ARVCF Genetic Influences on Neurocognitive and Neuroanatomical Intermediate Phenotypes in Chinese Patients With Schizophrenia

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ABSTRACT

Objective: There are notable similarities between velocardiofacial syndrome and schizophrenia in terms of neurocognitive deficits and brain structural abnormalities. These similarities have supported the role of the armadillo repeat gene deleted in velocardiofacial syndrome (*ARVCF*) as a susceptibility gene in schizophrenia. This study investigated the relationships between haplotypes of the *ARVCF* gene and specific intermediate phenotypes in schizophrenia. We hypothesized that *ARVCF* gene haplotypes influence caudate nucleus volume, fractional anisotropy, and neurocognitive functioning in schizophrenia.

Method: Between May 2006 and November 2009, 200 Chinese participants (125 patients with *DSM-IV* diagnosis of schizophrenia and 75 controls) were genotyped using blood samples, and a subset of 166 participants (99 patients with *DSM-IV* diagnosis of schizophrenia and 67 controls) underwent structural magnetic resonance imaging, diffusion tensor imaging, and completed neuropsychological testing.

Results: The haplotype T-G-A-T-T-G-G-C-T-G-T (*ARVCF-Hap1*) was significantly associated with fractional anisotropy of the caudate nucleus and executive functioning in patients. Specifically, patients with more copies of *ARVCF-Hap1* have lower white matter integrity in caudate nucleus ($P = .0008$) and greater perseverative errors ($P = .00003$) on the Wisconsin Card Sorting Test. A trend of lower caudate volume ($P = .015$) in patients with more copies of *ARVCF-Hap1* was also observed.

Conclusions: These findings are consistent with known *ARVCF* gene effects on neurodevelopment in terms of cellular arrangement, migration, and intracellular signaling involving the striatum and may involve interactions with other brain networks such as prefrontal cortex, and they underscore the importance of imaging-genetic studies to elucidate the genetic influences underlying intermediate phenotypes in complex neurobehavioral disorders.

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Velocardiofacial syndrome, also known as DiGeorge syndrome or 22q11.2 deletion syndrome, has been associated with high rates of psychiatric disorders, especially schizophrenia.[1,2] This association between velocardiofacial syndrome and schizophrenia has highlighted the possible role of the armadillo repeat gene deleted in velocardiofacial syndrome (*ARVCF*) as a susceptibility gene in schizophrenia.[3–7] Sanders et al[5] found a weak association between schizophrenia and rs165849 in *ARVCF* and a stronger association with a 3 marker haplotype spanning the 3′ portions of catechol-*O*-methyltransferase (*COMT)* and *ARVCF*. In a study involving different single-nucleotide polymorphisms (SNPs), Mas et al[7] found an association between a 4-SNP haplotype (rs165849, rs2518823, rs887199, and rs2239395) spanning part of the *ARVCF* gene and schizophrenia. Examining family trios within the Chinese population, Li et al[3] and Xie et al[6] found significant associations between schizophrenia and background haplotypes that included the *ARVCF* gene.

Extant imaging genetic studies in schizophrenia have focused on investigating associations of quantitative traits (such as imaging and neurocognitive measures) with SNPs,[8–10] and examination of such associations with haplotypes are considerably less common, especially haplotypes involving the *ARVCF* gene. Elucidation of complex traits and intermediate phenotypes with haplotypes has advantages, including biological plausibility and better statistical power,[11] and recent developments in processing algorithms have allowed for the robust reconstruction of haplotypes from genotyping data,[12,13] as well as the performance of quantitative trait associations, with haplotypes reconstructed from genotyping data in familial[14] and case-control[15–17] studies. In this regard, Cannon et al[18] employed haplotypes in their analyses and found that a 3-loci haplotype in *DISC1* and a 4-loci haplotype spanning *TRAX* and *DISC* genes demonstrated associations with reduced prefrontal gray matter density and short- and long-term memory impairments.

