Heritability and Linkage Analysis of Appendicitis Utilizing Age at Onset

Christopher Oldmeadow,[1] Kerrie Mengersen,[1] Nicholas Martin,[2] and David L. Duffy[2]

[1] *Department of Mathematical Sciences, Queensland University of Technology, Brisbane, Australia*
[2] *Genetic Epidemiology Unit, Queensland Institute of Medical Research, Brisbane, Australia*

Appendicitis usually afflicts the young, but there is a large tail in the distribution of onset age. The genetics of this disease are still not well understood. A heritability analysis and genome wide linkage analysis of a large twin dataset was undertaken. Treating age of onset of appendicitis as a censored survival trait revealed a heritability of 0.21, and found evidence of linkage to Chromosome 1p37.3.

Keywords: appendicitis, age at onset, linkage analysis, survival, heritability

Appendicitis, a condition characterized by inflammation of the appendix, affects roughly 5 to 14% of the population (Bierman, 1968) and its etiology is still largely unknown. The most common initiation of the disease is a blockage of the lumen (Horton, 1977), often by fecal matter; however, analyses of removed appendices reveals this is true in only approximately 30 to 40% of cases (Silen, 1998). Epidemiological studies have identified gender, age, year of birth, socio-economic status, low-fiber diet and smoking status as risk factors for the disease (Oldmeadow et al., 2008). Relatively little attention has been given to the possibilities of genetic contributions.

A complex segregation analysis of appendicitis in children and early adolescents estimated the heritability of the disease to be 50% (Basta et al., 1990), although this figure may have been overestimated as it did not take into account their respective common family environments. A twin study by the last authors of the current paper utilized data from the Australian Twin Registry (ATR) and estimated the heritability of liability to appendectomy at 27% and domesticity (shared environment) at 16%. While both these studies have noted a high proportion of cases under the age of 20, neither study effectively accounted for the variability in onset age.

The ATR appendectomy data has been analyzed by several others (Prentice & Hsu, 1997), (Dabrowska et al., 1998; Fan et al., 2000; Kooperberg, 1998) and all are consistent in finding evidence of an age-dependent genetic predisposition to appendicitis, where correlations between MZ twins are significantly larger than DZ twins, especially for those under the age of 20. While these results support the proposition that appendicitis may be genetically influenced, and its expression is related to the age of onset, no attempt has been made to give an estimate of the heritability accounting for these features. Furthermore, there has been no attempt to locate the genomic positions of any cosegregating genetic markers with the disease.

The analysis of the causes of variability in the age of onset of a disease is a challenging and important task, as disease heterogeneity is often manifested by the age of expression. Diseases with an early age of onset have been often thought to be under greater genetic influence than those with an older onset; but such a belief needs to be rigorously tested. Attempts to incorporate such information include modeling the correlation between liability to disease and age at onset (Neale et al., 1989), or modeling hazard rates where dependencies in survival times are modeled by a frailty parameter (Korsgaard & Andersen, 1998). Nonparametric measures of association within relative pairs have been developed for the analysis of diseases with age-dependent penetrances (Dabrowska et al., 1998; Fan et al., 2000; Kooperberg, 1998; Prentice & Hsu, 1997). These methods calculate a local measure of association weighted by the bivariate survival distribution.

For linkage analysis of disease data, it is common to consider only the affection status and study either the affected sibling pairs only (Whittemore & Halpern, 1994), or include the unaffected in a binary trait regression analysis (Dawson et al., 1990). Traditional nonparametric variance components (VC) linkage analysis methods (Almasy & Blangero, 1998; Amos, 1994) are more powerful than the methods mentioned above, but they rely heavily on the assumption of a normally distributed trait; violations of this assumption can lead to biased parameter estimates (Amos et al., 1996). The multifactorial threshold model (Duggirala et al., 1997) underlies one such method. Variance

Received 1 October, 2008; accepted 04 February, 2009.

Address for correspondence: Chris Oldmeadow, Queensland University of Technology, Mathematical Sciences, O Block, Gardens Point Campus, Brisbane, Australia. E-mail: c.oldmeadow@student.qut.edu.au

components are estimated for a latent liability trait (an unobserved random variable giving rise to the observed categorical trait) which is assumed to b normally distributed. However, this method and other sibling pair methods previously mentioned, neglect to include information on the age of onset.

Incorporating both disease status and age-at-onset data in parametric linkage analysis involves the specification of age-specific penetrances (Hasstedt et al., 1994) which can be estimated from the data. When the mode of inheritance is not specified, care must be taken as trait censoring can result in non-normal distributions.

There are a number of VC models for such data, but at this stage there is no 'gold standard'. Current methods used to incorporate censored observations in VC linkage analysis, include the traditional VC analysis on transformed residuals resulting from a Cox regression (Amos et al., 2001), the tobit model with random effects (Epstein et al. , 2003), the Cox proportional hazards model with random effects following Normal (Pankratz et al., 2005) or gamma distributions (Zhong & Li, 2004), and those completely nonspecified transformation models with random effects (Diao & Lin, 2005; 2006).

