·Review·

The role of heredity in pterygium development

Peter Anguria[1], James Kitinya[2], Sam Ntuli[3], Trevor Carmichael[1]

[1]Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand Johannesburg, 7 York Road, Park Town 2193, South Africa
[2]Department of Anatomic Pathology, University of Limpopo Polokwane Campus, Private Bag X9316 Polokwane 0700, South Africa
[3]Department of Public Health Medicine, University of Limpopo Polokwane Campus, Private Bag X9316 Polokwane 0700, South Africa
Correspondence to: Peter Anguria. Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand Johannesburg, Polokwane 55226, South Africa. irarak58@gmail.com
Received: 2013-12-09 Accepted: 2014-03-01

Abstract

• Several risk factors, which include heredity, ultra-violet (UV) light and chronic inflammation, contribute to pterygium development. However, there is no report integrating these factors in the pathogenesis of pterygium. The aim of this review is to describe the connection between heredity, UV, and inflammation in pterygium development. Existing reports indicate that sunlight exposure is the main factor in pterygium occurrence by inducing growth factor production or chronic inflammation or DNA damage. Heredity may be a factor. Our studies on factors in pterygium occurrence and recurrence identify that heredity is crucial for pterygium to develop, and that sunlight is only a trigger, and that chronic inflammation promotes pterygium enlargement. We propose that genetic factors may interfere with the control of fibrovascular proliferation while UV light or (sunlight) most likely only triggers pterygium development by inducing growth factors which promote vibrant fibrovascular proliferation in predisposed individuals. It also just triggers inflammation and collagenolysis, which may be promoters of the enlargement of the fibrovascular mass. Pterygium probably occurs in the presence of exuberant collagen production and profuse neovascularisation.

• **KEYWORDS:** pterygium; fibrovascular proliferation; heredity; sunlight; inflammation; growth factors
DOI:10.3980/j.issn.2222-3959.2014.03.31

Anguria P, Kitinya J, Ntuli S, Carmichael T. The role of heredity in pterygium development. *Int J Ophthalmol* 2014;7(3):563-573

INTRODUCTION

Pterygium, which is a wing-shaped fibrovascular growth of the conjunctiva across the limbus onto the cornea[1,2], is divided into the head that invades the anterior cornea, the neck that includes the superficial limbus, and the body that overlies the sclera [3]. The cap, which is the first sign of pterygium [4,5] is the halo infront of the pterygium head. It is deep to the epithelium [6]. Pterygium can impair vision and it can be cosmetically unacceptable [7]. Recurrent pterygium after surgery may be more aggressive than the primary growth [8]. Despite the problems related to pterygium [7,8] prevention of its occurrence and its recurrence after surgery have not been successful because the pathogenesis of pterygium is not clear.

This review describes the ocular surface anatomy relevant to pterygium, and it discusses the literature related to the current theories on pterygium pathogenesis, and it outlines the modes of genetic inheritance. It summarizes our recent studies on factors in pterygium occurrence and post-surgical recurrence and it concludes by proposing a model of pterygium development.

Ocular Surface Anatomy Relevant to Pterygium The bulbar conjunctiva is loosely attached to the underlying Tenon's fascia before the surgical limbus, thereafter the conjunctiva and Tenon's fascia, fused, adhere to the episclera [9]. The superficial episcleral plexus is found in the surgical limbus [10]. From the limbus, centrally, the tissues are compact [11]. Fibroblasts and blood vessels as well as inflammatory cells are located in the conjunctival stroma, which is at the same plane as the limbal stroma and Bowman's membrane of the cornea[3,6,12,13,16].

Current Theories on Pterygium Pathogenesis Inflammation [14] and fibrovascular proliferation [15-18] may be factors in pterygium occurrence. DNA damage [19-21] has been reported to initiate pterygium development. Hereditary predisposition [8,22-24] may be the underlying factor for pterygium occurrence. Ultra violet (UV) light has been shown to induce proinflammatory cytokines, chronic inflammatory cells, and growth factors [16,17,25,26]. It also may damage DNA in predisposed individuals [21,23,24,27]. However, integration of factors associated with pterygium occurrence has not been reported.

Sunlight Exposure All individuals may be exposed to UV light, which generates reactive oxygen species (ROS) from the ocular surface [28]. Excessive exposure is widely believed

to be the reason for pterygium to occur [29,30]. However, some studies have shown that pterygium may be infrequent in individuals highly exposed or, a low exposure may be frequent in pterygium patients [5.31-34]. Excessive sunlight exposure perhaps is also related to pterygium recurrence after surgery[35].

Excessive exposure to sunlight has been correlated with collagen degeneration although collagen degeneration has been discredited as a mechanism of pterygium pathogenesis[4]. Collagen degeneration may be present in primary pterygia but, some primary pterygia may not show collagen degeneration histologically [36,37]. This degeneration is not manifested in recurrent pterygia, suggesting short durations of exposure to UV light[4,38]. It seems that the level of sunlight exposure may not be important for pterygium to occur or to recur.

Inflammation It has been shown that UV light induces pro-inflammatory cytokines in pterygia, however, the degree of induction varied in pterygia exposed to the same level of UV light, suggesting that the level of exposure to sunlight may not be important for inflammation to be severe [25,39]. It might be that the severity of inflammation is genetically controlled. Some individuals may be deficient of T-lymphokine activated killer cell-originated protein kinase (TOPK) and its deficiency appears to increase sunlight induced inflammation[40].

Reactive oxygen species phosphorylate cell membrane lipids, which manifests as increased products of lipid metabolism [41,42]. These lipid products include prostaglandin E-2 (PGE-2), which has been reported in pterygia [18]. Oxidized phospholipids stimulate production of cyclooxygenase-2 (COX-2) enzyme and interleukin-8 (IL-8), which are pro-inflammatory [43,44]. Inflammatory cells are present in all pterygium samples, which indicates inflammation[5,14,15,45,46].

Inflammation has been proposed to be the final step in the formation of pterygia, however, that inflammation was thought to be a type of hypersensitivity because the leukocytes were mainly located in the epithelium [5]. It is not clear whether hypersensitivity is crucial for pterygium to be formed. Although inflammatory cells are present in pterygia older studies [5,14] did not indicate whether the inflammatory cell infiltration was related to the severity of inflammation or to the size of pterygium or to the level of exposure to sunlight. It is not clear how inflammation may be the final step in pterygium formation.

Inflammation activates transforming growth factor-beta (TGF –β) thereby stimulating the fibroblasts to synthesize collagen [47-50]. Transforming growth factor-beta also inhibits MMPs[51,52]. Collagen is deposited randomly (fibrosis), which causes tissues to become opaque[53]. Inhibition of MMPs tends to decrease collagenolysis, however, collagen degeneration characterizes pterygium[12,15,36]. Collagen degeneration is a sign of prolonged collagenolysis, which may be caused by ROS[41,54]. Although one previous study failed to detect MMPs in pterygium fibroblasts several studies have reported MMP expression, which seems to suggest that collagen degeneration in pterygia is due to MMPs [3,20,55,56]. As all pterygia have inflammatory cell infiltrations it may be that MMPs are not expressed in pterygium fibroblasts despite limbal stem cell damage, but, this needs to be corroborated. Inflammation also induces angiogenic growth factors[18,55,57,58]. Moreover, TGF –β up-regulates vascular endothelial growth factor (VEGF), which in a frame-work of fibronectin stimulates neovascularisation as collagen synthesis proceeds[53,59,60].

Fibrovascular proliferation Ultraviolet light, even of a short duration may induce growth factors such as basic fibroblast growth factor (bFGF), TGF-β, platelet derived growth factor (PDGF), VEGF, connective tissue growth factor (CTGF) and heparin binding epidermal growth factor-like epidermal growth factor (HB-EGF)[15-17]. Oxidative stress induces those growth factors in the fibroblasts, endothelial cells and inflammatory cells in the stroma. It also induces those growth factors in the conjunctival epithelium [15-18,61]. Growth factors promote vibrant proliferation of fibroblasts in pterygia but in controls, the same level of growth factor proteins causes sluggish mitosis [62,63]. This seems to suggest that vibrant fibroblast mitosis is unlikely to be due to overexpression of these proteins in pterygia. It may be due to an abnormal phenotype of pterygium fibroblasts that causes fibroblasts to respond energetically to growth factors [4,62]. Fibroblast abnormality might arise from sunlight damage, which causes these to over-express MMPs [20,56]. However, acquired fibroblast damage fails to explain why pterygium occurs in patients whose fibroblasts do not express MMPs[55].

