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ORIGINAL ARTICLE

TGFB-induced factor (TGIF): a candidate gene for psychosis on chromosome 18p

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Schizophrenia (SC) and bipolar disorder (BP) share many clinical features, among them psychosis. We previously identified a putative gene locus for psychosis on chromosome 18p in a sample from the Central Valley of Costa Rica (CVCR) population. The present study replicated the association to a specific allele of microsatellite marker D18S63 on 18p11.3, using a newly collected sample from the CVCR. A combined analysis of both samples, plus additional subjects, showed that this specific allele on D18S63, which lies within an intron on the TGFB-induced factor (*TGIF*) gene, is strongly associated (*P*-value = 0.0005) with psychosis. Eleven additional SNP markers, spanning five genes in the region, were analyzed in the combined sample from the CVCR. Only the four SNPs within the TGIF gene were in strong linkage disequilibrium with D18S63 (*D′* = 1.00). A specific haplotype for all five markers within the *TGIF* gene showed evidence of association (*P*-value = 0.011) to psychosis. A second, distinct haplotype, containing a newly identified nonsynonymous polymorphism in exon 5 of the *TGIF* gene, showed a nonsignificant trend towards association to psychosis (*P*-value = 0.077). TGIF is involved in neurodevelopment, neuron survival and controls the expression of dopamine receptors. Altogether, our results point to the possible involvement of TGIF in the pathophysiology of psychotic disorders in the CVCR population.
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Introduction

Schizophrenia (SC) and bipolar disorder (BP) are two of the most prevalent psychiatric diseases.[1] According to the 10th revision of the International Classification of Diseases (ICD-10)[2] and the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV),[3] SC and BP should be classified as separate clinical entities. This is Kraepelin's traditional dichotomous classification of the so called functional psychosis and it has been used for both clinical and genetic research.[4] However, the current evidence of overlap of genetic susceptibility for SC and BP has led to the question of whether the understanding of a common pathophysiology of psychotic-related disorders will lead to a change in the way we classify these disorders.[5–7]

We have previously used psychosis as a phenotype for association studies of different regions of the genome[8–10] using subjects from the Central Valley of Costa Rica (CVCR). We have found in this population evidence of association of psychosis with three loci on chromosome 18.[10] One of the regions, 18p11.3, has been reported as a potential susceptibility locus for psychotic disorders in different populations, but a putative candidate gene or genes in this region has not been identified.[11–13]

In this current study, we sought to confirm our previous finding of association of a specific allele of marker D18S63, on 18p11.3, with psychosis, using an independent population from the CVCR. We also performed fine mapping of the region to determine which genes were in strong linkage disequilibrium (LD) with the D18S63 marker. We identified a specific haplotype formed by several markers that is associated with psychosis, all in strong LD with D18S63, and all within the TGFB-induced factor (*TGIF*) gene. TGIF is a member of the three-amino-acid loop extension (TALE) superclass of homeobox proteins and acts as an inhibitory transcription factor by repressing transcription of several genes. TGIF regulates normal neuronal development, neuron survival[14] and controls the expression of the dopamine receptors in neurons.[15] Based on our results we

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propose *TGIF* as a candidate gene for psychosis on Ch18p11.3.

Methods

Sample population

All subjects were recruited in accordance with the principles of the Declaration of Helsinki with approval from the Institutional Review Boards of the University of Costa Rica and the University of Texas Health Science Center at San Antonio.

In our previous report from our ongoing study of Schizophrenia in the CVCR, we analyzed 376 subjects, 154 of whom had a psychotic disorder (Sample 1), which was previously described in Walss-Bass *et al*.[10] For the first part of the current report, we analyzed 322 new families drawn from the CVCR (Sample 2), consisting of 793 subjects, 308 with history of psychosis. Probands from both Samples 1 and 2 were recruited from psychiatric hospitals and clinics in the CVCR, using the same protocol, and all probands had a documented diagnosis of schizophrenia by either inpatient or outpatient records. Once consented, DNA was drawn from probands and available parents. If parents were not available, siblings were recruited to help reconstruct parental genotypes. In each family, if additional subjects were identified who had a history of psychosis, attempts were made to recruit them as well. All affected subjects (those with a history of psychosis by medical record or family history interview) were interviewed by a psychiatrist using the Diagnostic Interview for Genetics Studies (DIGS). In addition, information was gathered on each affected subject by interviewing a close relative, using the Family Interview for Genetic Studies (FIGS), and all available psychiatric records were gathered. The three sources of information (DIGS, FIGS and available records) were analyzed by bilingual psychiatrists to obtain a lifetime consensus diagnosis for each affected subject, using a process of best estimation described in previous studies of the CVCR.[10–16] A consensus diagnosis of lifetime history of psychosis was also arrived at, utilizing the methodology described in Escamilla *et al*.[17] Assessors and best estimate teams were the same for Samples 1 and 2, and there was no change in protocol, as Sample 2 was technically the next group of families recruited after Sample 1.

