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Brief Research Communication

Association Study of the Estrogen Receptor Alpha Gene (*ESR1*) and Childhood-Onset Mood Disorders

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Depressive disorders are heterogeneous psychiatric disorders involving deficits in cognitive, psychomotor, and emotional processing. Depressive disorders have a significant genetic component, with severe, recurrent and early-onset forms demonstrating elevated heritability. In this study we genotyped eleven single nucleotide polymorphisms (SNPs) spanning the estrogen receptor alpha gene (*ESR1*) in a large family-based childhood-onset mood disorder (COMD) sample. None of the individual SNP or global haplotype analyses was significant in the entire COMD sample, but haplotype analysis of three SNPs in strong linkage disequilibrium (rs746432, rs2077647, and rs532010) uncovered an association with COMD, specifically in females. Our data are consistent with previous studies demonstrating a female-specific association between *ESR1* and neurobehavioral phenotypes. These results suggest the existence of sex-specific etiological factors in depressive disorders, related to estrogen, with onset in childhood.

KEY WORDS: estrogen receptor alpha gene (ESR1); association study; sex-effect; depression; childhood-onset mood disorders (COMD)

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Depressive disorders are highly heterogeneous psychiatric disorders involving deficits to cognitive, psychomotor and emotional processing. They are extremely common, ranking second in the global burden of disease in developed countries [Murray and Lopez, 1997]. Depressive disorders strongly aggregate in families [Sullivan et al., 2000], and twin studies demonstrate that this familial clustering contains a significant genetic component, with severe, recurrent and early-onset forms of the disorder demonstrating elevated heritability [Thapar and McGuffin, 1994; Rice et al., 2002; Thapar and Rice, 2006].

Estrogen is known to interact with the central nervous system, and has been shown to influence anxiety and depressive behaviors [Walf and Frye, 2006]. The estrogen receptor alpha gene (*ESR1*), highly expressed in the amygdala and hypothalamus, encodes a ligand-activated transcription factor that mediates the action of estrogen [Ostlund et al., 2003]. Of the two estrogen receptors, the alpha receptor is the predominant form expressed in the amygdala, indicating a role in affective, emotional, and motivational behavior [Osterlund and Hurd, 2001]. Estrogen also regulates serotonergic and norepinephrinergic neurotransmission [Ostlund et al., 2003], both integral to affective processing in the brain. Only a few studies have previously investigated *ESR1* in depression [Tsai et al., 2003; Tiemeier et al., 2005; Kravitz et al., 2006]. Tsai et al. [2003] observed strong evidence for a female-specific association between a Pvu*II* restriction enzyme site (corresponding to rs2234693) and major depressive disorder (MDD), although neither Tiemeier et al. [2005] nor Kravitz et al. [2006] found an association between *ESR1* and depression. It is worth noting, however, that *ESR1* polymorphisms have been associated with several etiologically-related phenotypes including anxiety disorder [Tiemeier et al., 2005], personality traits [Westberg et al., 2003], cognitive functioning [Kravitz et al., 2006], and premenstrual dysphoric disorder [Huo et al., 2007]. A striking observation is that, as in the study of Tsai et al. [2003] of MDD, these associations were predominantly found in female samples; a conclusion also apparent for the other, primarily physiological, phenotypes studied in relation to *ESR1*. Taken together, these data strongly suggest that *ESR1* has a female-specific association with pathophysiology.

Whilst results from previous studies investigating *ESR1* in depression have been mixed, Tsai et al.'s [2003] strong findings of a female-specific association with MDD suggest that further investigation of this gene is warranted. The aim of this study was to investigate whether *ESR1* single-nucleotide polymorphisms (SNPs) are associated with childhood-onset mood disorders (COMD), and if any sex-specific effects are present.

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Unlike prior studies, our study utilized a larger number of polymorphisms spanning the entire *ESR1* gene. Furthermore, unlike previous studies, our goal was to focus on childhood-onset depression; by testing a phenotype with higher heritability we hoped to increase our power of detecting an association.

