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The association between endophthalmitis risk following a variety of intra-ocular procedures and defensin gene profile

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ABSTRACT

Objective: Endophthalmitis is a sight threatening complication of intra-ocular procedures. It has been suggested that a predisposition to endophthalmitis is linked to a specific genotype in the human beta-defensin 1 (HBD-1) gene when previously analysed in a small UK cohort undergoing cataract surgery. We sought to test out this hypothesis in a larger international case: control study (centres in UK, Netherlands and Canada) following a variety of intraocular procedures.

Design: International case: control study

Participants & Methods: 660 individuals undergoing an intraocular procedure were recruited (165 cases of endophthalmitis and 495 controls). DNA was extracted. Single nucleotide polymorphisms, SNPs, rs11362, rs1800972 and rs2702877 in the HBD-1 gene were analysed (both individual genotypes and haplotypes were obtained).

Results: No associations were found when individual SNPs were analysed across the combined international cohort. However, analysis of the Toronto sub-cohort, showed a statistically significant association between the endophthalmitis cohort and the rs1800972 C allele (OR: 3.18, CI: 1.32 - 7.68, $p = 0.01$) and rs2702877 G allele (OR: 3.06, CI: 1.35 - 6.95, $p = 0.017$). No haplotype association was identified with endophthalmitis compared to control in both institution sub-cohorts and combined cohort analysis. A strong trend associating the rs1800972, rs11362 GG mini-haplotype with culture positive endophthalmitis groups across all groups was noted, but this did not reach statistical significance.

Conclusions: This is the first study to demonstrate a genetic link between a certain genetic profile (HBD-1 gene SNP variation) and endophthalmitis. Future pre-operative genetic testing could help identify patients at risk of endophthalmitis to guide invasive treatment options.

Introduction

Endophthalmitis is a sight threatening infection that can occur after any intra-ocular procedure, including cataract surgery, pars plana vitrectomy (PPV), intravitreal injections, trabeculectomy, corneal transplant surgery and lens exchange surgery.

Previous retrospective studies have investigated the incidence and risk factors for endophthalmitis following intraocular surgery.[1–16] For cataract surgery[1,2], PPV[15,16] and intravitreal injections[17] there have also been multi-centre prospective studies investigating the incidence,

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risk factors, clinical presentation, microbiology, management and outcome of endophthalmitis.

Intraocular surgery is very commonly performed, so although endophthalmitis is a rare complication, the number of patients per year acquiring endophthalmitis is significant.[1,2,16–22] The outcome following endophthalmitis is poor[1,2,15,17] so this devastating complication causes substantial morbidity at a population level.

Previous studies investigating risk factors for endophthalmitis have reviewed environmental risk factors, including both endogenous factors such as immunosuppression and external factors such as surgical complications.[1-14,16-22] Genetic risk factors have not received much attention. In 2007, a case report was published of an 80-year-old gentleman who developed endophthalmitis in both eyes following sequential cataract surgery.[23] This patient with bilateral endophthalmitis was shown to have the −44CC genotype in defensin,[23] which has been linked to increased susceptibility to infection.[24,25]

Beta-defensins are small antimicrobial peptides that are found in ocular tissues and have been demonstrated to be critical for a healthy, innate immune response to prevent infection [26–28]. Human β-defensin 1 (HBD-1), the most extensively studied β-defensin, is expressed by leukocytes and a range of epithelial cells including the pancreas, kidney, skin, gut and respiratory epithelium [29,30]. HBD-1 has also been identified in a variety of ocular tissues, including the lacrimal gland, conjunctiva, cornea, aqueous and vitreous [26,28]. The variability of defensin expression has led to the hypothesis that genetic alterations may confer susceptibility to infection.

“Genotype” refers to the genetic make-up of an individual. A genotype is the DNA sequence of an individual which determines (along with environmental influences) the specific observable characteristics (phenotype) of that person. A more specific concept of a genotype is a scoring of the type of variant present at a given location in the genome (hence genotypes can be represented by the actual DNA sequence at a specific location).

“Single nucleotide polymorphisms”, SNPs, are a DNA sequence variation that occurs when a single nucleotide (either adenine, thymine, cytosine or guanine) is different from the reference sequence – they are usually present in at least 1 % of the population and are the most common type of genetic variation among people and may increase the risk of developing certain diseases. A “haplotype” is a physical grouping of genomic variants (or SNPs) that tend to be inherited together, since they are often close to each other on a chromosome. An “allele” is a variant of the SNP sequence at a particular location (locus) on a DNA molecule in the chromosome.