After analyzing Sample 2 independently, we combined all available subjects in order to increase the power of the sample for fine mapping. All subjects from Samples 1 and 2 were included in this combined analysis, in addition to 77 additional relatives (28 with a history of psychosis) of the families in Sample 1. The final combined sample then consisted of 476 unique families (1246 subjects, 490 with history of psychosis). The DSM-IV consensus diagnoses for these 490 affected subjects were as follows: 244 had a diagnosis of SC, 90 had a diagnosis of BP, 95 had a diagnosis of Schizoaffective (SA), 28 had a diagnosis of Psychosis not otherwise specified (PNOS), 20 had a diagnosis of Major Depression Episode (MDE) with psychosis, eight had a diagnosis of substance-induced psychosis and five had other disorders with history of psychosis.

Genotyping

Genomic DNA was extracted from blood samples using the Puregene DNA purification kit (Gentra. Minneapolis, MN, USA). DNA from 793 subjects of the independent sample from CVCR and the 77 newly collected relatives of families from Sample 1 were genotyped using the fluorescently labeled microsatellite marker D18S63 (Applied Biosystems; Foster City, CA, USA). Standard polymerase chain reaction (PCR) was performed using the GeneAmp PCR system 9700 (Applied Biosystems). Amplified fragments were analyzed on the ABI 3100 Genetic Analyzer (Applied Biosystems) and genotypes were assigned using GeneMapper v3.5 (Applied Biosystems). Two individuals blind to the diagnosis scored each genotype separately. Discrepancies were discussed with review of the peaks of the original run to obtain a final genotype.

Given our limited resources, we were not able to examine all variants in the genes surrounding D18S63. We focused instead on selecting single nucleotide polymorphisms (SNPs) that would help us to determine which genes were in tight LD with the microsatellite marker D18S63. We selected 10 SNPs in this region from the SNPbrowser database (Applied Biosystems) based on the location and heterozygosity of the SNPs. The 10 SNPs span a region of 0.8 Mbp and cover five genes that surround D18S63 (Figure 1). DNA from 1246 subjects of the combined sample of the CVCR was genotyped using these 10 SNP markers (rs660716, rs729673, rs6506081, rs1785243, rs4798096, rs3866990, rs238132, rs381234, rs2072389, rs658857). Standard PCR was performed using the Taqman 5′ nuclease assay (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed using the ABI 7900HT SDS 2.2.2 software adapted in the ABI 7900HT Sequence Detection System (Applied Biosystems).

Statistical analysis

For each marker genotyped, any families showing Mendelian errors using the program INFER in PEDSYS (Southwest Foundation for Biomedical Research; San Antonio, TX, USA) were removed from further analyses. All association analyses were performed using the Family Based Association Test (FBAT) (http://www.biostat.harvard.edu/~fbat/default.html), with the following settings: additive model, bi-allelic test (which provides asymptotic *P*-values of the *Z* score function, which looks at the transmitted alleles to affected offspring) and minimum size 10 (only alleles that were present in at least 10 informative families were tested for association). Alleles from affected subjects were compared to non-transmitted parental alleles. When both parents were not available, additional siblings of the affected