Heparin binding epidermal growth factor-like epidermal growth factor, a fibrogenic growth factor may be available for at least 48h in pterygia after exposure to UV light has stopped[17,63]. Fibrogenic growth factors such as PDGF are not over-expressed in controls [15,62]. The expression of bFGF in some controls may be the same as in cases, which suggests that over-expression of angiogenic growth factors is not the reason for vibrant fibroblast mitosis or for pterygium to occur [15,62]. Rather, the up-regulation of fibrogenic growth factors is most likely to be the reason for fibroblast proliferation and for pterygium to occur [15,62,63]. Nevertheless, angiogenic growth factors such as bFGF and VEGF are up-regulated in pterygia mainly *via* ROS [15,16,18,64]. Reactive oxygen species in addition directly stimulate capillary growth[65]. Bevacizumab, which is anti-VEGF fails to abolish pterygium recurrence after surgery[66]. Since pterygia occur in the presence of fibrogenic growth factors failure of bevacizumab to abolish post-surgical pterygium recurrence

may be due to its lack of inhibition of fibrogenic growth factors [15]. This seems to suggest that pterygium occurs because fibrogenic growth factors are not inhibited, however, there is no literature that lack of inhibition of fibrogenic growth factors occurs in pterygium.

A fibrogenic growth factor binds to its receptor at the fibroblast cell membrane to form a complex which is internalised to form specific endocytic vesicles [67]. Receptor-regulated sma (small) and mad (mothers against decapentaplegic) proteins abbreviated smad, *via* a series of steps including smad1 and 5 activate the receptor thereby translocating the growth factor to the nucleus [67-70]. A signal for the transcription of genes for fibroblast mitosis is initiated [71]. After adequate signals a different type of specific endocytic vesicles is formed [67]. Inhibitory smad proteins (smad7) in these vesicles terminate the signal for genetic transcription [72,73]. Inhibitory smad proteins stimulate smad ubiquitin regulatory factor-1 (smurf-1), which may compete with smurf-2 to deactivate growth factor receptors [74,75]. The action of smad7 is independent of smurf proteins [72]. Inhibitory smads and smurf proteins are genetically determined [76,77]. Growth factors generate ROS at the cell membrane and TGF-β inhibits antioxidant enzymes[78].

DNA damage Ultraviolet light may damage DNA [20,21,27] irrespective of the dose of radiation, race, or age of the individual [21,27,79]. DNA damage might cause localised limbal stem cell deficiency probably due to migration of both the reserve stem cells and transient amplifying cells[56]. Damaged cells perhaps migrate in all directions [80] assisted by MMPs, which may degrade collagen and fragment Bowman's membrane[20,56]. Pterygium occurs maybe as a result of corneal conjunctivalisation [56]. Migration might be promoted by inflammation whereby epithelial mesenchymal transition occurs to the cells which migrate to the stroma in individuals predisposed to a deficiency of discs large factor-5 (Dlg-5)[81-83]. This may cause a fibrotic mass [80]. The wing-like shape of pterygium may be calculated as due to more epithelial cell loss centrally than at the limbus[84]. However, this theory fails to explain pterygium shape peripheral to the limbus. The theory of DNA damage fails to explain why pterygium develops in those having no evidence of DNA damage [27,85]. Moreover, some pterygium patients do not have predisposition to DNA damage[23,24].

Hereditary predisposition Hereditary predisposition to pterygium development has been acknowledged, but, it has been underemphasized [22,86-89]. The mode of inheritance has been reported to be autosomal dominant based on a study of one [87,88] or two families [86] however, a large sample is necessary to increase credibility [90] since alleles may or may not be transmitted. Autosomal recessive mode might also be possible however, because the original report in French is difficult to find it is difficult to ascertain whether pterygium patients were compared with unaffected individuals or, how many pedigrees or patients were considered[87]. Multifactorial mode is likely but, it was determined using self-reported family histories which were not tested for independence of association with pterygium occurrence [89]. Knowledge of the mode of inheritance facilitates determination of the possible mechanism of pterygium development[91,92].

Modes of Inheritance These may be Mendelian or non Mendelian. Mendelian inheritance may be autosomal dominant, autosomal recessive or sex linked. Non Mendelian inheritance may be multifactorial or mitochondrial. Mendelian and multifactorial modes of inheritance involve genes that are located in the nucleus.

The phenotype in autosomal dominant inheritance is determined by a single defective allele, which is dominant[91,93]. According to Mendelian principles, whether one or both parents are affected the offspring have a 50% chance of being affected as individuals who are homozygous defective are so severely affected that they perish before birth or early in life [91]. Incomplete penetrance sometimes occurs thereby causing a skipped generation[90].

Inheritance in autosomal recessive mode occurs due to two ineffective alleles, which are recessive [40,94]. It may be homozygous recessive [40] or double heterozygous whereby two heterozygous recessive genes that code for the same phenotype are located in different loci [94]. The risk of the offspring becoming affected is 100% if both parents are homozygous recessive while it is 50% if one parent is homozygous and the partner is heterozygous recessive and it is 25% if both parents are heterozygous[40]. There is no risk of having an affected offspring if one parent has normal paired alleles [40]. According to Mendelian principles [91,93] if both parents are double heterozygotes the unaffected to the affected ratio of the offspring is 5:11.

Mendelian principles require that sex linked conditions are always recessive [95] because males lack one pair of alleles in the Y chromosome, which causes inheritance of a defective dominant allele in the Y chromosome to be lethal [91]. Males may become affected when they inherit a recessive allele from an affected or heterozygous mother and the affected fathers may transmit their recessive gene only to their daughters who may become affected only if the mother is homozygous or heterozygous recessive[95].

In multifactorial inheritance genes may interact with environment [96], or, two or more genes coding for different proteins and in different loci may modify one another's effect [97,98] to produce a phenotype. Genes are not defective[91,93] however, genes have to be activated before transcription to messenger RNA, after which protein synthesis may occur[67,71,99]. One active allele is sufficient for the gene to be effective[97], which allows a protein to be synthesized [40]. The risk of transmission to subsequent offspring, which can be predicted

using Mendelian principles [91,93] depends on the genotypes of the mating partners. Affected individuals tend to cluster in families[100].

The polygenic model specifies that the more inactive (determinant) alleles interacting the severer the phenotype[92] and the higher the risk of transmission to subsequent generations [100]. The threshold model requires that the contributions by genes and environment reach a threshold that leads to phenotype whereby sometimes, genes are the main contributor and other times, the environment is the main contributor to the threshold[96].

Recent Studies on Factors in Pterygium Occurrence

These were undertaken in Mankweng Hospital, a tertiary referral centre in Limpopo Province of South Africa, which is mainly rural and it is bisected by the tropic of Capricorn (23.5^0 south of the Equator). The Province is sunny and dry[101] and it is inhabited mainly by the Pedi, Tsonga, Venda, and Tswana. These groups of Bantu people have been reported to practice first degree cousin marriage [102], in which reproduction may promote the occurrence of hereditary conditions [103]. The population of Limpopo in 2009 when these studies begun was estimated at 5227200, of which 2436400 (46.6%) was 20-64y old, and 87% of the population was rural [104]. The methods and results of these studies have been reported[105-108].

The pterygium cases and control patients whose conjunctivas were investigated by immunohistochemistry were selected from the Eye Clinic, and the unaffected individuals matched with pterygium cases were selected from the refraction Clinic. Two hundred and thirty cases and 150 controls matched for age and sex with the first 150 cases, as well as seven unmatched controls whose eyes had been irreparably injured were interviewed and their eyes examined. Data from 150 case-control pairs were analyzed as pre-calculated to give a 20% difference in family history at a power of 80%, assuming a base rate of 10% in controls and P-value of 5%. Of the 300 participants whose data were analyzed the age range was 22-65y, modal range was 40-49y; the females were 3.5 times more frequent than males [106]. Pterygium surgery was done on 200 cases, which had indications for surgery. The indications included corneal astigmatism, pterygium obstructing or threatening to obstruct the visual axis, frequent pterygium inflammation, and disfigurement by pterygium [108]. Interviews were conducted, a full eye examination done, and the pterygia in patients having indications for surgery were excised. The 59 pterygium specimens and 7 control nasal conjunctivas were investigated by immunohistochemistry [107]. The control specimens were obtained from males who were aged 23-51y old. Follow-up family visits were conducted on selected cases and controls[106]. Because alleles may or may not be transmitted a large sample that is necessary for the calculation of a credible mode of inheritance was obtained by combining the relatives of cases and controls into 2 separate families [90,108]. There were 382 combined relatives of pterygium probands and 394, of unaffected probands; their age range was 10-86y, and 275 of 382 relatives of cases (71.9%) were ≥40y old compared with 284 of 394 relatives of controls (72%)[106]. The ratio between the unaffected and pterygium-affected relatives in the combined families of cases was calculated, which was used to determine the likely mode of inheritance based on Mendelian principles[40,91,93,94]. As that ratio was not Mendelian the equivalent ratio was computed and estimated because decimals of individuals do not exist. The estimated ratio predicted the likely genotypes of a mating couple whose offspring are expected to be in the proportion of the estimated ratio. Mendelian principles[93,94] were applied to that mating to depict genotypes of the offspring. One pedigree was used to demonstrate the likely mode of inheritance[87,88].