Our sample is part of a multidisciplinary program project researching risk factors in COMD and comprised of 460 affected children (246 male and 214 female) recruited from 23 mental health facilities across Hungary. A full description of sample recruitment and assessment can be found in Kiss et al. [2007]. Briefly, the probands and affected siblings met DSM-IV criteria for either depressive or bipolar disorders with onset prior to 14.9 years. The Interview Schedule for Children and Adolescents Diagnostic Version (ISCA-D), which is an extension and modification of the ISCA [Sherrill and Kovacs, 2000], was the instrument used for diagnosis. The child and the parent informants were interviewed individually on two separate occasions approximately 1 month apart by two different trained clinicians. A best-estimate consensus diagnosis taken from both clinicians was used as the final diagnosis. At the time of diagnosis, 0.8% of the children met the criteria for bipolar disorder; we decided not to exclude these cases from the study because quantitative genetic studies indicate bipolar and depressive disorders share substantial genetic overlap [McGuffin et al., 2003]. Further, based on previous longitudinal studies, we predict that ~15–30% of the children that are currently diagnosed with a depressive disorder in childhood will develop bipolar disorder as they enter young adulthood [Strober and Carlson, 1982; Kovacs et al., 1994; Kovacs, 1996, 1997; Geller et al., 2001]. Because it is not possible to predict which of these children will develop bipolar disorder they cannot be excluded from our sample. Written informed consent for adults and assent for children was obtained from all

TABLE I. Single Marker and Haplotype Results for TDT Analysis on *ESR1* SNPs

	Location (UCSC co-ordinate[a])	Allele	Freq	T:NT full sample	T:NT males	T:NT females
rs9478244	5′ promoter (chr6:152,163,730)	A	0.78	127:117	74:68	53:49
		G	0.22	117:127	68:74	49:53
rs488133	5′ promoter (chr6:152,167,137)	C	0.68	148:139	83:84	65:55
		T	0.32	139:148	84:83	55:65
rs2071454	5′ promoter (chr6:152,168,517)	G	0.13	76:85	47:50	29:35
		T	0.87	85:76	50:47	35:29
rs2077647	Exon 1 (chr6:152,170,770)	G	0.53	176:175	106:95	70:80
		A	0.47	175:176	95:106	80:70
rs746432	Exon 1 (chr6:152,171,001)	C	0.90	56:42	30:28	26:14
		G	0.10	42:56	28:30	14:26
rs532010	Intron 1 (chr6:152,172,611)	C	0.37	166:156	85:88	81:68
		T	0.63	156:166	88:85	68:81
rs17081698	Intron 1 (chr6:152,173,792)	G	0.11	61:70	39:41	22:29
		T	0.89	70:61	41:39	29:22
rs10484922	Intron 1 (chr6:152,174,010)	C	0.90	82:68	45:42	37:26
		T	0.10	68:82	42:45	26:37
rs2234693	Intron 1 (chr6:152,205,028)	C	0.45	142:146	77:82	65:64
		T	0.55	146:142	82:77	64:65
rs1801132	Exon 4 (chr6:152,307,215)	C	0.79	107:110	58:56	49:54
		G	0.21	110:107	56:58	54:49
rs2228480	Exon 8 (chr6:152,461,788)	A	0.16	82:100	41:57	41:43
		G	0.84	100:82	57:41	43:41
rs2077647-rs746432-rs532010	—	G-C-T	0.53	108:102	60:53	48:49
		A-C-C	0.37	107:88	51:50	56:38
		A-G-T	0.10	31:47	20:25	**11:22**
		Global		$\chi^2 = 5.31$, 2 df, $P = 0.07$	$\chi^2 = 1.03$, 2 df, $P = 0.60$	**$\chi^2 = 6.02$, 2 df, $P = 0.05$**
rs746432-rs532010	—	C-C	0.37	114:101	57:59	57:42
		C-T	0.53	125:121	73:69	52:52
		G-T	0.10	38:54	28:29	**10:25**
		Global		$\chi^2 = 3.04$, 2 df, $P = 0.22$	$\chi^2 = 0.12$, 2 df, $P = 0.94$	**$\chi^2 = 6.12$, 2 df, $P = 0.05$**
rs2077647-rs532010	—	A-T	0.53	124:119	73:63	51:56
		G-C	0.37	120:101	58:58	62:43
		G-T	0.10	**37:60**	23:32	**14:28**
		Global		$\chi^2 = 5.36$, 2 df, $P = 0.07$	$\chi^2 = 1.49$, 2 df, $P = 0.47$	$\chi^2 = 5.59$, 2 df, $P = 0.06$

Bold denotes two-tailed $P < 0.05$. T, transmitted; NT, not-transmitted.
[a]March 2006 assembly.

participants as required by the Institutional Review Boards of the University of Pittsburgh, The University of Toronto, and in Hungary.