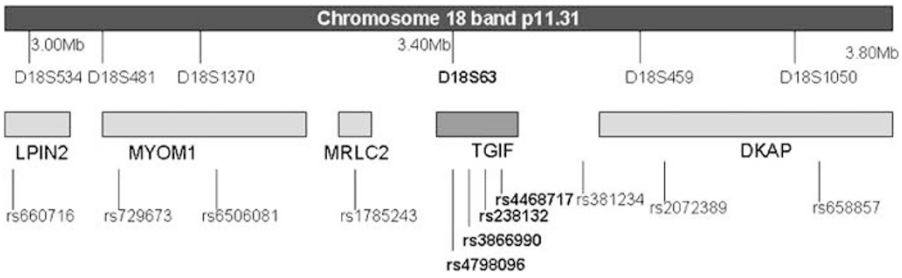

Figure 1 Distribution of markers and genes in chromosome 18 band p11.31. Schematic distribution of genes in region 18p11.31 in close proximity to D18S63. SNPs used in the association analysis are shown in relation to each gene. The markers within TGIF are highlighted in bold.

subjects had been genotyped to permit inference of the parental alleles. As the analyses for D18S63 conducted in this report focused on replication of one specific allele (allele 11) that had previously shown association in the CVCR, our criteria for significant association in Sample 2 was a *P*-value less than 0.05 for the bi-allelic FBAT test of allele 11 with the phenotype of psychosis. Linkage disequilibrium was evaluated between the SNP markers and D18S63 using the command hapfreq – d implemented in FBAT. Association to psychosis was evaluated with all markers individually. Once we determined that only the three SNPs in the *TGIF* gene were in strong disequilibrium with D18S63 (see Results), we analyzed all possible haplotypes of four markers within the gene *TGIF* for association with psychosis using the HBAT test (bi-allelic and global tests, with minimum informative family counts of 10).

FBAT was selected because this software provides methods for a wide range of situations that arise in family-based association studies, like complex family structures, which we had, as many families had more than one affected subject. FBAT provides options to test linkage or association in the presence of linkage, using marker or haplotype data. The expected distribution is derived using Mendel's law of segregation and conditioning on the sufficient statistics for any nuisance parameters.

Sequencing

The five exons of *TGIF* were sequenced in 30 subjects who had a consensus diagnosis of schizophrenia and who carried the four-marker *TGIF* haplotype (H1 in Table 5) that was associated with psychosis (see Results). The sequencing was performed by Polymorphic DNA technologies (Alameda, CA, USA). In order to confirm a novel SNP found in this sample, we performed the sequencing of a 397 bp region of exon 5 containing the novel polymorphism in eight subjects. PCR was performed using the primers: 5′-TTTTG CCTGAAGCTCCATCT-3′ and 5′-GGAGACCCCATTT CATTCCT-3′ under the following conditions (95°C for 5 min; then 50 cycles of 95°C for 30 s, 53°C for 30 s and 72°C for 30 s; finally 72°C for 10 min) in a PTC-200 Peltier Thermal Cycler (MJ Research; Miami, FL, USA). This PCR product was sequenced using the ABI 3100 Genetic Analyzer (Applied Biosystems).

SNaPshot reaction

DNA from 1246 subjects of the combined sample of the CVCR was genotyped for the novel polymorphism in *TGIF* using the ABI SNaPshot Multiplex Kit (Applied Biosystems). A PCR template of 600 bp containing this polymorphism was obtained using the primers: 5′-ACATTCTCAGAACCGTTGG-3′ and 5′-CGTTTGAGTGCAACATCCAC-3′, under the following conditions: 95°C for 5 min; then 50 cycles of 95°C for 30 s, 53°C for 30 s and 72°C for 30 s; finally 72°C for 10 min; in a PTC-200 Peltier Thermal Cycler. A 30 bp primer was designed for single-nucleotide extension of the polymorphism: 5′-GGGAGGCCACT GTCTCCTAAGCCGTCATCC-3′, using the ABI SNaPshot Multiplex kit under standard conditions in a PTC-200 Peltier Thermal Cycler. The primer extension products were analyzed on the ABI 3100 Genetic Analyzer (Applied Biosystems) and genotypes were assigned using GeneMapper v3.5 (Applied Biosystems).

Once this genotyping information was obtained, we analyzed for association of this SNP individually (using FBAT), analyzed whether it was in LD with D18S63 and the other 10 SNPs (using FBAT), and performed five-marker haplotype analyses of D18S63, this novel SNP and the other three SNPs within the TGIF gene region using the command hbat in FBAT.