Heredity is fundamental for pterygium to occur Family history, which implicates heredity [22,89] was associated with pterygium occurrence [105,106] independent of the use of traditional eye medication and of the unstable tear film[106]. Having diagnosed pterygium-affected relatives, which implicates heredity [8,86-88] was associated with pterygium occurrence hence confirming familial occurrence of pterygium [106]. Pterygium patients with the unaffected individuals had similar exposure to sunlight, which suggests that familial occurrence was due to heredity rather than environment[106].

Traditional eye medication was the only environmental factor associated with pterygium however, the controls also had used this medication in a similar way, obtained from the same practitioners, in the same period, which suggests that traditional eye medicine was not a direct cause of pterygium [106]. Individuals who use African traditional medication are likely to follow certain traditions [109] such as first degree cousin marriage [102]. Reproduction between cousins increases the risk of occurrence of hereditary conditions present in an extended family[103]. The following of the tradition of first degree cousin marriage [102] is the reason for the association of traditional eye treatment with pterygium and so, the use of traditional medication may implicate heredity in pterygium occurrence[106].

The ratio between the unaffected and pterygium-affected individuals in the combined family of pterygium probands was 9:7[106], which suggests digenic inheritance (the simplest form of multifactorial inheritance [97]). Table 1 shows the depicted genotypes of affected and unaffected offspring. A and B indicate active alleles whereas a and b indicate inactive alleles. Bold font indicates predicted genotypes of a pterygium patient. The appearance of only two letters represents predicted alleles in the gametes of the parents. The genotypes of the two parents are AaBb and AaBb. Each letter

Table 1 Depicted genotypes of affected and unaffected offspring

Genotypes	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AAbB	AAbb	AabB	Aabb
aB	aABB	aABb	aaBB	aaBb
ab	aAbB	aAbb	aabB	aabb

whether capital or small represents one type of gene. This table suggests that pterygium cases have at least one type of gene having both alleles inactive (determinant gene). The signals for fibroblast mitosis, generated by fibrogenic growth factors are regulated by inhibitory smad proteins [72,73] and smurf proteins [74,75]. These proteins are genetically determined[76,77] and so it is possible that pterygium fibroblasts undergo vibrant mitosis [62] because of genetic lack of inhibitory smads or smurf proteins.

Figure 1 depicts a pedigree of a pterygium proband. Oval empty drawings illustrate unaffected females and the shaded oval drawings illustrate affected females. Rectangular empty drawings illustrate unaffected males and the shaded rectangular drawing indicates an affected male. The arrow points at the proband. As 2 generations were affected autosomal dominant with incomplete penetrance in the first generation is likely [87]. As only the second and third generations had pterygium patients it is possible that this was due to autosomal recessive mode of inheritance whereby the first generation and the spouse of the proband were carriers[40]. It is also possible that sex linked inheritance was the mode of inheritance since the proband, a male, might have inherited a recessive gene from his carrier mother and he transmitted this gene to his daughter whose mother was a carrier [95]. Because this pedigree does not show a consistent Mendelian pattern Mendelian inheritance is unlikely in pterygium occurrence. Rather, the most likely mode of inheritance is multifactorial because it was determined from a large sample [90,110]. The proband had a short recurrence time (Less than 3mo after surgery [108]) and his daughter was 16 years old. These observations indicate that multifactorial mode of inheritance follows the polygenic model [92]. A short recurrence time and an early onset are signs of severe pterygium [8]. The skipping of the first generation and only one individual in the third generation being affected might suggest the presence of few genes[92]. However, the small size of a pedigree[90] most likely caused it to appear that all siblings in the second generation and only one in the third generation were pterygium patients because alleles may or may not be transmitted. All pterygium patients are predisposed and they may have unaffected relatives [106]. The proportion of the pterygium-affected relatives seems to depend on the proportion of determinant genes (polygenic model [100]). These findings suggest that predisposition to pterygium is unlikely to be the deficiency of Dlg-5[82] because more than one gene seems to be involved in

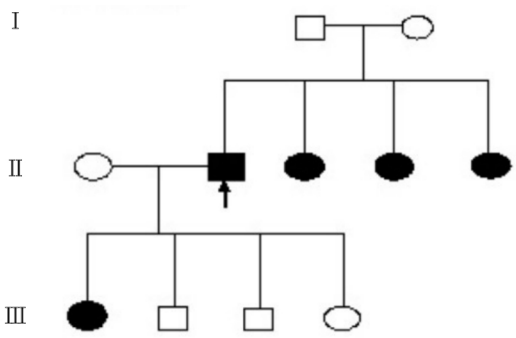

Figure 1 Pterygium pedigree.

pterygium development whereas Dlg-5 deficiency involves only one gene.

Age and small pterygium extent seem to be associated with pterygium recurrence after surgery perhaps due to the patients' selection criteria [108]. Also, large pterygium extent seems to be associated with recurrence perhaps due to inadequate treatment[111]. It is possible that pterygium size has no relationship with post-surgical recurrence. Pterygium fleshiness appears to be associated with pterygium recurrence after excision probably because excision was not followed by adjunctive treatment [112] otherwise, fleshiness has no relationship with post-surgical recurrence[108]. Since it is likely that pterygium occurrence is due to dormant genes post-surgical recurrence (pterygium progression [108]) can be explained by continued genetic inactivity [113]. The patient's age, pterygium size, and its fleshiness most likely depend on pterygium progression to be associated with post-surgical recurrence. However, genes controlling pterygium occurrence have yet to be established.

Sunlight exposure is only a trigger for pterygium to occur All the participants had been exposed to sunlight and excessive exposure had no relationship with pterygium occurrence [106], which is similar to recent reports [32,34]. Since sunlight damage may induce chronic inflammatory cell infiltration in the conjunctival stroma [26] the presence of chronic inflammatory cells in all pterygium samples, and the inhibition of MMPs in all pterygia and controls[107] support the finding that all pterygium patients as well as controls had been exposed to sunlight [106]. As the inflammatory cell infiltrate varied in pterygia that had collagen degeneration (sign of prolonged UV radiation[54]) this shows that the degree of infiltration was not related to the level of exposure to the sun. Sunlight irrespective of its degree of exposure may be only a trigger for pterygium occurrence in those predisposed to pterygium formation [106]. This may occur by inducing oxidative stress at the ocular surface[28]. Also, sunlight may be only a trigger for pterygium recurrence after excision[108].

Chronic inflammation is only a promoter of pterygium enlargement Inflammatory cell infiltration in pterygium samples is a sign of inflammation [46]. Although the inflammatory cell count was correlated with pterygium size,

which suggests that inflammation may contribute to pterygium enlargement inflammation is unlikely to be a determinant of enlargement because pterygia irrespective of their size tended to have a low count [107]. The degree of the inflammatory cell infiltration may indicate the severity of inflammation rather than pterygium size [107]. Inflammation irrespective of its severity may be just a promoter of pterygium enlargement. Because pterygia tended to be mildly inflamed this suggests that epithelial mesenchymal transition is unlikely to be the mechanism for pterygium to occur as epithelial mesenchymal transition requires that inflammation be severe for it to occur[81].

Inhibition of MMPs in the fibroblasts and stroma of all pterygium samples and controls is most likely to be due to inflammation [107]. Inflammation activates TGFβ [47,48], which stimulates the fibroblasts to synthesize collagen [49,50]. In addition, TGFβ inhibits MMPs [51,52]. The synthesized collagen is deposited randomly, which causes previously transparent tissues to become opaque [53]. Collagen is the reason that pterygia are fleshy and it is the reason for the cap. Inhibition of MMPs minimizes collagenolysis [12] and it suggests that the collagen degeneration which was present in most of the pterygia and controls was not due to MMPs. This contradicts previous studies [20,56] perhaps because the participants in the present study had not used spectacles[107]. Transforming growth factor-beta up-regulates VEGF [59], which stimulates neovascularisation[60] hence, inflammation in addition promotes pterygium neovascularisation.

