A new paradigm for delivering personalised care: integrating genetics with surgical interventions in *BEST1* mutations

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Introduction

Personalised medicine (PM) is the translational goal at the core of current molecular medical research.[1] How to effectively, efficiently and economically integrate genetic information into the medical management of patients is the biggest challenge in frontline innovation strategies.[2,3] Using genetic information to guide the selection of surgical candidates has now been reported in cancer therapy[4] and neurosurgery,[5] with likely applications in more specialties.

Best disease (BD) was first described by Adams in 1883, and named by Best in 1905.[6] It is an inherited, macular dystrophy characterised by vitelliform macular lesions (egg yolk appearance), a normal electroretiongram (ERG) and loss of the electo-oculogram (EOG) light-rise. The aetiology of Best disease was first linked to the *BEST1* (*VMD2*) gene in 1998.[7] BD is classically an autosomal dominant disorder (ADB). In 2008, a more devastating and under-diagnosed form, autosomal recessive bestrophinopathy (ARB), was discovered, with a previously unknown risk of angle-closure glaucoma (ACG), which was present in at least 50% of patients with ARB, [8]

all of whom required glaucoma surgery. We present a translational disease paradigm where genetically informed personalised surgical intervention can directly improve clinical outcomes, thereby coining the term “surgico-genetics”.

At two tertiary referral centres for glaucoma, we observed a series of three patients with ARB (cases 1, 2 & 3) and one with ADB (case 4, the only autosomal dominant case with angle-closure glaucoma reported in the literature at the time of this report), who developed a sight threatening complication after first-line glaucoma surgery, known as malignant glaucoma (MG). MG is also known as vitreous block or aqueous misdirection, more frequently occurring in nanophthalmic eyes than those with shallow iridocorneal angles alone. We observed that all our cases, except case 5, developed MG. This was similar to the 100% penetrance of MG in Zhong *et al*’s report of 8 Chinese patients with *BEST1* mutations (15 eyes) that underwent trabeculectomy. The surgical management of case 5 in our paper was performed with the hypothesis that *BEST1* mutations causes an inherent vitreopathy that increases the risk of MG. We devised a suitable surgical intervention of combining primary pars plana vitrectomy with a pars plana Baerveldt tube insertion to circumvent the devastating visual loss of MG in Case 5. This patient is the only case reported to date that did not develop MG after having glaucoma surgery, and managed to achieve optimal IOP control after 5 years. Full details with the key clinical signs for all our 5 cases are presented below, with a supplementary PowerPoint presentation available online.

Methods

Genome sequencing and assembly

Bidirectional Sanger sequencing of *BEST1* in affected individuals was performed at the National Genetics Reference Library in Manchester, UK.

Ethical approval

Patients 1 to 4 and their family members were participants of research studies approved by Moorfields Eye Hospital NHS Foundation Trust Ethics Committee. Patient 5 and her family members were treated as National Health Service patients and genotyping performed based on clinical need. All subjects received education and counselling and provided informed consent.

Case Reports

Case 1

A 44-year old Caucasian female previously diagnosed with bilateral Autosomal Recessive Best disease (ARB) presented with bilaterally closed angles, right glaucomatous optic neuropathy (GON), intraocular pressures (IOPs) of 34mmHg right eye (RE) and 26mmHg left eye (LE), best corrected visual acuity (BCVA) was 6/60 RE and 6/36 LE. She underwent bilateral YAG laser iridotomies followed by right mitomycin augmented trabeculectomy. One week post-operatively, the patient presented with right severe ocular pain, shallow right anterior chamber (AC) and right IOP 47mmHg. MG was diagnosed and she underwent right pars-plana vitrectomy (PPV) with subsequent AC reformation. 6 months post-operatively, right BCVA had deteriorated to HM due to right cystoid macular oedema (CMO) despite being on long term oral acetazolamide 500 mg bd, her IOPs were 11 mmHg RE (on

cyclopentolate 1% nocte) and 16 mmHg LE (on timolol 0.25% bd, latanoprost 0.005% nocte) with significant cupping of the right optic disc. Fundus examination also showed bilateral juxtapapillary disc drusen. Molecular genetic analysis revealed two heterozygous variants: c.102C>T p.Gly34Gly and c.572T>C p.Leu191Pro at the *BEST1* gene.

