SUPPLEMENTAL MATERIAL, ONLINE-ONLY

INVENTORY

e1 -- Participant Ascertainment, Characterization, Image Acquisition & Processing

Participants and Their Ascertainment Those with MDD in G1 were selected from an outpatient clinic for the pharmacologic treatment of depression, and they had moderate to severe MDD with impairment in functioning. The study was initiated in 1982, and the fourth wave of assessments was completed in 2002. The fifth wave, a partial sample of the second and third generation who agreed to MRI scanning, began in 2002 and ended in 2007. Full methodological details for the assessments performed at wave 1 (baseline), wave 2 (year 2), wave 3 (year 10), and wave 4 (year 20) follow-ups have been described previously.[1,2] Assessment procedures were kept similar across the waves, with few exceptions, to avoid introducing bias from method variation. G1 participants and their spouses, offspring, and grandchildren (if applicable) were interviewed independently, with the interviewers blind to the clinical status of participants in the previous generations. After Wave 2, the spouses of two participants in the G1 control group developed a first major depression, as determined by an independent best-estimate diagnostic procedure undertaken by clinicians blind to data from other members of the cohort. These 2 spouses and their 4 offspring were reassigned to the depressed group in G1. We did not remove or reassign group membership for any participants in Generation 2 (G2) or Generation 3 (G3) if they developed any clinical disorders, because their diagnoses were the study outcomes.

Wave 1 and 2 assessments were approved by the Human Investigation Committee at Yale University School of Medicine. Wave 3 and 4 assessments were approved by the Institutional Review Board at New York State Psychiatric Institute/Columbia University. The fifth wave was approved by both of these review boards, as the MRI scanning was conducted at Yale. Written informed consent was obtained at each wave, and children provided written assent. Inclusion criteria for each of the five waves of assessment was age 6 years or over, and absence of psychotic symptoms or other extremely severe symptoms that would interfere with the capacity to provide informed consent to participate. Entry into the MRI study in Wave 5 in addition required a negative pregnancy test for females who were post-menarchal and pre-menopausal, and the absence of ferromagnetic implants.

G2 participants in Wave1 assessments consisted of 220 offspring, from 91 G1 families, who were between 6 and 23 years of age. These participants were reassessed in Waves 2, 3, and 4. Additional G2 members who were assessed in these subsequent waves were offspring who did not meet age criteria in previous waves but did meet them subsequently, as well as those who refused assessment in previous waves but agreed in subsequent waves. With these additions, the augmented total G2 sample available at Wave 5 was 256. The G3 participants available at Wave 5 consisted of 211 biological grandchildren of G1 members, of whom 157 had been assessed at Waves 3 or 4.

We identified the participants at "high risk" for developing MDD as those members of G2 and G3 who were biological descendants of the MDD group in G1 and those at "low risk" as the G2 and G3 biological descendants of the unaffected control group in G1. Initially, the sample targeted for MRI scanning in Wave 5 consisted of G2 members who had been interviewed in Wave 4 and G3 members who had been interviewed in Waves 3 or 4, and who were the biological offspring of G1 members. These Waves were selected to provide the most current and complete diagnostic information possible. This sample consisted of 196 G2 members who were interviewed in Wave 4 and 157 G3 members who were interviewed in either of Waves 3 or 4, totaling 353 individuals descended from 76 distinct G1 families (83.5% of all 91 G1 families). Of this target sample, 266 (75.3%) were located and 153 (57.5%) gave written consent to be scanned. Of these 153 who consented, 150 were actually scanned. In addition to this initially targeted sample, we also recruited from eligible G2 and G3 members those who were biological offspring of G1 members but who had not been interviewed in Wave 4 (for G2) or in either Wave 3 or 4 (G3). This sample consisted of 60 G2 and 54 G3 members, totaling 114 eligible participants descended from 57 distinct families of G1. Of these, 78 were located, 74 (65%) provided written consent to be scanned, and 24 (34%) actually were scanned (the number scanned being limited by available funding). The combined sample therefore consisted of 174 participants from G2 and G3 who were scanned. Of these 174 scanned participants, we obtained usable functional MRI scans in 143 individuals, ages 7 to 54 years, from 58 different families. The HR group contained 83 individuals (comprising 14 children, defined as younger than

18 years of age, and 69 adults). The LR group contained 60 individuals (26 children, 34 adults) (Main Text Table 1). Functional MRI scans were attempted but unsuccessful in another 20 individuals, 10 of whom were in the LR group (8 children, 2 adults) and 10 in the LR group (9 children, 1 adult). For technical reasons, fMRI scans were not attempted in an additional 5 individuals from the HR group and 4 from the LR group.

Additional Assessments and Best-Estimate Diagnostic Procedures

Wave 5 assessments also included the DuPaul-Barkeley ADHD rating scale, the Children's Depression Rating Scale-Revised (CDRS-R) for children or the Hamilton Depression Rating Scale (HAM-D) for adults, and the Revised Children's Manifest Anxiety Scale (RCMAS) for children or Hamilton Anxiety Rating Scale (HAM-A) in adults. We constructed an index of the severity of either depressive or anxiety symptoms across children and adults for use in correlation analyses by converting the respective measure in each age group into a Z-score for each participant and then combining those Z-scores across age groups into a single variable for each symptom domain. Thus, "Z-depression" was constructed using Z-scores from the CDRS-R and HAM-D, and "Z-anxiety" was constructed using Z-scores from the RCMAS and HAM-A. The Global Assessment Scale assessed functional impairment at the time of scan.

