FULL LENGTH ARTICLE

Prevalence and gene frequency of color vision impairments among children of six populations from North Indian region

Mohd Fareed a,*, Malik Azeem Anwar a,b, Mohammad Afzal a,*

a Human Genetics and Toxicology Laboratory, Section of Genetics, Department of Zoology, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, 202002, Uttar Pradesh, India

b Department of Molecular Genetics, The Ohio State University, Columbus, OH, USA

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Abstract X-linked red—green color blindness is the most widespread form of vision impairment. The study aimed to determine the prevalence and gene frequencies of red—green color vision impairments among children of six different human populations of Jammu province. A total of 1028 healthy subjects (6–15 years of age) were selected from five Muslim populations and the color vision impairments were determined using the Ishihara’s test of color deficiency. The gene frequency was calculated using Hardy–Weinberg equilibrium method. The prevalence of color vision deficiency (CVD) ranged from 5.26% to 11.36% among males and 0.00%–3.03% among females of six different populations. The gender based differences in the frequency of CVD was found to be statistically significant (p < 0.0001), with a higher prevalence among male (7.52%) as compared to female (0.83%) children. We observed high frequency of deutan as compared to protan defects. The incidences of deuteranomaly (5.68%) and deuteranopia (2.27%) were higher among male children of Syed population while the frequencies of protanomaly (1.94%), protanopia (1.28%) and achromacy (2.27%) were the highest among male subjects of Khan, Malik and Syed populations, respectively. The allele and genotype frequencies showed cogent differences among six populations. The population based assessment of CVDs help patients to follow adaptive strategies that could minimize the risks of the disease.

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* Corresponding authors.
E-mail addresses: mohdfareedk@gmail.com (M. Fareed), afzal1235@rediffmail.com (M. Afzal).

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Introduction

Color blindness, or color vision deficiency (CVD), is the inability or decreased ability to perceive color differences under normal lighting conditions. CVD can be classified as congenital or acquired. The prevalence of congenital color blindness is about 8% in males and 0.4% in females, results either from alterations or absence in the absorption spectrum of photopigment. The frequency of color blindness vary among different ethnic populations across the world. A recent study from Eastern India has reported 8.73% of males and 1.69% of females as color blind.1

The discrimination of color in humans depends on unequal stimulation of three (red, green and blue) cone types to lights of different wavelengths. Normal human color vision is trichromatic, based on the presence of three spectrally-distinct types of cone photoreceptors in the retina that are maximally sensitive to light at 420, 530 and 560 nm (short, middle and long wavelength sensitive cones; S, M and L, respectively).2 Mutations and rearrangements in the genes encoding the long, middle, and short wavelength sensitive cone pigments are responsible for color vision deficiencies.4 A recent study has revealed the missense mutation in a hybrid L/M cone opsin gene leading to X-linked cone dystrophy and color vision deficiency.5

The blue pigment gene is located on chromosome 7, while the red and green pigment genes are located on long arm of the X-chromosome (Xq28). The mothers who are carriers of the abnormal gene have a chance of 50% abnormal color vision for sons. The CVD fathers transmit their X-chromosomes to daughters only, which leads to all daughters as carriers and sons with normal color vision. A simplified inheritance pattern of sex-linked red–green color blindness is shown in Fig. 1.

The two broad categories of ‘red–green’ defects are protan and deutan. The protan defects are characterized by an absence or anomaly of L-cone function, while deutan defects are characterized by an absence or anomaly of M-cone function. Deuteranopia or protanopia arises due to the absence of photopigment of the green or red cone, whereas the photopigment response of the green cones is shifted towards that of the red cones and leads to deuteranomaly or vice versa (protanomaly).6

Estimating the CVD phenotypes and gene frequencies among different populations has several benefits in occupations or jobs and in routine life that involve precise color matching. These include telecommunication and electrical mechanics, seamen, train drivers, air traffic controllers, painters and several other jobs as well as daily routine work deemed of color recognition. The North Indian human populations and populations from Jammu and Kashmir (J&K) have historical, linguistic, cultural, and socio-religious significance for the Indian subcontinent.7 The aim of this preliminary study was to estimate the phenotype and gene frequencies of color vision impairments among the children of six different Muslim populations of Jammu region.

Materials and methods

Ethics statement

The study was approved by Institutional Ethics Committee of Jawaharlal Nehru Medical College (JNMC), Aligarh Muslim University, India. We obtained the written informed consent from the parents, caretakers, or guardians on behalf of the minors/children participants involved in our study.

