



# Pedigree Analysis of inheritance of hazel eye colour in humans in North India

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## ABSTRACT

The eye colour in humans is a complex and polygenic trait. The hazel eyes, which are a variant of brown eye colour, are found in Indian subcontinent, but their occurrence is comparatively lesser than brown or black eyes. Our pedigree study suggested that the hazel eye colour inheritance does not follow the Mendelian inheritance patterns. It also showed that the hazel eye colour is not sex-linked and moreover, that it is neither autosomal dominant nor autosomal recessive. One case from Kangra, Himachal Pradesh, a region with mild temperate climate, showed higher occurrence of hazel eyes in the family, with many lineages showing continuous expression of hazel eyes for three generations and in some for even four generations. The other two cases involving families from Gorakhpur, Uttar Pradesh, and Darbhanga, Bihar, regions with humid subtropical climate had comparatively lesser number of persons with hazel eyes, showing continuous expression of hazel eyes for only two generations in few lineages. Thus, climate might have a role in the eye pigmentation due to differential expression of the genes responsible for eye colour. Since, there are many genes responsible for production of eye colour like *OCA2*, *BEY2*, *GEY*, *HERC2* etc., primarily the expression of these genes might produce the eye colour but the climate/environment might have its own influence on the expression of these genes. The finer mechanisms by which different genes interact with each other to produce variations in the eye colour and the role of the climate on the production of eye colour needs further investigation.

## LIST OF ABBREVIATIONS USED

*OCA2*: Oculocutaneous albinism II; SNP: Single nucleotide polymorphism; UTR: Untranslated region; *GEY*: green eye gene; *BEY*: Brown eye gene; EYCL1: eye colour 1 gene locus; EYCL3: eye colour 3 gene locus

Keywords: Autosomal, dominant, genotype, Hazeleyes, pedigree, phenotype, polymorphic, recessive

## INTRODUCTION

The existence of variation in the skin colour is quite common around the world. But the hair and eye colour are not that common in all kinds of populations, with variation in hair colour

more prevalent in populations of European origin (5).

The most common eye colour in the world is brown and the least common is green. There are a variety of eye colour shades in between, many of which are not studied in detail. The eye colour of the child depends on the genetic material that the child receives from the parents. However, the genes of the parents can mix and develop variations in the eye colour of the child. In Caucasian population, blue is the most common eye colour when the child is born but as the child grows the colour may change due to pigmentation. However, in Asian and African population, the brown and black eye colour is more prevalent.

The human eye colour is a polymorphic trait. Till date eight genes have been identified that play a role in imparting eye colour (6). The *OCA2* gene on chromosome 15 plays a major role in controlling blue/brown eye colour. It produces a P protein that is involved in formation and distribution of the pigment melanin. The human P-gene transcript is divided into 24 exons, out of these 23 exons contain 826 amino acid coding region and exon 1 represents noncoding region with 5' UTR (3). The genes for eye colour are found to be associated with SNPs in *OCA2* and adjacent *HERC2* genes (upstream of *OCA2* gene) (5). The three SNPs in intron 1 of *OCA2* gene are most strongly associated with blue/non-blue eye colour. The eye colour of a person can be predicted reasonably well by the person's genotype at the *OCA2* locus (10). There are at least 35 variants of the *OCA2* gene that are non-pathogenic.

The genes for eye colour have been mapped using linkage studies. For example, green or blue eye colour locus is eye colour 1 (*EYCL1/GEY*, chromosome 19) and brown/blue eye colour locus is eye colour 3 (*EYCL3/BEY2*, chromosome 15) (8). The *BEY2* locus has been identified as the gene *OCA2*. There are two known alleles for *BEY2* locus: dominant allele for brown eye colour (B) and recessive one for blue eye colour (b). The *GEY* locus also has two alleles: dominant allele for green eye colour (G) and recessive allele for blue eye colour (g). The brown alleles at *BEY2* are epistatic to green alleles at *GEY*, thus producing brown eyes. Thus only one allele at *BEY2* is necessary to produce brown eyes. Four blue alleles are required to produce blue eyes, two alleles in each locus i.e. *BEY2* & *GEY* (bb;gg). Green eyes are produced if there are two blue alleles for *BEY2* and at least one green allele for *GEY* (2).

