GENETIC STUDY OF MYP 2, MYP 15, MYP 16 AND MYP 17 LOCI IN MYOPIA AFFECTED FAMILIES IN THE PUNJAB

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Abstract

Myopia is a common cause of impaired vision and visual disability and highly prevalent eye disease in Pakistan. It is multi factorial disease and 19 loci identified up to date. Seven myopic families were identified and selected for this study from different areas of Punjab province. Linkage analysis of these families was done by MYP2, MYP15, MYP16 and MYP17 loci (each consisting of a set of 3 microsatellite markers) of myopia that were selected from the panel of 19 loci. After DNA extraction and PCR amplification, linkage analysis was carried out by genotyping through PAGE and haplotypes were constructed for the families. This study leads us to find out the molecular basis of this disease for better understanding. Further study at broader scale with more number of families including more affected and normal individuals may identify any new locus involved.

Introduction

Myopia is a Greek word which means “close sightedness” or "narrow sightedness". The eye has a defect of refraction in which image formed “in front” of the retina instead of “on” the retina. Myopia is a condition in which the distant objects and sometimes closer ones too are not focused by the eye. Myopia has many clinical symptoms like blurred vision and has a problem to see the distant objects. There are three types of myopias; low myopia is a refraction error equal or below -3.00D, the diopter of medium starts from -3.00D and ends at -6.00D and high myopia is equal or above -6.00D. All over the world, eye has a common problem of myopia and a major issue of vision loss in the world (Fredrick, 2002). Myopia is caused by the genes, environment and nutritional deficiencies (Lougheed, 2014). Currently, there is a high prevalence of myopia in the whole world (Dunaway and Berger, 2006; Norton, *et al*. 2005). According to the National Blindness and Visual Impairment Survey in Pakistan, myopia is found up to 37.23% (Shah *et al* ., 2008). A baby girl / boy with myopic mother / father have myopia than with non- myopic parents. The refractive errors look to be higher in the off springs than the parent (Krause, *et al* . 1993)**.** However, it is difficult to separate hereditary factors from environmental factors such as similar work patterns in parents and their children.

The salient symptoms found in a myopic person are as follows: weakness and continuous pain of head, dim vision, watching very closely at TV and computer work, shapes of eyes are also disturbed or abnormal shape of the eyes, watery and swollen eyes. The World Health Organization (WHO) promoted the goal for eradication of blindness by the year 2020 called as “Vision 2020 initiative”. Refractive errors are kept on the priority for vision 2020 initiative and eye sight glasses give a cure that is less expensive, effective and linked with improvement (Ackland, 2012). It was known in one research study that the refractive errors account for 8% cases of uniocular blindness in Khyber Pakhtunkhwa province of Pakistan (former North West Frontier Province) (Khan *et al* ., 1994). The students are considered to be at a high risk because myopia can seriously affect their physical and mental development (Gilbert and Foster, 2001) as well as their educational abilities (Negrel *et al* ., 2000).
Advanced research on Myopia revealed that there is a great role of genetics in developing the Myopia (Dirani *et al* ., 2008; Zadnik *et al* ., 1994). The percentage of occurrence of Myopia in off springs from one affected parent is 23-40% and the percentage of occurrence of Myopia in off springs from one normal parent is minimized from 5-15% (Zadnik *et al* ., 1994). Women have more chances to be myopic than men but the exact reason is still unknown (Wang *et al* ., 2003). Myopia is inherited by Mandel laws of Inheritance and may be inherited from parents to off springs by autosomal dominant, autosomal recessive and X-linked patterns. The frequently found pattern of myopic inheritance is autosomal dominant (Jacobi and Pusch, 2010). MYP1 was the First discovered myopic locus which was later mapped in an X-linked recessive myopic family on Xq28 (chromosome). Three advance studies mapped to chromosome 18q11.3 (MYP2), chromosome 12q21-23 (MYP3) and chromosome 17q21-22.

Recent myopic research showed that 12 loci have been involved for high myopia and 7 for common myopia. Out of these 19 loci 5 have been confirmed through replication studies (Nurnberg *et al* ., 2008). Linkage studies can be performed using genetic markers particularly micro-satellite markers that are also called short tandem repeats (STRs). Genetic markers or primers are successful tool to investigate linkage analysis in humans and animals.

Total 19 Myopia loci (MYP1, MYP2, MYP3, MYP4, MYP5, MYP6, MYP7, MYP8, MYP9, MYP10, MYP11, MYP12, MYP13, MYP14, MYP15, MYP16, MYP17, MYP18 and MYP19) have been genetically mapped. In past, no study was carried out in Pakistan on myopic families for finding any locus responsible for myopia.

