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Risk for Premenstrual Dysphoric Disorder is Associated with Genetic Variation in ESR1, the Estrogen Receptor Alpha Gene

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Abstract

Background—Premenstrual dysphoric disorder (PMDD) is a heritable mood disorder that is triggered by gonadal steroids during the luteal phase in susceptible women.

Methods—We performed haplotype analyses of estrogen receptors alpha and beta (ESR1 and ESR2) in 91 women with prospectively confirmed PMDD and 56 controls to investigate possible sources of the genetic susceptibility to affective dysregulation induced by normal levels of gonadal steroids. We also examined associations with the Val158Met SNP of the gene for COMT, an enzyme involved in estrogen metabolism and prefrontal cortical activation.

Results—Four SNPS in intron 4 of ESR1 showed significantly different genotype and allele distributions between patients and controls. Significant case-control differences were seen in sliding-window analyses of two, three, and four marker haplotypes, but only in those haplotypes containing SNPs in intron 4 that were positive in the single-locus analysis. No significant associations were observed with ESR2 or with the COMT Val158Met polymorphism, although the significant associations with ESR1 were observed only in those with the Val/Val genotype.

Conclusion—These are the first positive (albeit preliminary) genetic findings in this reproductive endocrine-related mood disorder and involve the receptor for a hormone that is pathogenically relevant.

Keywords

premenstrual dysphoric disorder; PMDD; ESR1; estrogen receptor; alpha gene

Premenstrual Dysphoric Disorder (PMDD) is a reproductive endocrine-related mood disorder that affects approximately 5%–8% of women and is associated with substantial morbidity. While affective disturbance in this condition has been presumed to be linked to hormonal changes over the course of the menstrual cycle, the exact role of hormones has been elusive. PMDD is not characterized by abnormal gonadal steroid levels or disordered hypothalamic-pituitary-ovarian axis function (Rubinow 1992). Nonetheless, we have demonstrated that exposure to normal levels of gonadal steroids can trigger depressed mood in women with

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PMDD, but not in those without such history (Schmidt et al 1998). Consequently, any pathophysiological explanation for PMDD must account for both the triggering of mood symptoms by gonadal steroids and the vulnerability to experience affective disturbance in association with levels of gonadal steroids that are without effect on mood in women lacking a history of PMDD.

Genotypic differences are likely to mediate a differential behavioral response to gonadal steroids. In both animals and humans, polymorphic variants in genes encoding gonadal steroid receptors associate with both altered sensitivity and differential response to gonadal steroids. These gonadal steroid receptor polymorphisms have been shown to alter receptor transcriptional efficacy (e.g., CAG repeat in exon 1 of the androgen receptor; progins insertion in intron 7 of the progesterone receptor) and are associated with differential illness risk (i.e., prostate cancer, breast cancer) (Zhang et al 2004; Beilin et al 1999; Giovannucci et al 1997; Wang-Gohrke et al 2000). Additionally, the susceptibility to the disruptive effects of estradiol on reproductive development differs enormously (up to 100-fold) between mouse strains, with genotype controlling more of the variance than the dose of estradiol employed (Spearow et al 1999). There is precedent, then, for inferring that polymorphisms in genes in the gonadal steroid signaling pathway or in gonadal steroid-regulated genes may alter the nature or strength of the steroid signal as well as the clinical and behavioral phenotype. Moreover, a genetic contribution to PMDD is supported by the results of both family and twin studies (Wilson et al 1991; Kendler et al 1992; Condon 1993).

Several genetic polymorphisms have been tested for possible association to PMDD (Melke et al 2003; Damberg et al 2005) without any positive reports thus far [e.g., variants in the serotonin transporter (SLC6A4) including the 5′HTTLPR polymorphism (Heils et al 1995), an intron 2 VNTR (Ogilvie et al 1996), and a G to T single nucleotide polymorphism (SNP) in the 3′ UTR region (Battersby et al 1999); and an intron 2 VNTR in the gene for transcription factor activating protein 2beta (AP-2beta) (Damberg et al 2005)]. In the absence of validated functional polymorphisms in potentially causative genes, genetic association relies on typing marker alleles across samples as potential proxies for causative mutations. The extent of gene sequence diversity, however, has made apparent the limited power of single SNP-based candidate gene studies and consequently raised questions about their validity (Hoehe 2003). Gene-based haplotype analysis employs a set of SNPs (or other markers) that when analyzed together can have substantially greater power to capture the genetic diversity of the sample compared to single SNPs.

In this study, we initiated gene-based haplotype analyses of estrogen receptors (ER) alpha and beta in women with PMDD and controls. We selected these genes for two reasons: 1) our prior demonstration of the triggering of affective dysregulation in women with PMDD when exogenous estradiol was administered in the context of GnRH agonist-induced hypogonadism (Schmidt et al 1998); 2) recent studies identifying the importance of ER beta in animal models of anxiety and depression (Walf et al 2004; Krezel et al 2001; Rocha et al 2005) and of estrogen receptor alpha in arousal (Garey et al 2003). In addition, we examined associations with the Val158Met SNP (rs4680) in the gene for catechol-O-methyltransferase (COMT), an enzyme involved in estradiol metabolism, implicated in sex hormone-mediated cancer (Tanaka et al 2006; Sazci et al 2004), and observed in other studies to regulate activity in the prefrontal cortex, a brain region implicated as dysfunctional in PMDD (Rubinow et al in press).

