The Heritability of Dry Eye Disease in a Female Twin Cohort

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Purpose. We estimated the relative importance of genes and environment in dry eye disease (DED) using a classic twin study.

Methods. A large sample of 3930 female monozygotic and dizygotic twins from the UK Adult Twin Registry (TwinsUK) was questioned about the presence of a DED diagnosis and about DED symptoms in the preceding 3 months. In addition, a subset of 606 twins was examined for several dry eye signs. Genetic and environmental effects were estimated using maximum likelihood structural equation modeling.

Results. All DED outcome variables showed higher correlation in monozygotic twin pairs than in dizygotic twin pairs, suggesting genes have a contributory role in DED. The DED symptoms showed a heritability of 29% (95% confidence interval [CI], 18%–40%). A clinician's diagnosis of DED with concurrent use of artificial tears showed a heritability of 41% (95% CI, 26%–56%). Estimates of the heritability of DED signs were 25% (95% CI, 7%–42%) for interblink interval, 58% (95% CI, 43%–70%) for Schirmer value, 40% (95% CI, 25%–53%) for tear osmolarity, and 78% (95% CI, 59%–90%) for the presence of blepharitis. The unique environment explained the remainder of the variance. We found no significant heritability for tear breakup time.

Conclusions. Genetic factors contribute moderately to the diagnosis, symptoms, and the signs of DED. Compared to other ocular phenotypes, the lower heritability might reflect some of the difficulties in objective phenotyping of DED in a population-based sample. However, future genetic studies are now justified and may help in unraveling the pathophysiology of DED.

Keywords: dry eye disease, DED, heritability, environment, genetics, sicca syndrome, OSDI

Dry eye disease (DED) is defined as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface.[1] It is a common problem, affecting 7% to 33% of middle-aged and elderly subjects, depending on the definition used and population studied.[2] Despite its high prevalence and impact on quality of life, the etiology of DED still is poorly understood.[1] Its research is complicated by the lack of a simple diagnostic test or highly specific and sensitive diagnostic criteria.[3] Dry eye disease is likely to be a complex disease with many underlying factors.[4] To date, most epidemiologic research into the etiology of DED has concentrated on environmental risk factors, such as diet and drugs. Age and Sjögren's disease are well-known risk factors, and women are at increased risk compared to men.[2,4–7]

However, little is known about the role of genetic factors in DED. Some small candidate gene studies on non-Sjögren dry eye have been performed and show a possible role for polymorphisms in proinflammatory cytokine genes,[8] and killer cell immunoglobulin-like receptor and human leukocyte antigen-C genes,[9] but these results have not yet been replicated. In addition, genome wide association studies (GWAS) on Sjögren's syndrome in general showed shared susceptibility loci in immune-related genes with other immune-mediated inflammatory disease, such as systemic lupus erythematosus, but these studies have found no genes encoding for lacrimal components, secretion machinery, or neuronal proteins involved in innervation of the glands.[10]

As far as we know, the relative importance of genes compared to environmental factors for DED is not known, and other genetic studies, such as GWAS, have not been performed for DED to date. Therefore, we conducted a classical twin study to examine the heritability of DED and the phenotypic variation of some of its measured components. Dry eye diagnosis, symptoms, and signs were evaluated in a large unselected sample of middle-aged and elderly female twin pairs.

Methods

Subjects

Subjects for this study were twins recruited from the TwinsUK Adult Twin Registry, held at King's College London, United Kingdom.[11] This registry has been ascertained from the general population through national media campaigns. Twins from this

registry have been shown to be comparable to the age-matched general population singletons for a broad variety of medical and behavioral traits.[12] For historical reasons, most enrolled twins are female. Local ethics committee approval was obtained for the study, and twin volunteers gave informed consent, but were unaware of the precise hypotheses being tested. The research followed the tenets of the Declaration of Helsinki. Zygosity had been determined from standardized questionnaires and confirmed with genome-wide analyses.

