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Steroid-Induced Ocular Hypertension/Glaucoma: Focus on Pharmacogenomics and Implications for Precision Medicine

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Abstract

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Elevation of intraocular pressure (IOP) due to therapeutic use of glucocorticoids is called steroid-induced ocular hypertension (SIOH); this can lead to steroid-induced glaucoma (SIG). Glucocorticoids initiate signaling cascades ultimately affecting expression of hundreds of genes; this provides the potential for a highly personalized pharmacological response. Studies attempting to define genetic risk factors were undertaken early in the history of glucocorticoid use, however scientific tools available at that time were limited and progress stalled. In contrast, significant advances were made over the ensuing years in defining disease pathophysiology. As the genomics age emerged, it appeared the time was right to renew investigation into genetics. Pharmacogenomics is an unbiased discovery approach, not requiring an underlying hypothesis, and provides a way to pinpoint clinically significant genes and pathways that could not have been discovered any other way. Results of the first genome-wide association study to identify polymorphisms associated with SIOH, and follow-up on two novel genes linked to the disorder, GPR158 and HCG22, is discussed in the second half of the article. However, knowledge of genetic variants determining response to steroids in the eye also has value in its own right as a predictive and diagnostic tool. This article concludes with a discussion of how the Precision Medicine Initiative®, announced by U.S. President Obama in his 2015 State of the Union address, is beginning to touch the practice of ophthalmology. It is argued that SIOH/SIG may provide one of the next opportunities for effective application of precision medicine.

Keywords

Ocular hypertension; glaucoma; glucocorticoid; pharmacogenomics; precision medicine; ophthalmology

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1. Introduction

Elevated intraocular pressure (IOP) is a major risk factor for glaucoma, a group of eye diseases characterized by a progressive loss of retinal ganglion cells (Kwon et al., 2009; Quigley, 1999). Glaucoma is the second leading cause of visual impairment and blindness worldwide, affecting about 70 million people, and is the leading cause of blindness among African-Americans (Quigley, 1996; Quigley and Broman, 2006). Primary open-angle glaucoma (POAG) is the most common form of the disease, accounting for approximately 70% of all cases (Kwon et al., 2009). It has been increasingly recognized that a significant percentage of these cases are characterized by normal IOP (Anderson, 2011; Kass et al., 2002; Tielsch et al., 1991). Interestingly however, reducing IOP is an effective treatment for both high tension and normal tension forms of glaucoma (Gordon et al., 2002; Kass et al., 2002; Leske et al., 2003), and in fact, is currently the *only* treatment for glaucoma.

Ocular hypertension due to secondary factors can also lead to glaucoma. One form is initiated by glucocorticoids, which are one of two types of natural corticosteroid hormones produced by the adrenal glands (the other being mineralocorticoids). Released in response to stress, glucocorticoids regulate a natural feedback mechanism that turns down the inflammatory response. As such, they are useful pharmacologically for treating a wide variety of diseases (Rhen and Cidlowski, 2005). Cortisol is the most important human glucocorticoid, essential for life. Hydrocortisone is the name used for pharmaceutical

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preparations. By diffusing through the plasma membrane and binding to intracellular receptors, glucocorticoids initiate signaling cascades that ultimately affect expression of hundreds of genes. This means the potential for a highly individualized response to therapeutic use of glucocorticoids, including adverse effects in susceptible patients. Elevation of IOP due to glucocorticoid use in the eye is called steroid-induced ocular hypertension (SIOH). If IOP elevation is of sufficient magnitude and is not treated, glaucomatous optic neuropathy can develop. This is called steroid-induced glaucoma (SIG).

In this perspectives/review article we begin with a short history of the field, which originated in 1948 with the first therapeutic use of glucocorticoids. Shortly thereafter, in 1950, the Nobel Prize for Physiology or Medicine was awarded to Philip Hench, Edward Kendall and Tadeus Reichstein for "discoveries relating to the hormones of the adrenal cortex, their structure and biological effects". By the early 1960s it seemed clear that both ocular hypertension leading to POAG and SIOH were influenced by genetics. Studies attempting to define genetic risk factors were undertaken, however the scientific tools available at that time were limited, thus progress stalled.

In contrast, much progress was made over the ensuing years in defining the nature of steroid-induced changes to the conventional outflow pathway, which is responsible for elevated IOP. Steroid-induced changes in outflow tissues were first described in morphological studies, and then using the tools of cell biology and molecular biology, as they emerged. Most recently, the use of new methods to determine the biomechanical properties of tissues, as well as the introduction of SIOH/SIG animal models, has provided additional insight. As will be discussed below, while SIOH leading to SIG bears many similarities to POAG, there are also striking differences. Thus the comparison between the two diseases may provide important clues to understanding normal outflow mechanisms and outflow resistance.

By the 1990s, new molecular tools were available for genetic analysis, and family studies began to appear in the literature identifying chromosomal loci causally associated with POAG (e.g., (Richards et al., 1994; Wirtz et al., 1997); reviewed in (Fan et al., 2006; Fingert, 2011)). This progressed rapidly to fine-mapping studies. The first disease-causing POAG mutation was reported in 1997, identified in the MYOC gene, which encodes a protein known as myocilin (Stone et al., 1997). Coincidentally, MYOC had previously been identified as a glucocorticoid-induced protein in trabecular meshwork cell cultures, and given the name TIGR (trabecular meshwork-inducible glucocorticoid response protein) (Nguyen et al., 1998; Polansky et al., 1997). However, subsequent analyses revealed no evidence for a causal link between POAG-causing MYOC mutations and SIOH (Fingert et al., 2001), and whether MYOC has any role in SIOH remains unresolved to this day.

The invention of microarray technology provided an important step forward for genetic analysis. The original HuSNP™ assay (Affymetrix, Santa Clara, California, USA) was designed to genotype nearly 1500 human single nucleotide polymorphisms (SNPs) on a single chip (Wang et al., 1998). Two major events led to further advances. First was the Human Genome Project, completed in 2001, which gave us the ability to read the complete genetic blueprint of a human being for the very first time (Venter et al., 2001). The

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International HapMap Consortium and its 1000 Genomes Project followed in 2002, initiating a comprehensive effort to identify SNPs and determine their frequencies in individuals of various ancestries. Both Affymetrix and Illumina (San Diego, California, USA) now make chips with millions of SNPs and applications have expanded to include copy number variations (reviewed in (LaFramboise, 2009)). This has led to identification of numerous genetic variants that increase risk for ocular hypertension leading to ocular hypertension POAG (e.g., IOP (Gao et al., 2015); reviewed in (Burdon et al., 2011; van Koolwijk et al., 2012; Wiggs et al., 2013; Wiggs et al., 2012). It appeared the time was right to renew investigations into the genetics of SIOH.

The National Institutes of Health (NIH) *Genetics Home Reference* website defines pharmacogenomics as "the study of how genes affect a person's response to drugs" (https://ghr.nlm.nih.gov/). For the biomedical research scientist interested in disease mechanisms, genomics and pharmacogenomics offer an unbiased discovery approach, not requiring an underlying hypothesis. The advantage is the opportunity to pinpoint genes and pathways that could not have been discovered in any other way. These leads are then followed-up functionally to determine how the identified genetic variants increase risk for drug response, and how the associated genes and pathways might cause disease. Genomics and pharmacogenomics are hypothesis-generating approaches, and ultimately the functional studies provide the most important information about the significance of the identified gene.

In the second half of this article, we demonstrate the power of pharmacogenomics with functional follow-up. We describe the first genome-wide study to identify polymorphisms associated with SIOH (Jeong et al., 2015), and our functional findings on the two novel linked genes, GPR158 (Patel et al., 2013; Patel et al., 2015) and HCG22 (Jeong et al., 2015). There is a great need to identify new drug targets for ocular hypertension, as many patients remain refractory to existing medications and eventually may become blind. The novel genes identified in our study are promising in this regard.

In addition, knowledge of genetic variants determining steroid sensitivity also has value in its own right, because it provides a predictive and diagnostic tool for use of drugs. For a pharmacologist, the primary goal of pharmacogenomics is to develop more effective, safer medications and doses by taking into consideration a person's genetic makeup. This important goal was recently recognized as part of the new U.S. Precision Medicine Initiative® announced by President Obama in his State of the Union address on January 20, 2015 (https://www.nih.gov/precision-medicine-initiative-cohort-program), and launched by an article in the *New England Journal of Medicine* co-authored by current NIH director Francis S. Collins and past director Harold E. Varmus (Collins and Varmus, 2015). We end this article with a discussion of how this new initiative is beginning to touch the practice of ophthalmology, and we argue that SIOH may provide one of the next opportunities in ophthalmology for effective application of precision medicine.

2. Clinical History and Genetics

Benedek reviewed the history of corticosteroid therapy for human ailments (Benedek, 2011). The first clinical evidence that an extract of animal adrenocortical tissue could counteract

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human adrenal failure was reported in 1930. Chemical analyses of tissue extracts revealed the presence of multiple steroid hormones with different activities. By 1940 these hormones had been defined as belonging to two classes: mineralocorticoids that cause sodium and fluid retention and glucocorticoids that counteract shock and inflammation. In 1948, the therapeutic value of cortisone was discovered, when it was used for the first time to treat a patient with rheumatoid arthritis. This first administration was intramuscular. Oral and intra-articular administration of cortisone and hydrocortisone began in 1950–51, and it also began to be administered topically to the eye. Beginning around that time, reports rapidly accumulated to support a connection between glucocorticoid therapies and IOP elevation.

In 1962, Bernstein and Schwartz reported that patients on long-term systemic glucocorticoid therapy showed consistently higher IOP when compared with normal individuals (Bernstein and Schwartz, 1962). The next year, several studies appeared in the literature linking topical glucocorticoid treatment in the eye with elevated IOP and glaucoma (reviewed in (Jones and Rhee, 2006; Kersey and Broadway, 2006)). Becker and Mills observed that glaucoma patients and glaucoma suspects exhibited a marked IOP increase following topical treatment with glucocorticoids for several weeks (Becker and Mills, 1963). However, IOP rose much less in the normal group. Armaly also reported greater response to glucocorticoid treatment in eyes of glaucoma patients versus normal subjects and it was further noted that the effect was greater in older versus younger eyes (Armaly, 1963a, b).

In the Becker and Mills study, it was also observed that normal subjects fell into two distinct subgroups: those exhibiting a moderate increase and those exhibiting no increase (Becker and Mills, 1963). Subsequently, Armaly (Armaly, 1965) and Becker (Becker, 1965) refined characterization of the response in normal subjects and demonstrated three distinct subgroups: those exhibiting a high response (a change >15 mm Hg), those exhibiting a moderate response (a change of 6–15 mm Hg), and those exhibiting a low response (a change < 6 mm Hg).

We now know that there is considerable inter-individual variability in glucocorticoid response (Berry et al., 2010; Chriguer et al., 2005; Donn et al., 2007; Huizenga et al., 1998; Stevens et al., 2004). Systemic administration to sensitive individuals can lead to adverse events such as visceral obesity-related insulin resistance and ischemic heart disease (Franchimont et al., 1999; Kino and Chrousos, 2001; van Rossum and Lamberts, 2004). Those with a predisposition to develop elevated IOP when treated with glucocorticoids systemically or locally in the eye are referred to as “steroid responders”, a term first used by Armaly and Becker (Armaly and Becker, 1965). When glucocorticoid treatment is discontinued, the IOP usually returns to normal within a few weeks, although a few patients in each group may have a continued elevation of IOP (Armaly, 1963a, b, 1965; Becker, 1965). As noted above, glaucoma patients and glaucoma suspects are much more likely to be steroid responders. Interestingly, the converse is also true: responders are more likely to develop POAG as compared to non-responders (Kitazawa and Horie, 1981; Lewis et al., 1988). Moreover, relatives of POAG patients have higher rates of steroid responsiveness (Bartlett et al., 1993; Becker and Chevrette, 1966; Davies, 1968; Paterson, 1965).

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It was conjectured early on that genetics was the basis for susceptibility to glucocorticoids (Armaly, 1963a, b; Becker and Mills, 1963). In 1964, Becker and Hahn suggested that patient response to glucocorticoids in the eye could be explained by variation in a single gene (Becker and Hahn, 1964). In 1965, Armaly (Armaly, 1965) and Becker (Becker, 1965) further suggested that moderate responders were heterozygotes while high responders were homozygotes. Other studies did not support this idea however. Schwartz and colleagues found no significant difference in the frequency of glucocorticoid response between monozygotic and dizygotic twin groups (Schwartz et al., 1973). Palmberg and colleagues (Palmberg et al., 1975) offered a possible explanation for the apparent discrepancy in findings by investigating the reproducibility of the glucocorticoid response. While topical testing had limited effectiveness in whole populations, it did show good reproducibility in the high-response group. It was suggested that the less-than-perfect reproducibility might explain to an extent the low concordance identified in Schwartz's twin study. However, Francois proposed that children of glaucoma patients should all be moderate responders if this were correct, but only 29 out of 87 tested exhibited a response (Francois, 1977). Subsequent studies found an association between POAG and the histocompatibility loci HLA-A and HLA-B (Aviner et al., 1976; Bigger et al., 1972; Bigger et al., 1975), and between high response to steroids (> 31 mm Hg) and HLA-B12 (Becker et al., 1977; Becker et al., 1976). However, several other studies could not confirm the POAG findings (Damgaard-Jensen and Kissmeyer-Nielsen, 1978; Mayr and Grabner, 1978; Ritch et al., 1978). A reassessment of some of the original data concluded that the associations were less impressive than previously proposed (Kass et al., 1978; Leopold, 1979).

3. Current Clinical Perspective

Currently, the most common ocular use of glucocorticoids is topical application to control postoperative inflammation. Topical glucocorticoids are also the mainstay for preventing and treating corneal transplant rejection and for treating ocular conditions involving immune hyper-reactivity (e.g., noninfectious uveitis, and allergic disorders such as atopic or vernal keratoconjunctivitis). The principal drawbacks include SIOH and the potential for SIG. The incidence (penetrance) of these varies with dosing frequency and treatment duration, as well as with the type of glucocorticoid and its formulation.

Treatment durations range from a few weeks, for controlling postoperative inflammation, to a year or longer, for preventing corneal transplant rejection (Price et al., 2009). Studies by this group (Vajaranant et al., 2009) as well as others (Ayyala, 2000; Kirkness and Moshegov, 1988) have shown that up to 35% of patients without a prior glaucoma diagnosis and up to 80% of those with preexisting glaucoma experience clinically significant post-keratoplasty IOP elevation with long-term topical glucocorticoid use.

The most commonly used topical glucocorticoid in the U.S. has been prednisolone acetate 1% (Pred Forte®, Allergan, Irvine, California, USA), which was only approved for treating conjunctivitis but is routinely used off label for other indications. Some of the more recent Food and Drug Administration (FDA) approvals, i.e. loteprednol etabonate 0.5% gel (Lotemax®, Bausch & Lomb, Bridgewater, New Jersey, USA) and difluprednate 0.05% ophthalmic emulsion (Durezol®, Alcon, Fort Worth, Texas, USA), are for treating post-

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operative inflammation, based on clinical trials involving short-term use after cataract surgery.

Corneal transplantation offers a unique opportunity to evaluate how long-term use of topical glucocorticoids affects IOP without the confounding effects seen with immune hyper-reactivity disorders. The relative efficacy at preventing transplant rejection episodes is a sensitive measure of steroid potency. Traditional penetrating keratoplasty replaces all layers of the cornea (epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium) regardless of the underlying condition. Recently, selective replacement of diseased corneal layers has become increasingly popular. The technique called Descemet's membrane endothelial keratoplasty (DMEK), which only replaces dysfunctional endothelium and Descemet's membrane, has the lowest risk of rejection (Anshu et al., 2012). The reduced rejection risk led to a unique set of studies from our group, which compared the rates of IOP elevation and rejection episodes with use of different topical glucocorticoids.

The first study was a retrospective analysis of 400 eyes in 400 patients (Vajaranant et al., 2009), some results from which are depicted graphically in Figure 1. It was found that 30% of eyes without a history of glaucoma developed ocular hypertension (defined in this study as IOP 24 mm Hg or a relative increase of 10 mm Hg over the baseline preoperative IOP) within 1 year when dosed with prednisolone acetate 1% qid for 4 months, then tapered over 2 to 3 months to once or twice daily (Vajaranant et al., 2009). To manage IOP, glaucoma medications were initiated in 18% of patients and topical glucocorticoids were reduced earlier than planned in 22% of patients (Vajaranant et al., 2009). In 2 subsequent prospective studies, DMEK patients were treated with prednisolone acetate 1% qid for 1 month and then 325 eyes were randomized to remain on prednisolone or switch to fluorometholone 0.1% (FML®, Allergan Pharmaceuticals, Republic of Ireland) and 233 eyes were randomized to remain on prednisolone or switch to loteprednol etabonate 0.5% gel (dosing: tid for months 2 and 3, bid month 4, and once daily months 5 through 12) (Price et al., 2015; Price et al., 2014). The incidence of ocular hypertension as defined above was 24%, 11% and 8% with prednisolone acetate 1%, loteprednol 0.5% gel, and fluorometholone 0.1% respectively ($p=0.0005$ for prednisolone vs. fluorometholone and $p=0.013$ for predinisolone vs. loteprednol), while the incidence of rejection episodes were 0%, 0% and 1.4% respectively ($p=0.17$ for prednisolone vs. fluorometholone and $p=1$ for prednisolone vs. loteprednol) (Price et al., 2015; Price et al., 2014). In the latter two studies, approximately 9% of patients had a preoperative diagnosis of glaucoma. In those without pre-existing glaucoma, the incidence of postoperative IOP elevation (defined as 10 mm Hg over the baseline preoperative IOP) was 18% in the prednisolone group vs. 4% in the loteprednol group ($p=0.0045$) (Price et al., 2015). Notably, the glucocorticoid dosing was tapered more quickly in the latter 2 prospective studies than it was in the initial retrospective study (Price et al., 2015; Price et al., 2014; Vajaranant et al., 2009); a smaller proportion of patients exceeded the defined IOP elevation threshold with the faster glucocorticoid taper. Importantly, the IOP elevation threshold was not reached until the 1-year exam in some patients (Price et al., 2015; Vajaranant et al., 2009). Thus this series of studies clearly illustrate how the response to glucocorticoids depends upon the glucocorticoid potency,

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formulation (which influences ocular penetration), dosing frequency, and duration of exposure.

Glucocorticoids have been reported beneficial for many retinal diseases. Schwartz and colleagues recently reviewed the literature on the variety of delivery systems for introducing glucocorticoids into the vitreous cavity (Schwartz et al., 2013). There are currently three different synthetic corticosteroids in clinical use for this purpose. Off-label intravitreal triamcinolone acetonide (IVTA) has been used for many years in the treatment of various diseases, including diabetic macular edema (DME) (Schwartz et al., 2010), branch retinal vein occlusion (BRVO) (Jonas et al., 2005), central retinal vein occlusion (CRVO) (Gregori et al., 2006), and others. A prepackaged, single-dose preparation for specific intravitreal use (Triesence®, Alcon, Fort Worth, Texas, USA) is FDA approved for visualization during pars plana vitrectomy (Dyer et al., 2009) and the treatment of certain posterior segment inflammatory disorders, although not for DME, BRVO, or CRVO. The dexamethasone delivery system (Ozurdex®, Allergan, Irvine, California, USA) is a bioerodable insert, which is injected in a clinic setting and is FDA approved for the treatment of DME (Boyer et al., 2014), noninfectious posterior segment uveitis (Lowder et al., 2011), and macular edema secondary to BRVO and CRVO (Haller et al., 2011). A nonbioerodable fluocinolone acetonide implant (Retisert®, Bausch & Lomb, Bridgewater, New Jersey, USA), which is surgically implanted under aseptic conditions in an operating room, is FDA approved for the treatment of chronic noninfectious posterior segment uveitis (Jaffe et al., 2006). Most recently, a smaller, nonbioerodable fluocinolone acetonide insert (Iluvien®, Alimera, Alpharetta, Georgia, USA), which is injected in a clinic setting, achieved FDA approval for the treatment of DME in patients previously treated with glucocorticoids without a clinically significant increase in IOP (that is, patients without a history of steroid response) (Campochiaro et al., 2012).

Similar to topical glucocorticoids, intravitreal glucocorticoids are also associated with risk of elevated IOP, which may be severe. In the Standard of Care versus Corticosteroid for Retinal Vein Occlusion (SCORE) Study, a prospective, randomized clinical trial of IVTA, the cumulative rates of IOP elevation greater than 10 mm Hg above baseline within 36 months were 2% for “standard of care” (no injection), 9% for patients treated with IVTA, 1 mg, and 45% for patients treated with IVTA, 4 mg (Aref et al., 2015). In a prospective, randomized clinical trial comparing IVTA, 4 mg versus other (non-glucocorticoid) treatments for DME, the cumulative rates of IOP elevation of 10 mm Hg from baseline within 1 year were 3%–5% for patients treated with non-glucocorticoid therapies and 38% for patients treated with IVTA, 4 mg (Diabetic Retinopathy Clinical Research et al., 2010). In a phase III study for the dexamethasone delivery system, among patients treated with the 0.7-mg insert (which was eventually FDA approved), 41.5% required IOP-lowering medication and 0.3% required incisional surgery within 36 months (Maturi et al., 2016). In the phase III study for the fluocinolone acetonide insert, 38.4% of patients treated with the low-dose insert (which was eventually FDA approved) required IOP-lowering medication and 4.8% required incisional glaucoma surgery within 36 months (Campochiaro et al., 2012).

The steroid response represents a major clinical challenge, which most likely limits the use of these medications today. This concern is especially relevant to the fluocinolone acetonide

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insert, which releases medication for up to three years following its insertion, and thus presents the greatest safety concerns.

4. Physiology and Pathogenesis

IOP represents the outcome of four physiological processes: aqueous humor formation, trabecular meshwork outflow, uveoscleral outflow and episcleral venous pressure (Brubaker, 2004). It is well established that all forms of ocular hypertension in eyes with an open angle are caused by an increase in outflow resistance in the trabecular outflow pathway, also called the “conventional” outflow pathway (Alvarado et al., 1984; Alvarado et al., 2005; Epstein, 1996; Grant, 1951b, a). The trabecular meshwork is a spongy connective tissue in the iridocorneal angle of the eye that spans from the scleral spur to Schwalbe’s line around the entire circumference. Aqueous humor flows from the anterior chamber of the eye through the trabecular meshwork, emptying into Schlemm’s canal. Much of the resistance to outflow putatively resides within 7 to 14 um of the inner wall of Schlemm’s canal in a region known as the cribriform or juxtacanalicular region (Acott and Kelley, 2008; Ethier, 2002; Johnson, 2006; Maepea and Bill, 1992).

Healthy aqueous outflow through the trabecular meshwork has been linked to robust extracellular matrix turnover (reviewed in (Vranka et al., 2015)). Elevation of IOP stretches the extracellular matrix and attached cells of the trabecular meshwork and Schlemm’s canal, initiating a cascade of events that ultimately increases matrix metalloproteinase (MMP) activity. Keller and Acott recently proposed the concept of “maintenance remodeling”, whereby certain MMPs are constitutively expressed and active, functioning to maintain open outflow pathways (Keller and Acott, 2013). Imbalances in extracellular matrix turnover can result in elevated IOP. MMP9-deficient mice were found to have increased IOP compared to wild-type littermates (Robertson et al., 2013). Other proteinases such as PLG (plasminogen) and plasminogen activators (PLAT and PLAU), which serve to convert pro-MMPs to their active form, have also been implicated (Candia et al., 2014; Gerometta et al., 2013).

Anterior ciliary muscle tendons are anchored within the cribriform plexus, a network of elastic-like sheathed fibrils that connect with the endothelium of Schlemm’s canal. Contraction of the ciliary muscle influences the dimensions of the cribriform layer and changes the flow passageways of that area, thereby influencing outflow resistance (Gabelt and Kaufman, 2005).

In closed angle forms of ocular hypertension, elevated IOP develops due to anatomical factors that physically block conventional outflow. In contrast, the progressive malfunction of the conventional outflow pathway in ocular hypertension leading to POAG – or in SIOH leading to SIG – appears to be caused by alterations at the cellular and molecular levels.

A substantial fraction of POAG has a genetic basis (Fingert, 2011). Various forms of POAG are inherited in families as Mendelian traits with a very high likelihood of penetrance. Idiopathic forms of POAG are more complex, caused by the combined effects of many genetic and environmental risk factors. These factors are more frequently detected in patients with POAG, but are also common in normal subjects. A growing number of

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mutations causing inherited POAG, as well as risk-associated polymorphisms for idiopathic POAG have been identified in extracellular matrix genes and genes that modulate extracellular matrix (reviewed in (Vranka et al., 2015)).

