

Heritability of Major Depressive and Comorbid Anxiety Disorders in Multi-Generational Families at High Risk for Depression

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Family studies have shown that MDD is highly transmittable but have not studied its heritability. Twin studies show heritability of about 40% and do not include anxiety disorders. We assessed heritability of MDD and comorbid anxiety disorders in a multigenerational study of family members at high risk for MDD. In addition, we tested the hypothesis that examined clinical subtypes of MDD defined by early and late age of onset would be under relatively stronger genetic control than broadly defined DSM-IV MDD. The first generation with moderate to severe MDD was recruited from an ambulatory psychiatric treatment setting, and their descendants in the second, third, and fourth generation, were interviewed by clinicians up to six times during a 30-year period. Lifetime rates of MDD and anxiety disorders were collected for 545 participants from 65 multigenerational families. The heritability (h^2) of MDD in this high risk sample was estimated at 67%. Anxiety and sequential comorbidity of anxiety disorders and MDD revealed h^2 of 49% and 53%, respectively, and strong positive genetic correlation ($\rho_g = 0.92, P = 7.3 \times 10^{-7}$). Early onset MDD did not appear to be under greater genetic control than broadly defined DSM-IV MDD. Individuals who are direct descendants of subjects ascertained for moderate to severe MDD have strong genetic vulnerability to develop anxiety or MDD. Our findings support family based studies as appropriate and useful design to understand the heritability of common disorders such as MDD.

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INTRODUCTION

Major Depressive Disorder (MDD) is highly familial [Tsuang et al., 1980; Gershon et al., 1982; Weissman et al., 1984, 1993, 2005; Maier et al., 1993]. Twin studies estimated heritability (h^2) to be around 40% in the general population [Sullivan et al., 2000]. Despite the

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enthusiasm generated by large-scale genome-wide association studies for other psychiatric disorders such as schizophrenia, this approach did not yield the same promising results for MDD [Wray et al., 2012; Levinson et al., 2014] until very recently with the first genome-wide significant results in a MDD study of Chinese women [Converge, 2015]. Heterogeneity of the clinical manifestation of the disorder, along with limited sample size (hundred thousands of subjects are required for this type of genetic

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studies), has been indicated as one of the most probable causes of this unexpected failure [Levinson et al., 2014].

Clinical heterogeneity of MDD has been extensively studied over the years [Weissman et al., 1986; Goldberg, 2011]. To overcome this issue, one option is to collect extremely large samples to increase the chances of identifying common subgroups of depressive patients with similar clinical manifestations and genetic configuration. An alternative and possibly complementary approach is to focus on the identification of features that allow reducing the heterogeneity of the clinical manifestations. There are several sources of evidence suggesting that some clinical features might be markers of part of the genetic heterogeneity of MDD. First, different patients with same number of symptoms required for DSM diagnosis display opposite behavior, for example, psychomotor retardation opposed to arousal, hypersomnia opposed to insomnia [Lux and Kendler, 2010]. Second, even within subjects with symptoms in the same range of behaviors, subtypes of depressive symptoms, for example, cognitive or neurovegetative, have been identified [Lux and Kendler, 2010]. Third, more importantly, different subtypes display different heritabilities (see Jang et al. [2004]). Taken together these findings these lead us to hypothesize that more clinically homogeneous subtypes of MDD, for example, defined by more severe clinical course such as early onset or recurrence of multiple episodes, might have a stronger genetic loading than broadly based DSM-IV MDD diagnosis [Kendler et al., 1993, 1999; Levinson et al., 2007]. This hypothesis was corroborated by findings by Olvera et al. [2011] in a community-based sample of multigenerational Mexican-American families.