Case 2

A 41-year-old Caucasian male, phakic with bilateral advanced ACG, bilateral ARB, exfoliation syndrome on the anterior chamber lens surface, and optic discs drusen presented with bilateral ocular pain and blurred vision for 12 hours, having previously had mitomycin augmented trabeculectomies in both eyes. BCVA was 6/60 RE and 3/60 LE, examination revealed shallow ACs (right>left), flat hyperemic blebs, IOPs measuring 43 mmHg and 25 mmHg, axial lengths (ALs) measuring 21.45 mm and 21.32 mm and AC depths (ACDs) measuring 2.05 mm and 2.36 mm RE and LE respectively. Bilateral MG was diagnosed and both eyes were firstly commenced on topical atropine 1% tds, then underwent cyclodiode laser ciliary body ablation (CLCBA) 1 week and 9 months after trabeculectomy, RE and LE respectively. 8 weeks after left CLCBA, IOPs measured 22 mmHg RE and 9 mmHg LE with partial recovery of BCVA in the right eye to 6/24. Molecular genetic analysis revealed a heterozygous mutation c.602T>C p (Ile201Thr) in exon 5 of the *BEST1* gene. Malignant glaucoma recurred after 4 months of CLCBA, and he also developed an allergy to atropine eye drops used for the treatment of MG.

Case 3

A 24-year-old student bilaterally phakic with bilateral ARB and bilateral ACG had left eye mitomycin-C augmented trabeculectomy, and presented with progressive blurred central vision in her right eye over 6 months. On examination, BCVA was 6/24 RE and CF LE, IOPs were 46 mmHg and 16 mmHg respectively, examination showed LE MG (-6.75 spherical equivalent of the myopic shift in the left eye + left AC depth < 2 mm) and bilateral peripheral anterior synechiae. The patient initially underwent right iridotomy and iridoplasty, then right clear lens extraction with intraocular lens insertion (IOL) two weeks later, with subsequent right aqueous misdirection 1 week post-operatively. The RE IOP progressively increased from 14 to 35 mmHg with a flat AC. She was initially commenced on g. atropine 1% to both eyes, then underwent right CLCBA and left clear lens extraction with IOL. The left trabeculectomy bleb failed, and she underwent left CLCBA. 4 months post-operatively, BCVA was 6/36 in both eyes, her IOPs 16 and 26 mmHg respectively on dorzolamide 2%/timolol 0.5% bd and latanoprost 0.005% nocte to both eyes with a left fully cupped disc. Molecular genetic analysis revealed the c.454 C>G and the c.481+1 G>T p (mutations at the *BEST1* gene.

Case 4

A 42-year-old Caucasian female bilaterally phakic, with bilateral ADB, bilateral ACG and previous left eye multiple filtering surgeries which led to corneal endothelial damage and opacities presented for a second opinion about her right eye. Her right IOP was 43 mmHg on 4 topical lowering-IOP drugs, she initially underwent right clear lens extraction + IOL and developed aqueous misdirection 1 week post-operatively. Despite a deepening of her right AC with topical atropine 1%, right IOP remained 40 mmHg requiring right CLCBA, and right IOP normalized to 10 mmHg on

no topical/systemic anti-glaucoma treatment 9 months post-CLCBA. EOG showed neither dark troughs nor light-rise in both eyes, confirming the ADB diagnosis. Molecular genetic analysis the coding region (exon 8), c.914T>C, p.Phe305Ser.

Case 5

A 23-year-old lady with deteriorating vision for four years, best corrected visual acuity (BCVA) 6/60 right eye (RE), hand motion left eye (LE), was found to have elevated IOP of 38mmHg RE and 48mmHg LE, and bilateral vitelliform lesions at the macula. Her optic disc and anterior segment examination showed end stage angle-closure glaucoma, with disc cupping >0.9 in both eyes. Due to her young age and significant risk of complete visual loss, a multi-disciplinary approach was taken while temporizing surgery with cycloablation was performed, and genetic testing to confirm her *BEST1* disease status.

Her IOPs remained uncontrolled with maximal medical therapy and acetazolamide, so definitive surgery with primary pars plana vitrectomy (complete vitreous gel removal) and a glaucoma drainage implant (Baerveldt tube) directly through the pars plana into the posterior chamber was performed sequentially in both eyes – see also supplementary material.

Two months later, her IOPs normalized to 12 mmHg RE and 17 mmHg LE. After 5 years, her IOPs were 16mmHg in both eyes with no medications required in both eyes. There was reversal of her optic disc cupping to 0.7 in the right eye, and stable at 0.9 in her left eye. She achieved the best IOP and vision outcome, and avoided the complication of MG throughout her 5-year treatment course. This was a direct

result of selecting primary vitreous removal surgery, combined with a pars plana drainage implant, when we recognised her genetic risk of refractory MG that was observed in the other patients who had first line surgery not personalised to their underlying genetic condition (See Figure 1).