Questionnaires to Evaluate Dry Eye

We asked 3930 female twin pairs the following two questions by a postal questionnaire as proxy for having DED: "Have you ever been diagnosed (by a clinician) as having dry eye syndrome?" and "Do you currently use artificial tear eye drops or gel?"[13,14] We applied a strict definition of DED to reduce the possibility of misclassification. If a participant answered "Yes" to both questions, she was assigned as having DED. If both questions were answered with "No" she was assigned as having no DED. Subjects who answered "Yes" to only one of the questions were excluded from analysis, as were subjects who answered "Don't know." In addition to a diagnosis, symptoms were evaluated by the question "For the past three months or longer, have you had dry eyes? (This is described as a foreign body sensation with itching and burning, sandy feeling, not related to allergy)."[8]

Tests to Evaluate Dry Eye

A subset of 606 twins that collaborated in a substudy[15] also were evaluated for dry eye signs. Dry eye signs were measured in the following order: interblink interval, tear osmolarity, Schirmer value, tear breakup time (TBUT), and the presence of signs of anterior or posterior blepharitis. These tests were all performed by the same trained research nurse (DK).

Interblink interval was measured by counting the amount of blinks in 30 seconds while the participant was reading a logMAR chart. The participant was unaware of the investigator counting the blinks. The interblink interval subsequently was calculated by dividing 30 seconds by the amount of blinks in that period.[3] Tear osmolarity was measured in both eyes from the inferior lateral meniscus with a laboratory-on-a-chip by the TearLab Osmolarity System (San Diego, CA, USA) following standard protocols.[3] An unanesthetized Schirmer-1 value after 5 minutes (mm/5 min) using sterile strips was measured in both eyes following standard protocols.[3] When the Schirmer strip was fully wet (5 mm) before the 5 minutes were passed, this 5 mm was multiplied such that an estimated Schirmer value after 5 minutes was obtained. The TBUT was measured in both eyes by instilling a drop of 2% sodium fluorescein and counting the seconds before the fluorescinized tear was broken up after a blink, following standard protocols.[3] Each eye was measured three consecutive times and the average value per eye was used. Signs of blepharitis (anterior or posterior) were evaluated by looking at foamy meibomian gland discharge, crusts, collarettes, or scales by slit-lamp examination. Presence of any of these signs led to a positive diagnosis of blepharitis.

Analytical Approach

Heritability is the proportion of phenotypic variation in a population that is attributable to genetic variation among individuals. The aims of our analyses were to estimate the relative influence of genetic (heritability) and environmental factors on the observed phenotypic variance in each dry eye outcome variable.

The rationale of the twin design is to compare the degree of similarity of resemblance among monozygotic (MZ) twins, who share 100% of their genetic make-up, and dizygotic (DZ) twins, who share on average 50% of their segregating genes. Twin models assume that MZ and DZ twins share roughly the same common family environment (the equal environment assumption [EEA]). Relative differences between MZ and DZ within-pair correlations then are used to estimate the relative contributions of the additive genetic effects (A, heritability), the shared environmental effects (C), and the nonshared environmental effects, which also include measurement error (E).[16] Confidence intervals (CI) of parameter estimates were obtained by maximum likelihood.[17] Age was entered into the model as a covariate.

The prevalence of dichotomous outcomes (dry eye diagnosis and concurrent use of artificial tears, symptoms of dry eye in preceding three months, presence of blepharitis) was calculated and proband-wise concordance rates in MZ and DZ twin pairs were calculated. Similarity between twins was examined further using tetrachoric correlations with a liability-threshold model,[18] performed in the program OpenMx.[19] The liability-threshold model assumes an underlying continuous liability that follows a normal distribution. The threshold is estimated from the population frequency of the phenotype.[20] The mean or median values of the continuous outcome variables (interblink interval, tear osmolarity, Schirmer value, TBUT) and their correlations in MZ and DZ pairs were calculated. The average value of both eyes was used for all analyses. The distribution of these variables was evaluated for normality and checked for outliers. If traits were not normally distributed, the exclusion of outliers and/or transformation to a normal distribution was considered. If normality could not be achieved, the traits were ordinalized into quartiles and used in liability-threshold models.

