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HLA and Genomewide Allele Sharing in Dizygotic Twins

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Gametic selection during fertilization or the effects of specific genotypes on the viability of embryos may cause a skewed transmission of chromosomes to surviving offspring. A recent analysis of transmission distortion in humans reported significant excess sharing among full siblings. Dizygotic (DZ) twin pairs are a special case of the simultaneous survival of two genotypes, and there have been reports of DZ pairs with excess allele sharing around the HLA locus, a candidate locus for embryo survival. We performed an allele-sharing study of 1,592 DZ twin pairs from two independent Australian cohorts, of which 1,561 pairs were informative for linkage on chromosome 6. We also analyzed allele sharing in 336 DZ twin pairs from The Netherlands. We found no evidence of excess allele sharing, either at the HLA locus or in the rest of the genome. In contrast, we found evidence of a small but significant ($P = .003$ for the Australian sample) genomewide deficit in the proportion of two alleles shared identical by descent among DZ twin pairs. We reconciled conflicting evidence in the literature for excess genomewide allele sharing by performing a simulation study that shows how undetected genotyping errors can lead to an apparent deficit or excess of allele sharing among sibling pairs, dependent on whether parental genotypes are known. Our results imply that gene-mapping studies based on affected sibling pairs that include DZ pairs will not suffer from false-positive results due to loci involved in embryo survival.

Gametic selection during fertilization or the effects of specific genotypes on the viability of embryos may cause skewed transmission of chromosomes to surviving offspring.[1,2] Significant excess allele sharing was reported among siblings in 148 Hutterite nuclear families.[3] The effect was modest but highly significant and appeared to be spread across many chromosomes. The distortion showed similar maternal and paternal patterns of allele sharing,[3] suggesting selection during embryo development rather than gamete selection.

Genotypic effects on embryo survival are possible, since humans are relatively infertile and most human conceptions fail to reach term.[4–6] Most losses occur in the early stages of pregnancy. It is difficult to estimate these rates of early embryo loss, but most studies suggest up to 75% of conceptions are lost during early development.[5,6] A large proportion of these losses result from chromosomal abnormalities,[7] but some fraction of the losses may be due to genetic factors influencing embryo viability and to interactions between the mother and fetus.[3,8] There is also evidence of selection of maternal gametes that is based on a large study of recombination rate.[9] Rates of recombination increased significantly with maternal age, which suggests that a high recombination rate increased the chances for an oocyte to give rise to a live birth.[9] Mothers with a high oocyte recombination rate also tend to have more children than mothers who do not.

DZ twins (MIM 276400) represent a special case of selection of oocytes and survival of embryos. Embryo losses occur in twin pregnancies and result in the birth of a single offspring ("the vanishing twin syndrome")[10] or loss of both twins (complete loss of pregnancy). Observations with use of Doppler ultrasound suggest that the loss rate per embryo is the same for twins and singletons.[11] Other estimates from survival curves for twin and single pregnancies suggest the losses may be much higher for twins.[12,13] Whatever the true value, live DZ-twin births represent some fraction of the potential number of twins, where two ova were both fertilized. An important question is whether selection mechanisms operate in successful twin pregnancies so that DZ twins are more alike than siblings from individual pregnancies.

Twins are widely used in genetic epidemiological studies.[14,15] Designs of genetic studies with twins assume that DZ twins are no more similar than siblings and therefore share 0, 1, or 2 alleles identical by descent (IBD) with probabilities of 25%, 50%, and 25%, respectively. However, typing of the HLA locus suggested increased sharing of HLA haplotypes in DZ twins.[16,17] Embryonic or maternal factors influencing survival of some twin pregnancies (including mechanisms associated with the immune function) could lead to selection for greater similarity between twins at specific loci, such as the HLA region, although one study failed to replicate increased HLA sharing in twins.[18]

It is important to clarify the level of sharing at the HLA locus and other regions of the genome in twins, because it impacts both genetic studies and our understanding of mechanisms contributing to the birth of twins. If DZ twins share, on average, >50% of their genes IBD at the HLA

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locus and/or at other loci in the genome, then gene-mapping studies that include DZ twins in an affected sibling–pair design may falsely conclude that regions of increased sharing harbor disease-susceptibility loci.[3,19]

We have conducted genome scans in large samples of DZ twins from Australia and The Netherlands. The aim of the present study was to estimate allele sharing in DZ twins, to determine whether there is evidence of transmission distortion around the HLA locus or in other chromosomal regions.