Results

Disease variants

Mutations in *BEST1* have now been found to be causative for a number of distinctive retinal dystrophies, including circumferential retinitis pigmentosa,[9] autosomal dominant vitreoretinochoroidopathy,[10], and ARB.[8]

When both alleles of the *BEST1* gene carry mutations, the null phenotype, ARB is observed. Biallelic mutations in *BEST1* produces a retinopathy distinct from Best disease, with global changes in the autofluorescence pattern. Unlike BD where the ERG function is normal, patients with ARB also have reduced full-field ERGs with delayed responses for both rods and cones due to widespread photoreceptor cell death, characterised by irregular, small pale subretinal deposits on fundal examination. Diffuse RPE changes, including dispersed punctate flecks, are also observed. Phenotypically patients have marked central visual disturbance with up to 50% of ARB cases complicated by ACG.[8] We have shown in a previous publication that the non-disease carrying allele in autosomal dominant best disease (patient 1) can alter the EOG phenotype.[11]

Personalised surgery

The *BEST1* gene encodes bestrophin-1, a transmembrane protein located in the basolateral membrane of the retinal pigment epithelium (RPE).[12,13,14,15] The light-rise of the EOG is thought to result from chloride conductance across the basolateral membrane of the RPE.[16,17] Exogenous expression of bestrophin-1 produces a chloride ion conductance which suggests that bestrophin-1 may function as a calcium-sensitive chloride channel directly responsible for generating the light-rise of the EOG.[18]

Half of all mutations described are found in the C-terminal region of bestrophin-1, which interacts with serine/ threonine protein phosphatase2A (PP2A), indicating that these residues may be important in the regulatory interaction between bestrophin-1 and phosphatase2A.[11] The protein PP2A is important in the regulation of photoreceptors in the human retina, which might explain the ERG abnormalities noted in ARB. Phosphorylation and dephosphorylation of bestrophin-1 may also act as the on/off light switch for the amplitude and timing of the EOG response.[11]

Choroidal expansion is the most likely mechanistic explanation underlying ARB-associated ACG[19], and defective homeostasis of the fluid exchange chloride pump is also highly probable.[20] Since the embryologic origin, development and differentiation of RPE and the ciliary body are under the same control mechanisms, this lends support to the hypothesis that ARB may be classified as a vitreo-retinopathy. In case 5, personalised surgical treatment of vitrectomy disrupted the anterior hyaloid face, released any pockets of aqueous sequestered within the vitreous gel and re-estabilished communication between anterior and posterior chamber. This avoided development of malignant glaucoma during her clinical course. Interestingly, a case

series of angle-closure glaucoma in Chinese patients with *BEST1* mutations also identified that 'selection of filtering surgery should be very careful' and to 'avoid trabeculectomy'[21]. Our paper presents an alternative surgical method with primary pars plana vitrectomy and posterior chamber drainage tube implantation, that successfully bypassed the complication of MG, and remained functional after 5 years. We thereby highlight the translational implications of genetic information guiding surgical selection (surgico-genetics).

Discussion

The functional role of bestrophin-1 in the RPE is still largely elusive, although the clinical features suggest the gene also influences other intraocular tissues such as the ciliary body, vitreous, choroid and peripheral retina. To date, key information remains unidentified, such as: the functioning of key proteins; regulation by associated transcription factors; and alteration via miRNA or epigenetic modification.

Due to paucity of molecular knowledge, identification of potential therapeutic targets within affected pathways is limited, making personalised medical interventions for the retinal findings of bestrophinopathies unlikely at present. It is of considerable translational significance then, particularly in ARB, that a personalised surgical approach to deal with the secondary effects (avoiding malignant glaucoma in these patients) provided treatment benefits despite limitations in our mechanistic understanding of its pathophysiology. This underscores the potential utility of personalised surgery, especially when combined with detailed clinical observations and prospective evaluation of surgery techniques and outcomes.

Actionability of genetic data is defined by Snyder et al as the interpretation of molecular events to guide therapeutics and clinical care. The first paper describing genetic diagnosis changing the medical treatment of an individual patient was published in 2012.[22] Since then, genomics based treatments have become a reality in modern medicine. Notable success stories, however, all involve pharmaco-genetic or gene-therapy interventions. To date, the translational impact of genetic and molecular developments has been entirely within medical, not surgical, disciplines. This report describes genetic analysis directly informing the selection of surgical options, not directly for the primary retinal disorder, but to manage and prevent devastating sight threatening complications from the associated glaucoma. As such, there is currently no recognition, regulatory framework or funding for the field which we call surgico-genetics.

