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A pilot genome wide association and gene expression array study of suicide with and without major depression

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Abstract

Objectives—Suicide is partly heritable but the responsible genes have not been identified. We conducted a gene-centric, low coverage single nucleotide polymorphism (SNP) pilot genome-wide association study (GWAS) seeking new candidate regions in suicides with and without depression, combined with gene expression assay of brain tissue.

Methods—Ninety-nine Caucasian subjects, including 68 who completed suicide and 31 who died suddenly from other causes, were genotyped postmortem using GeneChip® Mapping 50K Xba. Clinical data were obtained from relatives. SNPs with Hardy – Weinberg equilibrium *P* values below 0.001 were excluded from analysis. Illumina chip expression arrays assayed the transcriptome in prefrontal cortex in a drug-free subgroup.

Results—GWAS analysis (cutoff *P* < 0.001) yielded 58 SNPs, 22 of them in or near 19 known genes, with risk allele-associated odds ratios between 2.7 and 6.9. Diagnosis of mood disorder did not explain the associations. Some of the SNPs matched into four functional groups in gene ontology. Gene expression in the prefrontal and the anterior cingulate cortex for these 19 genes was measured on a separate, though overlapping, sample of suicides and seven of 19 genes showed altered expression in suicides as compared with controls, especially in immune system related genes.

Conclusions—Matching GWAS findings with expression data assesses functional effect of new candidate genes in suicide, and is an alternative form of confirmation or replication study. Results highlight a role for neuroimmunological effects in suicidal behaviour.

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Statement of interest

The authors declare no competing interests other than unrelated grants to JJM from Novartis and GSK.

Keywords

Suicide; genetics; mood disorders; single nucleotide polymorphism; psychological autopsy

Introduction

Genetic factors contribute to the risk of suicidal behaviour (Roy et al. 1991; Brent et al. 1996; Mann 2003). Concordance rate for suicide is higher in monozygotic (13.2%) compared with dizygotic twins (0.7%) (Roy et al. 1991). Adoption studies reveal higher suicide rates in biological parents of adoptees who died by suicide (Roy 1983). However, the specific genes that contribute to vulnerability for suicidal behaviour are unknown despite numerous association studies of candidate genes (Mann 2003). Most studies have examined a limited number of polymorphisms in a few candidate genes and report inconsistent results.

Single nucleotide polymorphisms (SNPs) when studied in an unbiased genome-wide fashion may identify associations with novel candidate regions of the genome that might include previously unsuspected pathogenic genes (Matsuzaki et al. 2004). Genome wide association studies (GWAS) using SNP microarrays (“SNPs chips”) permit genetic screening of larger sample sizes (Lipshutz et al. 1999; Bunney et al. 2003; Kennedy et al. 2003). A recent study (Perlis et al. 2010) analyzed data on lifetime suicide attempts from genome-wide association studies of bipolar I and II disorder as well as major depressive disorder. Strongest evidence of association with suicide attempt in bipolar disorder was observed in a region without identified genes (rs1466846), although five other loci also showed suggestive evidence of association.

Defining the phenotype of suicidal behaviour as precisely as possible is essential. Suicidal behaviour ranges from low-lethality, low-intent impulsive acts to high-lethality high-intent suicidal acts. High lethality suicidal acts correlate more consistently with biological abnormalities in the brain (Mann 2003). Completed suicides represent the most severe behavioural phenotype, and therefore may offer a higher probability of detecting a genetic association.

Suicide is associated with a psychiatric disorder in 90% or more of cases in most published series (see Mann et al. 1999 for review). The most commonly associated diagnosis in suicides is a mood disorder (about 60% in most studies), but most patients with these disorders never attempt suicide. Thus suicide is considered to require a predisposition to suicidal acts (diathesis) as well as an acute trigger (stress) that has been conceptualized in a stress-diathesis model of suicidal behaviour (Mann et al. 1999; Mann et al. 2005; Mann and Currier 2006). Both major psychiatric diagnoses such as mood disorders, alcoholism and schizophrenia (McGuffin et al. 2001) and the diathesis for suicide (Mann 2003; Mann and Currier 2006) have strongly heritable components. The challenge is to find the responsible genes and to separate genes associated with the major psychiatric disorders from genes associated with the diathesis. Candidate gene studies have tended to focus on the serotonin system since it has been most closely identified with suicide (see Mann 2002 for review). However, looking beyond the serotonin system is important for both understanding causes of suicide (Rujescu et al. 2007; Rujescu and Giegling 2010) and prevention (Kasper and Hamon 2009). Genome-wide SNP arrays have made possible a gene-centric, low coverage hypothesis-free survey of most genes (Lipshutz et al. 1999; Bunney et al. 2003; Kennedy et al. 2003). Such studies have the potential of finding new unsuspected candidate genes or regions in the genome closer to functionally important candidate genes with a pathogenic role (Matsuzaki et al. 2004). We found only three studies in the literature using GWAS in suicidal subjects (Zubenko et al. 2004; Laje et al. 2009; Perlis et al. 2010) and none in

