **structural colour.** Structural colours have also been observed in the colouring of many insects and birds. In some cases of albinism, the iris has lost all its melanin which leads to red eyes because of the blood vessels in the iris.

Eye colour genetics

In 1907 Charles and Gertrude Davenport developed the dominant model of brown eyes. They suggested that blue eyes were caused by a single recessive gene and blue-eyed parents could never give a brown-eyed child.

Today, we know this dominant model is very simplistic, and that many genes determine eye colour. Although we can predict a child's eye colour based on the parent's eye colour, other genetic and non-genetic factors can alter the outcome.

Human eye colour is a **polygenic trait** – many genetical factors are affecting it. However, the most important ones are *OCA2* and *HERC2*. They are neighbouring genes, and their loci are on the long arm of chromosome 15.

OCA2 (*oculocutaneous albinism 2 gene*) codes the P-protein – integral membrane protein, which transports tyrosine – a melanin precursor. Mutation in this gene can lead to oculocutaneous albinism. *HERC2* (*HECT and RLD domain containing E3 ubiquitin protein ligase 2 gene*), on the other hand, codes for an E3 ubiquitin ligase – a group of proteins that has no role in eye colour formation. The role of *HERC2* in eye colour is because it contains regulatory elements of *OCA2*. A recessive allele or of *HERC2* (a mutation in the regulatory elements of *OCA2*) leads to lower expression of *OCA2* hence light-coloured eyes, while a dominant allele leads to darker eye phenotype as the *OCA2* gene is normally expressed

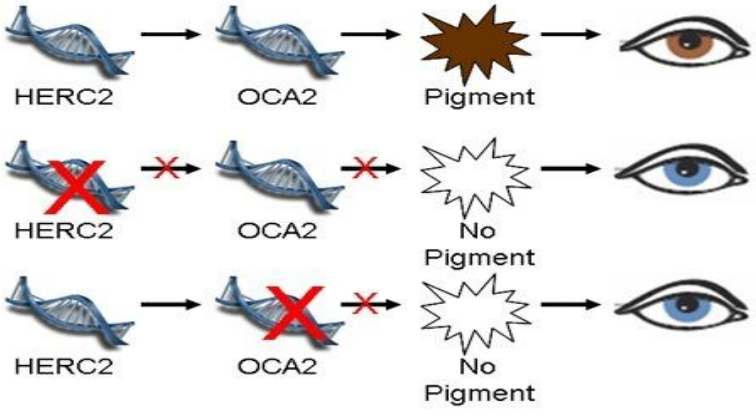


Fig. 1. A simplified scheme representing the roles of *OCA2* and *HERC2* in eye colour formation (Dr. Barry Starr, Stanford University, „How Blue Eyed Parents Can Have Brown Eyed Children. Two Different Ways to Get Blue Eyes ", July 27, 2012, Stanford at The Tech Understanding Genetics, https://genetics.thetech.org/how-blue-eyed-parents-can-have-brown-eyed-children)

The transcription factors HLTF (helicase-like transcription factor), LEF1 (lymphoid enhancer-binding factor 1) and MITF (microphthalmia-associated transcrip-

tion factor) attach to HERC2 (Mijke Visser et al., 2012). They enhance OCA2 transcription by converting heterochromatin into euchromatin, which makes DNA more accessible to RNA polymerase. A specific SNP (Single Nucleotide Polymorphism) in *HERC2* could prevent those transcription factors to attach properly to the gene which leads to a lower rate of transcription of OCA2 and the development of blue eyes. The name of this SNP is rs12913832 and it has a key role for the blue-eyed population in Europe. It has developed around 8000 years ago north from the Black Sea.