There are notable similarities between patients with velocardiofacial syndrome and those with schizophrenia in terms of neurocognitive deficits and brain structural abnormalities. Henry et al[19] found that adults with velocardiofacial syndrome have neurocognitive impairments, particularly in visuoperceptual ability, working memory, and executive function. Similar patterns of neurocognitive deficits have been identified in patients with schizophrenia and have been shown to have a certain degree of heritability.[20–23] Recent work revealed caudate volume abnormalities in patients with schizophrenia,[24–26] offsprings,[27] and siblings of patients with schizophrenia,[24,26] indicating potential genetic influences on caudate volume. Caudate volume abnormalities have also been reported in children with velocardiofacial syndrome,[28,29] although the data are less consistent with adults.[30] These findings suggest that neurocognitive abilities, including working memory, executive function, and caudate volume and integrity, are suitable intermediate phenotypes for examining their relationships with genetic variations of the *ARVCF* gene in schizophrenia. This study extends our understanding of the relatively understudied

ARVCF gene within schizophrenia in at least 2 ways. First, on the basis of previous literature, we investigated the *ARVCF* gene haplotypes and their relationships with specific intermediate phenotypes (neurocognitive measures of working memory and executive functioning, caudate nucleus indices) in schizophrenia. Second, in addition to structural volume, we also employed diffusion tensor imaging to derive an integrity metric (fractional anisotropy) of the caudate nucleus to better appreciate the neural basis of any *ARVCF*-related genetic effects in this potentially devastating illness. On the basis of extant data, we hypothesized that *ARVCF* haplotypes influence caudate nucleus volume and fractional anisotropy and neurocognitive functioning.

Clinical Points

- There is evidence for the role of the armadillo repeat gene deleted in velocardiofacial syndrome (*ARVCF*) as a susceptibility gene in schizophrenia.
- Patients with more copies of the haplotype T-G-A-T-T-G-G-C-T-G-T (*ARVCF-Hap1*) have lower white matter integrity in caudate nucleus and greater perseverative errors.
- These findings highlight the importance of imaging-genetic studies to clarify genetic influences underlying intermediate phenotypes in complex neurobehavioral disorders.

METHOD

Study Participants

The study sample comprised 200 Chinese participants (138 men and 62 women) recruited between May 2006 and November 2009. Of these participants, 125 were patients from the Institute of Mental Health, Singapore, who met the *DSM-IV*[31] diagnosis of schizophrenia. The majority of patients (85%) were recruited during the latter half of their inpatient stay just prior to their discharge from the hospital. The administration of rating scales and neuroimaging procedures were completed at the outpatient setting within 1 week of their discharge from the hospital. Confirmation of the diagnosis was made for all patients by a psychiatrist using information obtained from the clinical history, existing medical records, and interviews with significant others as well as the administration of the Structured Clinical Interview for *DSM-IV* Axis I Disorders-Patient Edition (SCID-I/P).[32] The patients were maintained on a stable dose of antipsychotic medications for at least 2 weeks prior to the study and did not have their medication withdrawn for the purpose of the study. There was no history of any significant neurologic illness, such as seizure disorder, head trauma, or cerebrovascular accident, and no subject met *DSM-IV* criteria for alcohol or other substance abuse in the preceding 3 months. The remaining 75 participants were healthy controls recruited either from the staff population at the hospital or from the community by advertisements. They were screened using the Structured Clinical Interview for *DSM-IV* Axis I Disorders–Non-patient Edition (SCID-I/NP)[33] and deemed not to be suffering from any Axis I psychiatric disorder and had no history of any major neurologic or medical illnesses, substance abuse, or psychotropic medication use.

Blood samples were drawn from all 200 participants and subsequently genotyped. One hundred sixty-six participants (99 patients and 67 controls) received structural magnetic resonance imaging and diffusion tensor imaging, and completed the full neuropsychological assessments. These participants were administered by a trained psychometrist the following neuropsychological assessments: (1) Raven's Progressive Matrices (nonverbal intelligence),[34] (2) Digit and Spatial Span subtest of the Weschsler Adult Intelligence Scale-III (verbal and spatial memory span),[35] and (3) Wisconsin Card Sorting Test (executive functioning).[36] Psychopathology and symptom severity of patients were also assessed by psychiatrists using the Positive and Negative Syndrome Scale (PANSS).[37] Written informed consent was obtained from all the participants after a detailed explanation of the study procedures. Ethical approvals were obtained from the institutional review boards of the Institute of Mental Health, Singapore, as well as the National Neuroscience Institute, Singapore.