Nucleotide and amino-acid sequences accession numbers

The accession number of the nucleotide and amino-acid sequences for *TGIF* used in this study in the GenBank database is BC031268. The accession number for the variation in *TGIF* that causes holoprosencephaly type 4 (HPE4) in the UniProtKB/Swiss-Prot database is Q15583 (variant: VAR_009964).

Results

We first sought to replicate our previous finding of association to a specific allele of D18S63 with psychosis in the CVCR[10] using an independent

sample from the CVCR (Sample 2: 322 families, 793 subjects, 308 with history of psychosis). From the genotyping of our sample set, we obtained 14 different alleles for marker D18S63; the sizes of alleles 1 through 14 range from 78 to 104 bp (at 2 bp intervals). Using FBAT in the bi-allelic mode, allele 11 (product size 98) of D18S63 showed association with psychosis (*P*-value 0.0278) (Table 1). This allele was the same allele that showed association to psychosis in the original study.[10] We then combined both samples from the CVCR plus 77 additional individuals (476 families, 1246 subjects, 490 with history of psychosis) to analyze the association of marker D18S63 with psychosis. In this combined sample set, allele 11 continued to show strong evidence of association with psychosis (*P*-value 0.0005) (Table 2).

D18S63 lies in the second intron of the *TGIF* gene. Next, we completed fine mapping analyses to identify whether other SNPs in *TGIF* or surrounding genes were in strong LD with D18S63. Analyses of (LD) between these 10 SNPs, which span five genes in this region (Figure 1), using FBAT showed that D18S63 was in complete LD ($D' = 1.00$) with the three SNPs within *TGIF*, and none of the other markers showed strong LD with D18S63 (Table 3).

Aside from allele 11 of D18S63, none of the additional SNPs showed independent association to the phenotype of psychosis in this population (Table 4). We then proceeded to analyze all possible haplotypes of the microsatellite marker D18S63 and the three SNPs that were in complete LD with this marker. We obtained 10 haplotypes that were informative for the association test (only haplotypes present in at least 10 informative families were tested for association). A specific haplotype (H1 in Table 5) formed by markers D18S63–rs47988096–rs3866990–rs238132 (Alleles: 11–A–T–C) showed evidence of association with psychosis (bi-allelic *P*-value = 0.005) (Table 5), although the global haplotype test was nonsignificant.

The H1 'high-risk' haplotype (D18S63(11)–rs47988096(A)–rs3866990(T)–rs238132(C)) was found

Table 1 Association analysis of D18S63 with psychosis in the independent CVCR sample

Allele[a]	*Allele frequency*	P-*values*
2	0.208	0.3126
8	0.071	0.8389
9	0.098	0.6860
10	0.288	0.4884
11	0.082	**0.0278**
12	0.084	0.5485
13	0.046	0.6095
14	0.028	0.8185

Abbreviation: CVCR, Central Valley of Costa Rica.
[a]Only informative alleles are shown.
P-value < 0.05 in bold.

Table 2 Association analysis of D18S63 with psychosis in the combined sample from the CVCR

Allele[a]	*Allele frequency*	P-*values*
2	0.20	0.2423
3	0.02	0.5637
4	0.02	1.0000
6	0.01	0.8612
7	0.01	0.9210
8	0.07	0.7992
9	0.12	0.6427
10	0.27	0.7057
11	0.08	**0.0005**
12	0.07	0.4658
13	0.04	0.8244
14	0.02	0.7236

Abbreviation: CVCR, Central Valley of Costa Rica.
[a]Only informative alleles are shown.
P-value < 0.05 in bold.

Table 3 Intermarker linkage disequilibrium (D')

Marker[a]	*SNP1*	*SNP2*	*SNP3*	*SNP4*	*D18S63*	*SNP5*	*SNP6*	*SNP7*	*SNP8*	*SNP9*
SNP2	0.03									
SNP3	0.14	0.06								
SNP4	0.05	0.04	0.21							
D18S63	0.12	0.10	0.41	0.31						
SNP5	0.02	0.07	0.06	0.18	**1.00**					
SNP6	0.04	0.05	0.07	1.13	**1.00**	0.88				
SNP7	0.13	0.15	0.05	0.09	**1.00**	0.04	0.06			
SNP8	0.07	0.17	0.04	0.14	0.01	0.38	0.32	0.06		
SNP9	0.21	0.08	0.09	0.25	0.06	0.24	0.22	0.16	0.12	
SNP10	0.02	0.12	0.04	0.01	0.34	0.22	0.18	0.14	0.02	0.007