One approach for understanding the clinical heterogeneity of major depression has been the longitudinal study of biological relatives who directly descend from MDD affected probands [Weissman et al., 1986]. This design has subsequently become known as “high risk design.” This type of study provided two main findings. First, relatives of probands affected by MDD display simultaneously increased prevalence of anxiety and major depressive disorders, that is, comorbidity of the two types of symptomatology [Tsuang et al., 1980; Gershon et al., 1982; Weissman et al., 1984, 1993, 2005; Maier et al., 1993]. Second, longitudinal design allowed the identification of an unusually high rate of early onset anxiety disorders in families at high risk for depression, followed in most cases by onset of depression in adolescence and early adulthood [Weissman et al., 1997, 2005]. Comorbidity of anxiety and major depressive disorders (MDD) is common [Kessler, 1995]. Epidemiological research of anxiety disorders has consistently shown that the occurrence of one anxiety disorder increases up to sixfold the risk of having an additional anxiety disorder [Kessler, 1995]. High comorbidity might be explained by common etiology underlying shared genetic and environmental factors. Common genetic influences underlying both anxiety disorders and MDD have been reported in the general population [Hettema, 2008; Mosing et al., 2009] as well as for anxiety and MDD separately [Kendler et al., 1992, 2007; Roy et al., 1995; Hudziak et al., 2000; Hettema et al., 2001, 2005; Zavos et al., 2010; Franz et al., 2011; Loken et al., 2014], corroborating the idea of common genetic etiology. In addition, several types of evidence suggest that a history of anxiety disorders during childhood might predict or contribute

to depression later in life, introducing the concept of “sequential comorbidity” of anxiety and depression [Bittner et al., 2004]. Whether anxiety disorders cause or simply precede depression remains to be established. Several potential pathways have been proposed [Schleider et al., 2014]. The first posits that anxiety symptoms act as a psychological mediator leading children to think and behave in ways that precipitate the onset of depression [Warner et al., 2008; Schleider et al., 2014]. The second posits that anxiety and major depressive disorders represent a single disorder with common etiology and shared risk factors that trigger anxiety earlier than depression, and that both of these are different manifestations of the same disorder [Schleider et al., 2014].

Taken together, the findings of familial studies demonstrate that anxiety and depressive disorders aggregate in families and are transmitted. Assessment of familial aggregation per se, however, does not enable us to explicitly distinguish the influence of genetic as compared to environmental factors. Although several twin studies have demonstrated the importance of genetic influences contributing to familial aggregation of major depression [Kendler et al., 1995; McGuffin et al., 1996; Lyons et al., 1998; Bierut et al., 1999; Kendler and Prescott, 1999], its heritability has never been estimated in the context of a longitudinal multi-generational study. Inflation of heritability estimates by the effect of environment is less likely to occur with multigenerational families [Falconer and Mackay, 1996; Pettay et al., 2005; Gur et al., 2007]. By increasing possible genetic homogeneity of familial aggregation of major anxiety disorders [Hettema et al., 2001], high-risk designs provide an optimal setting to better understand the extent to which genetic vulnerabilities play a role in different patterns of risk and comorbidity of anxiety and depressive disorders.

We propose to assess the genetic component of familiarity of MDD and anxiety disorders, as well as their sequential comorbidities with early onset anxiety, and early and late onset of MDD in a multigenerational study of family members at high risk for MDD [Weissman et al., 2005]. In addition, we propose to evaluate the genetic correlations between anxiety disorders highly comorbid with MDD to elucidate the relationship underlying their genetic risk.

While estimates of the heritability of MDD and related comorbidity in a high-risk study cannot be extended to the general population, it will provide a framework to identify heritable phenotypes that are more likely to be substantially affected by genetic variation. If patterns of comorbidity and symptom trajectories are associated with genetic liability, which are expected to increase with severity of genetic loading displayed in families at high risk for MDD.

METHODS

Ascertainment

Design and recruitment procedures of the High Risk Study sample are fully described elsewhere [Weissman et al., 2005]. Briefly, the High Risk Study sample is the result of a 30-year longitudinal study designed to investigate transmission of depression from affected parents to their offspring. The study was initiated in 1982 and is now in its sixth wave of data collection. The depressed participants

of the first generation G1 were recruited from an ambulatory treatment setting at Yale University Depression Research Unit (New Haven, CT), where they were receiving pharmacological treatment. They were required to have definite moderate to severe MDD and no lifetime history of other psychiatric illnesses or primary substance use disorders. All participants belonged to the same ethnic group (Caucasian) and had similar social background (predominantly from the Italian and/or Irish community). The families included in this study consist of four generations comprising the original sample probands, defined as G1, their offspring in the second generation, G2, the third generation, G3, and the fourth generation G4. G2, G3, and G4 participants were identified as individuals at high risk for MDD based on the clinical diagnosis of MDD of the G1 probands from which they descend.