A retrospective case-control study of 28 patients who developed endophthalmitis following cataract surgery (compared to 75 controls) confirmed that the −44CC genotype in HBD-1 was more prevalent in the endophthalmitis cohort.[31] Furthermore, a 3 SNP haplotype −688C / −44C / −20A was associated with endophthalmitis (odds ratio = 8.8, 95 % confidence interval 1.74 – 45.42, $p = 0.0095$).[32]

The conjunctiva typically has commensal bacterial flora present at the time of intraocular surgery, which is the likely source of bacterial inoculation responsible for endophthalmitis following intraocular surgery.[32,33] At the end of routine cataract surgery, 3–14 % of eyes with an anterior chamber tap of aqueous fluid demonstrate positive bacterial cultures.[32,33] Despite this bacterial inoculation occurring at the time of surgery, most eyes do not suffer endophthalmitis. We hypothesise that if a patient has an altered copy of the HBD-1 gene, they will have an abnormal or reduced HBD-1 related immune peptide function resulting in poor innate ocular immunity, and hence a genetic predisposition to endophthalmitis.

If it is established that a specific HBD-1 genotype or haplotype is associated with an increased risk of endophthalmitis, then pre-operative genetic testing which is now simple, cost-effective, and accessible could be used to identify patients who are genetically predisposed to endophthalmitis.

Identification of patients with a genetic risk for endophthalmitis will allow improved informed consent and allow other clinical options to be considered, such as less invasive treatment options or deferring surgery until the need is greater. It would also improve patient follow-up after intra-ocular surgery since patients with an increased genetic risk of endophthalmitis could be reviewed earlier to monitor and promptly treat for endophthalmitis. It would also help with decisions relating to bilateral intervention, such as intra-vitreal injections and immediate sequential bilateral cataract surgery). This could be an unacceptable risk for patients who are genetically predisposed to endophthalmitis.

The aim of this study is to determine whether a specific HBD-1 genetic profile is associated with endophthalmitis following a range of intraocular procedures. The primary objective is to compare the incidence of specific HBD-1 alleles between cases and controls, to establish genetic profiles that predispose to endophthalmitis following intraocular surgery.

Material and methods

The study design was an international case-control study comparing specific HBD-1 genotype in cases of presumed infectious endophthalmitis following intraocular procedures with controls (patients having an intraocular procedure with no subsequent endophthalmitis). The three sub-cohorts included patients recruited by the UK (Bristol Eye Hospital, Musgrove Park Hospital, Taunton and Sunderland Eye Infirmary), Canada (Toronto Western Hospital & Sunnybrook Health Sciences Centre) and The Netherlands (Radboud University Medical Centre).

A power calculation was completed to demonstrate that recruitment of 383 individuals formed of 96 cases of endophthalmitis following ocular procedures and 287 controls would achieve a 60 % power for the −44CC genotype (>80 % for the CCA haplotype) with a 95 % two-sided confidence level. To improve further analysis between sub-groups, the final study size chosen ($n = 660$) was larger than this (see Results).

Inclusion criteria for cases and controls were being at least 18 years old and being able to provide written informed consent to engage with the study. To avoid the effect of inter-racial variability, only individuals who self-reported their ethnicity as Caucasian were recruited. Cases included patients who underwent an intra-ocular procedure and then developed a presumed infectious endophthalmitis (including both culture negative and positive cases, to be consistent with previously published studies.[1,2,15–17] Controls included patients who underwent an intra-ocular procedure but did not go on to develop endophthalmitis. Cases were recruited prospectively and retrospectively (provided their procedure was within one year of the start date of study recruitment, to ensure adequate available clinical information) and controls were recruited prospectively.

Patients were excluded if there was the presence of any environmental risk factors that might independently increase the risk of endophthalmitis, such as intraocular surgery performed for open globe trauma or infection, active systemic infection, or immunosuppression (including active malignancy, renal failure requiring dialysis or immunomodulatory treatments).

All participant data was pseudo-anonymised and ethical approval was obtained from the relevant organisations for each of the countries involved (IRAS project ID 145,301).

Case and control information relating to the event (age at endophthalmitis onset or sample taken for controls, gender, surgery details, microbiology, outcome and medical history) were obtained retrospectively from the medical notes.