2. Materials and Methods

2.1. Subjects and recruitment procedures

We studied 91 women with PMDD and 56 women who served as an asymptomatic comparison group. Medication-free Caucasian women with regular menstrual cycles were selected from

respondents to newspaper advertisements for volunteers with a history of PMDD or without any history of menstrual cycle-related mood changes. Subjects from both groups had similar demographic and socio-economic characteristics. Before entry into the study, prospective participants were screened with a daily visual analogue scale of self-ratings of affective symptoms (i.e., a 100 mm line bracketed by severity extremes [none to worst ever] for sadness and irritability/anxiety) (Rubinow et al 1984). In at least two of three menstrual cycles, women diagnosed with PMDD (n = 91, age = 39.5. yrs, SD = 5.9) showed a 30% (or greater) higher mean level (adjusted for the range of the scale employed) of sadness and/or irritability/anxiety symptoms in the week before menses than in the week following the end of menses. (Adjustment consisted of dividing 30% by the percent of the scale spanned by the extreme high and low ratings (Smith et al 2003).) This criterion operationalizes the DSM-IV severity and cyclicity criteria for PMDD (Smith et al 2003), and all patient volunteers met DSM-IV criteria. On the daily ratings control women without PMDD (n = 56, age = 40.6 yrs, SD = 9.0) showed no evidence of mood changes related to menstrual cycle phase. All subjects were given a Structured Clinical Interview for DSM-III-R (SCID) (Spitzer et al 1990) and a modified Schedule for Affective Disorders and Schizophrenia-Lifetime (SADS-L) (Spitzer et al 1979); women with PMDD were required to have no current or recent (< 2 years) Axis I condition, and control women without PMDD were required to have no current or past history of an Axis I condition. The two year requirement eliminated subjects with significant current or recent comorbidities, which could serve as confounds, but did not eliminate the majority of subjects with PMDD with more remote histories of Axis I disorders. All subjects provided written informed consent, and the protocol was approved by the National Institute of Mental Health institutional review board. All subjects reported menstrual cycles of regular length, ranging between subjects from 21 to 33 days, and none had any significant medical illness either at intake or at time of testing or within the previous year.

2.2. Genotyping

Genomic DNA was extracted from peripheral lymphocytes from 20 ml of whole blood with the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN), used according to the manufacturer's instructions.

A total of 24 SNPs were selected from the Celera and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) databases to test for association of PMDD with estrogen receptor alpha (ESR1) and estrogen receptor beta (ESR2). Initially we examined 10 SNPs that spanned a 295.7 kilobase (kb) genomic DNA interval encompassing the *ESR1* transcript (GenBank NM_000125) and attempted 8 SNPs that spanned the *ESR2* transcript (GenBank NM_001437). One ESR2 SNP failed, and primers and probes for another could not be successfully produced; therefore, 6 ESR2 SNPs were genotyped. We selected SNPs that are at roughly 30 kb intervals for ESR1 or 10 kb intervals for ESR2, spanned the whole gene coding region, and, with two exceptions (low frequency SNPs previously validated in the literature), had a minor allele frequency > 10%. Regions identified as showing positive associations with diagnosis (PMDD vs. controls) were further interrogated with additional SNPs. This resulted in the genotyping of six additional SNPs in ESR1. The COMT Val158Met polymorphism was also genotyped; primer and probe were designed by Applied Biosystems, Assay-by-Design as previously described (Chen et al 2004). The map positions and polymorphism information for the SNPs tested are shown in Tables 1 and 2. The Haploview 3.2 program was used to determine which of these SNPs were also ht-SNPs (Barrett et al 2005).

Amplification reactions were performed in a 384-well format in a total reaction volume of 10 μl with 7.5 ng of dried genomic DNA, 5 μl of 2 × AmpliTag Gold® PCR master mix (Applied Biosystems), 0.1 μl (1000 nM) of each primer, 0.02 μl (100 nM) of each probe, and 3.76 μl of 1 × TE buffer. The plates were then placed in a thermal cycler (PE 9700; Applied Biosystems)

and were heated at 50°C for two minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 30s and 60°C for one minute. Plates were then transferred to the Prism 7900HT (Applied Biosystems), in which the fluorescence intensity in each well of the plate was read. Water was used as a negative control. Greater than 96% of genotypes were called. In addition, consistency of genotyping was determined by loading four case and four control samples in duplicate in each genotyping plate; reliability of genotyping was 99.6%. Interplate and intraplate duplicate testing of known DNAs was performed.

2.3. Statistical analysis

The 25 SNPs were tested for Hardy-Weinberg equilibrium in the Caucasian individuals. Differences in genotype distributions between cases and controls were tested for significance using a χ^2 test (unadjusted for multiple comparisons). The standardized measure of linkage disequilibrium (LD), denoted as D', was estimated with software (Haploview 3.1) from haplotype frequencies based on alleles at all possible pairs of SNP loci. A graphical overview of the LD plot was constructed using Haploview software (see Figure 1).

COCAPHASE, a method of standard unconditional logistic regression, was used to test for single-locus association with our case-control data (http://www.hgmp.mrs.ac.uk/~fdudbrid/software/unphased/). The SNPHAP program (version 1.0, http://www.gene.cimr.cam.ac.uk/clayton/software/) was used to infer ESR1 haplotype frequencies in case and control samples. Haplotype frequencies were compared more rigorously using the GENECOUNTING program (version 2.0, 1/04; (Zhao et al 2002)). This program, which in our sample can be used for up to 10 SNP haplotypes, assesses the significance of each haplotype individually by permutation (here, n = 1000), producing empirical p-values, and also performs a global measure of association. We used this program to estimate multi-marker haplotype frequencies and to test each haplotype for differences in frequency between our subject groups. In addition to the single-locus analysis, then, haplotype analysis was performed using two, three, and four marker moving-windows. The association between individual SNPs and diagnosis was compared across COMT genotypes with the Cochran-Mantel-Haenszel odds ratio test (SPSS version 14.0.2, SPSS, Inc, Chicago, IL). Additionally, to rule out the possible contribution to the associations observed of a mood disorder in general (vs PMDD), single-locus associations were recalculated after the 29 PMD subjects with a past history of MDD were deleted.

3. Results

3.1. Genotype distribution

The marker to marker linkage disequilibrium map constructed by the program HAPLOVIEW is shown in Figure 1. All 16 SNPs tested in ESR1 (see Table 1 and Figure 2) were in Hardy–Weinberg equilibrium in both PMDD and control groups. Four SNPs, all located in intron 4 showed significantly different allele and genotype frequencies between patients and controls (Table 3A), and for each SNP the 1 was the risk allele. Reanalysis of the data after excluding the 29 PMD subjects with a past history of MDD produced identical genotypic results (despite the decreased sample size) and nearly identical allelic results, with the exception that the contiguous intron 4 SNPs L0058 + L0057 also demonstrated significant case control associations. No significant association was observed with any of the eight SNPs (see Table 3B) in ESR2 (estrogen receptor beta) or with the COMT Val158Met polymorphism (data not shown).

