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Rare Functional Variants Associated with Antidepressant Remission in Mexican-Americans
Short title: Antidepressant remission and pharmacogenetics in Mexican-Americans

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ABSTRACT

Introduction: Rare genetic functional variants can contribute to 30-40% of functional variability in genes relevant to drug action. Therefore, we investigated the role of rare functional variants in antidepressant response.

Method: Mexican-American individuals meeting the Diagnostic and Statistical Manual-IV criteria for major depressive disorder (MDD) participated in a prospective randomized, double-blind study with desipramine or fluoxetine. The rare variant analysis was performed using whole-exome genotyping data. Network and pathway analyses were carried out with the list of significant genes.

Results: The Kernel-Based Adaptive Cluster method identified functional rare variants in 35 genes significantly associated with treatment remission (False discovery rate, FDR <0.01). Pathway analysis of these genes supports the involvement of the following gene ontology processes: olfactory/sensory transduction, regulation of response to cytokine stimulus, and meiotic cell cycleprocess.

Limitations: Our study did not have a placebo arm. We were not able to use antidepressant blood level as a covariate. Our study is based on a small sample size of only 65 Mexican-American individuals. Further studies using larger cohorts are warranted.

Conclusion: Our data identified several rare functional variants in antidepressant drug response in MDD patients. These have the potential to serve as genetic markers for predicting drug response. Trial registration: ClinicalTrials.gov NCT00265291

Abbreviations: Chr, Chromosome; Ejc, Exon junction complex; Eqtl, Expression quantitative trait loci; Expr id, Expression identification; Fdr, False discovery rate; Go, Gene ontology; Ham-d, Hamilton depression rating scale; Kbac, Kernel-based adaptive cluster; Maf, Minor allele frequency; Mdd, Major depressive disorder; Rsid, Reference snp identification number; Snp, Single nucleotide polymorphism

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1. Introduction

Major depressive disorder (MDD) represents a severe public health challenge in the U.S. and worldwide, with a lifetime prevalence of 10-20% in the general population (Wong and Licinio, 2001). Currently, antidepressants are among the top prescribed drugs (Larura et al., 2011). Thus, the ability to predict antidepressant response could have a significant and substantial public health impact. However, recent meta-analyses on pharmacodynamic pharmacogenetics of antidepressants in MDD failed to show any variation of genome-wide significance (Fabbri et al., 2013; Fabbri and Serretti, 2015; GENDEP et al., 2013; Niitsu et al., 2013).

Common and rare variants contribute to common complex disease heritability, and expanding evidence supports the significant role of rare genetic variants in the etiology of complex diseases (Cohen et al., 2004; Leblond et al., 2012; Liu and Leal, 2010b; Yu et al., 2018). Human populations possess an abundance of rare genetic variants (once every 17 bases) that cluster geographically; consequently, assembling a complete rare variant catalog will be a formidable feat (Nelson et al., 2012). An incipient drug response literature supports the notion that the genetic architecture of drug efficacy likely mirrors that of complex traits (Zhou and Pearson, 2013). Rare genetic variants have shown to reduce protein levels by more than 70%, be responsible for drug-induced severe toxicity/lethality, and contribute to 30-40% of functional variability in pharmacogenetics genes, such as nuclear receptors, metabolic enzymes, and cellular transporters (Del Re et al., 2016; Kozyra et al., 2016; Wobst et al., 2016). Therefore, previously unreported rare functional variants may exert further impact on the phenotype than common variations and may be crucial in the problematic genotype/phenotype discordance influencing the analysis of pharmacogenetic screening (Maggo et al., 2016).

Up until now, no gene has been identified to have a major effect in antidepressant drug response in MDD. Using transcriptomics Hodgson et al. (Hodgson et al., 2016) have recently shown that alterations in the expression of the MMP28 and KXD1 genes in the blood were associated with better response to nortriptyline. Polygenic risk scores have been recently used to test whether they could predict symptom improvement or remission. No significant predictive effect was found using polygenic risk scores in pharmacogenetic studies and the genetic liability to MDD (Garcia-Gonzalez et al., 2017).

Functional single nucleotide polymorphisms (SNPs) that result in amino acid, regulatory, epigenetic, or splicing changes can have a considerable impact on phenotype (Ng et al., 2009). In fact, significant portions of the current missing heritability of complex traits may be due to genetic interactions (Zuk et al., 2012). Here, we explored the premise that rare functional SNPs influence to antidepressant drug response by performing whole-genome screening. The goal of this line of study is to provide a basis for future pharmacogenetics approaches aimed at using genetic markers to predict treatment outcomes and to pave the foundation for future personalized MDD treatment.

2. Materials and methods

2.1. Clinical study

Institutional Review Boards and Human Research Ethics Committees approved the study protocol at the University of California Los Angeles and the University of Miami in the USA, the Australian National University and Bellbery Ltd in Australia, and the State University of New York Upstate Medical University. The study was registered in the public database clinicaltrials.gov, NCT00265291 (Dong et al., 2009; Wong et al., 2012; Wong et al., 2014). We obtained written informed consent from all participants after oral and written explanations of all study procedures were furnished to participants in their preferred language (English or Spanish).