Univariate model comparisons were conducted using χ^2 tests.[21] Akaike's information criterion (AIC) was used for the model selection. The AIC, an index of goodness of fit and parsimony, is calculated by minus twice the log of the maximized value of the likelihood function of the tested model minus two times the degrees of freedom (df) of the tested model.[22] The model with the minimum AIC is the preferred model and reflects the best balance between explanatory power and parsimony. The program OpenMx[19] was used for all twin modeling. Data handling and preliminary analyses were performed with SPSS (version 22.0; SPSS, Inc., Chicago, IL, USA).

Results

Characteristics of the study population are shown in Table 1. Mean age (± SD) was 57.1 (±13.1) years for the population that completed the DED questionnaire, and 61.2 (±9.7) years for those who completed the DED tests. All participants were female and 98.4% of the participants were Caucasian. Not all twins participated in all DED tests, leading to a slightly different number of complete twin pairs for the various analyses. The overall prevalence of DED (diagnosed by a clinician and using artificial tear eye drops or gel) was 9.4% and the prevalence of DED symptoms in the preceding three months was 20.4%. The prevalence and outcomes of DED diagnosis, symptoms, and signs were similar for MZ and DZ twins for all measurements ($P > 0.05$ for all variables), although the DZ twins who answered the questionnaire were slightly older (mean, 59.5 vs. 54.5 years; $P < 0.0005$). Tear osmolarity and TBUT showed normal distributions after the exclusion of one DZ twin pair outlier. Interblink interval and Schirmer test results could not be transformed to a normal distribution and were analyzed ordinally using quartiles.

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Table 1. Characteristics of the Study Population, and Prevalence and Outcomes of DED Measurements for All Participants, and Separated by Zygosity

Variables	All	MZ Twins	DZ Twins	*P* Value for Difference MZ vs. DZ
DED questionnaire, *n*	3930	2183	1747	
Age, mean, y (SD)	57.1 (13.1)	55.4 (14.9)	59.5 (11.6)	<0.0005
Dry eye symptoms preceding 3 mo, %				
Yes	20.4	20.7	20.2	0.73
No	79.6	79.3	79.8	
DED diagnosis by a clinician, %				
Yes	11.2	11.4	11.0	0.21
No	88.4	88.3	88.4	
Don't know	0.4	0.2%	0.6	
Current use of artificial tears, %				
Yes	14.8	14.5	14.8	0.78
No	83.8	84.0	83.5	
Don't know	1.6	1.5	1.8	
DED diagnosis and current use of artificial tears				
Cases, both present, %	9.4	9.4	9.4	0.96
Controls, both absent	81.2	81.4	81.0	
DED tests, *n*	606	244	362	
Age, mean, y (SD)	61.2 (9.7)	62.9 (8.6)	60.0 (10.3)	<0.0005
Tear osmolarity, *n* = 490, mean, mOsm/L (SD)	299.1 (10.5)	299.4 (10.6)	299.0 (10.4)	0.72
TBUT, *n* = 534, mean, s (SD)	3.2 (1.1)	3.1 (1.1)	3.3 (1.1)	0.09
Schirmer value, *n* = 522, median, mm/5 min (range)	6.5 (0–460)	7 (0–210)	6.3 (0–460)	0.38
Interblink interval, *n* = 548, median, s (range)	4.3 (1.2–30)	4.4 (2–30)	4.3 (1.2–30)	0.23
Presence of blepharitis, %, *n* = 547	73.7%	74.5%	73.6%	0.77

Table 2 shows the proband-wise concordance rates and phenotypic correlations in MZ and DZ twin pairs and the age-adjusted model-fitting results of the genetic (A) and environmental (C and E) effects for all DED variables. The correlation within pairs was higher for MZ pairs than for DZ pairs for all variables, suggesting that genes have a contributory role in DED. For example, for a diagnosis of DED with concurrent use of artificial tears MZ pairs showed a phenotypic correlation of 44% versus 12% in DZ pairs, and for dry eye symptoms this was 30% versus 13%.