3.2. Haplotype analysis

Significant case-control differences were seen in the two-, three-, and four-marker haplotypes analyses (Tables 4A, 4B, and 4C, respectively) in those haplotypes containing the SNPs in

intron 4 that were positive in the single-locus analysis. In all three analyses, the first significant haplotypes (global and empirical p values) contained SNPs L0025 or L0060 or both, and the last significant two and three marker haplotypes began with L0026. Almost every significant association consisted of the common 11, 111 and 1111 haplotypes as risk and the 22, 222, and 2222 haplotype as protective. No significant associations were observed upstream of the L0025 locus or downstream of the L0026 locus. L0026 (rs1884051) and L0060 (rs3020314) are ht-SNPS in block 6, and the only other ht-SNP that we genotyped in ESR1 was the slightly upstream L0058 (rs1801132).

3.3 Effect of COMT genotype on ESR1 association results

The significant differences in the allele frequencies between patients and controls of four SNPs in intron 4 of ESR1 were observed only in subjects with the COMT Val/Val homozygote genotype (Table 5). No associations were observed in Met/Met homozygotes or in Val/Met heterozygotes, and the Cochran-Mantel-Haenszel test showed the single SNP associations to be significantly different in the Val/Val compared with the combined Val/Met and Met/Met groups for markers 25, 26, 60, 61, as well as for 57 and 21 (odds ratios varied between 1.7 and 3).

Discussion

Our demonstration of an association between allelic variants in the estrogen receptor alpha gene and PMDD is the first positive genetic finding in this disorder, a condition estimated to affect 5–8% of reproductive age women and to be responsible for 14.5 million disability adjusted life-years in the United States (Halbreich et al 2003). The gene for ER alpha (ESR1), a 595 amino acid protein, contains 8 exons, which code for two activation domains, a DNA binding domain, and a ligand binding domain. ESR1 was cloned in 1985 (Walter et al 1985), and a little over a decade later a second ER, ER beta (ESR2), was cloned (Kuiper et al 1996). ER beta is a 530 amino acid protein that is 95% homologous with ER alpha in the DNA binding domain and 53% homologous in the E/F transactivation/ligand binding domain, but is otherwise poorly conserved (Ogawa et al 1998). As ligand-dependent transcription factors, ER alpha and beta regulate the synthesis and metabolism of multiple neurotransmitters and neuropeptides, their receptors, and transporters (McEwen et al 1999). Additionally, both receptors have been shown to play a role in acute, non-genomic cell signaling (Razandi et al 1999; Razandi et al 2002; Kim et al 1999). Through these manifold actions, ER alpha and beta impact on virtually all elements of CNS development and function – neuronal and glial proliferation, migration, differentiation, activation, survival, and death.

The results of twin and family studies suggest that PMDD is a heritable disorder (Kendler et al 1992; Condon 1993; Wilson et al 1991). Kendler et al, for example, observed that premenstrual, menstrual and neurotic symptoms had different genetic and environmental determinants (Kendler et al 1992) and that the heritability of PMS was about 56% (Kendler et al 1998). These studies provide a rational basis for the search for genetic contributions to PMDD. As with all complex genetic disorders, the contribution of any single gene is likely to be quite small. Nonetheless, demonstration of a relationship between genetic variations in ESR1 and PMDD is very promising for several reasons. First, ER alpha plays a major role in arousal (Garey et al 2003), dysfunction of which could underlie somatic, cognitive and affective symptoms of PMDD. Second, ER alpha regulates the signaling of neurotransmitter systems implicated in both the etiopathogenesis and treatment of PMDD. For example, extensive links exist between estrogen and serotonin function, with the latter involved in mood regulation and the selective therapeutic effects of SSRIs in PMDD (Rubinow et al 1998). At least some of the effects of estradiol are mediated through serotonin 1A receptors, which are upregulated through nuclear factor-kappa B (NF-kB) by ER alpha but not ER beta (Wissink et al 2001). Third, the

estrogen receptor has clear physiologic relevance in PMDD as the receptor for a hormone that can trigger the onset of symptoms of the disorder (Schmidt et al 1998). Fourth, and perhaps most important, PMDD has been shown to be a disorder of hormone sensitivity; i.e., individuals with the disorder display an abnormal behavioral response to normal levels of estradiol and progesterone (Schmidt et al 1998). Examples of differential sensitivity to the actions of hormones exist in both the human and animal literatures, with both environmental (Meaney et al 2005; Meaney et al 1991; Ladd et al 1996), and genetic sources identified (Bailey et al 2002; Svare 1998; Spearow et al 1999). Of particular note are demonstrations of polymorphic variants in genes encoding reproductive steroid hormone receptors that are associated with both altered transcriptional activity and phenotypic variation. VNTRs in the androgen receptor influence the transcriptional efficiency of the activated receptor (Beilin et al 1999) and are associated in some (Giovannucci et al 1997) but not all (Zeegers et al 2004) studies with increased susceptibility to the development of prostate cancer. Similarly, the progins insertion in the progesterone receptor leads to increased transcriptional effects and diminished susceptibility to the development of breast cancer (Wang-Gohrke et al 2000).

The SNPs in ESR1 that are positively associated with PMDD are located within a 16 kb region of intron 4. While this corresponds to a non-coding region of the gene, the findings nonetheless may be of physiological significance: 1) the SNPs may be in linkage disequilibrium with a nearby “causative” polymorphism; 2) intronic SNPs may be functional, involving regulatory sequences (e.g., enhancers) that can regulate expression levels (Cai et al 2003); 3) intronic SNPs have been shown to alter mRNA folding and hence a range of mRNA processing events, including splicing (Shen et al 1999); 4) intronic SNPs in the GR are associated with altered glucocorticoid sensitivity (Stevens et al 2004; Wust et al 2004; Stevens et al 2004).