Aging is a major risk factor for idiopathic POAG (Barany, 1966; Rohen et al., 1973; Rohen et al., 1967; Rohen, 1982). Progressive accumulation of damaged and cross-linked proteins is a hallmark of aging tissues and has been proposed to play a causal role in the tissue abnormalities associated with aging and age-related diseases. With regard to idiopathic forms of POAG, it has been proposed that damaged proteins accumulate in the trabecular meshwork cells due to an increase in oxidative and other stresses, as well as from a functional decline in the cellular proteolytic machinery that eliminates damaged proteins (Liton et al., 2009). In addition, a recent study revealed that autophagy is impaired in trabecular meshwork cells isolated from glaucomatous eyes (Porter et al., 2015). A study using a mouse model of SIOH/SIG provides evidence for similar mechanisms (Zode et al., 2014).

5. Morphology

The literature comparing ultrastructure of the conventional outflow pathway in young eyes, to aging eyes and eyes with POAG has been reviewed in several comprehensive articles (Gabelt and Kaufman, 2005; Overby and Clark, 2015; Tektas and Lutjen-Drecoll, 2009). Changes are seen in both cases, but appear earlier in POAG. Thus there is a progressive decrease in the number of trabecular meshwork cells, as well as an increase in cell pigmentation, cell detachment from trabecular beams, and fusion between adjacent beams. The number of sheath-derived- or “SD”-plaques, which are thickenings associated with the sheathed fibers of the cribriform plexus, increase with aging and POAG. This is due to fine fibrils and other components of the extracellular matrix that adhere to the sheaths of the elastic fibers and their connections to the inner wall endothelium of Schlemm’s canal. The plaques consist of a core of elastic fibers surrounded by a sheath containing FBN1 (fibrillin-1) and MFAP1 (microfibrillar associated protein-1) (Gong et al., 1989; Lutjen-Drecoll et al., 1986; Ueda et al., 2002).

In POAG eyes, there is a significant correlation between the area occupied by SD-plaques in the trabecular meshwork and axon loss in the optic nerve. Interestingly however, there is no correlation between SD-plaques and increase in IOP. One possible explanation for these findings is that common factors are involved in SD-plaque formation in the trabecular meshwork and optic nerve pathology. However the evidence suggests that increased SD-plaque formation is secondary to the factors causing ocular hypertension in POAG, thus not causative itself (Tektas and Lutjen-Drecoll, 2009).

Ultrastructural changes in the conventional outflow pathways of aging or POAG eyes were also compared to changes in eyes diagnosed with SIG (reviewed in (Tektas and Lutjen-Drecoll, 2009). Differences were described as “profound” (Johnson et al., 1997). Like POAG, a fine fibrillar material is seen to accumulate in the subendothelial region of Schlemm’s canal, however these fibrils do not adhere to the sheath of the elastic fibers to form SD-plaques, but are deposited underneath the inner wall endothelium. Most prominent

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is an accumulation of basement membrane-like material deposited in a "fingerprint" pattern in the outer trabecular meshwork beams, but not underneath the inner wall of Schlemm's canal endothelium. Immunohistochemical analysis later showed that this material contained type IV collagen, heparin sulfate proteoglycan and fibronectin (Tawara et al., 2008). Most recently, an increased basement membrane length was described underlying the inner wall endothelium of Schlemm's canal, and a similar change was observed in a mouse model of SIOH that positively correlated with decreased outflow resistance (Overby et al., 2014).

Another interesting observation is that many cells in the juxtacanalicular trabecular meshwork of eyes diagnosed with SIG had an activated appearance resembling "myofibroblasts" (Johnson et al., 1997). Characteristic of fibrotic wounds, myofibroblasts express a specialized form of actin called ACTA2 (α-smooth muscle actin) that is also expressed by smooth muscle cells. ACTA2-positive cells were also observed along the outer wall of Schlemm's canal in the mouse model of SIOH mentioned above (Overby et al., 2014). Myofibroblasts are a hallmark of fibrosis (discussed more in the next section).

It should be emphasized that eyes analyzed in the studies reported above were all diagnosed with SIG. In fact, in most cases, withdrawal of glucocorticoids reverses the elevation of IOP that occurs in sensitive individuals. This suggests that the fibrotic phenotype described above may be reserved for the most serious cases. A clinical impression from our team is the observation of complete fibrosis of the trabecular meshwork and inner wall of Schlemm's canal when this area is un-roofed during non-penetrating filtration surgery in human eyes with long standing steroid induced glaucoma after corneal transplantation (F. Price, unpublished).

6. Cell Biology

Cultured primary trabecular meshwork cells and immortalized cell lines (e.g., (Filla et al., 2002; Pang et al., 1994)) have been used to investigate the cell biology of POAG and SIOH. For functional follow-up, the human anterior segment perfusion model, which recapitulates outflow regulation *in vivo* (Johnson and Tschumper, 1987), has been employed. Many of the changes seen in the trabecular meshwork of human eyes treated with glucocorticoids are also seen in eyes perfused with glucocorticoids *in vitro* (Clark et al., 1995b) and can be mimicked in cultured trabecular meshwork cells (Filla et al., 2011; Gagen et al., 2014; Gagen et al., 2013). This provides evidence for a direct action of glucocorticoids on the trabecular meshwork (rather than a systemic action).

Numerous studies have demonstrated that treatment of cultured trabecular meshwork tissues or cells with glucocorticoids directly stimulates expression and deposition of specific basement membrane type extracellular matrix. These findings are consistent with the morphological changes of basement membrane type extracellular matrix deposition in SIOH leading to SIG discussed above in the Morphology section. Proteins deposited include LAMA1 (laminin-1) (Dickerson et al., 1998), LAMA5 (laminin-5) (Filla et al., 2014) and COL4A1 (collagen type IV A1) (Zhou et al., 1998), as well as other extracellular matrix components including FN1 (fibronectin) (Steely et al., 1992; Zhou et al., 1998), TSP1 (thrombospondin-1) (Flugel-Koch et al., 2004), and ELN (elastin) (Yun et al., 1989).

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Glucocorticoid treatment further increases the resistance of glycosaminoglycans to enzymatic degradation (Johnson et al., 1990) and reduces hyaluronan synthesis (Engelbrecht-Schnur et al., 1997).

Correspondingly, glucocorticoid treatment inhibits expression of several MMPs and other proteinases that have been implicated in the normal turnover of the extracellular matrix thought to be important for regulation of aqueous outflow (el-Shabrawi et al., 2000; Samples et al., 1993; Snyder et al., 1993). Borrás and colleagues reported a gene therapy strategy for IOP reduction in a sheep model for SIOH/SIG that makes use of a recombinant adenoviral vector for the production of steroid-induced MMP1 in the trabecular meshwork (Spiga and Borras, 2010). More recently, they conducted a trial transferring the same transgene cassette to a clinically safe adeno-associated viral vector, and extended the therapeutic outcome to longer periods of times (Borras et al., 2016).

Together these findings provide support for the idea that dysregulated and excessive extracellular matrix deposition is a pathophysiological factor in SIOH/SIG.

In 1994, Clark and colleagues (Clark et al., 1994) reported their observation that glucocorticoids also affect the organization of the actin cytoskeleton and induce the formation of cross-linked actin networks in trabecular meshwork cells in culture. They called these structures “CLANs” (reviewed in (Clark and Wordinger, 2009; Wordinger and Clark, 1999)). CLANs form, not only in glucocorticoid-treated trabecular meshwork cells in culture, but also in glucocorticoid-treated perfused human anterior segments as they develop increased outflow resistance (Clark et al., 2005). CLANs have also been found in POAG eyes. Interestingly, CLANs are more prevalent in trabecular meshwork cells isolated from glaucomatous eyes versus normal eyes, and glucocorticoid-induced CLAN formation is higher in trabecular meshwork cells isolated from glaucomatous eyes versus normal eyes (Clark et al., 1995a). CLAN-like structures are found in the trabecular meshwork and inner wall of Schlemm’s canal in situ, with higher levels in glaucomatous eyes (Hoare et al., 2009; Read et al., 2007). They can also be found in cells of the lamina cribrosa of glaucomatous optic nerve as well as dexamethasone-treated dissected tissue (Job et al., 2010). These observations suggest a role in glaucoma pathology.

Peters and colleagues have published a number of sequential reports defining CLAN formation and structure (reviewed in (Filla et al., 2016; Gagen et al., 2014). CLANs are likely to be the same as the actin geodesic dome structures, first described by Lazarides and Burridge (Lazarides and Burridge, 1975), and may be a precursor structure to stress fibers. These cross-linked actin networks contain a nidus of signaling proteins including SDC4 (syndecan-4), PIK3R1 (Phosphatidylinositol 3-kinase regulatory subunit α) and PDLIM1 at the regions where actin filaments intersect, suggesting that they are more than a structural component and may represent a signaling mechanism to sense changes in IOP. CLANs are regulated by interactions with the extracellular matrix through integrins especially the αvβ3 integrin (Filla et al., 2011; Filla et al., 2009; Filla et al., 2006), which is upregulated and activated by dexamethasone (Clark et al., 2013; Faralli et al., 2013) and syndecans (Filla et al., 2014) in cultured trabecular meshwork cells. This group showed that a number of integrin binding matrix proteins upregulated by glucocorticoid treatment of trabecular

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meshwork cells increase CLAN formation (Filla et al., 2006). Among these proteins are TSP1 and FN1 (Filla et al., 2009) as well as LAMA5 (Filla et al., 2014). These studies demonstrate that changes in extracellular matrix deposition stimulated by glucocorticoids control CLAN formation, providing support for the idea that CLANs contribute to the development of ocular hypertension.

It is also likely that at least some of the cells in which CLANs are visualized may evolve into the myofibroblast-like cells identified in trabecular meshwork of eyes diagnosed with SIG (Johnson et al., 1997) (as introduced in the preceding section). Characteristic of fibrotic wounds, myofibroblasts contain more stress fibers (containing bundles of actin filaments and non-muscle myosin) than other fibroblasts, and exert more tension on the surrounding extracellular matrix. Myofibroblasts transdifferentiate from the fibroblasts that proliferate and deposit extracellular matrix in repairing wounds; they are thought to play an important role in wound contracture (Gabbiani et al., 1972; Tomasek et al., 2002). It is suggested that the myofibroblast-like cells in eyes with SIOH leading to SIG may transdifferentiate from normal trabecular meshwork cells. This transition may be the result of changes in the composition of focal adhesions and actin binding proteins associated with stress fibers, both of which are major targets for dexamethasone induced changes in protein expression (Clark et al., 2013).

Although, stress fibers are normally thought to transverse the cell and are anchored at one or both ends by focal adhesions, another type of stress fiber is also found in endothelial cell types such as Schlemm's canal cells. These stress fibers are similar to those found in fibroblasts except that – rather than inserting into focal adhesions – they are inserted into the adherens junctions that link endothelial cells together (Millan et al., 2010). These junctional stress fibers are postulated to help cells withstand mechanical stress and regulate endothelial permeability. The association of actin filaments with cell-cell junctions in endothelia is critical for the regulation of barrier function (discussed in (Burridge and Wittchen, 2013)). Some years ago, glucocorticoid treatment was shown to increase barrier function of a cultured trabecular meshwork cell monolayer through effects on cell-cell junctions (Underwood et al., 1999). Much more recently, it was shown that the tight junction-associated protein ZO1 and the gap junction protein GJA1 were also increased in glaucomatous trabecular meshwork cells in culture (Zhuo et al., 2010).

Stress fibers are prominent in cells in culture, but are only rarely seen in tissues. As noted above, they are found in wound tissues, and they have also been observed in endothelial cells, particularly those lining arteries exposed to high velocity flow (Wong et al., 1983) or in hypertensive animals (White et al., 1983). Cells in these situations are all experiencing mechanical forces (discussed in (Burridge and Wittchen, 2013)). This raises the question as to whether actin stress fibers and CLANs are normal features of conventional outflow cells, but increase in prominence as a result of the ocular hypertension, which would be consistent with the greater mechanical stress that develops with steroid treatment; or whether CLANs are part of the mechanism *causing* ocular hypertension.

A number of studies have shown that treatment with cytoskeletal-altering agents that affect cell shape, adhesion, and focal adhesions of cells in culture, also enhances aqueous outflow

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and lowers IOP. These agents include actin microfilament disrupting agents such as cytochalasin and latrunculin, nitric oxide (Chang et al., 2015; Dismuke et al., 2014; Stamer et al., 2011), and protein kinase inhibitors such as H7 (14667835) or Rho kinase inhibitors (Cai et al., 2000; Honjo et al., 2001; Sabanay et al., 2004; Tian et al., 2000; Vittitow et al., 2002). Microtubule-disrupting agents, such as ethacrynic acid, have similar effects (Erickson-Lamy et al., 1992; Johnson and Tschumper, 1993; Liang et al., 1992; Melamed et al., 1992; O'Brien et al., 1996; Tingey et al., 1992). Several Rho kinase inhibitors are FDA approved or are currently in clinical trials for ocular hypertension (Kopczynski and Epstein, 2014). Recently a gene therapy approach was demonstrated to be effective. Thus inhibition of the RHOA pathway in trabecular meshwork by a dominant negatively acting RHOA gene delivered via recombinant viral vector reduced nocturnal elevated IOP in a living rat (Borras et al., 2015a).

7. Biomechanics

The past decade has seen increasing interest in the biomechanical properties of the trabecular meshwork with relation to outflow resistance in ocular hypertension and glaucoma (reviewed and discussed in (Braunger et al., 2015; Stamer et al., 2015)). Organ stiffness can be evaluated using atomic force microscopy, which measures the indentation of the surface of tissue sections or whole mounts by a cantilever (Engler et al., 2007). Soft tissues are relatively elastic in behavior, and can be characterized by the Young's elastic modulus E, i.e., the force per area (stress) that is required to strain an elastic material (Janmey et al., 2007). Using atomic force microscopy, it was demonstrated that trabecular meshwork tissue in POAG eyes is stiffer than in normal human eyes (Last et al., 2011; Russell and Johnson, 2012). Relevant to SIOH, it was shown that treatment of normal human eyes with glucocorticoids in vitro resulted in a 2-fold increase in trabecular meshwork cell stiffness, and the matrix deposited by these cells was approximately 4-fold stiffer (Raghunathan et al., 2015). Similarly, topical glucocorticoid treatment in live rabbits resulted in a 3.5-fold increase in trabecular meshwork stiffness.

A defining characteristic of the fibrotic scar that develops in soft organs is its high stiffness compared to the surrounding tissue. This is determined by cytoskeletal reorganization, the deposition of extracellular matrix, and myofibroblast differentiation (discussed in (Hinz, 2015)). The actin geodesic dome structures that form in cultured cells were recognized by Ingber in his tensegrity model (Ingber, 2003), which is based on the architectural principles articulated by R. Buckminster Fuller. Tensegrity architecture can produce exceptionally rigid structures for their mass and for the cross section of the components. Myofibroblasts play a crucial role in maintaining constructive tension in a tissue. It seems likely that trabecular meshwork tissue stiffening in POAG, and in response to glucocorticoids, might be similarly determined (discussed in (Pattabiraman and Rao, 2010)).

Another factor in tissue biomechanics is exemplified by the aging vasculature, where stiffening occurs through a process of calcification. Interestingly, POAG patients exhibit elevated activity of ALP (alkaline phosphatase), a calcification marker, and reduced levels of MGP (matrix gla protein), a potent inhibitor of calcification. Treatment of normal trabecular meshwork with glucocorticoids also increased ALP activity and decreased MGP levels;

siRNA-mediated knockdown of MGP mRNA resulted in a significant increase in ALP activity (Xue et al., 2007). These findings suggest that a process of calcification may contribute to stiffening of the trabecular meshwork tissue in both POAG and SIOH (Borras et al., 2015b).

8. Intracellular Signaling

A hallmark of ocular hypertension leading to POAG is dysregulation of intracellular signaling networks controlled by secreted cytokines. Three major pathways have been implicated: TGF-β superfamily signaling, Wnt signaling, and inflammatory cytokine signaling. As discussed below, excessive signaling by these pathways can lead to ocular hypertension in perfused human anterior segments or in mouse models, but this does not prove that they are involved in the pathogenesis of ocular hypertension. To establish their significance to POAG or SIOH will require knockdown of pathway components in the perfused human anterior segment model, and knockdown or knockout in a mouse model.

Several comprehensive reviews discuss the evidence implicating signaling by members of the TGF-β superfamily in POAG (Fuchshofer and Tamm, 2009, 2012; Wordinger et al., 2014). TGF-β signaling was first proposed based on the observation that TGFB2 protein is elevated in the aqueous humor of POAG eyes (Lutjen-Drecoll, 2005; Picht et al., 2001). More recently, the TGF-β superfamily member BMP4 has been proposed as a TGFB2 antagonist in POAG pathogenesis (Wordinger et al., 2007).

TGFB2 is one of three human genes encoding TGF-β family proteins; TGFB2 is the family member characteristic of the eye (discussed in (Fini and Stramer, 2005)). Trabecular meshwork cells express *TGFB2* (Fuchshofer et al., 2005), which regulates a number of cell behaviors in the eye linked to wound healing and fibrosis, such as extracellular matrix deposition and myofibroblast transformation (e.g., (Fini and Stramer, 2005; Stramer et al., 2003). Treatment of trabecular meshwork cells with a dose of TGFB2 comparable to the level associated with POAG increased expression of the extracellular matrix protein FN1 (fibronectin), as well as PAI, a proteinase inhibitor that prevents activation of pro-MMPs (Fuchshofer et al., 2003). In one study, TGFB2 treatment of perfused human anterior segments increased deposition of extracellular matrix in the trabecular meshwork and increased outflow resistance (Gottanka et al., 2004) and in a second study it induced CLAN formation (Yuan et al., 2013). Transfer of a TGF-β gene to the trabecular meshwork of perfused human anterior segments (Fuchshofer and Tamm, 2012) or live mice (Robertson et al., 2010; Shepard et al., 2010) reduced aqueous outflow and increased IOP.

More recent studies implicate Wnt signaling (Mao et al., 2012; Wang et al., 2008). Expression of the canonical Wnt pathway antagonist SFRP1 was increased in trabecular meshwork cell lines from POAG patients and transfer of an SFRP1 gene to perfused human anterior segments increased IOP. Also importantly, Wnt pathway inhibition was shown to induce a persistent increase in intrinsic stiffness of human trabecular meshwork cells (Morgan et al., 2015). Treatment of trabecular meshwork cells with glucocorticoids stimulates expression of the non-canonical Wnt ligand WNT5A and treatment of trabecular meshwork cells with WNT5A induces CLAN formation (Yuan et al., 2013). Wnt signaling

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and TGF-β signaling are known to crosstalk (Attisano and Wrana, 2013), suggesting interaction of these pathways could be important in the pathogenesis of ocular hypertension.

Human aging is characterized by a chronic, low-grade inflammation, a phenomenon that has been termed "inflammaging" (Baylis et al., 2013; Franceschi and Campisi, 2014). Most, if not all age-related diseases share an inflammatory pathogenesis. About 15 years ago, our team identified expression of the inflammatory marker endothelial leukocyte adhesion molecule-1 (ELAM-1), also known as SELE (e-selectin), as a defining feature of the diseased phenotype of the trabecular meshwork in both open and closed angle forms of high-tension glaucoma (Wang et al., 2001). We further showed that SELE expression is activated by an IL1A/Nuclear Factor κB (NF-κB) inflammatory pathway that is constitutively active in trabecular meshwork tissue from eyes with high-tension glaucoma. We subsequently showed that the response further involves constitutive activation of MAP kinases downstream of the IL-1 receptor (Zhang et al., 2006b).

Interleukin 1 (IL-1) (i.e., IL1A or IL1B) had previously been demonstrated to stimulate aqueous outflow in rat, rabbit, and human models (Bradley et al., 1998; Kee and Seo, 1997). It has been suggested that this occurs through stimulation of MMP expression (Bradley et al., 1998; Samples et al., 1993), or by directly increasing paracellular permeability across Schlemm's canal (Alvarado et al., 2005). Thus activation of inflammatory signaling is compensatory, acting to reverse the increase in IOP. We further demonstrated that binding of IL1A to its cell surface receptor protects trabecular meshwork cells against oxidative stress (Wang et al., 2001). However, we proposed that chronic activation of the pathway would lead to pathology, as occurs in so many other age-related diseases. In contrast to acute inflammatory reactions, characterized by neutrophil infiltration of the inflamed tissue, chronic inflammation usually leads to fibrosis (reviewed in (Wynn, 2008)). This suggests a model in which chronic low-grade inflammation elevates TGF-b levels and deposition of fibrotic extracellular matrix, thus decreasing aqueous outflow and leading to ocular hypertension.

Our findings about IL-1, inflammatory signaling pathway activation and SELE have been confirmed in several other labs (Birke et al., 2011; Diskin et al., 2006; Li et al., 2007; Liton et al., 2006; Suarez and Vecino, 2006). Significance was recently extended to inflammatory cytokine IL20 and signaling through Jak/Stat, identified as causal in an inherited form of POAG (Keller et al., 2014; Wirtz and Keller, 2016).

As should be evident from the discussion above, IL-1 and TGF-β typically have opposing actions on the extracellular matrix; the former stimulates matrix degradation, while the latter stimulates matrix deposition (e.g., (Fini et al., 1987; West-Mays et al., 1999)). As a natural feedback system controlling the inflammatory response, glucocorticoids inhibit the expression and action of most inflammatory cytokines. However, glucocorticoids have a variable effect on "repair phase" cytokines like TGF-β and PDGF, expression and activity of which may remain unaffected, or may even be up-regulated (Brattsand and Linden, 1996). These observations suggest that exploration of the effects of glucocorticoids on the balance between IL-1 and TGF-β signaling in the trabecular meshwork could yield information useful to our understanding of SIOH.

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9. Glucocorticoid Receptor

Over the years since glucocorticoids were first identified, an increasingly complex network of protein-protein interactions mediating their mechanism of action has been uncovered, including both classic actions at the genomic level, as well as more direct, non-canonical actions. For a comprehensive update, the reader is referred to a recently published review (Ramamoorthy and Cidlowski, 2016). The classic, genomic actions of glucocorticoids are mediated through the glucocorticoid receptor, a member of the nuclear receptor family of ligand-activated transcription factors. The gene NR3C1 encodes the glucocorticoid receptor. The receptor predominantly resides in the cytoplasm of cells as part of a large multiprotein complex that includes chaperone proteins (HSP90, HSP70, and PTGES3) and immunophilins (FKBP4 and FKBP5). On binding to its ligand, the receptor undergoes a conformational change. This results in the dissociation of the multiprotein complex and rapid translocation into the nucleus through nuclear pores. The activated receptor binds to the glucocorticoid response element in the promoter/enhancer regions of many genes. Binding induces conformational changes in the receptor leading to recruitment of co-regulator and chromatin-remodeling complexes, activating gene transcription.

Apart from driving gene transcription by binding onto glucocorticoid response elements in regulatory regions of particular target genes, the activated glucocorticoid receptor can also inhibit gene expression through transrepression (reviewed in (Ramamoorthy and Cidlowski, 2013)). Most of the anti-inflammatory effects of glucocorticoids appear to occur through this important negative regulatory mechanism, in which the activated glucocorticoid receptor is recruited to chromatin by protein-protein interactions with DNA-bound transcription factors, particularly NF-κB and AP-1 (activating protein-1). The activated glucocorticoid receptor can further modulate TGF–β signaling both positively (e.g., (Peltier et al., 2003)) and negatively (e.g., (Song et al., 1999)) through protein-protein interactions.

The glucocorticoid receptor can exist in multiple isoforms generated via alternative splicing (Oakley and Cidlowski, 2011). The classic cytoplasmic receptor subtype is called glucocorticoid receptor-α (GRα). A second subtype called GRβ does not bind ligand or activate glucocorticoid-responsive reporter genes. In the presence of GRα, GRβ functions as a dominant negative inhibitor and antagonizes GRα activity on many glucocorticoid-responsive target genes. GRβ can also directly induce and repress a large number of genes independent of its interaction with GRα. The GRβ-GRα ratio may be regulated by the splicosome (Zhu et al., 2007). Elevated GRβ has been associated with systemic resistance to glucocorticoids in connection with inflammatory conditions and mood disorders, and GRβ levels can be increased by inflammatory cytokines (reviewed by (Lewis-Tuffin and Cidlowski, 2006)). In contrast, trabecular meshwork cell strains from POAG patients have a lower GRβ-GRα ratio compared to their normal counterparts, correlating with increased glucocorticoid responsiveness; over-expression of GRβ rendered them less responsive (Jain et al., 2012; Zhang et al., 2006a; Zhang et al., 2005). Thus a lower than normal GRβ-GRα ratio may contribute to the greater sensitivity to glucocorticoids exhibited by POAG patients, as recently reviewed (Jain et al., 2014).

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10. Myocilin

Since the primary action of glucocorticoids is to control gene expression, identification of major induced genes could provide insight into the pathophysiology of SIOH and SIG, which could potentially be extended to idiopathic POAG. The trabecular meshwork-inducible glucocorticoid response (TIGR) gene product was isolated by Polansky and colleagues through the use of differential cDNA library screening using selection criteria based on the gene expression induction pattern after prolonged but not brief exposure to glucocorticoids (Polansky et al., 1997). The very large, progressive induction of TIGR, combined with specific structural features of its cDNA, suggested that TIGR should be considered as a candidate gene for outflow obstruction/regulation in glaucoma (Nguyen et al., 1998).

