Cataractogenesis essentially involves damage of lens proteins leading to loss of transparency of lens. It is brought about by either quantitative or qualitative alterations in lens components such as macromolecules, small molecules (water and metabolites) or the cells themselves. Although knowledge of cataract and its remedy is centuries old, understanding of its pathogenesis has been possible only during the last century. Insight into the basis for the lens transparency came initially from biochemical and biophysical studies of the lens, followed by molecular genetic approaches during the later part of this century, which aimed at identifying the genetic defects associated with hereditary cataracts.

This review attempts to provide an insight on how epidemiological risk factors, oxidative stress, and molecular genetic markers play their role in the development of cataract.

3.1 EPIDEMIOLOGY

Epidemiological studies of any condition including age-related cataracts (ARCs) adds to the knowledge about the condition and helps in estimating the magnitude of the problem, determining its prevalence and incidence in different regions/ethnic groups.

3.1.1 Prevalence

Age-related eye diseases are the leading cause of vision impairment and blindness worldwide (Thylefors et al, 1995; Thylefors, 1999). The most prevalent among age-related eye diseases is cataract formation which accounts for almost 42% of the total blindness (Brian and Taylor, 2001). There are about 16 million cases of blindness worldwide, with approximately half of all cases originating from Africa and Asia affecting nearly 20.5 million Americans who are above 65yrs (Anonymous, 1998). The number of people blind due to cataract is increasing by approximately one million a year which is an alarming issue (Foster, 1999).

Considering different types of age-related cataracts (ARC), nuclear cataract (NC) is by far the most prevalent ARC in tropical or subtropical regions of the world, followed by cortical (CC) and posterior subcapsular (PSC) cataracts. Moderate-to-severe nuclear cataracts are found in 20-60% of the population aged ≥70 in tropical or subtropical regions,
and 10-15% in temperate regions (Sasaki et al, 2002). About 80% of rural populations in the USA are affected by NC (Klein et al, 1992). Based on the same age group, moderate-to-severe cortical cataract has a prevalence of about 45% in tropical or subtropical regions based on observations in Iceland, Japan and Singapore and 30-40% in temperate regions (Sasaki et al, 2002). Cortical cataract affects nearly 20% of the population aged 30 years and older living in rural China (Xin Rong Duan et al, 2013). PSC is much less common, with 10–15% in tropical or subtropical regions and 2–3% in temperate regions (Sasaki et al, 2002).

Small dot-like and spoke-like cortical opacities were found in 20% in the age group of 31–45 years and in 30% in the age group 76–90 years. Larger segmental and annular cortical opacities were found in 10% in the age group 31–45 years and in 45% in the age group 76–90 years (Vrensen and Willekens, 1989). While these cortical opacities can be either restricted to a sector or quadrant or found around the entire circumference of the lens, their extent is not directly related to the lens age (Klein et al, 1992). The segmental cortical cataract is found more frequently in the inferior nasal sectors of the lens (Klein et al, 1992).

In India 40% of all blindness is estimated due to cataract (Specter, 1974), about 27 million people are blind due to cataract in one or both eyes (Liu et al, 1977), and the prevalence of cataract is reported as 7% by Venkataswamy, (1975). Surveys in different climatic zones in Northern India revealed cataract prevalence of 4-10% and senile cataract is found to increase steadily after the age of 30 yrs and the prevalence being 13-36% among persons aged 30 years and older (Chatterjee, 1973; Mehrotra and Maheswari, 1976). Prevalence rates adjusted for age and sex have been reported for the Punjab plains of India that were almost three times higher than the comparable Framingham eye study (FES) rates. For persons in the Punjab aged 75-85 years, more than 80% had age-related cataracts with vision reduced to 6/9 or worse (Chatterjee, 1982). The Andhra Pradesh Eye Diseases Study (APEDS) reported that cataract alone contributes to 44% of the total blindness in India (Dandona et al, 2001). A study by Balasubramanian, (2002) has reported that 9.5 million people had impaired vision due to cataract in India during the year 2000 and another 3 million people could not see because they have not corrected their ‘power’. The actual number of people affected is estimated to rise from 12.5 million in 2000 to about 18.8
million in the year 2020. A recent study on prevalence of ARCs in an older Indian population, conducted by Praveen et al, (2011) showed that the prevalence of both operated and unoperated cataracts was similar in North (73.8%) and South India (71.8%). The unoperated cataract cases were increased with age and were higher in women than men.

3.1.2 Risk Factors
As cataract is a complex multi-factorial condition, several epidemiological factors are expected to play their roles in increasing risk for the condition, apart from the major etiological factors.

3.1.2.1 Age
Ageing is by far a major known risk factor for cataract (Jessica et al, 2011) and older age is an independent baseline risk factor for incidence of nuclear and cortical lens opacities (Richter et al, 2012). An unpublished data by Bhagyalaxmi, (2009) showed that about 67% of cases were of ≥ 50 years of age indicating higher risk for older age group. An Aravind Comprehensive Eye Study conducted on the population from three districts in Southern India also showed that the prevalence of age-related cataract was increased significantly with an increasing age, from 15.7% among those aged 40–49 years to 79.4% among those aged ≥70 years (Nirmalan et al, 2004). Data from the Model Reporting Area suggested that the prevalence of cataract blindness increased from 17/100,000 for persons aged 45-64 years to 488/100,000 for persons over the age of 85 years (Kahn and Moorhead, 1973). In the Framingham Eye Study, the prevalence of cataract augmented from 4% at age 52-64 years to 50% at age 75-85 years (Kahn et at, 1977). More than 90% of persons aged 75-85 years have age-related lens changes (Sperduto and Seigel, 1980). Age is the most important associated factor of cataracts among adult Chinese residents in Beijing (Xu et al, 2006).