Population and study design

The J&K is the Northern most state of India, situated between 32.17 and 36.58 North latitude and 37.26 and 80.30 East longitude. To its North is China, Russia and Turkistan and on its East is Chinese Tibet and on the West are the north western frontier provinces of Pakistan.8 The study was conducted in Rajouri and Poonch districts of J&K, during April 2013 through December 2013. The incidence of consanguineous marriages ranges from 35% to 50% among these populations.9,10 Family pedigrees were made up to five generations back (volunteered by the parents) helped in ascertaining the consanguinity status of their marriage. The information provided by the parents was also cross-checked by seeking help from the elder members of the family. Only non-consanguineous families were taken in our study to follow Hardy–Weinberg equilibrium for gene frequency analysis. A total of 1028 healthy children (6–15 years of age) were selected from 815 families of six Muslim populations viz., Gujjar and Bakarwal (n = 184), Mughal (n = 160), Khan (n = 190), Malik (n = 167), Mir (n = 173), and Syed (n = 154). Care was taken to avoid the selection of two or more children of the same sex for each family. The family based children sample include: (a) single child (n = 602) from 602 families and (b) two children (n = 426) of different sex from 213 families. Fig. 2 depicts the steps involved in the recruitment process. The details of sample size under study are presented in Table 1.

Measure and procedure

The color vision impairment or CVD was determined using the Ishihara’s Test of Color deficiency (38-plate edition). The plates make up several different test designs as follows:

(a) Transformation plates: The CVD patients recognize a different figure from individuals with normal color vision.
(b) Vanishing plates: Only normal color vision individuals could recognize the figure.
(c) Hidden digit plates: Only CVD patients were able to spot the sign.
(d) Diagnostic plates: These were used to differentiate red–green color blindness (protan and deutan defects).

The subjects were approached through their parents (at residence) and their name, age, sex, class and school
attended with parental consanguinity were entered in the record form. The color vision testing plates were held at 75 cm from the children and tilted at right angle to the line of vision. The screening test was performed under sun light. An assessment of the readings of plates 1 to 21 determines the normality or defectiveness of color vision. If 17 or more plates were read normally, the color vision was regarded as normal. If only 13 or less than 13 plates were read normal, the color vision was regarded as deficient. However, in reference to plates 18, 19, 20, and 21, only those who read the numerals 5, 2, 45, and 73 and read them easier than those on plates 14, 10, 13 and 17 were recorded as abnormal. The plates 22, 23, 24 and 25 were used to differentiate protan and deutan types of color vision.

Figure 1  Inheritance pattern of X-linked red—green color blindness. The genes for protan (L-cone) and deutan (M-cone) phenotypes are located on X-chromosome. The single X-chromosome in males is predominant to color blindness, while females with two X-chromosomes can act as dosage compensation and decrease the risks.
efficiency. An overview of the discrimination of color in deuteranope and protanope from normal vision individuals has been presented in Fig. 3.

Statistical analysis

Statistical analysis was conducted using GraphPad InStat 3.0 (USA). The chi-square ($\chi^2$) test was used to determine the significant differences.

$$\chi^2 = \sum \frac{(Observed \ Number - Expected \ Number)^2}{Expected \ Number}$$ (1)

Gene frequency analysis

Considering $C$ allele = $p$, and $c$ allele = $q$, then gene frequencies for color blindness were calculated by Hardy–Weinberg method ($p^2 + q^2 + 2pq = 1$). The allele frequencies for male, female and combined group were calculated as follows:

(a) For male:

$$c = \frac{\% \ color \ blind \ phenotype}{100}$$ (2)

Now, $C = 1-c$

Table 1  Characteristics of sample size under study.

| Populations          | Male     | Female   | Total (n) |
|----------------------|----------|----------|-----------|
| Gujjar and Bakarwal  | 102 (55.43) | 82 (44.57) | 184       |
| Mughal               | 76 (47.50)  | 84 (52.50)  | 160       |
| Khan                 | 103 (54.21) | 87 (45.79)  | 190       |
| Malik                | 78 (46.71)  | 89 (53.29)  | 167       |
| Mir                  | 98 (56.65)  | 75 (43.35)  | 173       |
| Syed                 | 88 (57.14)  | 66 (42.86)  | 154       |
| Combined             | 545 (53.02) | 483 (46.98) | 1028      |

Values shown in table indicate number (%) for children. Total sample size 1028, $n$ = number of subjects.