Eleven *ESR1* SNPs were genotyped in affected children and their parents using the TaqMan System (Applied Biosystems, Foster City, CA). All data were screened for Mendelian errors using PEDSTATS, and MERLIN to detect for crossovers between markers [Abecasis et al., 2002]. All genotypes were in Hardy–Weinberg equilibrium (HWE) in both males and females; this contrasts to a previous study that reported a departure from HWE in females, suggesting the possibility of sex-specific selection at this locus [Prichard et al., 2002]. Single-marker and multi-marker haplotype association analyses were performed using UNPHASED [Dudbridge, 2003], and employed the transmission disequilibrium test (TDT) [Spielman et al., 1993]. For haplotype analysis haplotypes with a frequency less than 5% were combined for the analyses. Linkage disequilibrium (LD) between the markers was calculated using Haploview v 3.2 [Barrett et al., 2005].

Our study of *ESR1* is, to our knowledge, the most thorough yet performed for a depression-related phenotype, and the first using a family-based sample that avoids the population stratification problems that can affect case-control samples. Table I contains single marker and haplotype transmission disequilibrium test (TDT) results for the SNPs genotyped. None of the individual SNP or global haplotype analyses was significant in the entire COMD sample (males and females combined). Given previous reports of female-specific association with this gene, TDT analyses were repeated stratified by sex (males and females separately). In single-marker analyses, a strong trend was found for the C allele of a synonymous SNP in exon 1 (rs746432) to be over-transmitted to affected females (26T vs. 14T; TDT = 3.6, $P = 0.055$, OR = 1.86 (95% CI = 0.93–3.76)). More strikingly, multi-marker analysis of rs746432 and two adjacent SNPs, rs2077647 (another synonymous SNP in exon 1) and rs532010 (located in intron 1), all in strong linkage disequilibrium ($D' > 0.95$ for all two-marker combinations), indicates that haplotypes across this region were associated with COMD, specifically in females (global haplotype TDT analysis for the three SNPs: $\chi^2 = 6.02$, 2 df, $P = 0.05$). In particular, two- and three-marker haplotype analysis across these three SNPs consistently identified a haplotype (A-G-T), present at ~10% frequency, that is significantly under-transmitted to affected females ($P = 0.01$–0.05, depending on SNPs included in analysis). The strongest protective effect is seen for the rs746432-rs532010 G-T haplotype (10T vs. 25NT; TDT = 6.43, $P = 0.01$, OR = 0.40 (95% CI = 0.17–0.86)). Interestingly, SNPs in this region have been previously associated with depression and anxiety [Tsai et al., 2003; Tiemeier et al., 2005]. Previous studies have predominantly focused on a *Pvu*II restriction enzyme site; whilst this SNP was included in our study (rs2234693) and was in strong LD with markers in the significant haplotype ($D' > 0.95$), we did not find it to be individually associated with childhood-onset mood disorders. We found no associations in the male samples; our data are thus consistent with previous studies demonstrating a female-specific association between *ESR1* and neurobehavioural phenotypes.

The prevalence of childhood depressive disorders is approximately the same in both sexes. After puberty, however, there is a clear discordance in prevalence, with women having approximately twice the lifetime risk than men [Kuehner, 2003]. This may in part be due to hormonal changes, particularly increased estrogen levels, occurring in females during adolescence. Our results are intriguing because they suggest the existence of sex-specific etiological factors in depressive disorders, related to estrogen, with onset in childhood/early adolescence. It is known that estrogen plays an important role in brain development during prenatal and neonatal growth, providing a possible explanation for sex-differences in behavior in children and early adolescents [Collaer and Hines, 1995]. Furthermore, there is a rise in estrogen levels prior to the actual physical signs of puberty [Blogowska et al., 2003] which, given the average age of onset of females enrolled in this study (11.03 ± 2.30 years), and the average age for the onset of thelarche (10.8–11.2) and menarche (12.5–13.5 years) in Europe [Parent et al., 2003], could also explain the female-specific association of *ESR1*. Interestingly, a recent study from our group reported female-specific association between a HPA-axis gene postulated to interact with estrogen, the vasopressin V1B receptor gene (*AVPR1B*), and COMD [Dempster et al., 2007]. Both sets of data suggest that estrogen-related genetic variation may be associated with COMD specifically in females but not males, and that gender-specific factors are in operation prior to the major sexual dichotomy in depression prevalence observed after puberty. Whilst our data are preliminary, and should be thus treated with caution, they suggest that future studies should further investigate the sex-specific role of *ESR1*, and other estrogen-associated genes, in mediating susceptibility to depressive disorders.

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