Excitingly, even before publication of the report on its cloning, the gene encoding the TIGR protein was determined to be identical to the gene mutated at the GCL1A locus identified by genetic linkage analysis in POAG family studies (Stone et al., 1997). The gene is now called *MYOC* (myocilin). The known disease-causing mutations are catalogued in an online database (http://www.myocilin.com) (Hewitt et al., 2008). At the time this article was submitted for publication, 273 variants were recognized. It has been estimated that "myocilin glaucoma" accounts for 2–4% of all cases of POAG (Alward et al., 1998; Fingert et al., 1999; Gong et al., 2004; Tamm, 2002).

As the first gene for which coding mutations were linked with POAG, *MYOC* stimulated a surge of activity in the research community. The function of MYOC protein in normal biology was not known when its causative role in POAG was discovered, and frustratingly, its normal function in cells remains poorly understood to this day. *MYOC* is expressed in several tissues of the body, but to our knowledge, mutations cause disease only in the eye (Borras, 2014; Tamm, 2002). Haploinsufficiency has been excluded as the primary mechanism for how *MYOC* mutations cause POAG (Fingert et al., 2002). Moreover, polymorphisms that increase or decrease expression of *MYOC* do not cause POAG (Gould et al., 2004; Pang et al., 2002). Therefore, a gain-of-function disease mechanism was hypothesized, meaning the mutation creates new activities that cause pathology (Kim et al., 2001; Lam et al., 2000; Wiggs and Vollrath, 2001). Consistent with this idea, most mutant MYOC protein forms are misfolded and aggregate in the endoplasmic reticulum, activating the unfolded protein response. This suggests that activation of this stress response is the gain of function that leads to elevated IOP (Joe et al., 2003; Kwon et al., 2009). Specific MYOC mutations may lead to different amounts of MYOC misfolding, with corresponding varying degrees of recognition by the ubiquitin degradation pathway.

In seeming contradiction to these results discussed above, transgenic expression of mouse *Myoc* with a mutation orthologous to the non-secreted human Y437H mutation (also non-secreted in mice) was not very effective in causing elevated IOP (Gould et al., 2006; Senatorov et al., 2006). This argues against non-secretion as the fundamental mechanisms leading to pathology. Interestingly, mice over-expressing the mouse version of the Y437H mutant develop ocular hypertension and glaucoma (Zhou et al., 2008). Therefore, Clark and colleagues hypothesized that comparison of the human and mouse sequences might be

revealing of mechanism. Indeed, it was found that human MYOC, unlike mouse, contains a cryptic peroxisomal targeting signal type 1 (PTS1). POAG-associated mutations activate the signal, which is critical for mutant MYOC-induced toxicity in trabecular meshwork cells (Shepard et al., 2007). One theory for the toxicity mechanism is that interaction of human MYOC Y437H protein with the PTS1 receptor results in poorer clearance from the endoplasmic reticulum, leading to greater trabecular meshwork cell dysfunction and culminating in a higher IOP phenotype (Shepard et al., 2007). A caveat is that fold-changes in expression between different MYOC genes in different mouse lines cannot be directly compared, thus confounding analysis. Alternative hypotheses have been proposed to explain the deleterious cellular effects of MYOC mutations, such as altered association kinetics and co-localization with proteins in the endocytic pathway (e.g. (Hardy et al., 2005; McKay et al., 2013)).

As mentioned above, the late glucocorticoid-induction pattern for *MYOC* matches the time course of IOP elevation in patients receiving glucocorticoid treatment. Peters and colleagues have published evidence that the delayed glucocorticoid induction of the normal *MYOC* gene appears to be due to secondary activation of an inflammatory signaling pathway involving the protein phosphatase PPC3C (calcineurin) and transcription factor NFATC1 (Faralli et al., 2015). Moreover, our team showed that over-expression of both wild type *MYOC* and *MYOC* mutants induces inflammatory cytokine expression (Itakura et al., 2015) and over-expression of wild type *MYOC* and *MYOC* mutants also stimulates Wnt signaling (Shen et al., 2012). Thus *MYOC* is linked to activation of signaling pathways altered in both POAG and SIOH. It should be emphasized however, that no evidence has ever been found to implicate POAG-causing *MYOC* mutations in risk for SIOH and SIG (Fingert et al., 2001). In fact, many investigators regard *MYOC* as a “red herring”. Comparison of the results of glucocorticoid treatment in wild type and *MYOC* knockout mice could provide a more definitive answer to the question about whether MYOC protein plays any role in SIOH or SIG.

11. Gene Expression Profiling

Gene expression profiling is a technique that can be used to compare untreated and steroid-treated cells or tissues with the goal of revealing differences that might be clinically significant. Once gene expression microarrays became commercially available, publications began to appear comparing expression profiles in normal and glucocorticoid-treated trabecular meshwork cells and tissues (for example, (Fan et al., 2008; Ishibashi et al., 2002; Leung et al., 2003; Lo et al., 2003; Rozsa et al., 2006); reviewed in (Borras, 2008)). Results of these early profiling attempts were quite variable. This might be attributed to differences in the microarrays used (which were not very comprehensive at that time), differences in the individual responsiveness of each cell strain, and differences in the particulars of glucocorticoid treatment

A variety of synthetic glucocorticoids, some far more potent than the biologic standard, cortisol, have been created for therapeutic use, and they differ in both pharmacokinetics and pharmacodynamics. A study from the lab of one of our team members, funded by Allergan Pharmaceuticals (Republic of Ireland), used gene expression microarrays to compare the

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effects of dexamethasone, fluocinolone acetonide and triamcinolone acetonide of two primary human trabecular meshwork cell strains (Nehme et al., 2009). It was found that the subtly different chemical structures of the various glucocorticoids regulated common and unique gene subsets, which presumably would reflect different biological responses. A second study funded by GlaxoSmithKline (Brentford, London, U.K.) compared effects of dexamethasone, prednisolone and GW870086X on expression of the specific genes relevant to outflow resistance regulation: *FN1*, *MYOC*, *PLAT*, and *MMP2*. Again, the different compounds had different effects on gene expression (Stamer et al., 2013). Significantly, GW870086X is a selective glucocorticoid receptor agonist, having greater trans-repressive versus transactivation effects than traditional compounds like dexamethasone.

A recent study used gene expression profiling and Ingenuity Pathway Analysis to identify gene networks that are affected in the TBM when IOP is elevated by glucocorticoid perfusion of bovine anterior segments (Danias et al., 2011). The canonical pathway that came out on top was integrin signaling, including a number of ECM genes. This fits with observational and functional studies described in previous sections of this article. Two of the top ten networks contained genes that have been previously linked to OH in glaucoma. For example, one of the genes was *MAPK14* (p38 MAPK), a protein kinase, which our own group found to be activated in glaucomatous TBM cells, associated with constitutive inflammatory cytokine signaling (Zhang et al., 2006b). Interestingly, there were also genes associated with pathways involved in normal regulation of aqueous outflow. An example is *ADRB2*, a GPCR whose primary ligand, epinephrine, stimulates aqueous outflow facility (Alvarado et al., 1998; Erickson-Lamy and Nathanson, 1992; Wang et al., 2002).

The steroid responder rate has been reported as 30% for perfusion cultured human anterior segments, similar to the eye *in situ* (Clark et al., 1995b). Similarly a 40% steroid responder rate was observed for bovine anterior segments (Mao et al., 2011). However, as handled by this group, the perfusion cultured bovine anterior segments exhibited a 100% steroid responder rate (This was also found to be the case for sheep (Gerometta et al., 2009) and for specific inbred mouse lines (e.g., (Overby et al., 2014)). The discrepancy is unexplained at this time. With a 100% response rate however, we can be assured that the genes identified as induced by glucocorticoids are genes induced in eyes that experience an IOP increase. Nevertheless, as discussed above, gene expression studies such as this one still cannot distinguish which of the steroid effects on gene expression are *causative* of IOP elevation. Regardless, the results are valuable, as defining these networks provides a framework for confirming that individual genes are involved in a process, for exploring how related genes are also affected, and ultimately for pinpointing key molecules that might affect the behavior of the whole network.

12. Pharmacogenomics

12.1. Cohort Enrollment and Quantitative Trait

The limitation of expression profiling is that it provides only correlative associations as only a subset of the genes whose expression changes are likely responsible for outflow facility impairment and IOP elevation. In contrast, genetic approaches make a direct link between the gene and the pathology. About a decade ago, our group began to co-enroll patients

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treated with IVTA for retinal disease into a genetic study aimed at understanding why some individuals are steroid responders. This became known as the “Florida-1” cohort.

To phenotype Florida-1 subjects, IOP was recorded in the study eye prior to IVTA injection (baseline IOP). On each subsequent clinic visit, IOP was also recorded (follow-up IOP). This was done for up to 1 year, or until the study eye met any of several pre-specified termination criteria, which included the initiation of a drug intended to lower IOP. A blood sample was collected from each of the research subjects and DNA was extracted and stored for subsequent analysis. The change in IOP following glucocorticoid treatment (“ IOP”, defined as the maximum post-treatment IOP minus the baseline IOP, with a positive value indicating a rise in IOP following injection of IVTA) was determined for all subjects. For this study, a “steroid responder” was defined, according to Armaly and Becker (Armaly and Becker, 1965) as an individual with normal baseline IOP (12–22 mm Hg) with a positive IOP > 6 mm Hg above baseline IOP following injection of IVTA. By this criterion, 51% of the subjects enrolled in the Florida-1 cohort were steroid responders. However, splitting patients into cases and controls is an artificial determination, as steroid response occurs on a continuous spectrum. Figure 2 demonstrates for patient data from the Florida-1 cohort (this is the first time the data are presented graphically, but were previously published (Gerzenstein et al., 2008)). Steroid response (i.e., IOP) is a quantifiable trait (QT). This provides an important advantage in genetic association studies, as the phenotype can be treated as a continuous variable, and a cohort study design can be employed, which is statistically powerful (Plomin et al., 2009).

12.2. Candidate Gene Analysis

When we began our analysis of the Florida-1 cohort, there had been no reports on genetic variants associated with steroid response in the eye since the late 1970s. However, studies on systemic sensitivity to steroid response had been ongoing. As an initial approach, candidate gene strategy was used to analyze polymorphisms in genes known to be associated with systemic steroid response. Many polymorphisms in NR3C1 have been linked to systemic steroid resistance (Stevens et al., 2004; van Rossum and Lamberts, 2004). It was hypothesized that these polymorphisms could also predispose to SIOH. Our team performed statistical association tests for six candidate polymorphisms. All results were negative (Gerzenstein et al., 2008). A similarly designed study utilizing a cohort treated with glucocorticoids following photorefractive keratectomy, which was published as ours was in press, reported a correlation between N363S heterozygosity and glucocorticoid response in a subgroup treated with 0.5% prednisolone acetate (Szabo et al., 2007). The size of the subgroup was extremely small however. A study published two years later (Fingert et al., 2010), utilizing a case-control design with 400 normal controls and 107 steroid responders, similarly analyzed known protein coding polymorphisms in the glucocorticoid receptor NR3C1 as well as in the genes encoding the immunophilins FKBP4 and FKBP5 and the Wnt ligands SFRS5 and SFRS9. Again no significant associations were found. A later study analyzing SFRS3 and FKBP4 also found no association (Hogewind et al., 2012). Most recently, a study analyzing SNPs in NR3C1, FKBP5 and MYOC (myocilin) reported negative results again (Hogewind et al., 2015). This does not mean that the products of these candidate genes are not involved in the pathogenesis of glucocorticoid-induced ocular

hypertension, but it does not appear that major heritable risk alleles in these genes define a responder population.

12.3. Genome Wide Association Study

In contrast to candidate gene studies, genome wide association studies (GWASs; (defined as at least 100,000 SNPs) examine hundreds of thousands, to millions of SNPs densely spaced across the entire human genome. The large number of hypothesis tests in GWASs creates a burden for multiple testing adjustments, and typically requires a stringent cutoff for declaring statistical significance. A well-accepted genome-wide significance level is a p-value of 5×10^{-8}, assuming one million independent tests in the human genome (Pe'er et al., 2008; Risch and Merikangas, 1996). As a result, GWASs of complex human diseases usually require large cohorts (Gao, 2011), because these diseases involve many different genes, each responsible for only a small genetic effect. Nevertheless, while we were conducting our candidate gene analysis, the very first success in GWAS was published (Klein et al., 2005). A polymorphism in the *CFH* (complement factor H) gene associated with age related macular degeneration was identified in a small patient cohort of only 96 cases and 50 controls. The explanation for why the small cohort was sufficient is that the polymorphism in the *CFH* accounts for 40–50% of the population-attributable risk (Gorin, 2005).

While familial forms of high tension POAG can have quite large IOPs, idiopathic forms of POAG typically have much smaller increases. Existing GWAS data suggest that POAG is more complex genetically than age related macular degeneration, with no common risk alleles of large effect (Scheetz et al., 2013). Compounding this, there is variance in IOP among healthy individuals and an even larger variance among patients with glaucoma (Liu et al., 2011; Pekmezci et al., 2011). Thus, like most complex diseases, GWASs on ocular hypertension and POAG have required large cohorts to detect significant differences. Meta-analysis to combine multiple cohorts is also used, although this also introduces variability, including the use of different IOP measurement methods (Hysi et al., 2014; Ozel et al., 2014).

In contrast, drug response often manifests a large genetic effect (Harper and Topol, 2012), thus amplifying factors determining complex disease that might be hard to detect otherwise. Pharmacogenomics studies of only around 100 cases have detected polymorphisms of genome-wide significance (Daly, 2010). This is the case with SIOH. When glucocorticoids are administered in the eye, IOP increases substantially in a significant proportion of subjects. In our Florida-1 cohort (data graphed in Fig. 2), mean baseline IOP was 15.15±2.68 mm Hg and the mean maximum treated IOP was 23.69±7.35 mm Hg, representing a 56% increase. The highest responders (top 11.1%) exhibited a IOP range of 14–28 mm Hg with the maximum IOP recorded being 45 mm Hg. The lowest responders exhibited no response to glucocorticoid treatment, or a slightly negative response. Thus the genetic effect size for this cohort is quite large. Power analysis of the Florida-1 cohort supported the possibility that we could achieve a statistically significant result using a GWAS approach.

Florida-1 DNA samples were genotyped using the only GWAS platform available at the time, the original Affymetrix GeneChip® Mapping 500K Set. In a preliminary statistical

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analysis, a single SNP was identified with near genome-wide significance located in the first intron of a novel orphan G protein coupled receptor (GPCR) called GPR158. About 88% of GWAS hits are intronic or intergenic, concentrated in gene regulatory regions (Hindorff et al., 2009). In a follow-up functional analysis, it was found that GPR158 is expressed in trabecular meshwork cells and that glucocorticoid treatment stimulates expression. This was consistent with the idea that the identified SNP, or a genetic variant in linkage disequilibrium (LD) with the identified SNP, might alter GPR158 response to glucocorticoids, thus affecting the magnitude of glucocorticoid-induced IOP response.

GPR158 was of particular interest because several different GPCRs serve as targets for IOP lowering drugs (e.g., timolol, brimonidine, latanoprost) (Toris, 2010) and potentially, GPR158 could provide a new target of value. Once quality control was performed on our GWAS data, the p-value on the SNP in GPR158 was no longer significant at the GWAS level. Nevertheless it remained suggestive. In fact, GWAS is primarily a hypothesis-generating exercise and the most important evidence for a gene's role is provided by functional studies. Because the initial findings on GPR158 expression were consistent with SIOH, and because any characterization of a novel GPCR would be broadly valuable, we undertook more extensive molecular studies. We showed that over-expression of GPR158, by transient transfection of cultured trabecular meshwork cells, enhanced barrier function of a cell monolayer and increased tight junction proteins, similar to glucocorticoid treatment (Patel et al., 2013). These results are consistent with the proposed role in IOP regulation and ocular hypertension.

GPCRs sense information about the environment by binding extracellular ligands, and then transmitting this information into the cell by interaction with heterotrimeric G proteins, which affect the production of two principal 2nd messengers. GPCRs coupled to Gαs or Gαi/o proteins regulate adenylyl cyclase, activating (s) or inhibiting (i/o), respectively to alter levels of cAMP. GPCRs act as Guanine Nucleotide Exchange Factors (GEFs) for their cognate G proteins. GTPase-Accelerating Proteins (GAPs) are a family of regulatory proteins whose members can bind to activated G proteins and stimulate their GTPase activity, terminating the signaling event much more rapidly. GAPs are crucial for the very rapid signaling required for processes like visual transduction. GPCR activity is also terminated by endocytosis.

Ligand binding stabilizes the active conformation that allows the GPCR to activate its cognate Gα protein. Unliganded GPCRs constantly shift back and forth between an inactive and active conformation in the plasma membrane, meaning there is always a basal level of activity. Thus over-expression of a GPCR can be used to study its signaling when the ligand is unknown (Bond and Ijzerman, 2006; Menzaghi et al., 2002). However, there are now a number of examples of ligand-independent activity of orphan GPCRs (Levoye et al., 2006; Milligan, 2009). In fact, it appears that GPR158 is quite unusual in having two different unconventional activities that may be ligand-independent.

Martemyanov and colleagues identified the first novel activity (Orlandi et al., 2012). Working independently of our group, they discovered GPR158 as part of a screen for proteins interacting with members of the R7 family of GTPase Activating Proteins (GAPs)

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involved in visual transduction. They determined that GPR158 forms complexes in the brain with a dominant GAP protein called RGS7. The complex further contains the unusual Gβ protein encoded by GNB5, which is an obligatory subunit of RGS7, needed to maintain stability of both proteins. Among Gβ isoforms, GBN5 protein is unique in its ability to bind to R7 family GAPs. Interestingly, a similar complex also exists in retinal bipolar neurons, where closely related orphan receptor, GPR179 binds to RGS7 or its homolog RGS11. GPR179 recruits RGS7/11-GBN5 complexes to the plasma membrane at the dendritic tips of retinal bipolar cells (Orlandi et al., 2012), where it is positioned to rapidly inactivate signaling by nearby GPCRs that couple with Gαi/o proteins. In their most recent paper, the Martemyanov lab showed that GPR158 is necessary for the plasma membrane recruitment of RGS7 in the brain (Orlandi et al., 2015). These studies demonstrated a novel activity for GPR158 and GPR179 as plasma membrane scaffold proteins interacting with conventional GPCRs to modify their signaling (Orlandi et al., 2012). This could make GPR158 a uniquely effective drug target for ocular hypertension and glaucoma. One caveat for pharmaceutical targeting is that mutations in GPR179 have been demonstrated to cause night blindness (Audo et al., 2012; Peachey et al., 2012). It seems unlikely that GPR158 is also involved in visual transduction, as it does not appear to be expressed in the retina or optic nerve (Orlandi et al., 2012). Nevertheless, possible off-target effects must be kept in mind.

Our group identified the second novel activity for GPR158 (Patel et al., 2013). We demonstrated that newly synthesized GPR158 traffics to the plasma membrane, as is typical of GPCRs, but it is rapidly endocytosed and then exhibits the unusual behavior of translocation to the nucleus. Nuclear translocation is mediated via a classical bipartite nuclear localization signal (NLS) in GPR158's 8th helix, which is located in the intracellular domain (ICD) that projects into the cytoplasm. This is shown on the schematic diagram of the *GPR158*-encoded protein shown in Figure 3. Mutation of the NLS abrogated nuclear localization of GPR158 and also abrogated the GPR158-mediated enhancement of cell proliferation (Patel et al., 2013).

Sequence analysis places GPR158 in the GPCR glutamate family (Family C), which contains 22 receptor subtypes, many with important functions in the central nervous system (Bjarnadottir et al., 2005). Seven glutamate family receptors are orphans with no known ligands, including GPR158, which is one of three orphans of the GABAB-like branch of the family. Online datasets revealed that GPR158 is highly expressed in the brain, but also at lower levels in other organs.

We performed bioinformatics searches to find other steroid-regulated disorders that might involve GPR158. Analysis of datasets from the Oncomine bioinformatics website revealed 14 PCa expression microarray studies in which changes in GPR158 mRNA were identified. In *every study*, a positive correlation was observed between GPR158 expression and PCa progression. Analysis of datasets from 216 human PCa specimens in the cBio Cancer Genomics Portal revealed that GPR158 mRNA was altered in 9% of cases and upregulated in 8%. Up-regulation was associated with significantly lower rates of disease-free survival. We went on to characterize the role of GPR158 in prostate cancer experimentally. These findings on GPR158 in PCa were recently published (Patel et al., 2015). The paper was

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spotlighted in *Nature Reviews Urology*, chosen because of the promise of GPR158 as a potential new drug target (Fenner, 2015).

Following our analysis of the Florida-1 cohort, a 2nd group of patients treated with glucocorticoids was enrolled and named the "Florida-2" cohort. Phenotyping criteria were applied as for the original patient group. This time DNA samples were analyzed using the Illumina HumanOmni2.5-Quad (Omni2.5) BeadChip, which comprises a much larger set of SNPs (2.5 million) in comparison to the original Affymetrix GeneChip® Mapping 500K Set (~1 million). Data from 64 research subjects survived quality control. Association analysis was performed (as described above), and then combined by a meta-analysis with the 49 subjects of the Florida-1 cohort, for a total of 113 subjects (Jeong et al., 2015). A quantitative trait locus (QTL) was identified at chromosomal position 6p21.33, as shown in Figure 4. The QTL is defined by a common SNP that attained GWAS significance, and a second common SNP in high LD (r^2 0.8), that came close to meeting this bar. The QTL is located within a group of three mucin genes: *MUC21*, *MUC22*, and *HCG22*.

At the time we were conducting our study, both *MUC22* and *HCG22* had only just been discovered (Hijikata et al., 2011), and *MUC21* had never been studied in the eye. However, it was known that several MUC-type mucin genes are expressed at the ocular surface (Argueso and Gipson, 2001). In contrast, mucins had not previously been associated with cells of the aqueous outflow pathways. We compared expression of *MUC21*, *MUC22*, and *HCG22* in cultured human trabecular meshwork cells and corneal epithelial cells. Results are shown in Figure 5. *MUC21* mRNA was specific for corneal epithelial cells, but *MUC22* and *HCG22* were expressed in both corneal epithelial and trabecular meshwork cells. Significantly, the two lead SNPs we identified map to the intergenic region between *MUC22* and *HCG22*, ~1.8 kb from the start site for HCG22 transcription (Fig. 4).

If altered transcription of *HCG22* is a causal factor in steroid-induced OH, we reasoned that the gene would be regulated by glucocorticoids and/or cytokines linked to ocular hypertension. We found this to be the case: *HCG22* expression was stimulated by treatment with IL-1 and inhibited by treatment with TGF-β or the glucocorticoid, triamcinolone acetate in cultures of trabecular meshwork cells. To identify possible causal SNPs in the QTL, we used information from the 1000 Genomes Project. An additional eight common SNPs were found to be in high LD (r^2 0.8) with the two lead SNPs identified by our GWAS. Four of the ten SNPs in the LD block (including our two lead SNPs) were cited in the National Center for Bioinformatics Information (NCBI) GTEx browser (http://www.gtexportal.org/home/) as significant modifiers of *HCG22* expression. Further analysis revealed that most of the SNPs in our LD block have the potential to alter binding motifs for various transcription factors. Most interesting was a SNP predicted to disrupt a binding motif for the glucocorticoid receptor.

As summarized in Figure 6A and 6B, the predicted amino acid sequence of the HCG22 coding transcript revealed features consistent with a small, secreted, N- and O-glycosylated mucin-like protein. In transient expression studies, we showed for the first time that this transcript can be translated, O-glycosylated, and exported outside the cell as an ~ 70–75 kDa mucin protein. Bioinformatics analysis revealed that HCG22 is most closely related to

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peritrophin-A, a mucin protein found in the peritrophic matrix of the insect digestive tract. The peritrophic matrix functions as a molecular sieve to partially digested protein and carbohydrate, as a barrier to ingested pathogens, as a scaffold for proteases and glycosidases, and as a sink for toxic substances. We speculate that disrupted binding of the glucocorticoid receptor in individuals with the minor genotype may prevent the glucocorticoid-mediated inhibition of HCG22 expression and contribute to SIOH. Further studies will be needed to test this hypothesis.

For expression analysis of the encoded protein, total RNA purified from a human bronchial epithelial cell line was used to synthesize cDNA, from which HCG22 cDNA (GenBank AB560771) was amplified by polymerase chain reaction. When DNA sequencing was performed, two nucleotide substitutions were discovered corresponding to two SNPs present in the NCBI dbSNP database, rs3873352 and rs2523855, each of which predicts an amino acid substitution in the signal peptide. This is shown in Figure 6C. The SNP rs3873352 would cause a change from arginine to glycine at amino acid 3. This represents a change from a positive charge and hydropathy index of −4.5, to a neutral charge and hydropathy index of 0.4. Such a change is likely to affect secretion. The SNP rs2523855 would result in a glutamic acid to glutamine substitution at amino acid 18, a conservative change that is less likely to have functional consequences. Candidate gene analysis revealed that the SNP leading to a non-conservative change was also significantly associated with IOP. The second SNP (which was not in LD with the first) was not significantly associated. Identification of two independent QTLs that could affect expression of the *HCG22* mucin gene product via two different mechanisms (transcription or secretion) is highly suggestive of a role for this protein in SIOH.

