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Non-Random Mating, Parent-of-Origin, and Maternal-Fetal Incompatibility Effects in Schizophrenia

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Abstract

Although the association of common genetic variation in the extended MHC region with schizophrenia is the most significant yet discovered, the MHC region is one of the more complex regions of the human genome, with unusually high gene density and long-range linkage disequilibrium. The statistical test on which the MHC association is based is a relatively simple, additive model which uses logistic regression of SNP genotypes to predict case-control status. However, it is plausible that more complex models underlie this association. Using a well-characterized sample of trios, we evaluated more complex models by looking for evidence for: (a) non-random mating for HLA alleles, schizophrenia risk profiles, and ancestry; (b) parent-of-origin effects for HLA alleles; and (c) maternal-fetal genotype incompatibility in the HLA. We found no evidence for non-random mating in the parents of individuals with schizophrenia in terms of MHC genotypes or schizophrenia risk profile scores. However, there was evidence of non-random mating that appeared mostly to be driven by ancestry. We did not detect over-transmission of HLA alleles to affected offspring via the general TDT test (without regard to parent of origin) or preferential transmission via paternal or maternal inheritance. We evaluated the hypothesis that maternal-fetal HLA incompatibility may increase risk for schizophrenia using eight classical HLA loci. The most significant alleles were in *HLA-B*, *HLA-C*, *HLA-DQB1*, and *HLA-DRB1* but none was significant after accounting for multiple comparisons. We did not find evidence to support more complex models of gene action, but statistical power may have been limiting.

Keywords

Schizophrenia; human leukocyte antigens; non-random mating; maternal-fetal incompatibility

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1. Introduction

Common genetic variation in the major histocompatibility complex (MHC) on 6p22.1 is a risk factor for many complex human diseases. The association of common genetic variation in the extended MHC region with schizophrenia is the most significant yet discovered ($P \sim 10^{-12}$) (Ripke et al., 2011) and meets community standards in human genetics for replication (Chanock SJ, 2007). However, the MHC region is one of the more complex regions of the human genome, with unusually high gene density and long-range linkage disequilibrium. As a result, the genome-wide significant evidence for association involves more than 100 SNPs, extends a very large distance (26–33 Mb), and encompasses around 300 genes.

The statistical test on which the MHC association is based is simple, using logistic regression of SNP genotypes to predict case-control status under an additive model. It is plausible that more complex models underlie this association. First, non-random mating (i.e., the tendency for mating partners to have greater phenotypic similarity than expected by chance) occurs for many physiological traits (Merikangas, 1982) as well as schizophrenia (Lichtenstein et al., 2006). Non-random mating can lead to complex biases in genomic studies (Redden and Allison, 2006) and may even be driven by genetic variation in the MHC region (Havlicek and Roberts, 2009). Second, parent-of-origin effects (variable genetic risk depending on the parent from which an allele is inherited) can occur in the MHC (Bassett, 2011; Chao et al., 2010). If this mechanism is operative, statistical models explicitly including such effects could assist in refining the currently broad and ill-defined MHC-schizophrenia association. Finally, maternal-fetal genotype incompatibility occurs when specific combinations of maternal and fetal genotypes yield an adverse prenatal environment (Childs et al., 2011). During pregnancy, maternal antibodies to paternal HLAs can be detected (Palmer, 2010). Since maternal antibodies to fetal antigens have been observed in a large proportion of healthy pregnancies, it is possible that maternal recognition or sensitization of paternally-derived fetal HLAs dissimilar to maternal HLAs may be beneficial for implantation and maintenance of pregnancy (Palmer et al., 2006). If paternally-derived fetal HLAs are similar to the maternal HLAs, maternal sensitization can fail to occur and lead to adverse fetal outcomes. Maternal-fetal genotype incompatibility may increase the risk of prenatal/obstetric complications (Cowan et al., 1994; Ober et al., 1998; Schneider et al., 1994; Verp et al., 1993), and there is some evidence that risk of schizophrenia may also be elevated (Palmer, 2010; Palmer et al., 2006).