Briefly, in this single site treatment trial all subjects had comprehensive psychiatric and medical assessments, and if enrolled, participated in two consecutive study phases: 1) An initial 1-week single-blind placebo lead-in phase to mitigate placebo responders, followed by 2) Random assignment to fluoxetine 10-40 mg/day or desipramine 50-200 mg/day treatment. As antidepressant medications are efficacious MDD treatment, a placebo lead-in period followed by active treatment for all patients was used to minimize human participants at risk (Baldwin et al., 2003; Kim and Holloway, 2003). Participants had weekly follow-up visits to assess their clinical status using clinical observations, interviews, and self-reports.

2.1.1. Participants

All subjects met four inclusion criteria: 1) Age between 18-70 years. 2) ≥ 3 out of 4 grandparents were born in Mexico (Hazuda et al., 1986). 3) Diagnostic and Statistical Manual-IV diagnosis of a current unipolar major depressive episode (APA, 1994). 4) 21-Item Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960) score of ≥17 with item #1 (depressed mood) rated ≥2 (Dong et al., 2009; Wong et al., 2012; Wong et al., 2014). Our exclusion criteria included: 1) Active medical illnesses that could be related to the current depressive episode. 2) Axis I disorder other than major depressive disorder or primary anxiety disorders. 3) Active suicidal ideation or severe recent suicide attempt. 4) Lack of contraception use in women of childbearing age, or pregnant or lactating. 5) Electroconvulsive treatment within the last six months; 6) Use of other antidepressant treatment or medications that interfere with EEG or antidepressant treatment in last 2 weeks. 7) Previous unsatisfactory response to desipramine or fluoxetine treatment. 8) Illicit drug or alcohol abuse within the previous three months. 9) Presently receiving counseling or psychotherapy (Dong et al., 2009; Wong et al., 2012; Wong et al., 2014).

2.1.2. Treatment

All subjects received an initial one-week of single-blind placebo to reduce placebo responders (defined as subjects with a ≥ 25% decrease in HAM-D score compared to the screening visit and or HAM-D score less than 18). Remaining participants were then randomly assigned to receive an 8-week double-blinded treatment with either fluoxetine or desipramine in a 1:1 ratio. Initially, participants received either fluoxetine 10 mg/day or desipramine 50 mg/day; doses increased respectively to 20 mg/day or 100 mg/day at week 2 of active treatment. Dose escalation schedule occurred as follows in subjects who tolerated the previous dose: 1) The dose was increased to 30 mg/day for fluoxetine, and 150 mg/day for desipramine at week 4 for participants who showed less than a 25% decrease in their HAM-D scores. 2) A maximum dose of 40 mg fluoxetine/200 mg desipramine was increased in participants with HAM-D scores <12 at week 6. Each subject was assessed according to the reduction in HAM-D score from week 0 to week 8. The categorical outcome measures were as follow: remission for HAM-D scores <8 and nonresponse for HAM-D score reduction of <50%. After the 8-week double-blinded phase, participants were referred to a psychiatric clinic of their choice to follow up treatment.

2.1.3. Clinical outcome measures

We determined the presence of a current major depressive episode using the Structured Clinical Interview for Diagnosis (First et al., 1994) either in English or in Spanish, and the DSM-IV diagnoses were further confirmed by a research psychiatrist. The mean Kappa score among raters ranged from 0.84-0.85 for sensitivity and specificity.

Experienced bilingual clinical personnel rated MDD symptom severity weekly using the Spanish or English versions of the HAM-D (Hamilton, 1960), Hamilton Anxiety Rating Scale (Hamilton, 1959), Global Assessment Scale (Endicott et al., 1976), Beck Depression Inventory (Beck et al., 1961), and the Center for Epidemiological Depression Rating Scale (Roberts, 1980). Antidepressant drug levels were obtained randomly to confirm treatment adherence.

2.1.4. Genomic DNA collection

Blood samples from participants were collected into BD Vacutainer EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Genomic DNA was extracted using the Gentra Puregene Blood Kit (Qiagen, Germantown, MD, USA).

2.2. Pharmacogenetics Procedures

2.2.1. Whole-exome genotyping

Whole-exome genotyping was performed using the Illumina® HumanExome BeadChip-12v1_A, which has > 250,000 markers from diverse populations and common conditions, including psychiatric disorders, diabetes, and cancer. Genotyping reliability and quality were tested using a duplicated sample was duplicated.

2.2.2. Quality Control and Rare and Functional Variants filtering

Samples with SNP call rates below 99% were excluded. We estimated and used for quality control the Identity by Descent between all pairs of individuals. Illumina's GenomeStudio data was used in SVS 7.6.7, Golden Helix® (Golden Helix, Inc. Bozeman, MT, USA (http://www.goldenhelix.com), to extract genotyping data (such as genotype, log ratio, computed copy number variation values, allele frequency, etc). The following exclusion criteria were used: (i) deviations from the Hardy-Weinberg equilibrium using P-values $< 0.05/m$ (m is the number of markers included in the analyses), (ii) a genotype call rate of less than 90%% and (iii) number of alleles different from 2. We used maximum likelihood to estimate genotype and allelic frequencies; only markers with minor allele frequency (MAF) < 0.01 in a larger ascertained cohort of Mexican-Americans were used to define the criteria of rareness for subsequent analysis (Wong et al., 2017).

2.2.3. Genetic Stratification Analysis

The inbreeding coefficient was estimated to detect the presence of hidden biological relatives in the sample, as this may reduce the independence of the data. We estimated to evaluate the potential presence of genotype stratification (micro-differentiation) using the fixation index between pairs of subpopulations, case subjects, and controls, by employing a set of autosomal independent markers. Additional correction of putative population stratification was applied using 10 principal component analyses to normalize genotypic data by its actual standard deviation.