suicide. None of these studies used an expression array to assess gene function of the genes identified by the GWAS.

Therefore, we conducted a pilot study of sudden death suicides and controls who died from other causes (e.g., accidents or sudden natural death) that we could both genotype and measure gene expression in a relevant brain region. Detailed clinical data, including Axis I and II diagnoses were acquired for all cases and comparison subjects entered into this study. The suicides were further subdivided into those with and without a mood disorder (50%). We compared suicides to non-suicide subjects without a psychiatric disorder to detect suicide-related genes, and then compared suicides with and without a mood disorder separately to the comparison cases to eliminate genes associated with mood disorders. Genotyping over 58,000 SNPs presents an opportunity for a pilot whole-genome association study in this uniquely well-characterized but small set of brain samples (Bunney et al. 2003; Matsuzaki et al. 2004). Gene expression was assayed in dorsal lateral prefrontal cortex (Brodmann area 9) and anterior cingulate (Brodmann area 24) cortex in an overlapping sample of suicides and non-suicides. We then compared our results with genes identified in published expression array studies (Sequeira et al. 2007; Thalmeier et al. 2008).

Materials and methods

Subjects

Ninety-nine Caucasian subjects, including 68 suicides and 31 non-suicide deaths (death by accident, or natural causes) were genotyped post-mortem. Tissue and clinical data were obtained after written informed consent was given by the next-of-kin. For each subject, one or more informants were interviewed to obtain a comprehensive demographic and psychiatric history regarding the deceased. Interviews with next-of-kin or close friends were done by a psychiatrist or clinical psychologist. Axis I and II diagnoses were validly determined using the Structured Clinical Interview for DSM (SCID I or P and II) and all available information from medical records and the autopsy at a consensus conference with a psychiatrist (JJM), as reported previously (Kelly and Mann 1996). Non-suicides subjects had no detected Axis I psychiatric disorders. In the suicide group, 46 (68%) had a diagnosis of major depression disorder or bipolar disorder with depression, 13 (19%) had schizophrenia, 13 (19%) had an alcohol use disorder (alone or in addition to other Axis I diagnoses), and 5 (7%) had no Axis I psychiatric disorder (Table I).

Sample preparation

DNA was extracted from frozen cerebellar tissue or from occipital cortex by a previously published method (Huang et al. 1999). Genomic DNA fractions were suspended in 10 mM TE buffer. The yield and purity of each DNA sample was assessed using UV spectrophotometry.

Genotyping

We used the GeneChip® Mapping 50K Xba Arrays (one of two chips of the 100K pair) on which about 58,900 SNPs are represented including numerous markers including many genes known to be expressed in the brain. Samples were processed according to the GeneChip Mapping 100k Assay Manual (Version 1, Affymetrix). Arrays were scanned and geno-type calls were made by the Affymetrix proprietary software (GeneChip® Genotyping Analysis Software GTYPE). Twenty-eight samples were genotyped twice because of low call rate (< 90%) on the first chip. One sample was excluded from the study due to low call rate on both occasions. The mean call rate for the 71 remaining chips was $97.8 \pm 1.8\%$, the lowest was 90.1%.

Statistical analysis

All statistical analysis (described below) was performed in the statistical language R (<http://www.r-project.org/>). SNPs with less than 90% group call rate and those with minor allele frequency below 10% were excluded from the analysis. Hardy – Weinberg equilibrium was checked in the comparison sample for all SNPs and SNPs with *P* values below .001 were excluded from further analysis leaving 37,344 (63%) of the SNPs. Next, SNPs that were no more than 10 kb from a known gene (*N*= 15,521 SNPs) were flagged as of primary interest, but all analyses were performed on the all SNPs that passed screening.