Evaluation of these potentially more complex, MHC-themed models is difficult or impossible to do in case-control studies. Using a well-characterized sample of parent-affected offspring trios, we evaluated the evidence for: (a) non-random mating for HLA alleles and MHC SNPs, schizophrenia risk profiles, and ancestry; (b) parent-of-origin effects for HLA alleles and MHC SNPs; and (c) maternal-fetal genotype incompatibility in the HLA.

2. Materials and Methods

2.1. Subjects and genotyping

The study sample comprised 698 parent-offspring trio families from Bulgaria with 727 affected offspring (50.2% male). All subjects were genotyped with Affymetrix 6.0 chips at the Broad Institute(Ruderfer et al., 2011). We performed quality control (QC) steps in which we removed subjects with high genotype missing rates (> 2%) or high Mendelian errors per individual (> 2000 SNPs) along with SNPs with high missing rates (> 2%), strong deviation from Hardy-Weinberg Equilibrium ($p < 1\times10^{-6}$ in parents although there were none in the MHC region), frequency difference to HapMap3 CEU (> 0.15)(Altshuler et al., 2010), or high Mendelian errors per SNP (> 4). After QC, there were 642 probands in 624 complete trios (607 families with 1 proband, 16 families with 2 probands, and 1 family with 3 probands). Of the 642 probands, 544 (85%) had a diagnosis of schizophrenia and 98 (15%) schizoaffective disorder (Table S1), and 12 (1.0%) fathers and 36 (2.9%) mothers were diagnosed with schizophrenia or schizoaffective disorder. We kept 657,466 successfully genotyped SNPs which contain 1,704 SNPs in the extended MHC region (chr6: 26–33Mb).

In order to impute classical HLA alleles, we used a dataset created by the MHC Working Group of the Type 1 Diabetes Genetics Consortium (Pereyra et al., 2010). This dataset contains genotypes (MHC tag SNPs and direct determination of alleles for eight MHC genes at four digit resolution) for 2,767 unrelated individuals of European ancestry. The eight MHC genes are *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1* (Brown et al., 2009), and the dataset enables imputation of 377 HLA alleles, 3,852 SNPs, and 372 amino acid changing polymorphisms. We used Beagle (Browning and Browning, 2009) to phase and impute classical HLA alleles (Robinson et al., 2011) for these subjects. We selected 189 HLA alleles imputed with high confidence (imputation quality > 0.8) and no Mendelian inheritance errors for further analysis.

2.2. Non-Random mating

We evaluated non-random mating in three ways. First, to evaluate non-random mating for HLA alleles and MHC SNPs, we used methods described in Chaix et al. (Chaix et al., 2008). The genetic similarity of a mating pair *c* at a genetic marker in the HLA region was estimated as *R*, the ratio of probabilities of identity in state: $R=(Q_c - Q_m) / (1-Q_m)$, where Q_c is the proportion of identical variants in the mating pair (0 if different alleles, 0.5 if one allele identical, or 1 if both alleles were the same) and Q_m is the mean proportion of identical variants over all possible pairs of individuals in the sample. We summarized *R* by computing its mean for mating pairs across the imputed HLA alleles or across SNPs with $R > 0$ indicating genetic similarity and $R < 0$ indicating genetic dissimilarity between mating pairs relative to random mates in the sample. The significance of *R* was assessed using permutation and random shuffling of mating pairs 1,000 times.