2.2.4. Functional Variants Filtering and Classification

We used the information from the dbNSFP_NS_Functional_Predictions annotation track (GRCh_37) to identify variations with potential functional effect (Davydov et al., 2010) employing SIFT, PolyPhen-2, Mutation Taster, Gerp++, and PhyloP (Adzhubei et al., 2010; Ng and Henikoff, 2003; Schwarz et al., 2010), to consider which variants were most likely to have functional effects. The SVS 7.6.6, Golden Helix® Variant Classification module was used to investigate the interactions among variants and gene transcripts in order to categorize variants.

2.2.5. Rare Variant Analysis

We used the Kernel-Based Adaptive Cluster (KBAC) method, implemented in Golden Helix®, which relates variant classification and association testing. The rare variant data are collapsed within each of a number of chromosomal regions into multi-marker genotypes. Because variants are rare, only a relatively few different multi-marker genotypes will be found in any given region, and their counts are used to perform a distinct multivariate case/control test to ascertain their association with the phenotype (Liu and Leal, 2010a, b). In the KBAC method, genotypes with high sample risks are given higher weights, which can potentially separate causal from non-causal genotypes. A one-sided test was used due to the weighting procedure, the P-values were estimated using 10,000 permutations, and correction for multiple comparisons was performed using FDR (False discovery rate). Missing data were imputed as a homozygous major allele (wild-type). This method is used as implemented in SVS 7.6.7. that also implemented the mixed model to the KBAC analysis to correct for genetic relationships among the samples. The test works as the KBAC with Regression but adapted to a logistic mixed-model equation. For the Kinship information, we estimated the identity by state matrix (IBS) with 83,937 common, non-functional, and redundant markers that were excluded from the final analysis.

2.3. Pathway and Network Analyses

We used ConsensusPathDB (Release 30) (Kamburov et al., 2011; Kamburov et al., 2013), which is a freely available molecular functional interaction database with a web interface, to evaluate potential common ontogenetic or cellular processes and build networks with the list of genes that were significantly associated to remission in the rare variants analyses.

2.4. Brain Expression Quantitative trait loci (eQTL) Analyses

The UK Brain Expression Consortium (http://www.braineac.org/) web-based server Brain eQTL Almanac was used to find a significant statistical association between genetic polymorphisms and gene expression levels in the hippocampus and other brain areas (Ramasamy et al., 2014).

3. Results

3.1. Participant characteristics and response of a prospective pharmacogenetics

Our participants were mostly first-generation Spanish-speaking-only Mexican-Americans of Los Angeles; a detailed description of this cohort and treatment outcomes can be found elsewhere (Dong et al., 2009; Wong et al., 2012; Wong et al., 2014). Briefly, no statistically significant differences in demographic and baseline clinical characteristics were found between desipramine- and fluoxetine-treated patients (Table S1). In this study, 72% of patients (N = 166) completed 8 weeks of treatment, while 66 participants did not complete the study after receiving at least one week of medication. A higher dropout rate was found in patients treated with desipramine. Fluoxetine and desipramine were effective in this population; however, fluoxetine-treated participants had better scores at the end of the 8-week treatment in several rating scales (HAM-D, Hamilton Anxiety Rating scale, Depression Inventory, and Global Assessment Scale) than desipramine-treated patients. We obtained and analyzed Illumina® HumanExome BeadChip-12v1_A data from 65 participants - 36 remitters (17 desipramine- and 19 fluoxetine-treated), and 29 non-responders (14 desipramine- and 15 fluoxetine-treated). Demographic data and antidepressant treatment of remitters and non-responders are provided in Table S2.

3.2. Power analysis

Consistent with our hypothesis that varianst of large effect (i.e. mutations) modify antidepressant response in MDD, we performed a power analyses to detect any such large effects (as defined by the Cohen's d parameter). This analysis used small sample sizes (Vélez et al., 2016). In previous work, we showed that 72 individuals would be sufficient to detect up to 80% true positives with effect size $d = 0.82$ when $m = 100{,}000$ variants are tested for association to a quantitative trait (Fig. S1); this power analysis extrapolates to a categorical trait as demonstrated in Velez et al., supplementary material (Vélez et al., 2016). The final number of tested variants was significantly lower than 100,000, due to pruning of non-informative, and common variants.