In this study the myopic families in Punjab, Pakistan were randomly selected and were checked for susceptibility for *MYP2, MYP15, MYP16 and MYP17* by using synthesized primers, through linkage analysis. After the study of my seniors in University of Veterinary and Animals Sciences, Lahore in 2012, this is the second study on Myopia in Pakistan. There are various approaches to identify the myopia producing genes like parametric linkage, non-parametric linkage analysis and genetic studies. In this study, microsatellite markers (STRs) were selected for linkage analysis and genetic characterization of myopic patients through genotyping was carried out.

Advantages of STRs markers are that we can genotype easily with the help of STRs rather than sequencing. The product size of these STRs is very small so it can be amplified by forward and reverse primer only. High polymorphism of STRs has magnificent ability to detect linkage. STRs have low rate of mutation (Sunnucks, 2000)

The aims of this research were to search out myopic families and linkage analysis to see responsible locus for myopia and linkage analysis of myopic patients through genotyping technique PAGE (Poly acrylamide gel electrophoresis). Further emphasis of this research was to generate a database to keep Pakistani myopic records and perseverance of their DNA for future research purposes.

These investigations will lead to develop a reliable group of micro-satellite markers to be used in linkage analysis, individual identification and genetic characterization of myopic patients. This work will also help in developing a pioneering database of myopia pedigreed families in Pakistan. The present research will also help to know something new of any novel locus responsible for myopia.

Materials and Methods

Selection of Families: The Myopic families were identified and selected from different areas of Punjab province of Pakistan. Their pattern of inheritance was studied and pedigrees were drawn. Their disease history especially for diabetes, cardiac problems high blood pressure and other diseases of past and present was recorded.

Enrollment of families: The families were given a Performa for recording all their current and previous histories. The wanted information were comprised of the following things: Name, Sex, Age, Cousin marriage or out of families marriage, Number of Offspring, information about forefathers, Home addresses, Affected persons, other diseases and eye sight history. When enrollment of myopic patients was completed, then their pedigrees were drawn by using Macro media free hand software or Cyrillic® program (Cyrillic for Windows 3.1).

Sample Collection and Preservation: Total 40 blood samples were collected as 8 myopic families (each family consisted of father, mother and their children) from different areas of Punjab. Blood samples were collected from myopic patients by 10 µL disposable syringes in 50ml falcon tubes having 200 µL (0.5M) EDTA as anticoagulant. EDTA rate is 200µl EDTA per 10ml of blood (Sheridan and Douthwaite, 1989). Falcon tubes were shaken gently for appropriate length of time to ensure complete mixing of anticoagulant to prevent blood clotting and blood samples were stored in refrigerator (-20 °C).

Storage of blood specimen: Blood specimens were safe in refrigerator at -18°C to -20°C (Green and Sambrook, 2012).

DNA Extraction from blood (inorganic method): Inorganic method (Green and Sambrook, 2012) was used for genomic DNA extraction from blood samples.

DNA Quantification: By using 0.8% Agarose gel, DNA quantification was carried out.

PCR Optimization: Primers were optimized to find out the annealing temperatures for successful Polymerase Chain Reaction (PCR). To achieve this all the primers were amplified by a temperature gradient PCR in which a

range 53°C - 60°C was used in the pEQ-Lab thermocycler. All the microsatellite markers showed the best results at 54°C.

Haplotype Analysis: A haplotype is a set of alleles inherited one per locus from the parent. In the present study alleles were arranged for various markers and Haplotypes were created that showed the inheritance pattern of the disease. With the help of this analysis we could track the inherited alleles with reference to each parent. After running PAGE, haplotype were constructed manually and were analyzed to identify any inherited pattern found in any of the family that was screened for four loci, MYP2, MYP15, MYP16 and MYP17.

Lod Score Calculation: Likelihood of odds is called LOD. The LOD score is a kind of statistical calculation to know where the two genes, a normal gene and a diseased gene located on chromosomes. They exist on the chromosomes near each other. They are inherited. For Mendelian linkage occurrence, the LOD score should be greater than +3. Against linkage the LOD score should be less than -2 (Andrew *et al* ., 2008). LOD score of the families screened on the four loci was calculated to identify and probable linkage after constructing haplotype. Also to see the chances of linkage present in the families screened for the four loci.