The purpose of this study is twofold. The first aim is to derive an estimate of the heritability of appendicitis, accounting for age of onset and all known environmental risk factors. We are using survey data made available from and collected by the 1980 Australian Twin Registry (ATR). This contains information on the appendectomy status and the age at surgery of 3808 twin pairs. The second aim is to conduct a genome-wide linkage analysis, utilizing genotypic data available from a subset of individuals who participated in the above questionnaire. In this analysis, we exploit the methodologies and available models needed to consider both the survival and binary nature of the data, in estimating heritability and conducting a genome-wide nonparametric linkage analysis.

Materials

Phenotype Data

The Australian NHMRC Twin Registry (ATR) is a volunteer twin registry established in 1979. The data used in the present study are derived from a questionnaire mailed to 5967 ATR twin pairs on November 14th 1980 and returned over a period of 2 years (Duffy et al., 1991). Both members of 3808 pairs (aged 18 and over) completed and returned the questionnaires, and only these respondents were considered (see Table 1).

The questionnaire included items on age, sex, zygosity, tobacco use, a disease checklist comprising some questions on common medical procedures; including whether the subject had undergone an appendectomy and if so their age at operation. A total of 1718 appendectomies were recorded which constitutes 21% of respondents. Of these cases, 96 women reported a hysterectomy or cholecystectomy on the same year as an appendectomy and were censored at age of survey. Previously, analysis of these data showed appreciable genetic and common environment influences on risk of appendectomy (Duffy et al., 1991; Oldmeadow et al., 2008).

Genotype Data

The data used for this research, represent a compilation of data from several microsatellite genome scans undertaken for particular phenotypic studies at QIMR. A detailed discussion of these data is found in Cornes (Cornes et al., 2005).

We compared twin self-reported zygosity to their classification through genotyping and found 23 pairs, originally stated as being MZ twins were actually DZ. Analysis using the RELPAIR package (Epstein et al., 2000) identified two pairs of twins originally labeled DZ were actually MZ. These cases were rectified and this updates earlier reported analyses. The genotype data were checked for pedigree errors by RELPAIR and Mendelian inconsistencies by SIB-PAIR (Duffy, 1997). The markers themselves were checked for genotyping errors using MERLIN (Abecasis et al., 2002), and when identified were removed from the analysis. This left a total of 1376 unique markers spaced fairly evenly across the genome from 915 pairs of DZ twins, of which there were 235 families with discordant pairs, 87 families with affected pairs, and 593 concordant unaffected pairs available for linkage analysis.

Methods

Heritability Analysis

We treated disease status as a binary variable and undertook separate analyses incorporating the age at

Table 1

Breakdown of Zygosity by Sex for the 3808 Twin Pairs in the 1980 Survey

Zygosity group	*N* pairs	Concordant affected pairs	Discordant pairs	Correlation of appendectomy status	SE
MZ female	1212	143	303	0.51	0.04
DZ female	767	75	125	0.39	0.08
MZ male	563	35	128	0.41	0.08
DZ male	354	20	69	0.46	0.10
DZ opposite-sex	912	58	235	0.32	0.06

https://doi.org/10.1375/twin.12.2.150 Published online by Cambridge University Press

onset with an indicator for censoring. Both MZ and DZ twins were used to decompose the variance (Falconer & Mackay, 1996), into that due to additive genes (A), common environment (C) and unique environment (E). Tetrachoric correlations were calculated using an R script (http://www.qimr.edu.au/davidD/R/polyr.R). The full ACE model (as well as the three nested submodels –CE, AE and E) was fitted using SOLAR (Duggirala et al., 1997). The multifactorial threshold model (MFT) (Falconer & Mackay, 1996) was employed to estimate the heritability of liability with gender, birth category (defined as year of birth from 1946 to 1962, 1926 to 1945, or before 1925), and smoking status as fixed effects. The previously published analysis of this data did not include smoking status.

To examine the causes of variation in age of onset, rather than disease status, a Cox proportional hazards model was fitted to the data using the same covariates. The components of variance of the rank normal residuals from this fit were estimated under an ACE (as well as AE, CE and E) model assumption, and parameters were estimated using a variance component analysis in SOLAR.

The overall fit of the models mentioned above was assessed using the chi-square goodness-of-fit statistic. Nested models were compared using the chi-square difference test, where differences in chi-square values of nested models were compared using the difference in degrees of freedom for the two models.

Tests of Genetic Linkage

Affected DZ pairs were analyzed using the methods of Kong and Cox (Kong & Cox, 1997) with the software MERLIN (Abecasis et al., 2002). All DZ pairs were then analyzed using the Haseman-Elston regression (Haseman & Elston, 1972) procedure implemented in MERLIN-REGRESS (Abecasis et al., 2002), where affected and unaffected individuals were given trait values of zero and one, respectively.