Second, we evaluated evidence for non-random mating that might be driven by sub-clinical phenotypes genetically related to schizophrenia by using risk profile scores (Purcell et al., 2009) genome-wide, genome-wide excluding MHC, and MHC only. There are robust and replicable findings that liability to schizophrenia is distributed across the genome and can be

assessed by a weighted sum of the number of associated risk alleles (Purcell et al., 2009; Ripke et al., 2011; Ruderfer et al., 2011). We used the PGC schizophrenia sample (Ripke et al., 2011) as the discovery sample (excluding the subjects from this study) to generate a set of markers for generating risk profiles in these trios. The risk profile set contained 64,254 SNPs and was a subset of the full GWAS results file after filtering for allele frequency 0.02–0.98, imputation INFO score > 0.9, approximate linkage equilibrium ($r^2 \leq 0.25$ within 500kb windows), and association threshold $P_T < 0.2$ (see Figure S6 in reference (Ripke et al., 2011) for justification for selection of $P_T < 0.2$). Using this risk profile, we calculated the schizophrenia risk profile scores for each of the parents in these Bulgarian trios, and assessed non-random mating via the correlation between mothers and fathers. As a comparison, we performed the same analysis for the HapMap3 CEU founders. Third, we evaluated evidence for non-random mating based on ancestry as determined using multidimensional scaling (MDS) dimensions (Purcell et al., 2007; Sebro et al., 2010) and again used HapMap3 CEU founders for comparison.

2.3. Parent-of-origin effects

We used the transmission disequilibrium test (Spielman and Ewens, 1993) implemented in PLINK (Purcell et al., 2007) to determine whether any HLA allele was more frequently transmitted to the affected offspring than the other allele without regard to the gender of the transmitting parent. We then refined this general analysis by separately considering transmissions from heterozygous fathers versus heterozygous mothers to affected offspring to assess whether the transmission of risk alleles was biased towards one parent.

2.4. Maternal-fetal genotype incompatibility

To evaluate the presence of maternal-fetal genotype incompatibility on risk for schizophrenia, we used “MFG” option in Mendel (Childs EJ, 2010; Sinsheimer et al., 2003). The MFG option implements an affected-only likelihood ratio test relying on the joint estimation of offspring allelic effects, maternal allelic effects, and interactions between maternal and offspring genotypes. As the Mendel MFG implementation can handle only biallelic loci, we converted multi-allelic HLA types into binary format and tested for MFG incompatibility at each biallelic HLA marker. Over all alleles at each HLA locus, we designated an index allele as “G” (e.g. *HLA-A*0101*), and “A” for all the other alleles at the locus. For example, an individual with two *HLA-A*0101* alleles would be assigned “G/G” at marker *HLA-A*0101*, and an individual with genotype *HLA-A*0301/0302* would be assigned “A/A” at the same marker. With our genotype notation of biallelic HLA marker, there are 7 possible maternal-child genotype combinations between mother and child (Table S2). Childs et al. (Childs et al., 2011) and Palmer et al. (Palmer et al., 2006) defined offspring as being MFG incompatible for a polymorphism if mother and child have identical genotypes or if the mother is heterozygous and the offspring is homozygous. Following their definition, we defined the following two combinations as MFG incompatible: (g_m= G/G, g_c=G/G), (g_m= A/G, g_c=G/G) where g_m=maternal genotype and g_c=child genotype. We did not consider (g_m= A/G, g_c=A/A), (g_m= A/A, g_c=A/A), or (g_m= A/G, g_c=A/G) as incompatible since “A” denotes other alleles at the index allele being considered. Mendel maximizes the log-likelihoods under our MFG incompatibility model and a null model in which all mother-offspring genotype combinations confer the same risk of disease. For each

biallelic HLA marker, Mendel calculates a likelihood ratio test statistic and reports an asymptotic p-value.