The diagnostic assessments were administered by clinically experienced masters- and doctoral-level mental health professionals who had been trained in the specific study assessments and who were blind both to the clinical status of members of the prior generations and to the previous assessments of the participant being interviewed. Multiple sources of information were obtained. The final diagnosis for all participants was based on a best-estimate procedure that involved an independent review of all assessment materials by two experienced clinicians, a child psychiatrist and a psychologist, who were not involved in the interviewing and who were blind to the diagnostic status of the previous generations and prior assessments.[3]

At Wave 4, materials from 178 participants randomly selected from all generations were co-rated by the two diagnosticians. Inter-rater Kappa reliability scores were good to excellent (MDD, 0.82; dysthymia, 0.89; anxiety disorder, 0.65; alcohol abuse/dependency, 0.94; and drug abuse/dependency, 1.00). At Wave

5, an additional 24 cases were randomly selected and co-rated, with comparable results. Diagnoses were cumulative across all waves. DSM-IV diagnoses at the probable or definite level of certainty were used for the G3 members because of their young age, and diagnoses at the definite level were used for G1 and G2 members.

Image Acquisition Images were acquired over 4 years, with full interleaving across risk groups, on a single Siemens Sonata 1.5 Tesla scanner (Siemens, Germany) equipped with a quadrature head coil. Functional images were acquired using a single shot gradient echo planar pulse sequence with TR=1650ms, TE=30ms, flip angle=90°, matrix=64x64, field of view=20cmx20cm, slice thickness=8mm, skip=0.5mm, receiver bandwidth=62.5 kHz, in-plane resolution=3.125mmx3.125mm, 16 axial slices per volume and 102 volumes per run. We acquired 10 runs for each participant, each run lasting 2.97 minutes.

The fMRI Task The Simon Spatial Incompatibility task belongs to a general class of neuropsychological interference tasks, including the Stroop, in which reaction times are slowed when irrelevant features of the task are inconsistent with task-relevant ones.[4] Reaction times in the Simon task are slowed when the target stimulus feature (the direction in which an arrow points) conflicts with the (task-irrelevant) spatial location of the arrow (the side of the screen on which the arrow appears). Because reaction times are generally faster when responding to the spatial location of an arrow than when responding to the direction of the arrow, responses to spatial location are considered to be more automatic than are responses to direction. The need to respond to the direction of a rightward pointing arrow presented on the left side of the screen therefore requires inhibition of the more automatic response in order to provide a correct, less automatic response, a requirement widely regarded as representing the exertion of self-regulatory control over conflicting stimulus features and response tendencies and as relying upon the mobilization of broadly distributed, top-down control systems in the brain,[5] thereby distinguishing this class of self-regulatory interference tasks from Continuous Performance Tasks, which require only sufficient vigilance and attention to respond correctly to one among two or more possible stimuli within a stimulus train.

Image Acquisition Head positioning in the magnet was standardized using the canthomeatal line. Images were acquired over a span of 4 years, with full interleaving across risk groups. A T_1-weighted sagittal localizing scan was used to position the axial functional images parallel to anterior commissure-posterior commissure line. In all participants, a 3D spoiled gradient recall image was acquired for co-registration with the axial functional images and with the MNI (Montreal Neurological Institute) coordinate system.

Behavioral Data Analysis Reaction times (RTs) and accuracy scores on each trial of the Simon task were entered as dependent variables in separate repeated measures, linear mixed models in SAS version 9.2 (SAS Institute Inc, Carey, NC) with risk group (high, low), stimulus congruence (incongruent, congruent), age, sex, and run number (0-10) included as independent variables. The difference in performance (RTs or accuracy) on congruent and incongruent trials between risk groups was assessed by the statistical significance of the group-by-stimulus interaction. We used an ANCOVA to assess the differences across groups in interference scores, calculated as the difference in mean RTs during correct performance on the incongruent and congruent stimulus trials. Post-error adjustment in performance was also calculated for both groups as the difference of (1) the mean RT on trials immediately following erroneous responses to conflict trials, and (2) the mean RT on trials immediately following correct responses to conflict trials.[6,7] These subsequent trials were always congruent stimuli because incongruent trials were never preceded by incongruent trials in this implementation of the Simon task. We also implemented these same models in a subset of the cohort that had no lifetime history of MDD or anxiety disorder to determine the behavioral profile on the task that is trait-related, rather than possibly related to a previous or current history of illness.

Image Preprocessing We ran in batch mode a preprocessing software package running under MATLAB (Mathworks, Natick, MA, USA) that was developed in-house by integrating processing functions from SPM http://www.fil.ion.ucl.ac.uk/spm/) and FSL(http://www.fmrib.ox.ac.uk/fsl/) for the preprocessing of functional images. Slice timing was corrected using the middle slice as the timing reference. Slice timing-corrected functional images were then realigned to the middle image of the middle run for motion correction. Images with motion greater than one voxel were excluded from all subsequent analyses. Motion-

corrected images of each participant were co-registered to the corresponding T1-weighted high-resolution anatomical image, which in turn was spatially normalized to the MNI template ICBM152 with voxel dimensions of $3x3x3mm^3$. The normalization parameters were then used to warp the functional images into the same MNI template. A spatial smoother with a Gaussian kernel of 8 mm Full Width at Half Maximum was applied to the functional images, which were then temporally filtered using a Discrete Cosine Transform high-pass filter with a cutoff frequency of 1/128Hz to remove low frequency noise such as scanner drift.