Many other genes like *TRYP1*, *ASIP*, *SLC24A5* and *ALC42A5* are also found to play a role in melanin pathway and decide the total amount of melanin present in the eyes resulting in either brown, hazel, blue eyes etc. (9). Hazel eye colour is mid way between the lightest blue and the darkest brown eyes. In hazel eyes there is a large amount of melanin in the anterior border of the iris.

Recently it has been found that there are three new significant loci associated with quantitative eye colour. One of the three genes is the *LYST* gene which was previously considered a pigmentation gene in mice and cattle, whereas the other two genes had no previous association with pigmentation. These three genes along with previously identified genes explain over 50% of eye colour variance, representing the highest accuracy achieved so far in genomic prediction of complex and quantitative human traits (4).

The eye colour in humans should be inherited according to the Mendelian principles as it is controlled by specific genes. The studies concerning the pedigree analysis of the eye colour inheritance are lacking in human population in India. So, the present study was undertaken to collect the relevant information pertaining to the eye colour in the families and to look into the possible inheritance mechanisms of the hazel eyes.

## METHODOLOGY

The study was performed by collecting the data from few families in North India. An individual having hazel eye colour was selected and her family members were included in the study. Prior consent was taken from the persons for the study. The symbols used for making the pedigree follow the guidelines of the Pedigree Standardization Task Force (PSTF) of National Society of Genetic Counselors (NSGC) [1995].

A total of four families were studied and they were divided into three cases as follows:

CASE I: Family A (22 members) + Family B (38 members) from Gorakhpur, Uttar Pradesh, India (Geographical coordinates: 26°45'18" North and 83°22'26" East). The data was collected in the months of September -October, 2014. These two families were kept in one group because one of the families i.e. Family A, represents the paternal side and the other family *viz.* Family B represents the maternal side of the female selected for the study (IV-3).

CASE II: Single family of 53 members from Kangra, Himachal Pradesh, India (Geographical coordinates: 32°6'0" North and 76°16'0" East). The data was collected in the months of December, 2014 & January, 2015. The female selected for the study was IV-19.

CASE III: Single family of 21 members from Darbhanga, Bihar, India (Geographical coordinates: 26°10'0" North and 85°54'0" East). The data was collected in the month of March, 2015. The individual selected for the study was IV-3.

The eye colour of each family member was noted down vigilantly. A great care was taken to record all the members in a family to the maximum extent possible. The care was also taken to record the members up to a minimum of four generations.

The pedigree was constructed using the information provided by the members of the families about their eye colour. It was analyzed for the inheritance pattern of the hazel eyes. Further, a comparison was carried out between the occurrences of brown and hazel eye colour among the members of the families studied.

## RESULTS

### CASE I:

Two families were studied under this section. Both the families originally belong to the Gorakhpur region of east Uttar Pradesh, India. However, many of them have relocated themselves to other parts of the country. The female who was selected for the study had hazel eyes and her immediate family resides in the NCR (National Capital Region) region, Delhi, India, for the past few years. The climate of the Gorakhpur region is humid subtropical with dry winter.

In this Case (Figure-I), the female IV-3 was selected for the study as she had hazel eyes and both her paternal and maternal family members were taken into consideration. The female IV-3 had hazel eyes while her sister (IV-4) had brown eyes. To look for the possibility that the hazel eye colour inheritance might have followed any of the Mendelian inheritance patterns, the eye colour data of the family members of both her paternal side (Family A) and maternal side (Family B) was recorded.