To incorporate age-specific effects we performed the methods of Kong and Cox on subsets of those DZ pairs, with onset ages of appendectomy less than 30, 25 and 20. Similarly, we consider appendectomy status at 20, 25 and 30 and analyze all DZ pairs in MERLIN-REGRESS. A variance components nonparametric linkage analysis was performed on the rank-normal martingale residuals from a Cox proportional hazards model, accounting for sex, smoking status prior to appendectomy and year of birth. A Cox proportional hazards model fitting was performed using the Survival package in R (Therneau & Lumley, 2008) and rank-normal residuals were calculated using standard methods.

The genome wide statistical significance of the maximum LOD score was calculated using gene-dropping simulation methods (Sawcer et al., 1997) via the Simulate option in MERLIN. Allele frequencies, marker positions and missing genotype patterns of the real data set were used to simulate data under a model that assumes random linkage between genotype and phenotype. One thousand such data sets were simulated and analyzed using the same methods as for the original data. The empirical p-value for a LOD score was defined as the proportion of simulated genomes for which the LOD score in question was reached or exceeded.

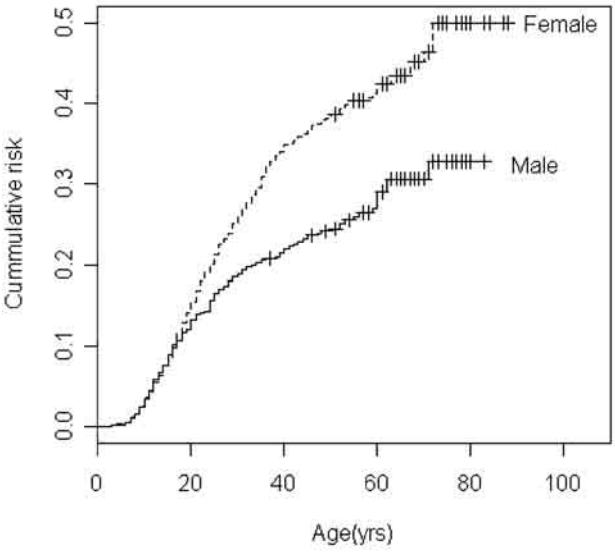

Figure 1
Cumulative risk of appendicitis for the 3080 twin pairs.

Results

As previously reported, approximately 21% of all respondents had undergone appendectomy by time of survey. The plot of the cumulative risk of appendicitis for all pairs by sex, seen in Figure 1, shows a noticeable increase in risk of appendicitis after the age of 10 years. Median age at appendectomy in the sample was 18 years for all twins combined. The differences in survival curves for males and females are negligible before the age of 20. Higher risks for females after this age are most likely due to a number of prophylactic appendectomies that escaped detection.

MZ female twins had significantly higher correlations for appendectomy status than DZ females (Table 2); whereas no significant differences in correlation between MZ and DZ male pairs were found, suggesting that genetic factors may be responsible for appendicitis in females only. However, a log-linear analysis indicated that there was no significant heterogeneity of variation between the sexes. MZ twins had a higher correlation in appendectomy status at age 20 (Table 2) than DZ twins. This suggests that genetic factors may be involved in appendicitis leading to appendectomy (in both sexes).

Conditional on twin 1 being affected, we have plotted the co-twin survival probabilities by zygosity, with separate plots for male and female twin pairs (Figure 2). The differences between MZ and DZ twins

https://doi.org/10.1375/twin.12.2.150 Published online by Cambridge University Press