Assessment

G1 probands and their spouses as well as G2 and G3 18 years of age or older were directly and independently interviewed with the Schedule for Affective Disorders and Schizophrenia Lifetime (SADS-L) version modified to include DSM-IV criteria [Mannuzza et al., 1986]. For the purpose of this study, we focused on the following diagnoses: Major Depressive Disorder (MDD), anxiety disorders including panic disorder, agoraphobia, generalized anxiety disorder, separation anxiety disorder, posttraumatic stress disorder, obsessive-compulsive disorder, social phobia, specific phobia, and anxiety disorder not otherwise specified (NOS), collectively referred to as “any anxiety disorders.” G2, G3, and G4 younger than 18 years old were interviewed using the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS) Epidemiologic or Present Lifetime [Kaufman et al., 1997; Orvaschel, 2006] and child versions of the Schedule for Affective Disorders and Schizophrenia Lifetime version (SADS-LA) [Endicott and Spitzer, 1978; Mannuzza et al., 1986]. The SADS-LA was used for all subjects aged 18 years or older. These instruments allowed interviewing the children directly as well as the parents about the child. An experienced clinician administered interviews independently and blind to diagnostic status of the parent (and/or grandparent) and the previous assessments.

Diagnosis of G2, G3, and G4 were based on best-estimate procedure [Leckman et al., 1982]. To derive best-estimate diagnoses, an experienced clinician who was not involved in the interviewing, independently and blind to the diagnostic status of the parent (and/or grandparent) and the previous assessments, reviewed all available information and assigned a *DSM-IV* diagnosis for each offspring. Sequential comorbidity of anxiety and major depressive disorders was defined as the temporal sequence of onset of anxiety disorders (any anxiety disorders) followed by a subsequent onset of MDD. Independent analysis of age of onset of MDD was also tested stratifying the sample based on the onset before or after 25 years.

Statistical Genetic Analysis

Pedigree structure descriptive statistics and relative pairs were assessed using PedWiz, a web-based tool for pedigree informatics [Song and Elston, 2013]. Heritability was assessed using variance

component analysis implemented in the software SOLAR [Almasy and Blangero, 1998]. Variance component analysis is the equivalent pedigree-based method of quantitative genetics used for twin studies. This analysis assumes a polygenic model in which a phenotype is determined by the additive effects of multiple unmeasured genes of small effects (polygenes), measured covariates, and unmeasured individual environmental effects. The goal of the analysis is to decompose the phenotypic variance of a trait into its additive genetic (σ_g^2) and non-genetic, environmental, components (σ_e^2). The genetic variance contributing to the total phenotypic variance is defined as heritability (h^2), estimated in the narrow sense as $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$. SOLAR allows the calculation of an additional variance component parameter to account for any shared environment; hereafter defined “household” effect. Covariate effects are modeled as linear fixed effects through the mean function of the phenotype. Significance of heritability is assessed through a likelihood ratio test comparing a model with the heritability parameter included and a model with the heritability parameter constrained to zero.

We estimated heritability in the whole sample including parents (G1), children and grand-children (G2, G3, and G4) conditioning on the proband’s MDD diagnosis and introducing correction for ascertainment bias based on the algorithm implemented in the original program FISHER [Lange et al., 1988]. The goal for the correction of ascertainment bias is to find the correct sampling distribution under the ascertainment bias. The correction is achieved by conditioning the likelihood for each pedigree on the trait values of the pedigree probands. We estimated models using age and gender as covariates (adjusted) and without covariates (unadjusted). In order to evaluate the effect of correction for ascertainment bias, we also estimated heritability in the subgroup of offspring and grand-children (G2, G3, and G4), which corresponds to remove from the analysis both the proband and her/his spouse belonging to G1. The analyses were also repeated without ascertainment bias correction to assess the impact of this correction (data not shown). We also evaluated heritability taking into account the effect of two types of shared environment, or household: (i) having the same mother and (ii) belonging to the same family. For age of onset of MDD, we estimated heritability using the approach previously described in the study by Olvera et al. [2011], which estimated heritability in a sample of multigenerational families similar to ours, with the exception that it was community-based sample. Briefly, this approach consists of two independent analyses. In the first analysis, subjects that do not meet the criterion for onset of depression before 25 years old were coded as unaffected. We referred to this approach as Method 1, which uses the full sample of 545 individuals. In the second analysis, subjects that do not meet the criteria for onset of depression before 25 years old were simply excluded. We referred to this approach as Method 2, which uses a subset of the total sample. We repeated the same approach, including Methods 1 and 2, to estimate the heritability of subjects with onset of depression after 25 years old.