3.1.2.2 Sex
Several studies suggested that there is a small excess risk of cataract for women compared to men (Sperduto and Seigel, 1980; Klein et al, 1992; Leske et al, 1997; Mitchel et al, 1997; Leske et al, 1999; Cheng et al, 2000; Tsai et al, 2003). 10-20% excess risk of
cataract among women was reported by Hiller et al, (1983). The 10 year, age-adjusted incident rates of nuclear and cortical opacities were higher in females than males in a study on Caucasian populations of Blue Mountain, Australia. Similarly, 10 and 15 year, age-adjusted incidence of nuclear opacities were higher in females in Beaver Dam study (West, 2010). Female gender was an independent risk factor for PSC lens opacities (Richter et al, 2012). A study by Duan et al, (2013) reported that the incidence of cataract was more in women than in men. Bhagyalaxmi, (2009) showed a significant increase in the frequency of females (55.7%) as compared to male patients (44.3%) of cataract.

3.1.2.3 Body mass index (BMI)
A study by Lu et al, (2012) showed risk for developing ARC in case of males who are both overweight and obese. A large-cohort study by Yoshida et al, (2010) showed a U-shaped association between BMI and the incidence of cataracts in Japanese men and women. In the Shihpai Eye Study on the population of 65 years and older persons living in Shihpai, Taipei demonstrated that the risk for developing nuclear opacity decreases with a gradual increase in BMI from 21. This protective effect ceases when BMI approaches 28, and the risk of nuclear opacity increases as BMI progresses to obese levels. Conversely, risk of cortical opacity increases as BMI increases from 21, and when BMI is greater than 28, risk of cortical opacity decreases (Kuang et al, 2005). Two hospital-based case-control studies found that a low BMI was associated with nuclear opacity (Mohan et al, 1989; Leske et al, 1991). A study in Punjab, India, found that a higher prevalence of cataract was associated with short height, low weight, and low weight-height ratio (Chatterjee, 1982).

3.1.2.4 Family history
Family studies have shown that hereditary factors play an important role in the occurrence of ARC (Italian and American study group, 1991; Leske et al, 1991). The strongest evidence came from twin studies demonstrating a heritability of 48% for nuclear cataract and 59% for cortical cataract (Hammond et al, 2000 & 2001). Sibship analysis showed strong association for nuclear and posterior subcapsular cataracts (Framingham Eye Study; 1994). In Beaver Dam Eye Study, the segregation analysis suggested the contribution of single major genes in the development of cortical and nuclear cataracts. One single major gene may account for 58.0% of the variable risk for cortical cataract (Heiba et al, 1995).
and another single major gene may account for 35.0% for nuclear cataract (Heiba et al, 1993). Discussing genetic contribution to cataracts, McCarty et al, (2000) and Taylor, (2001) suggested that 50% of the age-related cataracts can be explained based on genetic reason while the remainder attributes to ageing and other environmental factors.

3.1.2.5 Nutrition
Two of three large case-control studies to estimate dietary intake of selected nutrients reported a decreased risk of cataracts in persons with a higher intake of a number of micronutrients (Leske et al, 1991; Mohan et al, 1989). Study of Leske et al, (1991) also reported a decreased risk of cataracts among persons who used multiple vitamin supplements regularly. A smaller case-control study found fewer cataracts in persons taking supplementary vitamin C or supplementary vitamin E, but no protective effect for persons taking multivitamin preparations (Robertson et al, 1989). One additional observational study reported that subjects with "high" plasma levels of at least two of three vitamins with antioxidant characteristics (vitamin E, vitamin C, or carotenoids) were at reduced risk of cataract compared with subjects with low levels of these vitamins (Jacques et al, 1988). Long-term use of multivitamins B and A supplements were reported to be associated with reduced prevalence of either nuclear or cortical cataract (Kuzniarz et al, 2001).

3.1.2.6 Radiation exposure
Ultraviolet radiation, a part of the sunlight spectrum that is absorbed efficiently by the lens, was used to produce cataracts in laboratory animals (Pitts et al, 1977; Zigman et al, 1974). Several ecologic studies reported an association between cataracts and sunlight or ultraviolet light exposure (Hiller et al, 1977; Hollows and Moran, 1980; Tarwadi and Agte, 2011). One nonecologic study that attempted to quantify individual lifetime UV exposure reported that fishermen with the highest amount of exposure were at increased risk of cortical and posterior subcapsular cataracts (Taylor, 1988; Bochow et al, 1989). Damage by energy from electromagnetic spectrum to the lens was suggested by Leske and Sperduto, (1983). High dose exposures to ionizing, infrared, and microwave radiation are capable of inducing lens opacities. The cumulative effect of low-dose exposures to such forms of radiation, which occur far more frequently than high-dose exposures, is unknown.
The difficulty in measuring individual long-term exposure to the various energy sources and the absence of unique features for cataracts induced by such exposures has complicated this type of studies.