DNA was extracted from either a buccal swab / saliva sample or a blood sample, depending on the infra-structure available at the local units. The DNA was then analysed for SNPs in the HBD-1 gene. Genotyping was performed by Sanger sequencing by capillary electrophoresis on Applied Biosystems ABI 3730XL Genetic Analyzer instrument at the Clinical Genomics Centre.

Statistical analysis

Case-control statistical analysis compared the incidence of HBD-1 SNP alleles to determine whether individual genetic profiles predispose to endophthalmitis. Multiple SNPs combined in haplotypes and their estimated population frequencies were inferred using the PHASE program v. 2.1.

Cochran-Mantel-Haenszel and Cochran Q tests for heterogeneity were used to compare the endophthalmitis and control groups for possible associations between SNP genotype, allele or haplotype frequency and disease state. This analysis was done at different institutions for each of the sub-cohorts. As such, statistical adjustments were made to account for site differences in analysis methodologies. A sub-analysis of culture-positive cases was also conducted for the SNP genotype, allele and haplotype frequency.

The Bonferroni corrected / adjusted p values were used to reduce the instance of a false positive by dividing the original α-value by the number of analyses on the dependent variable. Statistical significance was assumed at corrected $p < 0.05$. Odds ratios (ORs) were also calculated, adjusted by Haldane's correction where necessary. Overall p values for combined data sets across institutions were calculated using the Cochran-Mantel-Haenszel tests and Cochran Q test for heterogeneity.

Results

A total of 660 subjects, including 165 cases with endophthalmitis and 495 controls were included in the analysis. Of these 165 cases with presumed infectious endophthalmitis, 96 cases (58 %) had a positive culture (culture positive rate of 58 % which is comparable to other studies[1,2,17,19]) and sub-group analysis of these culture positive cases is detailed below.

The patient population consisted of a sub-cohort of 79 from Toronto ($n = 19$ cases, $n = 60$ controls), 200 from United Kingdom ($n = 50$ cases, $n = 150$ controls), and 381 from Netherlands ($n = 96$ cases, $n = 285$ controls). Patient demographics and the types of intra-ocular procedures are displayed in Table 1.

In our analysis we investigated 3 SNPs (rs11362, rs1800972 and rs2702877), all of which have been reported previously. Minor allele frequencies (MAF) were obtained from the 1000 Genomes Project Phase 3 (Appendix 1). The MAF reflects how frequently the allele being considered occurs in the general population. The MAF is a fraction where the numerator is the number of positive alleles and the denominator is the total number of alleles screened. Appendix 2 demonstrates the haplotype frequency in the three cohorts (Toronto, UK and the Netherlands) for both cases and controls.

Table 1
The number of cases of endophthalmitis and controls, demographics and procedures. Note the total percentage of procedures for cases is above 100 %, since some patients had combined procedures prior to developing endophthalmitis.

	Cases	Controls
Number (Total)	165	495
Number (Netherlands)	96	285
Number (UK)	150	50
Number (Toronto)	19	60
Age at endophthalmitis onset	72.9 (71.1–74.6)	N/A
Male	56 %	51 %
Female	44 %	49 %
Procedure		
Cataract extraction	86.00 %	46.60 %
Intravitreal injection	28.70 %	29.30 %
Vitreo-retinal	10.00 %	19.40 %
Corneal transplant	2.30 %	3.10 %
Secondary lens implant	0.70 %	1.00 %
Glaucoma surgery	0.70 %	0.50 %

Individual SNP analysis

Table 2 illustrates allele pairing at each SNP, minor allele at each SNP and MAF in a reference European population.

No associations were found when individual SNPs were analysed across the combined international cohort. However, analysis of the Toronto sub-cohort, showed a statistically signification association between the endophthalmitis cohort and the rs1800972 C allele (OR: 3.18, CI: 1.32 - 7.68, $p = 0.01$) and rs2702877 G allele (OR: 3.06, CI: 1.35 - 6.95, $p = 0.017$).

This is in keeping with the linkage disequilibrium observed between the C and G allele where two SNP's (rs1800972 and rs2702877) are in high linkage disequilibrium (LD) with D' equal to 1 and r2 equal to 0.8. For clarification, LD is a measure of the non-random association between alleles at different loci (positions on chromosome). LD compares the frequency at which two alleles are together (at the same loci) with the frequency at which each allele is detected at that loci overall. Loci demonstrate LD when the frequency of being detected together is different (either higher or lower) to what would be expected if the loci were independent and associated randomly.