Limbal stem cell damage was not associated with pterygium[107]. This suggests that DNA damage[19,29] is unlikely to be a factor in pterygium development. The predisposition to DNA damage [23,24] is unlikely to be the predisposition to pterygium occurrence.

Proposed Model of Pterygium Development Figure 2 shows the proposed model of pterygium development, which is a flow chart showing that pterygium development is influenced by heredity in conjunction with sunlight exposure. Sunlight exposure, *via* oxidative stress induces growth factor production, angiogenesis, chronic inflammation, and collagenolysis. Bold black font indicates determinant factors, bold black font in italics indicates promoting factors, and bold black arrows show the determinant pathway. Normal black font in italics indicates a trigger and bold yellow arrows show the triggering pathway. Heredity[105,106] influences growth factors to cause vibrant fibroblast mitosis[63]. Sunlight induced oxidative stress triggers growth factors [28,61] and it triggers angiogenesis by directly stimulating capillaries to grow [65]. Oxidative stress triggers inflammation[18,41] in sunlight exposed conjunctivas [106], and it causes collagenolysis also [41,107]. Vibrant mitosis produces many fibroblasts [62], which are stimulated by inflammation [47] to collectively synthesize collagen [49] exuberantly. Excessive collagen is deposited in

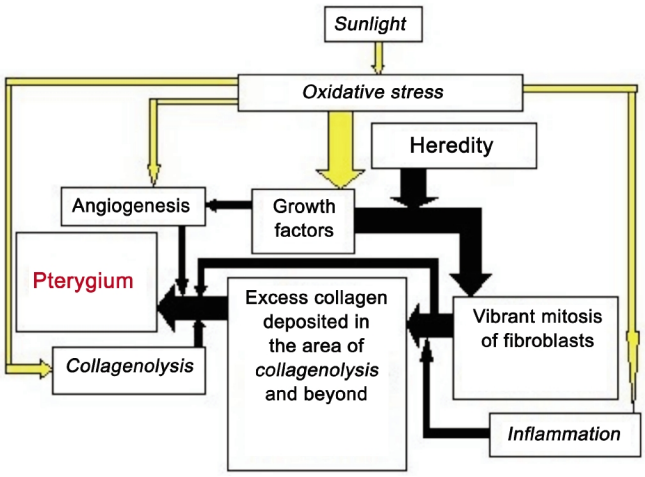

Figure 2 Proposed model of pterygium development.

the damaged area and beyond the margins of the damaged matrix to develop the pterygium cap. The excessive collagen is invaded by fibroblasts, and new blood vessels stimulated by growth factors and ROS to develop pterygium. Cap collagenolysis [41] facilitates the fibroblasts and new blood vessels to invade the stroma. Bowman's membrane probably gets fragmented due to the location of the cap in it [4,6]. Pterygium onset is at the surgical limbus probably because of the numerous endothelial cells [10], which generate abundant ROS after sunlight exposure[28].

Figure 3 depicts sub-model 1, which is a flow chart showing that heredity sustains pterygium development *via* oxidative stress generated by proliferating fibroblasts and endothelial cells. Bold font indicates determinant factors, bold font in italics shows promoting factors, and orange arrows indicate pathways involving ROS, and a plain arrow indicates a subsidiary pathway for growth factor production. After sunlight has triggered pterygium onset the proliferating fibroblasts generate ROS, through which production of fibrogenic and angiogenic growth factors is sustained [61]. Through ROS the proliferating fibroblasts sustain inflammation [18,39,41,42]. Matrix damage [41,107] and angiogenesis (by directly stimulating capillary growth [65]) are also sustained. The replicating endothelial cells generate oxidative stress thereby stimulating endothelial cells and fibroblasts to produce fibrogenic and angiogenic growth factors [61]. Also, capillary growth is directly stimulated [65]. Angiogenic growth factors are also induced by inflammation [57,58]. It seems that sustenance of pterygium development can be terminated if hereditary predisposition is halted, perhaps by activation of previously dormant genes[99,108].

Because the conjunctiva and Tenon's fascia are loosely attached before the surgical limbus, thereafter the two, fused, are firmly attached to the episclera [9], and the limbus and cornea are compact[11], it is most likely that there is increasing centripetal resistance to the expanding fibrovascular mass, which causes it to be shaped like a wing. The role of

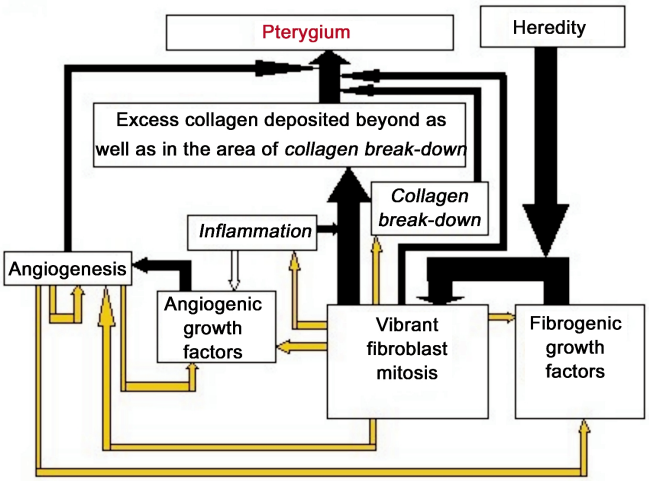

Figure 3 Sub-model 1.

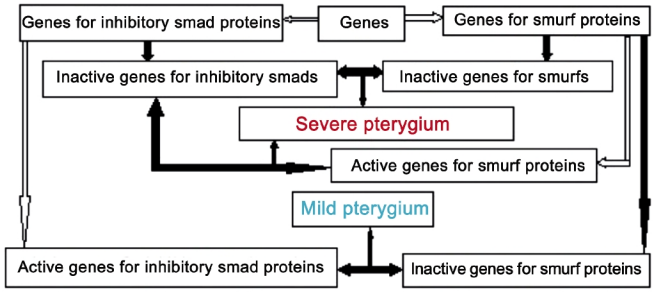

Figure 4 sub-model 2.

pterygium inflammation is to promote pterygium enlargement [107] and fleshiness by stimulating collagen synthesis[49,50] and its conservation[107].

Figure 4 depicts sub-model 2, which is a flow chart showing that heredity determines pterygium severity. Bold font indicates determinant factors. Double arrows indicate interaction. Normal arrows show normal outcome and bold single arrows show an abnormal outcome. The genes involved in pterygium occurrence may be those for inhibitory smad proteins [76] or for smurf proteins [77]. Because the inhibition of signals for mitosis, generated by growth factors is independent of receptor inhibition [72] it may be that interaction between inactive genes for inhibitory smads and inactive genes for smurfs or, between inactive genes for inhibitory smads and active genes for smurf proteins causes severe pterygium to occur. Because degradation of growth factor type 1 receptors depends on smad 7 [74] it may be that a mild pterygium occurs if active genes for smad 7 proteins interact with inactive genes for smurf proteins.

Since numerous fibrogenic growth factors are present in pterygium[15-17] it is possible that pterygium size is determined by the proportion of growth factors lacking inhibitory smads or smurf proteins (polygenic model[100]). Severity in large pterygia may be determined by the proportion of growth factors lacking inhibitory smad proteins (polygenic model[92]).

CONCLUSION

Hereditary predisposition is fundamental for the onset and sustenance of pterygium. Pterygium size and severity are most likely to be determined by hereditary factors. Predisposition to pterygium occurrence most likely follows multifactorial mode of inheritance, which is of the polygenic model. It is possible that two types of genes, one for inhibitory smad proteins and the second for smurf proteins are inactive thereby predisposing to pterygium occurrence. It seems that fibrogenic growth factors are crucial for pterygium to develop, and it seems that pterygium angiogenesis follows fibroblast proliferation, collagen synthesis, and collagenolysis.

Sunlight is only a trigger for pterygium to occur, perhaps *via* reactive oxygen species. It appears that inflammation and collagen damage, which are most likely to be due to oxidative stress only promote pterygium enlargement.

Recommendations Genetic counselling to advise family members regarding risks for pterygium development seems far off at present although it might play a role with further investigation in high risk communities. Studies to determine the molecular nature of predisposition are recommended.