In order to further investigate the genetic basis of comorbidity patterns among highly correlated phenotypes, we performed bivariate analysis, also called bivariate heritability, which yields estimate of the total overlapping genetic and environmental component (correlation) of the traits of interest. A high, positive genetic

correlation would imply that the genetic influences on one trait tend to overlap with the genetic influences on the second trait, independent of the actual magnitude of the genetic influence for either trait. As such, genetic correlation provides an estimate of the proportion of the phenotypic correlation, that is, comorbidity, which is due to shared genes. Two statistical tests are performed to assess the significance of the genetic correlation: (i) to test the independence of the traits in the bivariate analysis (ρ different from 0), and (ii) to test the direction of the correlation (ρ different from 1 or -1).

RESULTS

A total of 545 participants from 65 multigenerational families at high risk for MDD from the High Risk Study were included in the analysis. There are 65 probands and 479 non-proband subjects including members of G1, G2, G3, and G4 as well as their spouses. The mean number of participants per family was 8.3, with a total of 278 females (51%) in HR families. The 544 individuals for this study included the following types of relatives: 678 parent-offspring, 338 full siblings, 14 half sib-pairs, 186 cousin pairs, 270 grandparent-grandchild and 417 avuncular pairs.

Two hundred forty-five individuals (44.95%) of the sample met criteria for MDD based on DSM-IV, of which 61% were female. Fifty-four percent of female subjects had a diagnosis of MDD compared to 35% of male participants ($\chi^2 = 19.05$, $df = 1$, $P < 10^{-4}$). Two hundred seventy-five individuals (50.46%) of the sample met criteria for diagnosis of any anxiety disorders. Fifty-nine percent of female subjects had a diagnosis of any anxiety disorders compared to 41% of male subjects ($\chi^2 = 18.35$, $df = 1$, $P < 10^{-4}$). One hundred fifty-nine individuals (29.17%) of the sample met the criteria for sequential comorbidity of any anxiety and MDD. Only 27 individuals (5%) of the sample met criteria

for onset of MDD followed by onset of any anxiety disorders. Thirty-seven percent of female subjects compared to 20% of male subjects had diagnosis of sequential comorbidity. One hundred sixty individuals (29.36%) of the sample met criteria for early onset of MDD and 85 individuals (15%) for late onset of MDD. For further details on the study, we refer the reader to the original article by Weissman et al. [2005].

The heritabilities of MDD, anxiety disorders, and sequential comorbidity of anxiety and depression unadjusted and adjusted for age and gender are presented in Table I. Without adjustment for any covariates, heritability of MDD was 68%. Adjustment for age and gender yielded an estimate of heritability of 67%. Table I also reports the heritability estimated without inclusion of probands and their spouses (i.e., the first generation, G1). Heritability of MDD in the offspring generations (without G1 participants) was 56%. In an effort to elucidate the impact of shared environment compared to non-shared environment we repeated the analysis adjusting for two different household effects of having the same mother and belong to the same family (data not shown). Heritability estimates were substantially unchanged and the household effects negligible.

Heritability of anxiety disorders was 50% in the unadjusted model and 49% after adjustment for age and gender. In the offspring generations, heritability of anxiety disorders was estimated at 56% as for MDD in the offspring generations. Heritability of sequential comorbidity of anxiety disorders and major depressive disorders was 57% in the unadjusted model, 53% when adjusting for age and gender, and 57% in the offspring generations, the same estimate obtained with the unadjusted model.