Image Analysis A first-level analysis used a General Linear Model with a weighted least-squares algorithm to model the fMRI time series for each participant. A second-level analysis detected functional activation associated with the task within and between groups using a Bayesian-based group analysis.[8,9] We also conducted various regressions that correlated the first-level beta estimates with behavioral and clinical data. All analyses were performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) operating under MATLAB 2009B.

First-Level In each design matrix, except the constant, we had 6 independent functions (regressors), that represented the trial types of Fixation, Congruent Correct (congruent trials were performed correctly), Incongruent Correct (incongruent trials were performed correctly), Congruent Incorrect (congruent trials were performed incorrectly), Incongruent Incorrect (incongruent trials were performed incorrectly), and Missing Response (participants did not respond). We obtained each of these regressors by convolving the boxcar function derived from the onset and duration of the corresponding condition with a canonical hemodynamic response function. We removed the serial correlations in the fMRI time series and the regressors of the design matrix simultaneously using the first-order autoregressive model and the Restricted Maximum Likelihood algorithm. Different t-contrasts of interest, such as Incongruent Correct vs. Congruent Correct (interference effects) and Incongruent Incorrect vs. Incongruent Correct (error-related activity) were then used in second-level analyses.

Second-Level Analyses: Identification of Risk, Resilience and Illness Effects In the masking operations use for second-level analyses, we used spatial clustering to avoid edge artifacts, removing cluster by cluster the effect X (e.g., Risk effects) from effect Y (e.g., Resilience effects), as follows: Let Cluster-X denote a significant cluster of voxels for effect X, and let Cluster-Y denote a significant cluster of voxels for effect Y. When Cluster-X and Cluster-Y had the same sign and the total number of significant voxels in Cluster-Y overlapped in space with more than 50% of the significant voxels in Cluster-X, we removed Cluster-Y from the map for effect Y. In all other cases, we removed from effect Y all the voxels where Cluster-X and Cluster-Y were both significant.

e2 – Main Findings for All Slices and Activation Table

Supplement eFigures 1 & 2: All Slices Acquired for All Participants

The risk, resilience, and lifetime illness effects identified in brain activation during the task are shown in all slices acquired while covarying for age, sex, and interference scores in 143 participants (83 HR, 60 LR) (Supplement eFigures 1-2).

eFigure 1, Panels 1-2: Interference effects

eFigure 2: Error-related activity

Abbreviations: ***dACC*** dorsal anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MFG*** middle frontal gyrus; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***pgACC*** pregenual anterior cingulate cortex; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus; ***vACC*** ventral anterior cingulate cortex; ***Thal*** thalamus; ***MGB*** medial geniculate body; ***Hippo*** hippocampus; ***Caud*** caudate; ***CerV*** cerebellar vermis.

Supplemental eTable 1: The locations of activation for risk, resilience, and lifetime illness effects for all slices acquired and all participants while covarying for age, sex, and interference scores

Activated Area	Location		Peak location			Z
	Side	BA	x	y	z	statistic
All Participants HR vs LR: Interference						
Insula	L	13	-30	24	3	+5.77
	R	13	51	12	-3	+5.42
Middle Temporal Gyrus	R	21	63	-48	3	+3.82
Superior Temporal Gyrus	R	22	60	-51	6	+3.92
	L	39	-54	-57	27	+2.92
Lateral Prefrontal Cortex	L	46	-43	41	16	+4.59
	R	46	43	42	15	+2.39
Superior Parietal Lobule	L	7	-13	-70	40	+4.91
	R	7	15	-66	57	+4.32
Precuneus	L	7	-12	-69	33	+5.07
Parahippocampus (or Retrosplenial Cingulate Cortex)	L	29	-6	-48	9	-4.51
Cerebellar Vermis (or Retrosplenial Cingulate Cortex)	L	29	-4	-49	5	-4.11
Caudate Nucleus	R	NA	9	0	15	-2.68
Risk Effects: Interference						
Insula	L	13	-30	24	3	+5.53
Superior Temporal Gyrus	R	13	48	-48	18	+4.57
Lateral Prefrontal Cortex	L	46	-43	41	16	+5.11
	R	46	42	41	6	+2.89
Superior Parietal Lobule	R	7	33	-63	54	+3.84
Precuneus	L	7	-12	-69	30	+5.60
Posterior Cingulate Cortex	R	31	18	-60	21	+4.10
Resilience Effects: Interference						
Middle Temporal Gyrus	R	21	57	-57	0	+3.46
Dorsolateral Prefrontal Cortex	R	9	33	36	30	+3.91
	L	9	-36	33	36	+3.35
Pregenual Anterior Cingulate Cortex	R	33	3	21	21	+3.84
Dorsal Anterior Cingulate Cortex	R	32	7	15	35	+3.27
Superior Frontal Gyrus	R	10	3	57	15	+3.29
Resilience Effects: Error-Related						
Insula	R	13	47	18	-6	+4.25
	L	13	-39	17	-2	+2.46
Pregenual Anterior Cingulate Cortex	L	33	-5	19	23	+2.37
Ventral Anterior Cingulate Cortex	R	24	3	40	8	+2.49
Lifetime Illness Main Effect: Interference						
Dorsolateral Prefrontal Cortex	R	9	30	36	35	-3.30
	L	9	-33	33	30	-3.08
Posterior Cingulate Cortex	L	31	0	-36	33	-6.13
Middle Frontal Gyrus	R	8	27	33	45	-3.75
	L	8	-24	30	48	-3.44
Inferior Parietal Lobule	L	40	-36	-54	42	-3.96
	R	40	45	-54	45	-4.35
Parahippocampus (or Retrosplenial Cingulate)	L	30	-12	-54	12	-3.56
Thalamus	L	NA	-3	-21	0	-3.82
Medial Geniculate Body (or Retrosplenial Cingulate)	L	29	-6	-39	6	-3.30
Risk Group-By-Lifetime Illness Interaction: Interference						
Insula	R	13	42	15	-6	-3.85