Control of sunlight exposure by use of spectacles/sunglasses in predisposed individuals is encouraged. Control of collagen synthesis seems to be an attractive option to minimize enlargement of the fibrovascular mass in those predisposed.

ACKNOWLEDGEMENTS

The authors thank the study participants for accepting to participate. We also thank the Provincial Department of Health for granting permission to conduct Research in Limpopo Province.

Conflicts of Interest: Anguria P, None; **Kitinya J,** None; **Ntuli S,** None; **Carmichael T,** None.

REFERENCES

1 Jaros PA, DeLuise VP. Pingueculae and pterygia. *Surv Ophthalmol*1988; 33(1): 41-49

2 Bai H, Teng Y, Wong L, Jhanji V, Pang CP, Yam GH. Proliferative and migratory aptitude in pterygium. *Histochem Cell Biol* 2010; 134 (5): 527-535

3 Seet LF, Tong L, Su R, Wong TT. Involvement of SPARC and MMP-3 in the pathogenesis of human pterygium. *Invest Ophthalmol Vis Sci*2012; 53 (2): 587-595

4 Cameron ME. Histology of pterygium: an electron microscopic study. *Br J Ophthalmol* 1983; 67(9): 604-8

5 Hill JC, Maske R. Pathogenesis of pterygium. *Eye (Lond)*1989; 3(P62): 218-226

6 Dushku N, Hatcher SL, Albert DM, Reid TW. P53 expression and relation to human papillomavirus infection in pingueculae, pterygia, and limbal tumours. *Arch Ophthalmol*1999; 117(12): 1593-1599

7 Eze BI. Audit of Ophthalmic Surgical interventions in a resource-deficient tertiary eye care facility in Sub-Saharan Africa. *J Health Care Poor Underserved*2013; 24(1): 197-205

8 Islam SI, Wagoner MD. Pterygium in young members of one family. *Cornea*2001; 20(7): 708-710

9 Grehn F, Mauthes S, Pfeiffer N. Limbus-based versus Fornix-based

conjunctival flap in filtering surgery. *Int Ophthalmol* 1989; 13 (1-2): 139-143

10 Raviola G. Conjunctival and episcleral blood vessels are permeable to blood-borne horseradish peroxidase. *Invest Ophthalmol Vis Sci* 1983; 24 (6): 725-736

11 Butler TKH, Dua HS, Edwards R, Lowe JS. *In vitro* model of infectious grystalline keratopathy: tissue architecture determines pattern of microbial spread. *Invest Ophthalmol Vis Sci* 2001; 42(6): 1243-1246

12 Guo P, Zhang SZ, He H, Zhu YT, Tseng SC. PTX3 controls activation of matrix metalloproteinase 1 and apoptosis in conjunctivochalasis fibroblasts. *Invest Ophthalmol Vis Sci* 2012; 53(7): 3414-3423

13 De Faria NV, Russ HH, Rose P, Noronha L, Mello PA, Montiani-Ferreira F, Sobrinho SC. Conjunctival changes and inflammatory aspects in rabbits' conjunctivas induced by fixed combinations of prostaglandin analogues and timolol maleate. *J Ophthalmic Inflamm Infect* 2013; 3(1): 22

14 Golu T, Mogoanta L, Streba CT, Pirici DN, Malaescu D, Mateescu GO, Mutiu G. Pterygium: histological and immunohistochemical aspects. *Rom J Morphol Embryol* 2011; 52(1): 153-158

15 Kria L, Ohira A, Amemiya T. Immunohistochemical localization of basic fibroblast growth factor, platelet derived growth factor, transforming growth factor-beta and tumor necrosis factor-alpha in the pterygium. *Acta Histochem* 1996; 98(2): 195-201

16 Van Setten G, Aspiotis M, Blalock TD, Grotendorst G, Schultz G. Connective tissue growth factor in pterygium: simultaneous presence with vascular endothelial growth factor-possible contributing factor to conjunctival scarring. *Graefes Arch Clin Exp Ophthalmol* 2003; 241 (2): 135-139

17 Nolan TM, Di Girolamo N, Sachedev NH, Hampartzoumian T, Coroneo MT, Wakefield D. The role of ultraviolet irradiation and heparin-binding epidermal growth factor-like growth factor in the pathogenesis of pterygium. *Am J Pathol* 2003; 162(2): 567-574

18 Bianchi E, Scarinci F, Grande C, Plateroti R, Plateroti P, Plateroti AM, Fumagalli L, Capozzi P, Feher J, Artico M. Immunohistochemical profile of VEGF, TGF-β and PGE_2 in human pterygium and normal conjunctiva: experimental study and review of the literature. *Int J Immunopathol Pharmacol* 2012; 25(3): 607-615

19 Pelit A, Bal N, Akova YA, Demirhan B. P53 expression in pterygium in two climatic regions in Turkey. *Indian J Ophthalmol* 2009; 57(3): 203-206

20 Tsai YY, Chiang KC, Lin CL, Lee H, Tsai FJ, Cheng YW. Effect of TIMP-1 and MMP in pterygium invasion. *Invest Ophthalmol Vis Sci* 2010; 51(7): 3462-3467

21 Cimpean AM, Sava MP, Raica M. DNA damage in human pterygium: one-shot multiple targets. *Mol Vis* 2013; 19: 348-356

22 Booth F. Heredity in one hundred patients admitted for excision of pterygia. *Aust NZ J Ophthalmol* 1 985; 13(1): 59-61

23 Tsai YY, Bai DT, Chiang CC, Cheng YW, Tseng SH, Tsai FJ. Pterygium and genetic polymorphism of DNA double strand break repair gene Ku70. *Mol Vis* 2007; 13: 1436-1440

24 Young CH, Lo YL, Tsai YY, Shih TS, Lee H, Cheng YW. CYP1A1 gene polymorphism as a risk factor for pterygium. *Mol Vis* 2010; 16: 1054-1058

25 Di Girolamo N, Wakefield D, Coroneo MT. UVB-mediated induction of cytokines and growth factors in pterygium epithelial cells involves cell surface receptors and intracellular signaling. *Invest Ophthalmol Vis Sci* 2006;47(6): 2430-2437

26 Gaton DD, Lichter H, Avisar I, Slodovinic D, Solomon AS. Lymphocytic reaction to ultraviolet radiation on rabbit conjunctiva. *Ann Ophthalmol (Skokie)* 2007; 39(2): 128-133

27 Ateenyi-Agaba C, Dai M, LeCalvez F, Katongole-Mbidde E, Smek A, Tommasino M, Franceschi S, Hainaut P, Weiderpass E. TP53 mutations in squamous-cell carcinomas of the conjunctiva: evidence for UV-induced mutagenesis. *Mutagenesis* 2004; 19(5): 395-401

28 Kau HC, Tsai CC, Lee CF, Kao SC, Hsu WM, Liu JH, Wei YH. Increased oxidative DNA damage, 8-hydroxy-guanosine, in human pterygium. *Eye* 2006; 20: 826-831

29 Gazzard G, Saw SM, Farook M, Koh D, Widjaja D, Chia SE, Hong CY, Tan DTH. Pterygium in Indonesia: prevalence, severity and risk factors. *Br J Ophthalmol* 2002; 86(12): 1341-1346

30 Nemesure B, Wu SY, Hennis A, Leske MC; Barbados Eye Study Group. Nine-year incidence and risk factors for pterygium in the Barbados Eye Studies. *Ophthalmology* 2008; 115(12): 2153-2158

31 Forsius H, Maertens K, Fellman J. Changes of the eye caused by the climate in Rwanda, Africa. *Ophthalmic Epidemiol*1995; 2(2): 107-113

32 Akinsola FB, Mbadugha CA, Onakoya AO, Adefule-Ositelu AO, Aribaba OT, Ratomi-Samuel A. Pattern of conjunctival masses seen at Guinness Eye Centre Luth Idi-Araba. *Nig Q J Hosp Med* 2012; 22 (1): 39-43

33 Ajayi Iyiade A, Omotoye Olusola J. Pattern of eye diseases among welders in a Nigerian community. *Afr Health Sci*2012; 12(2): 210-216

34 Zhao L, You QS, Xu L, Ma K, Wang YX, Yang H, Jonas JB. 10-year incidence and associations of pterygium in adult Chinese: the Beijing Eye Study. *Invest Ophthalmol Vis Sci* 2013; 54(2): 1509-1514