Material and Methods

Australian Adolescent Twins

Twins were recruited through primary schools in the greater Brisbane area as part of an ongoing study of the development of melanocytic naevi (moles), and the clinical protocol has been described in detail elsewhere.[20] The project was approved by the Human Research Ethics Committee of the Queensland Institute of Medical Research, and all participants gave their signed informed consent. It is estimated that ~50% of the eligible birth cohort was recruited into the study and that they were typical of the population, with respect to other variables tested.[20]

Genomewide genotype data from families with adolescent twins were available for 2,019 individuals from 503 families, with a total of 419 DZ twin pairs (table 1). Overlapping parts of the sample received a 10-cM scan with use of the ABI-2 marker set (400 markers) at the Australian Genome Research Facility,[21] a 10-cM scan with use of the Weber marker set at the Center for Inherited Disease Research in the United States, or both. Only 30 markers were common to both marker sets; they were used for quality control. Details of the mean numbers of markers available per individual are given in table 1. For the HLA locus, we investigated an 8-cM region (48–56 cM) on chromosome 6 between markers *D6S276* (at 46.7 cM) and *D6S1610* (at 59.2 cM). The only families to receive only one scan had both parents genotyped and so had higher information content. The average heterozygosity of markers was 0.78, and the mean information content was 0.77. The genotyping, error checking, and cleaning of these data have been described in detail elsewhere.[20]

Australian Adult Twins

Adult twins born before 1971 were recruited from a volunteer twin register (Australian Twin Registry) for studies at the Queensland Institute of Medical Research. Twins and additional family members were recruited using clinical protocols described elsewhere,[22–24] and blood samples were collected for genetic analysis. Projects were approved by the Human Research Ethics Committee of the Queensland Institute of Medical Research, and all participants gave their signed informed consent.

Genomewide genotype data for families with adult twins were available for 7,055 individuals from 2,091 families with (1) 1,142 DZ twin pairs who were informative for linkage on chromosome 6 and (2) 961 pairs informative for all autosomes (table 1). The genotype data were combined from genome scans undertaken at the Australian Genome Research Facility in Australia,[21] Gemini Genomics in the United Kingdom,[25] Leiden University Medical Centre in The Netherlands,[26] and the Mammalian Genotyping Service (Marshfield) and Sequana Therapeutics in the United States.[27] Details of individual data sets, genotyping, error checking, and cleaning of these data have been described in detail elsewhere.[25] The average number of markers genotyped per twin on chromosome 6 was 31 (table 1).

Table 1. Study Design for Genomewide and HLA Analysis of Genotype Data from Twin Samples

Data and Twin Sample	Quantity by Region	
	HLA[a]	Genomewide
No. of informative DZ pairs:		
Australian adolescent	419	418
Australian adult	1,142	961
Dutch		336
Mean no. of markers per DZ individual:		
Australian adolescent	28	588
Australian adult	31	697
Dutch		394

[a] The HLA region was not tested in the Dutch sample.

Dutch Twins

DNA samples from Dutch DZ twins were collected as part of two studies on cardiovascular risk factors [28,29] and studies of anxiety/depression[30] (MIM 607834) and smoking and nicotine addiction[31] (MIM 188890). Participants gave their signed informed consent, and the projects were approved by the Ethics Committee of the Vrije Universiteit Hospital. Genotyping for 400 subjects from the two cardiovascular studies was performed by the Molecular Epidemiology Section, Leiden University Medical Centre, The Netherlands,[32] with a 395-marker genome scan (~10-cM spacing). Genome scans for the anxiety-depression and smoking studies were conducted by the Mammalian Genotyping Service (Marshfield) in two batches.