Hippocampus	L	NA	-18	-39	-3	+2.77
Parahippocampus (or Retrosplenial Cingulate Cortex)	L	29	-12	-51	6	+3.60
Caudate Nucleus	R	NA	12	9	9	-3.88
Ventral Anterior Cingulate Cortex	L	24	-3	42	9	-3.76
Pregenual Anterior Cingulate Cortex	R	33	3	21	21	-3.12
Dorsal Anterior Cingulate Cortex	R	32	9	14	37	-2.95
Superior Frontal Gyrus	R	9	0	48	15	-3.39
Superior Temporal Gyrus	R	40	63	-33	24	-4.10
Lateral Prefrontal Cortex	L	46	-48	27	21	+5.18
	R	46	48	33	15	+3.37
Dorsolateral Prefrontal Cortex	L	9	36	33	36	-3.95
	R	9	-33	33	36	-3.26
Inferior Parietal Lobule	L	40	-48	-42	36	+3.40
Superior Parietal Lobule	L	7	-28	-61	44	+2.22
Posterior Cingulate Cortex	L	23	-3	-48	21	-2.91
HR-Specific Illness Effects: Error-Related						
Insula	R	13	47	17	-6	-4.44
Ventral Anterior Cingulate Cortex	R	24	3	45	0	-4.08
Pregenual Anterior Cingulate Cortex	L	33	-1	23	19	-2.29
Dorsal Anterior Cingulate Cortex	L	32	0	33	30	-2.92
Superior Temporal Gyrus	R	22	63	-42	21	-3.37

NA = Not applicable

All coordinates are in the Montreal Neurological Institute ICBM 152 template.

e3 – Identifying Endophenotypes for Lifetime MDD and Anxiety Disorders Separately

We reran our primary analyses while accounting for the effects of lifetime MDD and anxiety disorder separately. In one analysis we identified risk, resilience, and illness effects for MDD while covarying for the presence of any lifetime anxiety disorder, and in another we identified these for any lifetime anxiety disorder while covarying for lifetime MDD. Our findings were unchanged for both analyses from our primary analyses in which we combined lifetime MDD and anxiety disorders into a single variable. The exception was substantially attenuated lifetime illness effects for anxiety disorder (Supplemental eFigure 3). The overall similarity in findings across these analyses suggests that risk and resilience endophenotypes are similar for MDD and anxiety disorders, providing further justification for combining MDD and anxiety disorders in our primary analyses.

Supplement eFigure 3, Panels 1-3: Assessing Risk, Resilience, and Illness Effects for MDD and Anxiety Disorder Separately Risk, resilience, and illness effects were identified using regression analyses while ccovarying for age, sex, and interference effects

"MDD" Column: covarying for 62 Participants (44 HR, 18 LR) who had a lifetime history of anxiety disorder

"Anxiety" Column: covarying for 59 Participants (47 HR, 12 LR) who had a lifetime history of MDD

Abbreviations: ***dACC*** dorsal anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MFG*** middle frontal gyrus; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***pgACC*** pregenual anterior cingulate cortex; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus.

e4 – Covarying for Generation 2 or 3

Supplement eFigures 4 & 5: Findings while Covarying for Generation 2 or 3

Activations did not change appreciably when covarying for generation 2 or 3 ("wG", where the column without the label "wG" shows findings without covarying for generation) when all participants were included (N=143, 83 HR, 60 LR) (Supplement eFigures 4-5). Age, sex, and interference scores were also included as covariates.

Abbreviations: ***dACC*** dorsal anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MFG*** middle frontal gyrus; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***pgACC*** pregenual anterior cingulate cortex; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus; ***vACC*** ventral anterior cingulate cortex; ***Thal*** thalamus; ***MGB*** medial geniculate body; ***Hippo*** hippocampus; ***Caud*** caudate; ***CerV*** cerebellar vermis.

e5 – Use of Psychotropic Medications

Excluding the 25 participants (HR=20, LR=5) who were taking a psychotropic medication at the time of scan while covarying for age, sex, and interference effects (Supplement eTable 2) had little effect on our findings, although the lifetime illness effects were somewhat weakened by removing this large number of people who were or had been recently ill (Supplement eFigure 6).