Neuroimaging Procedures

Brain imaging was performed at the National Neuroscience Institute, Singapore, on a 3 Tesla whole body scanner (Philips Achieva, Philips Medical System, Eindhoven, The Netherlands) with a SENSE head coil. Diffusion-encoded images were acquired using a single-shot echo-planar sequence (repetition time, 3,725 milliseconds; echo time, 56 milliseconds; flip angle = 90°; b factor, 800 s/mm^2) from 15 different nonparallel directions, with the baseline image being obtained without diffusion weighting (b factor = 0). Images were acquired parallel to the anterior-posterior commissures. Each volume consisted of 42 axial 3.0-mm slices with no gap (field of view, 230 mm × 230 mm; acquisition matrix, 112 × 109 pixels, reconstructed to 256 × 256 pixels). Three volumes (number of excitations, 3) were obtained to improve signal-to-noise ratio of the scans. Stability of a high signal-to-noise ratio was assured through a regular automated quality control procedure. Diffusion tensors were reconstructed from the 3 × 16 pixels (15 gradients and 1 b factor = 0 images) diffusion-weighted images, and fractional anisotropy maps were obtained using DTIStudio software.[38]

A semiautomated procedure based on a commercially available, electronic Talairach-Tournoux atlas—the Cerefy Brain Atlases[39–41] (see also http://www.cerefy.com)—was employed to define the contours of the caudate nucleus: First, neuroanatomical landmarks including the anterior commissure, posterior commissure, and brain extents in left, right, anterior, posterior, dorsal, and ventral directions were manually identified for each image. To enhance the accuracy of the Talairach transformation, 2 additional landmarks were

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defined, ie, the top of the corpus callosum and the most ventral point of the orbitofrontal cortex on the midsagittal slab.[42] The electronic atlas was then warped against each individual's scans using a landmark-based, piecewise linear approach with 12 degrees of freedom. Finally, contours of the caudate nuclei were visually inspected for all participants and manually adjusted when necessary to improve accuracy. Mean fractional anisotropy of the caudate nuclei was obtained by averaging the fractional anisotropy across all voxels that lie within the contours. Caudate nucleus volumes were obtained by counting the number of voxels within the contours and multiplying by the volume of each voxel.

Table 1. Demographic and Clinical Characteristics of Participants

Characteristic	Patients (n = 125)[a]	Controls (n = 75)[a]	Statistic	*P* Value
Age, y	34.3 (9.2)	31.9 (10.1)	$t = 1.72$	.088
Gender, n			$\chi^2 = 1.40$	.236
Male	90	48		
Female	35	27		
Handedness, n			$\chi^2 = 0.03$	.866
Right	114	68		
Left	11	7		
Education, y	11.3 (2.4)	13.9 (2.0)	$t = 7.65$	< .001
Raven's Progressive Matrices score	48.4 (8.2)	50.2 (7.4)	$t = 1.23$	.220
Duration of illness, y	7.9 (8.0)	…		
Age at onset, y	26.3 (7.9)	…		
Duration of untreated psychosis, y	1.6 (2.4)	…		
Medication (daily chlorpromazine mg equivalents)	233 (188)	…		
PANSS positive subscale score	11.1 (4.2)	…		
PANSS negative subscale score	9.3 (3.3)	…		
PASS general subscale score	20.2 (3.9)	…		

[a]Numbers represent mean (SD) unless otherwise stated.
Abbreviation: PANSS = Positive and Negative Syndrome Scale.

Genotyping Methods

Genotyped data were obtained through an ongoing genetic association study using the Illumina HumanHap Beadchips (Illumina Inc, San Diego, California). In brief, the deoxyribonucleic acid (DNA) sample was first isothermally amplified and then fragmented by a controlled enzymatic process that does not require gel electrophoresis. The DNA was then alcohol precipitated and hybridized. Allelic specificity is conferred by enzymatic single-based extension reaction followed by fluorescence staining. The intensities of the beads' fluorescence were read by the Illumina BeadArray Reader and analyzed using Illumina BeadStudio software. For quality control, SNPs were excluded if they had a call rate lower than 90%, a minor allele frequency < 5%, and/or significant deviation from Hardy-Weinberg equilibrium (thresholded at $P < 10^{-5}$). Similarly, all the samples with genotyping rate less than 98% were removed from further analysis.