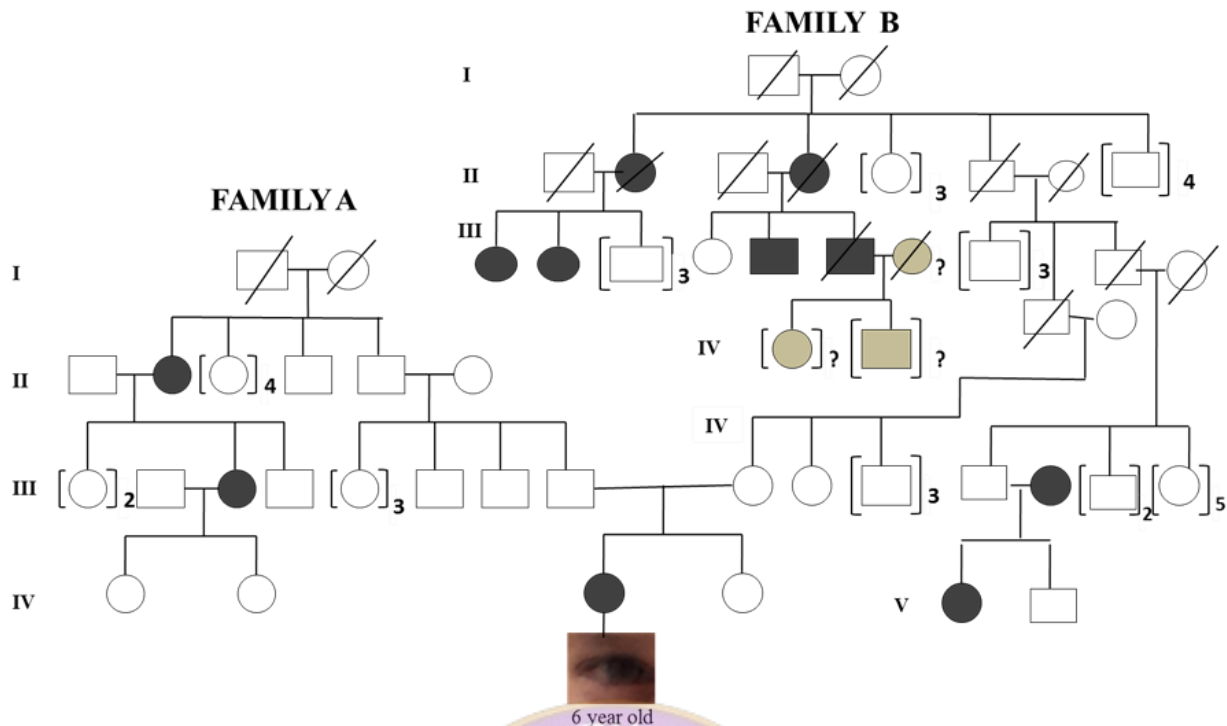


Figure-I: Pedigree of inheritance of hazel eye colour in Case I

Family A (The paternal side): For family A, it was possible to record the eye colour information of the four generations. As per the pedigree constructed for Family A, the founders had brown eyes. They produced seven children in generation II. Out of the seven offspring produced, one daughter (II-2) expressed hazel eyes, while her siblings had brown eyes. This might indicate that the parents in generation I might be the carriers of the genes for the hazel eyes. The female (II-2) married a brown eyed male (II-1) and together they produced four children in generation III. So, in generation III, it was observed that one female (III-4) had hazel eyes, while the other two females had brown eyes (III-1 & III-2) and the male (III-5) also had brown eyes. The female (III-4) married a male with brown eyes and they produced two daughters (IV-1 & IV-2), both of whom had brown eyes.

One of the males in generation II (II-8) who had brown eyes married a female with brown eyes. They produced three daughters (III-6, III-7 & III-8) and three sons (III-9, III-10 & III-11), all of whom had brown eyes.

Family B (The maternal side): In Family B, information about the eye colour could be recorded for five generations and it was arranged in the form of a pedigree. If one looks at the pedigree, it may be observed that the founders of the pedigree did not have hazel eyes. They produced ten children which included two daughters who had hazel eyes (II-2 & II-4). The rest of the children had brown eyes. The first daughter with hazel eyes (II-2) got married to a brown eyed male (II-1) and they produced five children in generation III which included two daughters with hazel eyes (III-1 & III-2) and three sons having brown eyes (III-3, III-4 & III-5). Further, the second daughter having hazel eyes (II-4) also married a male with brown eyes and they produced one daughter and two sons. Both of their sons in generation III (III-7 & III-8) had hazel eyes while the daughter (III-6) had brown eyes.