Supplement eTable 2: Psychotropic Medication Use at the Time of MRI Scanning

CURRENT MEDICATION (n=25)	Anticonvulsant/ Mood Stabilizer	Antidepressant					Benzodiazepine	Stimulant
		SSRI	NRI	Tricyclics	Atypical	(Subtotal)		
High Risk (N=20)	5	9	1	3	3	16	4	2
Low Risk (N=5)	0	4	1	0	0	5	1	0
Total	5	13	2	3	3	21	5	2

Supplement eFigure 6: Findings when Excluding 25 Participants (N=20 HR, N=5 LR) Taking Psychotropic Medications at the Time of Scan

Risk, resilience, and illness effects were identified using regression analyses while covarying for age, sex, and interference effects

Abbreviations: ***dACC*** dorsal anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MFG*** middle frontal gyrus; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***pgACC*** pregenual anterior cingulate cortex; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus.

e6 – Participants with a History of Substance Abuse

Excluding the 34 participants (HR=21, LR=13) who had a lifetime history of abusing or dependence on either drugs (N=23; HR 17, LR 6) or alcohol (N=24; HR 24, LR 8) when covarying for age, sex, and interference effects had little effect on our findings (Supplement eFigure 7).

Supplement eFigure 7, Panels 1-10: Findings when Excluding Participants Who Had a Lifetime History of Abuse or Dependence on Either Drugs or Alcohol Risk, resilience, and illness effects were identified using regression analyses while covarying for age, sex, and interference effects

Lifetime history of abuse or dependence on either drugs or alcohol: N=34 (HR=21, LR=13)

Lifetime history of abuse or dependence on drugs: N=23 (HR 17, LR 6)

Lifetime history of abuse or dependence on alcohol: N=24 (HR 16, LR 8)

<u>Covarying for alcohol or drug history</u>

Panels 1-3: Interference-Related Activity for Risk Effects (panel 1), Resilience Effects (panel 2), and Lifetime Illness Effects (panel 3)

Panels 4-5: Error-Related Activity for Resilience Effects (panel 4) and Lifetime Illness Effects in the HR group only (panel 5)

<u>Excluding participants with alcohol or drug history</u>

Panels 6-8: Interference-Related Activity for Risk Effects (panel 6), Resilience Effects (panel 7), and Lifetime Illness Effects (panel 8)

Panels 9-10: Error-Related Activity for Resilience Effects (panel 9) and Lifetime Illness Effects in the HR group only (panel 10)

Abbreviations: ***dACC*** dorsal anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MFG*** middle frontal gyrus; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***pgACC*** pregenual anterior cingulate cortex; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus; ***vACC*** ventral anterior cingulate cortex.

e7 – Assessing the Imbalance in Sex Distribution Across Illness Groups as a Potential Confound

As expected from the population-based preponderance of females who have MDD or anxiety disorder, members of the HR group who had a lifetime history of MDD or anxiety disorder were predominantly female (40 of 61, 66%), compared with the HR group who did not have lifetime illness (6 of 22, 27%). Although we covaried for sex in all of our analyses, we conducted additional analyses that ensured this imbalance in sex distribution was not responsible for our findings. Eliminating all 80 females from the analysis would leave a total sample of N=63 and would reduce statistical power greatly, making it difficult to assess whether differences in our findings are attributable to differences in statistical power or to the elimination of sex effects from the findings. Therefore, we have adopted a 2-step approach to assessing the possible confound of sex effects on our findings:

1. We selected a random subgroup of 6 females from the "HR with History of MDD/Anx" group to match demographically the 6 females in the "HR No MDD/Anx" group and then ran the regressions to identify each of the 3 endophenotypes while covarying for age and interference effects. This left us with a sample of 109 participants (49 HR, 60 LR). We repeated this random sampling 9 times, yielding a total of 10 maps for each of the endophenotypes. We averaged these 10 maps and compared them to our original findings and found them to be unchanged (Supplement eFigure 8, middle column). This analysis demonstrated quite conclusively that sex effects were not responsible for our findings.
2. We eliminated all females from our analysis, leaving a total of N=63 males, reran the analyses while covarying for age and interference effects, and compared the findings with the original. This analysis, as expected because of the reduce statistical power, yielded statistically weaker effects, but their overall pattern was similar to the pattern of activations in the full sample for the risk and lifetime illness effects, although the resilience effects did not reach the level of statistical significance (Supplement eFigure 8, right-most column).

Supplement eFigure 8: Addressing Imbalance in Sex Distribution Identification of risk (panel 1), resilience (panel 2), and lifetime illness (panel 3) effects while covarying for age and interference effects

Abbreviations: ***dACC*** dorsal anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MFG*** middle frontal gyrus; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***pgACC*** pregenual anterior cingulate cortex; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus.

e8 – Participants Older than 25 Years

Analyses identifying risk, resilience, and lifetime illness effects were conducted for participants who were 25 years of age or older (N=84; 58 HR, 26 LR) while covarying for age, sex, and interference effects so as to ensure that they had passed through the age of maximum risk for new onset MDD. Findings in general were statistically even more significant than when including all participants in the analyses, despite the lower statistical power to detect real effects associated with fewer participants, probably because the effects were not diluted by those youngest participants who had yet to become ill (Supplement eFigures 9-10). Moreover, the fact that the findings persisted in this all-adult sample in which demographic characteristics were similar across the HR and LR groups (Supplement eTables 3 & 4) indicates that differences in age and developmental stage in the overall sample are not confounding our analyses and findings.