Figure 3  Color vision discrimination in deuteranope and protanope from normal individuals.
For female:

\[ c = \frac{\text{\% color blind phenotype}}{100} \]  

Now, \( C = 1 - c \)

(c) For combined group:

\[ c = \frac{1}{3} \times c \text{(Male)} + \frac{2}{3} \times c \text{(Female)} \]

Now, \( C = 1 - c \)

The homozygosity \( (H_o) \) and heterozygosity \( (H_t) \) was determined using the formula:

\[ H_o = \sum P_i^2 \]  

Where \( P_i \) represents the alleles (C or c).

Now, \( H_t = 1 - \sum H_o \)

Results

Population and sex based differences of color vision impairment

Table 2 presents the detailed phenotypic frequencies (shown in number and percentage) of color blindness among males and females of six populations. The combined results of color blindness depict the highest frequency among Syed (7.79%) population and the least was observed among Mughals (2.50%), and frequencies of CVD for the remaining four populations lie between these two. The difference among populations was statistically insignificant \( (\chi^2 = 6.505, df = 5, p = 0.2601) \). The general CVD frequency followed the trend: Syed (7.79%) > Khan (4.74%) > Gujjar and Bakarwal (4.35%) > Mir (4.05%) > Malik (2.99%) > Mughal (2.50%). However, gender based differences for CVD shows significant values \( (\chi^2 = 27.42, df = 1, p < 0.0001) \). In 1028 child cohort, the CVD frequency was found higher of about 41 (7.52%) among males and least as 4 (0.83%) among females.

Table 3 presents the prevalence of achromacy, deutan and protan defects among male and female children of six human populations.

Results presented are n (%); n = number of individuals.
Gender based differences of protan and deutan defects among six populations

Table 3 presents the frequency of red–green color vision impairments. The frequency of achromacy for male was about 0.55%, while female showed zero frequency for the same. Only males of Syed (2.27%) and Khan (0.97%) populations showed achromacy phenotypes. The prevalences of protan and deutan defects were higher among males as compared to female children. The highest phenotypic frequency of protanopia was found among males of Malik (1.28%) and none of female was found among all populations. The highest frequency (1.94%) of protanomaly was observed among males of Khan population, while only females of Syed (1.51%) population showed the protanomaly phenotype. The phenotypic frequency of deuteranopia among male children observed for Syed was 2.27% of the population and among females only single phenotype from Khan (1.15%) population was observed. The deuteranomaly phenotypes showed the higher frequency among all vision defects under study. The Syed population depicts highest deuteranomaly phenotypes among males (5.68%) and females (1.51%).

Allele frequency of color blindness among six populations

Fig. 4 presents the allele frequencies of color blindness among six populations. The frequency of allele C for male children was found the highest among Mughal (0.95) and the least accounts for Syed (0.89) and the other four populations lie between these two. The frequency of allele c for

![Figure 4](image)

Figure 4  Allele frequencies of red–green color blindness among (a) male, (b) female and (c) combined groups of six human populations. Populations presented as, P1 = Gujjar and Bakarwal, P2 = Mughal, P3 = Khan, P4 = Malik, P5 = Mir, and P6 = Syed.
male children was found the highest among Syed (0.11) and the least accounts for Mughal (0.89). Among females, the frequency of allele c was found 100% among Mughal, Malik and Mir (i.e., 1.00) populations and zero frequency of allele c for the same populations, while other populations showed little differences for the two alleles. The combined results for allele C follow the trend: Mughal > Malik > Mir > Gujjar and Bakarwal > Khan > Syed and vice versa for allele c.

Genotypic frequency and heterozygosity of color blindness among six populations

Table 4 presents the genotypic frequencies among male and female children of six populations. Since male with single X-chromosome, the genotypic frequencies are the same as of the allelic frequencies (allele C and allele c). The females with two X-chromosomes present the three types of genotypes: homozygous dominant (CC), homozygous recessive (cc) and heterozygous (Cc). The Mughal, Malik and Mir present 100% of the CC genotypic frequency (i.e., 1.00) and accounts 0.00% for Cc and cc genotypes. The heterozygosity (Cc) was found the highest among Syed (28.76%) population, while the least among Gujjar and Bakarwal (19.64%) and Khan (19.14%) and accounts zero for Mughal, Malik and Mir populations.