Several other QTLs with suggestive p-values were also identified in this study (Jeong et al., 2015). The top SNP of a 2nd peak on chromosome 9 ($P = 1.56 \times 10^{-7}$) is located in an intragenic region upstream of *TLE4*. Two SNPs in perfect LD ($P = 4.09 \times 10^{-6}$ for each) on chromosome 10 are located in an intron of *ADARB2*. A SNP on chromosome 20 ($P = 1.03 \times 10^{-5}$) is located in an intron of *EYA2*.

12.4. Pathways

In the preceding sections, we have discussed how both high tension POAG and SIOH have been linked by observational and functional studies to changes in the ECM and cytoskeleton of TBM cells, and their connection through integrins. We have also discussed the connection to dysregulation of specific intracellular signaling pathways. There are now 70 genetic variants associated by GWAS with POAG, and these have provided considerable evidence in support of these functional pathways (Iglesias et al., 2015; Wiggs, 2015). Similarly, genes identified in our pharmacogenomics analysis can be linked to pathways identified for high tension POAG.

The function of the novel mucin protein encoded by *HCG22* is currently unknown, but it could be a component of the TBM's ECM. Our bioinformatics analysis revealed that HCG22 is most closely related to peritrophin-A, an ECM protein found in the insect digestive tract (Jeong et al., 2015). The peritrophic matrix functions as a molecular sieve for partially digested protein and carbohydrate, as a barrier to ingested pathogens, as a scaffold

for proteases and glycosidases, and as a sink for toxic substances. We hypothesize that disrupted binding of the activated GCR to *HCG22's* transcriptional promoter in individuals with the genetic variant may prevent glucocorticoid-mediated inhibition of *HCG22* expression, perhaps necessary to prevent excessive ECM accumulation.

The proteins encoded by *TLE4* and *EYA2* are part of signaling pathways disrupted in POAG and SIOH. Members of the TLE gene family encode transcriptional corepressors that interact with the Wnt/beta-catenin signaling pathway implicated in POAG, and more recently in SIOH (Mao et al., 2012; Wang et al., 2008; Yuan et al., 2013). Similarly, *EYA2* interacts with signaling pathways implicated in OH, e.g., TGF-β (Farabaugh et al., 2012). Mutations in *EYA2* cause anterior segment dysgenesis in the eye, resulting in anatomic defects that lead to congenital OH. Chemical inhibitors of EYA2 activity were recently described (Krueger et al., 2014), providing a useful tool for evaluating the role of the gene product, as well as a potential new drug for treating OH.

In the Gene Expression Profiling section above, we noted a recent study in which pathways functionally involved in normal outflow regulation were associated with SIOH (Danias et al., 2011). We noted that one associated gene, *ADRB2*, encodes a GPCR whose primary ligand, epinephrine, stimulates aqueous outflow facility. Intriguingly, two of the genes identified in our GWAS can be similarly linked to GPCR signaling. *GPR158* and *ADARB2*. The signaling connection for *GPR158* is obvious, as it is a GPCR itself. ADARB2 (not to be confused with ADRB2, an entirely different gene) encodes a truncated, non-functional version of the protein encoded by *ADAR2*. The truncated protein is proposed to act dominant-negatively to inhibit ADAR2 action. ADAR2 is an RNA-editing enzyme responsible for most known codon-altering post-transcriptional adenosine to inosine modifications in mammals. One target of RNA editing by ADAR2 is GPCRs of the serotonin receptor family. RNA editing alters activity of these GPCRs (Slotkin and Nishikura, 2013), which are known to control aqueous outflow (Sharif, 2010). In fact, post-transcriptional RNA editing has the potential to modify activity of many genes that affect IOP. An analysis of RNA editing in glucocorticoid-treated TBM cells could provide another important approach to pathway discovery in SIOH.

In summary, our approach of pharmacogenomics with functional follow-up has been very fruitful this far. First, we have linked two novel genes to risk for SIOH. New genes are exciting, as there is potential for significant innovation in our understanding with elucidation of their function. We also have support for three known genes. We have connected these genes to know pathways functionally linked to POAG and SIOH, and have new support for the idea that GPCR signaling may be a factor in SIOH.

13. Precision Medicine in Ophthalmology

The U.S. Precision Medicine Initiative® acknowledges that every medical condition, disease susceptibility, or response to treatment is caused, regulated or influenced by genes (Kotze et al., 2015). Precision medicine incorporates the earlier concept of "personalized medicine", which refers to the tailoring of medical treatment and prevention to fit the genetic and other characteristics of the individual patient. However, personalized medicine is already here,

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e.g., matching therapy to specific genetic mutation is common in oncology. The goal of the precision medicine initiative is to move beyond such islands of personal therapy, to develop a systematic plan for all illness, including eye disease.

A good example in ophthalmology of a disease that can be diagnosed and treated best using precision medicine is inherited retinal degeneration, also called retinitis pigmentosa (Daiger et al., 2013). Currently, mutations in at least 100 genes are known to cause this disease, and nearly 3100 disease-causing mutations are reported in mutation databases. Genes responsible for the related disorders of Usher syndrome and Bardet-Biedl syndrome, account for at least another 1200 mutations. The retinal degenerations differ substantially depending on their genetic mutation, and the treatments for preserving vision in affected individuals will also be dependent on the specific gene mutation.

Precision medicine leverages advances in genomics and data science (Green et al., 2011). The ability to provide precision medicine to patients in routine clinical settings depends on the availability of molecular profiling tests (Kohn and Ivy, 2016). Genetic testing for individual inherited eye diseases, for example, myocilin glaucoma and various retinal degenerations, has been available for some time (Stone, 2007). However, our understanding of the genes involved in inherited eye disease is now sufficiently advanced, and molecular technology is sufficiently powerful, to make possible the more comprehensive testing required for application of precision medicine (Stone et al., 2012; Wiggs and Pierce, 2013).

A step forward for precision medicine in ophthalmology was recently made with introduction of a new and comprehensive diagnostic test, the Genetic Eye Disease Panel (GEDi). This test includes all of the genes known to harbor mutations that cause inherited retinal degenerations, optic atrophy and early onset glaucoma (Consugar et al., 2015). In the past, gene patents have often resulted in companies having sole ownership of genetic testing for patented genes. This arguably insurmountable barrier was removed by a 2013 Supreme Court ruling that DNA is a “product of nature” and therefore human genes cannot be patented. More than 4,300 human gene patents were invalidated by this decision, making the DNA sequences freely available (https://ghr.nlm.nih.gov/primer/testing/genepatents).

Our ability to use the information provided by the GEDi panel to treat patients received a tremendous boost with the introduction of a gene editing technology called CRISPR-Cas9. Gene editing is not new overall, but the innovative CRISPR-Cas9 technology provides the significant advantage of being inexpensive, quick and easy to use. As a result, applications have exploded in labs around the world. At the 2016 annual meeting of the Association for Research in Ophthalmology, Val Sheffield from the University of Iowa Carver College of Medicine presented results from a series of preclinical studies, providing proof-of-principle that gene editing using CRISPR-Cas9 can prevent or treat myocilin glaucoma. The strategy is to create insertions or deletions resulting in frame-shift mutations that attenuate translation of *MYOC* transcripts. Transgenic mice harboring the *MYOCY437H* mutant that causes myocilin glaucoma were given gene therapy using the Ad-5-CRISPR-Cas9 adenovirus with guide RNAs targeting exon 1 of the *MYOC* gene before they developed elevated IOP. This treatment was shown to relieve endoplasmic reticulum stress in the trabecular meshwork

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cells and mitigate elevations in IOP that occurred in animals treated with CRISPR-Cas9 with control guide RNA (reported in *Ophthalmology Times*, May 2, 2016).

Pharmacotherapy is another aspect of precision medicine that can be individualized based on the patient's genome and other characteristics. Inter-individual variation in drug response and adverse drug reactions are well known in medicine (Shastry, 2011). At the present time, physicians must use a trial-and-error method to determine whether a medication is right for a specific patient. This approach is inefficient and often costly and can on some occasions be life-threatening. Application of precision medicine could make a major impact in this area. Therapeutic responses to drugs varies over time and are influenced by age, sex, environmental, and genetic factors (Shastry, 2011).

A variable and unpredictable rate of nonresponse to glaucoma medications is a problem for both adrenergic and prostaglandin agents used as glaucoma medications (Allen et al., 1986). There are currently a few documented examples of genetic polymorphisms that affect a patient's response to these drugs (reviewed in (Ayala-Haedo et al., 2009; Schwartz et al., 2008). Relevant to adrenergic agents is a coding region polymorphism at Arg389 affects receptor functionality and the homozygous genotype had been associated with systemic hypertension (Bengtsson et al., 2001). Hypertensive patients with the Arg389 homozygote genotype demonstrate a greater reduction in diastolic blood pressure than Gly389 carriers when treated with metoprolol tartrate, a selective beta1-adrenergic receptor blocker (Johnson et al., 2003). We hypothesized that the same polymorphisms could also explain ocular non-response to betaxolol hydrochloride, which is also a selective β1-adrenergic receptor-blocking agent. This was confirmed in a study of normal volunteers, which revealed that Arg389 homozygotes had a higher baseline IOP and a greater magnitude of response to betaxolol treatment, as compared with Gly389 carriers (Schwartz et al., 2005). More recently, a similar association was documented between response to latanoprost and polymorphisms in MMP1, a latanoprost target (Ussa et al., 2015).

The clinical utility of this information has been limited for several reasons. The beta-blockers timolol, levobunolol, metipranolol and carteolol are nonselective (active against β1- and β2-adrenergic receptors), while betaxolol is cardioselective (β1-receptor selective) (Brooks and Gillies, 1992). While betaxolol has fewer side effects, it is prescribed infrequently because of the fact that some patients are non-responders. A genetic test could make betaxolol a preferred choice for clinicians. However, given the availability of other effective medications – in particular the prostaglandin analogues, which also have reduced side effects over non-selective β-blockers – it seems unlikely that clinicians would find the test worthwhile. In fact, all glaucoma medications are associated with variable response rates and standard clinical practice is to select a medication and then observe the patient for several weeks to determine if the medication is efficacious; if not, the original medication can be discontinued and a new medication selected. With topical medications, this "trial and error" approach is probably more efficient than the use of a pharmacogenetic test.

In contrast, prior knowledge about an individual patient's sensitivity to steroids could be highly valuable to the clinician. Thus far in this article, we have discussed how the pharmacogenomics approach can be used to identify genes involved in normal aqueous

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outflow and its inhibition in pathological conditions. However, the ability to predict an individual patient's sensitivity to steroids is important in its own right, as steroid sensitivity severely limits the usefulness of a class of drugs that is very effective in blunting the inflammatory response (Rhen and Cidlowski, 2005).

At this time there are no tests to determine whether an individual will respond to glucocorticoids with a rise in IOP. All treated patients must be followed to determine their response, which at the very least leads to additional clinic visits and exposure to additional medications in at least some patients. The clinician can stop the treatment if a response occurs, but some patients exhibit very rapid IOP elevation that is refractory to glucocorticoid withdrawal or pharmacotherapy. A minor percentage of steroid responders develop elevated IOP that is intractable, requiring treatment with incisional surgery. This is associated with risks of infection, hemorrhage, visual loss, and other adverse outcomes. Pharmacogenomics may provide some assistance with this problem, as we have previously discussed (Schwartz, 2010). Should a pharmacogenetic test become validated and available, it could be used to identify responders before injecting a long-acting medication such as the fluocinolone insert (Iluvien), thus greatly increasing patient safety.

In 2013, the FDA issued a guidance document on clinical pharmacogenomics (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm337169.pdf) intended to assist entities engaged in new drug development to determine how variations in the human genome that affect pharmacokinetic and pharmacodynamics properties of drugs should be considered during drug development and regulatory review. The guidance observes that genetic differences likely to be of most relevance fall into four broad categories: 1) genes relevant to the drug's pharmacokinetics and 2) genes relevant to the drug's pharmacodynamics (effects) including, a) genes that code for drug targets and other pathways related to the drug's pharmacologic effect, b) genes that can predispose to toxicities and c) genes that influence disease susceptibility or progression. In the pharmacogenomics guidance, the FDA recommends that "when possible under applicable laws, regulations, and ethics committee polices", DNA should be collected from all participants for exploratory analyses. The ultimate goal would be to use the information gained for better design of clinical trials, and also for safer use of the therapeutic, once approved.

The journal, *The Ocular Surface*, publishes a quarterly column on new ophthalmics called "Pipeline", which is introduced by an editorial opinion. In a recent 2015 installment (Novack, 2015), our team's GWAS findings on the "intergenic quantitative trait locus" associated with glucocorticoid responsiveness were discussed with relation to precision medicine. It was observed that the "impact of this on therapy is not yet clear". This is quite accurate; our publication (Jeong et al., 2015) represents the first genome-wide effort to identify genetic polymorphisms associated with steroid response in the eye. The two statistically significant QTLs associated with *HCG22* that we identified are located at chromosomal locus 6p21.33. It is fascinating to note that this is within the 3.6 Mb major histocompatibility complex class I locus, between the genes for HLA-A and HLA-B, a little more than 300 Kb telomeric of HLA-B. As discussed in the "History and Genetics" section of this article, studies published when the field of glucocorticoid therapeutics was still young

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identified an association between response to glucocorticoids and HLA-B12. Given the low resolution of genetic association mapping at the time, our results provide new support for the historic finding, bringing us full circle (Becker et al., 1977; Becker et al., 1976). The genetic variants we identified have been submitted to and accepted by the Pharmacogenomics Knowledgebase (PharmGKB) (https://www.pharmgkb.org/index.jsp), a database that organizes information about genetic variants affecting drug response. Further investigations will now be needed to evaluate their predictive value. Establishing such clinical significance will require an "it-takes-a-village" approach, involving the entire community of ophthalmic health care professionals, and eye and vision scientists.

14. Future Directions

In this perspectives article, we describe work from our group over the past decade that led to the first GWAS for SIOH, and identification of the first genomic variants associated with the disease. While our patient cohort was small, the genetic effect is large, which explains our ability to detect statistically significant variants that affect phenotype. However, a larger cohort powered for more moderate effect sizes could provide much additional new information. It is hoped that the discussion herein leads the reader to appreciate that many different genes and polymorphisms are likely to be involved in determining the magnitude of response to glucocorticoids in the eye. The variability of SIOH disease progression among different individuals, and the fact that a subset of individuals does not improve with steroid withdrawal, argues for the existence of disease subtypes. In fact, the extracellular matrix deposits and cellular changes identified in patients diagnosed with SIG may be representative of this more severe disease subtype rather than all types of SIOH and SIG. Nevertheless, cell biological studies discussed in this article demonstrate that deposition of extracellular matrix is a common response to steroids in different cell lines and tissues. Thus, severity of response to glucocorticoids might be more a matter of quantity than quality. It is noted that genomics has proven a very effective means for characterizing disease subtypes (e.g., (Cancer Genome Atlas Research, 2012) to enable more effective treatment and might be similarly valuable in the case of SIOH and SIG.

The major reason why GWASs on SIOH had not been previously performed prior to the study we report here is that recruitment of a research subject cohort of substantial size is a major challenge. Clinical trials of new steroid therapeutics invariably turn up safety issues, leading to curtailment of patient enrollment. Cobbling together of cohorts from various studies and practices is less than optimal because of differences in patient management. The best study design would employ a large cohort of patients that have been consistently treated with glucocorticoids in the same way, by the same physicians, in the same practice. Such an optimal situation is now within our reach. The Cornea Research Foundation of America (CRFA) (http://www.cornea.org/) maintains a registry of patients that underwent corneal transplantation at the Price Vision Group in Indianapolis, and then received steroid treatment according to a highly uniform protocol. The registry currently contains almost 4500 cases of corneal transplantation followed by treatment with glucocorticoids (1664 unilateral and 1412 bilateral transplants, i.e. ~3076 patients) going back to 2003, with complete patient contact information, patient characterization and follow-up data. This represents a unique and

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powerful resource. We are now beginning to enroll patients from the registry into our new study.

In our next step analyses, we plan to add DNA sequencing to the previously used microarray-based genotyping to enhance discovery opportunity. First introduced to the market in 2005 (Morozova and Marra, 2008), next-generation sequencing (NGS) technology is now commonly used for deep sequencing of the DNA in a key genomic region, across multiple individuals, to find rare variants not present on SNP chips, but which may be responsible for the genetic effect. In the near future, it is likely that genomic DNA sequencing will replace SNP microarrays altogether, as the cost has been steadily decreasing.

The International Genome Sample Resource (IGSR) (http://www.1000genomes.org/) tells us that the frequency of some of the genetic variants associated with IOP response to glucocorticoids identified in our studies varies substantially in the different population groups that IGSR is examining. To avoid the confounding effects of genetic differences across races we enrolled only Caucasians. It should be noted that there is currently no known racial predilection for steroid-induced OH, however, as mentioned at the beginning of this article, the incidence of POAG is higher in African-Americans. Thus, future studies that incorporate racial ancestry as a variable may provide additional information of value to our understanding of ocular hypertension.

As discussed above, GWAS is inherently a hypothesis-generating exercise. A statistically significant association of a genetic variant and a disease endophenotype is a strong argument linking a gene with a disease. However, functional validation of any gene implicated in disease should ultimately be the goal of all laboratories. To our knowledge, none of the currently ongoing GWA studies for POAG, ocular hypertension, or related disorders have identified either GPR158 or HCG22 as candidate genes, making our results quite novel. We are currently conducting studies using knockout mice to further examine the role of GPR158 in ocular hypertension and prostate cancer. We also plan to carry out mechanistic studies for HCG22. Both genes represent promising drug targets for controlling IOP in glaucoma.

It seems pertinent to conclude this article with the emerging approaches of epigenetics. Epigenetics describes stable modifications to the chromosome that result in genetic effects not encoded in the DNA sequences of the genome (Wu and Morris, 2001). Current usage of the term by molecular biologists includes mechanisms for modification of DNA and histones by acetylation and methylation, as well as noncoding RNA transcripts, and regulation of chromatin accessibility by nucleosome positioning (Kouzarides, 2007). Epigenetic mechanisms play an important part in determining which genes in an organism are expressed in different cell types and under specific conditions. Such changes are influenced by a variety of factors external to the cell, thus contributing to the ability of an organism to adapt to its environment.

A recent review article discusses intriguing links between epigenetic mechanisms and various ocular diseases, including optic nerve degeneration in glaucoma (He et al., 2013). The *CDKN2BAS*, recently associated with POAG and normal tension glaucoma (Wiggs,

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2012), is regulated by epigenetic mechanisms (Yap et al., 2010), although whether this contributes to glaucoma is not known.

Relevant to ocular hypertension is the emerging role of epigenetic processes in the progression of fibrotic disease (e.g., (Coward et al., 2009; Kaminski et al., 2000; Robinson et al., 2012). A recent review article discusses the evidence for epigenetic mechanisms in response to hypoxia, implicated as a factor in development of ocular hypertension, and in regulation of fibrotic signaling pathways, including direct effects on expression of genes encoding TGF-βs (McDonnell et al., 2014). A recent study demonstrated that inhibition of histone deacetylation significantly increased TGFB2 mRNA and protein expression in cultured human TBM cells, and elevated IOP in perfusion-cultured bovine eyes (Bermudez et al., 2016). Also, hypoxia was shown to induce changes in DNA methylation that alter TGFB1 Expression in cultured human TBM cells (McDonnell et al., 2016). Whether epigenetic mechanisms are involved in the pathogenesis of ocular hypertension remains to be shown.

With specific relevance to SIOH/SIG is epigenetic regulation of the glucocorticoid response. Dysregulation of the hypothalamic-pituitary-adrenal axis is associated with the pathophysiology of stress-related diseases. The evidence to date supports the idea that exposure to prolonged levels of glucocorticoids stimulated by stress can induce epigenetic modifications at key regions on the gene for the glucocorticoid receptor, lead to alterations in expression and function (Farrell and O'Keane, 2016). Specific involvement of epigenetic mechanisms in modifying the glucocorticoid response in SIOH/SIG has yet to be explored.

Thus, investigations on a role for epigenetics in glaucoma are still at an early stage, however, we predict that epigenetics of SIOH/SIG will be an area of intensive investigation in the next few years. As just one example, while our pharmacogenomics study discussed above was under review, sequences of three long non-coding RNAs transcribed from the HCG22 gene were submitted to Genbank (Jeong et al., 2015). These are epigenetic regulators. Importantly, a number of therapeutic drugs targeting epigenetic mechanisms are already in use for other diseases (discussed in (He et al., 2013)). Re-purposing of such therapeutics holds distinct promise for future treatment of ocular hypertension and glaucoma.

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Abbreviations

HUGO nomenclature is used for genes and their products

AP-1 activating protein-1

BRVO branch retinal vein occlusion

CLAN cross-linked actin network

CRVO central retinal vein occlusion

DME diabetic macular edema

DMEK Descemet's membrane endothelial keratoplasty

ECD extracellular domain

ELAM-1 endothelial leukocyte adhesion molecule-1

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eQTL	expression quantitative trait locus
FDA	Food and Drug Administration
GAP	GTPase-Accelerating Protein
GEDi	Genetic Eye Disease Panel
GEF	Guanine Nucleotide Exchange Factor
GRα	glucocorticoid receptor-α
GRβ	glucocorticoid receptor-β
IL-1	Interleukin 1 (i.e. IL1A, IL1B)
LD	linkage disequilibrium
GPCR	G protein-coupled receptor
GWAS	genome wide association study
ICD	intracellular domain
IOP	intraocular pressure
IVTA	intravitreal triamcinolone acetonide
MMP	matrix metalloproteinase
NCBI	National Center for Bioinformatics Information
NIH	National Institutes of Health
NF-κB	Nuclear Factor-κB
NGS	next-generation sequencing
NLS	nuclear localization signal
QTL	quantitative trait locus
PTSI	peroxisomal targeting signal type 1
POAG	primary open angle glaucoma
SIOH	steroid-induced ocular hypertension
SIG	steroid-induced glaucoma
SNP	single nucleotide polymorphism
TGF-β	Transforming Growth Factor-β (i.e., TGFB1, TGFB2, TGFB3)
TIGR	trabecular meshwork-inducible glucocorticoid response protein.

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References

Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. Experimental eye research. 2008; 86:543–561. [PubMed: 18313051]

Allen RC, Hertzmark E, Walker AM, Epstein DL. A double-masked comparison of betaxolol vs timolol in the treatment of open-angle glaucoma. American journal of ophthalmology. 1986; 101:535–541. [PubMed: 3518464]

Alvarado J, Murphy C, Juster R. Trabecular meshwork cellularity in primary open-angle glaucoma and nonglaucomatous normals. Ophthalmology. 1984; 91:564–579. [PubMed: 6462622]

Alvarado J, Murphy C, Polansky J, Juster R. Age-related changes in trabecular meshwork cellularity. Investigative ophthalmology & visual science. 1981; 21:714–727. [PubMed: 7298275]

Alvarado JA, Alvarado RG, Yeh RF, Franse-Carman L, Marcellino GR, Brownstein MJ. A new insight into the cellular regulation of aqueous outflow: how trabecular meshwork endothelial cells drive a mechanism that regulates the permeability of Schlemm's canal endothelial cells. The British journal of ophthalmology. 2005; 89:1500–1505. [PubMed: 16234461]

Alvarado JA, Murphy CG, Franse-Carman L, Chen J, Underwood JL. Effect of beta-adrenergic agonists on paracellular width and fluid flow across outflow pathway cells. Investigative ophthalmology & visual science. 1998; 39:1813–1822. [PubMed: 9727404]

Alvarado JA, Wood I, Polansky JR. Human trabecular cells. II. Growth pattern and ultrastructural characteristics. Investigative ophthalmology & visual science. 1982; 23:464–478. [PubMed: 7118506]

Alward WL, Fingert JH, Coote MA, Johnson AT, Lerner SF, Junqua D, Durcan FJ, McCartney PJ, Mackey DA, Sheffield VC, Stone EM. Clinical features associated with mutations in the chromosome 1 open-angle glaucoma gene (GLC1A). The New England journal of medicine. 1998; 338:1022–1027. [PubMed: 9535666]

Anderson DR. Normal-tension glaucoma (Low-tension glaucoma). Indian J Ophthalmol. 2011; 59(Suppl):S97–101. [PubMed: 21150042]

Anshu A, Price MO, Price FW Jr. Risk of corneal transplant rejection significantly reduced with Descemet's membrane endothelial keratoplasty. Ophthalmology. 2012; 119:536–540. [PubMed: 22218143]

Aref AA, Scott IU, Oden NL, Ip MS, Blodi BA, VanVeldhuisen PC, Group SSI. Incidence, Risk Factors, and Timing of Elevated Intraocular Pressure After Intravitreal Triamcinolone Acetonide Injection for Macular Edema Secondary to Retinal Vein Occlusion: SCORE Study Report 15. JAMA ophthalmology. 2015; 133:1022–1029. [PubMed: 26086920]

Argueso P, Gipson IK. Epithelial mucins of the ocular surface: structure, biosynthesis and function. Experimental eye research. 2001; 73:281–289. [PubMed: 11520103]

Armaly MF. Effect of Corticosteroids on Intraocular Pressure and Fluid Dynamics. I. The Effect of Dexamethasone in the Normal Eye. Archives of ophthalmology. 1963a; 70:482–491. [PubMed: 14078870]

Armaly MF. Effect of Corticosteroids on Intraocular Pressure and Fluid Dynamics. Ii. The Effect of Dexamethasone in the Glaucomatous Eye. Archives of ophthalmology. 1963b; 70:492–499. [PubMed: 14078871]

Armaly MF. Statistical Attributes of the Steroid Hypertensive Response in the Clinically Normal Eye. I. The Demonstration of Three Levels of Response. Investigative ophthalmology. 1965; 4:187–197. [PubMed: 14283012]

Armaly MF, Becker B. Intraocular pressure response to topical corticosteroids. Federation proceedings. 1965; 24:1274–1278. [PubMed: 5853148]

Attisano L, Wrana JL. Signal integration in TGF-beta, WNT, and Hippo pathways. F1000Prime Rep. 2013; 5:17. [PubMed: 23755364]

Audo I, Bujakowska K, Orhan E, Poloschek CM, Defoort-Dhellemmes S, Drumare I, Kohl S, Luu TD, Lecompte O, Zrenner E, Lancelot ME, Antonio A, Germain A, Michiels C, Audier C, Letexier M, Saraiva JP, Leroy BP, Munier FL, Mohand-Said S, Lorenz B, Friedburg C, Preising M, Kellner U, Renner AB, Moskova-Doumanova V, Berger W, Wissinger B, Hamel CP, Schorderet DF, De Baere E, Sharon D, Banin E, Jacobson SG, Bonneau D, Zanlonghi X, Le Meur G, Casteels I, Koenekoop

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

R, Long VW, Meire F, Prescott K, de Ravel T, Simmons I, Nguyen H, Dollfus H, Poch O, Leveillard T, Nguyen-Ba-Charvet K, Sahel JA, Bhattacharya SS, Zeitz C. Whole-exome sequencing identifies mutations in GPR179 leading to autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet. 2012; 90:321–330. [PubMed: 22325361]

Aviner Z, Henley WL, Fotino M, Leopold IH. Histocompatibility (HL-A) antigens and primary open-angle glaucoma. Tissue antigens. 1976; 7:193–200. [PubMed: 941127]

Ayala-Haedo J, Schwartz SG, Kishor KS, Fini ME. Pharmacogenomics of Ophthalmic Beta Blockers (solicited). International Journal of Medical and Biological Frontiers. 2009; 14:139–152.