Results of the single-locus analyses were strengthened in the haplotype analyses. In the haplotypes generated from the moving window analyses, the 2-allele containing haplotype was strongly over-represented in the controls (e.g., case-control ratio of .39, empirical $p = 0.0000$ for L0057/L0026). All haplotypes containing intron 4 loci L0025, L0060, L0061, or L0026 significantly distinguished patients from controls, in marked contrast to intron 4 haplotypes containing none of the four significant SNPs. Given that the SNPs tested spanned from intron 1 to the 3′ UTR of ESR1, these data suggest that the 5′ region of intron 4 is particularly and perhaps selectively associated with the susceptibility to experience PMDD.

As mentioned above, psychiatric disorders are not inherited in simple Mendelian pattern and instead probably represent low penetrance effects of common variations of multiple interacting genes that confer increased susceptibility (Weinberger et al 2006). As such, we were interested in examining the significantly associated variants in ESR1 (multi-SNP association) against the background of gene polymorphisms that do not appear associated with the diagnosis of PMDD but may nevertheless be involved. The Val158Met polymorphism in the COMT gene was selected for the following reasons: 1) COMT is involved in estrogen metabolism, performing O-methylation of 2- and 4-hydroxy estrogen metabolites (catecholestrogens); 2) the COMT gene contains estrogen response elements (Xie et al 1999), consistent with its regulation (decrease) by estradiol in vitro (Jiang et al 2003), an effect that is presumed to be mediated by ER alpha (Jiang et al 2003); 3) COMT is implicated in sex steroid associated cancers (Tanaka et al 2006; Sazci et al 2004); 4) COMT is responsible for regulating dopamine levels in the PFC, which are critical for modulation of cognitive function and “tuning” of the PFC (the ratio of task-related to task-unrelated neuronal firing), and the PFC is a brain region in which estradiol has been shown to regulate cerebral blood flow and function in humans (Berman et al 1997; Keenan et al 2001); 5) the COMT Val/Met polymorphism has been linked to the predisposition to several psychiatric disorders, including schizophrenia and OCD (Tunbridge et al in press; Karayiorgou et al 1997) and has been shown to moderate the effects of environmental factors in determining disease expression (Caspi et al 2005). The manifold

interactions of COMT and estradiol and their convergence in areas of the brain critical in regulating mood and hypothesized as dysfunctional in PMDD (Rubinow et al in press) provided compelling justification for investigation of possible epistasis (gene-gene interactions). We found that the intron 4 SNPs in ESR1 were significantly associated with PMDD only in those individuals with a Val/Val genotype; patient groups with Val/Met or Met/Met genotypes were not associated, either individually or when combined.

The Val/Val genotype has been tentatively associated with decreased PFC dopamine and decreased tuning efficiency and signal to noise of prefrontal related circuitry. Estradiol regulates PFC blood flow in humans (Berman et al 1997), and Korol (2002; 2004) has demonstrated that E2 biases toward or against the activation of circuits mediating different forms of cognition: high estrogen favoring place-activated learning and low estrogen levels response-dependent learning. It is tempting to speculate, therefore, that disturbed ER alpha signaling in the luteal phase may interact with decreased PFC efficiency in those with the Val/Val genotype so as to permit the expression of a dysphoric state suggestive of disinhibited subcortical (e.g., amygdala) activity. Indirect support for this speculation is derived from unpublished data (Dancer et al., unpublished) showing luteal phase-related compromise of PFC function in women with PMDD but not in control women.

A strength of our study is the prospective demonstration of the presence or absence of luteal phase-specific symptoms in patients and controls, respectively. Nonetheless, several caveats should be noted. First, ascertainment of large samples of such carefully screened and evaluated individuals is arduous, and, consequently, our sample sizes are not large. Further, the strength of the single-locus associations is such that they would not survive correction for multiple testing. Replication, therefore, is clearly required, and our results must be viewed as preliminary until such replication occurs. Second, given the relative uniqueness of our controls in having neither a past psychiatric history nor any menstrual cycle-related symptoms, our findings may reflect the existence of protective alleles in the controls rather than susceptibility alleles in the patients. Indeed, our controls may be “supernormal” and unrepresentative of the overall population without PMDD. Third, the SNPs that we selected did not comprehensively interrogate the genes studied, perhaps resulting in our failure to find associations with SNPs in ESR2. One can certainly not conclude the lack of involvement of this gene on the basis of our data, and replication studies are underway employing ht-SNPs that better assay the haplotypic complexity in each gene. Fourth, as befits a complex genetic disorder, the contribution of the ESR polymorphisms to the expression of PMDD is not large. These caveats notwithstanding, identification of a significant association between PMDD and polymorphic variants of the gene for a physiologically relevant steroid receptor is a significant step forward in the effort to discover the genetic underpinnings of PMDD.