35 Tananuvat N, Martin T. The results of amniotic membrane transplantation for primary pterygium compared with conjunctival autograft. *Cornea* 2004; 23(5): 458-463

36 Austin P, Jakobiec A, Iwamoto T. Elastodysplasia and elastodystrophy as the pathologic bases of ocular pterygia and pinguecula. *Ophthalmology* 1983; 90(1): 96-109

37Ansari MW, Rahi AHS, Shukla BR. Pseudoelastic nature of pterygium. *Br J Ophthalmol* 1970; 54(7): 473-476

38 Sherratt MJ, Bayley CP, Reilly SM, Gibbs NK, Griffiths CE, Watson RE. Low-dose ultraviolet radiation selectively degrades chromophore-rich extracellular matrix components. *J Pathol* 2010; 222(1): 32-40

39 Di Girolamo N, Kumar RK, Coroneo MT, Wakefield D. UVB-Mediated Induction of Interleukin-6 and -8 in Pterygium Epithelial Cells. *Invest Ophthalmol Vis Sci* 2002; 43: 3430-3437

40 Li S, Zhu F, Zykova T, Kim MO, Cho YY, Bode AM, Peng C, Ma W, Carper A, Langfeld A, Dong Z. T-LAK cell-originated protein kinase (TOPK) phosphorylation of MKP1 protein prevents solar ultraviolet light-induced inflammation through inhibition of the p38 protein signaling pathway. *J Biol Chem* 2011; 286(34): 29601-29609

41 Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. *Lab Invest* 1982; 47: 412-426

42 Peiretti E, Dessi S, Mulas MF, Abete C, Galantuomo MS, Fossarello M. Fibroblasts isolated from human pterygia exhibit altered lipid metabolism characteristics. *Exp Eye Res* 2006; 83(3): 536-542

43 Bochkov VN, Philippova M, Oskolkova O, Kadl A, Furnkranz A, Karabeg E, Afonyushkin T, Gruber F, Breuss J, Michenko A, Mechtcheriakova D, Hohensimer P, Rychli K, Wojta J, Resink T, Erne P, Binder BR, Leitinger N. Oxidized phospholipids stimulate angiogenesis *via* autocrine mechanisms, implicating a novel role for lipid oxidation in the evolution of artherosclerotic lesions. *Circ Res* 2006; 99: 900-908

44 Koshima H, Kondo S, Mishima S, Choi HR, Shimpo H, Sakai T, Ishiguro N. Expression of interleukin-1beta, cyclooxygenase-2, and prostaglandin E2 in a rotator cuff tear in rabbits. *J Orthop Res* 2007; 25(1): 92-97

45 Clear AS, Chirambo MC, Hutt MS. Solar keratosis, pterygium, and squamous cell carcinoma of the conjunctiva in Malawi. *Br J Ophthalmol* 1979; 63(2): 102-109

46 Binnebosel M, Rosch R, Junge K, Lynen-Jansen P, Schumpelick V, Klinge U. Macrophage and T-lymphocyte infiltrates in human peritoneal adhesions indicate a chronic inflammatory disease. *World J Surg* 2008; 32 (2): 296-304

47 Koli K, Saharinen J, Hyytiainen M, Penttinen C, Keski-Oja J. Latency, activation, and binding proteins of TGF-beta. *Microsc Res Tech* 2001; 52 (4): 354-362

48 Kim CS, Joo SY, Lee KE, Choi JS, Bae EH, Ma SK, Kim SH, Lee J, Kim SW. Paricalcitol attenuates 4-hydroxy-2hexanal-induced inflammation and epithelial-mesenchymal transition in human renal proximal tubular epithelial cells. *PLoS One* 2013; 8(5): e63186

49 Khalil N, Xu YD, O'Connor R, Duronio V. Proliferation of pulmonary interstitial fibrosis is mediated by transforming growth factor-beta1-induced release of extracellular fibroblast growth factor-2 and phosphorylation of p38 MAPK and JNK. *J Biol Chem* 2005; 280(52): 43000-43009

50 Tomcik M, Zerr P, Pitkowski J, Palumbo-zerr K, Avouac J, Distler O, Becvar R, Senolt L, Schett G, Distler JH. Heat shock protein 90 (Hsp90) inhibition targets canonical TGF-β signalling to prevent fibrosis. *Ann Rheum Dis* 2014;73(6):215-222

51 Ma C, Nasser C. Regulation of matrix metalloproteinases (MMPs) and their tissue inhibitors in human myometrial smooth muscle cells by TGF-β 1. *Mol Hum Reprod* 1999; 5(10): 950-954

52 Sun DX, Liu Z, Tan XD, Cui DX, Wang BS, Dai XW. Nanoparticle-mediated local delivery of an antisense TGF-β 1 construct inhibits intimal hyperplasia in autogenous vein grafts in rats. *PLoS One* 2012; 7(7): e41857

53 Stramer BM, Zieske JD, Jung JC, Austin JS, Fini ME. Molecular mechanisms controlling the fibrotic repair phenotype in cornea: implications for surgical outcomes. *Invest Ophthalmol Vis Sci* 2003; 44 (10): 4237-4246

54 Bae JY, Choi JS, Kang SW, Lee YJ, Park J, Kang YH. Dietary compound ellagic acid alleviates skin wrinkle and inflammation induced by UB-B irradiation. *Exp Dermatol* 2010; 19(8):e 182-190

55 Di Girolamo N, McCluskey P, Lloyd A, Coroneo MT, Wakefield D. Expression of MMPs and TIMPS in human pterygia and cultured pterygium epithelial cells. *Invest Ophthalmol Vis Sci* 2000; 41(3): 671-679

56 Dushku N, John MK, Schultz GS, Reid TW. Pterygia pathogenesis: corneal invasion by matrixmetalloproteinase expressing altered limbal epithelial basal cells. *Arch Ophthalmol* 2001; 119(5): 695-706

57 Ning Y, Manegold PC, Hong YK, Zhang W, Pohl A, Lurie G, Winder T, Yang D, LaBonte MJ, Wilson PM, Ladner RD, Lenz HJ. Interleukin-8 is associated with proliferation, migration, angiogenesis and chemosensitivity *in vitro* and *vivo* in colon cancer cell line models. *Int J Cancer* 2011; 12 (9): 2038-2049

58 Park CY, Choi JS, Lee SJ, Hwang SW, Kim E-J, Chuck RS. Cyclooxygenase-2-expressing macrophages in human pterygium co-express vascular endothelial growth factor. *Mol Vis* 2011; 17: 3468-3480

59 Nagineni CN, Samuel W, Nagineni S, Pardhasaradhi K, Wiggart B, Detrick B, Hooks JJ. Transforming growth factor-beta induces expression of vascular endothelial growth factor in human retinal pigment epithelial cells: involvement of mitogen-activated protein kinases. *J Cell Physiol* 2003; 197(3): 453-462

60 Wijelath ES, Rahman S, Murray J, Patel Y, Ishida A, Strand K, Aziz S, Cardona C, Hammond WP, Savidge GF, Rafi S, Sorbel M. Novel vascular endothelial growth factor binding domains of fibronectin enhance vascular endothelial growth factor biological activity. *Circ Res* 2002; 91(1): 25-31

61 Eyries M, Collins T, Khachigian L. Modulation of growth factor gene expression in vascular cells by oxidative stress. *Endothelium* 2004; 11(2): 133-139

62 Kria L, Ohira A, Amemiya T. Growth factors in cultured pterygium fibroblasts: immunohistochemical and ELISA analysis. *Graefes Arch Clin Exp Ophthalmol* 1998; 236(9): 702-708

63 Nolan TM, Di Girolamo N, Coroneo MT, Wakefield D. Proliferative effects of heparin-binding epidermal growth factor-like growth factor on pterygium epithelial cells and fibroblasts. *Invest Ophthalmol Vis Sci* 2004; 45(1): 110-113

64 Shafer G, Cramer T, Suske G, Kemmner W, Wiedenmann B, Hocker M. Oxidative stress regulates vascular endothelial growth factor-A gene transcription through sp1- and sp3-dependent activation of two proximal GC-rich promoter elements. *J Biol Chem* 2003; 278(10): 8190-8198

65 Song H, Yin W, Zeng Q, Jia H, Lin L, Liu X, Mu L, Wang R. Haemokinins modulate endothelium function and promote angiogenesis through neurokinin-1 receptor. *Int J Biochem Cell Biol* 2012; 44 (9): 1410-1421

66 Bayar SA, Kucukerdonmez C, Oner O, Akova YA. Subconjunctival bevacizumab in the impending recurrent pterygia. *Int Ophthalmol* 2014;34 (3):541-547