Ayyala RS. Penetrating keratoplasty and glaucoma. Survey of ophthalmology. 2000; 45:91–105. [PubMed: 11033036]

Barany EH. The mode of action of miotics on outflow resistance. A study of pilocarpine in the vervet monkey Cercopithecus ethiops. Trans Ophthalmol Soc U K. 1966; 86:539–578. [PubMed: 4959867]

Bartlett JD, Woolley TW, Adams CM. Identification of high intraocular pressure responders to topical ophthalmic corticosteroids. Journal of ocular pharmacology. 1993; 9:35–45. [PubMed: 8463731]

Baylis D, Bartlett DB, Patel HP, Roberts HC. Understanding how we age: insights into inflammaging. Longev Healthspan. 2013; 2:8. [PubMed: 24472098]

Becker B. Intraocular Pressure Response to Topical Corticosteroids. Investigative ophthalmology. 1965; 4:198–205. [PubMed: 14283013]

Becker B, Chevrette L. Topical corticosteroid testing in glaucoma siblings. Archives of ophthalmology. 1966; 76:484–487. [PubMed: 5928135]

Becker B, Hahn KA. Topical Corticosteroids and Heredity in Primary Open-Angle Glaucoma. American journal of ophthalmology. 1964; 57:543–551. [PubMed: 14139296]

Becker B, Mills DW. Corticosteroids and Intraocular Pressure. Archives of ophthalmology. 1963; 70:500–507. [PubMed: 14078872]

Becker B, Palmberg F, Shin DH. Glucocorticoid responsiveness associated with HLA-B12. Investigative ophthalmology & visual science. 1977; 16:61–63. [PubMed: 832966]

Becker B, Shin DH, Palmberg PF, Waltman SR. HLA antigens and corticosteroid response. Science. 1976; 194:1427–1428. [PubMed: 1006308]

Benedek TG. History of the development of corticosteroid therapy. Clin Exp Rheumatol. 2011; 29:S-5–12.

Bengtsson K, Melander O, Orho-Melander M, Lindblad U, Ranstam J, Rastam L, Groop L. Polymorphism in the beta(1)-adrenergic receptor gene and hypertension. Circulation. 2001; 104:187–190. [PubMed: 11447084]

Bermudez JY, Webber HC, Patel GC, Liu X, Cheng YQ, Clark AF, Mao W. HDAC Inhibitor-Mediated Epigenetic Regulation of Glaucoma-Associated TGFbeta2 in the Trabecular Meshwork. Investigative ophthalmology & visual science. 2016; 57:3698–3707. [PubMed: 27403998]

Bernstein HN, Schwartz B. Effects of long-term systemic steroids on ocular pressure and tonographic values. Archives of ophthalmology. 1962; 68:742–753. [PubMed: 13967694]

Berry A, Matthews L, Jangani M, Plumb J, Farrow S, Buchan N, Wilson PA, Singh D, Ray DW, Donn RP. Interferon-inducible factor 16 is a novel modulator of glucocorticoid action. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2010; 24:1700–1713. [PubMed: 20086048]

Bigger JF, Palmberg PF, Becker B. Increased cellular sensitivity to glucocorticoids in primary open-angle glaucoma. Investigative ophthalmology. 1972; 11:832–837. [PubMed: 5071841]

Bigger JF, Palmberg PF, Zink HA. In vitro corticosteroid: correlation response with primary open-angle glaucoma and ocular corticosteroid sensitivity. American journal of ophthalmology. 1975; 79:92–97. [PubMed: 1110208]

Birke MT, Birke K, Lutjen-Drecoll E, Schlotzer-Schrehardt U, Hammer CM. Cytokine-dependent ELAM-1 induction and concomitant intraocular pressure regulation in porcine anterior eye perfusion culture. Investigative ophthalmology & visual science. 2011; 52:468–475. [PubMed: 20861478]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bjarnadottir TK, Fredriksson R, Schioth HB. The gene repertoire and the common evolutionary history of glutamate, pheromone (V2R), taste(1) and other related G protein-coupled receptors. Gene. 2005; 362:70–84. [PubMed: 16229975]

Bond RA, Ijzerman AP. Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. Trends in pharmacological sciences. 2006; 27:92–96. [PubMed: 16406086]

Borras, T. Chapter 11: What is Functional Genomics Teaching us about Intraocular Pressure Regulation and Glaucoma?. Elsevier Inc; 2008.

Borras T. The effects of myocilin expression on functionally relevant trabecular meshwork genes: a mini-review. Journal of ocular pharmacology and therapeutics: the official journal of the Association for Ocular Pharmacology and Therapeutics. 2014; 30:202–212.

Borras T, Buie LK, Spiga MG. Inducible scAAV2.GRE.MMP1 lowers IOP long-term in a large animal model for steroid-induced glaucoma gene therapy. Gene therapy. 2016; 23:438–449. [PubMed: 26855269]

Borras T, Buie LK, Spiga MG, Carabana J. Prevention of nocturnal elevation of intraocular pressure by gene transfer of dominant-negative RhoA in rats. JAMA ophthalmology. 2015a; 133:182–190. [PubMed: 25412195]

Borras T, Smith MH, Buie LK. A Novel Mgp-Cre Knock-In Mouse Reveals an Anticalcification/Antistiffness Candidate Gene in the Trabecular Meshwork and Peripapillary Scleral Region. Investigative ophthalmology & visual science. 2015b; 56:2203–2214. [PubMed: 25711639]

Boyer DS, Yoon YH, Belfort R Jr, Bandello F, Maturi RK, Augustin AJ, Li XY, Cui H, Hashad Y, Whitcup SM, Ozurdex MSG. Three-year, randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with diabetic macular edema. Ophthalmology. 2014; 121:1904–1914. [PubMed: 24907062]

Bradley JM, Vranka J, Colvis CM, Conger DM, Alexander JP, Fisk AS, Samples JR, Acott TS. Effect of matrix metalloproteinases activity on outflow in perfused human organ culture. Investigative ophthalmology & visual science. 1998; 39:2649–2658. [PubMed: 9856774]

Brattsand R, Linden M. Cytokine modulation by glucocorticoids: mechanisms and actions in cellular studies. Aliment Pharmacol Ther. 1996; 10(Suppl 2):81–90. discussion 91–82. [PubMed: 8899106]

Braunger BM, Fuchshofer R, Tamm ER. The aqueous humor outflow pathways in glaucoma: A unifying concept of disease mechanisms and causative treatment. Eur J Pharm Biopharm. 2015; 95:173–181. [PubMed: 25957840]

Brooks AM, Gillies WE. Ocular beta-blockers in glaucoma management. Clinical pharmacological aspects. Drugs Aging. 1992; 2:208–221. [PubMed: 1351412]

Brubaker RF. Goldmann’s equation and clinical measures of aqueous dynamics. Experimental eye research. 2004; 78:633–637. [PubMed: 15106943]

Burdon KP, Macgregor S, Hewitt AW, Sharma S, Chidlow G, Mills RA, Danoy P, Casson R, Viswanathan AC, Liu JZ, Landers J, Henders AK, Wood J, Souzeau E, Crawford A, Leo P, Wang JJ, Rochtchina E, Nyholt DR, Martin NG, Montgomery GW, Mitchell P, Brown MA, Mackey DA, Craig JE. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. Nature genetics. 2011; 43:574–578. [PubMed: 21532571]

Burridge K, Wittchen ES. The tension mounts: stress fibers as force-generating mechanotransducers. The Journal of cell biology. 2013; 200:9–19. [PubMed: 23295347]

Cai S, Liu X, Glasser A, Volberg T, Filla M, Geiger B, Polansky JR, Kaufman PL. Effect of latrunculin-A on morphology and actin-associated adhesions of cultured human trabecular meshwork cells. Molecular vision. 2000; 6:132–143. [PubMed: 10930474]

Campochiaro PA, Brown DM, Pearson A, Chen S, Boyer D, Ruiz-Moreno J, Garretson B, Gupta A, Hariprasad SM, Bailey C, Reichel E, Soubrane G, Kapik B, Billman K, Kane FE, Green K, Group FS. Sustained delivery fluocinolone acetonide vitreous inserts provide benefit for at least 3 years in patients with diabetic macular edema. Ophthalmology. 2012; 119:2125–2132. [PubMed: 22727177]

Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012; 489:519–525. [PubMed: 22960745]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Candia OA, Gerometta RM, Danias J. Tissue plasminogen activator reduces the elevated intraocular pressure induced by prednisolone in sheep. Experimental eye research. 2014; 128:114–116. [PubMed: 25304217]

Chang JY, Stamer WD, Bertrand J, Read AT, Marando CM, Ethier CR, Overby DR. Role of nitric oxide in murine conventional outflow physiology. American journal of physiology Cell physiology. 2015; 309:C205–214. [PubMed: 26040898]

Chriguer RS, Elias LL, da Silva IM Jr, Vieira JG, Moreira AC, de Castro M. Glucocorticoid sensitivity in young healthy individuals: in vitro and in vivo studies. The Journal of clinical endocrinology and metabolism. 2005; 90:5978–5984. [PubMed: 16091495]

Clark AF, Brotchie D, Read AT, Hellberg P, English-Wright S, Pang IH, Ethier CR, Grierson I. Dexamethasone alters F-actin architecture and promotes cross-linked actin network formation in human trabecular meshwork tissue. Cell Motil Cytoskeleton. 2005; 60:83–95. [PubMed: 15593281]

Clark AF, Miggans ST, Wilson K, Browder S, McCartney MD. Cytoskeletal changes in cultured human glaucoma trabecular meshwork cells. Journal of glaucoma. 1995a; 4:183–188. [PubMed: 19920666]

Clark AF, Wilson K, de Kater AW, Allingham RR, McCartney MD. Dexamethasone-induced ocular hypertension in perfusion-cultured human eyes. Investigative ophthalmology & visual science. 1995b; 36:478–489. [PubMed: 7843916]

Clark AF, Wilson K, McCartney MD, Miggans ST, Kunkle M, Howe W. Glucocorticoid-induced formation of cross-linked actin networks in cultured human trabecular meshwork cells. Investigative ophthalmology & visual science. 1994; 35:281–294. [PubMed: 8300356]

Clark AF, Wordinger RJ. The role of steroids in outflow resistance. Experimental eye research. 2009; 88:752–759. [PubMed: 18977348]

Clark R, Nosie A, Walker T, Faralli JA, Filla MS, Barrett-Wilt G, Peters DM. Comparative genomic and proteomic analysis of cytoskeletal changes in dexamethasone-treated trabecular meshwork cells. Molecular & cellular proteomics: MCP. 2013; 12:194–206. [PubMed: 23105009]

Collins FS, Varmus H. A new initiative on precision medicine. The New England journal of medicine. 2015; 372:793–795. [PubMed: 25635347]

Consugar MB, Navarro-Gomez D, Place EM, Bujakowska KM, Sousa ME, Fonseca-Kelly ZD, Taub DG, Janessian M, Wang DY, Au ED, Sims KB, Sweetser DA, Fulton AB, Liu Q, Wiggs JL, Gai X, Pierce EA. Panel-based genetic diagnostic testing for inherited eye diseases is highly accurate and reproducible, and more sensitive for variant detection, than exome sequencing. Genet Med. 2015; 17:253–261. [PubMed: 25412400]

Coward WR, Watts K, Feghali-Bostwick CA, Knox A, Pang L. Defective histone acetylation is responsible for the diminished expression of cyclooxygenase 2 in idiopathic pulmonary fibrosis. Molecular and cellular biology. 2009; 29:4325–4339. [PubMed: 19487460]

Daiger SP, Sullivan LS, Bowne SJ. Genes and mutations causing retinitis pigmentosa. Clin Genet. 2013; 84:132–141. [PubMed: 23701314]

Daly AK. Genome-wide association studies in pharmacogenomics. Nature reviews Genetics. 2010; 11:241–246.

Damgaard-Jensen L, Kissmeyer-Nielsen F. HLA histocompatibility antigens in open-angle glaucoma. Acta ophthalmologica. 1978; 56:384–388. [PubMed: 581129]

Danias J, Gerometta R, Ge Y, Ren L, Panagis L, Mittag TW, Candia OA, Podos SM. Gene expression changes in steroid-induced IOP elevation in bovine trabecular meshwork. Investigative ophthalmology & visual science. 2011; 52:8636–8645. [PubMed: 21980000]

Davies TG. Tonographic survey of the close relatives of patients with chronic simple glaucoma. The British journal of ophthalmology. 1968; 52:32–39. [PubMed: 5635901]

Elman MJ, Aiello LP, Beck RW, Bressler NM, Bressler SB, Edwards AR, Ferris FL 3rd, Friedman SM, Glassman AR, Miller KM, Scott IU, Stockdale CR, Sun JK. Diabetic Retinopathy Clinical Research N. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology. 2010; 117:1064–1077. e1035. [PubMed: 20427088]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Dickerson JE Jr, Steely HT Jr, English-Wright SL, Clark AF. The effect of dexamethasone on integrin and laminin expression in cultured human trabecular meshwork cells. Experimental eye research. 1998; 66:731–738. [PubMed: 9657905]

Diskin S, Kumar J, Cao Z, Schuman JS, Gilmartin T, Head SR, Panjwani N. Detection of differentially expressed glycogenes in trabecular meshwork of eyes with primary open-angle glaucoma. Investigative ophthalmology & visual science. 2006; 47:1491–1499. [PubMed: 16565384]

Dismuke WM, Liang J, Overby DR, Stamer WD. Concentration-related effects of nitric oxide and endothelin-1 on human trabecular meshwork cell contractility. Experimental eye research. 2014; 120:28–35. [PubMed: 24374036]

Donn R, Berry A, Stevens A, Farrow S, Betts J, Stevens R, Clayton C, Wang J, Warnock L, Worthington J, Scott L, Graham S, Ray D. Use of gene expression profiling to identify a novel glucocorticoid sensitivity determining gene, BMPRII. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2007; 21:402–414. [PubMed: 17185747]

Dyer D, Callanan D, Bochow T, Abraham P, Lambert HM, Lee SY, Schneiderman T, Potts SL, Walker TM. Clinical evaluation of the safety and efficacy of preservative-free triamcinolone (triesence [triamcinolone acetonide injectable suspension] 40 mg/ml) for visualization during pars plana vitrectomy. Retina. 2009; 29:38–45. [PubMed: 18827733]

el-Shabrawi Y, Eckhardt M, Berghold A, Faulborn J, Auboeck L, Mangge H, Ardjomand N. Synthesis pattern of matrix metalloproteinases (MMPs) and inhibitors (TIMPs) in human explant organ cultures after treatment with latanoprost and dexamethasone. Eye. 2000; 14(Pt 3A):375–383. [PubMed: 11027004]

Engelbrecht-Schnur S, Siegner A, Prehm P, Lutjen-Drecoll E. Dexamethasone treatment decreases hyaluronan-formation by primate trabecular meshwork cells in vitro. Experimental eye research. 1997; 64:539–543. [PubMed: 9227271]

Engler AJ, Rehfeldt F, Sen S, Discher DE. Microtissue elasticity: measurements by atomic force microscopy and its influence on cell differentiation. Methods Cell Biol. 2007; 83:521–545. [PubMed: 17613323]

Epstein, DL.; Allingham, RR.; Schuman, JS. Chandler and Grant's Glaucoma. 4. Williams and Wilkins; Baltimore, Maryland: 1996.

Erickson-Lamy K, Schroeder A, Epstein DL. Ethacrynic acid induces reversible shape and cytoskeletal changes in cultured cells. Investigative ophthalmology & visual science. 1992; 33:2631–2640. [PubMed: 1639610]

Erickson-Lamy KA, Nathanson JA. Epinephrine increases facility of outflow and cyclic AMP content in the human eye in vitro. Investigative ophthalmology & visual science. 1992; 33:2672–2678. [PubMed: 1353486]

Ethier CR. The inner wall of Schlemm's canal. Experimental eye research. 2002; 74:161–172. [PubMed: 11950226]

Fan BJ, Wang DY, Lam DS, Pang CP. Gene mapping for primary open angle glaucoma. Clin Biochem. 2006; 39:249–258. [PubMed: 16332362]

Fan BJ, Wang DY, Tham CC, Lam DS, Pang CP. Gene expression profiles of human trabecular meshwork cells induced by triamcinolone and dexamethasone. Investigative ophthalmology & visual science. 2008; 49:1886–1897. [PubMed: 18436822]

Farabaugh SM, Micalizzi DS, Jedlicka P, Zhao R, Ford HL. Eya2 is required to mediate the pro-metastatic functions of Six1 via the induction of TGF-beta signaling, epithelial-mesenchymal transition, and cancer stem cell properties. Oncogene. 2012; 31:552–562. [PubMed: 21706047]

Faralli JA, Clark RW, Filla MS, Peters DM. NFATc1 activity regulates the expression of myocilin induced by dexamethasone. Experimental eye research. 2015; 130:9–16. [PubMed: 25450062]

Faralli JA, Gagen D, Filla MS, Crotti TN, Peters DM. Dexamethasone increases alphavbeta3 integrin expression and affinity through a calcineurin/NFAT pathway. Biochimica et biophysica acta. 2013; 1833:3306–3313. [PubMed: 24100160]

Farrell C, O'Keane V. Epigenetics and the glucocorticoid receptor: A review of the implications in depression. Psychiatry Res. 2016; 242:349–356. [PubMed: 27344028]

Fenner A. Prostate cancer: Orphan receptor GPR158 finds a home in prostate cancer growth and progression. Nature reviews Urology. 2015; 12:182.

Filla MS, Clark R, Peters DM. A syndecan-4 binding peptide derived from laminin 5 uses a novel PKCepsilon pathway to induce cross-linked actin network (CLAN) formation in human trabecular meshwork (HTM) cells. Experimental cell research. 2014; 327:171–182. [PubMed: 25128150]

Filla MS, Faralli JA, Peotter JL, Peters DM. The role of integrins in glaucoma. Experimental eye research. 2016

Filla MS, Liu X, Nguyen TD, Polansky JR, Brandt CR, Kaufman PL, Peters DM. In vitro localization of TIGR/MYOC in trabecular meshwork extracellular matrix and binding to fibronectin. Investigative ophthalmology & visual science. 2002; 43:151–161. [PubMed: 11773026]

Filla MS, Schwinn MK, Nosie AK, Clark RW, Peters DM. Dexamethasone-associated cross-linked actin network formation in human trabecular meshwork cells involves beta3 integrin signaling. Investigative ophthalmology & visual science. 2011; 52:2952–2959. [PubMed: 21273548]

Filla MS, Schwinn MK, Sheibani N, Kaufman PL, Peters DM. Regulation of cross-linked actin network (CLAN) formation in human trabecular meshwork (HTM) cells by convergence of distinct beta1 and beta3 integrin pathways. Investigative ophthalmology & visual science. 2009; 50:5723–5731. [PubMed: 19643963]

Filla MS, Woods A, Kaufman PL, Peters DM. Beta1 and beta3 integrins cooperate to induce syndecan-4-containing cross-linked actin networks in human trabecular meshwork cells. Investigative ophthalmology & visual science. 2006; 47:1956–1967. [PubMed: 16639003]

Fingert JH. Primary open-angle glaucoma genes. Eye. 2011; 25:587–595. [PubMed: 21562585]

Fingert JH, Alward WL, Wang K, Yorio T, Clark AF. Assessment of SNPs associated with the human glucocorticoid receptor in primary open-angle glaucoma and steroid responders. Molecular vision. 2010; 16:596–601. [PubMed: 20376328]

Fingert JH, Clark AF, Craig JE, Alward WL, Snibson GR, McLaughlin M, Tuttle L, Mackey DA, Sheffield VC, Stone EM. Evaluation of the myocilin (MYOC) glaucoma gene in monkey and human steroid-induced ocular hypertension. Investigative ophthalmology & visual science. 2001; 42:145–152. [PubMed: 11133859]

Fingert JH, Heon E, Liebmann JM, Yamamoto T, Craig JE, Rait J, Kawase K, Hoh ST, Buys YM, Dickinson J, Hockey RR, Williams-Lyn D, Trope G, Kitazawa Y, Ritch R, Mackey DA, Alward WL, Sheffield VC, Stone EM. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. Human molecular genetics. 1999; 8:899–905. [PubMed: 10196380]

Fingert JH, Stone EM, Sheffield VC, Alward WL. Myocilin glaucoma. Survey of ophthalmology. 2002; 47:547–561. [PubMed: 12504739]

Fini ME, Karmilowicz MJ, Ruby PL, Beeman AM, Borges KA, Brinckerhoff CE. Cloning of a complementary DNA for rabbit proactivator. A metalloproteinase that activates synovial cell collagenase, shares homology with stromelysin and transin, and is coordinately regulated with collagenase. Arthritis and rheumatism. 1987; 30:1254–1264. [PubMed: 2825726]

Fini ME, Stramer BM. How the cornea heals: cornea-specific repair mechanisms affecting surgical outcomes. Cornea. 2005; 24:S2–S11. [PubMed: 16227819]

Flugel-Koch C, Ohlmann A, Fuchshofer R, Welge-Lussen U, Tamm ER. Thrombospondin-1 in the trabecular meshwork: localization in normal and glaucomatous eyes, and induction by TGF-beta1 and dexamethasone in vitro. Experimental eye research. 2004; 79:649–663. [PubMed: 15500824]

Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. The journals of gerontology Series A, Biological sciences and medical sciences. 2014; 69(Suppl 1):S4–9.

Franchimont D, Louis E, Dupont P, Vrindts-Gevaert Y, Dewe W, Chrousos G, Geenen V, Belaiche J. Decreased corticosensitivity in quiescent Crohn's disease: an ex vivo study using whole blood cell cultures. Digestive diseases and sciences. 1999; 44:1208–1215. [PubMed: 10389698]

Francois J. Corticosteroid glaucoma. Annals of ophthalmology. 1977; 9:1075–1080. [PubMed: 143229]

Fuchshofer R, Birke M, Welge-Lussen U, Kook D, Lutjen-Drecoll E. Transforming growth factor-beta 2 modulated extracellular matrix component expression in cultured human optic nerve head

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

astrocytes. Investigative ophthalmology & visual science. 2005; 46:568–578. [PubMed: 15671284]

Fuchshofer R, Tamm ER. Modulation of extracellular matrix turnover in the trabecular meshwork. Experimental eye research. 2009; 88:683–688. [PubMed: 19385040]

Fuchshofer R, Tamm ER. The role of TGF-beta in the pathogenesis of primary open-angle glaucoma. Cell and tissue research. 2012; 347:279–290. [PubMed: 22101332]

Fuchshofer R, Welge-Lussen U, Lutjen-Drecoll E. The effect of TGF-beta2 on human trabecular meshwork extracellular proteolytic system. Experimental eye research. 2003; 77:757–765. [PubMed: 14609564]

Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR, Majno G. Granulation tissue as a contractile organ. A study of structure and function. The Journal of experimental medicine. 1972; 135:719–734. [PubMed: 4336123]

Gabelt BT, Kaufman PL. Changes in aqueous humor dynamics with age and glaucoma. Progress in retinal and eye research. 2005; 24:612–637. [PubMed: 15919228]

Gagen D, Faralli JA, Filla MS, Peters DM. The role of integrins in the trabecular meshwork. Journal of ocular pharmacology and therapeutics: the official journal of the Association for Ocular Pharmacology and Therapeutics. 2014; 30:110–120.