For the present study, individuals were selected using at least 200 markers and a maximum 18-cM spacing on each chromosome (table 1). All available parental marker data were included, and only families with DZ twins were selected for analysis. The number of complete DZ pairs was 336, with parental information available for 232 DZ pairs. An empirical evaluation of the genetic similarity of samples from the Australian and Dutch twin registries with use of 359 STRP markers showed that there is negligible evidence of population differentiation.[33]

Statistical Analysis

The combined data sets were cleaned for errors. Self-reported pedigree relations were compared with the genotypic data with use of GRR.[34] Pedigree misspecifications, incorrect zygosity calls for twins, sex misspecifications, and sample mix-ups were identified and resolved. Mendelian inconsistencies were identified using Pedstats[35] and were cleaned in the data. Unlikely recombinant genotypes were identified and omitted from further analyses with use of Merlin version 1.01.[36] Map positions were in Kosambi centimorgans and were estimated via locally weighted linear regression from the National Center for Biotechnology Information build 34.3 physical map positions and from published deCODE and Marshfield genetic map positions.[37] This map was converted to Haldane centimorgans before Merlin analyses, and the same genome maps were used for both the Dutch and the Australian sample. Allele frequencies were calculated for all markers with all genotype data.

Our design is analogous to a linkage study of affected pairs of relatives, with "being a DZ twin" the phenotype. Multipoint IBD probabilities were calculated on a 1-cM grid with use of Merlin.[36]

The hypothesis that there was excess IBD sharing among DZ twin pairs at each centimorgan was tested using the NPL test, as implemented in Merlin. This test compares the mean IBD sharing at each location with the expected value of 0.5, under the null hypothesis of no excess sharing, and uses the Kong and Cox method[38] to account for (lack of) marker informativeness. Since we are effectively dealing with small nuclear families with a single set of "affected" relatives, the test statistic from the NPL method is almost equivalent to a *Z*-test that uses the mean multipoint IBD proportion across the pairs and the empirical variance of that mean. This was verified empirically (results not shown). We used such a *Z*-statistic to test whether the proportion of two alleles shared IBD (IBD2) differs from the expected value of 0.25. For the HLA region, we calculated allele-sharing tests at each centimorgan and also for the mean IBD-sharing proportions across the 8-cM interval. In addition to a test for location-specific excess allele sharing among DZ twin pairs and for the average at the HLA locus, we calculated a genomewide average mean IBD–sharing statistic for each DZ twin pair by averaging all estimated 1-cM IBD proportions across the genome. The hypothesis that there was genomewide excess allele sharing was tested using a *Z*-test and the empirical variance of the mean-sharing statistic.

Power calculations were performed with the assumption that the deviation from 0.5 of the mean multipoint IBD proportion across twin pairs divided by its SD is normally distributed with unit variance. Under the null hypothesis, the mean is 0.5; the alternative hypothesis is that the mean is >0.5. For the power calculations, we therefore performed a one-sided test. Since we were testing at only one specific location (HLA) and the genomewide average, we performed the calculations at a type I error rate of 0.05. For a single location (HLA) and a fully informative marker, so that the variance of the proportion of alleles shared IBD is 1/8, we have 80% power to detect a true mean of 0.5225 for a sample size of 1,561 (the combined Australian sample). For genomewide mean IBD sharing, under the assumption of an SD of 0.038,[39] we have 80% power to detect a true mean of 0.5025 for a sample size of 1,561. Hence, we have sufficient power to detect the deviations from expectation that have been reported in the literature.