The list of SNPs associated with the *ARVCF* gene was obtained from the Database of Single Nucleotide Polymorphisms (available at http://www.ncbi.nlm.nih.gov/SNP/).[43] This list was then matched with the marker list from Illumina to obtain 14 SNPs that were genotyped and associated with the *ARVCF* gene. Two SNPs (rs2239395 and rs1058399) had minor allele frequency of less than 5% and were excluded from further analyses.

Statistical Analyses

Categorical and continuous variables were analyzed using χ^2 tests and Student *t* tests, respectively. The 12 SNPs were analyzed for linkage disequilibrium using Haploview 4.1.[13] Blocks of SNPs were defined using the four gamete rule[44] and used in haplotype-quantitative trait analysis. By using the general linear model framework,[16] a model based on comparing differential effects of haplotype by diagnosis ("D × H" model) was applied to each specific neuropsychological (digit span, spatial span, and perseverative errors on the WCST) and neuroimaging measure (mean values of caudate fractional anisotropy (FA) and caudate volume), controlling for age, gender, education, and antipsychotic dose in daily chlorpromazine equivalents (in mg/d only for patients). Intracranial volume was also included as a covariate during the analysis of caudate volume. Model deviance was first inspected to determine whether to use additive, dominant, or recessive model in the general linear model. False discovery rate procedure was used to adjust for multiple comparisons. For haplotypes showing a significant interaction, post hoc (simple effects) analyses were done by separately fitting general linear models to patients and controls. Analysis was done using the Haplo Stats package version 1.4.0[45] in R (http://www.r-project.org).

RESULTS

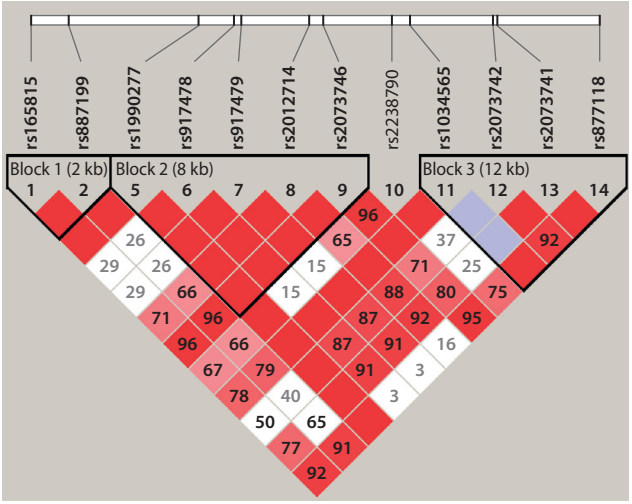
Sociodemographic, Clinical Features, and Haplotype Analyses

The sociodemographic and clinical features of the entire cohort (N = 200) are shown in Table 1. The pattern of sociodemographic characteristics did not differ for those who underwent further neuroimaging and neuropsychological evaluations (n = 166). Figure 1 shows the linkage disequilibrium (LD) plot from Haploview. Three blocks were defined: rs165815-rs887199, rs1990277-rs917478-rs917479-rs2012714-rs2073746, and rs1034565-rs2073742-rs2073741-rs877118; and Table 2 shows the estimated haplotype frequencies for each of these blocks.

Effects of Haplotype and Diagnosis

Based on the model deviance, the dominant effect model was used for all response variables except for digit span (additive model) and perseverative errors (recessive model). A total of 33 interactions was tested, and Table 3 shows the interactions between haplotype and intermediate phenotypes within the general linear model. Based on false discovery rate calculations, all interactions with *P* value less

Figure 1. Linkage Disequilibrium (LD) Plot From Haploview 4.1 for 12 Single Nucleotide Polymorphisms (SNPs) Associated With *ARVCF* Gene[a]



[a]Blocks were defined using the four gamete rule. The deeper the shade of red the stronger the LD is between the 2 SNPs that are at the root of the respective diagonals. The numbers indicate the measurement of LD (D′) multiplied by 100. D′ is a haplotypic measure of the relationship between 2 alleles on a haplotype.