3. Results

3.1. Non-random mating

First, we tested for genetic similarity in founders of HapMap2 CEU, HapMap3 CEU, and our trio sample using imputed HLA alleles (Table S3) and SNPs (Table S4). To calibrate the method described in (Chaix et al., 2008) and replicate their finding, we repeated the non-random mating analysis of 5,708 MHC SNPs (chr6: 29.6 −33.3 Mb) with MAF ≥5% in HapMap2 CEU founders and confirmed the previously reported results (a slight but statistically significant dissimilarity in the MHC region, R=−0.064, p=0.016). We applied these procedures to 1,175 MHC SNPs (chr6: 29.6 −33.3 Mb) with MAF ≥1% and to imputed 4-digit HLA alleles across class I and II genes in 42 mating pairs from HapMap3 CEU. However, unlike the HapMap2 CEU results, there was no evidence for genetic similarity in HapMap3 CEU based neither on SNPs (R=−0.047, p=0.175) nor on imputed HLA alleles (six HLA genes, R=0.009, p=0.589; eight HLA genes, R=0.003, p=0.521). Derti et al. (Derti et al., 2010) suggest that the HapMap2-HapMap3 discrepancy could be due to influential outliers. Next, we applied similar approaches to 1,082 MHC SNPs (chr6: 29.6 −33.3 Mb) with MAF≥1% and class I and II HLA genes in our sample. Although we observed marginally significant similarity among parents of schizophrenic offspring using SNPs (R=0.0082, p=0.052), there was no evidence for similarity using HLA genes (six HLA genes, R=0.002, p=0.534; eight HLA genes, R=0.004, p=0.804). In summary, we failed to find evidence to support the hypothesis that parents of schizophrenic offspring are more likely to be genetically similar in MHC than parents of healthy offspring.

Second, we evaluated non-random mating in regard to risk profile scores derived from PGC schizophrenia results. As higher risk profile scores have been highly significantly but weakly correlated with schizophrenia, parents of offspring with schizophrenia are, in general, likely to have higher risk profile scores than parents of healthy children (Figure S1). Moreover, if parental mating was influenced by similarity for phenotypic traits related to risk profile scores, an appreciable positive correlation between parents might be observed. The genome-wide correlation in risk profile scores between parents was highly significant (Figure 1, Pearson's r=0.174, asymptotic p=1.3×10^{-5}, and permutation p < 1×10^{-4}, Figure S2). However, the paternal-maternal risk profile non-parametric correlation was not significant (Spearman's ρ=0.065, p=0.105) (Table 1), suggesting that the Pearson correlation could be unduly influenced by outliers. After removing 137 ancestral outliers detected by smartpca in EigenSoft (Price et al., 2006), we found that Pearson's correlation was no longer significant (Pearson's r=−0.07, p=0.11, Figure 1, Table 1). Of the 137 ancestral outliers, 130 individuals were control and 7 were diagnosed with schizophrenia. Correlations excluding MHC (Pearson's r=0.16, p=6.1×10^{-5} ; Spearman's ρ=0.05, p=0.24) were quite similar to those including MHC. Correlations in MHC only were null (Pearson's r=0.01, p=0.76 ; Spearman's ρ=−0.03, p=0.52). We applied the same risk score profile to the HapMap3 CEU founders, and observed non-significant results genome-wide (Pearson's r=−0.04, p=0.80; Spearman's ρ=−0.03, p=0.81; Figure 1, Table 1). Correlations excluding MHC (Pearson's r=

−0.02, p=0.90; Spearman's ρ=0.02, p=0.91) were insignificant, similar to genome-wide results. Although non-parametric correlations in MHC only were significant (Spearman's ρ= −0.32, p=0.01), parametric correlations in MHC were insignificant (Pearson's r=−0.23, p=0.08). Taken together, we conclude that there is no strong evidence to support non-random mating based on schizophrenia risk profile scores calculated across genome-wide or across MHC in either Bulgarian sample or HapMap3 CEU.

Finally, we assessed non-random mating in regard to genetic ancestry. The first multidimensional scale score was positively correlated between the parents of probands with schizophrenia (Pearson's r=0.92, $p<2.2\times10^{-16}$, and Spearman's ρ=0.23, $p=7.6\times10^{-9}$), suggesting ancestry-related non-random mating. After removing 137 ancestry outliers detected by smartpca, the maternal-paternal correlation was greatly attenuated (r=0.11, p=0.01, Figure 1, Table 1). There were no significant correlations between intra-parental scores on the second or third multidimensional scale scores. We also applied the same procedure to the HapMap3 CEU founders, and observed significant correlations in the first three MDS values, indicating that there is ancestral non-random mating among the HapMap3 CEU founders. Even after removing 7 outliers detected by EigenSoft (Price et al., 2006), correlations in the first three MDS values remained strongly significant. We conclude that there is supportive evidence for non-random mating with respect to genetic ancestry in these Bulgarian trios as well as HapMap 3 CEU.