Anxiety and MDD liabilities showed very high positive genetic correlation ($\rho_{g} = 0.92$, $P = 7.3 \times 10^{-7}$), which suggests that the vast part of the phenotypic correlation between these traits is due to common genetic factors.

TABLE I. Heritability (h^2) of Major Depressive Disorder, Any Anxiety Disorders, and Sequential Comorbidity of Any Anxiety and Major Depressive Disorders in the Sample of Subjects at High Risk for Major Depression

Sample	Model*	Major depressive disorder			
		N	h^2	SE	P (**)
Proband and offspring	Unadjusted	545	0.68	0.120	<0.00001
Proband and offspring	Adjusted for age and gender	545	0.67	0.152	<0.00001
Offspring only	Adjusted for age and gender	412	0.56	0.175	<0.001
		Any anxiety disorders			
Proband and offspring	Unadjusted	545	0.5	0.120	<0.001
Proband and offspring	Adjusted for age and gender	545	0.49	0.134	<0.001
Offspring only	Adjusted for age and gender	412	0.56	0.163	<0.001
		Sequential comorbidity of any anxiety and major depressive disorder			
Proband and offspring	Unadjusted	545	0.57	0.131	<0.001
Proband and offspring	Adjusted for age and gender	545	0.53	0.145	<0.001
Offspring only	Adjusted for age and gender	412	0.57	0.184	<0.001

Heritability estimates are reported for the sample including probands (G1) and their descendants (G2–G4) without and with adjustment for age and gender and in the sample including only the descendant's generations (G2–G4).

*All models of analyses including parent and offspring were adjusted for bias ascertainment.

**Likelihood ratio test for testing the significance of an additive genetic effect.

Heritability of early onset of MDD (<27 years old) ranged between 25% and 30% depending on whether the model was unadjusted, adjusted for gender or for age and gender (Table II) using Method 1, which codes as unaffected subjects that do not meet criteria for early onset of MDD. The application of Method 2, which excludes subjects that do not meet criteria for early onset of MDD, yielded an estimate of heritability ranging between 59% and 62% depending on whether the model was unadjusted, adjusted for gender or for age and gender (Table II). The same analytical strategy was implemented for late onset of MDD (>27 years old). Method 1 yielded estimate of heritability of late onset of MDD ranging between 14% and 31%, while Method 2 ranging between 26% and 46% depending on whether the model was unadjusted, adjusted for gender or for age and gender (Table II). For late onset MDD, the inclusion of age as covariate in addition to gender yielded the highest heritability estimates both for Methods 1 and 2 with 31% and 46%, respectively.

DISCUSSION

We examined the heritability of MDD and anxiety disorders along with sequential comorbidity of early onset anxiety and MDD, and early and late onset of MDD in families ascertained through probands with a diagnosis of MDD. This is the first multi-generational family study that examines and demonstrates heritability of MDD in offspring at high risk for depression.

The adjusted heritability of MDD is 67% after constraining on proband ascertainment, which was modeled to allow an unbiased estimation of parameters relevant to the general population. This estimate is larger than the heritability estimate of 30–40% in the general population [Sullivan et al., 2000], although this reflects only a point estimate difference, but confidence intervals are likely to overlap. Differences between heritability rates found in this and previous studies may be attributed to study design. First, high MDD heritability may be due to the inclusion of G1 individuals

who had definite moderate to severe MDD. Second, it might be related to the uniformity of the exposure to the same familial environment in participants at high risk for depression. This ultimately might have decreased the proportion of phenotypic variation attributable to environment, while increasing the estimated genetic component. When we further tested whether there was a significant effect of shared environment, we noticed that heritability estimated did not vary. This is consistent with a great body of the literature that reported consistently that shared environment does not account for much of the variance not even in twin studies [Plomin, 2011]. To further investigate the effect of the high-risk design through ascertainment of MDD probands on the estimate of heritability, we set out to estimate the heritability in the generations of descendants of MDD probands independently. We hypothesized that the heritability would be lower as we are substantially removing the bulk of the genetic load carried by the first generation responsible for the transmissibility of the disorder. An alternative is also that phenotypic variance in offspring at risk is more likely explained by environment effects. We found that heritability of MDD in the descendants of MDD probands decreases from 67% to 56%. In the absence of direct measurement of the individual or shared environment, all these findings suggest that MDD heritability is high in families at risk for depression and that the effect of environment may be more relevant at younger ages.