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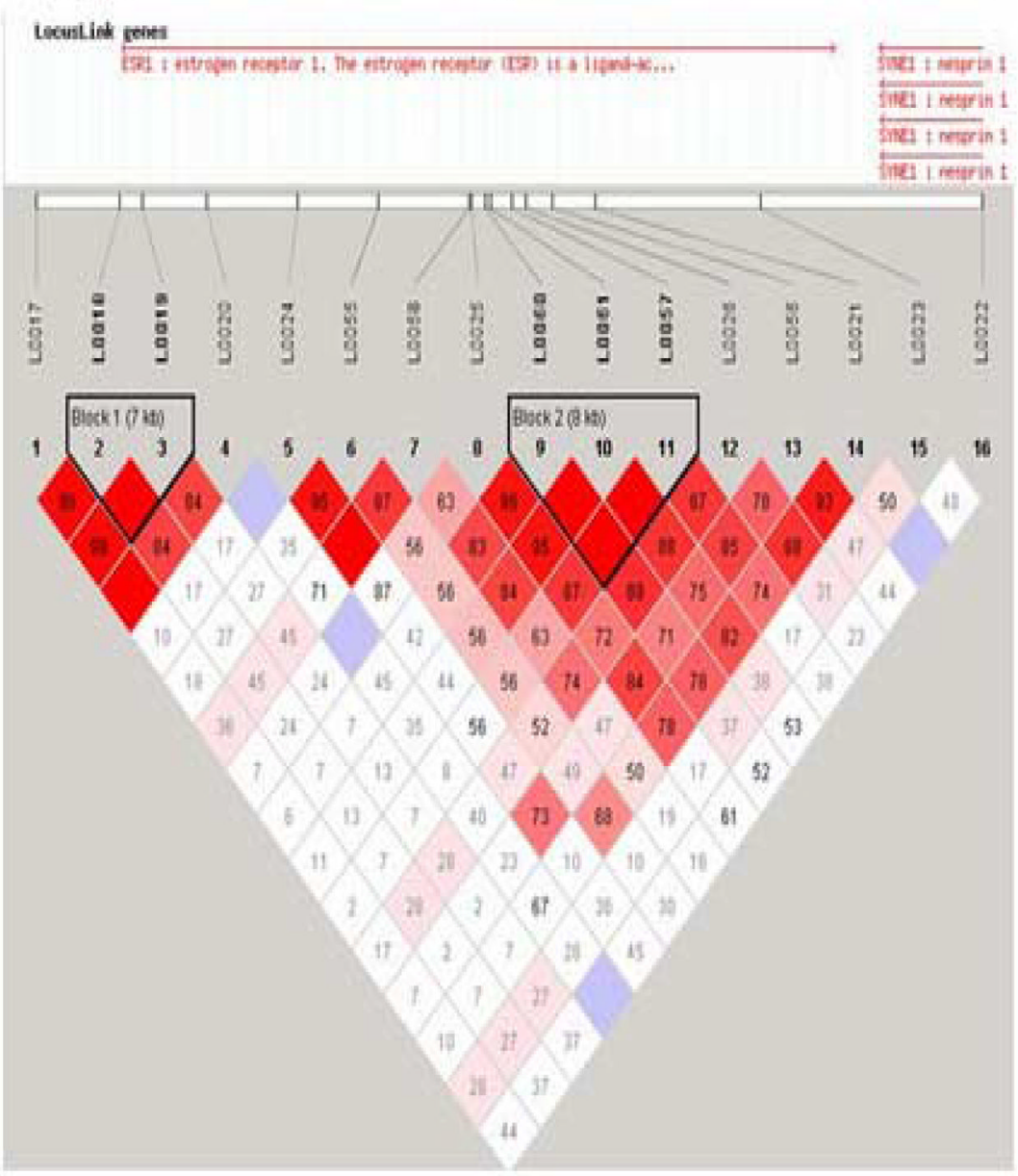


Figure 1.
Marker to Marker Linkage Disquillibrium - HAPLOVIEW D′

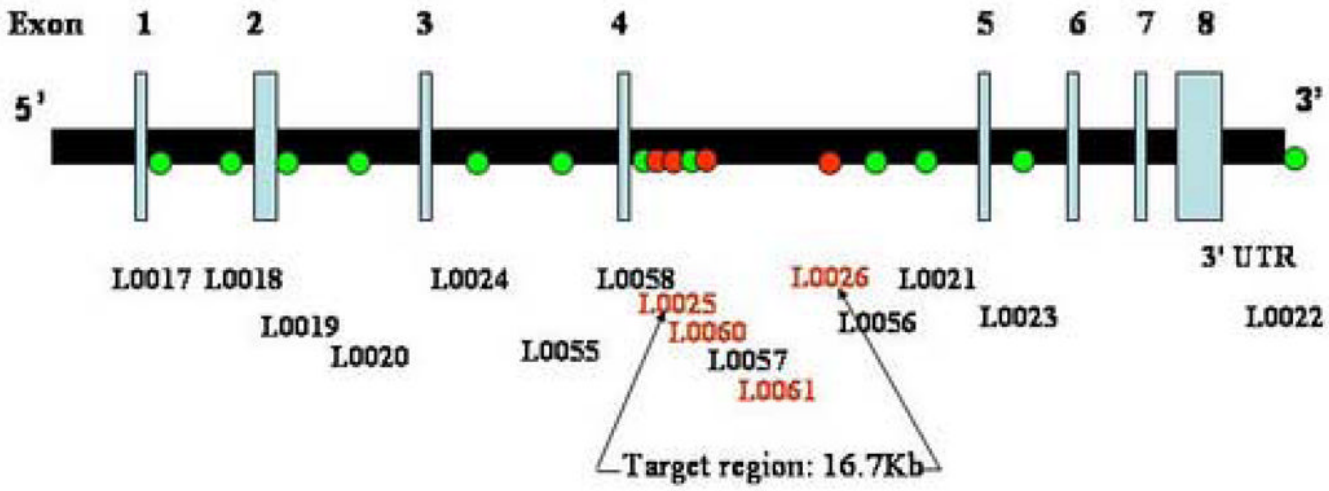


Figure 2.
SNPs genotyped in ESR1 (6q25.1)

Table 1

ESR1 marker and map information

SNP	NCBI dbSNP rs#	Coding Strand Alleles[1]	MAF[2]	Location	Chromosomal Position[3]	Intermarker Distance[4]	Distance from M01[4]
L0017	rs6920483	G/A	0.487	intron 1	152223435	0	0
L0018	rs827421	T/C	0.447	intron 1	152249236	25801	25801
L0019	rs7774230	C/T	0.447	intron 2	152256353	7117	32918
L0020	rs11155818	A/G	0.041	intron 2	152276244	19891	52809
L0024	rs7761846	T/C	0.117	intron 3	152304622	28378	81187
L0055	rs4870062	T/G	0.378	intron 3	152329732	25110	106297
L0058	rs1801132	C/G	0.229	intron 4	152357636	27904	134201
L0025	rs3003917	A/G	0.220	intron 4	152358582	946	135147
L0060	rs3020314	C/T	0.423	intron 4	152362786	4204	139351
L0061	rs3020377	G/A	0.416	intron 4	152364512	1726	141077
L0057	rs3020317	T/C	0.209	intron 4	152370855	6343	147420
L0026	rs1884051	A/G	0.373	intron 4	152375393	4538	151958
L0056	rs1884054	A/C	0.423	intron 4	152383680	8287	160245
L0021	rs932477	G/A	0.095	intron 4	152396710	13030	173275
L0023	rs926779	G/A	0.366	intron 5	152448034	51324	224599
L0022	rs2813543	G/A	0.156	3′ UTR	152516592	68558	293157