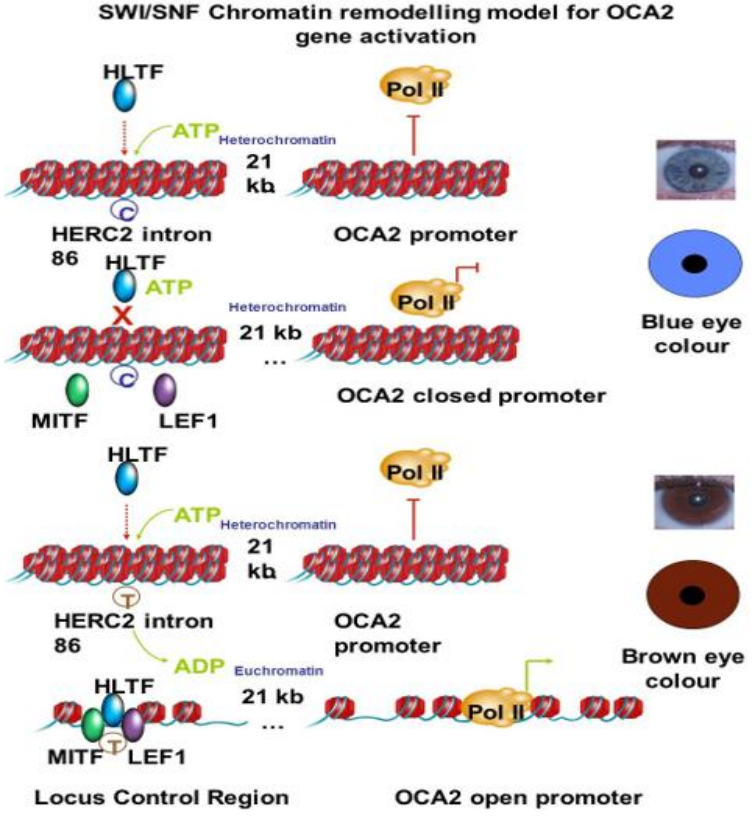


Fig. 2. Model for the determination of the blue-brown eye colour based upon regulation of *OCA2* gene expression (Sturm et al., 2009).

Experimental analysis of eye colour heredity

A family and one control subject outside of it have been analysed to explore the heredity of eye colour and apply the theoretical principles of eye colour genetics discussed before.

Genealogical pedigree

A genealogical pedigree of the family going back three generations has been constructed using the pedigree software Progeny (https://www.progenygenetics.com/online-pedigree/). Such analysis has its disadvantages as eye colour is a polygenic trait and the phenotype might not correlate to the genotype of one or even two genes. However, because *HERC2* has the greatest impact in the European population, the genotype of *HERC2* can be hypothesised based on the phenotypical knowledge from the pedigree and such hypothesis could support following experiments.

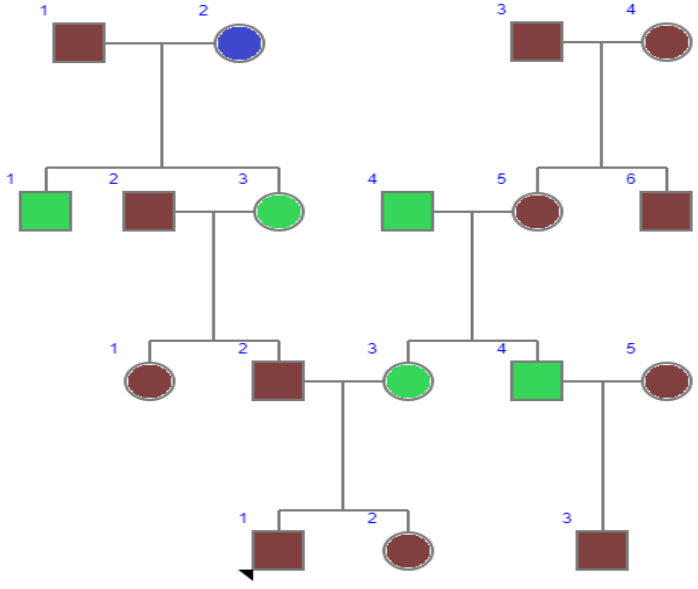


Fig. 3. Genealogical pedigree of the analysed Bulgarian family – constructed using Progeny (the proband – the starting point of the pedigree, is marked with a black triangle)

Light coloured eyes (blue, green, hazel, etc.) are phenotype of the recessive allele of *HERC2*. Based on that it can be hypothesised that people with light coloured eyes in the pedigree should be homozygous for the recessive allele of *HERC2* and the brown-eyed children of light-eyed parent should be heterozygous as well as brown-eyed parents who have light-eyed children. However, it still not excluded that another gene might be responsible for those phenotypes and the genotype of *HERC2* is different.

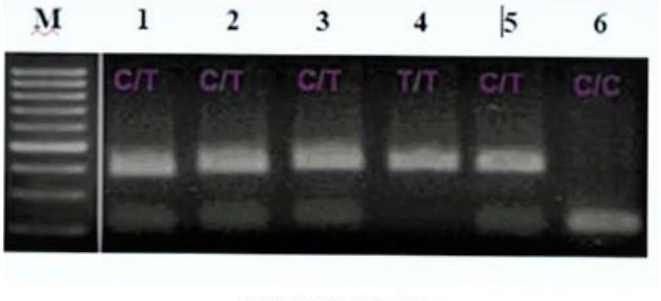
Molecular experiments

Molecular experiments have been done to explore the genotype of *HERC2* and *OCA2* as well as the presence of rs12913832. The DNA of five members of the family

(the proband, its sister and parents and its grandmother (the mother of the mother)) and the control subject has been extracted. First, the samples are tested for kinship using STR fingerprint testing. The test shows 99.9% probability of paternity between the parents and their children, 99% probability of relationship between the grandmother the mother and the children and no relatedness with the person outside the family as expected. The kinship between the family members is fully proven.