Given the high degree of comorbidity between anxiety disorders and MDD observed in the High Risk Study sample, we estimated the bivariate heritability or genetic correlation between these two traits. The observed significant genetic correlation of 92% supports the hypothesis that anxiety and MDD are part of the same genetic liability that comprehensively captures the dimension of emotional/behavioral disorders characterized by internal suffering and includes MDD, generalized anxiety disorders, and phobias [Kendler et al., 2003]. This finding is consistent with evidence from twin studies that support common genetic influences underlying the

TABLE II. Heritability (h^2) of Age of Onset of Major Depressive Disorder Before 25 Years Old and After 25 Years Old in the Sample of Subjects at High Risk for Major Depression

		Age of onset of major depressive disorder <25 years old							
		Method 1				Method 2			
Sample	Model*	N	h^2	SE	P (**)	N	h^2	SE	P (**)
Proband and offspring	Unadjusted	545	0.29	0.118	0.0026	460	0.59	0.174	4.6×10^{-5}
Proband and offspring	Adjusted for age and gender	545	0.25	0.119	0.007	460	0.59	0.226	7.4×10^{-5}
Proband and offspring	Adjusted for gender	545	0.30	0.122	0.002	460	0.62	0.165	1.8×10^{-5}
		Age of onset of major depressive disorder >25 years old							
Proband and offspring	Unadjusted	545	0.14	0.124	0.1069	385	0.26	0.166	0.0422
Proband and offspring	Adjusted for age and gender	545	0.31	0.181	0.0293	385	0.46	0.236	0.0215
Proband and offspring	Adjusted for gender	545	0.15	0.1274	0.0974	385	0.28	0.169	0.0371

Heritability estimates are reported for the sample including probands (G1) and their descendants (G2–G4) without and with adjustment for age and gender and gender only.

*All models of analyses including parent and offspring were adjusted for bias ascertainment.

** Likelihood ratio test for testing the significance of an additive genetic effect.

genetic architecture of anxiety and depression, with genetic correlation estimated to vary between 0.86 and 1.00 [Kendler et al., 1992, 2007; Roy et al., 1995; Thapar and McGuffin, 1997; Eley and Stevenson, 1999; Zavos et al., 2010; Franz et al., 2011]. Overall, our findings are consistent with and further support an early intuition by Kendler et al. that indicated that depression is a highly heritable trait of low reliability, rather than a moderately heritable trait of high reliability [Foley et al., 1998].

Common genetic etiology has been reported consistently, but the effect of environmental etiologic factors might be different along developmental progression from childhood to adulthood. The same stressors might determine anxiety if experienced in childhood, but lead to depression if occurring during adolescence [Garber and Weersing, 2010]. In this study, as previously commented for the heritability of MDD, the environmental exposure is highly homogeneous. This aspect, in addition to the substantial overlap among genetic factors, might explain the very high genetic correlation. This finding further supports the idea to consider these two disorders for the purpose of genetic investigation as two manifestations of the same broad phenotypic category. Consistently, we found that the adjusted heritability of sequential comorbidity of early onset anxiety followed by onset of MDD was between 53% and 57%.

The analysis of onset of MDD revealed strong genetic component underlying early onset of MDD with heritability estimates up to 62%. Heritability of late onset of MDD revealed a lesser degree of genetic contribution in this sample ($h^2 = 46\%$) with respect to early onset of MDD. Both sequential comorbidity of early onset anxiety (<13 years old) followed by onset of MDD and early onset of MDD (<27 years old) rely on the identification of subjects with more severe clinical presentation. In addition, high risk design allows identifying more homogeneous genetic loading underlying MDD phenotypic subgroups due to the exclusion of any other psychiatric disorders in the proband generation. Both of these factors might explain high estimates of heritability of age of onset MDD phenotypes, supporting strong genetic contribution for both early and late onset depression. Although we cannot tease out whether early and late onset are genetically correlated.