3.1.2.7 Smoking

Smoking is a well-known risk factor for a wide range of diseases, such as vascular disease, lung cancer, and chronic obstructive pulmonary disease (Hecht, 2002; Shah and Cole, 2010; Taylor, 2010). Tobacco smoke contains hundreds of different substances, including nicotine, free radicals, and carbon monoxide, which can increase oxidative stress and have an important role in the pathogenesis of ARC (Truscott, 2005; Beebe et al, 2010). Recent study by Ye et al, (2012) explained that smoking was associated with increased risk of ARC, especially of NC and a marginally significant relationship with PSC type. Increased consumption of tobacco is considered as one of the risk factors for cataracts (Tarwadi and Agte, 2011) and current smoking also increases the risk for NC (Richter et al, 2012). Observational studies reported that current history of cigarette smoking increases the risk of nuclear, but not cortical cataract (West et al, 1989; Leske et al, 1991). Risk decreased among those who had stopped smoking. Review by Kelly et al, (2005) includes twenty seven studies which suggested 3-fold increased risk for nuclear cataract development. There was also evidence of dose response, temporal relationship, and reversibility of the effect observed. There was limited evidence of an association between smoking and posterior subcapsular cataract, but little or no association with cortical cataract. Thus, the literature review indicated a strong association between smoking and the development of cataract, particularly nuclear cataract. Study on cohort group smoking showed high frequency of smokers among cases (21.1%) as compared to controls (16.9%; Bhagyalaxmi, 2009).

3.1.2.8 Alcohol consumption

Alcohol is widely consumed through the ages because of its perceived benefits as a social lubricant and for relaxation. Consumption of alcohol is causally associated with a number of diseases such as liver cirrhosis, pancreatitis, and cardiomyopathy (Hiratsuka et al, 2009). A study by Harding, (1991) suggested that heavy drinkers develop opacities by the reaction of a product of alcohol metabolism, acetoaldehyde with lens protein leading to
protein modification. Daily use of ≥1 alcoholic drinks was associated with a modest increase of risk for cataract extraction and the risk increased with increasing alcohol consumption (Lindblad et al, 2007; Tarwadi and Agte, 2011). As per the report of Gowri et al, (2010) no significant associations were observed between alcohol consumption and long-term risk of nuclear, cortical, and posterior subcapsular cataract, but total alcohol consumption of over 2 standard drinks per day was associated with a significantly increased likelihood of cataract surgery, when compared to total daily alcohol consumption of 1 to 2 standard drinks.

3.1.2.9 Drugs
Many drugs are assumed to have cataractogenic potential. Implicating evidence is strong for some drugs such as corticosteroids, but less strong for others. Drugs showing cataractogenic potential include phenothiazines, miotic cholinergic compounds, cancer chemotherapy agents, various photosensitizing drugs, diuretics, major tranquilizers, gout medications, cholesterol-lowering medications, and many others (Leske and Sperduto, 1983; Harding and Heyningen, 1987). For the most part, the cataracts that are caused by these agents are similar in appearance to age-related cataracts. In contradiction to the above reports, use of aspirin or aspirin-like analgesics protect against the formation of cataract. While some observational studies have reported a protective effect for aspirin (Cotlier and Sharma, 1981; cotlier, 1981), other observational studies (west et al, 1987; klein et al, 1987) and two clinical trials of physicians (Peto et al, 1988; Sedddon et al, 1989) seem to have excluded any large protective effect of low aspirin usage on cataract formation.

Apart from the above factors, a large number of other factors were also reported which increases the risk of cataract. These include myopia, systemic hypertension, severe diarrhea, renal failure, low socio- economic status, illeteracy and various biochemical factors (Leske and Sperduto, 1983; Ughade et al, 1998).

3. 2 FACTORS CAUSING STRESS

3.2.1 Oxidative Stress and Anti-oxidant Enzymes
Oxidation is a key feature of cataract formation especially cortical and nuclear types. Free radicals, including numerous reactive oxygen species (ROS) such as superoxide anion
radical (·O$_2^-$), H$_2$O$_2$, and hydroxyl free radical (·OH), may lead to structural damage of the crystallin lens and contribute to cataract formation (Spector and Garner, 1981; Giblin et al, 1984). ROS may be generated exogenously after UV light and ionizing radiation exposure, or endogenously as a result of normal metabolism. Oxidative stress occurs when the level of pro-oxidants surpass the level of antioxidants. If the level of ROS is not finely regulated, it will lead to the mitochondrial damage and their accumulation in the lens (Berthoud and Beyer, 2009).

Antioxidant enzymes work with reducing systems and protein repair systems to protect against ROS-induced damage. MnSOD (SOD2) and CuZnSOD (SOD1) present in the mitochondria converts the superoxide anion, which is generated by the electron transport chain, into hydrogen peroxide. By regulating the level of SOD2, lens epithelial cells can overcome oxidative stress (Ott et al, 2002; Matsui et al, 2003). Elevated levels of SOD1 protect the lens from H$_2$O$_2$-mediated damage (Lin et al, 2005). As fibre cells of the lens grow, nuclei and mitochondria are lost leading to simultaneous loss of repair systems of the mitochondria.