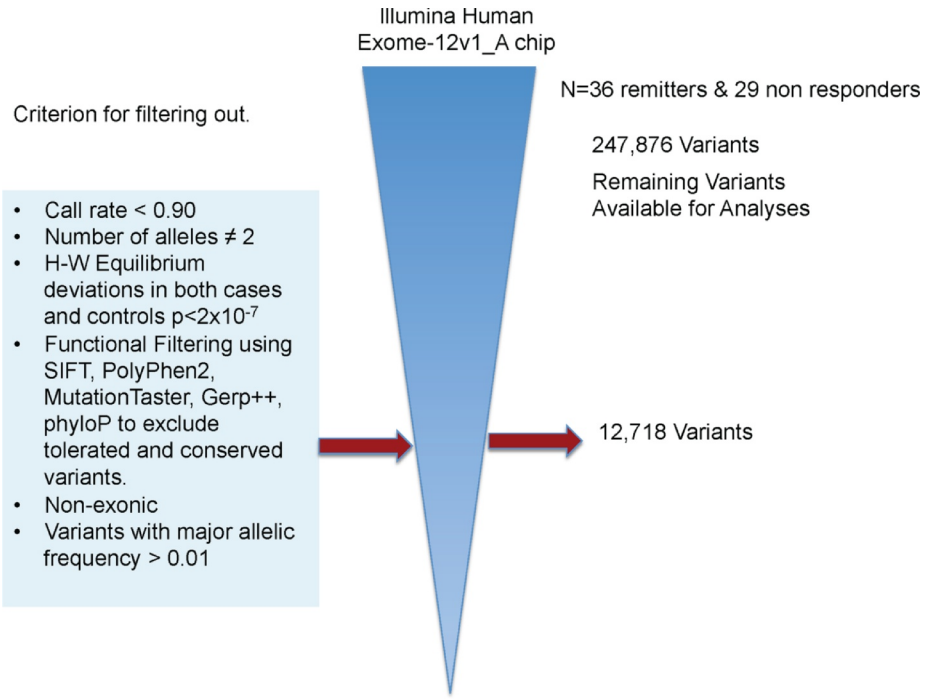

Fig. 1. The process used to filter out SNP exomic variants genotyped with the Illumina® HumanExome BeadChip-12v1_A to evaluate the role of rare functional variants in the pharmacogenetics of antidepressant response as remitters (n = 36) and non-responders (n = 29). SNPs were discarded because they were either monoallelic, had more than two alleles, had a call rate lower than 90%, or their genotype proportions deviated from the expected ones as defined by the Hardy-Weinberg equilibrium theorem, with P-values < 0.05/m (m is the number of markers included in the analyses), in both cases and controls. Common variants (allelic frequency of the minor allele higher than 0.01), non-exonic, non-functional variants, and tolerated and conserved variants were theoretically defined by the use of SIFT, PolyPhen2, MutationTaster, Gerp ++ and phyloP. The remaining 12,718 variants were used in the rare variant analysis.

3.3. Several rare variants were associated with antidepressant response

After filtering out markers that did not meet either quality control criteria or variability requirements, and polymorphisms without potential functional effect, 12,718 variants remained for rare variant analysis (Fig. 1). Those variants were anchored in 7,863 genes and categorized in 15 types according with their Sequence Ontology (Table S3).

Using the KBAC method, we reported that rare variants in 35 genes (Table 1) were significantly associated with remission of MDD symptoms (FDR ≤ 0.01). The rare variants examined that are likely to contribute to treatment outcomes are listed in Table S4.

3.4. Pathway and network analyses

Table 2 summarizes the pathway and network findings. The top gene ontology (GO) processes significantly associated with antidepressant remission were: 1) olfactory/sensory transduction, 2) regulation of response to cytokine stimulus, and 3) meiotic cell cycle process (Table 2 and Fig. 2). Enriched pathway-based sets were significant for olfactory/sensory transduction and signaling pathways and transport of glucose and other sugars, bile salts and organic acids, metal ions, and amine compounds (Table 2). The most significantly enriched neighborhood-based entity set included ALG6 (ALG6, alpha-1,3-glucosyltransferase, Table 2. ALG6 is an important post-translation modifier; it catalyzes the addition of the first glucose residue to the expanding lipid-linked oligosaccharide precursor of the N-linked glycosylation, a process by which glycosyl groups are added onto proteins in the endoplasmic reticulum membrane.

3.5. CWC22 variants significantly change eQTL in human brain areas

We considered that these results had strengthened the potential association between the CWC22 gene and antidepressant remission, as we previously listed that chromosome locus [Chr2q13.3/(exm) rs16867321] among the top common SNPs likely associated with antidepressant remission (Wong et al., 2014). As CWC22 variants may represent a valid signal associated with antidepressant remission and this gene is widely expressed in the brain (Allen Brain Atlas; www.brain-map.org) (Lein et al., 2007), we queried the CWC22 gene on the Braineac webserver and found that variants of this gene significantly changed eQTL in all ten brain areas listed (Table S5). Nine listed variants changed eQTL in the hippocampus (Table 3).

4. Discussion

Our rare variant analysis of whole-exome genotyping data identified 35 genes that were significantly associated with remission (FDR ≤ 0.01), which is surprising given the small number of remitters (n = 36) and non-responders (n = 29). Our Mexican-American cohort and HapMap Mexican-American cohort were recruited from the same location in Los Angeles; their estimated median ancestry proportions are 5% African, 45% Indigenous American, and 49% European, (Johnson et al., 2011). Therefore, they contain increased numbers of variants and rare variants because of their African and Spanish ancestries (Genomes Project et al., 2012; International HapMap et al., 2010).

We briefly summarized below the role of genes listed in Table 1 in MDD susceptibility or antidepressant response.

1) METTL3, methyltransferase like 3 - Located in chromosome 1q24 METTL3 is associated with methyltransferase activity, which participates in estrogen receptor-induced gene transcription (Green and Galea, 2008), and higher DNA methylation in that region has been observed in women with bipolar disorder and psychosis (Mill et al., 2008). Genome-wide linkage on chromosome 1q21.3-q32.1 suggests that this locus could modulate vulnerability to postpartum mood symptoms and postpartum psychosis (Mahon et al., 2009; Pinna and Zompo, 2012; Thippeswamy et al., 2017).
2) PSMD13, proteasome 26S subunit, non-ATPase 13 – Case-control studies suggest the involvement of PSMD13 in MDD treatment response (Wong et al., 2008; Minelli et al., 2015). Individuals with lower PSMD13 mRNA levels in fibroblasts may have a greater risk for treatment-resistant depression (Minelli et al., 2015).
3) TYK2, tyrosine kinase 2 – TYK2 gene variations have not been previously associated with MDD risk or antidepressant response. A body of literature supports the relevance of IL6 since it was first reported to be elevated in MDD (Alesci et al., 2005). Binding of IL6 to the IL6 receptor needs to associate with gp130, which associates

Table 1
Rare variant analysis: several genes were significantly associated with MDD treatment remission.