The AE-model turned out to be the best fitting model for all DED variables, with the exception of TBUT (see Appendix Table A1 for model fit statistics). This means additive genetic effects (A) and unique environmental effects (E) are responsible for most of the variation in these outcome measures, with only a minor role for the common environment (C). Estimates based on this AE-model showed a heritability of 29% (95% CI, 18%–40%) for the presence of dry eye symptoms and 41% (95% CI, 26%–56%) for a diagnosis of DED with concurrent use of artificial tears. Heritability estimates of DED signs varied from 25% (95% CI, 7%–42%) for interblink interval, 40% (95% CI, 25%–53%) for tear osmolarity, 58% (95% CI, 43%–70%) for Schirmer value, to 78% (95% CI, 59%–90%) for blepharitis. The TBUT was the only variable with the CE-model as best fitting model, reflecting no evidence of genetic effects (A) and having an estimate of 30% (95% CI, 18%–40%) for common environmental effects (C).

Twin modeling often is underpowered to find significant A and C estimates in a full Academic Center for Evidence-Based Practice (ACE)–model (San Antonio, TX, USA). Therefore, we also show the estimates of the full ACE-model as additional information, see Appendix Table A2. In addition to TBUT, the common environment also may have a minor role in blepharitis and Schirmer value.

Discussion

We believe this is the first population-based study to investigate the role of genetic effects on DED. We have demonstrated that genes are involved in DED and are moderately important, with a heritability of approximately 30% for DED symptoms and 40% for a DED diagnosis, and a varying heritability of 25% to 80% for the various DED signs in a cohort of British middle-aged and elderly female twins. Unique environmental effects explained the remainder of the variance of these variables. The TBUT showed no evidence of genetic effects in our study.

Other ocular phenotypes have been reported mostly to have higher heritability estimates; for example, 71% for late age-related macular degeneration, 55% for IOP, 71% for refractive error, 85% for central corneal thickness, and 89% for macular thickness.[23] The lower heritability of DED-associated phenotypes might reflect the high subjectivity in grading of DED and a relatively high measurement error, which increases the estimate of the unique environment (E) in twin modeling. This is possibly best reflected in our study by TBUT, which showed a complete lack of heritability and high unique environmental effects. The mean value of TBUT in our study population was only 3.2 seconds, which is very low and even below the threshold of defining DED (<5 seconds).[3] Although we had a middle-aged to older female cohort in which a lower than average value of TBUT might be expected, this low TBUT probably is not reliable for diagnosing DED. The low value in our study may be caused in part by the presence of strong air-conditioning in a relatively small test room, the relatively high (but standardized) temperature, and the fact that the dry eye tests were performed at the end of the day after a long battery of medical tests, including cognitive testing using computer monitors. The TBUT is known to have low specificity and sensitivity for diagnosing DED, to show high variability over time,[24] and to lack consistency in measurement technique,[3] making it a variable difficult to interpret and compare between

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TABLE 2. Proband-wise Concordance and Phenotypic Correlation in MZ and DZ Twin Pairs, and Estimated Genetic (A) and Environmental Effects (C and E) of the Best Fitting Model of Different DED Outcome Variables