Among ROS, H$_2$O$_2$ is the most stable oxygen species present in the aqueous humor and is threefold higher in cataract patients compared to normals. H$_2$O$_2$ can also diffuse into the interior lens. Lenticular epithelial cells have antioxidant defences (including catalase (CAT), GSH, and GSH peroxidase), which are capable of converting aqueous-derived H$_2$O$_2$ to H$_2$O and O$_2$ (Spector and Garner, 1981; Giblin et al, 1984; Halliwell and Gutteridge, 1990). ROS-induced destruction at different targets within the crystallin lens, such as proteins or lipids, is believed to underlie the pathogenesis of cataracts (Beal, 2005). Thioredoxin (Trx), another electron donor, is a small thiol protein with active-site dithiol, which reduces protein disulfide actively. Oxidized Trx is further reduced by Trx reductase in a NADPH-dependent manner. The production of NADPH increases under oxidative stress, making it important in redox control (Giblin et al, 1981).

To correlate these biochemical facts to lens morphology, the lens barrier may be of most importance. The barrier at the cortex/nucleus interface impedes the flow of molecules such as antioxidants into the nucleus and thus predisposes the lens center to oxidative damage.
(Sweeney and Truscott, 1998; Moffat et al, 1999). Many antioxidants, such as vitamins C and E and the carotenoids, are considered to work as ROS scavengers. In animal models, antioxidant levels are correlated with cataract progression.

### 3.2.2 Photo Oxidative Stress

The lens that works together with the cornea to focus radiation on the retina is constantly exposed to ambient radiation including UVA, UVB, and visible light, which lead to photochemical insult to the eye. As there is no turnover of proteins in the lens throughout life, the lens gets more vulnerable to photochemical insult. The differences in cataract risk in epidemiological assessments, particularly in geographic studies, indicate an association between cataract and UV light exposure (Hiller et al, 1977; Hollows and Moran, 1980; Tarwadi and Agte, 2011). The damaging wavelengths of UV radiation that reach the lens are in the range of 300-400 nm. 90-95% of this UV radiation is effectively absorbed by UV filters, which are low molecular mass lenticular UV absorbers. The UV filters present in the lens include Kynurenine, 3-Hydroxykynurenine (3OHKyn), and 3-OHkynurenine glucoside protects the lens from UV induced photodamage (Heyningen, 1973; Dillon and Atherton, 1990). In the lens compartment where the antioxidant (e.g., GSH) is depleted, 3OHKyn can act alternatively as an antioxidant (Truscott and Augusteyn, 1977). UV filters present in the human lens, react with lens proteins and affect lens coloration (Stutchbury and Truscott, 1993).

Exposure to UV also induces cross-linking of α, β and γ-crystallins, which lead to conformational and solubility change, resulting in light scattering in cataracts. The lens epithelium has anti-photooxidative enzymes, which prevent the lens from being damaged by photooxidation induced by UV (Reddan et al, 1988). Nevertheless, the α-crystallins themselves can act as chaperones against photodamage by UV irradiation (Borkman and McLaughlin, 1995).

### 3.2.3 Non-enzymatic Glycation

Non-enzymatic glycation is a condensation reaction between amino groups in proteins and reducing sugars. The glycated product, known as early glycation product, may undergo further reaction by oxidative or non-oxidative pathway. Under oxidative conditions, the
early glycation product does not undergo further reaction. Under non-oxidative conditions, the early glycation product can react with many amino groups to give rise to brown and cross-linked products called advanced glycation end products (Holmquist and Schroeder, 1966; Monnier and Cerami, 1981; Reddy et al, 1995). This mechanism may also contribute to high molecular weight aggregation and eventual protein insolubilization in lens. In addition to the lens protein, glycation also influences the lenticular membrane. Crosslinking of the intrinsic proteins in the membrane affects membrane rigidity and permeability, and may also lead to membrane opacity (Liang and Rossi, 1990).

3.3 MOLECULAR GENETICS OF CATARACTS

In 1968 an inherited form of isolated cataract, which had previously been linked to the Duffy blood group locus, became the first monogenic disorder in humans assigned to an autosome i.e chromosome 1 (Renwick, 1970). Inheritance of cataracts may follow Mendelian form in congenital (by birth) cataract cases or more commonly as a multifactorial condition in age-related cases. Half of congenital cataracts are developed due to Mendelian inheritance and the most frequent mode of inheritance is autosomal dominant, but also can be inherited in an autosomal recessive or X-linked fashion. Phenotypically identical cataracts can result from mutations at different genetic loci (locus heterogeneity) and may have different inheritance patterns, sometimes phenotypically variable cataracts occur in a single large family (Hejtmancik et al, 2001). Age-related cataract is less phenotypically variable than Mendelian forms of cataract, but the genetic complexity of this remains largely unknown. Genetic epidemiological studies of affected twins and siblings predict that genetic risk factors may account for 14%–48% of the heritability for nuclear cataract, and 24%–75% of the heritability for cortical cataract (Heiba et al, 1993; Hammond et al, 2000). So far, about 200 genes and loci for Mendelian and age-related forms of human cataract are estimated, and distributed on 22 chromosomes and the X-chromosome. Of these, more than 130 genes or loci are considered to be responsible for syndromic forms of cataract.