Chr.	Start	Stop	Gene Name	KBAC Score (One-Sided)	FDR < 0.01 Pc-Value (One-Sided)	# Markers	# Multi-Marker Genotypes
1	171750761	171766856	METTL13	4.79111958	0.001	4	4
4	147628179	147867034	TTC29	7.51030906	0.001	5	10
2	228474806	228498036	C2orf83	6.50475315	0.002	2	8
11	236808	252984	PSMD13	5.36578529	0.002	5	5
13	25742672	25745857	FAM123A	5.12291136	0.002	2	4
16	72152996	72206349	PMFBP1	6.45231158	0.002	3	7
1	100174259	100231349	FRRS1	6.22381351	0.003	2	5
2	180809604	180871780	CWC22	6.67081036	0.003	4	12
14	95883831	95942173	C14orf49	5.84237836	0.003	2	4
6	24495197	24537435	ALDH5A1	3.56923077	0.004	4	6
8	117962512	118188953	SLC30A8	5.67877054	0.004	1	3
11	58125598	58126542	OR5B17	5.54799654	0.004	2	5
5	118788138	118878030	HSD17B4	5.78600942	0.005	4	6
11	58189738	58190786	OR5B2	5.20040434	0.005	2	4
19	10461204	10491248	TYK2	5.87643803	0.005	5	9
1	57320443	57383894	C8A	4.10183042	0.006	4	4
19	16059818	16060768	OR10H4	5.35816149	0.006	3	4
1	179851420	179889211	TOR1AIP1	5.04115332	0.007	2	4
3	58549845	58563491	FAM107A	4.95954573	0.007	3	5
7	77428109	77586821	PHTF2	5.15205008	0.007	1	3
11	12695969	12966284	TEAD1	5.38681903	0.007	1	3
5	72416388	72427644	TMEM171	4.9260008	0.008	1	3
6	116421999	116566853	NT5DC1	3.12307692	0.008	1	2
9	79226292	79521003	PRUNE2	7.38514885	0.008	25	23
9	107509969	107522403	NIPSNAP3A	4.65492397	0.008	1	3
17	26691290	26692265	SEBOX	4.89169491	0.008	2	4
1	63833261	63904233	ALG6	2.87597633	0.009	3	5
1	150480487	150486265	ECM1	4.40944502	0.009	2	3
3	130064359	130203688	COL6A5	8.4624212	0.009	15	29
4	40337469	40356973	CHRNA9	3.89881189	0.009	3	4
12	70047389	70093196	BEST3	4.09028552	0.009	3	4
16	66788879	66835523	CCDC79	4.22956966	0.009	1	3
17	5404719	5487832	NLRP1	5.86664359	0.009	6	10
11	55587106	55588047	OR5D18	3.12307692	0.010	2	3
18	42792947	43263060	SLC14A2	5.32226339	0.010	5	7

The CWC22 gene appears in bold. Abbreviations: Chr, chromosome; KBAC, Kernel-Based Adaptive Cluster method; FDR, false discovery rate. Variants or genes positions are based on hg19.

exclusively to TYK2. Both IL6 classic and trans-signaling modes require TYK2 for downstream signaling (Hibi et al., 1990; Leitner et al., 2017). Therefore, selective TYK2 inhibition may represent a worthwhile target to block pathological IL6 trans-signaling (He et al., 2019; Kalkman, 2019; Leitner et al., 2017; Menet, 2014). The soluble gp130 protein is a recombinant derivative that acts as a specific inhibitor of IL6 trans-signaling, and it has shown efficacy in preclinical trials of inflammation models in CNS inflammation. However, the effects of depression models have not been tested.