Then the eye colour genes are amplified using PCR. The needed primers of *HERC2* and *OCA2* are constructed using the bioinformatical programs Primer-BLAST and Sequence Extractor and are provided by Dr. Dimitrina Miteva. A RFLP analysis is performed on the resulting amplified DNA sequences – the samples are treated with the restriction enzymes Apo I and Mfe I. Through this method and a following agarose gel electrophoresis, the *HERC2* and *OCA2* genotypes of the subjects as well as the presence of rs12913832 is revealed.

Fig. 4 shows the genotypes for *HERC2* – the mother is homozygous for the recessive allele, the control subject is homozygous for the dominant allele, while all the other subjects are heterozygous. Those results support the information from the pedigree and the homozygosity of the control subject further show the unrelatedness of the person to the analysed family.

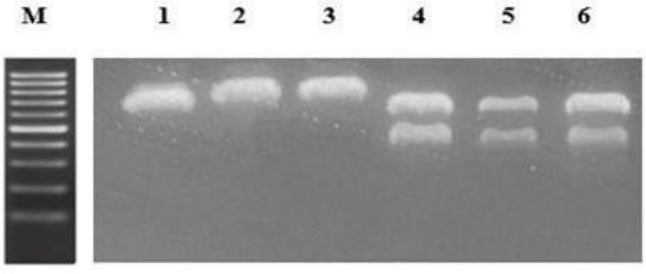


M:1Kb DNA Ladder
Lane 1: Father
Lane 2: Child 1
Lane 3: Child 2
Lane 4: Unrelated person
Lane 5: Grandmother
Lane 6: Mother

Fig. 4. RFLP results for *HERC2* genotype

The *OCA2* genotypes can be seen on Fig. 5 – the mother, the son (the proband/ Child 1) and the unrelated person are homozygous for the dominant allele while

the other subjects are heterozygous. Even though the mother has light eyes (hazel), her *OCA2* genotype has no recessive alleles which shows that the mechanism of *OCA2* is different as it shows generally the presence or absence of melanin in the eyes. However, in the iris of the mother (Fig. 6) can still be seen some small brown regions, which could result from the *OCA2* genotype.



M:1Kb DNA Ladder
Lane 1: Mother
Lane 2: Child 1
Lane 3: Unrelated person
Lane 4: Father
Lane 5: Child 2
Lane 6: Grandmother

Fig. 5. RFLP results for *OCA2* genotype

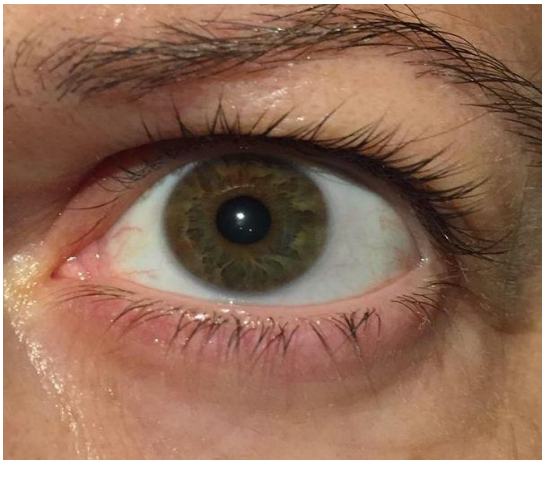


Fig. 6. Photo of the eye of the mother

Fig. 7 reveals that none of the subjects has the rs12913832. Even though the mother has light eyes they still are not blue, and the lack of this SNP presents that