Table 2. Haplotype Frequencies of 2-, 5-, 4-SNP Block of Markers Spanning *ARVCF* Gene

Block (SNPs)	Haplotype Frequency, %	Case Frequency, %	Control Frequency, %
Block 1 (rs165815-rs887199)			
C-A	49.7	50.4	48.7
T-G	50.0	49.2	51.3
T-A	0.3	0.4	0.0
Block 2 (rs1990277-rs917478-rs917479-rs2012714-rs2073746)			
G-T-T-G-A	41.2	43.5	37.3
A-T-T-G-G	36.2	35.9	36.7
G-C-G-A-G	16.3	15.3	18.0
G-C-G-G-G	6.3	5.2	8.0
Block 3 (rs1034565-rs2073742-rs2073741-rs877118)			
C-T-G-C	46.2	47.6	51.3
C-T-G-T	36.2	35.6	37.3
C-C-A-T	8.7	7.2	11.3
T-T-G-C	8.5	9.2	7.3
C-C-G-C	0.3	0.4	0.0

Abbreviations: *ARVCF* = armadillo repeat gene deleted in velocardiofacial syndrome, SNP = single nucleotide polymorphism.

than .0106 can be considered significant. In block 1, significant interactions were observed for perseverative errors and caudate FA, and a trend interaction was observed for caudate volume for T-G haplotype of block 1. In block 2, only A-T-T-G-G haplotype showed significant interactions for perseverative errors and caudate FA and a trend interaction for caudate volume. In block 3, the C-T-G-T haplotype similarly showed significant interactions in perseverative errors and caudate FA and trend effects for caudate volume.

Post hoc tests revealed significant dominant effects of the T-G haplotype in block 1 on caudate FA ($P = .003$) and trend effect of caudate volume ($P = .006$) for patients but not for controls after correction for multiple comparisons. On the other hand, the haplotype has a recessive effect on perseverative errors ($P = .00006$) for patients only. The A-T-T-G-G haplotype of block 2 also exhibited dominant effects on caudate FA ($P = .0009$) and a trend effect for caudate volume ($P = .009$) for patients but not for controls. Like the T-G haplotype of block 1, the A-T-T-G-G haplotype also showed a recessive effect on perseverative errors ($P = .00002$) for patients only. Finally, the C-T-G-T haplotype of block 3 has the same pattern of association as the A-T-T-G-G haplotype of block 2 in that dominant effects are seen on caudate FA ($P = .0006$), trend effect on caudate volume ($P = .008$), and recessive effects on perseverative errors ($P = .00003$) for patients only.

Considering the consistent effects of the 3 haplotypes on caudate FA and perseverative errors, we investigated the outcome for the combination of the 3 haplotypes (ie, T-G-A-T-T-G-G-C-T-G-T). This haplotype, which we shall refer to as *ARVCF-Hap1*, has an estimated haplotype frequency of 36.2% (case, 35.9%; controls, 36.7%; $\chi^2 = 0.02$, $P = .884$). Figure 2 shows the effects of the haplotype copy number on caudate volume, caudate FA, and executive functioning (perseverative errors). The same effects were found for patients and not for controls: a trend dominant effect on caudate volume ($P = .015$), a dominant effect on caudate FA ($P = .0008$), and a recessive effect on perseverative errors ($P = .00003$).

Clinical, Neurocognitive, and Neuroimaging Correlations

No significant correlations were found between the neuropsychological measures and the neuroimaging measures. However, the haplotype T-G of block 1 had a dominant effect on PANSS positive scores after adjustments were made for age and gender ($P = .037$).

DISCUSSION

Our findings associate sequence variations in the *ARVCF* gene with several intermediate phenotypes denoting processes thought to be involved in the pathophysiology of schizophrenia. These findings suggest that previous association with 22q11 deletion may be mediated through *ARVCF* influences on caudate nucleus volume, caudate FA, and executive functioning, and these findings were consistent with known *ARVCF* effects on neurodevelopment in terms of cellular arrangement, migration, and intracellular signaling.