4) ALG6, ALG6 alpha-1,3-glucosyltransferase – ALG6 mutations have been associated with congenital disorders with a primary neurological presentation, including epilepsy, ataxia, proximal muscle weakness, limb anomalies. Behavioral disorders included cyclic behavioral change, with depressive episodes and autistic features (Morava et al., 2016).
5) CWC22 spliceosome associated protein homolog gene – This gene located in chromosome locus 2q13.3/(exm) rs16867321, which has been previously listed to be amongst the top common SNPs likely to be associated with antidepressant remission (Wong et al., 2014). CWC22 is required for multiprotein exon junction complex (EJC) assembly (Barbosa et al., 2012), which forms upstream of exon-exon junctions that have produced during RNA splicing on mature mRNAs, and is involved in post-splicing mRNA processing. This gene is expressed in the CNS, and CWC22 variants significantly changed eQTL in the hippocampus and other brain areas. CWC22 gene expression was found to be increased in diabetic dorsal root ganglia, dysregulating Cajal bodies, nuclear substructure involved in RNA splicing, and impair neuronal function. CWC22 knockdown was associated with improved outgrowth and the number of processes in adult sensory neurons (Kobayashi et al., 2017; Lein et al., 2007). It is pertinent that this gene is expressed in several regions of the brain; however, further studies are needed to establish its association with antidepressant response fully, including independent replication studies.
6) NLRP1, NLR family pyrin domain containing 1 – This gene encodes for the sensor component of the NLRP1 inflammasome, which is present in neurons (Kaushal et al., 2015). Inflammasomes are cytosolic multiprotein complexes that respond to pathogens and other damage-associated signals playing crucial roles in the innate immune response and inflammation. Little is known about the role of the NLRP1 inflammasome in MDD or antidepressant response; however, growing evidence has implicated the role of the NLRP3 inflammasome activation in MDD and stress-induced depressive-like behaviors (Kaufmann et al., 2017; Wong et al., 2016).
7) Olfactory receptors (OR5B17, OR5B2, OR5D18) – Olfaction has long been postulated to be involved in depression (Croy and Hummel, 2017; Mac, 1949). Individuals with olfactory loss have an increased risk of depressive symptoms (Deems et al., 1991; Temmel et al., 2002). In rodents, olfactory bulbectomy results in neurochemical changes in the CNS that are suggestive of MDD and depressive-like behavior (Lumia et al., 1992; Song and Leonard, 2005; van der Stelt et al., 2005; Yuan and Slotnick, 2014), which can be reversed with chronic but not acute antidepressant treatment (Frazer and Morilak, 2005; Grecksch et al., 1997; Leonard and Tuite, 1981; Uzunova et al., 2004).

Our pathway and network analysis revealed the involvement of olfactory transduction, signaling pathway and chemical sensory of

Table 2
ConsensusPathDB analysis.

Enriched pathway-based sets					
Pathway	Members	Source	P-value	Q value	Candidates contained
Olfactory transduction (human)	OR5B2; OR5B17; OR10H4; OR5D18	KEGG	0.0028	0.025	4 (1.0%)
Olfactory Signaling Pathway	OR5D18; OR5B17; OR10H4; OR5B2	Reactome	0.0033	0.025	4 (0.9%)
Transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds	SLC30A8; SLC14A2	Reactome	0.0099	0.049	2 (2.0%)
Enriched gene ontology (GO)-based sets					
Term name; term GO ID	Members	Category, level	P value	Q value	Candidates contained
Odorant binding; GO:0005549	OR5D18; OR5B17; OR5B2	M, 2	0.00041	0.0066	3 (3.5%)
Detection of stimulus involved in sensory perception; GO:00050906	OR5D18; OR5B17; CHRNA9; OR10H4; OR5B2	B, 3	0.0018	0.015	5 (1.0%)
Olfactory receptor activity; GO:0004984	OR5D18; OR5B17; OR10H4; OR5B2	M, 5	0.0057	0.034	4 (0.9%)
Detection of chemical stimulus involved in sensory perception of smell; GO:0050911	OR5D18; OR5B17; OR10H4; OR5B2	B, 5	0.0058	0.25	4 (0.9%)
Detection of stimulus, GO:0051606	OR5D18; OR5B17; CHRNA9; OR10H4; OR5B2	B, 2	0.0080	0.29	5 (0.7%)
Detection of chemical stimulus involved in sensory perception; GO:0050907	OR5D18; OR5B17; OR10H4; OR5B2	B, 4	0.0081	0.69	4 (0.9%)
Regulation of response to cytokines stimulus; GO:0060759	TYK2; ECM1	B, 4	0.018	0.69	2 (1.6%)
Meiotic nuclear division; GO:0007126	PSMD13; CCDC79	B, 4	0.031	0.69	2 (1.2%)
Meiotic cell cycle process; GO:1903046	PSMD13; CCDC79	B, 4	0.032	0.69	2 (1.2%)
Meiotic cell cycle; GO:0051321	PSMD13; CCDC79	B, 4	0.046	0.69	2 (1.0%)
Enriched neighborhood-based sets					
Centers name/gene IDs	Members	Sources	P value	Q value	Candidates contained
ALG6/29929	ALG6; TMEM171	Biogrid; EHMN; KEGG; Reactome	0.00015	0.13	2 (18.2%)
SLC40A1/30061	NIPSNAP3A; NLRP1; CHRNA9	BIND; Biogrid; HPRD; IntAct; MINT; PDB; PID; PhosphoPOINT; Reactome; Spike	0.00027	0.98	3 (3.9%)
TUBD1/51174	NIPSNAP3A; ALDH5A1; TYK2	BIND; Biogrid; HPRD; IntAct; PDB; PhosphoPOINT; Reactome; Spike	0.00061	0.98	3 (3.0%)
(RefSeq) glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group) (EC:2.4.1.150)	NLRP1; COL6A5; CHRNA9; TMEM171	Biogrid; HPRD; IntAct; MINT; NetPath; PDB; React	0.00094	0.98	4 (1.5%)