Gagen D, Filla MS, Clark R, Liton P, Peters DM. Activated alphavbeta3 integrin regulates alphavbeta5 integrin-mediated phagocytosis in trabecular meshwork cells. Investigative ophthalmology & visual science. 2013; 54:5000–5011. [PubMed: 23821196]

Gao X. Genome-wide association studies: Where we are heading? World J Med Genet. 2011; 1:1–000.

Gao, X.; Nannini, D.; Torres, M.; Kristen, G.; Chen, Y-D.; Taylor, KD.; Rotter, JI.; Varma, R. Genetic variants associated with intraocular pressure in Latinos. Presented at the 2015 ARVO Annual Meeting; May 3–7; Denver, Colorado. 2015. Program #2170340

Gerometta R, Kumar S, Shah S, Alvarez L, Candia O, Danias J. Reduction of steroid-induced intraocular pressure elevation in sheep by tissue plasminogen activator. Investigative ophthalmology & visual science. 2013; 54:7903–7909. [PubMed: 24176900]

Gerometta R, Podos SM, Danias J, Candia OA. Steroid-induced ocular hypertension in normal sheep. Investigative ophthalmology & visual science. 2009; 50:669–673. [PubMed: 18824726]

Gerzenstein SM, Pletcher MT, Cervino AC, Tsinoremas NF, Young B, Puliafito CA, Fini ME, Schwartz SG. Glucocorticoid receptor polymorphisms and intraocular pressure response to intravitreal triamcinolone acetonide. Ophthalmic genetics. 2008; 29:166–170. [PubMed: 19005987]

Gong G, Kosoko-Lasaki O, Haynatzki GR, Wilson MR. Genetic dissection of myocilin glaucoma. Human molecular genetics. 2004; 13(Spec No 1):R91–102. [PubMed: 14764620]

Gong HY, Trinkaus-Randall V, Freddo TF. Ultrastructural immunocytochemical localization of elastin in normal human trabecular meshwork. Current eye research. 1989; 8:1071–1082. [PubMed: 2612196]

Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK 2nd, Wilson MR, Kass MA. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. Archives of ophthalmology. 2002; 120:714–720. discussion 829–730. [PubMed: 12049575]

Gorin MB. A new vision for age-related macular degeneration. European journal of human genetics: EJHG. 2005; 13:793–794. [PubMed: 15856069]

Gottanka J, Chan D, Eichhorn M, Lutjen-Drecoll E, Ethier CR. Effects of TGF-beta2 in perfused human eyes. Investigative ophthalmology & visual science. 2004; 45:153–158. [PubMed: 14691167]

Gould DB, Miceli-Libby L, Savinova OV, Torrado M, Tomarev SI, Smith RS, John SW. Genetically increasing Myoc expression supports a necessary pathologic role of abnormal proteins in glaucoma. Molecular and cellular biology. 2004; 24:9019–9025. [PubMed: 15456875]

Gould DB, Reedy M, Wilson LA, Smith RS, Johnson RL, John SW. Mutant myocilin nonsecretion in vivo is not sufficient to cause glaucoma. Molecular and cellular biology. 2006; 26:8427–8436. [PubMed: 16954374]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Grant WM. Clinical measurements of aqueous outflow. AMA Arch Ophthalmol. 1951a; 46:113–131. [PubMed: 14856471]

Grant WM. Clinical measurements of aqueous outflow. American journal of ophthalmology. 1951b; 34:1603–1605. [PubMed: 14885362]

Green ED, Guyer MS. National Human Genome Research I. Charting a course for genomic medicine from base pairs to bedside. Nature. 2011; 470:204–213. [PubMed: 21307933]

Gregori NZ, Rosenfeld PJ, Puliafito CA, Flynn HW Jr, Lee JE, Mavrofrides EC, Smiddy WE, Murray TG, Berrocal AM, Scott IU, Gregori G. One-year safety and efficacy of intravitreal triamcinolone acetonide for the management of macular edema secondary to central retinal vein occlusion. Retina. 2006; 26:889–895. [PubMed: 17031288]

Haller JA, Bandello F, Belfort R Jr, Blumenkranz MS, Gillies M, Heier J, Loewenstein A, Yoon YH, Jiao J, Li XY, Whitcup SM, Ozurdex GSG, Li J. Dexamethasone intravitreal implant in patients with macular edema related to branch or central retinal vein occlusion twelve-month study results. Ophthalmology. 2011; 118:2453–2460. [PubMed: 21764136]

Hardy KM, Hoffman EA, Gonzalez P, McKay BS, Stamer WD. Extracellular trafficking of myocilin in human trabecular meshwork cells. The Journal of biological chemistry. 2005; 280:28917–28926. [PubMed: 15944158]

Harper AR, Topol EJ. Pharmacogenomics in clinical practice and drug development. Nature biotechnology. 2012; 30:1117–1124.

He S, Li X, Chan N, Hinton DR. Review: Epigenetic mechanisms in ocular disease. Molecular vision. 2013; 19:665–674. [PubMed: 23559860]

Hewitt AW, Mackey DA, Craig JE. Myocilin allele-specific glaucoma phenotype database. Human mutation. 2008; 29:207–211. [PubMed: 17966125]

Hijikata M, Matsushita I, Tanaka G, Tsuchiya T, Ito H, Tokunaga K, Ohashi J, Homma S, Kobashi Y, Taguchi Y, Azuma A, Kudoh S, Keicho N. Molecular cloning of two novel mucin-like genes in the disease-susceptibility locus for diffuse panbronchiolitis. Human genetics. 2011; 129:117–128. [PubMed: 20981447]

Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:9362–9367. [PubMed: 19474294]

Hinz B. The extracellular matrix and transforming growth factor-beta1: Tale of a strained relationship. Matrix biology: journal of the International Society for Matrix Biology. 2015; 47:54–65. [PubMed: 25960420]

Hoare MJ, Grierson I, Brotchie D, Pollock N, Cracknell K, Clark AF. Cross-linked actin networks (CLANs) in the trabecular meshwork of the normal and glaucomatous human eye in situ. Investigative ophthalmology & visual science. 2009; 50:1255–1263. [PubMed: 18952927]

Hogewind BF, Micheal S, Bakker B, Hoyng CB, den Hollander AI. Analysis of single nucleotide polymorphisms in the SFRS3 and FKBP4 genes in corticosteroid-induced ocular hypertension. Ophthalmic genetics. 2012; 33:221–224. [PubMed: 22921020]

Hogewind BF, Micheal S, Schoenmaker-Koller FE, Hoyng CB, den Hollander AI. Analyses of Sequence Variants in the MYOC Gene and of Single Nucleotide Polymorphisms in the NR3C1 and FKBP5 Genes in Corticosteroid-Induced Ocular Hypertension. Ophthalmic genetics. 2015; 36:299–302. [PubMed: 24417561]

Honjo M, Inatani M, Kido N, Sawamura T, Yue BY, Honda Y, Tanihara H. Effects of protein kinase inhibitor, HA1077, on intraocular pressure and outflow facility in rabbit eyes. Archives of ophthalmology. 2001; 119:1171–1178. [PubMed: 11483085]

Huizenga NA, Koper JW, de Lange P, Pols HA, Stolk RP, Grobbee DE, de Jong FH, Lamberts SW. Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. The Journal of clinical endocrinology and metabolism. 1998; 83:47–54. [PubMed: 9435415]

Hysi PG, Cheng CY, Springelkamp H, Macgregor S, Bailey JN, Wojciechowski R, Vitart V, Nag A, Hewitt AW, Hohn R, Venturini C, Mirshahi A, Ramdas WD, Thorleifsson G, Vithana E, Khor

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

CC, Stefansson AB, Liao J, Haines JL, Amin N, Wang YX, Wild PS, Ozel AB, Li JZ, Fleck BW, Zeller T, Staffieri SE, Teo YY, Cuellar-Partida G, Luo X, Allingham RR, Richards JE, Senft A, Karssen LC, Zheng Y, Bellenguez C, Xu L, Iglesias AI, Wilson JF, Kang JH, van Leeuwen EM, Jonsson V, Thorsteinsdottir U, Despriet DD, Ennis S, Moroi SE, Martin NG, Jansonius NM, Yazar S, Tai ES, Amouyel P, Kirwan J, van Koolwijk LM, Hauser MA, Jonasson F, Leo P, Loomis SJ, Fogarty R, Rivadeneira F, Kearns L, Lackner KJ, de Jong PT, Simpson CL, Pennell CE, Oostra BA, Uitterlinden AG, Saw SM, Lotery AJ, Bailey-Wilson JE, Hofman A, Vingerling JR, Maubaret C, Pfeiffer N, Wolfs RC, Lemij HG, Young TL, Pasquale LR, Delcourt C, Spector TD, Klaver CC, Small KS, Burdon KP, Stefansson K, Wong TY, Group BG, Consortium N, Viswanathan A, Mackey DA, Craig JE, Wiggs JL, van Duijn CM, Hammond CJ, Aung T. Wellcome Trust Case Control C. Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. Nature genetics. 2014; 46:1126–1130. [PubMed: 25173106]

Iglesias AI, Springelkamp H, Ramdas WD, Klaver CC, Willemsen R, van Duijn CM. Genes, pathways, and animal models in primary open-angle glaucoma. Eye. 2015; 29:1285–1298. [PubMed: 26315706]

Ingber DE. Tensegrity I. Cell structure and hierarchical systems biology. Journal of cell science. 2003; 116:1157–1173. [PubMed: 12615960]

Ishibashi T, Takagi Y, Mori K, Naruse S, Nishino H, Yue BY, Kinoshita S. cDNA microarray analysis of gene expression changes induced by dexamethasone in cultured human trabecular meshwork cells. Investigative ophthalmology & visual science. 2002; 43:3691–3697. [PubMed: 12454038]

Itakura T, Peters DM, Fini ME. Glaucomatous MYOC mutations activate the IL-1/NF-kappaB inflammatory stress response and the glaucoma marker SELE in trabecular meshwork cells. Molecular vision. 2015; 21:1071–1084. [PubMed: 26396484]

Jaffe GJ, Martin D, Callanan D, Pearson PA, Levy B, Comstock T. Fluocinolone Acetonide Uveitis Study G. Fluocinolone acetonide implant (Retisert) for noninfectious posterior uveitis: thirty-four-week results of a multicenter randomized clinical study. Ophthalmology. 2006; 113:1020–1027. [PubMed: 16690128]

Jain A, Wordinger RJ, Yorio T, Clark AF. Spliceosome protein (SRp) regulation of glucocorticoid receptor isoforms and glucocorticoid response in human trabecular meshwork cells. Investigative ophthalmology & visual science. 2012; 53:857–866. [PubMed: 22205602]

Jain A, Wordinger RJ, Yorio T, Clark AF. Role of the Alternatively Spliced Glucocorticoid Receptor Isoform GRbeta in Steroid Responsiveness and Glaucoma. Journal of ocular pharmacology and therapeutics: the official journal of the Association for Ocular Pharmacology and Therapeutics. 2014; 30:121–127.

Janmey PA, Georges PC, Hvidt S. Basic rheology for biologists. Methods Cell Biol. 2007; 83:3–27. [PubMed: 17613302]

Jeong S, Patel N, Edlund CK, Hartiala J, Hazelett DJ, Itakura T, Wu PC, Avery RL, Davis JL, Flynn HW, Lalwani G, Puliafito CA, Wafapoor H, Hijikata M, Keicho N, Gao X, Argueso P, Allayee H, Coetzee GA, Pletcher MT, Conti DV, Schwartz SG, Eaton AM, Fini ME. Identification of a Novel Mucin Gene HCG22 Associated With Steroid-Induced Ocular Hypertension. Investigative ophthalmology & visual science. 2015; 56:2737–2748. [PubMed: 25813999]

Job R, Raja V, Grierson I, Currie L, O'Reilly S, Pollock N, Knight E, Clark AF. Cross-linked actin networks (CLANs) are present in lamina cribrosa cells. The British journal of ophthalmology. 2010; 94:1388–1392. [PubMed: 20693558]

Joe MK, Sohn S, Hur W, Moon Y, Choi YR, Kee C. Accumulation of mutant myocilins in ER leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. Biochemical and biophysical research communications. 2003; 312:592–600. [PubMed: 14680806]

Johnson D, Gottanka J, Flugel C, Hoffmann F, Futa R, Lutjen-Drecoll E. Ultrastructural changes in the trabecular meshwork of human eyes treated with corticosteroids. Archives of ophthalmology. 1997; 115:375–383. [PubMed: 9076211]

Johnson DH, Bradley JM, Acott TS. The effect of dexamethasone on glycosaminoglycans of human trabecular meshwork in perfusion organ culture. Investigative ophthalmology & visual science. 1990; 31:2568–2571. [PubMed: 2125032]

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Johnson DH, Tschumper RC. Human trabecular meshwork organ culture. A new method. Investigative ophthalmology & visual science. 1987; 28:945–953. [PubMed: 3583633]

Johnson DH, Tschumper RC. Ethacrynic acid: outflow effects and toxicity in human trabecular meshwork in perfusion organ culture. Current eye research. 1993; 12:385–396. [PubMed: 8344063]

Johnson JA, Zineh I, Puckett BJ, McGorray SP, Yarandi HN, Pauly DF. Beta 1-adrenergic receptor polymorphisms and antihypertensive response to metoprolol. Clin Pharmacol Ther. 2003; 74:44–52. [PubMed: 12844134]

Johnson M. What controls aqueous humour outflow resistance? Exp Eye Res. 2006; 82:545–557. [PubMed: 16386733]

Jonas JB, Akkoyun I, Kamppeter B, Kreissig I, Degenring RF. Branch retinal vein occlusion treated by intravitreal triamcinolone acetonide. Eye. 2005; 19:65–71. [PubMed: 15105817]

Jones R 3rd, Rhee DJ. Corticosteroid-induced ocular hypertension and glaucoma: a brief review and update of the literature. Current opinion in ophthalmology. 2006; 17:163–167. [PubMed: 16552251]

Kaminski N, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, Huang X, Sheppard D, Heller RA. Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97:1778–1783. [PubMed: 10677534]

Kass MA, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK 2nd, Wilson MR, Gordon MO. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. Archives of ophthalmology. 2002; 120:701–713. discussion 829–730. [PubMed: 12049574]

Kass MA, Palmberg P, Becker B, Miller JP. Histocompatibility antigens and primary open-angle glaucoma. A reassessment. Archives of ophthalmology. 1978; 96:2207–2208. [PubMed: 718510]

Kee C, Seo K. The effect of interleukin-1alpha on outflow facility in rat eyes. Journal of glaucoma. 1997; 6:246–249. [PubMed: 9264304]

Keller KE, Acott TS. The Juxtacanalicular Region of Ocular Trabecular Meshwork: A Tissue with a Unique Extracellular Matrix and Specialized Function. J Ocul Biol. 2013; 1:3. [PubMed: 24364042]

Keller KE, Yang YF, Sun YY, Sykes R, Gaudette ND, Samples JR, Acott TS, Wirtz MK. Interleukin-20 receptor expression in the trabecular meshwork and its implication in glaucoma. Journal of ocular pharmacology and therapeutics: the official journal of the Association for Ocular Pharmacology and Therapeutics. 2014; 30:267–276.

Kersey JP, Broadway DC. Corticosteroid-induced glaucoma: a review of the literature. Eye. 2006; 20:407–416. [PubMed: 15877093]

Kim BS, Savinova OV, Reedy MV, Martin J, Lun Y, Gan L, Smith RS, Tomarev SI, John SW, Johnson RL. Targeted Disruption of the Myocilin Gene (Myoc) Suggests that Human Glaucoma-Causing Mutations Are Gain of Function. Molecular and cellular biology. 2001; 21:7707–7713. [PubMed: 11604506]

Kino T, Chrousos GP. Glucocorticoid and mineralocorticoid resistance/hypersensitivity syndromes. The Journal of endocrinology. 2001; 169:437–445. [PubMed: 11375113]

Kirkness CM, Moshegov C. Post-keratoplasty glaucoma. Eye. 1988; 2(Suppl):S19–26. [PubMed: 3076147]

Kitazawa Y, Horie T. The prognosis of corticosteroid-responsive individuals. Archives of ophthalmology. 1981; 99:819–823. [PubMed: 7236083]

Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. Science. 2005; 308:385–389. [PubMed: 15761122]

Kohn EC, Ivy SP. Confronting the Care Delivery Challenges Arising from Precision Medicine. Frontiers in oncology. 2016; 6:106. [PubMed: 27200294]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Kopczynski CC, Epstein DL. Emerging trabecular outflow drugs. Journal of ocular pharmacology and therapeutics: the official journal of the Association for Ocular Pharmacology and Therapeutics. 2014; 30:85–87.

Kotze MJ, Luckhoff HK, Peeters AV, Baatjes K, Schoeman M, van der Merwe L, Grant KA, Fisher LR, van der Merwe N, Pretorius J, van Velden DP, Myburgh EJ, Pienaar FM, van Rensburg SJ, Yako YY, September AV, Moremi KE, Cronje FJ, Tiffin N, Bouwens CS, Bezuidenhout J, Apffelstaedt JP, Hough FS, Erasmus RT, Schneider JW. Genomic medicine and risk prediction across the disease spectrum. Crit Rev Clin Lab Sci. 2015; 52:120–137. [PubMed: 25597499]

Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128:693–705. [PubMed: 17320507]

Krueger AB, Drasin DJ, Lea WA, Patrick AN, Patnaik S, Backos DS, Matheson CJ, Hu X, Barnaeva E, Holliday MJ, Blevins MA, Robin TP, Eisenmesser EZ, Ferrer M, Simeonov A, Southall N, Reigan P, Marugan J, Ford HL, Zhao R. Allosteric inhibitors of the Eya2 phosphatase are selective and inhibit Eya2-mediated cell migration. The Journal of biological chemistry. 2014; 289:16349–16361. [PubMed: 24755226]

Kwon YH, Fingert JH, Kuehn MH, Alward WL. Primary open-angle glaucoma. The New England journal of medicine. 2009; 360:1113–1124. [PubMed: 19279343]

LaFramboise T. Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances. Nucleic acids research. 2009; 37:4181–4193. [PubMed: 19570852]

Lam DS, Leung YF, Chua JK, Baum L, Fan DS, Choy KW, Pang CP. Truncations in the TIGR gene in individuals with and without primary open-angle glaucoma. Investigative ophthalmology & visual science. 2000; 41:1386–1391. [PubMed: 10798654]

Last JA, Pan T, Ding Y, Reilly CM, Keller K, Acott TS, Fautsch MP, Murphy CJ, Russell P. Elastic modulus determination of normal and glaucomatous human trabecular meshwork. Investigative ophthalmology & visual science. 2011; 52:2147–2152. [PubMed: 21220561]

Lazarides E, Burridge K. Alpha-actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. Cell. 1975; 6:289–298. [PubMed: 802682]

Leopold IH. Ophthalmology-Epitomes of Progress: HLA Antigens and Glaucoma. West J Med. 1979; 130:542–543. [PubMed: 18748429]

Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L, Komaroff E. Early Manifest Glaucoma Trial G. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. Archives of ophthalmology. 2003; 121:48–56. [PubMed: 12523884]

Leung YF, Tam PO, Lee WS, Lam DS, Yam HF, Fan BJ, Tham CC, Chua JK, Pang CP. The dual role of dexamethasone on anti-inflammation and outflow resistance demonstrated in cultured human trabecular meshwork cells. Molecular vision. 2003; 9:425–439. [PubMed: 12963864]

Levoye A, Dam J, Ayoub MA, Guillaume JL, Jockers R. Do orphan G-protein-coupled receptors have ligand-independent functions? New insights from receptor heterodimers. EMBO reports. 2006; 7:1094–1098. [PubMed: 17077864]

Lewis JM, Priddy T, Judd J, Gordon MO, Kass MA, Kolker AE, Becker B. Intraocular pressure response to topical dexamethasone as a predictor for the development of primary open-angle glaucoma. American journal of ophthalmology. 1988; 106:607–612. [PubMed: 3189477]

Lewis-Tuffin LJ, Cidlowski JA. The physiology of human glucocorticoid receptor beta (hGRbeta) and glucocorticoid resistance. Annals of the New York Academy of Sciences. 2006; 1069:1–9. [PubMed: 16855130]

Li G, Luna C, Liton PB, Navarro I, Epstein DL, Gonzalez P. Sustained stress response after oxidative stress in trabecular meshwork cells. Molecular vision. 2007; 13:2282–2288. [PubMed: 18199969]

Liang LL, Epstein DL, de Kater AW, Shahsafaei A, Erickson-Lamy KA. Ethacrynic acid increases facility of outflow in the human eye in vitro. Archives of ophthalmology. 1992; 110:106–109. [PubMed: 1731701]

Liton PB, Gonzalez P, Epstein DL. The role of proteolytic cellular systems in trabecular meshwork homeostasis. Experimental eye research. 2009; 88:724–728. [PubMed: 19046967]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Liton PB, Luna C, Challa P, Epstein DL, Gonzalez P. Genome-wide expression profile of human trabecular meshwork cultured cells, nonglaucomatous and primary open angle glaucoma tissue. Molecular vision. 2006; 12:774–790. [PubMed: 16862071]

Liu JH, Realini T, Weinreb RN. Asymmetry of 24-hour intraocular pressure reduction by topical ocular hypotensive medications in fellow eyes. Ophthalmology. 2011; 118:1995–2000. [PubMed: 21676464]

Lo WR, Rowlette LL, Caballero M, Yang P, Hernandez MR, Borras T. Tissue differential microarray analysis of dexamethasone induction reveals potential mechanisms of steroid glaucoma. Investigative ophthalmology & visual science. 2003; 44:473–485. [PubMed: 12556371]

Lowder C, Belfort R Jr, Lightman S, Foster CS, Robinson MR, Schiffman RM, Li XY, Cui H, Whitcup SM, Ozurdex HSG. Dexamethasone intravitreal implant for noninfectious intermediate or posterior uveitis. Archives of ophthalmology. 2011; 129:545–553. [PubMed: 21220619]

Lutjen-Drecoll E. Morphological changes in glaucomatous eyes and the role of TGFbeta2 for the pathogenesis of the disease. Experimental eye research. 2005; 81:1–4. [PubMed: 15978248]

Lutjen-Drecoll E, Shimizu T, Rohrbach M, Rohen JW. Quantitative analysis of ‘plaque material’ in the inner- and outer wall of Schlemm’s canal in normal- and glaucomatous eyes. Experimental eye research. 1986; 42:443–455. [PubMed: 3720863]

Maepea O, Bill A. Pressures in the juxtacanalicular tissue and Schlemm’s canal in monkeys. Experimental eye research. 1992; 54:879–883. [PubMed: 1521580]

Mao W, Millar JC, Wang WH, Silverman SM, Liu Y, Wordinger RJ, Rubin JS, Pang IH, Clark AF. Existence of the canonical Wnt signaling pathway in the human trabecular meshwork. Investigative ophthalmology & visual science. 2012; 53:7043–7051. [PubMed: 22956608]

Mao W, Tovar-Vidales T, Yorio T, Wordinger RJ, Clark AF. Perfusion-cultured bovine anterior segments as an ex vivo model for studying glucocorticoid-induced ocular hypertension and glaucoma. Investigative ophthalmology & visual science. 2011; 52:8068–8075. [PubMed: 21911581]

Maturi RK, Pollack A, Uy HS, Varano M, Gomes AM, Li XY, Cui H, Lou J, Hashad Y, Whitcup SM, Ozurdex MSG. Intraocular Pressure in Patients with Diabetic Macular Edema Treated with Dexamethasone Intravitreal Implant in the 3-Year Mead Study. Retina. 2016

Mayr WR, Grabner G. HLA antigens-risk factors for primary open-angle glaucoma? Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie. Albrecht von Graefe’s archive for clinical and experimental ophthalmology. 1978; 207:291–297. [PubMed: 311598]

McDonnell F, Irnaten M, Clark AF, O’Brien CJ, Wallace DM. Hypoxia-Induced Changes in DNA Methylation Alter RASAL1 and TGFbeta1 Expression in Human Trabecular Meshwork Cells. PloS one. 2016; 11:e0153354. [PubMed: 27124111]

McDonnell F, O’Brien C, Wallace D. The role of epigenetics in the fibrotic processes associated with glaucoma. J Ophthalmol. 2014; 2014:750459. [PubMed: 24800062]

McKay BS, Congrove NR, Johnson AA, Dismuke WM, Bowen TJ, Stamer WD. A role for myocilin in receptor-mediated endocytosis. PloS one. 2013; 8:e82301. [PubMed: 24367514]