Nevertheless, our findings do not support the hypothesis that early onset of MDD has a higher genetic loading than the broad DSM-IV based diagnosis of MDD as previously proposed in several published works [Kendler et al., 1993, 1999; Levinson et al., 2007] and tested more recently by Olvera et al. [2011]. The authors reported that the genetic loading of early onset MDD was higher ($h^2 = 48\%$) in their sample than either MDD ($h^2 = 39\%$) or recurrent MDD ($h^2 = 46\%$). In our high risk sample the broadly defined DSM-IV MDD appeared to be just as heritable ($h^2 = 68\%$) as either early or late onset.

We also investigated a potential bias inherent in longitudinal high risk studies. Different rates of drop-out among the descendants' generations in the high risk group, for example, G2s with depression as compared to those without were more likely to participate in the subsequent waves estimates of transmission of genetic risk and consequently heritability might be biased. We found that, although a higher proportion of depressed G2s participated at almost every wave, the difference was not statistically significant. Therefore, we conclude that our heritability estimates

are not biased by high risk design and are generalizable to the general population with similar patterns of strong familiarity.

This study should be viewed within the context of several limitations. First, we modeled heritabilities as the summed effects of multiple unmeasured genetic risk factors and individual specific environmental effects. The additive genetic variance estimate does not take into account non-additive genetic variance, either derived by dominant genetic effects, gene-environment interactions or gene-gene interactions (epistasis). As a consequence, it is possible that the heritabilities reported do not reflect precisely the true underlying genetics of the phenotypes examined, although data and theory suggest that small proportions of non-additive trait variance are explained by dominant or epistatic effects in complex disorders [Hill et al., 2008; Maki-Tanila and Hill, 2014; Zhu et al., 2015]. We opted not to run further analyses to test non-additive effects as the expected coefficients of these effects in a variance component heritability analysis are small and prone to sampling errors even in study design based on large pedigrees. Second, heritability estimates of disorders that were diagnosed only starting from the second generation, for example, substance use and dependence, might be biased by sample ascertainment, since the first generation was screened and participants excluded from the study for any psychiatric disorder other than MDD. Third, sample size is limited compared to larger genetically informative samples used for previous population-based or family-based analysis, which might have affected our statistical power as reflected in very significant *P*-values and in wide confidence intervals associated with the heritability estimates. Fourth, it should be noted that although point estimates of heritability are higher in our family-based study compared to population-based studies, the confidence intervals most likely overlap, which does not support any statistical claim about our heritability estimate to be significantly different from those obtained using other study designs. Replication in an independent larger family-based study is warranted.

The heritability found in this multigenerational study of subjects at high risk for MDD indicates that the genetic make-up of individuals directly descending from subjects specifically ascertained for moderate to severe MDD confers strong genetic vulnerability to develop either anxiety or major depressive disorders. Furthermore, high genetic correlations of MDD, anxiety disorders, and their sequential comorbidity indicate that a large portion of genetic vulnerabilities is shared and seem to point to a pleiotropic effect of genetic risk factors underlying the genetic vulnerability of both disorders.

The high genetic heritability and shared vulnerability of anxiety disorders and MDD provide a promising phenotype for the identification of single gene effects in this and other populations with phenotypic assessment that include comorbid depressive and anxiety disorders, as illustrated by studies such as the aforementioned MDD GWAS in Chinese women [Converge, 2015], which allowed the inclusion of MDD cases with comorbid anxiety disorders. Even in the absence of actual genotypic data of parental generations, the inclusion in genetic association studies of unrelated depressive patients with comorbid symptoms is expected to increase the power to identify the specific genetic determinants of this specific subtype of MDD. Our findings support the idea that a

phenotype defined by comorbid depressive and anxiety disorders may decrease clinical phenotypic heterogeneity in GWAS of MDD.

Lastly, high-risk designs allowed identifying a trait that is highly heritable as a consequence of increased family aggregation. This further support family-based, and high-risk design, as a powerful tool to identify large effects of rare alleles complementing the power of genome-wide association studies in identifying weak effects of common alleles in large populations of unrelated subjects.

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