The male number 8 in generation III (i.e. III-8), having hazel eyes, married a female whose eye colour could not be confirmed. They together had two children: one daughter (IV-1) and one

son (IV-2), even their eye colour could not be confirmed.

The male number 8 in generation II (i.e. II-8), who had brown eyes, married a female with brown eyes and they together produced five sons, all of whom had brown eyes. The male, viz. III-13, married a brown eyed female (III-14) and both produced three sons and two daughters (IV-3 & IV-4), all having brown eyes. The male, viz. III-15, married a female with brown eyes and they produced five daughters and three sons, all having brown eyes. One of their son (i.e. IV-8) married a female having hazel eyes and they produced two children: a hazel eyed daughter (V-1) and a brown eyed son (V-2).

#### The relation between Family A and Family B

The brown eyed male (III-11), from Family A, married a brown eyed female (IV-3), from Family B. They produced two daughters: one hazel eyed daughter (IV-3) and a brown eyed daughter (IV-4).

#### CASE II:

The family that has been studied as Case II is native of Kangra region in Himachal Pradesh, India (Figure II). The climate in Kangra is warm and temperate. A lot of rain happens in this region even in the driest of the months. It was possible to collect data of this case for five generations.

The founder male of the pedigree had hazel eyes while the founder female had brown eyes. They produced four sons (II-1, II-2, II-3 & II-4) all of whom had hazel eyes. One of the sons (i.e. II-1) married a brown eyed female and they produced one son (III-1) who had hazel eyes and two daughters, one of whom had hazel eyes (III-6) while the other (III-4) had brown eyes. The hazel eyed son (III-1) married a brown eyed female and both their children were brown eyed. The brown eyed daughter (III-4) married a brown eyed man and they had three children, all having brown eyes. The daughter having hazel eyes (III-6) married a male with brown eyes. Both of their children had brown eyes.