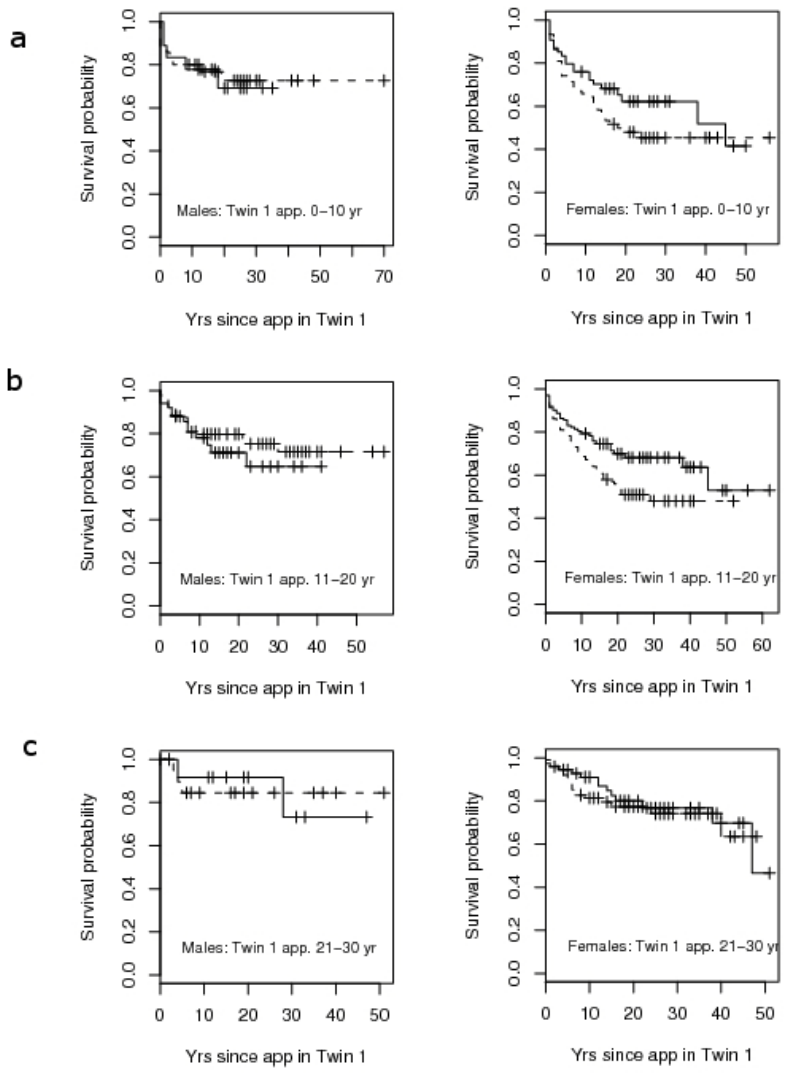

Figure 2

Survival probability conditional on twin 1 affected at age (a) 0–10 years, (b) 11–20 years, and (c) 21–30 years. The left column are for male pairs and the right female pairs. Dashed lines represent MZ twins, solid lines DZ twins. The greatres difference in survival probilities appears to be between when the onset age of the affected twin is between 11 and 20 years old. The difference is more pronounced in females.

Table 2

Correlation in Appendectomy Status at Age 20 for the 3808 Twin Pairs

Zygosity group	*N* pairs	Concordant affected pairs	Discordant pairs	Correlation of appendectomy status	SE
MZ	1775	85	312	0.47	0.04
DZ	2033	74	398	0.33	0.05

Table 3

Model Fitting for the MFT Model for the Trait Appendectomy Status

	h^2	c^2	e^2	Chi-square	Δdf	*p* value
ACE	0.21	0.28	0.51			
AE	0.53		0.67	10.55	1	<0.001
CE		0.43	0.57	4.15	1	0.02

Note: Covariates included year of birth, sex and smoking status.

https://doi.org/10.1375/twin.12.2.150 Published online by Cambridge University Press