Haplotype analysis

SNPs (DNA sequence variations when a single nucleotide is different from the reference sequence) are the most common type of genetic variation among people and may increase the risk of developing certain diseases. Since SNPs are often close to each other on a chromosome they are often inherited together, and this physical grouping of genomic variants (haplotypes) are therefore important to analyse for SNP driven diseases. If isolated SNPs only are considered, then important genetic risk factors could be missed. It is therefore necessary to analyse haplotypes involving the relevant SNPs for endophthalmitis.

Haplotype analysis is displayed by Table 3 which shows that in the combined international cohort there is a suggested association between endophthalmitis and the 3 SNP haplotypes (rs2702877, rs1800972, rs11362) CGG ($p = 0.07$), CCG ($p = 0.06$) GCA ($p = 0.03$). The statistical significances are lost however when the p values are corrected. The same is true for the smaller (rs1800972, rs11362) haplotype GG ($p = 0.09$).

Subgroup analysis

Subgroup analysis of the Toronto group (Appendix 2) shows that the CGG haplotype or the smaller rs1800972, rs11362 GG haplotype (SNPs: −44/−20) maintains this association even after the Bonferroni correction is applied (odds ratio [OR]: 3.24, CI: 1.47–7.16, $p = 0.06$)

Culture positive cases

Table 4 illustrates the haplotype analysis for the culture positive subgroup. Amongst the 96 culture positive cases (Toronto, $n = 8$ cases, $n = 60$ controls; UK, $n = 33$ cases, $n = 150$ controls; Netherland, $n = 55$ cases, $n = 285$ controls), no statistically significant haplotype association was identified with endophthalmitis compared to control in both institution sub-cohorts and combined cohort analysis. There is however a strong trend associating the rs1800972, rs11362 GG mini-haplotype with culture positive endophthalmitis groups across all groups.

Table 5 demonstrates the culture positive association analyses and combined meta-analyses results. These suggested significant findings for two SNPs including rs1800972e (chromosome 8, position: 6,877,901; DEFB1; 5 prime UTR variant, $p = 0.009$) and rs2702877f (chromosome 8, position: 6,878,545; DEFB1; 5 Upstream gene variant, $p = 0.016$). Notably, for rs1800972e, it was noted that the Toronto sub-cohort had greater mean adjusted frequency (OR=4.46, CI=1.38 - 14.5) of having the SNP in culture-positive endophthalmitis cases compared to controls (p value adjusted for multiple testing using the False Discovery Method = 0.014). Similarly, rs2702877f in the Toronto sub-cohort had greater

Table 2
Genotype data illustrating the allele pairing at each SNP, minor allele at each SNP and MAF in a reference European population. MAF (Comb.) is the MAF in the combined cohort for each centre.

SNP	Chromosome	Position	Gene	Cohort	Minor Allele	MAF (Comb.)	MAF (Cases)	MAF (Control)	OR (95 % CI)	P Value	FDR P Value
rs11362	8	6,877,877	DEFB1	TOR	T	0.37	0.26	0.41	0.47 (0.20 - 1.13)	0.08	0.08
				UK		0.37	0.34	0.38	0.83 (0.53 - 1.35)	0.43	0.43
				NETH		0.41	0.36	0.42	0.78 (0.55 - 1.09)	0.14	0.43
				Combined		0.39	0.34	0.41	0.91 (0.83 - 0.99)	0.03	0.10
rs1800972	8	6,877,901	DEFB1	TOR	C	0.24	0.42	0.18	**3.06 (1.35 - 6.95)**	**0.006**	**0.017**
				UK		0.24	0.19	0.26	0.68 (0.38–1.20)	0.17	0.52
				NETH		0.19	0.17	0.20	0.83 (0.54–1.29)	0.40	0.40
				Combined		0.22	0.21	0.22	0.98 (0.90 - 1.07)	0.66	0.99
rs2702877	8	6,878,545	DEFB1	TOR	G	0.30	0.47	0.25	**3.18 (1.32–7.68)**	**0.007**	**0.010**
				UK		0.29	0.25	0.31	0.76 (0.45–1.27)	0.29	0.43
				NETH		0.27	0.23	0.27	0.81 (0.54–1.21)	0.31	0.46
				Combined		0.28	0.27	0.28	0.98 (0.90–1.07)	0.68	0.68

Table 3
Haplotype analysis.