3.2. Association testing

We performed TDT and parent-of-origin analyses on 1704 SNPs in the extended MHC region (chr6: 26–33Mb) and on 189 HLA alleles with imputation quality > 0.8. TDT analysis revealed that 4 SNPs (rs2894249, rs2107191, rs3129932, rs3117194), *HLA-DPB1*0201*, and *HLA-DPB1*1101* were significant (Table 2, Figure S3 for 2-digit HLA alleles; Figure S4 for 4-digit HLA alleles). However, after multiple testing adjustment using false discovery rate method (Benjamini, 1995), all the adjusted p-values were greater than 0.6 and hence we concluded that no SNP or HLA allele was significantly over-transmitted to affected offspring without regard to parents. Parent-of-origin analysis, which separately considered paternal and maternal over-transmission of risk allele to affected offspring, suggested that 2 SNPs (rs2107191, rs3117194), *HLA-A*0301*, *HLA-C*1203*, and *HLA-DPB1*0201* were over-transmitted paternally whereas 3 SNPs (rs886403, rs1632857, rs1634717), *HLA-A*1101*, and *HLA-C*0401* were over-transmitted maternally (Table 3, Figure S3 for 2-digit HLA alleles; Figure S4 for 4-digit HLA alleles). Again, no SNP or HLA alleles survived after multiple testing adjustments.

3.3. Maternal-fetal genotype incompatibility

We tested the hypothesis of maternal-fetal HLA incompatibility effects on schizophrenia. Of the 189 HLA alleles tested, 7 alleles in *HLA-C*, *HLA-B*, *HLA-DRB1*, *HLA-DQB1* loci appeared to be associated with schizophrenia (Table 4, Figure S3 for 2-digit HLA alleles; Figure S4 for 4-digit HLA alleles). Due to previous findings that *HLA-B* matching effect on schizophrenia was exclusive to female offspring (Childs et al., 2011; Palmer et al., 2006), we tested for the MFG incompatibility effects on female probands by performing analyses on families with female probands. Although we found significant MFG incompatibility

effects at *HLA-B*56* and *HLA-B*5601* with all probands, we failed to detect such effects at *HLA-B* in female probands (p =0.2 for *HLA-B*56* and *HLA-B*5601*). The MFG incompatibility effect at *HLA-DRB1*01* and *HLA-DRB1*0101* remained significant in both all and female probands. However, after multiple testing correction using false discovery rate method (Benjamini, 1995), all the adjusted p-values were 1.0.

4. Discussion

Using a relatively large and well-characterized trio sample, our study looked for evidence of non-random mating, parent-of-origin effects for HLA alleles, and maternal-fetal genotype incompatibility in the HLA. The results are consistent with three conclusions.

First, there was evidence of non-random mating by ancestry that appeared mostly to be driven by a subset of subjects who were ancestry outliers. We speculate that this could reflect within-group mating by minority groups within Bulgaria (e.g., Turks or Roma). Without data from additional trios sampled without respect to affection status, we cannot know whether this is a risk factor for schizophrenia or the result of chance, geographic propinquity, or some other process mechanistically unrelated to schizophrenia. However, we found no compelling evidence for non-random mating in the parents of individuals with schizophrenia in terms of MHC genotypes or schizophrenia risk profile scores.

Second, after correcting for multiple testing, we did not detect over-transmission of HLA alleles to affected offspring via the general TDT test (without regard to parent of origin) or preferential transmission via paternal or maternal inheritance.