Melamed S, Kotas-Neumann R, Barak A, Epstein DL. The effect of intracamerally injected ethacrynic acid on intraocular pressure in patients with glaucoma. American journal of ophthalmology. 1992; 113:508–512. [PubMed: 1575224]

Menzaghi F, Behan DP, Chalmers DT. Constitutively activated G protein-coupled receptors: a novel approach to CNS drug discovery. Current drug targets. CNS and neurological disorders. 2002; 1:105–121. [PubMed: 12769637]

Millan J, Cain RJ, Reglero-Real N, Bigarella C, Marcos-Ramiro B, Fernandez-Martin L, Correas I, Ridley AJ. Adherens junctions connect stress fibres between adjacent endothelial cells. BMC Biol. 2010; 8:11. [PubMed: 20122254]

Milligan G. G protein-coupled receptor hetero-dimerization: contribution to pharmacology and function. British journal of pharmacology. 2009; 158:5–14. [PubMed: 19309353]

Morgan JT, Raghunathan VK, Chang YR, Murphy CJ, Russell P. Wnt inhibition induces persistent increases in intrinsic stiffness of human trabecular meshwork cells. Experimental eye research. 2015; 132:174–178. [PubMed: 25639201]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Morozova O, Marra MA. Applications of next-generation sequencing technologies in functional genomics. Genomics. 2008; 92:255–264. [PubMed: 18703132]

Nehme A, Lobenhofer EK, Stamer WD, Edelman JL. Glucocorticoids with different chemical structures but similar glucocorticoid receptor potency regulate subsets of common and unique genes in human trabecular meshwork cells. BMC Med Genomics. 2009; 2:58. [PubMed: 19744340]

Nguyen TD, Chen P, Huang WD, Chen H, Johnson D, Polansky JR. Gene structure and properties of TIGR, an olfactomedin-related glycoprotein cloned from glucocorticoid-induced trabecular meshwork cells. The Journal of biological chemistry. 1998; 273:6341–6350. [PubMed: 9497363]

Novack GD. Do Genes Matter in Treating Eye Disease? The ocular surface. 2015; 13:346–349. [PubMed: 26272274]

O'Brien ET, Lee RE 3rd, Epstein DL. Ethacrynic acid disrupts steady state microtubules in vitro. Current eye research. 1996; 15:985–990. [PubMed: 8921220]

Oakley RH, Cidlowski JA. Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. The Journal of biological chemistry. 2011; 286:3177–3184. [PubMed: 21149445]

Orlandi C, Posokhova E, Masuho I, Ray TA, Hasan N, Gregg RG, Martemyanov KA. GPR158/179 regulate G protein signaling by controlling localization and activity of the RGS7 complexes. The Journal of cell biology. 2012; 197:711–719. [PubMed: 22689652]

Orlandi C, Xie K, Masuho I, Fajardo-Serrano A, Lujan R, Martemyanov KA. Orphan Receptor GPR158 is an Allosteric Modulator of Regulator of G Protein Signaling 7 (RGS7) Catalytic Activity with Essential Role in Dictating its Expression and Localization in the Brain. The Journal of biological chemistry. 2015

Overby DR, Bertrand J, Tektas OY, Boussommier-Calleja A, Schicht M, Ethier CR, Woodward DF, Stamer WD, Lutjen-Drecoll E. Ultrastructural changes associated with dexamethasone-induced ocular hypertension in mice. Investigative ophthalmology & visual science. 2014; 55:4922–4933. [PubMed: 25028360]

Overby DR, Clark AF. Animal models of glucocorticoid-induced glaucoma. Experimental eye research. 2015; 141:15–22. [PubMed: 26051991]

Ozel AB, Moroi SE, Reed DM, Nika M, Schmidt CM, Akbari S, Scott K, Rozsa F, Pawar H, Musch DC, Lichter PR, Gaasterland D, Branham K, Gilbert J, Garnai SJ, Chen W, Othman M, Heckenlively J, Swaroop A, Abecasis G, Friedman DS, Zack D, Ashley-Koch A, Ulmer M, Kang JH, Consortium N, Liu Y, Yaspan BL, Haines J, Allingham RR, Hauser MA, Pasquale L, Wiggs J, Richards JE, Li JZ. Genome-wide association study and meta-analysis of intraocular pressure. Human genetics. 2014; 133:41–57. [PubMed: 24002674]

Palmberg PF, Mandell A, Wilensky JT, Podos SM, Becker B. The reproducibility of the intraocular pressure response to dexamethasone. American journal of ophthalmology. 1975; 80:844–856. [PubMed: 1190278]

Pang CP, Leung YF, Fan B, Baum L, Tong WC, Lee WS, Chua JK, Fan DS, Liu Y, Lam DS. TIGR/MYOC gene sequence alterations in individuals with and without primary open-angle glaucoma. Investigative ophthalmology & visual science. 2002; 43:3231–3235. [PubMed: 12356829]

Pang IH, Shade DL, Clark AF, Steely HT, DeSantis L. Preliminary characterization of a transformed cell strain derived from human trabecular meshwork. Current eye research. 1994; 13:51–63. [PubMed: 8156826]

Patel N, Itakura T, Gonzalez JM Jr, Schwartz SG, Fini ME. GPR158, an orphan member of G protein-coupled receptor Family C: glucocorticoid-stimulated expression and novel nuclear role. PloS one. 2013; 8:e57843. [PubMed: 23451275]

Patel N, Itakura T, Jeong S, Liao CP, Roy-Burman P, Zandi E, Groshen S, Pinski J, Coetzee GA, Gross ME, Fini ME. Expression and Functional Role of Orphan Receptor GPR158 in Prostate Cancer Growth and Progression. PloS one. 2015; 10:e0117758. [PubMed: 25693195]

Paterson G. Studies of the response to topical dexamethasone of glaucoma relatives. Trans Ophthalmol Soc U K. 1965; 85:295–305. [PubMed: 5227191]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Pattabiraman PP, Rao PV. Mechanistic basis of Rho GTPase-induced extracellular matrix synthesis in trabecular meshwork cells. American journal of physiology Cell physiology. 2010; 298:C749–763. [PubMed: 19940066]

Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genetic epidemiology. 2008; 32:381–385. [PubMed: 18348202]

Peachey NS, Ray TA, Florijn R, Rowe LB, Sjoerdsma T, Contreras-Alcantara S, Baba K, Tosini G, Pozdeyev N, Iuvone PM, Bojang P Jr, Pearring JN, Simonsz HJ, van Genderen M, Birch DG, Traboulsi EI, Dorfman A, Lopez I, Ren H, Goldberg AF, Nishina PM, Lachapelle P, McCall MA, Koenekoop RK, Bergen AA, Kamermans M, Gregg RG. GPR179 is required for depolarizing bipolar cell function and is mutated in autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet. 2012; 90:331–339. [PubMed: 22325362]

Pekmezci M, Chang ST, Wilson BS, Gordon MO, Bhorade AM. Effect of measurement order between right and left eyes on intraocular pressure measurement. Archives of ophthalmology. 2011; 129:276–281. [PubMed: 21402981]

Peltier J, Perez J, Bellocq A, Escoubet B, Fouqueray B, Baud L. Transforming growth factor-beta 1 increases glucocorticoid binding and signaling in macrophages through a Smad- and activated protein-1-mediated process. Kidney Int. 2003; 63:2028–2036. [PubMed: 12753290]

Picht G, Welge-Luessen U, Grehn F, Lutjen-Drecoll E. Transforming growth factor beta 2 levels in the aqueous humor in different types of glaucoma and the relation to filtering bleb development. Graefes Arch Clin Exp Ophthalmol. 2001; 239:199–207. [PubMed: 11405069]

Plomin R, Haworth CM, Davis OS. Common disorders are quantitative traits. Nature reviews Genetics. 2009; 10:872–878.

Polansky JR, Fauss DJ, Chen P, Chen H, Lutjen-Drecoll E, Johnson D, Kurtz RM, Ma ZD, Bloom E, Nguyen TD. Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product. Ophthalmologica. Journal international d'ophtalmologie. International journal of ophthalmology Zeitschrift fur Augenheilkunde. 1997; 211:126–139. [PubMed: 9176893]

Porter K, Hirt J, Stamer WD, Liton PB. Autophagic dysregulation in glaucomatous trabecular meshwork cells. Biochimica et biophysica acta. 2015; 1852:379–385. [PubMed: 25483712]

Price FW Jr, Price DA, Ngakeng V, Price MO. Survey of steroid usage patterns during and after low-risk penetrating keratoplasty. Cornea. 2009; 28:865–870. [PubMed: 19654531]

Price MO, Feng MT, Scanameo A, Price FW Jr. Loteprednol Etabonate 0.5% Gel Vs. Prednisolone Acetate 1% Solution After Descemet Membrane Endothelial Keratoplasty: Prospective Randomized Trial. Cornea. 2015; 34:853–858. [PubMed: 26020827]

Price MO, Price FW Jr, Kruse FE, Bachmann BO, Tourtas T. Randomized comparison of topical prednisolone acetate 1% versus fluorometholone 0.1% in the first year after descemet membrane endothelial keratoplasty. Cornea. 2014; 33:880–886. [PubMed: 25062336]

Quigley HA. Number of people with glaucoma worldwide. The British journal of ophthalmology. 1996; 80:389–393. [PubMed: 8695555]

Quigley HA. Neuronal death in glaucoma. Progress in retinal and eye research. 1999; 18:39–57. [PubMed: 9920498]

Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. The British journal of ophthalmology. 2006; 90:262–267. [PubMed: 16488940]

Raghunathan VK, Morgan JT, Park SA, Weber D, Phinney BS, Murphy CJ, Russell P. Dexamethasone Stiffens Trabecular Meshwork, Trabecular Meshwork Cells, and Matrix. Investigative ophthalmology & visual science. 2015; 56:4447–4459. [PubMed: 26193921]

Ramamoorthy S, Cidlowski JA. Exploring the molecular mechanisms of glucocorticoid receptor action from sensitivity to resistance. Endocr Dev. 2013; 24:41–56. [PubMed: 23392094]

Ramamoorthy S, Cidlowski JA. Corticosteroids: Mechanisms of Action in Health and Disease. Rheum Dis Clin North Am. 2016; 42:15–31. vii. [PubMed: 26611548]

Read AT, Chan DW, Ethier CR. Actin structure in the outflow tract of normal and glaucomatous eyes. Experimental eye research. 2007; 84:214–226. [PubMed: 17219625]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. The New England journal of medicine. 2005; 353:1711–1723. [PubMed: 16236742]

Richards JE, Lichter PR, Boehnke M, Uro JL, Torrez D, Wong D, Johnson AT. Mapping of a gene for autosomal dominant juvenile-onset open-angle glaucoma to chromosome Iq. Am J Hum Genet. 1994; 54:62–70. [PubMed: 8279471]

Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science. 1996; 273:1516–1517. [PubMed: 8801636]

Ritch R, Podos SM, Henley W, Moss A, Southern AL, Fotino M. Lack of association of histocompatibility antigens with primary open-angle glaucoma. Archives of ophthalmology. 1978; 96:2204–2206. [PubMed: 718509]

Robertson JV, Golesic E, Gauldie J, West-Mays JA. Ocular gene transfer of active TGF-beta induces changes in anterior segment morphology and elevated IOP in rats. Investigative ophthalmology & visual science. 2010; 51:308–318. [PubMed: 19696167]

Robertson JV, Siwakoti A, West-Mays JA. Altered expression of transforming growth factor beta 1 and matrix metalloproteinase-9 results in elevated intraocular pressure in mice. Molecular vision. 2013; 19:684–695. [PubMed: 23559862]

Robinson CM, Neary R, Levendale A, Watson CJ, Baugh JA. Hypoxia-induced DNA hypermethylation in human pulmonary fibroblasts is associated with Thy-1 promoter methylation and the development of a pro-fibrotic phenotype. Respir Res. 2012; 13:74. [PubMed: 22938014]

Rohen JW, Linner E, Witmer R. Electron microscopic studies on the trabecular meshwork in two cases of corticosteroid-glaucoma. Experimental eye research. 1973; 17:19–31. [PubMed: 4356557]

Rohen JW, Lutjen E, Barany E. The relation between the ciliary muscle and the trabecular meshwork and its importance for the effect of miotics on aqueous outflow resistance. A study in two contrasting monkey species, Macaca irus and Cercopithecus aethiops. Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie. Albrecht von Graefe's archive for clinical and experimental ophthalmology. 1967; 172:23–47. [PubMed: 4967268]

Rohen, JWL-DE. Biology of the Trabecular Meshwork. Verlap; Stuttgart: 1982.

Rozsa FW, Reed DM, Scott KM, Pawar H, Moroi SE, Kijek TG, Krafchak CM, Othman MI, Vollrath D, Elner VM, Richards JE. Gene expression profile of human trabecular meshwork cells in response to long-term dexamethasone exposure. Molecular vision. 2006; 12:125–141. [PubMed: 16541013]

Russell P, Johnson M. Elastic modulus determination of normal and glaucomatous human trabecular meshwork. Investigative ophthalmology & visual science. 2012; 53:117.

Sabanay I, Tian B, Gabelt BT, Geiger B, Kaufman PL. Functional and structural reversibility of H-7 effects on the conventional aqueous outflow pathway in monkeys. Experimental eye research. 2004; 78:137–150. [PubMed: 14667835]

Samples JR, Alexander JP, Acott TS. Regulation of the levels of human trabecular matrix metalloproteinases and inhibitor by interleukin-1 and dexamethasone. Investigative ophthalmology & visual science. 1993; 34:3386–3395. [PubMed: 8225873]

Scheetz TE, Fingert JH, Wang K, Kuehn MH, Knudtson KL, Alward WL, Boldt HC, Russell SR, Folk JC, Casavant TL, Braun TA, Clark AF, Stone EM, Sheffield VC. A genome-wide association study for primary open angle glaucoma and macular degeneration reveals novel Loci. PloS one. 2013; 8:e58657. [PubMed: 23536807]

Schwartz JT, Reuling FH, Feinleib M, Garrison RJ, Collie DJ. Twin study on ocular pressure following topically applied dexamethasone. II. Inheritance of variation in pressure response. Archives of ophthalmology. 1973; 90:281–286. [PubMed: 4795755]

Schwartz SG, Ayala-Haedo J, Kishor KS, Fini ME. Pharmacogenomics of Open-Angle Glaucoma. Current Pharmacogenomics and Personalized Medicine. 2008; 6:121–125.

Schwartz SG, Flynn HW Jr, Beer P. Intravitreal Triamcinolone Acetonide Use in Diabetic Macular Edema: Illustrative Cases. Ophthalmic surgery, lasers & imaging: the official journal of the International Society for Imaging in the Eye. 2010:1–6.

Schwartz SG, Flynn HW Jr, Scott IU. Intravitreal Corticosteroids in the Management of Diabetic Macular Edema. Curr Ophthalmol Rep. 2013:1.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Schwartz SG, Puckett BJ, Allen RC, Castillo IG, Leffler CT. Beta1-adrenergic receptor polymorphisms and clinical efficacy of betaxolol hydrochloride in normal volunteers. Ophthalmology. 2005; 112:2131–2136. [PubMed: 16325708]

Schwartz, SGaF; ME. Pharmacogenomics for the retina specialist. Retina Today. 2010

Senatorov V, Malyukova I, Fariss R, Wawrousek EF, Swaminathan S, Sharan SK, Tomarev S. Expression of mutated mouse myocilin induces open-angle glaucoma in transgenic mice. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2006; 26:11903–11914. [PubMed: 17108164]

Sharif NA. Serotonin-2 receptor agonists as novel ocular hypotensive agents and their cellular and molecular mechanisms of action. Current drug targets. 2010; 11:978–993. [PubMed: 20426763]

Shastry BS. Pharmacogenomics in ophthalmology. Discov Med. 2011; 12:159–167. [PubMed: 21878193]

Shen X, Ying H, Yue BY. Wnt activation by wild type and mutant myocilin in cultured human trabecular meshwork cells. PloS one. 2012; 7:e44902. [PubMed: 23028669]

Shepard AR, Jacobson N, Millar JC, Pang IH, Steely HT, Searby CC, Sheffield VC, Stone EM, Clark AF. Glaucoma-causing myocilin mutants require the Peroxisomal targeting signal-1 receptor (PTS1R) to elevate intraocular pressure. Human molecular genetics. 2007; 16:609–617. [PubMed: 17317787]

Shepard AR, Millar JC, Pang IH, Jacobson N, Wang WH, Clark AF. Adenoviral gene transfer of active human transforming growth factor-{beta}2 elevates intraocular pressure and reduces outflow facility in rodent eyes. Investigative ophthalmology & visual science. 2010; 51:2067–2076. [PubMed: 19959644]

Slotkin W, Nishikura K. Adenosine-to-inosine RNA editing and human disease. Genome medicine. 2013; 5:105. [PubMed: 24289319]

Snyder RW, Stamer WD, Kramer TR, Seftor RE. Corticosteroid treatment and trabecular meshwork proteases in cell and organ culture supernatants. Experimental eye research. 1993; 57:461–468. [PubMed: 8282032]

Song CZ, Tian X, Gelehrter TD. Glucocorticoid receptor inhibits transforming growth factor-beta signaling by directly targeting the transcriptional activation function of Smad3. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:11776–11781. [PubMed: 10518526]

Spiga MG, Borras T. Development of a gene therapy virus with a glucocorticoid-inducible MMP1 for the treatment of steroid glaucoma. Investigative ophthalmology & visual science. 2010; 51:3029–3041. [PubMed: 20089870]

Stamer WD, Braakman ST, Zhou EH, Ethier CR, Fredberg JJ, Overby DR, Johnson M. Biomechanics of Schlemm's canal endothelium and intraocular pressure reduction. Progress in retinal and eye research. 2015; 44:86–98. [PubMed: 25223880]

Stamer WD, Hoffman EA, Kurali E, Krauss AH. Unique response profile of trabecular meshwork cells to the novel selective glucocorticoid receptor agonist, GW870086X. Investigative ophthalmology & visual science. 2013; 54:2100–2107. [PubMed: 23462748]

Stamer WD, Lei Y, Boussommier-Calleja A, Overby DR, Ethier CR. eNOS, a pressure-dependent regulator of intraocular pressure. Investigative ophthalmology & visual science. 2011; 52:9438–9444. [PubMed: 22039240]

Steely HT, Browder SL, Julian MB, Miggans ST, Wilson KL, Clark AF. The effects of dexamethasone on fibronectin expression in cultured human trabecular meshwork cells. Investigative ophthalmology & visual science. 1992; 33:2242–2250. [PubMed: 1607235]

Stevens A, Ray DW, Zeggini E, John S, Richards HL, Griffiths CE, Donn R. Glucocorticoid sensitivity is determined by a specific glucocorticoid receptor haplotype. The Journal of clinical endocrinology and metabolism. 2004; 89:892–897. [PubMed: 14764810]

Stone EM. Genetic testing for inherited eye disease. Archives of ophthalmology. 2007; 125:205–212. [PubMed: 17296896]

Stone EM, Aldave AJ, Drack AV, Maccumber MW, Sheffield VC, Traboulsi E, Weleber RG. Recommendations for genetic testing of inherited eye diseases: report of the American Academy

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of Ophthalmology task force on genetic testing. Ophthalmology. 2012; 119:2408–2410. [PubMed: 22944025]

Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC. Identification of a gene that causes primary open angle glaucoma. Science. 1997; 275:668–670. [PubMed: 9005853]

Stramer BM, Zieske JD, Jung JC, Austin JS, Fini ME. Molecular mechanisms controlling the fibrotic repair phenotype in cornea: implications for surgical outcomes. Investigative ophthalmology & visual science. 2003; 44:4237–4246. [PubMed: 14507867]

Suarez T, Vecino E. Expression of endothelial leukocyte adhesion molecule 1 in the aqueous outflow pathway of porcine eyes with induced glaucoma. Molecular vision. 2006; 12:1467–1472. [PubMed: 17167401]

Szabo V, Borgulya G, Filkorn T, Majnik J, Banyasz I, Nagy ZZ. The variant N363S of glucocorticoid receptor in steroid-induced ocular hypertension in Hungarian patients treated with photorefractive keratectomy. Molecular vision. 2007; 13:659–666. [PubMed: 17563720]

Tamm ER. Myocilin and glaucoma: facts and ideas. Progress in retinal and eye research. 2002; 21:395–428. [PubMed: 12150989]

Tawara A, Tou N, Kubota T, Harada Y, Yokota K. Immunohistochemical evaluation of the extracellular matrix in trabecular meshwork in steroid-induced glaucoma. Graefes Arch Clin Exp Ophthalmol. 2008; 246:1021–1028. [PubMed: 18386038]

Tektas OY, Lutjen-Drecoll E. Structural changes of the trabecular meshwork in different kinds of glaucoma. Experimental eye research. 2009; 88:769–775. [PubMed: 19114037]

Tian B, Geiger B, Epstein DL, Kaufman PL. Cytoskeletal involvement in the regulation of aqueous humor outflow. Investigative ophthalmology & visual science. 2000; 41:619–623. [PubMed: 10711672]

Tielsch JM, Katz J, Singh K, Quigley HA, Gottsch JD, Javitt J, Sommer A. A population-based evaluation of glaucoma screening: the Baltimore Eye Survey. American journal of epidemiology. 1991; 134:1102–1110. [PubMed: 1746520]

Tingey DP, Schroeder A, Epstein MP, Epstein DL. Effects of topical ethacrynic acid adducts on intraocular pressure in rabbits and monkeys. Archives of ophthalmology. 1992; 110:699–702. [PubMed: 1580849]

Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nature reviews Molecular cell biology. 2002; 3:349–363. [PubMed: 11988769]

Toris CB. Pharmacotherapies for glaucoma. Curr Mol Med. 2010; 10:824–840. [PubMed: 21091423]

Ueda J, Wentz-Hunter K, Yue BY. Distribution of myocilin and extracellular matrix components in the juxtacanalicular tissue of human eyes. Investigative ophthalmology & visual science. 2002; 43:1068–1076. [PubMed: 11923248]

Underwood JL, Murphy CG, Chen J, Franse-Carman L, Wood I, Epstein DL, Alvarado JA. Glucocorticoids regulate transendothelial fluid flow resistance and formation of intercellular junctions. The American journal of physiology. 1999; 277:C330–342. [PubMed: 10444410]

Ussa F, Fernandez I, Brion M, Carracedo A, Blazquez F, Garcia MT, Sanchez-Jara A, De Juan-Marcos L, Jimenez-Carmona S, Juberias JR, Martinez-de-la-Casa JM, Pastor JC. Association between SNPs of Metalloproteinases and Prostaglandin F2alpha Receptor Genes and Latanoprost Response in Open-Angle Glaucoma. Ophthalmology. 2015; 122:1040–1048. e1044. [PubMed: 25704319]

Vajaranant TS, Price MO, Price FW, Gao W, Wilensky JT, Edward DP. Visual acuity and intraocular pressure after Descemet's stripping endothelial keratoplasty in eyes with and without preexisting glaucoma. Ophthalmology. 2009; 116:1644–1650. [PubMed: 19643499]

van Koolwijk LM, Ramdas WD, Ikram MK, Jansonius NM, Pasutto F, Hysi PG, Macgregor S, Janssen SF, Hewitt AW, Viswanathan AC, ten Brink JB, Hosseini SM, Amin N, Despriet DD, Willemse-Assink JJ, Kramer R, Rivadeneira F, Struchalin M, Aulchenko YS, Weisschuh N, Zenkel M, Mardin CY, Gramer E, Welge-Lussen U, Montgomery GW, Carbonaro F, Young TL, Group DER, Bellenguez C, McGuffin P, Foster PJ, Topouzis F, Mitchell P, Wang JJ, Wong TY,

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Czudowska MA, Hofman A, Uitterlinden AG, Wolfs RC, de Jong PT, Oostra BA, Paterson AD, Mackey DA, Bergen AA, Reis A, Hammond CJ, Vingerling JR, Lemij HG, Klaver CC, van Duijn CM. Wellcome Trust Case Control C. Common genetic determinants of intraocular pressure and primary open-angle glaucoma. PLoS genetics. 2012; 8:e1002611. [PubMed: 22570627]

van Rossum EF, Lamberts SW. Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. Recent progress in hormone research. 2004; 59:333–357. [PubMed: 14749509]

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. Science. 2001; 291:1304–1351. [PubMed: 11181995]

Vittitow JL, Garg R, Rowlette LL, Epstein DL, O'Brien ET, Borras T. Gene transfer of dominant-negative RhoA increases outflow facility in perfused human anterior segment cultures. Molecular vision. 2002; 8:32–44. [PubMed: 11889464]

Vranka JA, Kelley MJ, Acott TS, Keller KE. Extracellular matrix in the trabecular meshwork: intraocular pressure regulation and dysregulation in glaucoma. Experimental eye research. 2015; 133:112–125. [PubMed: 25819459]

Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science. 1998; 280:1077–1082. [PubMed: 9582121]

Wang N, Chintala SK, Fini ME, Schuman JS. Activation of a tissue-specific stress response in the aqueous outflow pathway of the eye defines the glaucoma disease phenotype. Nature medicine. 2001; 7:304–309.