[1] major/minor

[2] minor allele frequency

[3] from the UCSC May 2004 freeze

[4] in base pairs

Table 2

ESR2 marker and map information

SNP	NCBI dbSNP rs#	Coding Strand Alleles[1]	MAF[2]	Location	Chromosomal Position[3]	Intermarker Distance[4]	Distance from M01[4]
L0032	rs1952586	A/G	0.139	5′ UTR	63829170	0	0
L0034	rs7159462	C/T	0.043	5′ UTR	63828629	541	541
L0031	rs6573553	G/T	0.441	5′ UTR	63824114	4515	5056
L0030	rs1256030	C/T	0.361	intron 1	63816915	7199	11714
L0033	rs1256048	G/T	0.281	intron 3	63798033	18882	26081
L0029	rs6573549	C/T	0.337	intron 5	63791402	6631	25513
L0028	rs8017441	A/G	0.21	intron 6	63785547	5855	12486
L0027	rs1256061	A/C	0.421	intron 6	63773346	12201	18056

[1] major/minor

[2] minor allele frequency

[3] from the UCSC May 2004 freeze

[4] in base pairs

Table 3

Table 3A. ESR1 - Single SNP analysis of association with PMDD

	PMDD (n=91)	Controls (n=56)	P values	
SNP	**MAF**	**MAF**	**Alleles**	**Genotypes**
L0017	0.443	0.473	0.626	0.8659
L0018	0.411	0.429	0.769	0.7016
L0019	0.411	0.429	0.769	0.7016
L0020	0.017	0.036	0.316	1.000
L0024	0.094	0.063	0.326	0.5672
L0055	0.317	0.352	0.552	0.7712
L0058	0.200	0.295	0.070	0.1814
L0025	0.125	0.246	0.009	0.0169
L0060	0.279	0.434	0.008	0.0223
L0061	0.276	0.425	0.011	0.0357
L0057	0.136	0.226	0.057	0.1212
L0026	0.287	0.420	0.020	0.0437
L0056	0.324	0.380	0.339	0.5855
L0021	0.062	0.130	0.053	0.1203
L0023	0.285	0.348	0.261	0.4103
L0022	0.182	0.173	0.845	0.5149

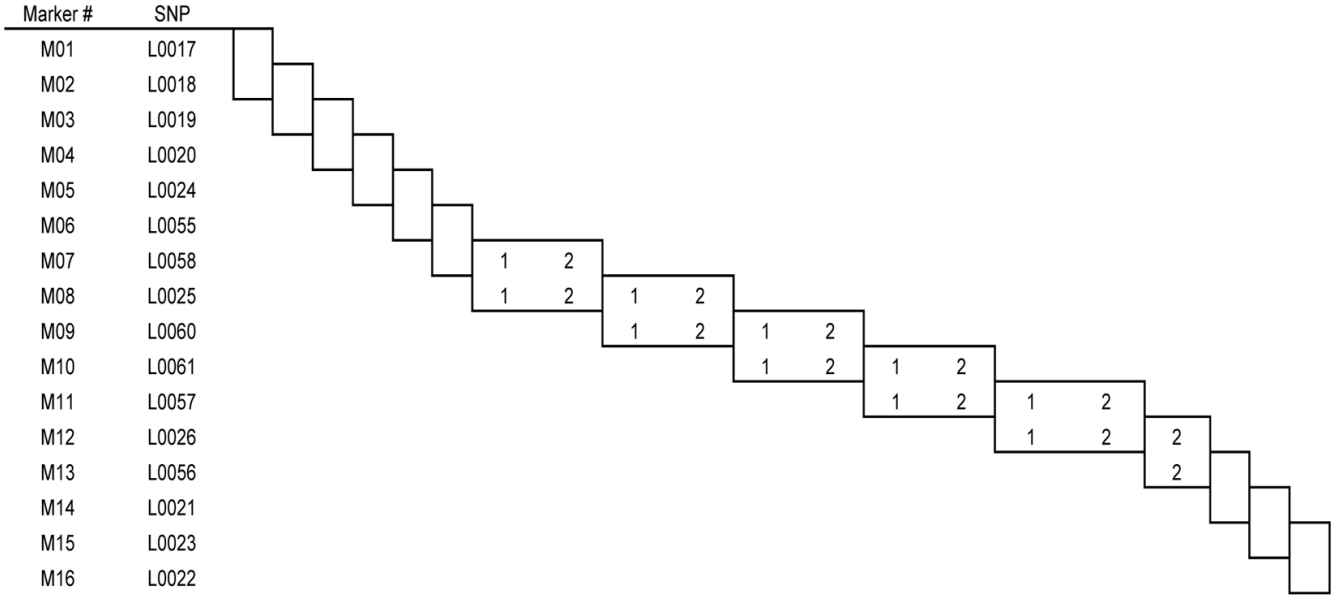
Table 3B. SNPs in ESR2 are not associated with PMDD: pairwise anaylsis

	PMS (n=91)	Controls (n=56)	*P* values	
SNP	**Minor allele frequency**	**Minor allele frequency**	**Alleles**	**Genotypes**
L0027	0.448	0.519	0.252	0.4627
L0028	0.138	0.098	0.338	0.442
L0030	0.461	0.391	0.241	0.493
L0031	0.476	0.422	0.382	0.245
L0032	0.135	0.147	0.779	0.224
L0034	0.074	0.127	0.138	0.283

note: *p values* were caculated using the χ^2 test.