Supplement eFigures 9 & 10: Findings when including only participants 25 years of age or older

Risk, resilience, and illness effects identified using regression analyses while covarying for age, sex, and interference effects (N=84; 58 HR, 26 LR)

eFigure 9, Panels 1-2: Interference effects

eFigure 10: Error-related activity

Abbreviations: ***ACC*** anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus; ***Thal*** thalamus; ***MGB*** medial geniculate body; ***Caud*** caudate; ***CerV*** cerebellar vermis.

Supplemental Materials Table 3: Demographic and Clinical Characteristics for Participants >25 Years of Age

Characteristic	All LR (N=26)	All HR (N=58)	Test Stat	df	P	LR No MDD/Anx (N=14)	HR No MDD/Anx (N=9)	Test Stat	df	P	LR Lifetime MDD/Anx (N=12)	HR Lifetime MDD/Anx (N=49)	Test Stat	df	P
Age	39.8±5.5	40.3±6.90	T 0.34	82	0.74	39.5±5.6	44.4±5.0	T 2.15	21	0.04	40.2±5.7	39.6±7.0	T -0.23	59	0.79
Z-Score Depression Severity	-0.31±0.71 (N=24)	0.13±1.12 (N=52)	T 1.81	74	0.07	-0.66±0.15 (N=13)	-0.53±0.36 (N=7)	T 1.1	18	0.29	0.10±0.89 (N=11)	0.24±1.17 (N=45)	T 0.39	54	0.70
Z-Score Anxiety Severity	-0.26±1.06 (N=24)	0.08±0.91 (N=52)	T 1.47	74	0.15	-0.69±0.20 (N=13)	-0.52±0.30 (N=7)	T 1.47	18	0.16	0.24±1.41 (N=11)	0.18±0.94 (N=45)	T -0.19	54	0.85
ADHD Severity	4.7±4.1 (N=22)	11.5±7.7 (N=52)	T 3.9	72	0.0002	4.6±2.8 (N=12)	8.6±4.5 (N=8)	T 2.48	18	0.02	4.9±5.4 (N=10)	12.0±8.0 (N=44)	T 2.65	52	0.01
GAS	81.5±6.7 (N=26)	75.1±9.7 (N=56)	T -3.04	80	0.003	85.4±3.7 (N=14)	86.9±2.5 (N=8)	T 1.01	20	0.32	77.0±6.6 (N=12)	73.1±9.1 (N=48)	T -1.38	58	0.17
Sex	F=15 M=11	F=37 M=21	χ^2 0.28	1	0.59	F=6 M=8	F=5 M=4	χ^2 0.35	1	0.55	F=9 M=3	F=32 M=17	χ^2 0.41	1	0.52
Generation	G2=26 G3=0	G2=55 G3=3	χ^2 1.39	1	0.24	G2=14 G3=0	G2=9 G3=0	na	na	na	G2=12 G3=0	G2=46 G3=3	χ^2 0.77	1	0.38
N age >18 years	26	58	**na**	na	na	14	9	na	na	na	12	49	na	na	na
N Lifetime MDD	9	42	χ^2 10.8	1	0.001	0	0	na	na	na	9	42	χ^2 0.81	1	0.37
N Current MDD	0	1	χ^2 0.45	1	0.50	0	0	na	na	na	0	1	χ^2 0.25	1	0.62
N Lifetime Anxiety Disorder	8	33	χ^2 4.9	1	0.03	0	0	na	na	na	8	33	χ^2 0.002	1	0.96
N Current Anxiety Disorder	2	4	χ^2 0.017	1	0.90	0	0	na	na	na	2	4	χ^2 0.79	1	0.38
N Lifetime ADHD	0 (of 22)	11 (of 52)	χ^2 5.47	1	0.02	0 (of 12)	1 (of 8)	χ^2 1.58	1	0.21	0 (of 10)	10 (of 44)	χ^2 2.79	1	0.09

Z-Scores for Severity: We constructed an index of the severity of either depressive (HAM-D) or anxiety (HAM-A) symptoms for use in correlation analyses by converting the respective measure into a Z-score for each participant.

ADHD Severity=Total score on the DuPaul-Barkley Rating Scale

GAS=Global Adjustment Scale

Supplemental Materials Table 4: Group Differences on Task Performance Measures for Participants >25 Years of Age

Measures	Mean(SD)		T	df	P	Mean(SD)		T	df	P	Mean(SD)		T	df	P
	All LR (N=26)	All HR (N=58)				LR No MDD/Anx (N=14)	HR No MDD/Anx (N=9)				LR Lifetime MDD/Anx (N=12)	HR Lifetime MDD/Anx (N=49)			
Reaction Time (msec)															
Incongruent	691.9 (6.4)	676.2 (5.5)	-0.11	82	0.91	711.3 (13.5)	666.9 (16.4)	-1.99	21	0.06	666.2 (11.3)	676.5 (5.8)	0.83	59	0.41
Congruent	493.8 (8.2)	492.7 (5.5)	-1.61	82	0.11	508.7 (13.4)	478.9 (16.4)	-1.33	21	0.20	471.7 (11.3)	493.7 (5.8)	1.78	59	0.08
Interference Reaction Time Effect	198.2 (4.3)	183.5 (2.7)	NA	82	0.005	202.6 (5.2)	188.0 (6.1)	NA	21	0.08	194.5 (6.4)	182.8 (3.2)	NA	59	0.11
Accuracy															
Incongruent	90.6 (0.64)	90.5 (0.4)	-0.13	82	0.90	94.1 (0.7)	93.7 (0.8)	-0.31	21	0.76	86.1 (0.96)	90.0 (0.49)	3.67	59	0.0005
Congruent	99.8 (0.64)	99.7 (0.4)	-0.13	82	0.90	98.9 (0.7)	100.8 (0.8)	1.74	21	0.10	99.7 (0.96)	99.6 (0.49)	-0.13	59	0.90
Interference Accuracy Effect (%)	9.2 (0.88)	9.2 (0.6)	NA	82	0.99	4.8 (0.9)	7.1 (1.1)	NA	21	0.13	13.6 (1.3)	9.6 (0.7)	NA	59	0.008