RS#	SNPs	Haplotype	Frequency	Frequency (Endophthalmitis)	Frequency (Control)	OR	L95	U95	P Value	Corrected P Value
rs2702877, rs1800972, rs11362	−688/−44/−20	CGG	0.26	0.30	0.24	1.31	0.98	1.75	0.07	0.92
rs2702877, rs1800972, rs11362	−688/−44/−20	CGA	0.23	0.21	0.23	0.85	0.62	1.17	0.33	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.19	0.17	0.20	0.79	0.56	1.11	0.11	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CCG	0.10	0.13	0.09	1.58	1.05	2.36	0.06	0.85
rs2702877, rs1800972, rs11362	−688/−44/−20	CCA	0.09	0.09	0.09	1.02	0.65	1.60	0.94	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCA	0.06	0.04	0.06	0.55	0.28	1.06	0.03	0.41
rs2702877, rs1800972, rs11362	−688/−44/−20	GGG	0.06	0.06	0.06	1.00	0.57	1.77	0.99	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGA	0.02	0.01	0.02	0.64	0.23	1.81	0.26	1.00
rs1800972, rs11362	−44/−20	GG	0.33	0.37	0.32	1.26	0.96	1.64	0.09	1.00
rs1800972, rs11362	−44/−20	CG	0.28	0.28	0.28	1.03	0.78	1.36	0.84	1.00
rs1800972, rs11362	−44/−20	GA	0.24	0.22	0.24	0.87	0.64	1.17	0.37	1.00
rs1800972, rs11362	−44/−20	CA	0.16	0.13	0.17	0.78	0.54	1.12	0.20	1.00

mean adjusted frequency (OR= 5.66, CI=1.43 - 22.4) of having the SNP in culture-positive endophthalmitis cases compared to controls (p value adjusted = 0.02, Table 5).

Discussion

For over 25 years it has been established that defensins are important peptides involved with the innate defence of the eye against infection.[27,28] Since genes code for peptides, it follows that those with certain genetic profiles may have a relatively weak inherent defence against endophthalmitis. This is the first large-scale clinical study to test this hypothesis.

Despite the relatively large-scale of this study, it is important to acknowledge that there were no significant findings across the whole group. This could potentially be due to the genetic association not being sufficiently strong to stand up across different international groups despite all self-reporting as Caucasian.

This is the first study to demonstrate a genetic link between an SNP and endophthalmitis, demonstrated by the analysis of the Toronto subcohort, showing a statistically significant association between the endophthalmitis cohort and the rs1800972 C allele (OR: 3.18, CI: 1.32–7.68, $p = 0.01$) and rs2702877 G allele (OR: 3.06, CI: 1.35 - 6.95, $p = 0.017$).

Despite statistical significance being demonstrated for an individual SNP and endophthalmitis risk, the study was not able to demonstrate any statistical significance of risk for haplotypes. A strong trend associating the rs1800972, rs11362 GG mini-haplotype with culture positive endophthalmitis groups across all groups was noted, but this did not reach statistical significance. This trend did not reach statistical significance, either because there is no haplotype link, or, the study size sample was too small to demonstrate the trend at a level of $p = 0.05$. This warrants further investigation since there is a possible biological plausible explanation for linking the GG mini haplotype to an increased risk of endophthalmitis since it has been demonstrated that the −44C/G SNP polymorphisms can affect the risk of type 2 diabetes, potentially by the C allele generating binding motifs for the ikaros transcription factor (IK).[34] IK is important in the immune response, so if the GG mini haplotype blocks the binding of IK it follows that this will reduce immunity and predispose to infections, such as endophthalmitis.

One limitation of this study could be the inclusion of culture negative cases. For this study to be consistent with previous landmark endophthalmitis studies, the clinical diagnosis of presumed infectious endophthalmitis was used and therefore included both culture positive and negative cases. However, since stronger associations were identified for the sub-group of culture positive cases, this would support the argument for future studies to focus more on culture positive cases. For studies assessing the clinical management of endophthalmitis the authors still support that a clinical diagnosis of presumed infectious endophthalmitis is required, since at the point of intention to treat, clinicians do not have the culture result. However, for this new and growing field of research investigating genetic associations with endophthalmitis this study supports the approach to primarily investigate culture positive cases.

Table 4
Haplotype analysis for the culture positive sub-group.