Results

For the Australian cohorts, there were no significant departures from the expected sharing frequencies at the HLA locus for 419 adolescent DZ twin pairs, the 1,142 adult pairs, or the combined sample. Figures 1 and 2 show the mean IBD- and IBD2-sharing proportions across the HLA region. For the combined sample, the mean (SE) IBD-sharing proportion across the 8-cM HLA locus was 0.5058 (0.0075), slightly above the expected value of 0.50, whereas the mean probability of IBD2 was 0.2470 (0.0090), slightly below the expected value of 0.25. The corresponding test statistics (either NPL or *Z*-test) all gave *P* values >.05. The average multipoint information content at the HLA locus, as measured by the observed variance in IBD sharing across pairs as a proportion of the variance expected with fully informative markers, was 0.75, 0.68, and 0.72 for the adolescent, adult, and combined sample, respectively. There was no evidence of significant departures in allele sharing in DZ twins anywhere on chromosome 6—in the adolescent, adult twins, or the combined sample—or anywhere else in the genome (fig. 3). The locations of genes with reported associations to pregnancy loss (*MTHFR*[8] [MIM 607093], *IL10* [MIM 124092], *IFNG*[40] [MIM 147570], and *CYP17A1*[41] [MIM 609300]) and genes associated with increased twinning in other species (*GDF9*[42] [MIM 601918], *BMPR1B*[43] [MIM 603248], and *BMP15*[44] [MIM 300247]) are also shown in figure 3. There was a minor peak of allele sharing in adult twins on the p arm of chromosome 6 near the telomere. However, this was not supported by any evidence of allele sharing in this region in the sample of adolescent twins.

Mean IBD sharing across the genome in the adolescent, adult, and combined DZ pairs was 0.4999 (n = 418), 0.4968 (n = 961), and 0.4975 (n = 1,379), respectively, with corresponding 2-sided *P* values of .570, .007, and 0.010, respectively. The corresponding genomewide-sharing proportions for IBD2 were 0.2489, 0.2458, and 0.2467, respectively, with *P* values of 0.615, 0.001, and 0.003, respectively. Hence, we find evidence of a small but significant deficit of genomewide allele sharing between DZ twin pairs that is driven by a deficit in the observed proportion having two alleles IBD.

Full parental genotyping information was available for only a subset of the data. Mean IBD sharing in the HLA region in the Australian adolescent, adult, and combined DZ pairs was 0.4966 (n = 304), 0.5103 (n = 208), and 0.5022 (n = 512), respectively, with corresponding 2-sided *P* values of .847, .617, and .870, respectively. Mean IBD sharing across the genome in this more informative subset of the adolescent, adult, and combined DZ pairs was 0.4992 (n = 303), 0.5005 (n = 195), and 0.4997 (n = 498), respectively, with corresponding 2-sided *P* values of .695, .832 and .871, respectively. We find no evidence of an excess or deficit in allele sharing across the HLA or genomewide in the restricted set of families with both parents genotyped.

In the Dutch genome scan, there was no evidence of increased sharing on chromosome 6 (fig. 3). Increased sharing was found on chromosome X, but this was not seen in the Australian data. Genomewide mean IBD sharing in 336 DZ twin pairs showed a slightly lower sharing of alleles, 0.4971, with a nonsignificant 2-sided *P* values of .945. The mean IBD2 was 0.2487, with a 2-sided *P* value of .738.

Discussion

Selection of gametes or variation in survival of embryos or fetuses during development could result in unequal representation of alleles in a population.[2,3] DZ twins represent a special case, since development and survival of twin embryos may be influenced by similarities and differences between the two embryos. By focussing on DZ twins in an affected-sibling-pair design, the phenotype of interest is a combination of survival and cosurvival with a cotwin, and the genotype of interest is that of the twins, not their mothers. If DZ twins both survived as embryos or fetuses

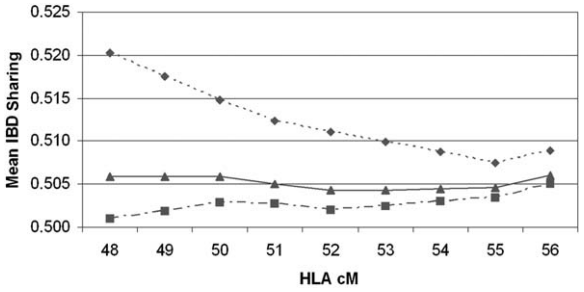

Figure 1. Mean IBD allele sharing for DZ twin pairs, calculated at 1-cM intervals across the HLA region on chromosome 6, for the Australian adolescent (♦), adult (■), and combined (▲) samples.