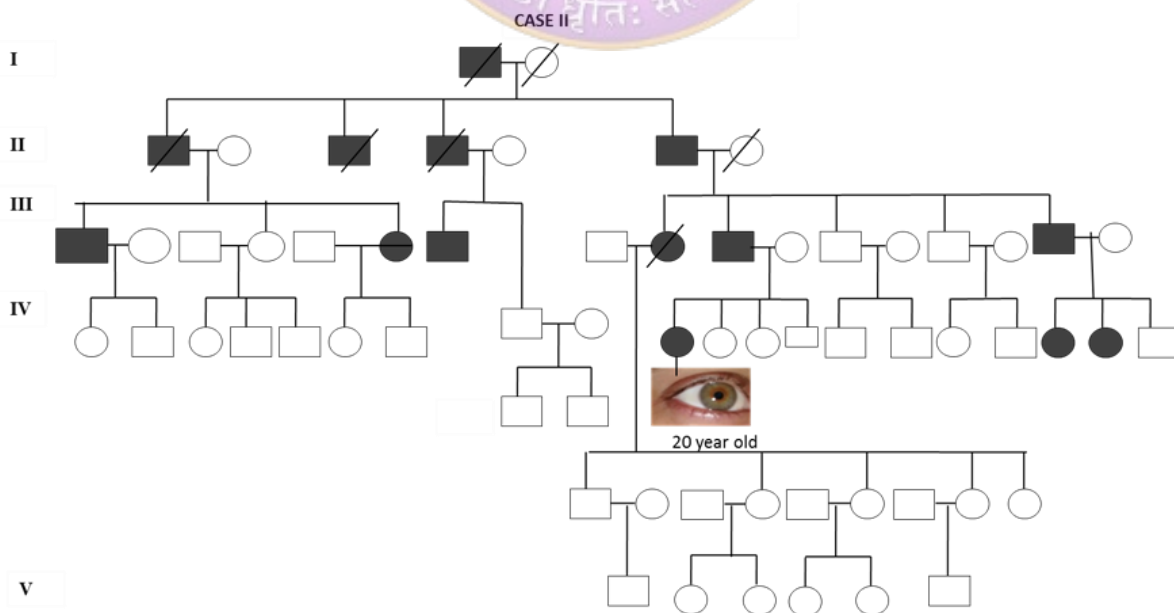


Figure-II: Pedigree of inheritance of hazel eyes in Case II

Another male (II-4), of the second generation, married a female with brown eyes. They produced two sons, one (III-7) had hazel eyes while the other (III- 8) had brown eyes. One of the sons i.e. III-8 married a female having brown eyes and both their sons (IV-8 & IV-9) had brown eyes.

The fourth son (II-6) of the founder couple, having hazel eyes, married a female with brown eyes. They produced one daughter (III-11) having hazel eyes and four sons- two sons (III-12 & III-18) having hazel eyes and two sons (III-14 & III-16) having brown eyes. The daughter (III-10) married a man with brown eyes and produced IV<sup>th</sup> generation, all having brown eyes. One of their sons with brown eyes (IV-10) married a brown eyed female and they produced a brown eyed son (V-1). Similarly, their daughters having brown eyes (IV-13, IV-15 & IV-17) married brown eyed men and all their progeny were brown eyed (V-2, V-3, V-4, V-5 & V-6 respectively).

One of the sons (III-12) married a female with brown eyes and they had one son (IV-22) having brown eyes and three daughters, of which one daughter (IV-19) had hazel eyes while others had brown eyes.

The other two men in generation III (III-14 & III-16) married brown eyed females and their next generation i.e. IV<sup>th</sup> generation had only brown eyes. One more hazel eyed male in generation III (III-18) married a brown eyed female. They had two daughters (IV-27 & IV-28) with hazel eyes and one brown eyed son (IV-29).

#### CASE III:

This case covers a family from Darbhanga in Bihar, India (Figure III). This region has a humid, subtropical hot summer climate with dry winters. The data could be collected for four generations.

The founder female (I-2) in this case had hazel eyes while the founder male (I-1) had brown eyes. They produced a brown eyed daughter (II-2) who married a brown eyed male. They produced two sons (III-1 & III-7) who had brown eyes and two daughters (III-4 & III-6) who also had brown eyes. One brown eyed son (III-1) married a brown eyed female and they had two brown eyed sons (IV-1 & IV-2).

One of the brown eyed daughters (III-4) married a brown eyed male and they had a brown eyed son (IV-5) and two daughters - one hazel eyed daughter (IV-3) and a brown eyed daughter (IV-4).