Third, we evaluated the hypothesis that maternal-fetal HLA incompatibility may increase risk for schizophrenia by examining the effect of maternal-fetal HLA matching in all probands, female probands using imputed alleles of eight classical HLA loci. The most significant alleles were in *HLA-B, HLA-C, HLA-DQB1, HLA-DRB1* but none was significant after accounting for multiple comparisons. In contrast to the more general and less assumption-laden approach we chose, Palmer et al. (Palmer, 2010; Palmer et al., 2006) and Childs et al. (Childs et al., 2011) applied a modified MFG test to three multi-allelic loci of *HLA-A*, *HLA-B*, and *HLA-DRB1*. They observed an association between *HLA-B* matching and schizophrenia in female offspring. Although we found significant *HLA-B* matching effect using families with all probands, we failed to replicate *HLA-B* matching effect using families with female probands only. Failure of replication could be due to methodological differences or different population (Finland versus Bulgaria) or HLA typing (imputed versus genotyped) or different sample sizes (274 families versus 624 families). Regarding methodological differences, they combined alleles to reduce the number of allele frequencies estimated at each locus and tested for HLA matching effect on schizophrenia at each HLA locus as a whole whereas we tested for HLA matching effect at each allele of each HLA locus. As discussed in their paper, however, combining alleles may result in binning low-risk mother-offspring combinations with high-risk ones. Different results may be obtained depending on how to combine the alleles.

These analyses must be viewed in context of several limitations. First, although the present sample is, to our knowledge, the largest trio sample with GWAS data available for

schizophrenia, it is possible that true effects could have gone undetected due to limited power. As an illustration, for one of our hypotheses involving the general TDT, we had 80% power to detect genotypic relative risks of 1.37 and 1.79 for minor allele frequencies of 0.30 and 0.05 (624 case-parent trios, α=0.00625 (8 tests), log-additive model, and 0.4% disease risk in the general population) (Gauderman, 2006). These effect sizes are quite large (Ripke et al., 2011) and thus power was probably insufficient, although we have no strong precedents with which to derive effect size expectations under the more complex models. Second, other models such as a model including gene by environment interaction are possible. Third, this Bulgarian sample is atypical in terms of frequency and effect size of the ancestral haplotype across European samples as the ISC study (Purcell et al., 2009) originally documented.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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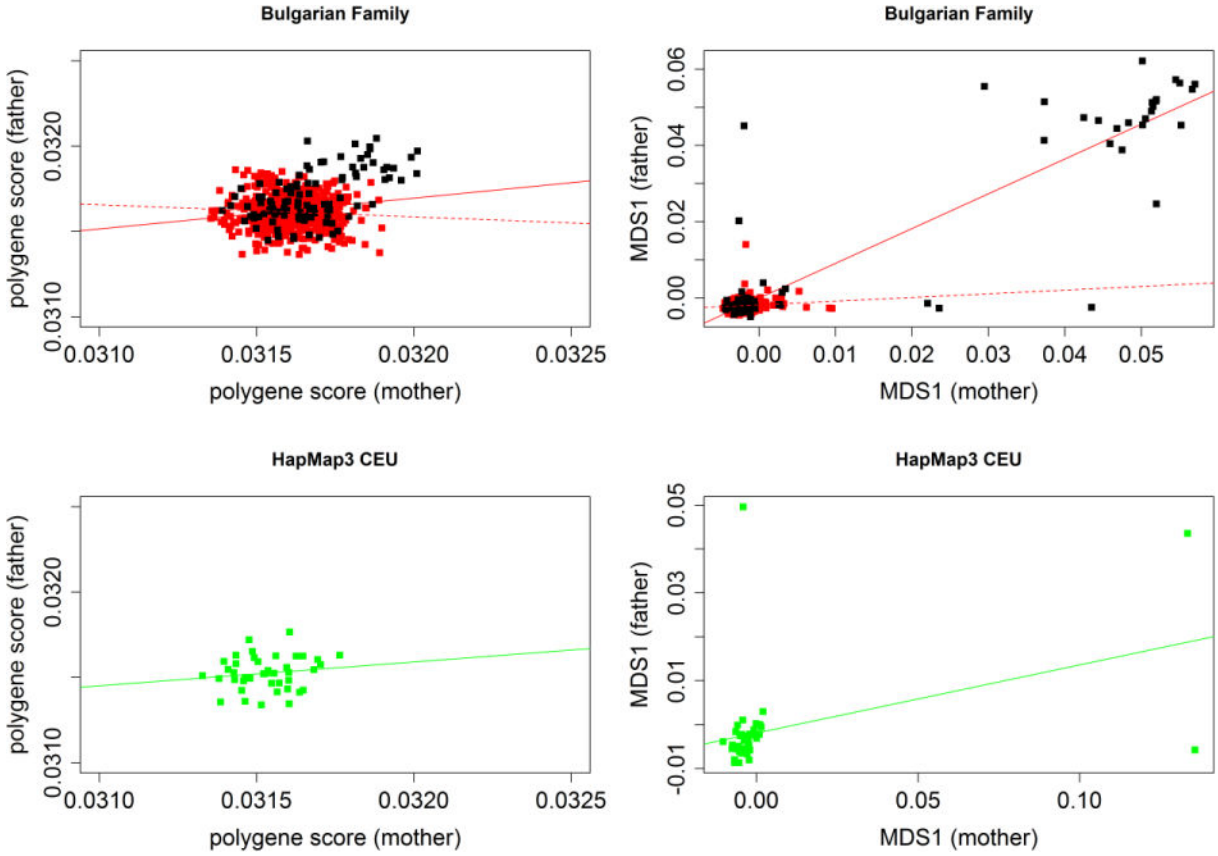