because they both inherited favorable alleles from their parents at a particular locus, this may be detected as excess IBD sharing around that locus. Analysis in our large sample of Australian twins found no evidence of significant excess IBD sharing in DZ twin pairs at either the HLA locus or at other regions of the genome, including locations of genes implicated elsewhere in embryo survival and twinning (fig. 3). Our results have important implications for linkage studies for disease phenotypes that employ DZ pairs in an affected-sibling design, because we can rule out a substantial excess IBD sharing for DZ twin pairs, either on chromosome 6 or in the rest of the genome.

HLA alleles may directly influence embryo viability through interactions at the maternal-fetal interface (for review, see the work of Choudhury and Knapp[45]). Embryo viability involves complex interactions, including genetic factors from both parents and interactions between the mother and fetus during implantation and pregnancy. The presence of twins adds further complexity. There is some evidence in genetically isolated groups to suggest that fetal loss and increased birth intervals may be associated with sharing of particular HLA loci or haplotypes, although this has not been demonstrated in other populations.[45] The limited evidence suggests that survival might be related to reduced rather than increased sharing at the HLA locus.

Genomewide mean IBD sharing (± SD) was 0.497 ± 0.036 for the combined Australian sample of 1,379 DZ twin pairs in the present study, which is similar to our previous estimate of 0.498 ± 0.036 from 4,401 pseudo-independent sibling pairs (which included a number of DZ twins).[39] Hence, we have consistently found evidence of a small but significant deficit in genomewide allele sharing between sibling pairs. The genomewide results are puzzling and need further investigation and replication by others. Since our data were combined from genome scans performed at several different genome centers, we considered the possibility that heterogeneity of typing between centers might have influenced our results. We, therefore, performed a new analysis using only marker data from a single source (Mammalian Genotyping Service, Marshfield) for 746 pairs with at least 200 markers spread over all chromosomes. Results were very similar to the adult scan for multiple genotyping sources that was based on 961 pairs and more markers.

Our test statistic was calculated from genomewide average IBD-sharing probabilities, and we used the empirical variance of the sharing statistics to calculate a *Z*-statistic. The assumption is that the distribution of the genomewide IBD-sharing statistic is normally distributed, which can be justified theoretically and was shown empirically.[39] Zöllner et al.[3] used the simulation option in Merlin to generate empirical *P* values. We note that this approach assumes a model for recombination and that violations of this assumption may yield inappropriate *P* values. When we restricted our analysis to those families in which both parents were genotyped, we found no evidence of significant departures in allele-sharing proportions, although power was reduced. It is unclear whether the difference in results between the analyses that included all DZ families and those that included DZ pairs and parental genotypes reflects a loss of power or more-accurate and unbiased IBD estimation when parental genotypes were known.

To further investigate the differences in reported genomewide IBD sharing, we performed a simple simulation study to quantify the effect of undetected genotyping errors on IBD sharing of sibling pairs. A marker with eight equifrequent alleles in Hardy-Weinberg equilibrium was simulated for independent nuclear families consisting of parents and two siblings. Genotyping errors were simulated at a rate of 1% per genotype by randomly changing one of the alleles to another allele. Mean IBD sharing for the siblings was calculated using standard conditional probabilities, under the assumption that the allele frequencies were known. IBD-sharing proportions were calculated either using or ignoring parental genotypes. When parental genotypes were used, IBD was calculated for the siblings only if there was no detectable Mendelian error. We ran one million replicates. Without parental genotypes, the mean (SE) IBD-sharing proportion was 0.4970 (0.0002). When parental genotypes were used, there were

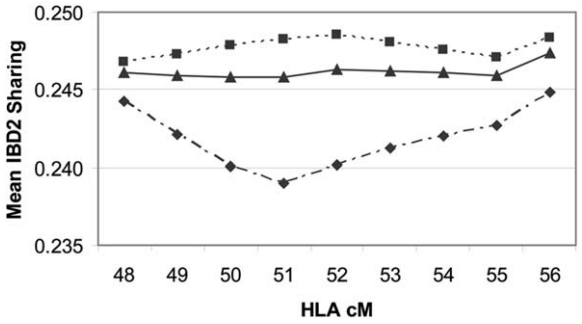

Figure 2. Mean probabilities of sharing 2 alleles IBD of DZ twin pairs, calculated at 1-cM intervals across the HLA region on chromosome 6, for the Australian adolescent (♦), adult (■), and combined (▲) sample.