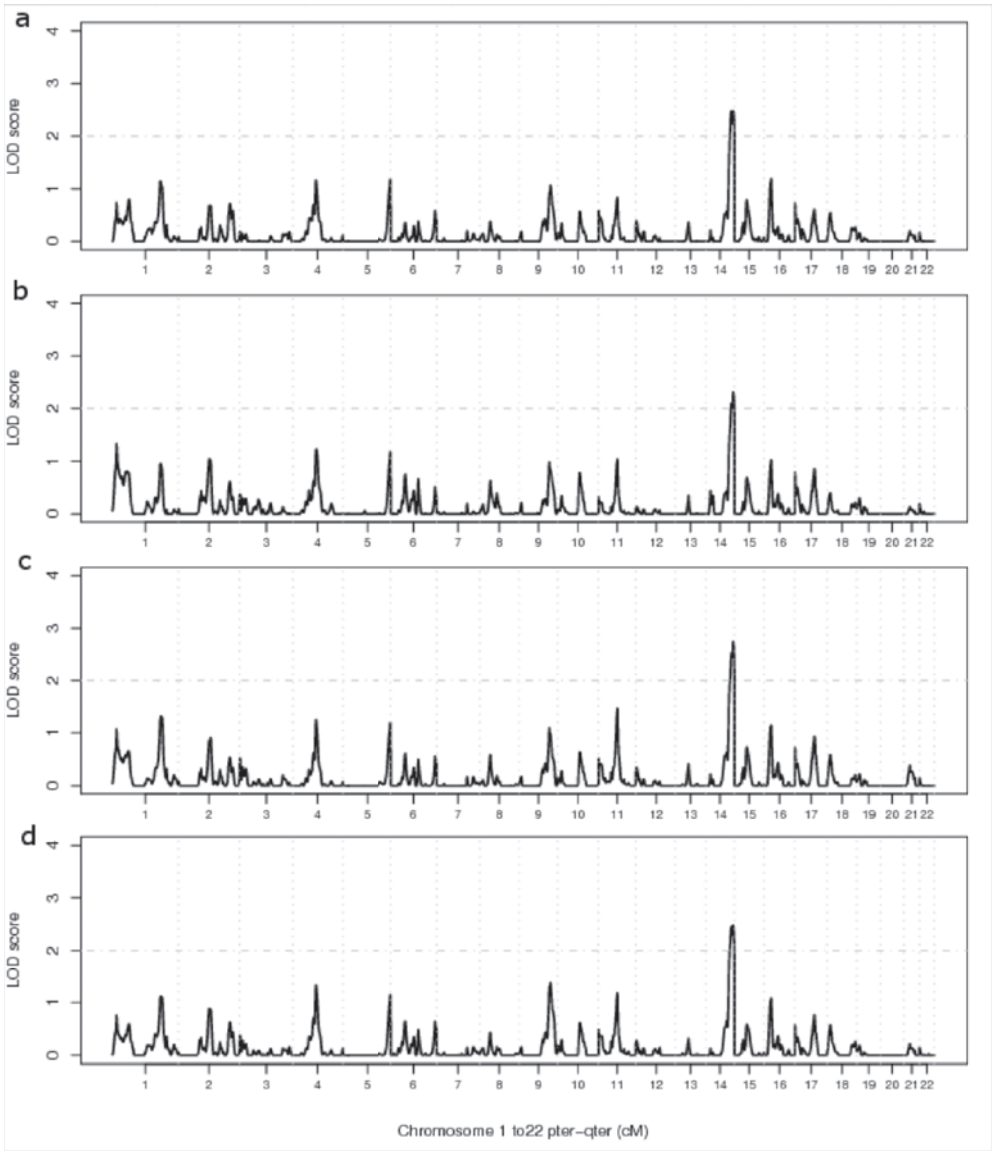

Figure 3

LOD scores for non-parametric Kong and Cox linkage analysis (a) all affected pairs, (b) pairs with onset < 20 years, (c) < 25 years and (d) < 30 years. The largest peak appears on chromosome 14 for cases with an onset < 25 years old. The peak is noticeably smaller for all ages and the other onset age categories.

are greatest when twin 1 had been affected by age 20. There was no noticeable difference after this age, and in general, the difference was largest for female twins.

The results for fitting the threshold model are given in Table 3, and variance components analysis of age of onset using the rank-normal martingale residuals are given in Table 4. In both these cases, we adjusted for covariates including sex, smoking status and birth category. For liability to appendectomy the ACE model provided a significant improvement in fit over both the AE ($\chi^2_{(1)} = 10.55$, $p < .005$) and CE ($\chi^2_{(1)} = 4.15$, $p = .02$) models. For age of onset we found similar results, with the ACE model providing a significant improvement over the AE ($\chi^2_{(1)}=11.45$, $p < .005$) and CE ($\chi^2_{(1)} = 14.71$, $p = < .005$) models. Note the common environmental effect is smaller in the residual analysis, indicating that there may have been some smoothing out of this effect.

Linkage Analysis

Analysis of the 84 affected DZ pairs alone, over all onset ages gave a maximum Kong and Cox LOD score of 1.9 on Chromosome 1 and a LOD score of 2.2 on Chromosome 14 (Figure 3). Seen on the same figure are the results for 64 DZ pairs with onset ages under 30, 49 pairs under 25 and 35 pairs under 20. The analysis of concordant and discordant pairs using the H-E regression, with affection status as a binary trait resulted in similar findings (Figure 4).

The results of the variance components linkage analysis based on the rank-normal Cox residuals are summarized in Figure 5. There is evidence of linkage at

https://doi.org/10.1375/twin.12.2.150 Published online by Cambridge University Press