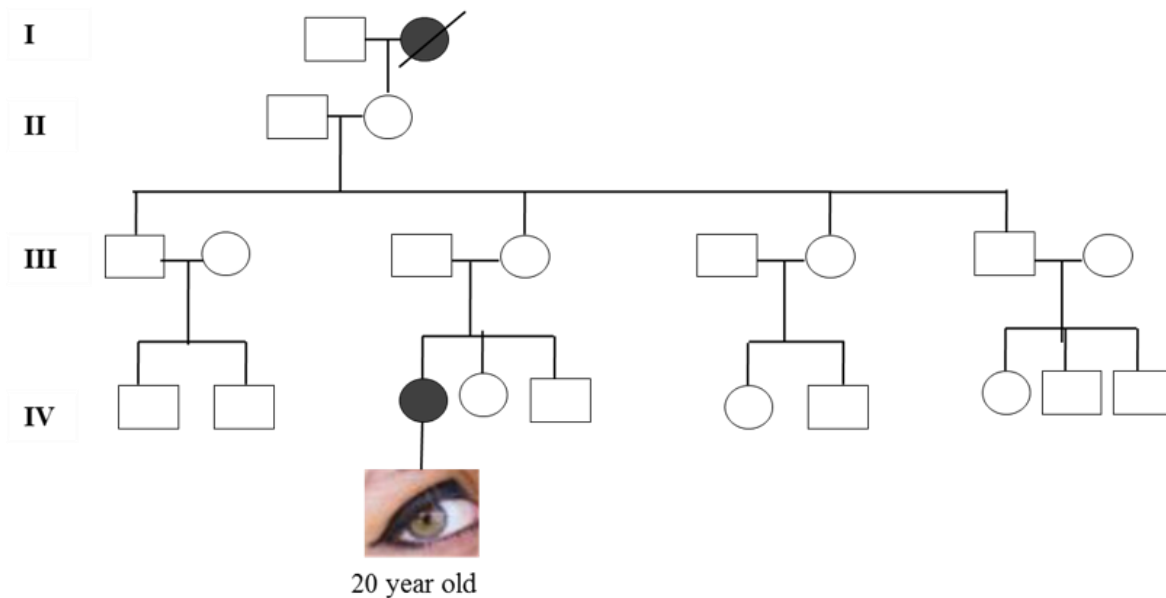


Figure-III: Pedigree of inheritance of hazel eyes in Case III

Another brown eyed daughter (III-6) married a brown eyed man and they had two brown eyed children (IV-6 & IV-7). The brown eyed son in generation III (III-7) married a brown eyed woman and they had three brown eyed children (IV-8, IV-9 & IV-10)

COMPARISON OF OCCURRENCE OF EYE COLOUR IN THE FAMILIES STUDIED AS CASES I, II & III

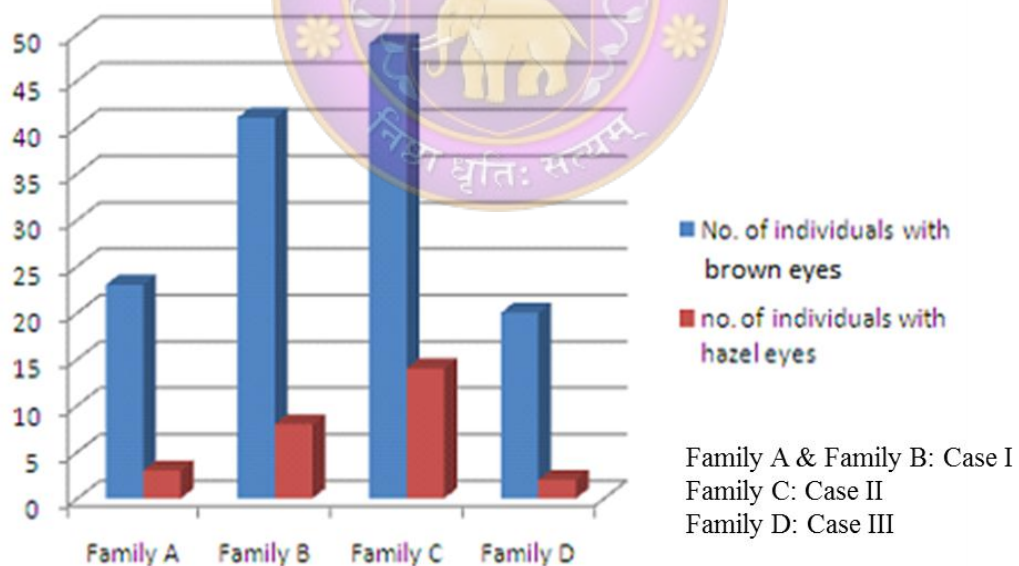


Figure-IV: Comparison of occurrence of hazel eyes in the three cases studied

A comparison was carried out in the families under study for the frequency of occurrence of brown eyes and hazel eyes. The graph (Figure IV) when plotted clearly showed that in all the families studied, the brown eye colour was predominantly seen more as compared to hazel eyes.

## DISCUSSION

The results of the above pedigrees lead us to many conclusions. It is evident from the pedigrees that both the sexes have expressed hazel eyes, hence we cannot call it a sex-linked character. The same has been proved by gene mapping studies that the gene for eye colour is present on chromosome 15 in humans (3), which means it is an autosomal trait.