3.4 GENES AND MUTATIONS CAUSING CONGENITAL CATARACTS

Of 200 genes around 35 independent loci have been identified for isolated or infantile cataracts. Genes mapped and the mutations identified in different genes related to
congenital cataracts are summarized in Annexure-I. Mendelian form of the congenital cataracts may be developed either as non-syndromic cataract or syndromic cataracts based on the absence or presence of associated ocular or systemic abnormalities, respectively.

3.4.1 Genes Causing Non-Syndromic Cataract

Non-syndromic cataracts generally develop without any associated ocular or systemic conditions. Several studies revealed that mutations in candidate genes lead to the development of non-syndromic cataract. These genes include crystallins, connexins, and genes for membrane or cytoskeleton proteins and transcription factor genes.

3.4.1.1. Crystallin genes

Crystallins constitutes more than 90% of the lens proteins. Around 60 different mutations segregating in some 98 families have been identified in 10 human crystallin genes, including those coding for both α-crystallins (CRYAA, 21q; CRYAB 11q), two acidic β-crystallins (CRYBA1, 17q; CRYBA4, 22q), three basic β-crystallins (CRYBB1, CRYBB2, and CRYBB3 all on 22q), and three γ-crystallins (CRYGC, 2q; CRYGD, 2q; and CRYGS, 3q). Generally, mutations in crystallin genes tend to cause nuclear or lamellar lens opacities. Most of the crystallin gene mutations are missense mutations, account for about 50% of non-syndromic familial cataract reported so far (Shiels and Hejtmancik, 2007; Hejtmancik, 2008).

3.4.1.2 Genes coding for Membrane or cytoskeleton proteins

Mutations in at least ten other genes that encode membrane proteins or cytoskeletal proteins have been linked with about 35% of non-syndromic cataract, mostly with autosomal dominant inheritance (Shiels and Hejtmancik, 2007; Hejtmancik, 2008). Mutations in genes coding for membrane protein, which include connexins (GJA3 and GJA8), Major intrinsic proteins (MIP, LIM2 and PMP22) are responsible for about 20% and 5% of the non-syndromic familial cataracts respectively (Shiels et al, 1998, Hansen et al, 2006). Several other genes like TMEM114, CHMP4B, ESCRTIII and EPHA2 involved in membrane associated signaling or transport processes are causative for about 5% of non-syndromic familial cataract (Shiels et al, 2008; Jun et al, 2009, Zhang et al, 2009; Kaul et
al, 2010). Mutations in the genes like BFSP1, BFSP2 and VIM coding for structural protein constitute about 4% of familial cataract (Bornheim et al, 2008; Muller et al, 2009).

3.4.1.3 Transcription factor genes
Mutations in the gene for heat-shock transcription factor-4 (HSF4, 16q) underlie about 6% of non-syndromic familial cataract may be inherited as an autosomal dominant or recessive cataract (Bu et al, 2002).

3.4.2 Genes Causing Syndromic Cataract
Syndromic forms of cataract appear as a secondary or variably associated symptom of a genetic syndrome (Werner syndrome, Down syndrome) or metabolic disorder that features other defining ocular and/or systemic abnormalities (Lin et al, 2010). Mutations in some transcription factor genes tend to cause extralenticular defects, they include homeobox genes, PAX6 (11q), FOXE3 (1q), PITX3 (10q) and VSX2 (14q), and the genes coding for bZIP transcription factor and V-MAF in some families (Gould and John, 2002, Jamieson et al, 2002). The X chromosome alone harbors over 15 syndromic forms of cataract (Coccia et al, 2009). As per Cat-Map there are at least 16 “orphan” loci for inherited forms of cataract at which the underlying genes remain to be discovered (Shiels et al, 2010).

3.5 GENES FOR AGE-RELATED (ARCs) CATARACTS
ARCs usually develop after the 4th decade of life and based on slit-lamp examination may be divided into three clinical types referred to as; nuclear cataract (NC), cortical cataract (CC), and posterior sub-capsular cataract (PSC). Each can occur separately or in combination referred as mixed cataract, and may progress to total opacification of the lens. In contrast to inherited congenital or childhood forms of cataracts, relatively few genes have been unambiguously associated with risk of age-related cataracts in humans (Annexure-II). Genes underlying Mendelian forms of cataract are considered plausible candidates for genetic determinants of ARCs (Moore, 2004). So far, variations in at least eight genes linked with inherited cataract have been associated with age-related cataract. These include EPHA2 (1p), GJA8 (1q), GALT (9p), SLC16A12 (10q), HSF4 (16q), GALK1 (17q), FTL (19q), and CRYAA (21q) (Okano et al, 2001; Karas et al, 2003; Shi et al, 2008; Shiels et al, 2008; Bhagyalaxmi et al, 2009; Faniello et al, 2009; Jun et al, 2009; Liu et al,
2010; Zuercher et al, 2010). “Osaka” variation (p.A198V) in the gene for autosomal recessive galactokinase-deficiency and congenital cataracts (GALK1) has so far been associated unambiguously with age-related cataracts, and this association appears to be limited to East Asian populations. Variations in the gene coding for Ephreceptor type-A2 (EPAH2), which functions in the ephrin cell signaling associated with cortical cataract was reported in an Italian population. Several sequence variations found in coding or intronic region of HSF4 were also associated with ARCs. A mutation screening study in the Chinese population with age-related cataracts revealed the presence of five novel variations in the genes GJA3, GJA8 and LIM2. They showed potential pathogenicity for the variant c.67A>C of the LIM2 gene (Zhou et al, 2011). The G>A transition at 6th position in exon-1 of CRYAA gene showed the risk for developing different types of ARCs (Bhagyalaxmi et al, 2010). A sporadic novel missense mutation p.F71L found in the CRYAA1 gene was found to show significant loss of chaperone like activity leading to cataract formation (Bhagyalaxmi et al, 2009). Study by Sireesha et al, (2012) showed the association of GSTM1 positive, GSTT1 null and double null (GSTM1 null, GSTT1 null) genotypes with ARCs.