67 DiGuglielmo GM, LeRoy C, Davidson AF, Wrana JL. Distict endocytic pathways regulate TGF receptor signaling and turnover. *Nat Cell Biol* 2003; 5(5): 410-421

68 Savage C, Das P, Finelli AL, Townsend SR, Sun CY, Baird SE, Padgett RW. Caenorhabditis elegans sma-3 and sma-4 genes define a novel conserved family of transforming growth factor beta pathway components. *Proc Natl Acad Sci USA* 1996; 93(2): 790-794

69 Sekelsky JJ, Newfeld SJ, Raftery LA, Chartoff EH, Gelbart WM. Genetic characterization and cloning of mothers against dpp: a gene required for decapentaplegic function in Drosophila melanogaster. *Genetics* 1995; 139 (3): 1347-1358

70 Zhang R, Huang H, Cao P, Wang Z, Chen Y, Pan Y. Sma- and ma-related protein 7 (smad 7) is required for embryonic eye development in the mouse. *J Biol Chem* 2013; 288(15): 10275-10285

71 Matsumoto T, Yamada A, Aizawa R, Suzuki D, Tsukasaki M, Suzuki W, Nakayama M, Maki K, Yamamoto M, Baba K, Kamijo R. BMP-2 induced expression of Alx3 that is a positive regulator of osteoblast differentiation. *PLos One* 2013; 18(6): e68774

72 Zhang S, Fei T, Zhang L, Zhang R, Chen F, Ning Y, Han Y, Feng XH, Meng A, Chen YG. SMAD7 antagonizes transforming growth factor beta signaling in the nucleus by interfering with functional Smod-DNA complex formation. *Mol Cell Biol* 2007; 27(12): 4488-4499

73 Wang T, Zhou XT, Yu Y, Zhu JY, Dai JH, Qu XM, Le QH, Chu RY. Inhibition of corneal fibrosis by smad7 in rats after photorefractive keratectomy. *Chin Med J (Engl)* 2013; 126(8): 1445-1450

74 Ebisawa T, Fukuchi M, Murakami G, Chiba T, Tanaka K, Imamura T, Miyazano K. Smurf 1 interacts with transforming growth factor-beta type 1 receptor through smad 7 and induces receptor degradation. *J Biol Chem* 2001; 276(16): 12477-12480

75 Hwang YS, Lee HS, Kamata T, Mood K, Choo HJ, Winterbottom E, Ji YJ, Singh A, Daar IO. The smurf ubiquitin ligases regulate tissue separation *via* antagonistic interactions with ephrin B1. *Genes Dev* 2013; 27 (5): 491-503

76 Zhou W, Zhu H, Zhao J, Li H, Wan Y, Cao J, Zhao H, Yu J, Zhou R,

Yao Y, Zhang L, Wang L, He L, Ma G, Yao Z, Guo X. Misexpression of Pknox2 in mouse limb bud mesenchyme pertubs zeugopod development and deltoid crest formation. *PLoS One* 2013 May 22; 8(5): e64237

77 Yamashita M, Ying S-X, Zhang G-M, Li C, Cheng SY, Deng C-X, Zhang YE. Ubiquitin ligase smurf 1 controls osteoblast activity and bone homeostasis by targeting MEKK2 for degradation. *Cell* 2005; 121 (1): 101-113

78 Islam KN, Kayanoki Y, Kaneto H, Suzuki K, Asahi M, Fuji J, Taniguchi N. TGF-beta1 triggers oxidative modifications and enhances apoptosis in HIT cells through accumulation of reactive oxygen species by suppression of catalase and glutathione peroxidase. *Free Radic Biol Med* 1997; 22(6): 1007-1017

79 Ouhtit A, Nakazawa H, Armstrong BK, Kricker A, Tan E, Yamasaki H, English DR. UV-radiation-specific p53 mutation frequency in normal skin as a predictor of risk of basal cell carcinoma. *J Natl Cancer Inst* 1998; 90 (1): 523-531

80 Dushku N, Reid TW. Immunohistochemical evidence that human pterygia originate from an invasion of vimentin-expressing altered limbal epithelial basal cells. *Curr Eye Res* 1994; 13(7): 473-481

81 Torok H-P, Glas J, Tonenchi L, Lohse P, Muller-Myhsok B, Limbersky O, Neugebauer C, Schnitzler F, Seiderer J, Tillack C, Brand S, Brunnler G, Jagiello P, Epplen JT, Griga T, Klein W, Schiemann U, Folwaczny M, Ochsenkuhn T, Folwaczny C. Polymorphisms in the Dlg5 and OCTN cation transporter genes in Chron's disease. *Gut* 2005; 54(10): 1421-1427

82 Kawakita T, Espana EM, He H, Li C-Y, Tseng SC. Instrastromal invasion by limbal epithelial cells is mediated by epithelial-mesenchymal transition activated by air exposure. *Am J Pathol* 2005; 167: 381-393

83 Sezaki T, Tomiyama Y, Ueda K, Kioka N. Dlg5 interacts with the TGF-β receptor and promotes its degradation. *FEBS Lett* 2013; 587(11): 1624-1629

84 Kwok LS and Coroneo MT. A model for pterygium formation. *Cornea* 1994; 13(3): 219-224

85 Tsai YY, Chiang KC, Lin CL, Lee H, Tsai FJ, Cheng YW, Tseng SH. P53 expression in pterygium by immunohistochemistry analysis: a series report of 127 cases and review of literature. *Cornea* 2005; 24(5): 583-586

86 Hilgers JH. Pterygium: its incidence, heredity and aetiology. *Am J Ophthalmol* 1960; 50: 635-644

87 Zhang JD. An investigation of aetiology and heredity of pterygium. Report of 11 cases in a family. *Acta Ophthalmol (Copenh)* 1987; 65(4): 413-416

88 Hecht F, Shoptaugh MG. Winglets of the eye: dominant transmission of early adult pterygium of the conjunctiva. *J Med Genet* 1990; 27 (6): 392-394

89 Carmichael TR. Genetic factors in pterygium in South Africans. *S Afr Med J* 2001; 91(4): 322

90 Defazio G, Martivo D, Aniello MS, Masi G, Abbruzzese G, Lamberti S, Valente EM, Brancati F, Livrea P, Beradelli A. A family study on primary blepharospasm. *J Neurol Neurosurg Psychiatry* 2006; 77(2): 252-254

91 Zhang Y, Zolov SN, Chow CY, Slutsky SG, Richardson SC, Piper RC, Yang B, Nau JJ, Westrick RJ, Morrison SJ, Meister MH, Weisman LS. Loss of Vac14, a regulator of the signaling lipid phosphatdylinositol 3, 5-biphosphate results in neurodegeneration in mice. *Proc Natl Acad Sci USA* 2007; 104(44): 17518-17523

92 Ganna A, Rivadeneira F, Hofman A, Uitterlinden AG, Magnusson PK, Pedersen NL, Ingelsson E, Tiemeier H. Genetic determinants of mortality. Can findings from genome-wide association studies explain variation in human mortality? *Hum Genet* 2013; 132: 553-561

93 Kawasaki S, Yamasaki K, Nakagawa H, Shinomiya K, Nakatsukasa M, Nakai Y, Kinoshita S. A novel mutation (pGlu1389AspfsX16) of the phosphoinositide kinase, FYVE finger containing gene found in a Japanese patient with fleck corneal dystrophy. *Mol Vis* 2012; 18: 2954-2960

94 Goldenberg-Cohen N, Banin E, Zalzstein Y, Cohen B, Rotenstreich Y, Rizel L, Basel-Vanagaite L, Ben-Yosef J. Genetic heterogeneity and consanguinity lead to a "double hit": homozygous mutations of MYO7A and PDE6B in a patient with retinitis pigmentosa. *Mol Vis* 2013; 19: 1565-1571

95 Shah A, Hussain R, Fareed M, Afzal M. Prevalence of red-green colour vision defects among Muslim males and females of Manipur, India. *Iran J Public Health* 2013; 42(1): 16-24

96 Fukumura T, Kose H, Takeda C, Kurita Y, Ochiai Y, Yamada T, Matsumoto K. Genetic interaction between hyperglycaemic QTL is manifested under a high calorie diet in OLETF-derived congenic rats. *Exp Anim* 2011; 60(2): 125-132