Like clinical medicine, surgery too can be optimised by considering individual variables, for example, age, co-morbidities, and psychological make-up. We believe surgico-genetics to be the next frontier in improving surgical outcomes for our patients. The logistics of achieving this within daily practice, are possibly what has halted the genesis and development of this idea so far. By working closely with our research partners, the potential gains of personalised surgery may shift this trend. Ophthalmology has an established record in the forefront of personalised medicine,[23] and with more molecular diagnoses being made, we anticipate further progress in surgico-genetics both from our field, and other surgical specialties.

Our experience with patients 1-4 suggested that trabeculectomy should be avoided in patients with *BEST1* mutations, and to perform lens extractions with caution. In

addition, we established that intraocular surgery with cataract or trabeculectomy was responsible for triggering episodes of MG. *BEST1* codes for a chloride channel that affects anion permeability. We propose that this genetic defect also produces an abnormal vitreopathy that gives rise to a much higher incidence of MG, compared to patients without *BEST1* mutations.

Since all patients had undergone genotyping, we were also able to recognise a correlation between an individual's *BEST1* gene mutation status and their clinical features. Consequently, when a fifth patient (case 5), presented with the identified at-risk phenotypes of bilateral macular lesions and angle-closure glaucoma, first line angle-closure glaucoma surgery (lens extraction or trabeculectomy) was avoided. We opted to remove the vitreous (primary pars plana vitrectomy) and implant an aqueous tube shunt (primary pars plana glaucoma drainage device) to control her eye pressure and prevent malignant glaucoma by directly addressing the vitreopathy. The novel use of this surgico-genetics approach was based on our understanding of the pathophysiology of MG, as well as the patient's *BEST1* mutation status.

BD is a rare monogenic disease affecting 1 in 10,000 individuals. We have presented our findings and expert opinion across 5 cases in this paper. In other disease models, clinicians must establish phenotypic accuracy, and surgical target efficacy in order to demonstrate effectiveness of surgico-genetic treatments. This will require the support of statistical methods for data aggregation and an information registry that can collect, categorise and make data on personalised surgical interventions more easily accessible. In a rare disorder like BD, the referring clinicians who first undertook lens extraction and trabeculectomy surgery were

unaware of the association between BD and angle-closure glaucoma. Scientific publications [8,11] and teaching symposia to disseminate findings are therefore required.

At present personalised surgery within the National Health Service (NHS) framework may only be accessible in a few specialist centres where appropriate genetic counselling can be offered to assist patients with treatment selection. Determining which information and surgical options will be reported back to a patient will require concerted multi-disciplinary efforts from clinicians, researchers and ethicists.

Combined pars-plana vitrectomy surgery and Baerveldt tube implantation for refractory glaucoma has already been shown,[24] and in this case applied so that the primary anatomical structure (vitreo-retinopathy) is treated when managing angle-closure glaucoma in a young patient with ARB, preserving her sight for more than 5 years to date, with no complications. For surgico-genetics to be integrated into clinical practice, significant overhauls to clinical guidelines, research, education funding and health-care planning will be required. The medico-legal and ethical consequences of offering, non-first line surgical treatments to patients will also need careful consideration.

Conclusion

In this era of genomics, omics[25] and scrutiny of surgical outcomes, our work is the first exemplar of a personalised surgical approach to achieve improved clinical outcomes. These cases provide a paradigm for how to respond to individual needs using multi-professional learning between geneticists, ethicists and surgical teams.

As we approach a new frontier of personalised care, surgico-genetics has wide implications across surgical specialities, especially in disease models where the molecular basis is poorly understood. Co-ordinated future efforts to advocate surgico-genetics will require wider awareness within the medical community, and funding support to realise and fully harness the potential benefits.

Summary

What was known before

- Best disease is a rare genetic disorder affecting the eye, mainly at the macula if autosomal dominant, and more severely affecting the macula and remaining fundus with autosomal recessive inheritance.

What this study adds

- Best patients have an additional risk of developing malignant glaucoma, which is particularly difficult to manage, and requires surgical intervention.
- Filtration surgery and trabeculectomy is not recommended in patients with bestrophinopathies.
- We demonstrate a solution describing a surgical technique based on an understanding of the molecular diagnosis.
- We provide proof-of-principle that genetic analysis can be used to inform the selection of surgical therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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