DED Outcome Variable	Distribution Used for Twin Modeling	No. of Complete Twin Pairs, MZ/DZ	Probandwise Concordance, %		Correlation (95% CI)		Additive Genetic Effects, A (95% CI)	Environmental Effects (95% CI)	
			MZ	DZ	MZ	DZ		Common, C	Unique, E
DED symptoms in preceding 3 mos	Dichotomous	838/622	34.1	26.7	0.30 (0.17 to 0.42)	0.13 (−0.02 to 0.28)	0.29 (0.18 to 0.40)	-	0.71 (0.60 to 0.82)
DED diagnosis and current use of artificial tears	Dichotomous	716/516	32.5	16.1	0.44 (0.27 to 0.58)	0.12 (−0.11 to 0.33)	0.41 (0.26 to 0.56)	-	0.59 (0.44 to 0.74)
Interblink interval, s	Ordinal, quartiles	108/175	-	-	0.30 (0.09 to 0.48)	0.06 (−0.13 to 0.24)	0.25 (0.07 to 0.42)	-	0.75 (0.58 to 0.93)
Tear osmolarity, mOsm/L	Normal	99/145	-	-	0.39 (0.23 to 0.53)	0.22 (0.04 to 0.38)	0.40 (0.25 to 0.53)	-	0.60 (0.47 to 0.75)
Schirmer-1 value, mm/5 min	Ordinal, quartiles	103/157	-	-	0.56 (0.39 to 0.69)	0.35 (0.17 to 0.50)	0.58 (0.43 to 0.70)	-	0.42 (0.30 to 0.57)
TBUT, s	Normal	104/162	-	-	0.33 (0.17 to 0.50)	0.27 (0.12 to 0.41)	-	0.30 (0.18 to 0.40)	0.71 (0.60 to 0.82)
Blepharitis signs	Dichotomous	83/164	88.2	83.0	0.75 (0.52 to 0.90)	0.51 (0.26 to 0.71)	0.78 (0.59 to 0.90)	-	0.22 (0.10 to 0.41)

different studies. In contrast, Schirmer value and tear osmolarity may be less influenced by subjectivity and measurement errors in clinical practice and showed relatively high heritability in our study. In this research study, observations were not made by an ophthalmologist, but by a research nurse. Therefore, we attempted to grade blepharitis using more objective criteria (instead of more subjective signs, such as eyelid thickening or hyperemia) to reduce measurement error, and this also may be reflected in our higher heritability estimate. The definition of blepharitis or meibomian gland dysfunction differs substantially among studies.[25] Using our criteria, our study sample showed a high prevalence of blepharitis (74%), which is comparable to other studies in similar age groups.[26,27] Having a diagnosis of DED by a clinician as outcome measure also may have suffered from bias; for example, from the likeliness of a participant to have visited a general practitioner or ophthalmologist, or from the presence of another diagnosis than DED with similar symptoms, such as allergic conjunctivitis. This bias also might reduce specificity of diagnosis and be expected to lead to lower heritability estimates, although both MZ and DZ twin concordances might be affected similarly. Sadly, there is no gold standard for defining DED.[3]

Twin studies rely on the EEA, which states that the effect of common family environment is constant regardless of zygosity. Although this assumption has been criticized, it generally has shown to be true when tested.[27] Since we had one research nurse performing the dry eye tests, another source of bias may be observer bias. This is potential bias, conscious or unconscious, when MZ twins are graded more similarly in subjective tests than DZ twins, because the examiner knows they are more similar, and this might lead to inflated heritability estimates. Obviously, the questionnaire-based analyses did not have observer bias, and these outcome variables showed very similar heritability estimates to the dry eye tests. In addition, TBUT, a very subjective test, did not show signs of heritability, suggesting no observer bias. In general, twins show morbidity and mortality similar to the rest of the population,[13] and have similar ocular phenotypes.[28] We studied only women, because DED is more prevalent in women and because this provided a more homogeneous group to study heritability (and the TwinsUK cohort is largely female). In this study, approximately 20% of females had DED symptoms and 10% had a diagnosis of DED, which is similar to other studies.[2] Therefore, we believe the conclusions from this twin study can be generalized to the whole population. However, it should be noted that heritability is population-specific and might be different for a different population; for example, one more exposed to low humidity. The twins in this study were volunteers, so there also is a potential for ascertainment bias, but this was minimized by the fact that twins were recruited largely for reasons other than eye studies, and were asked the DED questionnaire as part of their annual questionnaire. Similarly, the twins with ocular phenotyping attended for a variety of tests, not just eye studies.