Finally, variations in at least ten other genes not directly associated with inherited cataract have been tentatively implicated in age-related cataract. These include genes that function in antioxidant metabolism (GSTM1, 1p; GSTT1, 22q; Guven et al, 2007; Zhou et al, 2010), xenobiotic detoxification (NAT2, 8p; Tamer et al, 2005), DNA repair (ERCC2, 19q; Unal et al, 2007), folate metabolism (MTHFR, 1p; Zetterberg et al, 2005), lactose metabolism (LCT, 2q; Karas-Kuzelicki et al, 2008), RNA demethylation (FTO, 16q; Lim et al, 2009), lipid/cholesterol transport (APOE4, 19q; Utheim et al, 2008), kinesin/microtubule motor transport (KLC1, 14q; Andersson et al, 2007), and one of unknown identity (ARCC1, 6cen; Iyengar et al, 2004).

For the first time an association of c.422+90G>A; rs4613984 in intron down stream to the exon 4 of IDO gene with cataract formation among the aged has been reported by Mamata et al, (2011). Recently, Jiangsy Eye study showed the association of two WRN DNA repair gene polymorphisms (rs11574311 & rs2725338) with cortical and mixed types of cataracts (Su et al, 2013). The copy number variations present in the HSF4 and WRN genes were
shown to be involved in the pathogenesis of ARC in the Han Chinese population (Jiang et al, 2013).

3.5.1 Indoleamine 2,3-dioxygenase (IDO) and ARCs

IDO is the first rate limiting enzyme involved in synthesis of UV filters in the lens from L-tryptophan by kynurenine pathway (Van Heyningen, 1973; Figure-3.1). In humans over 90% of the tryptophan is catabolized by this pathway and it includes a number of other enzymes those ultimately forms nicotinamide adenine dinucleotide (NAD) end product.

The human lens UV filters (e.g. Kyn, 3OHKyn and 3OHKG) are capable of protecting lens from UV damage when they are free in solution. However, they are intrinsically unstable and can covalently bind to lens proteins. UV filters have been found to be unstable at physiological pH, resulting in deamination (Truscott et al, 1994; Garner et al, 1999). Deaminated Kyn and 3OHKyn are able to bind to nucleophilic amino acid residues, such as cysteine, histidine and lysine, on lens proteins in vitro, while in vivo studies suggest that preferential binding occurs at cysteine residues (Vazquez et al, 2002; Korlimbinis et al, 2006; Korlimbinis et al, 2007; Garner et al, 2000). Kynurenine-mediated modification could contribute to the lens protein modifications during aging and cataractogenesis. They may also reduce the chaperone function of α-crystallin, which is necessary for maintaining lens transparency (Bhattacharyya et al, 2006). In vivo study by Mailankot et al, (2009) showed that IDO over expression followed by kynurenine formation adversely affects lens development, causes massive apoptosis of fibre cells, and leads to poorly differentiated fibre cells and cataract formation.

3.5.1.1 IDO gene and its promoter

Indoleamine 2,3-dioxygenase (IDO), is a single copy gene present in humans. Other names of the IDO are IDOI and INDO (OMIM: 147435). The human IDO gene is mapped to the chromosome 8 at p12-p11. It spans 15kb with 10 exons. Transcription of the human IDO gene produces a full-length mRNA transcript of 1,572 base pairs. The open reading frame is preceded by a long, untranslated sequence. The mature mRNA codes for a full-length protein of 403 amino acids with a molecular weight of 45,332 Daltons. IDO is first translated as an apoenzyme, subsequently the heme prosthetic group is added to make the holoenzyme (Figure-3.2).
Figure-3.1: The kynurenine pathway of tryptophan metabolism (Curti et al, 2009)
Figure 3.2: Indoleamine 2,3-dioxygenase gene structure

The X-ray crystal structure of IDO (Figure 3.3) contains two alpha-helical domains, one large and one small distinct α-helical domain, with the heme prosthetic ring positioned between them. The heme is co-ordinated to the active site by a histidine imidazole as the proximal fifth ligand. Site-directed mutagenesis showed that His^{346} and Asp^{274} are required for maintaining heme binding in IDO. His^{346} and Asp^{274} may act as proximal and distal heme ligands in the binding of heme in IDO (Littlejohn et al, 2003). The enzyme indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.52) belongs to the family of heme-containing oxidoreductases. It catalyzes the first and rate-limiting step in the kynurenine pathway, the major pathway of tryptophan metabolism.