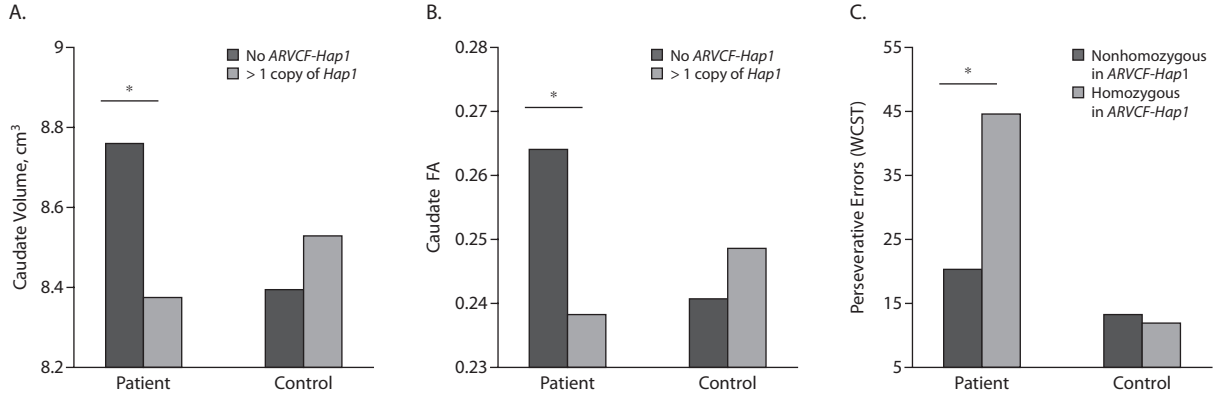
We found that *ARVCF* haplotypes influence caudate volume and fractional anisotropy in patients with schizophrenia. Ulfig and Chan[46] investigated the spatiotemporal expression of the *ARVCF* gene in the human fetal brain and found that the *ARVCF* gene is highly expressed in the ganglionic eminence of the developing neocortex, a structure known to contain precursor neurons of the striatum. The armadillo group of structural proteins belongs to the catenin-cadherin family, which is important in the

Table 3. Association of *ARVCF* Haplotypes With Neurocognitive and Neuroanatomical Measures

		Haplotype × Diagnosis Interactions (*P* value)[a]				
Block	Haplotype	Digit Span[b]	Spatial Span	Perserverative Errors[b]	Caudate Volume	Caudate FA
Block 1	T-G	1.99 (.049)	3.17 (.002)	−3.33 (.001)	2.14 (.034)	2.59 (.010)
	C-A[c]	…	…	…	…	…
Block 2	G-C-G-G-G	0.13 (.900)	0.45 (.656)	…	0.74 (.462)	−0.57 (.568)
	G-C-G-A-G	1.82 (.072)	1.65 (.101)	1.15 (.253)	0.86 (.390)	−0.09 (.923)
	A-T-T-G-G	1.04 (.302)	2.16 (.033)	−2.83 (.005)	1.74 (.084)	2.85 (.005)
	G-T-T-G-A[c]	…	…	…	…	…
Block 3	C-T-G-T	1.65 (.102)	1.88 (.062)	−2.83 (.005)	1.85 (.066)	3.12 (.002)
	C-C-A-T	2.02 (.046)	0.72 (.475)	1.15 (.253)[d]	0.14 (.891)	0.05 (.959)
	T-T-G-C	1.24 (.218)	1.46 (.147)		0.44 (.658)	1.91 (.057)
	C-T-G-C[c]	…	…	…	…	…

[a]Based on false discovery rate calculations, all interactions with *P* value less than .0106 can be considered significant.
[b]Recessive model fit for perserverative errors, additive model for block 1 and 3 of digit span, and dominant model fit for the rest of the response variables.
[c]Haplotypes in boldface font are the reference haplotypes in the general linear model analyses.
[d]Due to the low frequencies of the haplotypes, these 2 haplotypes were combined into a single category for the recessive model.
Abbreviations: *ARVCF* = armadillo repeat gene deleted in velocardiofacial syndrome, FA = fractional anisotropy.