Computing significance levels

Since the aim of this project was to identify suicide-related differences in genotype, the suicides were initially treated as a single group regardless of Axis I diagnosis. Thus, the 99 cases and control subjects were divided into two groups of 31 non-suicide control subjects and 68 suicides (i.e. the second and third groups in Table I were pooled). For each SNP, association between genotype and suicide status was tested using the Cochran – Armitage test for trends on genotype frequencies. Then significance levels for comparisons of the 31 comparison cases with (1) suicides with mood disorders and (2) suicides without a mood disorder were also calculated to screen the SNPs that may be primarily related to mood disorders from the list of suicide-related SNPs. For the significant SNPs, allelic odds ratios and the corresponding 95% confidence intervals were calculated as a marker of the magnitude of the association with suicide.

Determining significance with multiple testing

After pre-screening, about 16,000 SNPs were flagged as of primary interest, and another 21,000 as of secondary interest. The Benjamini – Hochberg linear step-up procedure (Hochberg and Benjamini 1990), calculating *Q* values and adjusted significance level to compute appropriate cutoffs for gene expression data is too stringent for identifying genes associated with more complex traits like psychiatric disease and suicide. Moreover, we used the expression data to refine the list of potential candidate genes identified initially from genotyping. Therefore, instead of The Benjamini – Hochberg Procedure, we used a cutoff of 0.001 as the criterion for significance, because we then followed with a comparison of data on the expression array in this pilot study. We judged it more important to avoid exclusion of potentially important SNPs than to be rigorous in the exclusion of false positives.

Gene expression analysis

To investigate whether the differences in genotype between suicides and controls are reflected in RNA expression, we examined gene expression data for the top 19 genes (associated with the 22 significant SNPs identified in the genotype analysis) identified by the GWAS, in Brodmann Areas 9 and 24, brain regions thought to be associated with suicide; and computed fold change values for each of them.

There were 39 subjects in the gene expression study who fulfilled the inclusion/exclusion criteria listed above for the GWAS. About half (19/39) overlapped with the 99 subjects in the GWAS study. Eighteen of the 39 subjects had MDD and died by suicide, 21 had no mood disorder diagnosis and had died suddenly from other causes. Mean age of the suicides was 55.8 ± 18.4 years, and controls was 51.5 ± 14.3 years; 44.4% of suicides and 66.7% of the controls were male. All other demographics remain comparable between the GWAS and expression samples (except for suicides without MDD diagnosis that were excluded).

Tissue samples from the prefrontal cortex, Brodmann Area 9 (BA9) and the dorsal anterior cingulate cortex, Brodmann Area 24 (BA24) were dissected from frozen brain sections that had been transferred from – 80 to – 20 °C for 2 h prior to gray matter sampling. Total RNA

was extracted by the guanidine thiocyanate method using the TRIZOL protocol (Invitrogen, Carlsbad, CA) and cleaned with Rneasy microcolumns (Qiagen GmbH, Germany). The RNA purity and integrity were assessed by optical densitometry, gel electrophoresis and subsequent array parameters. Gene expression in BA9 and 24 was analyzed separately using Affymetrix's GeneChip® Human Genome U133 Plus 2.0 Arrays. This array allows for the measurement of over 47,000 transcripts. Microarray samples were prepared according to the Affymetrix protocol (<http://www.affymetrix.com/support/>). Details of procedure and microarray quality control parameters are described elsewhere (Galfalvy et al., Gene expression in the ACC and PFC of suicide victims, in preparation). Probeset-level signal intensities were extracted with the Robust Multi-array Average (RMA) algorithm (Irizarry et al. 2003), which can be found in the R package *affy* that can be downloaded from the Bioconductor project website (<http://www.bioconductor.org>).

All the probesets from the HGU133Plus2.0 array that were associated with the 19 genes found to have different genotype distribution between suicides and controls were selected and the fold change values recorded for each of the probesets. Fold change is defined as the ratio of the average expression level in the experimental (suicide) group to the average expression level in the control group; equal group means are represented by a fold change of 1. Since each gene is represented by several probesets, we chose to report only the most extreme fold change per gene (i.e. the one farthest from a unit fold change in either direction). Significance levels are not reported since they test the differences in the intensity at the individual probeset level and not at the gene level.

It is of note that not all probesets from a specific gene will be expressed due to affinity differences. Similar studies report probeset-level significance values and focused on few probesets that were preselected and reported separately.