Haplotype association analysis in culture positive Toronto cohort 68 subjects (n = 8 cases, n = 60 controls)

Markers Used	SNPs	Haplotype	Freq	Freq Cases	Freq Controls	OR	L95	U95	*P*	*Bonf. P*
rs2702877, rs1800972, rs11362	−688/−44/−20	GCA	0.38	0.31	0.39	0.72	0.23	2.19	0.51	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.33	0.13	0.36	0.25	0.05	1.16	0.03	0.28
rs2702877, rs1800972, rs11362	−688/−44/−20	CGG	0.22	0.50	0.18	4.45	1.51	13.17	0.01	0.09
rs2702877, rs1800972, rs11362	−688/−44/−20	CCG	0.04	0.00	0.05	0.00	0.00	0.00	0.17	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CCA	0.02	0.06	0.02	3.28	0.30	36.03	0.29	1.00
rs1800972, rs11362	−44/−20	CG	0.40	0.37	0.41	0.87	0.30	2.55	0.78	1.00
rs1800972, rs11362	−44/−20	CA	0.38	0.13	0.41	0.21	0.05	0.95	0.02	0.15
rs1800972, rs11362	−44/−20	GG	0.22	0.50	0.18	4.45	1.51	13.17	0.01	0.09

Haplotype association analysis in culture positive UK cohort 183 subjects (n = 33 cases, n = 150 controls)

Markers Used	SNPs	Haplotype	Freq	Freq Cases	Freq Controls	OR	L95	U95	*P*	*Bonf. P*
rs2702877, rs1800972, rs11362	−688/−44/−20	CCA	0.36	0.38	0.35	1.10	0.63	1.94	0.75	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CCG	0.35	0.40	0.34	1.28	0.73	2.25	0.40	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGG	0.24	0.16	0.26	0.56	0.27	1.16	0.10	0.97
rs2702877, rs1800972, rs11362	−688/−44/−20	GCA	0.03	0.03	0.03	0.88	0.17	4.62	0.87	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.02	0.04	0.02	1.89	0.40	8.92	0.40	1.00
rs1800972, rs11362	−44/−20	CA	0.39	0.40	0.38	1.09	0.63	1.91	0.76	1.00
rs1800972, rs11362	−44/−20	CG	0.37	0.44	0.36	1.38	0.79	2.41	0.28	1.00
rs1800972, rs11362	−44/−20	GG	0.24	0.16	0.26	0.55	0.26	1.13	0.08	0.84

Haplotype association analysis in culture positive Netherland cohort 340 subjects (n = 55 cases, n = 285 controls)

Markers Used	SNPs	Haplotype	Freq	Freq Cases	Freq Controls	OR	L95	U95	*P*	*Bonf. P*
rs2702877, rs1800972, rs11362	−688/−44/−20	CGA	0.38	0.33	0.38	0.78	0.49	1.24	0.28	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CGG	0.35	0.42	0.34	1.42	0.91	2.23	0.12	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.20	0.14	0.21	0.64	0.34	1.19	0.13	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGA	0.04	0.03	0.04	0.80	0.24	2.72	0.69	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGG	0.04	0.08	0.03	2.69	1.08	6.72	0.05	0.48
rs1800972, rs11362	−44/−20	GA	0.41	0.36	0.42	0.76	0.49	1.17	0.21	1.00
rs1800972, rs11362	−44/−20	GG	0.39	0.48	0.37	1.55	1.02	2.36	0.04	0.40
rs1800972, rs11362	−44/−20	CG	0.20	0.16	0.20	0.75	0.43	1.31	0.29	1.00

Haplotype association analysis in combined data of 591 subjects (n = 96 cases, n = 495 controls)

Markers Used	SNPs	Haplotype	Freq	Freq Cases	Freq Controls	OR	L95	U95	*P*	*Bonf. P*
rs2702877, rs1800972, rs11362	−688/−44/−20	CGG	0.25	0.28	0.24	1.22	0.85	1.76	0.27	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CGA	0.23	0.20	0.23	0.81	0.54	1.22	0.32	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.19	0.13	0.20	0.62	0.39	0.99	0.01	0.20
rs2702877, rs1800972, rs11362	−688/−44/−20	CCA	0.10	0.13	0.09	1.47	0.90	2.41	0.21	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CCG	0.09	0.13	0.09	1.55	0.94	2.56	0.14	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGG	0.06	0.08	0.06	1.34	0.72	2.51	0.36	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCA	0.06	0.04	0.06	0.53	0.23	1.25	0.07	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGA	0.02	0.01	0.02	0.64	0.17	2.47	0.37	1.00
rs1800972, rs11362	−44/−20	GG	0.33	0.37	0.32	1.28	0.92	1.77	0.13	1.00
rs1800972, rs11362	−44/−20	CG	0.27	0.25	0.27	0.90	0.63	1.29	0.54	1.00
rs1800972, rs11362	−44/−20	GA	0.24	0.21	0.24	0.83	0.56	1.22	0.35	1.00
rs1800972, rs11362	−44/−20	CA	0.17	0.17	0.17	0.99	0.65	1.52	0.97	1.00