Figure 2. Plot of Estimated Effects of *ARVCF-Hap1*: Dominant Effect on Caudate Volume (A), Dominant Effect on Caudate Fractional Anisotropy (FA) (B), and a Recessive Effect on Perseverative Errors (C)



*$P < .05$.
Abbreviations: *ARVCF* = armadillo repeat gene deleted in velocardiofacial syndrome, *ARVCF-Hap1* = haplotype T-G-A-T-T-G-G-C-T-G-T, WCST = Wisconsin Card Sorting Test.

formation of adherent junctional complexes in cells during embryonic development, thus facilitating cell-to-cell and intracellular communication.[47–50] This is crucial to many neurodevelopmental processes, including cellular rearrangement and migration,[51] which were thought to be disrupted in schizophrenia.[52,53] These earlier findings provided some biological convergence to the associations found in our study between *ARVCF*-related haplotypes and caudate integrity and volume.

Animal studies involving transgenic mice demonstrated that aberrant expression of genes within the 22q11.2 segment can lead to variations in learning and memory,[54] and these observations were in line with our finding of increased perseverative errors in patients who are homozygous in haplotype *ARVCF-Hap1*. Although previous studies have implicated the caudate nucleus in perseverative responses in both animal models[55] and humans[56] and we found the *ARVCF-Hap1* to be associated with caudate nucleus integrity, we did not find significant correlations between caudate nucleus integrity and perseverative errors in patients. This finding did not support the notion that changes in caudate nucleus volume or fractional anisotropy mediated the effects of *ARVCF-Hap1* haplotype on executive functioning. A possible explanation may be that *ARVCF-Hap1* is associated with caudate nucleus changes as well as other involved parts of the brain (eg, prefrontal cortex) by being in linkage disequilibrium with *COMT* with possible gene-gene interactions,[5] which, in turn, affected executive functioning.

We found differential effects of *ARVCF* haplotypes on different neuroimaging and neurocognitive quantitative traits. Specifically, we found that patients, but not controls, with at least 1 copy of *ARVCF-Hap1* showed lower caudate volume and integrity, and patients with 2 copies of *ARVCF-Hap1* exhibited poorer executive functioning. In the context of

complex neuropsychiatric disorders such as schizophrenia, intermediate phenotypes reflecting processes between underlying molecular architecture and clinical features are useful in throwing light on genetic factors and their interactions with nongenetic factors underlying the pathophysiology of the illness.[57,58] The dominant effects of *ARVCF-Hap1* haplotype copy on caudate nucleus volume and integrity indicated possible gene dosing effects, which may interact with nongenetic factors in influencing brain structural changes in schizophrenia. In this regard, Caspi et al[59] showed that, specifically, only carriers of the *COMT* valine158 allele in their interaction with cannabis use are more likely to be associated with psychotic symptoms as adults. In addition, there could be gene-gene interactions in view of the proximity of *ARVCF* gene to other genes such as *COMT* and thioredoxin reductase (*TXNRD2*) within the 22q11 region.[54] Paterlini et al[60] showed that *COMT* is upregulated as a homeostatic response to enhanced dopaminergic signaling in the frontal cortex when there is a deficiency in the *PRODH* gene. It is possible that variable compensatory mechanisms, secondary pathology, and confounding influences associated with aberrant *ARVCF* expression may occur in patients compared with controls and affect the natural history of the illness.[61] Furthermore, the function of *ARVCF* in cellular scaffolding points toward its role in developmental patterning, which is consistent with the neurodevelopmental hypothesis of schizophrenia.[62,63]

There were several limitations in this study. First, we did not investigate the relationship of *ARVCF* haplotypes with those of other genes (such as *COMT*, *TXNRD2*) in close proximity within 22q11 region. This will be the subject of further study in terms of understanding specific 22q11 gene-gene interactions in increasing susceptibility toward schizophrenia. Second, we did not study interactions of *ARVCF* with nongenetic factors such as substance use, which can potentially throw light on genetic interactions with environmental factors in influencing liability to the illness. Third, this study was conducted on a relatively small number of subjects, and the findings need to be validated in a bigger sample. Fourth, the relatively lower mean medication dose and PANSS total and subscale scores may limit the generalizability of the neuroimaging and neurocognitive data in this study to a patient population with more severe psychopathology, as measured by PANSS scores.

In conclusion, through careful phenotyping and genotyping methods, we found differential effects of *ARVCF* haplotypes on quantitative traits previously implicated in schizophrenia, underscoring the importance of such studies to elucidate the genetic influences underlying intermediate phenotypes in complex neurobehavioral disorders such as schizophrenia.

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