The 5’ upstream of the ATG start codon constitutes promoter (1.3kb) of *IDO* gene. It includes transcription factor sites that confer responsiveness to type I and type II interferons (IFN-alpha / IFN-beta and IFN-γamma respectively), most potently to IFN-
γamma. Transcriptional induction of IDO gene is mediated through the Jak/Stat pathway particularly Jak1 and Stat1α (Du et al, 2000).

![X-ray crystal structure of human IDO](image)

(Adopted from Sugimoto et al, 2006)

**Figure-3.3:** X-ray crystal structure of human IDO, complexed with the ligand inhibitor 4-phenylimidazole and cyanide. Ribbon representation of the overall structure of human IDO. The small and large domains are represented by blue and green ribbons, respectively. The helices A–S are named in the order of appearance in the primary sequence. The connecting helices (K-L and N) are coloured in cyan. The long loop connecting the two domains is coloured in red. The heme (yellow), proximal ligand H346 (white), and heme inhibitor 4-phenylimidazole (white) are shown in a ball-and-stick model. The helices of the large domain create the cavity for the heme. The connecting loop (red) and small domain above the sixth-coordination site (heme distal side) cover the top of cavity on the heme.

Stat1α appears to act to induce IDO gene expression directly through binding of (Gamma activation sequences) GAS sites within the IDO promoter as well as indirectly through induction of Interferon regulatory factor 1 (IRF-1), which binds the IDO promoter at two interferon stimulated response elements (ISRE) sites. Stat and IRF transcription factors function cooperatively to mediate induction of IDO expression by IFN-γamma, and mice
lacking either IFN-γamma or IRF1 function are deficient in IDO expression during infections. Nuclear factor (NF)-kB also contributes to IDO induction (Du et al, 2000; Robinson et al, 2005). IDO is expressed intracellularly in a constitutive or inducible manner in placenta, lung, small and large intestine, colon, spleen, liver, kidney, stomach and brain. It can be induced by IFN-γ in myeloid lineage cells (dendritic cells, monocytes, macrophages, eosinophils), epithelial cells, fibroblasts, vascular smooth muscle and endothelial cells and certain tumour-cell lines (Thomas & Stocker, 1999; Grohmann et al, 2003; Mellor & Munn, 2004; Takikawa, 2005).

3.5.1.2 Genetic variants of IDO

Sequence variations are reported in the IDO gene in the NCBI database describing 43 variants in human IDO gene covering exon and intronic region and their boundaries but with no clinical associations including cataract. Arefayene et al, (2009) in their study using Coriell DNA samples (48 African Americans and 48 Caucasian Americans) found 24 IDO variants of which 17 were in exons, introns or exon/intron boundaries while 7 were within 1.3kb upstream of the translation start site. They identified 22 putative transcription binding sites within 1.3kb upstream of the translation site and two of the SNPs detected were located in GATA3 and FOXC1 sites (Arefayene, 2008).

A case-control study by Nishizawa et al, (2010) showed three variations (two in exonic region, one in promoter) in the IDO gene sequence. Amani et al, (2011) identified 10 SNPs; four exonic and six intronic regions of IDO gene in Iranian women with recurrent spontaneous abortions of which three have been registered with the NCBI single nucleotide polymorphism (SNP) database which were however not associated with recurrent spontaneous abortions. So far only one report from the present study claimed the involvement of IDO genetic variants in the development of ARC. This study revealed the presence of c.422+90 G>A in intron 4 of IDO gene in 5 of the 331 cataract cases may contribute to the development of ARC (Mamata et al, 2011).

3.5.1.3 Role of IDO as an antioxidant

IDO is a very efficient superoxide radical scavenger (Figure-3.4) and thus acts as an antioxidant enzyme by utilizing free oxygen radicals to cleave the pyrrole ring of the
tryptophan (Hirata and Hayaishi, 1975; Malina and Martin, 1996). IDO has been reported to be a better free radical scavenger than superoxide dismutase (SOD; Ohnishi et al, 1977). It is an enzyme containing ferrous ion in the active center. Oxidation of ferrous ions to ferric ions inhibits IDO activity (Hirata et al, 1977). Ascorbic acid is necessary to maintain the reduced form of the ferrous ion, and the reduction of ascorbic acid occurs due to the presence of reduced glutathione. Oxidized glutathione is reduced in the eye by NADPH-dependent glutathione reductase (Pirie, 1965). NAD is produced in the tryptophan degradation pathway beginning with IDO. Thus IDO activity seems to have a central role in the antioxidative process of the eye. Decrease of IDO activity should lead to oxidative stress.

The formation of one molecule of kynurenine or 3-OHkynurenine is associated with the covalent binding of two superoxide radicals. It has been reported that IDO is a much more efficient superoxide radical scavenger than SOD (Ohnishi et al, 1977). Consequently, when IDO is present the superoxide radicals will be scavenged by IDO and not by SOD. This process is very advantageous for the eye, as IDO activity leads to the production of UV filters in the eye in contrast to SOD which transforms superoxide radicals into harmful hydrogen peroxide (Van Heyningen, 1973).