Results

Table I provides descriptive statistics for three groups: sudden death non-psychiatric comparison subjects, suicides with a diagnosis of mood disorder and suicides without a mood disorder.

Suicides without a mood disorder diagnosis were the youngest group and non-suicide subjects the oldest. Mood disorder suicides had the highest proportion of females. Other psychiatric diagnoses such as alcoholism and psychosis were far more common in suicides without a mood disorder than in suicides with mood disorders. Brown – Goodwin Aggression History scores were significantly higher in the suicides without a mood disorder diagnosis compared with the other two groups, and this difference remained significant after adjustment for age and sex ($F = 3.4$, $df = 2,58$, $P = 0.040$ in the adjusted model).

SNP analyses

There were 36,048 SNPs that passed the screening procedure: they had less than 10% missing calls, minor allele frequency of at least 10% and passed the Hardy – Weinberg test ($P > 0.001$). When geno-type frequencies of suicide decedents and sudden death subjects were compared using the Cochran – Armitage test, 58 SNPs had significance levels below 0.001, and one (rs1926123, not associated with any known gene) was significant at the $\alpha = 0.15$ level after Bonferroni correction. The SNPs that showed significant differences were distributed fairly evenly across the chromosomes, the top two were from chromosome 10 (see the Manhattan plot of the P values in Figure 1).

When genotype frequencies were compared separately between depressed suicides and controls and non-depressed suicides and controls, the 58 SNPs identified above had an

average significance level of 0.002 and 0.0181, respectively, indicating that the differences between suicides and controls in the genotype frequencies persist regardless of AXIS I diagnosis. We also analyzed the association between genotype and aggression scores in a linear model adjusted for age and gender. None of the 58 selected SNPs had a significant association with lifetime aggression score. Twenty-two of the 58 significant SNPs were located on or close to a known gene (14,976 screened SNP out of a total of 36,048 were located on or near genes) (Table II). Among these 22 significant SNPs, there were three pairs of SNPs located on the same gene, so the list represents 19 different genes. Odds ratios for the risk alleles on these 22 SNPs were high (2.7 – 6.9), indicating moderate to strong association between genotype and suicide. Table II displays significance levels for two comparisons of genotype distribution: suicides with a mood disorders versus sudden death controls; and suicides without mood disorder versus controls. All but four of the *P* values were significant at the 0.05 level for both comparisons, indicating that these SNPs are associated with suicide independently of the Axis I diagnosis. Table III describes the genes in which the significant SNPs are located, their description and known role based on bioinformatics databases (OMIM).

Gene expression analysis

We have compared the gene expression levels in BA 9 and 24 between suicides and non-suicide controls for the 19 genes found to have SNPs associated with suicide. We identified 74 probesets from these genes, one gene had no probeset on the expression array, and the other eighteen had 1 to 17 probesets per gene. For each of these probesets, suicide to control fold changes were computed, and for each gene the one with maximum deviation (in percent change) from the unit fold change (equal average expression levels per group) was used in Table II. In four cases, the genes were under-expressed in suicides compared to the controls; the under-expression exceeded 20% for at least one brain area in three genes (CYP19A1, MBNL2, KTBBD2) and in both brain areas in one gene (CD44). There were two genes (FOXP3 and DSC2) with over-expression in one area and under-expression (fold change above 1.2) in the other. There was one additional gene, CD300LB with overexpression in one area and no difference in the other. No association was found with diagnosis of depression (data not shown).

RNA Integrity numbers (RIN) were calculated ($n = 19$) and the differences were not significant for both BA9 (Controls Mean 7.4, SD = 0.67 vs. suicides without depression 7.0 SD = 0.9; $F = 1.36$, $df = 1, 17$; $P = 0.25$) and BA24 (Controls Mean 7.3, SD = 0.58 vs. suicides without depression 7.4 SD = 0.76; $F = 0.01$, $df = 1, 17$; $P = 0.89$) indicating RNA quality was comparable between groups.

Discussion

This pilot study seeking candidate gene regions associated with the predisposition to suicide independently of the associated psychiatric diagnosis, has identified 22 SNPs in 19 genes using a SNP chip (Table II). Seven out of that set of 19 genes had altered expression associated with suicide (Table II) but not mood disorders.