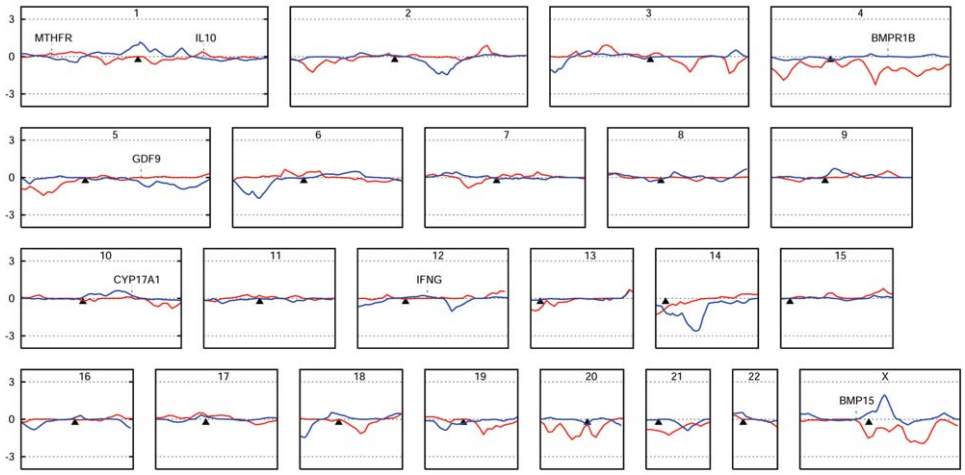

Figure 3. Affected-sibling-pair genome scan for the phenotype "being a DZ twin." Results for the combined sample of Australian twins are indicated with a red line and, for the Dutch twins, by the blue line. The locations of genes with reported associations to pregnancy loss (*MTHFR,*[8] *IL10, IFNG,*[40] and *CYP17A1*[41]) and genes associated with increased twinning in other species (*GDF9,*[42] *BMPR1B,*[43] and *BMP15*[44]) are indicated.

976,609 replicates for which a Mendelian error was not detected, and the mean IBD proportion for these families was 0.5018 (0.0003). Hence, our simple error model suggests that, with high information content (e.g., parental genotypes known), we can expect a slight excess of IBD sharing among sibling pairs and that, with low information content (e.g., parental genotypes unknown), a slight deficit can be expected. We suggest that undetected genotyping error rates explain the conflicting results in the literature. High-density SNP genotyping in families should resolve the question of whether mean genomewide IBD sharing deviates from its theoretical expectation.

One would expect natural selection to operate directly on variants that adversely affect reproductive fitness, so that any segregating variant that decreased embryo survival would be rare. Zöllner et al.[3] calculate that approximately six recessive lethal genotypes would explain their genomewide excess sharing of 0.43%. Rare variants can be detected by linkage if the effects are large or if multiple variants of moderate effects at the same locus are segregating in the population.

In conclusion, we have not found any evidence of increased IBD sharing by DZ twin pairs at the HLA locus or elsewhere in the genome. In contrast to previous findings, we report evidence of a small genomewide deficit in allele sharing among DZ twin pairs. The results are important for linkage studies of disease phenotypes that employ DZ pairs in an affected-sibling design, because we can rule out a substantial excess IBD sharing for DZ twin pairs.

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Web Resource

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for DZ twins, anxiety/depression, smoking and nicotine addiction, *MTHFR, IL10, IFNG, CYP17A1, GDF9, BMPR1B,* and *BMP15*)

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