A previous pilot study demonstrated a potential association between a certain genetic profile and endophthalmitis risk[31] and this research progressed this study and is the first to show a statistically significant link for a specific genotype and endophthalmitis. With future research, it would be possible to investigate this further such that genetic profiles and endophthalmitis risk can be more clearly understood. This would facilitate clinical decision making for many patients considering invasive ophthalmic procedures. It would help with informed consent for invasive procedures, aid targeted follow-up of those at risk after invasive procedures and help review the risk-benefit balance in patients considering bilateral invasive treatments (such as bilateral intra-vitreal injections or immediate sequential bilateral cataract surgery).

Conclusions

In summary, it was already known that Beta defensins are peptides that are important in protecting the eye against endophthalmitis and that a possible association between the risk of endophthalmitis and the HBD-1 profile had previously been demonstrated. This is the first study to demonstrate a statistically significant genetic link between a certain genetic profile (HBD-1) and cases of endophthalmitis. Studies investigating the clinical management of endophthalmitis typically include culture positive and culture negative cases, however, for this new field of genetic research this study suggests focusing on culture positive cases may be more successful at identifying associations in the future. This study can now facilitate further research to move towards genetic testing of our patients to help establish their endophthalmitis risk when faced

Table 5
Culture positive patients association analyses and combined meta-analyses results.

SNP	Position on Chr. 8	Gene	Functional annotation	Sample cohort[a]	Minor (Test) Allele	MAF	MAF (Cases)	MAF (Controls)	OR (95 % CI)	P value[b]	FDR p value[c]	Cochran Q p value[d]
rs11362	6,877,877	DEFB1	5 prime UTR variant	TOR	T	0.40	0.38	0.41	0.85 (0.27 - 2.69)	0.78	0.78	
				UK	T	0.38	0.39	0.38	1.03 (0.61 - 1.73)	0.92	0.92	
				NETH	T	0.41	0.35	0.42	0.72 (0.47 - 1.10)	0.13	0.38	
				Combined[d]	T	0.40	0.36	0.41	0.94 (0.84 - 1.04)	0.24	0.71	0.60
rs1800972[e]	6,877,901	DEFB1	5 prime UTR variant	TOR	G	0.22	0.50	0.18	4.46 (1.38 - 14.5)	**0.009**	**0.014**	
				UK	G	0.24	0.17	0.26	0.58 (0.29 - 1.19)	0.12	0.36	
				NETH	C	0.20	0.16	0.20	0.74 (0.42 - 1.32)	0.30	0.45	
				Combined	C	0.44	0.42	0.44	0.95 (0.85 - 1.06)	0.37	0.55	0.009
rs2702877[f]	6,878,545	DEFB1	Upstream gene variant	TOR	C	0.29	0.56	0.25	5.66 (1.43 - 22.4)	**0.007**	**0.020**	
				UK	G	0.30	0.26	0.31	0.79 (0.43 - 1.45)	0.44	0.65	
				NETH	G	0.27	0.25	0.27	0.88 (0.53 - 1.46)	0.61	0.61	
				Combined	G	0.33	0.27	0.34	1.00 (0.89 - 1.12)	1.00	1.00	0.016

[a] Culture positive patients: Toronto cohort 68 subjects ($n = 8$ cases, $n = 60$ controls), UK cohort 183 subjects ($n = 33$ cases, $n = 150$ controls) and Netherland cohort 340 subjects ($n = 55$ cases, $n = 285$ controls);.
[b] Nominal P value from logistic regression tests assuming an additive genetic model.
[c] FDR p value corresponds to p value adjusted for multiple testing using the False Discovery Method.
[d] Overall p values for combined TOR, UK and NETH data sets were calculated using the Cochran-Mantel-Haenszel tests and Cochran Q test for heterogeneity.
[e] &f: SNP rs1800972 and SNP rs2702877 are in high LD with r2 = 0.8, D' =1;.

with the option of invasive eye treatments.