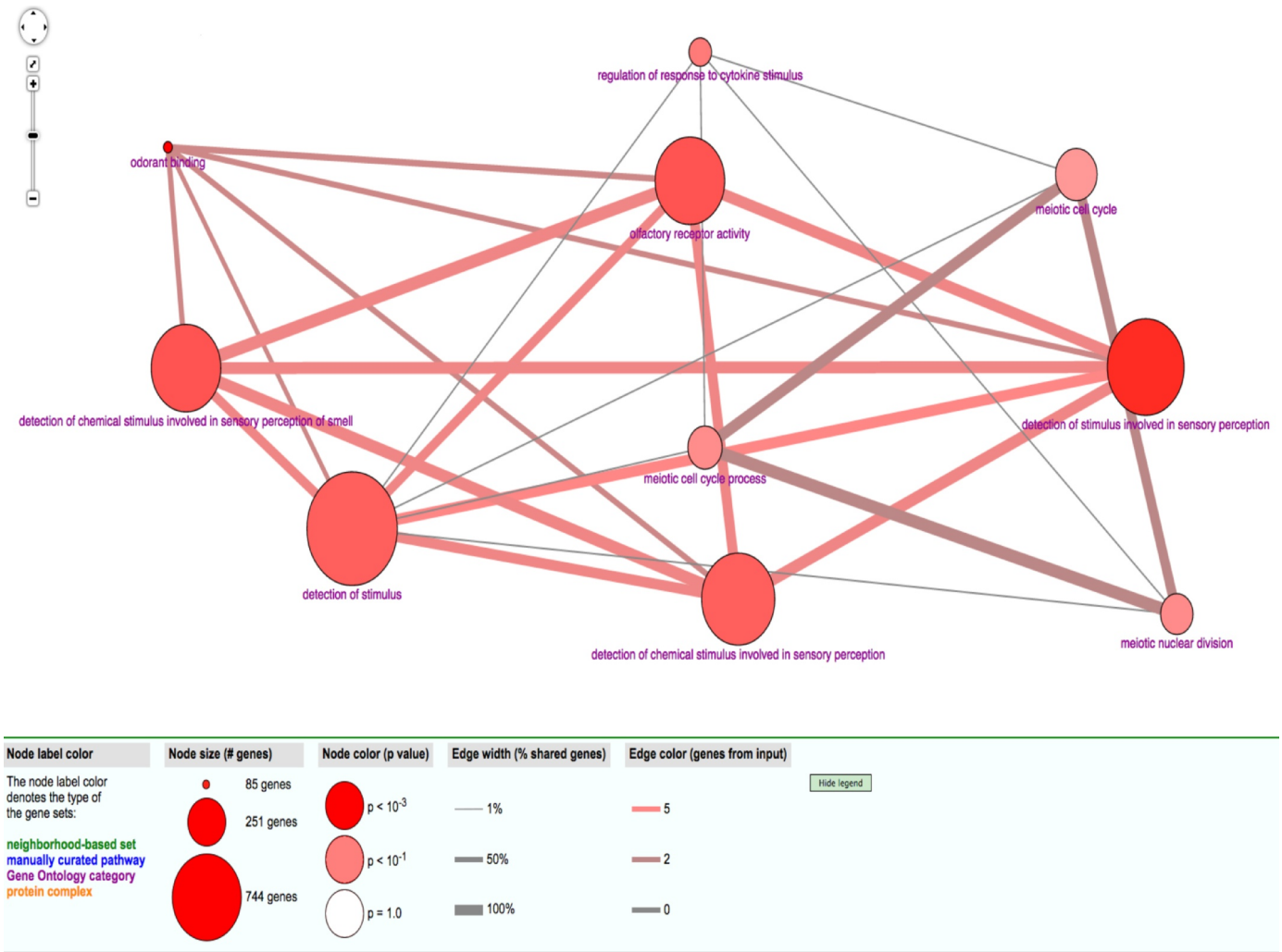

Fig. 2. Enriched gene ontology (GO) processes. Genes significantly associated with remission were analyzed using the ConsensusPathDB engine. Enriched GO processes with $P \leq 0.05$ are shown.

Table 3
Quantitative gene expression analyses for the CWC22 gene variants in the hippocampus extracted from the Braineac webserver.

rsID	Variant	Expr ID	P-value	Allele frequency
N/A	chr2:181492520:T_TA	2590216	4.40E-04	N/A
rs16867335	chr2:181458934	2590216	4.40E-04	C = 79.1% T = 20.9%
rs35579320	chr2:181463612	2590216	4.40E-04	T = 79.1% C = 20.9%
rs72884242	chr2:181465486	2590216	4.40E-04	A = 79.1% T = 20.9%
rs6739798	chr2:181465853	2590216	4.40E-04	T = 79.1% C = 20.9%
rs34842451	chr2:181480525	2590216	4.40E-04	T = 79.1% C = 20.9%
rs13424082	chr2:180227865	2590214	4.90E-01	G = 80.3% A = 19.7%
rs13424082	chr2:180227865	2590220	4.20E-04	G = 80.3% A = 19.7%
rs13424082	chr2:180227865	2590207	4.70E-01	G = 80.3% A = 19.7%

Abbreviations: rsID, reference SNP identification number; Expr ID, expression identification; N/A, not available; chr, chromosome. Variants or genes positions are based on hg19.

smell, transport of sugars, bile salts and organic acids, metal ions and amine compounds, regulation of response to cytokine stimulus, and meiotic cell cycle process. Notably, the central connections of the olfactory system are believed to play a significant role in affective behavior (see above). Neuroimmune mediation and neuroinflammation are active areas of investigation in MDD (Angelucci et al., 2005; Bhattacharya et al., 2016; Jansen et al., 2016; Wong et al., 2008).