Another information that may be interpreted from the pedigree is that the character sometimes skips generations, may be one or more generations, but many a times it doesn't skip generations. This may lead us to the conclusion that we cannot classify it as a true dominant character or a true recessive character. One possible reason for it may be that the eye colour is controlled by several genes. The most significant gene for eye colour is *OCA2*, mapped to chromosome 15 in humans. There are three SNPs in intron 1 of the *OCA2* gene that are involved in the production of blue or non-blue eye colour (5). There are other genes also that are part of the complex pathway through which the colour of the eye is established. For example, *EYCL1/GEY* gene on chromosome 19 controls the green or blue eye colour and *EYCL3/BEY2* gene on chromosome 15 regulates the brown or blue eye colour (8). Thus, it might be interpreted that the eye colour of an individual might be a complex interplay of all of these genes and/or expression of some genes and/or even the amount of expression is significant in production of eye colour.

The iris in human eyes consists of several layers including the anterior layer and the underlying stroma that play an important role in the appearance of the eyes. The number of melanocytes does not appear to differ in different eye colour but the quantity of melanin pigment produced, packing and the quality is behind various shades of eye colour. In hazel eyes there are less and scattered melanocytes and melanosomes in the anterior layer and stroma (8). Another factor that decides the colour is the varying amount of lipid pigment granules in the iris.

The hazel eye colour is a variant of the brown eye colour. The allele B (dominant allele of *bey2* gene) appears to not fully express itself, leading to scanty production of the pigment. All the cells in the iris may not be producing the pigment and thus scattered production may give the appearance of hazel eyes due to differential scattering of light rays.

However, there seems to be a pattern of expression of these genes that is inherited in some way within the families. For example, in Case I, the sudden appearance of hazel eyes in generation IV, skipping many generations, may be due to mutation or non-expression or partial expression of the alleles that produce brown eye colour.

The occurrence of black or brown eye colour is more common in the Indian subcontinent and the same has been shown, for the families studied, in Figure IV. Other eye colour like hazel, blue or green are less common, with hazel eyes showing more presence comparatively. The blue or green eye colour are rare in India. Unlike India, in the European and American populations, the blue or green eye colour is more common. This difference in the eye colour may be due to environmental influences (6). The melanin protects the cells from the harmful effects of the UV radiations from the sun. In countries like India, where temperature remains higher throughout the year in most parts of the country, this pigment might have a role in protection of our skin and eyes. In colder countries, where the sun rays do not fall direct, and the climatic conditions are mild when compared to other parts of the world at the same latitude, we come across people with lighter skin and eye pigmentation. However, the occurrence of eye colour has been reported to be sporadic in many other populations (10).

Another observation that may be extracted from the pedigree is that the other family members,



of the persons with hazel eyes, had brown eyes. There were no members with black eyes. In the family of Case II, one argument may be given that since the region has comparatively milder climate, so there might be chances of less pigmentation in eyes. The families in Case II showed higher occurrence of hazel eyes than in Cases I & III and also the trait was continuously expressed in four generations of two lineages in Case II. In rest of the lineages, the trait was expressed for three generations continuously. But, in the families of Case I and Case III, regions having humid, subtropical climate, the trait is seen usually skipping the generations. In only few lineages, the trait is continuously seen in two generations only. In the hazel eyed female (IV-3) selected for the study in Case I, the trait is seen to have skipped three generations on her paternal side and four generations on her maternal side in the lineage. Thus, it may be debated that the genes associated with the production of eye colour in humans may be under the influence of the climatic conditions to some extent. But, primarily it is the genes whose differential expression and complex interplay leads to the production of eye colour in humans.

### CONCLUSION

From the information obtained in the above pedigrees, it may be said that the eye colour in humans might be a trait that is dependent on the genetic composition of the person as well as the environmental/climatic conditions. It is not a trait that follows any of the Mendelian Inheritance patterns but still it is a genetic trait. This trait is also influenced by the climatic and environmental conditions. It is not clear yet what type of environmental conditions affects the expression of the genes which interact with each other and produce a particular eye colour. Hence, the exact molecular mechanisms need to be unearthed. The more detailed explanation for the occurrence of the hazel eyes and the possible means of inheritance may be understood by doing the molecular studies along with the pedigree analysis and the role of environmental factors as well.

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