Meetings

The results of this paper have also not yet been presented at any conference. However, during the data collection phase the profile of the study was highlighted by presentations at various international conferences to aid recruitment (such as the Canadian Ophthalmology Society annual general meeting in Canada and the British and Eire Association of Vitreo-Retinal Surgeons (BEAVRS) annual general meeting in the UK.

Synopsis

The new frontier of genetic research and endophthalmitis can progress further now a genetic link between a certain genetic profile (HBD-1 GG haplotype) and endophthalmitis has been demonstrated by this pioneering study.

Ethical approval

All participant data was pseudo-anonymised and ethical approval was obtained from the relevant organisations for each of the countries involved (IRAS project ID 145301).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1. For the single nucelotide polymorphisms (SNPs) the minor allele frequency (MAF) was obtained from the 1000 Genomes Project Phase 3

SNP	Reference	Minor Allele	MAF
rs11362	C/T	T	0.44
rs1800972	C/G	C	0.20
rs2702877	G/C	G	0.25

Appendix 2. The haplotype frequency in the three geographical cohorts (Toronto, UK and Netherlands) for both cases of endophthalmitis and controls

Toronto RS# (Toronto)	SNPs	Haplotype	Frequency	Frequency (Case)	Frequency (Control)	OR	L95	U95	P Value	Corrected P Value
rs2702877, rs1800972, rs11362	−688/−44/−20	GCA	0.35	0.24	0.39	0.49	0.21	1.12	0.05	0.52
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.34	0.29	0.36	0.72	0.33	1.60	0.39	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CGG	0.24	0.42	0.18	3.24	1.47	7.16	0.01	0.06
rs2702877, rs1800972, rs11362	−688/−44/−20	CCG	0.04	0.03	0.05	0.54	0.06	4.68	0.51	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CCA	0.02	0.03	0.02	1.38	0.13	14.92	0.76	1.00
rs1800972, rs11362	−44/−20	CG	0.39	0.32	0.41	0.67	0.31	1.45	0.30	1.00
rs1800972, rs11362	−44/−20	CA	0.37	0.26	0.41	0.52	0.23	1.16	0.08	0.81
rs1800972, rs11362	−44/−20	GG	0.24	0.42	0.18	3.24	1.47	7.16	0.01	0.06

UK RS# (UK)	SNPs	Haplotype	Frequency	Frequency (Case)	Frequency (Control)	OR	L95	U95	P Value	Corrected P Value
rs2702877, rs1800972, rs11362	−688/−44/−20	CCG	0.37	0.45	0.34	1.55	0.97	2.49	0.08	0.76
rs2702877, rs1800972, rs11362	−688/−44/−20	CCA	0.35	0.33	0.35	0.88	0.54	1.44	0.63	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGG	0.24	0.19	0.26	0.67	0.38	1.20	0.16	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCA	0.03	0.02	0.03	0.58	0.11	2.96	0.46	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.02	0.02	0.02	1.18	0.25	5.61	0.82	1.00
rs1800972, rs11362	−44/−20	CG	0.39	0.47	0.36	1.58	0.99	2.52	0.07	0.67
rs1800972, rs11362	−44/−20	CA	0.37	0.34	0.38	0.85	0.52	1.37	0.52	1.00
rs1800972, rs11362	−44/−20	GG	0.24	0.19	0.26	0.66	0.37	1.17	0.14	1.00

Netherlands RS# (Netherlands)	SNPs	Haplotype	Frequency	Frequency (Case)	Frequency (Control)	OR	L95	U95	P Value	Corrected P Value
rs2702877, rs1800972, rs11362	−688/−44/−20	CGA	0.37	0.34	0.38	0.83	0.58	1.20	0.32	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	CGG	0.36	0.42	0.34	1.41	0.98	2.01	0.06	0.61
rs2702877, rs1800972, rs11362	−688/−44/−20	GCG	0.20	0.16	0.21	0.76	0.48	1.21	0.24	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGA	0.04	0.03	0.04	0.76	0.29	1.99	0.52	1.00
rs2702877, rs1800972, rs11362	−688/−44/−20	GGG	0.03	0.04	0.03	1.46	0.59	3.65	0.42	1.00
rs1800972, rs11362	−44/−20	GA	0.41	0.37	0.42	0.82	0.58	1.15	0.25	1.00
rs1800972, rs11362	−44/−20	GG	0.39	0.45	0.37	1.38	0.98	1.93	0.07	0.68
rs1800972, rs11362	−44/−20	CG	0.20	0.17	0.20	0.83	0.54	1.28	0.38	1.00

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