No previous study has required the significant SNPs to show an association with suicide that is independent of diagnosis. This study is one of the first to use this methodology in a psychiatric sample (Malhotra and Goldman 1999; Bunney et al. 2003) and the first to use it specifically in suicide.

Table II lists the 19 genes detected. Previous candidate gene studies have not identified any of these genes; however, these genes overlap in terms of biological ontology with results from expression studies of suicides (Sequeira et al. 2007; Thalmeier et al. 2008). Thalmeier

et al. (2008) divided their highly significant genes into four main categories based on ontology of biological processes. As seen in Table III, eight out of the 19 significant genes in our analysis may fit into these categories: MBNL2 into “ CNS development ”, CD44, DSC2 and MARCH1 into “ homophilic cell adhesion ”, SFRS11 and TUBGCP3 into “ regulation of cell proliferation ” and LSAMP and SPTLC1 into “ transmission of nerve impulse ”.

Other similarities in results may be found in these two RNA expression studies (Sequeira et al. 2007; Thalmeier et al. 2008) and our study. As seen in Table III, CDH13, MYO3A and perhaps FOXN3 are related to CDH12, CDH22, MYR8 and the set of DNA migration genes, respectively, found by Thalmeier et al. (2008). The FLJ23312 gene is similar to FLJ21616 found by Sequeira et al. (2007). CD44 was found associated with suicide in our study and both these studies (Sequeira et al. 2007; Thalmeier et al. 2008).

Some of the genes that we and others have identified, are connected to the immune system (MARCH1, CD300LB and CD44). Of special interest is the CD44 gene since it was significant in our and the two other studies (Sequeira et al. 2007; Thalmeier et al. 2008) and its expression in both BA9 and BA24 was significantly low (0.78 and 0.47, respectively, see Table II). Immune system dysregulation is reported in major depression but little is known about suicide. Mendlovic et al. (1997, 1999) demonstrated immune activation in suicidal major depression subjects. Some studies have demonstrated a possible link between suicide and allergic reactions that may alter the function of the orbital prefrontal cortex (Postolache et al. 2005, 2007, 2010). Higher CSF interleukin-2 receptor concentration is reported in suicide attempters (Nassberger and Traskman-Bendz 1993; Rothenhausler et al. 2006). Tonelli et al. (2008) analyzed transcriptional regulation of immune system in rats with depressed-like behaviour and found higher cytokinines levels in several brain regions. CD44 play a key role in inflammatory process and is an inhibitor of TNF-driven joint destruction and inflammatory process (Hayer et al. 2005). Godar et al. (2008) have shown that under conditions of basal physiological and cell-culture stress, expression of the CD44 cell-surface molecule is inhibited. Recently, the inflammatory and neurodegenerative (I&ND) hypothesis of depression was formulated (Maes et al. 2009), proposing that neurodegeneration and reduced neurogenesis that characterize depression are caused by inflammation, cell-mediated immune activation and their long-term sequelae. Our findings suggest that disordered neuroimmune function may be present in suicide and have its pathogenesis related to these genes. It is of note that a meta-analysis (Perlis et al. 2010), including approximately 8,700 mood disorder subjects, identified four additional regions that met the threshold for suggestive association, including the locus containing the gene coding for protein kinase C-epsilon, another immune system protein, previously implicated in models of mood and anxiety.

Finally, two of the genes are associated with peripheral nervous system disorders: an SPTLC1 mutation was reported in hereditary sensory neuropathy, an autosomal dominant progressive sensory loss (Verhoeven et al. 2004), while MBNL2 may have a role in the pathogenesis of myotonic dystrophy (Hao et al. 2008). The latter gene was significantly under-expressed in both brain areas (BA9, 24).

None of the SNPs, genes or loci identified in our study or the other expression studies (Sequeira et al. 2007; Thalmeier et al. 2008) can be directly associated with our current knowledge of biological changes in the brain of suicides. The same is true of the single SNP (rs1466846) found in a recent large GWAS of mood disorders (Perlis et al. 2010). However, the power of a genome-wide association study is that it is hypothesis generating, and screening such a gene set for expression changes helps in the identification of new candidate

genes for suicide. Future work should determine which of the above genes have importance in the pathophysiology of suicide.