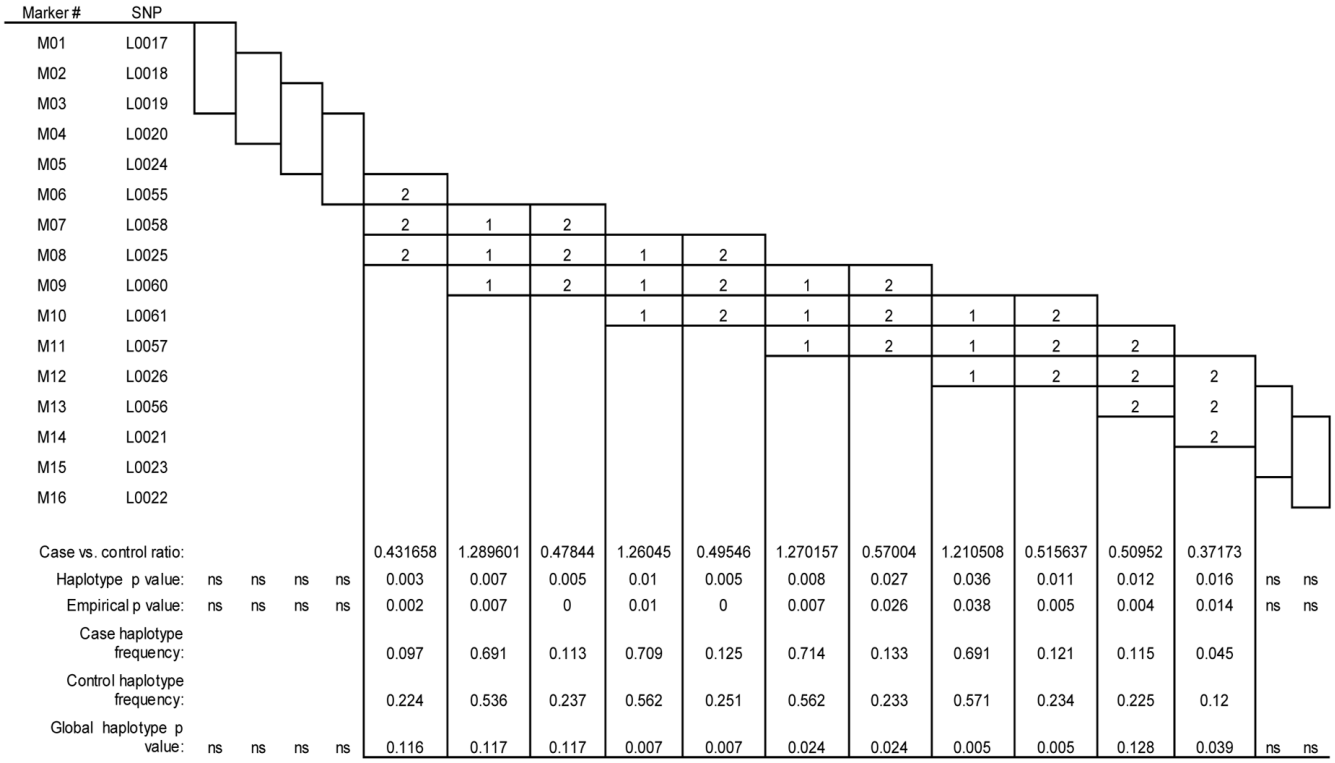
Table 4

Table 4A ESR1 two-marker haplotype results



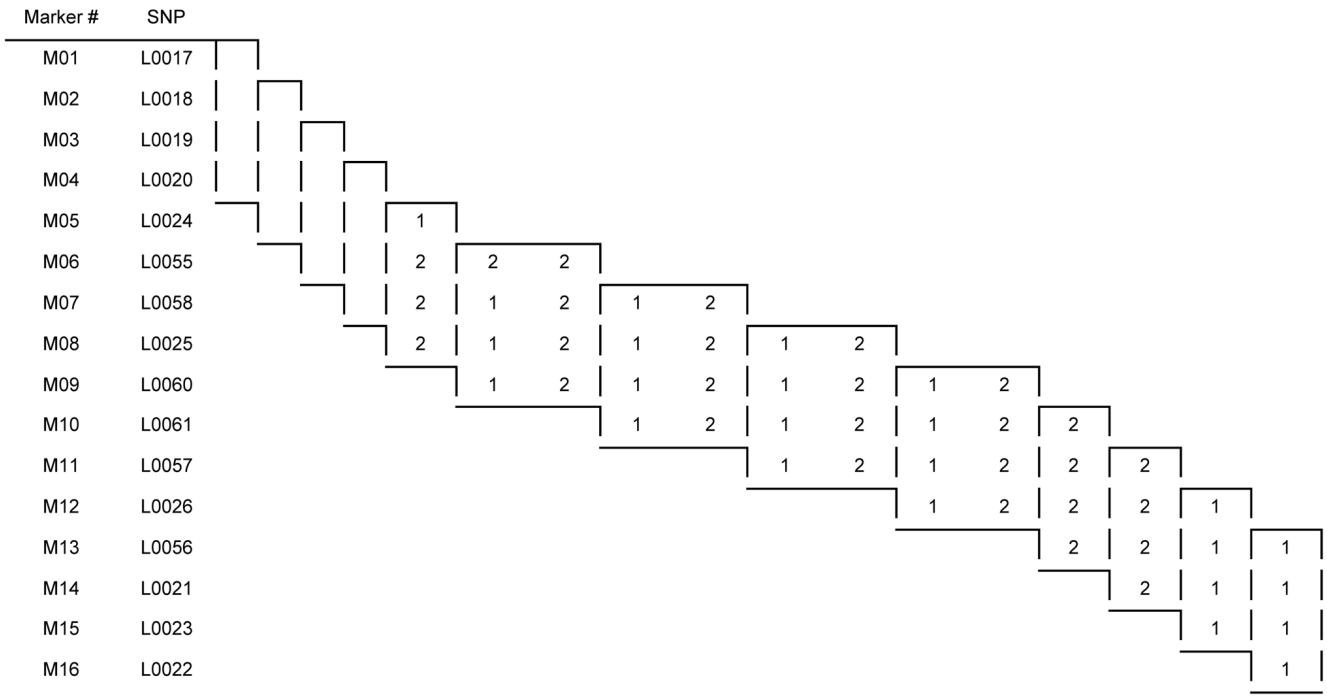
Marker #	SNP																			
M01	L0017																			
M02	L0018																			
M03	L0019																			
M04	L0020																			
M05	L0024																			
M06	L0055																			
M07	L0058							1	2											
M08	L0025							1	2	1	2									
M09	L0060									1	2	1	2							
M10	L0061											1	2	1	2					
M11	L0057													1	2	1	2			
M12	L0026															1	2	2		
M13	L0056																	2		
M14	L0021																			
M15	L0023																			
M16	L0022																			
Case vs. control ratio:		ns	ns	ns	ns	ns	ns	1.16	0.50	1.27	0.49	1.27	0.63	1.24	0.58	1.20	0.53	0.69		
Haplotype p value:		ns	ns	ns	ns	ns	ns	0.035	0.008	0.011	0.006	0.009	0.004	0.014	0.031	0.0384	0.0146	0.036	ns	ns	ns
Empirical p value:								0.031	0.009	0.010	0.01	0.011	0.003	0.014	0.036	0.033	0.019	0.026		
Case haplotype frequency:								0.796	0.119	0.719	0.120	0.713	0.275	0.722	0.134	0.6987	0.1249	0.255		
Control haplotype frequency:		ns	ns	ns	ns	ns	ns	0.686	0.236	0.566	0.245	0.562	0.437	0.582	0.233	0.580	0.2342	0.371		
Global haplotype p value:								0.078	0.078	0.023	0.023	0.007	0.007	0.043	0.043	0.060	0.060	0.044	ns	ns	ns