Values in the table are calculated using the least square means of the mixed model:

Reaction Time (or Accuracy) = age + diagnosis + sex + congruence + run + run*congruence + diagnosis*congruence + diagnosis*run*congruence over 10 runs

"Interference Reaction Time Effect": mean RT for incongruent trials - mean RT for congruent trials

"Interference Accuracy Effect (%)": accuracy during incongruent trials – accuracy during congruent trials, where "accuracy" is defined as percent correct trial responses

Some T-values are not available, because in the mixed model we use the lsmeans for the diagnosis*congruence term to obtain the T-value when congruence is fixed. The P-value for interference RT or accuracy effects are simply the p-value for the diagnosis*congruence term and no T-value are available.

Standard deviations are in parentheses

e9 Excluding Generation and the Genealogical Index of Familiality as Covariates

We ran our primary analyses for interference related-activity when not covarying for either generation (G2 or G3) or for the Genealogical Index of Familiality (GIF) to assess the stability of our findings without these variables in the statistical model. Findings were unchanged from analyses that included generation and GIF as covariates (eFigure 11).

Supplement eFigure 11: Interference-Related Activity without Covarying for Generation (G2 or G3) or the Genealogical Index of Familiality (GIF) Risk, resilience, and illness effects were identified while covarying for age, sex, and interference scores. N=143: 83 HR, 60 LR

e10 – Correlating fMRI Activation with the Degree of Right Hemisphere Cortical Thinning

We correlated fMRI measures of activation with the degree of right hemisphere cortical thinning within the location of the anatomical endophenotype we previously identified in this sample. This analysis required that we compute a single continuous measure for each participant that represented their average cortical thickness over the lateral convexity of the right hemisphere, where group differences in cortical thinning were greatest. We therefore identified all the voxels in template space where group differences over the lateral aspect of the right hemisphere were statistically significant in our prior publication, and then we calculated for each person in our sample the average thickness of the cortical mantle across all those significant voxels in template space. We then used conventional linear regression to correlate this single continuous variable for cortical thickness within the anatomical endophenotype with fMRI measures of brain activation averaged across voxels within regions identified in the map for the risk endophenotype for the 134 participants (78 HR, 56 LR) who had both anatomical and fMRI measures. We did not correct these p-values for multiple comparisons because the analyses were *post hoc*, intended to explore the association between structural and functional endophenotypes, not to test an *a priori* hypothesis. This analysis revealed significant correlations of thickness with activation within the lateral prefrontal and insular cortices in the HR group ($r=-0.26$, $p<0.02$; $r=-0.30$, $p<0.008$) but not in the LR group ($r=-0.04$, $p=0.78$; $r=0.01$, $p=0.93$, respectively) (Supplement eFigure 12), and a similar strong trend for the superior temporal gyrus (HR $r=-0.21$, $p<0.06$; LR $r=0.01$, $p=0.95$), indicating that HR participants who had the greatest degree of right hemisphere cortical thinning also activated most strongly the lateral prefrontal, insular, and superior temporal cortices, important components of the functional endophenotype for risk effects. Activations in the resilience and illness effects maps did not correlate significantly with thickness measures, either in the HR group alone, LR group alone, or in both groups combined. Finally, covarying for cortical thickness in the right hemisphere anatomical endophenotype did not appreciably alter risk, resilience, or lifetime illness effects (Supplement eFigure 13).

Supplement eFigures 12 & 13: Correlation of Activation in the Risk Effects Map with a Measure of Right Hemisphere Cortical Thickness N=134 (78 HR, 56 LR) participants who had both functional and anatomical measures

eFigure 12: scatterplots showing correlations of activation with cortical thickness in the DLPFC and insula in each risk group separately.

eFigure 13: risk, resilience, and lifetime illness effects while covarying for average right hemisphere cortical thickness

Abbreviations: ***dACC*** dorsal anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***IPL*** inferior parietal lobe; ***LPFC*** lateral prefrontal cortex; ***MFG*** middle frontal gyrus; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***pgACC*** pregenual anterior cingulate cortex; ***PH*** parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus.

e11 – Logical Conjunction Analyses

We cross-validated the regression analyses for detecting brain features associated with risk, resilience, and lifetime illness by conducting conjunction analyses on second-level Bayesian maps. We used the minimum of multiple posterior probability maps of group comparisons as their conjunction of effects (equivalent to a logical "AND" operation) across maps within each direction of activation, similar to the strategy used in SPM8 wherein the minimum of multiple statistical parametric maps are detected as their conjunction. Letting "&" denote the conjunction operation and "vs" denote the Bayesian statistical comparison across maps, we detected unique brain features for:

(1) Risk Effects: [HR_{Ill} & $HR_{Healthy}$] vs. [LR_{Ill} & $LR_{Healthy}$];

(2) Resilience Effects: [$HR_{Healthy}$ vs. $LR_{Healthy}$] and masking out Risk Effects (with Risk Effects defined in step 1 above);

(3) Illness Effects: [HR_{Ill} vs. $HR_{Healthy}$] & [LR_{Ill} vs. $LR_{Healthy}$].