Discussion

This study provides a detailed description of red—green color vision deficiency for the first time among male and female children of six populations of Jammu province (Northern India), and thus provides the basic epidemiology and genetics of color blindness in the region. The prevalence of CVD ranged from 5.26% to 11.36% among males and 1.15%—3.03% among female children of six populations. The average prevalence of CVD was 7.52% observed in males and 0.83% in female children. Male children tend to have higher CVD frequency which reinforces the fact of X-linked recessive nature of the trait (i.e., the single X-chromosome in males is predominant to color blindness, while females with two X-chromosomes can act as dosage compensation and decreases the risk of the disease). Some recent studies have reported the mutational mechanisms of different cone opsin genes that suggest their differential role in many disease mechanisms associated with various retinal phenotypes. Another study has revealed the functional analysis of OPN1LW (L-) and OPN1MW (M-) cone opsin genes and their genotype—phenotype correlation significantly associated with progressive retinal degeneration.

Studies among the populations worldwide depict the significant variation in the prevalence of color vision impairments. The frequency of red—green color blindness among the males of Libya (2.2%), Saudi Arabia (2.9%), Nepal (3.9%), Singapore (5.3%), Thailand (5.6%), Korea (5.9%), Turkey (7.3%), Iran (8.1%), Jordan (8.7%), and Eastern India (8.73%) were found higher than that among females.

The proton and deutan defects unmask the connotative use of color in humans. Our cohort of 1028 children depicts the trend: deutan > proton > achromatic, showing higher frequency among males as compared to females. Moreover, the details of these defects showed the trend: deuteranomaly > deuteranopia > protanomaly > protanopiaachromacy. Similar frequency trend of vision defects have been reported in our earlier study among different populations, while a Turkish study has reported about 5.10% protans and 2.23% deutans among males.

The significance of normal vision involve absolute color matching for many occupations. In traffic signals, for deuteranope and protanope persons, the signals are less obvious, making handicap to perceive the signals. Moreover, the deutan and proton individuals working with telecommunications and electric cables can recognize the blue and white wires but will be uncertain about the red, orange, brown and green. Nearly 30% of people with abnormal color vision report they have trouble judging the ripeness of fruit. Color perception is integral to an individual’s understanding the visual world, and those with these defects can experience hardships in everyday life. However, adaptive strategies and behaviors help to deal with potential difficulties they face in both their professional and personal lives.

Red—green color blindness being a genetic disorder, it is noteworthy to estimate the gene frequencies among populations for medical counseling purposes. This study provides the information about the pattern of dominant and recessive alleles among six populations and heterozygosity for the females. Identification of color deficient individuals among populations can help to stop or minimize the risk of transmitting the disorder to their offspring’s by preconception or premarital counseling and also through prenatal diagnosis strategies.

Conclusion

In summary, our comprehensive assessment of color vision impairments provides the significant gender based differences and the prevalence of CVD was found higher among males (7.52%) as compared to female (0.83%) children. The estimation of vision test in our study using Ishihara 38-plate edition provides efficient sensitivity and specificity in detecting proton and deutan CVDs. The distribution of CVD children showed an increasing order of achromatic, proton and deutan defects, respectively. Several occupations involve color matching, due to which CVD patients may be

| Populations     | Male   | Female  |
|-----------------|--------|---------|
|                 | C (Y)  | c (Y)   |
|                 | CC     | Cc      | cc     |
| Gujjar and Bakarwal | 0.9314 | 0.0686  | 0.7912 | 0.1964 | 0.0122 |
| Mughal          | 0.9474 | 0.0526  | 1.00   | 0.00   | 0.00   |
| Khan            | 0.9223 | 0.0777  | 0.7969 | 0.1914 | 0.0115 |
| Malik           | 0.9359 | 0.0641  | 1.00   | 0.00   | 0.00   |
| Mir             | 0.9286 | 0.0714  | 1.00   | 0.00   | 0.00   |
| Syed            | 0.8864 | 0.1136  | 0.6821 | 0.2876 | 0.0303 |
| Total           | 0.9248 | 0.0752  | 0.8261 | 0.1656 | 0.0083 |

In males, C (Y) and c (Y) present homozygous dominant and homozygous recessive genotypes respectively, where Y represents the Y-chromosome. In females, CC, Cc and cc represent the homozygous dominant, heterozygous and homozygous recessive genotypes, respectively.
handicapped in their job. The problems arise among CVD individuals working in industries (paint, textile, plastic, decorates, furniture), transport (rail, road, aviation, maritime), defense (police, armed force, fire and rescue services) and other occupations (electricians, technician, telecommunication, mechanics). Since, the red–green CVD is congenital, there is no cure for the patients. However, advice from optometrists to CVD patients at early age could help to find adaptive strategies, which enable to avoid disappointments in the choice of their future carrier. The parental education, awareness, genetic counseling strategies in the regions with high CVD incidence could help a lot in minimizing the occurrence of the disorder among their offspring.

Conflict of interest

The authors declare that there is no conflict of interest exists.

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