Limitations

This study is limited by the small sample size imposed by the use of postmortem cases, which was necessary in order to assay RNA expression in specific brain areas of relevance to suicide (BA24, BA9), and has the advantage of studying the most severe and homogeneous behavioural phenotype of suicide. The sample size and low statistical power increases the likelihood of a type II error and false positive and therefore our finding should be judged with caution as preliminary and are intended to generate hypotheses for larger scale analyses. Using completed suicides we also attempted to evaluate whether the genetic associations were with suicide or with a lifetime major psychiatric disorder. Furthermore, racial stratification effects were limited by studying only Caucasians of European origin. Because we also used altered expression level to screen the genotype identified genes, we chose to use a cut off significance level while other studies have favoured a more conservative approach such as Q values. Another limitation of this pilot study is that we did not validate the expression findings with another method (i.e. qRT-PCR), nor did we follow up on these findings with any further functional assay, and these strategies should be part of a future work.

Future directions

A complex behaviour like suicide is unlikely to be due to a single mutation at a single gene and it is probably multifactorial and attributable to polygenic and epigenetic factors (Bunney et al. 2003; Mann 2003) as well as environment. Arrays are now available with > 2,000,000 SNPs probes, and can be used to replicate our findings and to identify further genes in larger samples. Microarray technology is expected to become cheaper and may have potential diagnostic use (Lipshutz et al. 1999; Bunney et al. 2003; Kennedy et al. 2003). The next step should be to move from candidate genes from GWAS to deep sequencing or functional analysis. Matching GWAS findings with brain expression data may shed more light on functionality of new candidate genes but sample sizes will always be limited. Of interest are the genes that we and others are identifying are involved in the immune system and that should stimulate more research into neuroimmunology and suicidal behaviour.

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Figure 1. Manhattan plot of the P values in GWAS of postmortem brains of suicides vs. controls. GWAS, genome wide association study.

Table 1
Descriptive statistics for suicides with and without depression and sudden death controls.

Characteristic	Sudden death <i>N</i> = 31	Suicide with mood disorder <i>N</i> = 45	Suicide without mood disorder <i>N</i> = 23	<i>F</i> / ²	df	<i>P</i>
Age (years)	53 ± 18	48 ± 21	37 ± 14	7.2	2,98	0.001
Gender (%Male)	26 (83%)	23 (50%)	19 (83%)	11.9	2	0.003
Alcohol (abuse & dependence)	0 (0%)	2 (4%)	11 (48%)	32.9	2	<0.001
Psychosis	0 (0%)	7 (16%)	12 (52%)	23.6	2	<0.001
Brown–Goodwin Aggression Score	14 ± 4	14 ± 5	20 ± 9	6.1	2	0.004

Table II

Alleles frequency, *P* values and gene expression in suicides with and with out depression and controls.

SNP	Chro#	Gene	Risk allele	<i>P</i> value Sui vs. Cont ^a	<i>P</i> value Dep Sui vs. Cont ^a	<i>P</i> value Nondep Sui vs. Cont ^a	^a Allele frequency		^b Gene expression		
							Sui	Cont	BA24	BA9	BA9
rs1387381	16	CDH13	T	0.000538	0.000115	0.074898	55%	29%	0.99	1.03	1.03
rs8054845	16	CDH13	C	0.000442	0.000332	0.018859	54%	27%	0.99	1.03	1.03
rs3811966	5	NPR3	T	0.000438	0.001754	0.006439	83%	61%	1.08	0.99	0.99
rs10512596	17	CD300LB	A	0.000473	0.002737	0.006994	69%	44%	1.22	1.03	1.03
rs373655	14	FOXN3	A	0.000202	0.000282	0.003389	51%	21%	0.8	1.21	1.21
rs1073179	1	DISC1	T	0.000976	0.006102	0.007388	73%	47%	1.12	1.11	1.11
rs936306	15	CYP19A1	G	4.13E-05	0.000126	0.017254	91%	71%	0.86	0.89	0.89
rs1999240	10	MYO3A	G	0.000569	0.000796	0.02442	46%	23%	1.09	1.03	1.03
rs3767206	1	SFRS11	C	0.000452	0.008025	0.00387	97%	82%	0.91	1.2	1.2
rs10511351	3	LSAMP	G	6.53E-05	0.000306	0.007475	92%	69%	1.09	0.59	0.59
rs1790695	18	DSC2	C	0.000513	4.25E-05	0.104416	39%	18%	0.81	1.22	1.22
rs1970121	9	SPTLC1	C	0.000463	0.00136	0.011775	88%	66%	0.8	1.09	1.09
rs63953	17	ACCN1	A	0.00085	0.001341	0.052554	80%	56%	0.99	1.04	1.04
rs10515470	5	FLJ23312?	NA	0.000462	0.000381	0.06549	NA	NA	NA	NA	NA
rs4771992	13	MBNL2	A	0.000262	0.001815	0.005587	78%	50%	0.87	0.71	0.71
rs1467558	11	CD44	C	0.000431	0.004333	0.002193	93%	74%	0.47	0.78	0.78
rs9324313	13	TUBGCP3	C	0.00038	0.000363	0.01576	35%	13%	1.28	1.1	1.1
rs6462430	7	KBTD2	G	0.00043	0.008364	0.000317	73%	50%	0.84	0.75	0.75
rs2392147	7	KBTD2	T	0.000478	0.001137	0.005458	70%	45%	0.84	0.75	0.75
rs6469916	8	COL14A1	A	0.000296	0.000921	0.018992	96%	82%	1.09	0.85	0.85
rs4441122	13	MBNL2	A	0.000304	0.001096	0.009715	76%	48%	0.87	0.71	0.71
rs1039959	4	MARCH1	G	0.000294	0.002013	0.002192	57%	29%	1.33	0.93	0.93