We used *a posteriori* probability values >98.75% as the threshold of statistical significance for these conjunction maps. Logical conjunction analyses identified risk, resilience, and illness effects for all participants (N=143, 83 HR, 60 LR) (Supplement eFigure 14) and for participants older than 25 years of age (N=84; 58 HR, 26 LR) (Supplement eFigure 15).

Supplement eFigures 14-15: Logical Conjunction Analyses identifying risk, resilience, and illness effects

eFigure 14 all participants (N=143, 83 HR, 60 LR): Interference Effects (panel 1), Error-Related Activity (panel 2)

eFigure 15 participants older than 25 years of age (N=84, 58 HR, 26 LR): Interference Effects (panel 1), Error-Related Activity (panel 2)

Abbreviations: ***ACC*** anterior cingulate cortex; ***DLPFC*** dorsolateral prefrontal cortex; ***Ins*** insula; ***LPFC*** lateral prefrontal cortex; ***MTG*** middle temporal gyrus; ***PCC*** posterior cingulate cortex; ***PCu*** precuneus; ***PH***

parahippocampus; ***SPL*** superior parietal lobe; ***SFG*** superior frontal gyrus; ***STG*** superior temporal gyrus; ***vACC*** ventral anterior cingulate cortex; ***Caud*** caudate; ***CerV*** cerebellar vermis.

e12 -- SUPPLEMENTAL DISCUSSION

Interaction of Lifetime Illness with Risk Group Certain lifetime illness effects were significantly stronger in one or the other risk group (i.e., these were HR- or LR-specific illness effects, identified statistically as an interaction of lifetime illness with risk group). Those specific to the HR group included reduced activation of the insular and dorsolateral prefrontal cortices and disproportionately greater deactivation of the posterior cingulate cortex when responding correctly (Supplement eFigure 1), and reduced activation of the ventral, pregenual, and dorsal anterior cingulate, insular, and superior temporal cortices when responding incorrectly (Supplement eFigure 1). Reduced error-related activation of the anterior cingulate cortex in HR participants with a lifetime history of illness is consistent with prior evidence that deficient activation of the anterior cingulate disrupts attention and emotion regulation,[10,11] likely accounting for the significantly more frequent errors and impulsive responding during incongruent trials in HR participants who had a lifetime history of illness compared with those who did not have that history ($p<0.0001$) (Table 2 main text), and possibly for the greater prevalence of ADHD diagnoses and the more severe ADHD symptoms in the HR compared with LR group (Table 1 main text). Reduced anterior cingulate activation identified as HR-specific illness effects when responding incorrectly were the obverse of greater anterior cingulate activation that we identified as a resilience endophenotype in the HR group when responding correctly, supporting our theory that greater activation in the anterior cingulate during performance of this self-regulatory task helps to protect against developing MDD, whereas reduced anterior cingulate error-related activity helps to transform a familial risk into overt illness. The HR-specific greater deactivation of the posterior cingulate cortex during correct responses likely represents an exaggeration of the illness effects in default-mode circuits that were common to both risk groups, possibly representing in the HR group the more prominent presence of the self-referential thinking and processing of episodic memory that the posterior cingulate is thought to support.

Lifetime illness effects specific to the LR group included greater activation of the ventral, pregenual, and dorsal anterior cingulate cortex and caudate nuclei, reduced activation of the lateral prefrontal cortex,

and more prominent deactivation of the inferior parietal/posterior temporal cortex portion of the default-mode circuit and parahippocampus during correct responses (Supplement eFigure 2). The anterior cingulate findings were generally more ventral in the LR group than were the cingulate findings identified as a resilience endophenotype in the HR group, which together with greater activation of the caudate nucleus, suggests that the members of the LR group who became ill required greater activation of self-regulatory circuits, perhaps because they were unable to generate sufficient activation of their dorsolateral prefrontal cortex in the service of attentional and executive control. We did not detect significant LR-specific illness effects when responding incorrectly. Regardless of the interpretation of these LR-specific illness effects, our findings indicate that some portions of the neural correlates of lifetime illness are shared as a main effect across risk groups and some portions are unique to each risk group.

Limitations Our findings should be considered in light of several limitations of this study. Most importantly, the imaging component of this study was performed only in temporal cross-section. The conclusive demonstration of risk and resilience effects will require showing that the presence and magnitudes of activation in these brain-based endophenotypes predict relatively greater or lesser risks, respectively, for developing MDD, anxiety, and associated problems in future longitudinal follow-up of the sample. In addition, treatment studies are needed to determine whether the lifetime illness effects that we identified in default-mode systems will change with treatment and relate to clinical response.[12] Finally, our identification of risk, resilience, and lifetime illness effects were based on group statistics, whereas the clinical utility of these effects will require that their presence or absence is identified within single individuals, which in turn will require the development of new diagnostic algorithms that operate in individual fMRI maps.

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