Table 4B ESR1 three-marker haplotype results



Marker #	SNP															
M01	L0017															
M02	L0018															
M03	L0019															
M04	L0020															
M05	L0024															
M06	L0055				2											
M07	L0058				2	1	2									
M08	L0025				2	1	2	1	2							
M09	L0060					1	2	1	2	1	2					
M10	L0061							1	2	1	2	1	2			
M11	L0057									1	2	1	2	2		
M12	L0026											1	2	2	2	
M13	L0056													2	2	
M14	L0021														2	
M15	L0023															
M16	L0022															
Case vs. control ratio:					0.431658	1.289601	0.47844	1.26045	0.49546	1.270157	0.57004	1.210508	0.515637	0.50952	0.37173	
Haplotype p value:		ns	ns	ns	ns	0.003	0.007	0.005	0.01	0.005	0.008	0.027	0.036	0.011	0.012	0.016	ns	ns
Empirical p value:		ns	ns	ns	ns	0.002	0.007	0	0.01	0	0.007	0.026	0.038	0.005	0.004	0.014	ns	ns
Case haplotype frequency:						0.097	0.691	0.113	0.709	0.125	0.714	0.133	0.691	0.121	0.115	0.045		
Control haplotype frequency:						0.224	0.536	0.237	0.562	0.251	0.562	0.233	0.571	0.234	0.225	0.12		
Global haplotype p value:		ns	ns	ns	ns	0.116	0.117	0.117	0.007	0.007	0.024	0.024	0.005	0.005	0.128	0.039	ns	ns

Table 4C ESR1 four-marker haplotype results



Marker #	SNP																
M01	L0017																
M02	L0018																
M03	L0019																
M04	L0020																
M05	L0024				1												
M06	L0055				2	2	2										
M07	L0058				2	1	2	1	2								
M08	L0025				2	1	2	1	2	1	2						
M09	L0060					1	2	1	2	1	2	1	2				
M10	L0061							1	2	1	2	1	2	2			
M11	L0057									1	2	1	2	2	2		
M12	L0026											1	2	2	2	1	
M13	L0056													2	2	1	1
M14	L0021														2	1	1
M15	L0023															1	1
M16	L0022																1
Case vs. control ratio:					0.41	8.00	0.44	1.27	0.48	1.26	0.56	1.21	0.52	0.51	0.22	1.31	1.39
Haplotype p value:		ns	ns	ns	ns	0.002	0.003	0.003	0.011	0.005	0.010	0.028	0.042	0.011	0.011	0.002	0.030	0.028
Empirical p value:		ns	ns	ns	ns	0.001	0.010	0.002	0.009	0.000	0.008	0.009	0.034	0.007	0.003	0.001	0.057	0.074
Case haplotype frequency:						0.093	0.104	0.099	0.689	0.114	0.710	0.125	0.680	0.121	0.114	0.026	0.545	0.467
Control haplotype frequency:						0.225	0.013	0.227	0.542	0.238	0.562	0.222	0.562	0.234	0.225	0.117	0.415	0.337
Global haplotype p value:		ns	ns	ns	ns	0.122	0.052	0.052	0.105	0.105	0.078	0.078	0.005	0.005	0.014	0.056	0.031	0.637

Table 5

Significant ESR1 associations with PMDD are preserved in those with a COMT Val/Val genotype

Marker	*p* value in full sample (n=147)	*p* value in subjects with COMT Val/Val (n=38)	*p* value in subjects with COMT Val/Met (n=61)	*p* value in subjects with COMT Met/Met (n=43)	*P* value by Cochran - Mantel-Haenszel statistic (Val/Val vs grouped Val/Met and MetMet)
L0017	0.626	0.135	0.489	0.631	0.593
L0018	0.769	0.392	0.812	0.992	0.817
L0019	0.769	0.392	0.812	0.992	0.817
L0020	0.316	0.242	0.345	0.271	0.325
L0024	0.326	0.618	0.730	0.147	0.456
L0055	0.552	0.234	0.461	0.715	0.703
L0058	0.070	0.288	0.576	0.129	0.073
	0.009	0.026	0.397	0.092	0.010
L0060	0.008	0.019	0.309	0.202	0.011
L0061	0.011	0.022	0.310	0.137	0.009
L0057	0.057	0.003	0.873	0.369	0.033
L0026	0.020	0.059	0.512	0.199	0.033
L0056	0.339	0.101	0.777	0.946	0.288
L0021	0.053	0.072	0.161	0.250	0.017
L0023	0.261	0.166	0.528	0.204	0.318
L0022	0.845	0.4162	0.5947	0.5915	0.731

p values for the first four columns were calculated with the χ^2 test.