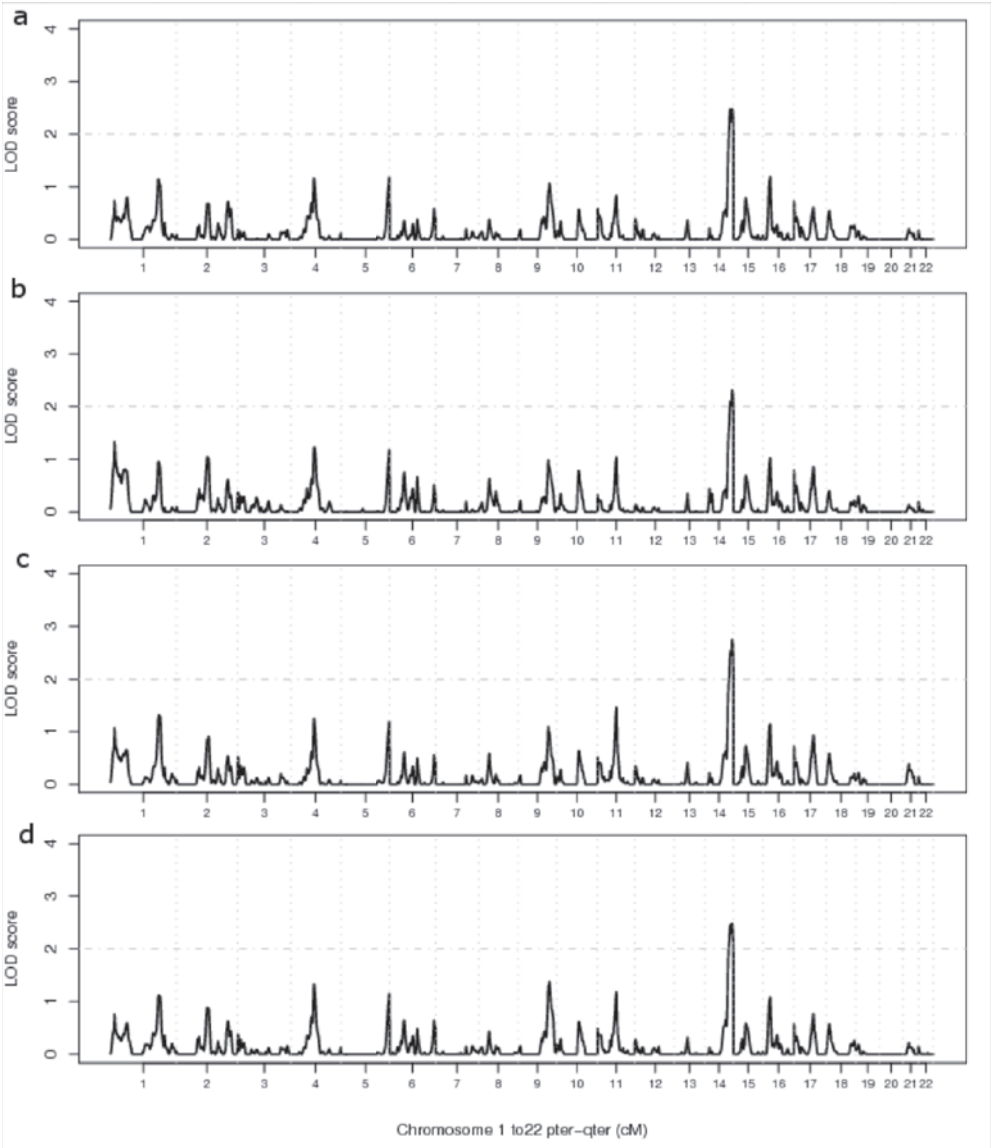

Figure 4

LOD scores for appendectomy status in merlin-regress using (a) all pairs, (b) appendectomy status defined at age < 20 years, (c) age < 25 years and (d) age < 30 years. The peak at chromosome 14 is largest for the onset < 25 years group.

Chromosome 1 (LOD = 3.78, 70.5cM, h^2 = 16.41%), and also at Chromosome 6 (LOD = 2.10) and Chromosome 9 (LOD = 2.25). Our maximum LOD score of 3.78 was declared not significant on a genome-wide scale, with 575 instances exceeding this value from the 1000 simulated data sets.

Discussion

To our knowledge, this is the first comprehensive study into the genetic etiology of appendicitis. In our sample of Australian twins, we found significant differences in the correlations between MZ and DZ female twins; no such differences in males were found. Nevertheless, this does not negate the findings of a possible genetic contribution to the disease. No significant differences in variation due to gender were found, suggesting that the failure to detect any effect in males may be due to a small sample size and high sample variability.

Known environmental effects were accounted for, and age at onset was utilized to derive estimates of heritability of appendicitis. The variables — disease status and age at onset were treated as censored survival data. Using the rank normal transformed residuals from fitting a Cox proportional hazards model to the 1980 ATR data, the ACE model was found to provide the best fit, with heritability of 21% and domesticity of 16%. The results of this study are in agreement with previous analyses of the same data, as discussed in the introduction.

Evidence of linkage was found between age at onset of appendicitis and the D1S255 marker on chromosome1 at location 1p34.3; although simulations

https://doi.org/10.1375/twin.12.2.150 Published online by Cambridge University Press

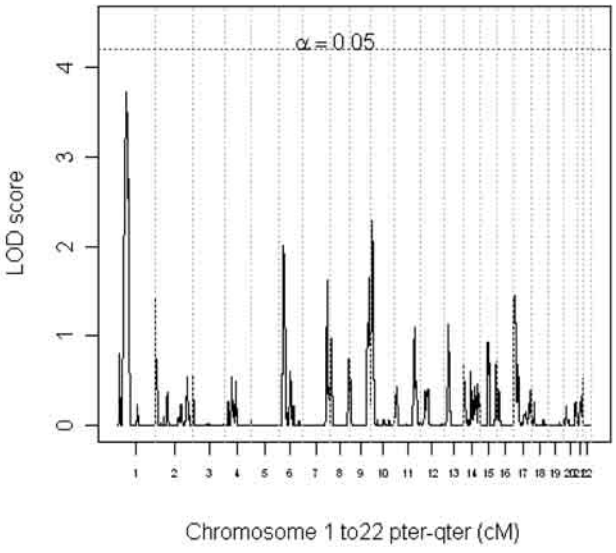

Figure 5

Variance component linkage analysis using the residuals from a Cox-regression as the trait. The dashed line indicates the genome-wide significance level, calculated through simulation.