Unexpectedly, our analysis showed the involvement of meiotic cell processes in antidepressant remission. Stress and emotional states may dysregulate reproductive functioning, as several neurotransmitters can modulate the hypothalamic-pituitary-gonadal axis and influence spermatogenesis or menstrual cycling (Hendrick et al., 2000). The correlation involving antidepressant medication and reproductive outcomes have been evaluated by sperm alterations and pregnancy outcomes (Riggin and Koren, 2015). A significant reduction in sperm viability, motility or volume has been reported for desmethylimipramine, trimipramine, clomipramine (Kurland et al., 1970; Levin et al., 1981; Maier and Koinig, 1994). Studies also support that selective serotonin reuptake inhibitors alter semen parameters reversibly, such as decreased sperm motility, concentration, sperm morphology or damaged

DNA (Koyuncu et al., 2011; Relwani et al., 2011; Safarinejad, 2008; Tanrikut et al., 2010; Tanrikut and Schlegel, 2007), which may negatively affect men's fertility (Tanrikut and Schlegel, 2007). Recently, in a cohort of females (N = 1,650) and males (N = 1,608), no antidepressant usage in women with active MDD symptoms (N = 72) was associated with a small increase in pregnancy rates and not associated with poorer fertility outcomes, in terms of miscarriage and percentage of live birth (Evans-Hoeker et al., 2018). However, men with active MDD episodes were less likely to achieve conception. Antidepressant use in women without current active MDD (N = 73) was associated with higher first-trimester miscarriage risk; this association was not shown in women with active MDD. No differences in live birth rates were shown in any groups of depressed women (Evans-Hoeker et al., 2018).

In rodent studies, paternal fluoxetine administration decreased spermatogenesis, sperm motility, and density, and decrease in pregnancy numbers and the number of viable fetuses (Alzahrani, 2012; Bataineh and Daradka, 2007). Citalopram treatment increased DNA strand breaks and oxidative DNA damage in sperm (Attia and Bakheet, 2013). A recent study suggests that antidepressants can influence sperm parameters differently; amitriptyline and venlafaxine administration respectively weaken and improve sperm parameters in rodents (Bandegi et al., 2018).

Rare variants are generally defined as variants with MAF < 1% in the population studied, which makes replication studies very challenging, as allele frequency varies amongst different populations. Two recent pharmacogenetics of antidepressant studies addressed rare variants in Caucasian and Han Chinese patients. A meta-analysis of European-ancestry data from the GENDEP-STAR*D (Genome-Based Therapeutic Drugs for Depression-Sequenced Treatment Alternatives to Relieve Depression) studies identified significant associations with variants in the ITGA9 (Integrin A9; rs116692768; P = 2.59e-08; MAF = 0.012) and NRXN3 (Neurexin 3; rs76191705; P = 1.80e-08; MAF = 0.033) genes with symptom improvement (Fabbri et al. 2018) by using MAGMA, a comprehensive gene-set analysis of GWAS data, that determine the combined effect of multiple genetic markers. This association was replicated only for the ITGA9 variant in the PGRN-AMPS (Pharmacogenomic Research Network Antidepressant Medication Pharmacogenomic Study) cohort, and no association was replicated in the NEWMEDS consortium (http://www.newmeds-europe.com) sample. However, neither of these SNPs adhere to the strict definition of rare variants. Xu et al. (2020) identified common variants associated with antidepressant response at a genome-wide significance level ($P < 5 \times 10^{-08}$) at 5 loci (IL1A, GNA15, PPP2CB, PLA2G4C, and GBA; respectively, Interleukin 1A, G protein subunit alpha 15, protein phosphatase 2 catalytic subunit beta, phospholipase A2 group IVC, and glucosylceramidase beta) in Han Chinese patients.

Our results strongly implicate the role of rare functional variants in antidepressant drug response. They also actively support the assumption that antidepressant drug response is a phenotype of significant genetic heterogeneity, since we have been able to identify several rare gene variants that may be associated with remission to antidepressants in Mexican-American individuals. Recent data from our lab supports the assumption that populations of European ancestry have drastically reduced numbers (50% less) of single nucleotide variants and small deletions and insertions in comparison to Mexican-Americans (Wong et al., 2017).

4.1. Limitations

The limitations of this clinical study include: 1) antidepressant blood levels were randomly collected at different times of the day, thus precluding their use as covariates. 2) There was no placebo arm; it was ethically problematic to justify a placebo arm because we used two antidepressant drugs with known efficacies. One week of single-blind placebo lead-in was given to participants, as it had been determined a priori that placebo responders would be removed from the study. The patients knew that they were going to receive one week of placebo at any point during the study; however, only the staff knew that placebo was always given in the first week of the study. 3) Our clinical treatment cohort was small; therefore, further replication studies with larger cohorts are warranted.

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Authorship contribution statement

Ma-Li Wong: Conceptualization, methodology, investigation, network and pathway analyses with table and figure, funding acquisition, supervision and writing - original draft. Mauricio Arcos-Burgos: Genetic statistics analyses with tables and figure, and writing. Sha Liu: Brain expression quantitative trait loci analysis with supplemental table. Alice W. Licinio: Brain expression quantitative trait loci analysis with table. Chenglong Yu: Statistics, writing, review and editing. Eunice W.M. Chin: Writing, review and editing. Wei-Dong Yao: Writing, review and editing. Xin-Yun Lu: Writing, review and editing. Stefan R. Bornstein: Conceptualization, methodology and funding, acquisition. Julio Licinio: Conceptualization, methodology, investigation, funding acquisition, supervision, writing review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests

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Supplementary materials

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