Subjects

Persons meeting a DSM-IV-TR (*1*) diagnosis of schizophrenia, schizoaffective disorder, or bipolar disorder with psychosis were rated on the Positive and Negative Syndrome (*2*), Young Mania Rating (*3*), Montgomery-Asberg Depression Rating (*4*), Schizo-Bipolar (*5*), and Birchwood Social Functioning (*6*) scales. They were also rated on the Hollingshead Index of Social Position (*7*).

Laboratory tasks

Brief Assessment of Cognition in Schizophrenia. This battery is a widely used test of global neuropsychological function. It covers multiple cognitive domains (Verbal Memory, Processing Speed, Reasoning and Problem Solving, Working Memory), although a global neuropsychological functioning composite score integrating over these domains yields the best measure of psychosis-related cognitive deviation (*8*); this measure was used in Biotype construction.

Pro- and Anti-Saccade Tasks. Eye movement recordings were analyzed using established methods (*9*). Saccade latency (time from peripheral cue onset to saccade onset) and percentage of error responses were recorded for each condition. The prosaccade task consisted of 3 blocks of 32 trials in which the timing of the central fixation crosshair was experimentally manipulated to extinguish simultaneously with (no gap condition), 200 ms before (gap condition) or 200 ms after (overlap condition) peripheral cue appearance. Subjects were instructed to make a saccade to the peripheral cue when it appeared. The antisaccade task consisted of 4 blocks of 20 overlap trials. The overlap condition was used because it is most sensitive to relatives' deficits (*10*). Subjects were instructed to not look to the peripheral cue but saccade to the mirrored location in the opposite visual field.

Stop Signal Task. Trials begin with a central fixation cross after which subjects were shown a Go cue to the left or right. On 40% of trials a Stop Signal was presented at central fixation (with delays between 50 and 282 ms after Go cue onset). Participants were to respond to the Go cue with a button press as quickly as possible unless they encountered the Stop Signal. Strategic slowing (difference between response latencies on baseline Go trials, a block of trials not interspersed with Stop Signal trials, and Go trials during Stop Signal performance) and proportion of Stop Signal errors were used in Biotype construction (see (*11*) for complete task and analysis details). All trials began with the presentation of a white central fixation crosshair for a random interval of 750-1500ms followed by a green circle (the Go cue) to the right or left of center for 650ms. On 40% of trials, a Stop Signal (red stop sign) was presented at the location of the central fixation crosshair at delays varying between 50-282ms after the Go stimulus was shown. The ordering of Stop Signal delays and occurrences of Stop trials varied pseudorandomly. Participants were instructed to respond as quickly and accurately as possible with a button press. The task was administered over four blocks of 63 trials each (38 Go; 25 Stop). A baseline task consisting of 50 consecutive Go trials, evenly and randomly distributed to cues on the left and right side of the screen, was administered to assess baseline reaction time to Go cues.

Auditory Paired-Stimuli and Oddball Evoked Brain Responses. For the paired stimuli task, subjects listened to 150 binaural broadband auditory click pairs (500-ms interclick interval) occurring an average of every 9.5s. For the oddball task, subjects listened to 567 standard (1000Hz) and 100 target (1500Hz) tones presented in pseudorandom order (1300ms inter-trial interval). Subjects were asked to press a button when a target was detected.

Electroencephalography data pre-processing was completed using previously published protocols (*12,13*). To maximize use of available spatial, temporal, and oscillatory information in the evoked auditory response, a frequency-wise principal component analysis of evoked power was first conducted across all subjects to define frequency bands for analysis: (a) LOW, 4–16 Hz; (b) BETA, 17–33 Hz; and (c) GAMMA, 34–55 Hz. Spatial principal component analysis (*12,13*) was completed on the broadband grand-averaged event-related potential waveforms (for traditional event-related potential analyses) and then once for each frequency band to define specific neural oscillatory activities. “Virtual sensors” were constructed based on the principal component analysis outcomes for the broadband event-related potentials and each frequency band (*12,13*). These analyses were performed separately for the paired-stimuli and oddball paradigms. Data from principal components capturing the majority of response variance were then analyzed. Data were analyzed over time (not just at voltage peaks) for both voltage amplitudes and powers in the empirically defined frequency bands. For each condition and subject, the individual principal component waveforms were analyzed in 10ms bins after adjusting for age effects (*12,13,14*). For each time bin, for each principal component, a one-way analysis of variance was conducted comparing DSM-diagnosed proband groups and healthy individuals, adjusting for multiple comparisons (*12,13*). The outcome was 31 electroencephalography variables in both time-voltage and time-frequency space that differentiated psychosis probands and healthy persons (see Table 2), and these variables were used in Biotype construction.

MRI acquisition and Voxel-Based Morphometry. T1-weighted Magnetization Prepared RApid Gradient Echo (MPRAGE) images using he Alzheimer's Disease Neuroimaging Initiative (ADNI) protocol (http://www.loni.ucla.edu/ADNI/Research/Cores/),

with sequence parameters standardized across sites. Images were processed in MATLAB2013a/SPM8/VBM8/DARTEL following standard steps. Modulated grey matter segments were smoothed with 8mm isotropic Gaussian kernel before group-level statistics (see (*15)* for complete details). Voxel-wise grey matter volume between-group differences were examined using full factorial design in SPM8 (analysis of covariance followed by pairwise *t*-contrasts), adjusted for site, age, sex, and handedness; correction for individual brain size was done during DARTEL segmentation/normalization step. To control for multiple testing, a cluster-level correction was employed with p = .05 FWE-R [Family Wise Error based on random field theory (*23*)], using an initial cluster-defining threshold of p = .001, uncorrected. Regional volume reduction analyses were based on Group ICA for fMRI Toolbox, GIFT1.3i, www.sourceforge.net) (*24*). Effect sizes for regional between-group grey matter volume differences were calculated using Cohen's *d* derived from *t* distribution statistics, similar to (*25*).

Medication and clinical effects on biomarkers

Most probands (>90%) and some relatives, were medicated, some with more than one agent, including mood stabilizers, antipsychotic, antidepressants and other psychotropic drugs (see Supplementary Table 2). Antipsychotic dose was estimated by chlorpromazine equivalents (*16*), benztropine (anti-cholinergic) dose, and the presence (vs. absence) of current antipsychotics, mood stabilizers, and antidepressants. For all subjects, medication status, prior history of substance abuse/dependence, and clinical symptom ratings were minimally related to biomarker variables (r^2's <0.04 (*8,9,11-13,15,17-19*)).

Kinship

Strong claims of traditional genetic heritability in the current sample are problematic given the absence of either monozygotic twin pairs or second-degree relatives (*20*), so the more conservative term “kinship” was chosen to refer to the degree to which biomarker measures are predicted by family membership. Kinship was assessed in proband-relative pedigrees via h^2r estimates calculated using SOLAR (Sequential Oligogenic Linkage Analysis Routines (*21*)). Total phenotypic variance was partitioned into additive polygenic and random environmental components. We assessed effects of age and sex on each phenotype and, when significant, adjusted for their effects in kinship analyses.

Imputation

The requirement for inclusion in this project was available data on a majority of the biomarker variables. Estimates of missing values were generated via a regression-based multiple imputation method (*22*) as implemented in SAS PROC MI using all available information from other biomarker variables. Multiple estimates from 1000 iterations were combined to provide final estimates of the missing values. Analyses for integrating data across measures and for generating Biotypes resulted in highly similar results when using imputed data versus when eliminating all cases with missing data.

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