showed this to be not significant on a genome scale. Other regions of interest were identified. The implementation of affection status alone produced a response on Chromosomes 14 and 11, with the signal being strongest using appendectomy status at age 25. The LOD peak on Chromosome 14 persisted with the inclusion of unaffected individuals using a regression linkage analysis.

Recent investigations into the nature of acute appendicitis show that it may be a result of an inappropriate immune response, which may have a genetic basis. This corresponds with the knowledge that the development of appendicitis can precede a colonization of the virulent *E. coli* bacteria in the appendix (SaxÈn et al., 1996). Protection against invading microbes is usually left to the innate immune system, which recognizes foreign molecules, and initiates a series of appropriate responses to them. The innate immune system has been highly conserved over evolutionary time (Martinelli & Reichhart, 2005), and there is evidence that variations in a gene that governs the inflammatory response (an immunity mechanism) is associated with acute appendicitis (Rivera-Chavez et al., 2004). To the authors' knowledge, the present article provides the first genome-wide linkage analysis of appendicitis.

The limitations of this study are also acknowledged. Measures of the key phenotypes have not been procured from accurate medical records, but are anamnestic. However, surgery is an important and often traumatic event likely to be remembered with some accuracy. The inherent limitations of using a phenotype that is the result of fitting a possibly incorrect model, and applying a transformation to the residuals, are also acknowledged.

With no benchmark for the analysis of censored survival traits, this paper has explored the various analytical methods for such data. We have used a challenging data set, and found only some evidence for the genetic influences of appendicitis.

References

Abecasis, G. R., Cherny, S. S., Cookson, W. O., & Cardon, L. R. (2002). Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. *Nature Genetics, 30*, 97–101.

Almasy, L., & Blangero, J. (1998). Multipoint Quantitative Trait linkage analysis in general pedigrees. *American Journal of Human Genetics, 62*, 1198–1211.

Amos, C., Shete, S., & Gu, X. (2001). Variance components analysis for genetic linkage of time to onset for disease. *Genetic Epidemiology, 21*, S768–773.

Amos, C., Zhu, D., & Boerwinkle, E. (1996). Assessing genetic linkage and association with robust components of variance approaches. *Annals of Human Genetics, 60*, 143–160.

Amos, C. I. (1994). Robust variance-components approach for assessing genetic linkage in pedigrees. *American Journal of Human Genetics, 54*, 535–543.

Basta, M., Morton, N. E., Mulvihill, J. J., Radovanovic, Z., Radojicic, C., & Marinkovic, D. (1990). Inheritance of acute appendicitis: familial aggregation and evidence of polygenic transmission. *American Journal of Human Genetics, 46*, 377–382.

Bierman, H. (1968). Human appendix and neoplasia. *Cancer, 21*, 109–118.

Cornes, B. K., Medland, S. E., Ferreira, M. A. R., Morley, K. I., Duffy, D. L., Heijmans, B. T., Montgomery, G. W., & Martin, N. G. Sex-limited genome-wide linkage scan for body mass index in an unselected sample of

Table 4

Model Fitting for the Transformed Cox Proportional Hazard Residuals, Treating Age at Appendectomy as a Censored Survival Trait

	h^2	c^2	e^2	Chi-square	Δdf	*p* value
ACE	0.21	0.16	0.63			
AE	0.21		0.79	11.45	1	<0.001
CE		0.15	0.85	14.71	1	<0.001

Note: Covariates included year of birth, sex and smoking status.

https://doi.org/10.1375/twin.12.2.150 Published online by Cambridge University Press

933 Australian twin families. *Twin Research and Human Genetics, 8*, 616–632.

Dabrowska, D. M., Duffy, D. L., & Zhang, Z. D. (1998). Hazard and density estimation from bivariate censored data. *Journal of Nonparametric Statistics, 10*, 67–93.

Dawson, D., Kaplan, E., & Elston, R. (1990). Extensions to sib-pair linkage tests applicable to disorders characterized by delayed onset. *Genetic Epidemiology, 7*, 453–466.

Diao, G., & Lin, D. Y. (2005). A Powerful and Robust Method for Mapping Quantitative Trait Loci in General Pedigrees. *American Journal of Human Genetics, 77*, 97–111.

Diao, G., & Lin, D. Y. (2006). Semiparamtric variance-components models for linkage and association analyses of censored trait data. *Genetic Epidemiology, 30*, 570–581.