Wang WH, McNatt LG, Pang IH, Millar JC, Hellberg PE, Hellberg MH, Steely HT, Rubin JS, Fingert JH, Sheffield VC, Stone EM, Clark AF. Increased expression of the WNT antagonist sFRP-1 in

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glaucoma elevates intraocular pressure. The Journal of clinical investigation. 2008; 118:1056–1064. [PubMed: 18274669]

Wang YL, Hayashi M, Yablonski ME, Toris CB. Effects of multiple dosing of epinephrine on aqueous humor dynamics in human eyes. Journal of ocular pharmacology and therapeutics: the official journal of the Association for Ocular Pharmacology and Therapeutics. 2002; 18:53–63.

West-Mays JA, Cook JR, Sadow PM, Mullady DK, Bargagna-Mohan P, Strissel KJ, Fini ME. Differential inhibition of collagenase and interleukin-1alpha gene expression in cultured corneal fibroblasts by TGF-beta, dexamethasone, and retinoic acid. Investigative ophthalmology & visual science. 1999; 40:887–896. [PubMed: 10102285]

White GE, Gimbrone MA Jr, Fujiwara K. Factors influencing the expression of stress fibers in vascular endothelial cells in situ. The Journal of cell biology. 1983; 97:416–424. [PubMed: 6684121]

Wiggs JL. The cell and molecular biology of complex forms of glaucoma: updates on genetic, environmental, and epigenetic risk factors. Investigative ophthalmology & visual science. 2012; 53:2467–2469. [PubMed: 22562842]

Wiggs JL. Glaucoma Genes and Mechanisms. Prog Mol Biol Transl Sci. 2015; 134:315–342. [PubMed: 26310163]

Wiggs JL, Hauser MA, Abdrabou W, Allingham RR, Budenz DL, Delbono E, Friedman DS, Kang JH, Gaasterland D, Gaasterland T, Lee RK, Lichter PR, Loomis S, Liu Y, McCarty C, Medeiros FA, Moroi SE, Olson LM, Realini A, Richards JE, Rozsa FW, Schuman JS, Singh K, Stein JD, Vollrath D, Weinreb RN, Wollstein G, Yaspan BL, Yoneyama S, Zack D, Zhang K, Pericak-Vance M, Pasquale LR, Haines JL. The NEIGHBOR consortium primary open-angle glaucoma genome-wide association study: rationale, study design, and clinical variables. Journal of glaucoma. 2013; 22:517–525. [PubMed: 22828004]

Wiggs JL, Pierce EA. Genetic testing for inherited eye disease: who benefits? JAMA ophthalmology. 2013; 131:1265–1266. [PubMed: 23949187]

Wiggs JL, Vollrath D. Molecular and clinical evaluation of a patient hemizygous for TIGR/MYOC. Archives of ophthalmology. 2001; 119:1674–1678. [PubMed: 11709019]

Wiggs JL, Yaspan BL, Hauser MA, Kang JH, Allingham RR, Olson LM, Abdrabou W, Fan BJ, Wang DY, Brodeur W, Budenz DL, Caprioli J, Crenshaw A, Crooks K, Delbono E, Doheny KF, Friedman DS, Gaasterland D, Gaasterland T, Laurie C, Lee RK, Lichter PR, Loomis S, Liu Y, Medeiros FA, McCarty C, Mirel D, Moroi SE, Musch DC, Realini A, Rozsa FW, Schuman JS, Scott K, Singh K, Stein JD, Trager EH, Vanveldhuisen P, Vollrath D, Wollstein G, Yoneyama S, Zhang K, Weinreb RN, Ernst J, Kellis M, Masuda T, Zack D, Richards JE, Pericak-Vance M, Pasquale LR, Haines JL. Common variants at 9p21 and 8q22 are associated with increased susceptibility to optic nerve degeneration in glaucoma. PLoS genetics. 2012; 8:e1002654. [PubMed: 22570617]

Wirtz MK, Keller KE. The Role of the IL-20 Subfamily in Glaucoma. Mediators Inflamm. 2016; 2016:4083735. [PubMed: 26903709]

Wirtz MK, Samples JR, Kramer PL, Rust K, Topinka JR, Yount J, Koler RD, Acott TS. Mapping a gene for adult-onset primary open-angle glaucoma to chromosome 3q. Am J Hum Genet. 1997; 60:296–304. [PubMed: 9012402]

Wong AJ, Pollard TD, Herman IM. Actin filament stress fibers in vascular endothelial cells in vivo. Science. 1983; 219:867–869. [PubMed: 6681677]

Wordinger RJ, Clark AF. Effects of glucocorticoids on the trabecular meshwork: towards a better understanding of glaucoma. Progress in retinal and eye research. 1999; 18:629–667. [PubMed: 10438153]

Wordinger RJ, Fleenor DL, Hellberg PE, Pang IH, Tovar TO, Zode GS, Fuller JA, Clark AF. Effects of TGF-beta2, BMP-4, and gremlin in the trabecular meshwork: implications for glaucoma. Investigative ophthalmology & visual science. 2007; 48:1191–1200. [PubMed: 17325163]

Wordinger RJ, Sharma T, Clark AF. The Role of TGF-beta2 and Bone Morphogenetic Proteins in the Trabecular Meshwork and Glaucoma. Journal of ocular pharmacology and therapeutics: the official journal of the Association for Ocular Pharmacology and Therapeutics. 2014

Wu C, Morris JR. Genes, genetics, and epigenetics: a correspondence. Science. 2001; 293:1103–1105. [PubMed: 11498582]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Wynn TA. Cellular and molecular mechanisms of fibrosis. The Journal of pathology. 2008; 214:199–210. [PubMed: 18161745]

Xue W, Comes N, Borras T. Presence of an established calcification marker in trabecular meshwork tissue of glaucoma donors. Investigative ophthalmology & visual science. 2007; 48:3184–3194. [PubMed: 17591888]

Yap KL, Li S, Munoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ, Zhou MM. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. Molecular cell. 2010; 38:662–674. [PubMed: 20541999]

Yuan Y, Call MK, Yuan Y, Zhang Y, Fischesser K, Liu CY, Kao WW. Dexamethasone induces cross-linked actin networks in trabecular meshwork cells through noncanonical wnt signaling. Investigative ophthalmology & visual science. 2013; 54:6502–6509. [PubMed: 23963164]

Yun AJ, Murphy CG, Polansky JR, Newsome DA, Alvarado JA. Proteins secreted by human trabecular cells. Glucocorticoid and other effects. Investigative ophthalmology & visual science. 1989; 30:2012–2022. [PubMed: 2674051]

Zhang H, Zhao GH, Zhang Q, Dong XC, Hu WK, Xie EJ, Li GG. Relationship between glucocorticoid receptors in the peripheral blood lymphocytes and trabecular meshwork and glucocorticoid induced glaucoma [Zhonghua yan ke za zhi]. Chinese journal of ophthalmology. 2006a; 42:431–434. [PubMed: 16762238]

Zhang X, Clark AF, Yorio T. Regulation of glucocorticoid responsiveness in glaucomatous trabecular meshwork cells by glucocorticoid receptor-beta. Investigative ophthalmology & visual science. 2005; 46:4607–4616. [PubMed: 16303956]

Zhang X, Schroeder A, Callahan EM, Coyle BM, Wang N, Erickson KA, Schuman JS, Fini ME. Constitutive signalling pathway activity in trabecular meshwork cells from glaucomatous eyes. Experimental eye research. 2006b; 82:968–973. [PubMed: 16516195]

Zhou L, Li Y, Yue BY. Glucocorticoid effects on extracellular matrix proteins and integrins in bovine trabecular meshwork cells in relation to glaucoma. International journal of molecular medicine. 1998; 1:339–346. [PubMed: 9852235]

Zhou Y, Grinchuk O, Tomarev SI. Transgenic mice expressing the Tyr437His mutant of human myocilin protein develop glaucoma. Investigative ophthalmology & visual science. 2008; 49:1932–1939. [PubMed: 18436825]

Zhu J, Gong JY, Goodman OB Jr, Cartegni L, Nanus DM, Shen R. Bombesin attenuates pre-mRNA splicing of glucocorticoid receptor by regulating the expression of serine-arginine protein p30c (SRp30c) in prostate cancer cells. Biochimica et biophysica acta. 2007; 1773:1087–1094. [PubMed: 17540466]

Zhuo YH, He Y, Leung KW, Hou F, Li YQ, Chai F, Ge J. Dexamethasone disrupts intercellular junction formation and cytoskeleton organization in human trabecular meshwork cells. Molecular vision. 2010; 16:61–71. [PubMed: 20090922]

Zode GS, Sharma AB, Lin X, Searby CC, Bugge K, Kim GH, Clark AF, Sheffield VC. Ocular-specific ER stress reduction rescues glaucoma in murine glucocorticoid-induced glaucoma. The Journal of clinical investigation. 2014; 124:1956–1965. [PubMed: 24691439]

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Highlights

- Glucocorticoids cause ocular hypertension/glaucoma, but only in a subset of patients.
- Glucocorticoids affect expression of many genes, variants in which could alter response.
- Pharmacogenomics can identify genetic variants affecting glucocorticoid sensitivity.
- The location of variants can pinpoint new causative genes and clinically relevant pathways.
- Testing for identified variants can be used to predict glucocorticoid sensitivity in patients.

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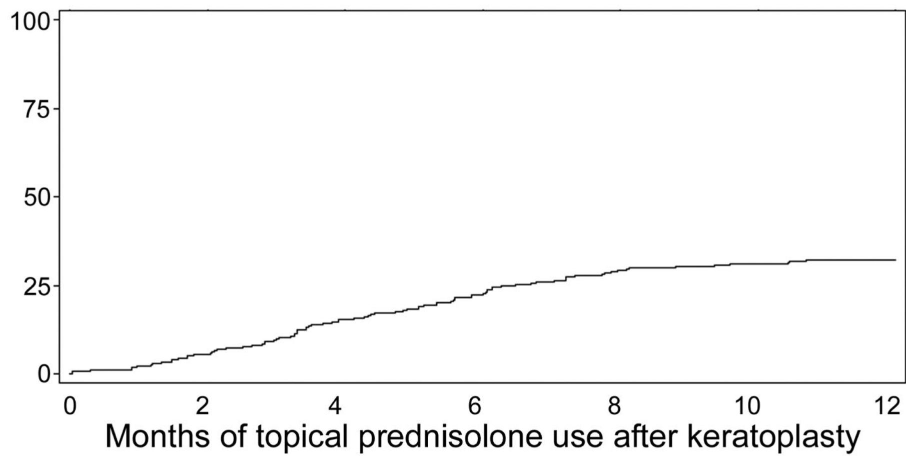

Figure 1. Cumulative probability of experiencing an IOP spike with glucocorticoid treatment

Kaplan-Meier analysis showing the cumulative probability of experiencing an IOP spike (defined as postoperative IOP 24 mm Hg or increased by 10 mm Hg over the baseline preoperative reading) when prednisolone acetate 1% ophthalmic suspension was dosed 4 times a day for 4 months then tapered over 2 to 3 months to once or twice daily dosing to prevent cornea transplant rejection.

Patient data from (Vajaranant et al., 2009); the graphic has not been previously published.

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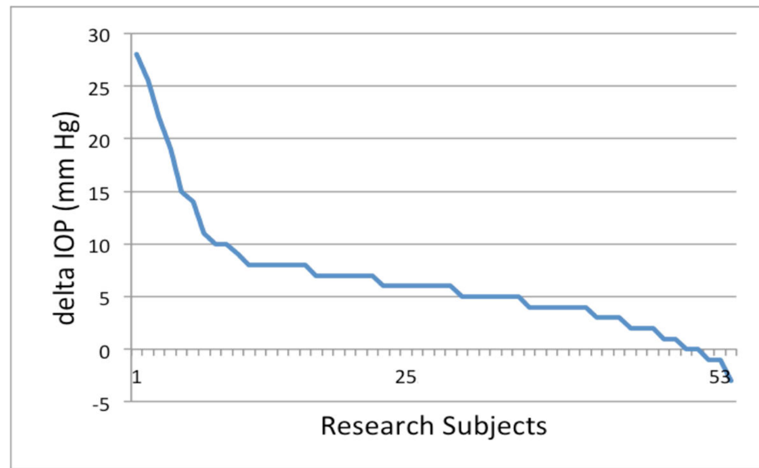

Figure 2. Quantitative trait distribution for the Florida-1 cohort

A graphic distribution of IOP values (y-axis) calculated for each of the research subjects comprising the Florida-1 cohort (x-axis) is shown.

Patient data from (Gerzenstein et al., 2008); the graphic has not been previously published.

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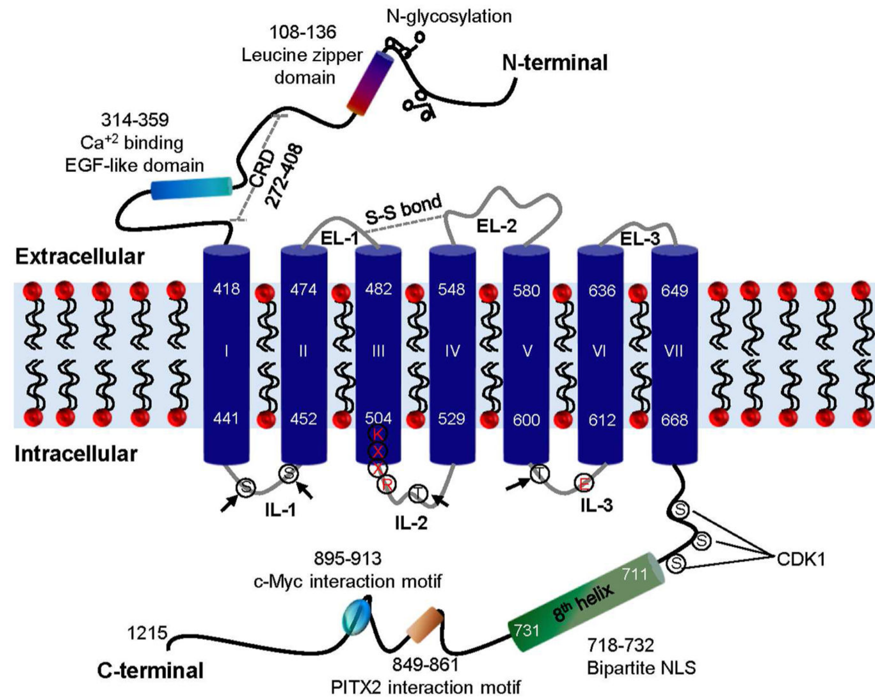

Figure 3. Predicted features of the protein encoded by *GPR158*

The schematic shown is based on sequence analysis of the conceptually translated product of *GPR158*. Three extracellular loops (ELs) and three intracellular loops (ILs) connect the seven TM (numbered I–VII). The arrow indicates putative PKC and PKA phosphorylation sites in the ILs. The cysteine residues in EL-1 and EL-2 involved in disulfide (S-S) bond formation are shown as a dotted grey double line. The eighth helix, bipartite NLS, c-Myc and PITX2 interaction motifs, and putative phosphorylation sites for kinases, such as CDK1, are indicated in the C-terminal tail. The leucine zipper domain, EGF like domain, N-glycosylation sites and putative CRD are shown in the N-terminal of GPR158. The conserved amino acid residues, KXXR and E, involved in G protein activation in class C GPCRs are marked in red color. CDK1, cyclin-dependent kinase 1; CRD, cysteine rich domain; EGF, epidermal growth factor; EL, extracellular loop; IL, intracellular loop; NLS, nuclear localization signal; PITX2, paired-like homeodomain transcription factor 2.

From: GPR158, an orphan member of G protein-coupled receptor Family C: glucocorticoid-stimulated expression and novel nuclear role. Patel N *et al.* PLoS One. 2013;8(2):e57843. doi: 10.1371/journal.pone.0057843 CC-BY; used with permission from the publisher.

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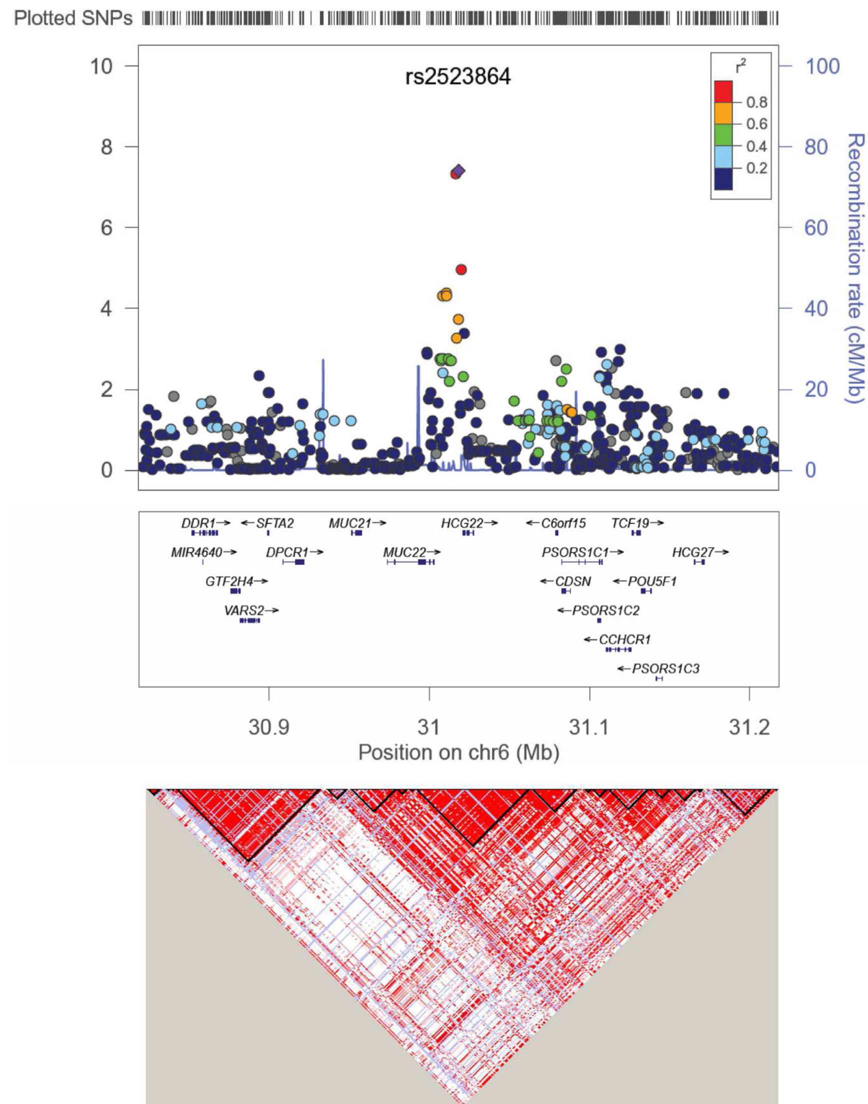

Figure 4. Regional Association Plot for lead SNP rs2523864 (marked as purple diamond) on Chr6

All SNPs shown as circles are plotted with their respective *P* values against their genomic location. The solid diamond indicates the top-ranked SNP rs2523864. The colored box at the right or left corner of each plot indicates the pairwise correlation (r^2) between the top SNP and the other SNPs in the region. The grey circles indicate the imputed SNPs from the CEU population of the HapMap. Each plot was created using LocusZoom (http://csg.sph.umich.edu/locuszoom/) for the top-ranked SNP in each region with a 400-kb region surrounding it. SNPs are plotted at the top of the figure. The box underneath each plot shows the gene annotations in the region, with the arrow indicated the DNA strand for transcription. The lower LD plot was created using Haploview (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview).

From: Identification of a Novel Mucin Gene HCG22 Associated With Steroid-Induced Ocular Hypertension. Jeong S, *et al.* Invest Ophthalmol Vis Sci. 2015 Apr;56(4):2737–48. doi: 10.1167/iovs.14-14803. Used with permission from the publisher. The authors acknowledge the Association for Research in Vision and Ophthalmology as the copyright holder.

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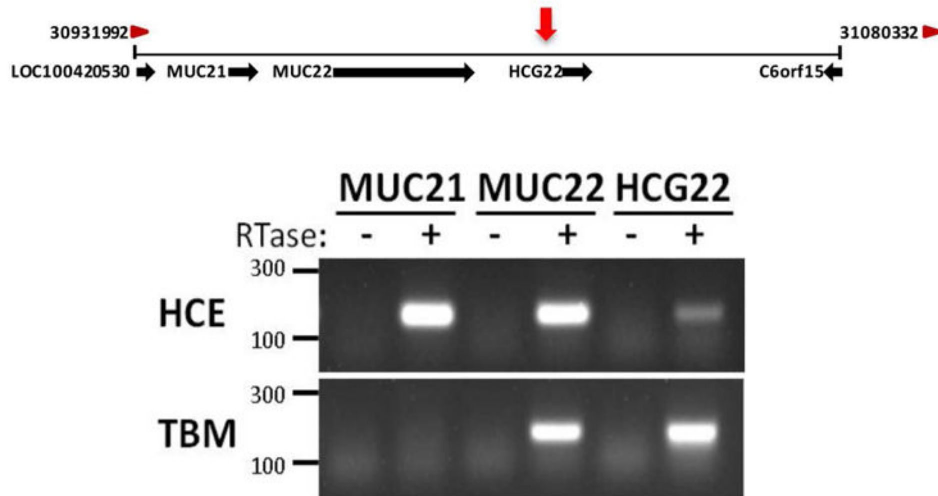


Figure 5. Location and expression of genes surrounding the QTL in chromosomal region 6p21.32-33

(Top panel) Schematic of chromosomal region 6p21.32-33 from the NCBI Gene website depicting annotated genes surrounding the identified QTL (red arrow).

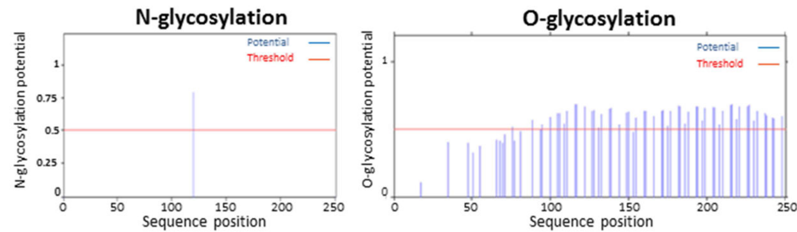
(Bottom panel) Total RNA was purified from cultured primary HCE and TM-1 cells, and used for cDNA synthesis. RT-PCR using cDNA was performed using gene-specific primers from MUC21, MUC22 and HCG22, and the products were resolved on a 1.5% agarose gel. Primers for HCG22 were designed to detect only the coding transcript. Similar results were obtained using three primary TBM cell lines (not shown). RTase: reverse transcriptase; HCE: primary corneal epithelial cells obtained from corneal rims; TBM: cells of the TM-1 line.

From: Identification of a Novel Mucin Gene HCG22 Associated With Steroid-Induced Ocular Hypertension. Jeong S, *et al.* Invest Ophthalmol Vis Sci. 2015 Apr;56(4):2737–48. doi: 10.1167/iovs.14-14803. Used with permission from the publisher. The authors acknowledge the Association for Research in Vision and Ophthalmology as the copyright holder.

(A) HCG22 deduced sequence

MPRYVPLLLLLLLLRCSERGGGVNFGEKDAKVPGTWRDGVRVPGEGASWDSDRASPERR
YGIVGLSQSISTKHPETSPKDSRIRENDVTADGRTTEDHITADPGTTEDSVTADPGTTE
DNVTVDPGTTEGSVTADPATTKDYVSADPGTTKDSVTADPGTTENFVTADPGTTKDSIT
ADPRTTEDSVTADPGTTKHSITVDPGTTEDSVTADPGTTKHSITADPGTTEDSVTADPG
TTEDETTKHGDTHLL

(B) Prediction of glycosylation



(C) Amino acid substitutions in the signal peptide

GenBank transcript AB560771
MP<u>R</u>YVPLLLLLLLLRCS<u>E</u>RGGG

Lung cell line cDNA
MP<u>G</u>YVPLLLLLLLLRCS<u>Q</u>RGGG

Figure 6. Conceptual Translation of *HCG22*

(A) HCG22 deduced sequence. Genbank transcript AB560771 is 1,671 nucleotides in length. The deduced amino acid sequence is 251 amino acids. A signal peptide of 22 amino acids at the N-terminus is predicted (underlined). The yellow highlights mark the start of each mucin-type repeat, which could be *O*-glycosylated. No transmembrane domain is found, suggesting that the protein is secreted.

(B) Prediction of glycosylation. N- and O-linked glycosylation patterns of *HCG22* were predicted by analysis of coding regions using web-based software analysis (N-glycosylation: http://www.cbs.dtu.dk/services/NetNGlyc/; O-glycosylation: http://www.cbs.dtu.dk/services/NetOGlyc/).

(C) Amino acid substitutions in the signal peptide. Signal peptide sequences deduced from GenBank transcript AB560771 and from cDNA prepared from human lung cell line BET1A are compared in the figure (relevant amino acids are in red and underlined).

This graphic has not been previously published.

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