Although DED is a difficult disease to phenotype with outcome variables that show relatively high measurement error, we still found consistent significant heritability in all outcome variables except TBUT. Dry eye disease is a multifactorial disease and this study shows evidence that genetic factors explain a significant proportion of DED variance. At the moment, however, the genetic component of DED is understudied. Two small candidate gene studies have suggested a role for immune-mediated genes,[9,10] but hypothesis-free GWAS are lacking. The GWAS may help unravel the pathophysiology by discovering unknown pathways and may help to identify potential disease-modifying agents that may

reduce DED. Our present findings are important and justify future studies searching for genes involved in DED, despite current suboptimal diagnostic classification in population-based studies.

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Appendix

Table A1. Model Fit Statistics

Model	−2ll	$\Delta\chi^2$	Δdf	*P*	ΔAIC
Dry eye diagnosis and current use of artificial tears					
1. ACE	2318.66	–	–	–	–
2. AE*	2318.66	0	1	1.00	−2.00
3. CE	2323.32	4.65	1	0.03	2.65
4. E	2344.28	25.62	2	0.00	21.62
Dry eye symptoms preceding 3 mos					
1. ACE	3914.08	–	–	–	–
2. AE*	3914.08	0	1	1.00	−2.00
3. CE	3916.75	2.67	1	0.10	0.67
4. E	3937.51	23.43	2	0.00	19.43
Interblink interval					
1. ACE	1488.78	–	–	–	–
2. AE*	1488.78	0	1	1.00	−2.00
3. CE	1491.01	2.23	1	0.14	0.23
4. E	1496.04	7.26	2	0.03	3.26
Tear osmolarity					
1. ACE	−387.54	–	–	–	–
2. AE*	−387.49	0.05	1	0.81	−1.95
3. CE	−385.21	2.33	1	0.13	0.33
4. E	−362.85	24.69	2	0.00	20.69
Schirmer value					
1. ACE	1386.86	–	–	–	–
2. AE*	1387.41	0.55	1	0.46	−1.45
3. CE	1390.28	3.42	1	0.06	1.42
4. E	1432.09	45.23	2	0.00	41.23
TBUT					
1. ACE	−2960.28	–	–	–	–
2. AE	−2958.65	1.63	1	0.20	−0.37
3. CE*	−2960.02	0.26	1	0.61	−1.74
4. E	−2935.78	24.50	2	0.00	20.50
Blepharitis					
1. ACE	556.45	–	–	–	–
2. AE*	557.51	1.06	1	0.30	−0.94
3. CE	558.94	2.49	1	0.11	0.49
4. E	597.54	41.09	2	0.00	37.09

For each model the minus two log-likelihood (−2ll), the change in χ^2 comparing submodel to full ACE-model ($\Delta\chi^2$), difference in df compared to full ACE-model (Δdf), corresponding probability (*P*), and difference in Akaike's information criterion compared to the full ACE-model (ΔAIC) are given.

* The best fitting model per variable.

Table A2. Estimated Genetic and Environmental Effects of the Full ACE-Model for Different DED Outcome Variables

	A (95% CI)	C (95% CI)	E (95% CI)
DED symptoms in preceding 3 mo	0.29 (0.00–0.40)	0.00 (0.00–0.00)	0.71 (0.60–0.82)
DED diagnosis and current use of artificial tears	0.41 (0.05–0.56)	0.00 (0.00–0.00)	0.59 (0.44–0.74)
Interblink interval	0.25 (0.00–0.42)	0.00 (0.00–0.25)	0.75 (0.58–0.93)
Tear osmolarity	0.35 (0.00–0.53)	0.04 (0.00–0.38)	0.61 (0.47–0.77)
Schirmer value	0.42 (0.00–0.69)	0.14 (0.00–0.48)	0.44 (0.31–0.61)
Blepharitis	0.48 (0.00–0.89)	0.27 (0.00–0.70)	0.25 (0.10–0.41)
TBUT	0.11 (0.00–0.47)	0.22 (0.00–0.40)	0.67 (0.53–0.81)

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