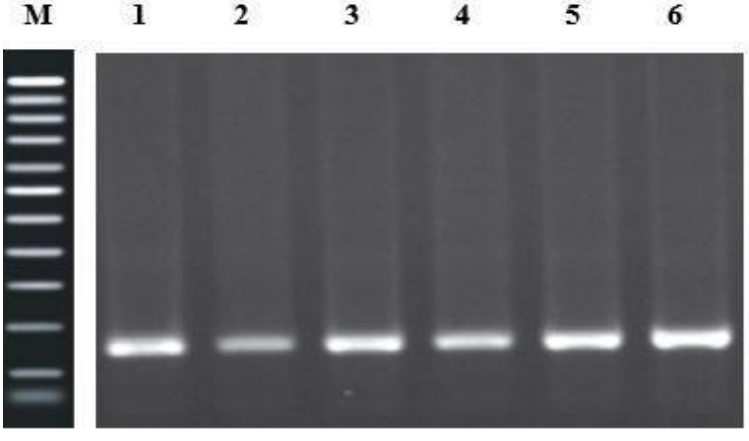


Fig. 7. RFLP results for the presence of rs12913832

Methods

Genealogical pedigree

This method aims to organise phenotype information of a trait in a family and to investigate what are the most probable genotypes of the trait. However, this method cannot precisely show exact genotypes as most of the time phenotype is not enough to do so. For the construction of the genealogical pedigree the software Progeny was used (https://www.progenygenetics.com/online-pedigree/).

Molecular methods

Those methods can reveal a precise genotype as well as the presence of specific DNA elements.

– **DNA extracting**

The first step of this method was to collect the DNA extraction source, i.e., lightly adherent superficial epithelial cells or naturally exfoliated cells of oral cavity which were from buccal mucosa. For the collection, a buffer for washing the oral cavity was prepared. The volunteers were asked to take 10 ml of buffer solution and swish the oral cavity for 1 min. They were instructed to intentionally bite on the buccal mucosa so that more cells could be exfoliated into the buffer. The swished-out buffer was then collected in a test tube.

The next step was to extract DNA from the collected epithelial cells present in buffer following the standard protocol of cell lysis, purification, and precipitation.

– **PCR (Polymerase Chain Reaction)**

The goal of this method is to amplify a specific gene or region from genomic DNA. The most important element of this reaction is Taq polymerase because of its ability to be functionally active in high temperatures, as this protein is extracted from thermophilic bacteria. This special property is essential because to replicate DNA, the two polynucleotide chains should be separated, and this is done by high temperature (95°C). However, as every DNA polymerase, it cannot start a new polynucleotide chain de novo. That is why the second key component of PCR are the primers – these are short polynucleotide chains used as a starting point of the polymerase to replicate DNA. Additionally, those primers define what part of the DNA will be replicated which is crucial for genetics and this project. Primers are constructed using bioinformatical programs. Thermo Scientific GeneJET Genomic DNA Purification Kit was used.

– **RFLP (Restriction Fragment Length Polymorphism)**

This method distinguishes differences in the sequence between two or more DNA fragments. Restriction endonucleases are essential for this technique – they cut DNA in specific locations based on the pattern of bases found at those locations. The presence or absence of those patterns can reveal the genotype of a given gene or even the presence of SNPs.

– **Agarose Gel DNA Electrophoresis**

This is one of the standard methods in molecular biology and genetics. Gel electrophoresis is a technique used to separate DNA fragments according to their size. DNA fragments are negatively charged, so they move towards the positive electrode. Because all DNA fragments have the same amount of charge per mass, small fragments move through the gel faster than large ones.

Agarose gels are produced from the natural polysaccharide polymers extracted from seaweed. Agarose gels have a different pore size, but are optimal for electrophoresis of DNA, RNA, and proteins. The distance between DNA fragments with different lengths is influenced by the percent agarose in the gel.

To visualise the DNA lanes ethidium bromide is put inside the gel. Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) for techniques such as agarose gel electrophoresis.

– **STR fingerprint analysis**

Short Tandem Repeat (STR) analysis is a common molecular biology method used to compare allele repeats at specific loci in DNA between two, three or more samples. STRs are a specific short sequence of DNA that are 2 to 7 base pairs in length, with the number of repeats varying among individuals, making STRs effective for human identification. This genetical trait changes rarely, which makes it a

perfect marker for such tests. Testing paternity or kinship is often needed in forensic genetics. However, it is also important for non-forensic studies like this project.

This method differs from restriction fragment length polymorphism analysis (RFLP) since STR analysis does not cut the DNA with restriction enzymes. Instead, polymerase chain reaction (PCR) is employed to discover the lengths of the short tandem repeats based on the length of the PCR product.

Conclusion

In this project the genotypes for OCA2 and HERC2 as well as the presence/absence of one of the main genetical factors leading to blue eye colour were revealed in a family. The significant role of HERC2 is confirmed and the function of OCA2 was also explored as a gene responsible for the general presence of melanin rather than a specific eye colour as the lack of dominant alleles could lead to oculocutaneous albinism. If a person with this type of albinism had been investigated, he should have been homozygous for the recessive allele. It has been shown that people without blue eyes lack rs12913832 even if they have light eyes (green, hazel, etc.)

Additionally, this project has applied and analysed some of the most essential biological methods used by scientists daily to also show their use in the context of a scientific research.

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