97 Savage DB, Agostini M, Barroso I, Gurnell M, Luan J, Meirhaeghe A, Harding A-H, Ihrke G, Rajanayagam O, Soos MA, George S, Berger D, Thomas EL, Bell JD, Meeran K, Ross RJ, Vidal-Pulg A, wareham NJ, O'Rahilly S, Chatterjee VKK, Schaffer AJ. Digenic inheritance of severe insulin resistance in a human pedigree. *Nat Genet* 2002; 31(4): 379-384

98 The International Schizophrenia Consortium, Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*2009; 460(7256:748-752

99 Melzner I, Scott V, Dorsch K, Fischer P, Wabitsch M, Bruderlein S, Hasel C, Moller P. Leptin gene expression in human preadipocytes is switched on by maturation-induced demethylation of distinct CpGs in its proximal promoter. *J Biol Chem* 2002; 277(47): 45420-45427

100 Allen HL, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, Willer CJ, Jackson AU, Vedantam S, Raychaudhuri S, Ferreira T, Wood AR, Weyant RJ, Segre AV, Speliotes EK, Wheeler E, Soranzo N, Park J-H, Yang J, Gudbjartsson D, Heard-Costa NL, Randall RC, Qi L, Smith AV, Magi R, Pastinen T, Liang L, Heid IM, Luan J, Thorleifsson G, Winkler TW, Goddard ME, Lo KS, Palmer C, Workalemahu T, Aulchenko YS, Johansson A, Zillikens MC, Feitosa MF, Esko T, Johnson T, Ketkar S, Kraft P, Mangino M, Prokopenko I, Absher D, Albrecht E, Ernst F, Glazer NL, Hayward C, Hottenga J-J, Jacobs KB, Knowles JW, Kutalik Z, Monda KL, Polasek O, Preuss M, Rayner NW, Robertson NR, Steinthorsdottir V, Tyrer JP, Voight BF, Wiklund F, Xu J, Zhao JH, Nyholt DR, Pellikka N, Perola M, Perry JRB, Surakka I, Tammesoo M-L, Altmaier EL, Amin N, Aspelund T, Bhangale T, Boucher G, Chasman DI, Chen C, Coin L, Cooper MN, Dixon AL, Gibson Q, Grundberg E, Hao K, Juhani Juntilla M, Kaplan LM, Kettunen J, Konig IR, Kwan T, Lawrence RW, Levinson DF, Lorentzon M, McKnight B, Morris AP, Muller M, Ngwa JS, Purcell S, Rafelt S, Salem RM, Salvi E, Sanna S, Shi J, Sovio U, Thompson JR, Turchin MC, Vandenput L, Verlaan DJ, Vitart V, White CC, Ziegler A, Almgren P, Balmforth AJ, Campbell H, Citterio L, De Grandi A, Dominiczak A, Duan J, Elliott P, Elosua R, Eriksson JG, Freimer NB, Geus EJC, Glorioso N, Haiqing S, Hartikainen A-L, Havulinna AS, Hicks AA, Hui J, Igl W, Illig T, Jula A, Kajantie E, Kilpelainen TO, Koiranen M, Kolcic I, Koskinen S, Kovacs P, Laitinen J, Liu J, Lokki M-L, Marusik AA, Maschio A, Meitinger T, Mulas A, Pare G, Parker AN, Peden JF, Petersmann A, Pichler I, Pietlainen KH, Pouta A, Ridderstrale M, Rotter JI, Sambrook JG, Sanders AR, Schmidt CO, Sinisalo J, Smit JH, Stringham HM, Walters GB, Widen E, Wild SH, Willemsen G, Zagato L, Zgaga L, Zitting P, Alavere H, Farrall M, McArdle WM, Nelis M, Peters MJ, Ripatti S, van Meurs JBJ, Aben KK, Ardlie KG, Beckmann JS, Beilby JP, Bergman RN, Bergmann S, Collins FS, Cusi D, den Heijer M, Eiriksdottir G, Gejman PV, Hall AS, Hamsten A, Huikuri

HV, Iribarren C, Kahonen M, Kaprio J, Kathiresan S, Kiemeney L, Kocher T, Launer LJ, Lehtimaki T, Melander O, Mosley TH, Musk AW, Nieminen MS, O'Donnell CJ, Ohlsson C, Oostra B, Palmer LJ, Raitakari O, Ridker PM, Rioux JD, Rissanen A, Rivolta C, Schunkert H, Shuldiner AR, Siscovic DS, Stumvoll M, Tonjes A, Tuomilehto J, van Ommen G-J, Viikari J, Heath AC, Martin NG, Montgomery GW, Province MA, Kayser M, Arnold AM, Atwood LD, Boerwinkle E, Chanock SJ, Deloukas P, Gieger C, Gronberg H, Hall P, Hattersley AT, Hengstenberg C, Hoffman W, Lathrop GM, Salomaa V, Schreiber S, Uda M, Waterworth D, Wright AF, Assimes TL, Barroso I, Hofman A, Mohlke KL, Boomsma DI, Caulfield MJ, Cupples LA, Erdmann J, Fox CS, Gudnason V, Gyllensten U, Harris TB, Hayes RB, Jarvelin M-R, Mooser V, Munroe PB, Ouwehand WH, Penninx BW, Pramstaller PP, Quertermous T, Rudan I, Samani NJ, Spector TD, Volzke H, Watkins H, Wilson JF, Groop LC, Haritunians T, Hu FB, Kaplan RC, Metspalu A, North KE, Schlessinger D, Wareham NJ, Hunter DJ, O'Connell JR, Strachan DP, Wichmann HE, Borecki IB, van Duijn CM, Schadt EE, Thorsteinsdottir U, Peltonen L, Uitterlinden A, Visscher PM, Chartterjee N, Loos RJF, Boehnke M, McCarthy MI, Ingelsson E, Lindgren CM, Abecasis GR, Stefansson K, Frayling TM, Hirschhorn JN. Hundreds of variants clustered in genomic loci and biological pathways affect height. *Nature* 2010; 467(7317): 832-838

101 The Climate of Limpopo Province-South Africa. *South African Weather Service*. <http://www.weathersa.co.za> [Accessed 26/02/2010]

102 Kromberg JG, Jenkins T. Prevalence of albinism in the South African Negro. *S Afr Med J* 1982; 61(11): 383-386

103 Jaouad IC, Elalaoui SC, Sbiti A, Elkerh F, Belmahi L, Sefiani A. Consanguineous marriages in Morocco and the consequence for the incidence of autosomal recessive disorders. *J Biosoc Sci* 2009; 41 (5): 575-581

104 Lehohla PJ. "Mid-year population estimates 2009". *Statistical release P0302* 2009 July 27. <http://www.statssa.gov.za> [Accessed 26/08/2013]

105 Anguria P, Ntuli S, Carmichael T. Relationships of heredity and dry eye with pterygia in black African patients. *S Afr Med J* 2011; 101(2): 110

106 Anguria P, Ntuli S, Interewicz B, Carmichael T. Traditional eye medication and pterygium occurrence in Limpopo Province. *S Afr Med J* 2012; 102(8): 687-690

107 Anguria P, Carmichael T, Ntuli S, Kitinya J. Chronic inflammatory cells and damaged limbal cells in pterygium. *Afr Health Sci* 2013; 13(3): 725-730

108 Anguria P Ntuli S, Carmichael T. Young patient's age determines pterygium recurrence after surgery. *Afr Health Sci* 2014;14(1): 72-76

109 Busia K. Medical provision in Africa-past and present. *Phytother Res* 2005; 19(11): 919-923

110 Lefevre JH, Bonilla C, Colas C, Winney B, Johnstone E, Tonks S, Day T, Hutnik K, Boumertit A, Soubrier F, Midgley R, Kerr D, Parcy Y, Bodmer WF. Role of rare variants in undetermined multiple adenomatous polyposis and early-onset colorectal cancer. *J Hum Genet* 2012; 57(11): 709-716

111 Yamada T, Mochizuki H, Ue T, Kiuchi Y, Takahaski Y, Oinaka M. Comparative study of different β -radiation doses for preventing pterygium recurrence. *Int J Radiat Oncol Biol Phys* 2011; 81(5): 1394-1398

112 Tan DT, Chee SP, Deer KB, Lim AS. Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. *Arch Ophthalmol* 1997; 115 (10): 1235-1240

113 Gan XT, Zhao G, Huang CX, Rowe AC, Purdham DM, Karmazyn M. Identification of fat mass and obesity associated (RTO) protein expression in cardiomyocytes: regulation by leptin and its contribution to leptin-induced hypertrophy. *PLoS One* 2013; 8(9): e74235