Sui, suicide; Dep, depression; Cont, controls; Nondep, non-depressed; SNP, single nucleotide polymorphism; OR, odds Ratio; Chro#, chromosome number.

^a *P*-value = 0.001 is considered significant threshold for SNPs in this study.

^b Bold fold change are significant at the 0.05 level at the probeset level.

Table III

Literature review for the 19 significant GWAS candidate genes in suicides (based on OMIM database^{*}).

Gene symbol	Chro.#	Description	Suggested clinical role [*]	Similar genes found by others in expression studies
<i>CDH13</i>	16	Cadherin 13, H-cadherin (heart)	Lung tumour recurrence?	CDH12, CDH22 (Thalmeier et al. 2008)
<i>NPR3</i>	5	Natriuretic peptide receptor C	Maintenance of blood pressure	
<i>CD300LB</i>	17	CD300 antigen-like family member b	Cell surface localization in B and NK cells	
<i>FOXN3</i>	14	Forkhead helix transcription	DNA damage correction?	ADAMTS1, IGF1, VIP, WDR39 (Thalmeier et al. 2008)
<i>DISC1</i>	1	Disrupted in schizophrenia 1	Susceptibility for schizophrenia (Millar et al. 2000)	
<i>CYP19A1</i>	15	Cytochrome P450, family 19, subfamily A, polypeptide 1	Aromatase deficiency	
<i>MYO3A</i>	10	Myosin IIIA	Autosomal recessive deafness	MYR8 (Thalmeier et al. 2008)
<i>SFRS11</i>	1	Arginine/serine-rich 11 splicing factors	Pre-mRNA splicing?	
<i>LSAMP</i>	3	Limbic system-associated membrane protein	Neuronal surface glycoprotein in limbic system (Pimenta et al. 1996)	
<i>DSC2</i>	18	Desmocollin 2	Ca-dependent glycoprotein important for cell adhesion	
<i>SPTLC1</i>	9	Serine palmitoyltransferase, long chain base subunit 1	Hereditary sensory neuropathy	
<i>ACCN1</i>	17	Amiloride-sensitive cation channel 1, neuronal (degenerin)	Neurodegeneration? KO-mice reduced sensitivity to mechanic sensation	
<i>FLJ23312</i>	5	Hypothetical protein	Not known	FLJ21616 (Sequeira et al. 2007)
<i>MBNL2</i>	13	Muscleblind-like 2	May be associated with myotonic dystrophy	
<i>CD44</i>	11	CD44 molecule	Migration, cell fusion, tumorigenesis?	(Thalmeier et al. 2008; Sequeira et al. 2007)
<i>TUBGCP3</i>	13	Tubulin, gamma complex associated protein 3	Associated with gamma-tubulin in cells and oocytes	
<i>KBTD2</i>	7	Kelch repeat and BTB (POZ)	Not known in humans	
<i>COL14A1</i>	8	Collagen, type XIV, alpha 1 (undulin)	Not known in humans	
<i>MARCH1</i>	4	Membrane-associated RING-CH protein I	Down-regulation of histocompatibility complex	

GWAS, genome wide association study.

^{*} OMIM, Online Mendelian Inheritance in Man.