Results and Discussion

The study of myopia impairments offers a unique canvas to draw two types of illustrations altogether, first, the identification of the causative genes and the underlying pathogenic process in myopia and Second, the elucidation of the molecular and cellular mechanisms of seeing the objects. A large assembly of proteins operates in the most integrated fashion to complements the function of sensory cells in the eye. This firmly synchronized system can be weaken by many factors, making myopia the most common form of low, medium and high myopia known to man. A remarkable progress has been made in search of loci/genes responsible for development of myopia that is unparalleled in human genetics.

This current study was done to observe the genetic reasons of refractive errors in the population of Pakistan. Eight families having three or more myopic individuals were ascertained from different cities of province of Punjab of Pakistan**.** Many experiments have been conducted for the genetic analysis of myopia loci in many parts of the world, but up till now, second study has been carried out for this purpose in Pakistan. So, in present study, a panel of twelve microsatellite markers belonging to *MYP2, MYP15, MYP16* and *MYP17* loci of myopia has been developed to carry out linkage analysis for these loci. To fulfill this purpose, eight myopic families, Myo-01, Myo-02, Myo-03 and Myo-06, Myo-09, Myo-10, Myo-11 and Myo-12 from province Punjab, were collected to find out that which locus is responsible for making them myopic. Commonly used approach to map the genes in myopia; is genetic linkage analysis (Scavello, *et al* ., 2004). Many chromosomal specific areas that are considered to be involved in refractive errors have been located by Genetic linkage analysis. Different tools, like individual identification by blood typing; identification based upon protein polymorphism; mitochondrial DNA analysis; analysis of variable nucleotide tandem repeats; detection of single nucleotide polymorphism and microsatellite analysis, have been deployed for linkage analysis and genetic characterization of myopic patients up till now (Fraser-Bell *et al* ., 2006)

All the families were identified and selected on the basis of their eye-sight. The Individuals having eye-sight greater than -1.0 D were considered myopic. The range of the spherical power in myopic patients of present study was from -1.00 D to -4.75 D. According to a study by (Llorente *et al* ., 2003) the increase in spherical power with age has been found to be directly proportional to increase in myopia pathogenesis and also its progression. Only those families having a minimum of three myopic individuals in them were involved in the study. The total individuals included in the study were 40, of which 29 were myopic and the rest were normal. Pedigree analysis showed that 6 out of 8 families have autosomal dominant mode of inheritance for the myopic alleles.

Further extending the study by more extensive pedigree analyses for confirmation of this locus would become helpful in mapping out those genes which are responsible for this condition and to find out its treatments methods. Moreover, unlinked loci may give information that there might be a novel locus existing. Then further experimentation is required linkage. Locus MYP17 showed no linkage in this research. The same locus was studied by (Ciner *et al* ., 2008) in African-American families and found linkage to the 7p15 region. In this research, Locus MYP2 was not linked in selected Pakistani families. A linkage found in seven American families with this locus MYP2 by (Young, 2009) but in that research MYP15 is not linked with selected families. Ma *et al* ., (2010) identified linkage of MYP15 on chromosome 10q21.1 in consanguineous Chinese family in an autosomal recessive pattern. Locus MYP16 showed no linkage in our research. The same locus was studied by (Lam *et al* ., 2008) in Chinese families on chromosome 5p15 and found linkage. The future scope of this study can be extended by working on other loci of myopia. The future cloning and mutational characterization of the genes for myopia will explain the molecular mechanisms underlying increased eye growth and lead to a better understanding of the clinical consequences of the mutations of these loci. This may

also lead to allow development of DNA based diagnosis including pre-symptomatic and post-natal diagnosis. It may suggest new therapies and may lead to understanding of other molecular diseases.

In this research, no gene linkage was found between the myopic alleles in the sample pool of eight families. The reasons for this non-linkage may be:

Other loci than MYP2, MYP15, MYP16 and MYP17 are responsible for myopia in these families.

Environmental factors (climate, medicines, diet and reading) may cause myopia in MYO-01, MYO-02, MYO-03, MYO-06, MYO-09, MYO-10, MYO-11 and MYO-12.

As scientists unlock the human genome, they are finding many ocular anomalies are actually multifactorial in nature. These findings also point to the involvement of multiple genetic loci in the genome related to the production of myopia. A multifactorial genetic defect occurs when multiple genetic expressions interact to produce a condition, or environmental factors interact with these existing genes to produce an anomaly. In description of complex disease myopia a single genetic locus is not responsible for production of the condition; rather a multifactorial inheritance pattern considers the combined effects of genetic predisposition, lifestyle, and environmental factors in evaluating the etiology of a condition.

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