Figure 1. Spousal correlation in Bulgarian sample and HapMap3 CEU using polygene scores

Upper panels depict polygene risk scores and MDS1 of Bulgarian founders. Black dots indicate genetic outliers detected by Eigenstrat. Solid red line represents a regression line when including all pairs. Dotted line represents a regression line after removing the genetic outliers. Lower panels show polygene risk scores and MDS1 of HapMap 3 CEU founders.

Table 1

Summary of non-random mating using risk profile scores and multidimensional scaling (MDS).

	Bulgarian Families		HapMap3 CEU	
	Pearson correlation (p-value)	**Spearman correlation (p-value)**	**Pearson correlation (p-value)**	**Spearman correlation (p-value)**
(a) Risk score profile				
Genome-wide	0.17 (1.3E-5)	0.07 (0.11)	−0.04 (0.80)	−0.03 (0.81)
Genome-wide (After removing outliers)	−0.07 (0.11)	−0.05 (0.28)	NA	NA
Excluding MHC	0.16 (6.1E-5)	0.05 (0.24)	−0.02 (0.90)	0.02 (0.91)
MHC only	0.01 (0.76)	−0.03 (0.52)	−0.23 (0.08)	−0.32 (0.01)
(b) MDS				
MDS1	0.92 (< 2.2E-16)	0.23 (7.6E-9)	0.41 (0.006)	0.45 (0.003)
MDS1 (After removing outliers)	0.11 (0.01)	0.12 (0.006)	0.54 (0.001)	0.52 (0.002)
MDS2	−0.02 (0.69)	0.08 (0.05)	0.90 (8.9E-16)	0.86 (< 2.2E-16)
MDS3	0.01 (0.73)	−0.004 (0.92)	0.98 (< 2.2E-16)	0.95 (< 2.2E-16)

Table 2

TDT results for 189 imputed HLA alleles with imputation quality > 0.8 and 1704 SNPs in extended MHC region.