![Figure-3-4](image)

**Figure-3-4:** IDO uses two superoxide radicals to form N-formyl kynurenine from tryptophan

### 3.6 IFN-GAMMA (IFN-γ) AND ITS GENETIC VARIANTS

IDO production is induced by several inflammatory mediators including interferons (IFN), the most potent of which is interferon-gamma (IFN-γ; OMIM: 147570). IFN-γ pathway is required for the normal upregulation of IDO expression during infection (Mellor and Munn, 2004). Interferon-gamma (IFN-γ), or type II interferon, is a cytokine responsible for innate and adaptive immunity against viral and intracellular bacterial infections and also
for tumor control. The gene sequence of IFN-γ is not related to the sequence of other interferons (α, β), contains 4 exons, a repetitive DNA element, and a low order of polymorphism. It is located on chromosome 12 and codes for a protein of 146 amino acids (Gray and Goeddel, 1982; Naylor et al. 1983).

Production of IFN-γ is genetically controlled, and has two well-known polymorphisms in the IFN-γ gene. It has been reported that the 12 CA repeat microsatellite allele in the noncoding region of the first intron is associated with a higher level of in vitro cytokine production (Pravica et al, 1999). The same group has also reported complete linkage disequilibrium between the 12 CA repeat allele and the presence of the T allele at the +874 position (+874 A>T) from the translation start site. This polymorphism lies within a binding site for the transcription factor NF-kB, and electrophoretic mobility shift assays showed specific binding of NF-kB to the allelic sequence containing the +874T allele (Pravica et al, 2000). As this transcription factor induces IFN-γ expression, +874T and A alleles probably correlate with high and low IFN-γ expression, respectively (Rossouw et al, 2003).

In inflammatory conditions such as uveitis, increases in IFN-γ levels are seen in the aqueous humor (Ongkosuwito et al, 1998). Thus, in chronic inflammation, lens IDO may be induced. In fact, chronic uveitis is a risk factor for cataract development in humans (Durrani et al, 2004; Hooper et al, 1990; Skarin et al, 2009; Azar and Martin, 2004). IFN-γ induces apoptosis in lens epithelial cells (Awasthi and Wagner, 2004; Egwuagu et al, 2006). In addition, transgenic mice over expressing IFN-γ in the lens have been reported to develop morphological abnormalities and cataracts (Egwuagu et al, 1994). A single nucleotide polymorphism in the IFN-γ receptor (IFNGR1), which increases the transcription of IFNGR1, also promotes cataract formation in humans (Matsuda et al, 2007). Mailankot et al, (2009) demonstrated that IFN-γ strongly induces IDO expression through the JAK–STAT1 pathway, which increases the levels of both intracellular and extracellular kynurenines, and that the production of 3OHKyn induces cataract formation in human lens.
The interaction of IFN-\(\gamma\) with its cell surface receptor leads to the activation of JAK1 and JAK2 (phosphorylation), which in turn phosphorylate and activate the transcription factor STAT1. Phosphorylated STAT1 forms a homodimer and translocates to the nucleus where it binds to GAS (gamma interferon activation site) to initiate the transcription of target genes (Decker et al, 1997; Kanno et al, 2005). Thus IFN-\(\gamma\) induces IDO expression via a GAS element. Kynurenine metabolites formed by the action of IDO are involved in modification of lens proteins, and they are easily oxidized and form free radicals like \(H_2O_2\) leading to oxidative stress in the lens, resulting in cataract formation (Mailankot and Nagaraj, 2010; Figure-3.5).

Figure-3.5: A conceptual view of the mechanism of IFN-\(\gamma\) induced cataract

IDO-inducing IFN-\(\gamma\) is a multifunctional cytokine that is essential in the development of T-helper 1 cells, defence against viruses and intracellular pathogens and in the induction of
immunemediated inflammatory responses. The CA repeat microsatellite polymorphism in the first intron of *IFN-γ* gene and the linked +874 A>T SNP have been associated with several autoimmune and chronic inflammatory conditions (Bream et al, 2000), e.g. higher production of IFN-γ *in vitro* (Pravica et al, 2000), allograft fibrosis in recipients of lung transplants (Award et al, 1999), the incidence and severity of acute kidney rejection (Asderakis et al, 2001), aplastic anaemia (Dufour et al, 2004), the development of tuberculosis (Lio et al, 2002a; Lopez-Maderuelo et al, 2003; Rossouw et al, 2003), symptomatic parvovirus infection (Kerr et al, 2003) and recurrent pregnancy loss (Daher et al, 2003). It has also been suggested that the presence of *IFN-γ* +874 A allele is advantageous for the longevity (Lio et al, 2002b). A meta-analysis of 11 studies using random effects models by Pacheco et al, (2008) explained that the *IFN-γ* +874 T allele has a significant protective effect against tuberculosis.

From few decades wide research is being carried out to explore the genes associated with ARCs and the search is still going on. Understanding the etiology of ARCs will help in developing the drugs to delay or prevent the occurrence of cataracts among elderly and can further reduce the burden to the individual families and also the government.