Duffy, D. (1997). Sib-pair: a program for non-parametric linkage/association analysis. *American Journal of Human Genetics, 61*, 197.

Duffy, D. L., Martin, N. G., & Matthews, J. D. (1990). Appendectomy in Australian Twins (Letter). *The American Journal of Human Genetics, 47*, 590–592.

Duggirala, R., Williams, J. T., Williams-Blangero, S., & Blangero, J. (1997). A variance component approach to dichotomous trait linkage analysis using a threshold model. *Genetic Epidemiology,* pp. 987–982.

Epstein, M. P., Duren, W. L., & Boehnke, M. (2000). Improved inference of relationship for pairs of individuals. *The American Journal of Human Genetics, 67*, 1219–1231.

Epstein, M. P., Lin, X., & Boehnke, M. (2003). A Tobit variance-component method for linkage analysis of censored trait data. *The American Journal of Human Genetics, 72*, 611–620.

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*. London: Longmann & Co.

Fan, J., Hsu, L., & Prentice, R. L. (2000). Dependence estimation over a finite bivariate failure time region. *Lifetime Data Analysis, 6*, 343–355.

Haseman, J., & Elston, R. (1972). The investigation of linkage between a quantitative trait and a marker locus. *Behavior Genetics, 2*, 3–19.

Hasstedt, S., Clegg, D., Ingles, L., & Ward, R. (1994). HLA-linked rheumatoid arthritis. *American Journal of Human Genetics, 55*, 738.

Horton, L. W. (1977). Pathogenesis of acute appendicitis. *British Medical Journal, 2*, 1672–1673.

Kong, A., & Cox, N. J. (1997). Allele-sharing models: LOD scores and accurate linkage tests. *The American Journal of Human Genetics, 61*, 1179–1188.

Kooperberg, C. (1998). Bivariate density estimation with an application to survival analysis. *Journal Of Computational And Graphical Statistics, 7*, 322–341.

Korsgaard, I. R., & Andersen, A. H. (1998). The Additive Genetic Gamma Frailty Model. *Scandinavian Journal of Statistics, 25,* 225–269.

Martinelli, C., & Reichhart, J. M. (2005). Evolution and integration of innate immune systems from fruit flies to man: Lessons and questions. *Journal of Endotoxin Research, 11*, 243.

Neale, M. C., Eaves, L. J., Hewitt, J. K., MacLean, C. J., Meyer, J. M., & Kendler, K. S. (1989). Analyzing the relationship between age at onset and risk to relatives. *American Journal of Human Genetics, 45*, 226.

Oldmeadow, C., Wood, I., Mengersen, K., Visscher, P., Martin, N., & Duffy, D. (2008). Investigation of the relationship between smoking and appendicitis in Australian twins. *Annals of Epidemiology, 18*, 631–636.

Pankratz, V. S., Andrade, M., & Therneau, T. M. (2005). Random effect Cox proportional hazards model: general variance components methods for time-to-event data. *Genetic Epidemiology, 28*, 97–109.

Prentice, R. L., & Hsu, L. (1997). Regression on hazard ratios and cross ratios in multivariate failure time analysis. *Biometrika, 84*, 349–363.

Rivera-Chavez, F., Peters-Hybki, D., Barber, R., Lindberg, G., Jialal, I., Munford, R., & O'Keefe, G. E. (2004). Innate immunity genes influence the severity of acute appendicitis. *Annals of Surgery, 240*, 269–277.

Sawcer, S., Jones, H. B., Judge, D., Visser, F., Compston, A., Goodfellow, P. N., & Clayton, D. (1997). Empirical genomewide significance levels established by whole genome simulations. *Genetic Epidemiology, 14*, 223–229.

SaxÈn, H., Tarkka, E., Hannikainen, P., Nikku, R., Rautio, M., & Siitonen, A. (1996). Escherichia coli and appendicitis: phenotypic characteristics of E. coli isolates from inflamed and noninflamed appendices. *Clinical Infectious Diseases, 23*, 1038–1042.

Silen, W. (1998). Acute Appendicitis. In E. Braunwald, K. J. Isselbacher, R. G. Petersdorf, J. D. Wilson, J. B. Martin & A. S. Fauci (Eds.), *Harrisons principles of internal medicine* (pp. 1304–1306). New York, McGraw-Hill Book Company.

Therneau, T. M., & Lumley, T. (2008). *Survival: Survival analysis, including penalised likelihood*. (R package version 2.34-1). Available at http://CRAN.R-project.org/package=survival

Whittemore, A. S., & Halpern, J. (1994). A class of tests for linkage using affected pedigree members. *Biometrics, 50*, 118–127.

Zhong, X., & Li, H. (2004). Score tests of genetic association in the presence of linkage based on the additive genetic gamma frailty model. *Biostatistics, 5*, 307.

https://doi.org/10.1375/twin.12.2.150 Published online by Cambridge University Press