Marker	Transmitted counts	Untransmitted counts	Odds ratio	Chisq	P-value	Empirical P-value	FDR adjusted P-value
(a) HLA alleles							
*HLA-DPB1*02*	200	163	1.23	3.77	0.052	0.076	0.96
*HLA-DPB1*0201*	197	161	1.22	3.62	0.057	0.090	0.96
*HLA-DPB1*11*	6	1	6.00	3.57	0.059	0.079	0.96
*HLA-DPB1*1101*	6	1	6.00	3.57	0.059	0.079	0.96
(b) SNPs							
rs2894249	161	114	1.41	8.03	0.005	0.005	0.67
rs2107191	347	277	1.25	7.85	0.005	0.006	0.67
rs3129932	161	117	1.38	6.96	0.008	0.010	0.71
rs3117194	344	279	1.23	6.78	0.009	0.008	0.71

Over-transmitted HLA alleles with p-value < 0.06 and over-transmitted SNPs with p-value < 0.01 are shown in this table. Empirical p-values were obtained via permutation in which transmitted/untransmitted status constantly was flipped for all SNPs for a given family, preserving the LD and linkage information between markers and siblings. FDR adjusted p-value was calculated using the method of Benjamini and Hochberg (1995).

Table 3

Parent-of-origin test results for 189 imputed HLA alleles with imputation quality > 0.8 and 1704 SNPs in extended MHC region.

HLA allele	T:U (PAT)	P (PAT)	T:U (MAT)	P (MAT)	Z (POO)	P (POO)	FDR adjusted P-value
(a) HLA alleles							
*HLA-A*03*	78:47	0.005	42:73	0.004	3.975	7.1E-5	0.28
*HLA-A*0301*	75:45	0.006	39:72	0.002	4.102	4.1E-5	0.28
*HLA-C*1203*	76:53	0.042	66:71	0.668	1.758	0.079	0.82
*HLA-DPB1*02*	104:77	0.045	96:86	0.459	0.902	0.367	0.82
*HLA-DPB1*0201*	104:76	0.036	94:86	0.549	1.062	0.288	0.82
(b) SNPs							
rs2107191	191:136	0.003	157:140	0.353	1.396	0.163	0.97
rs3117194	188:137	0.005	156:142	0.417	1.378	0.168	0.97
(c) HLA alleles							
*HLA-A*11*	47:55	0.426	58:34	0.012	−2.368	0.018	0.51
*HLA-A*1101*	47:55	0.426	58:34	0.012	−2.368	0.018	0.51
*HLA-C*04*	102:100	0.944	113:85	0.047	−1.37	0.167	0.75
*HLA-C*0401*	102:100	0.944	113:85	0.047	−1.37	0.167	0.75
(d) SNPs							
rs886403	139:144	0.767	157:110	0.004	−2.273	0.023	0.96
rs1632857	149:155	0.688	169:114	0.001	−2.592	0.009	0.96
rs1634717	156:154	0.909	174:129	0.012	−1.762	0.078	0.96

HLA alleles or SNPs with significantly more transmission to affected offspring paternally (highlighted with blue) or maternally (highlighted with pink) are listed. For HLA alleles, P(PAT) or P(MAT) <0.06 are shown in this table. For SNPs, P(PAT) or P(MAT) <0.01 are shown in this table. This test separately considers transmission of risk alleles from heterozygous fathers versus heterozygous mothers to affected offspring. Under the null, there is no differential transmission paternally or maternally. P(PAT) represents p-value for paternal over-transmission. T:U (PAT) means paternal transmitted counts versus untransmitted counts. Z (POO) represents Z score for difference in paternal versus maternal odds ratios to contrast parent-of-origin effects. P (POO) is the asymptotic two-sided p-value for Z (POO). FDR adjusted p-values for P(PAT) or P(MAT) were calculated using the method of Benjamini and Hochberg (1995).

Table 4

Summary of MFG incompatibility test.

HLA allele	P-value[1] (all probands)	P-value[2] (female probands)
*HLA-B*56*	0.047	0.201
*HLA-B*5601*	0.047	0.202
*HLA-C*03*	0.038	0.141
*HLA-C*0303*	0.029	0.153
*HLA-DRB1*01*	0.004	0.051
*HLA-DRB1*0101*	0.003	0.033
*HLA-DQB1*0301*	0.037	0.185

[1]: Families with all probands were included in analysis.

[2]: Families with female probands were included in analysis.