Abbreviation: SNP, single-nucleotide polymorphism.
[a]SNP1, rs660716; SNP2, rs729673; SNP3, rs6506081; SNP4, rs1785243; SNP5, rs4798096; SNP6, rs3866990; SNP7, rs238132; SNP8, rs381234; SNP9, rs2072389; SNP10, rs658857.
SNPs in complete D' (1.00) in bold.

Table 4 Association analysis for psychosis and individual SNPs in the combined sample from the CVCR

Marker	*Base position*[a]	*Gene*	*Alleles*	*Frequency*	P-*value for psychosis*
rs660716	2 956 840	LPIN2	G	0.517	0.134505
			A	0.483	0.134505
rs729673	3 057 733	MYOM1	G	0.688	0.709944
			C	0.312	0.709944
rs6506081	3 183 452	MYOM1	G	0.468	0.871253
			A	0.532	0.871253
rs1785243	3 264 253	MRLC2	G	0.289	0.648246
			C	0.711	0.648246
D18S63	3 428 542	TGIF			
rs4798096	3 430 997	TGIF	G	0.294	0.090959
			A	0.706	0.090959
rs3866990	3 432 399	TGIF	T	0.349	0.208815
			C	0.651	0.208815
rs238132	3 440 455	TGIF	C	0.818	0.260854
			A	0.182	0.260854
rs381234	3 454 650	None	C	0.490	0.433764
			A	0.510	0.433764
rs2072389	3 524 175	DKAP	G	0.757	0.526572
			A	0.243	0.526572
rs658857	3 572 605	DKAP	G	0.608	0.271253
			A	0.392	0.271253

Abbreviations: CVCR, Central Valley of Costa Rica; SNP, single-nucleotide polymorphism; TGIF, TGFB-induced factor.
[a]According to National Center for Biotechnology Information (NCBI) SNP database.

Table 5 Association analysis of haplotypes within TGIF with psychosis in the combined sample from the CVCR

Haplotypes[a]	*Markers*					P-*value*
	D18S63	rs4798096	rs3866990	rs238132	rs4468717	
H1	11	A	T	C	—	0.005
H2	11	A	T	C	C	0.011
H3	10	A	T	C	T	0.077

Abbreviations: CVCR, Central Valley of Costa Rica; TGIF, TGFB-induced factor.
[a]Only haplotypes showing association to psychosis (bi-allelic HBAT) at $P < 0.10$ are shown.

in 55 subjects with different consensus diagnoses, SC being the most abundant (30 subjects).The five exons of *TGIF* were then sequenced in these 30 SC subjects who carried the high-risk haplotype (H1). We found two known SNPs in the coding region present in our sample; rs238137 (four subjects) and rs238533 (six subjects). In addition, a novel SNP in exon 5 was found in four of these 30 subjects: a C/T polymorphism at position 1177 (GenBank accession no. BC031268) that produces a change of a conserved residue from proline (P) to serine (S) in position 292 (P292S) of the protein (Figure 2). All four subjects were found to be heterozygous for this SNP (Figure 2). This novel SNP is now listed in the National Center for Biotechnology Information (NCBI) SNP database with accession number rs4468717.

In order to test whether this novel variant (rs4468717) might be associated with psychosis, we analyzed this SNP in the combined sample of the CVCR using the ABI SNaPshot assay; this assay was chosen because we have found this region difficult to work with, and a Taqman (ABI) assay for this SNP is not available. Linkage disequilibrium analysis of this SNP using FBAT showed that it was in complete LD with the microsatellite marker D18S63 ($D' = 1.00$). The frequency of the novel SNP in our combined sample was 0.115. Sixty-five of the psychotic subjects (13%) had at least one copy of the novel T variant (62 C/T heterozygotes and three T/T homozygotes). Association analysis of this novel SNP using FBAT showed that, by itself, it is not associated with the phenotype of psychosis in our sample (multiallelic *P*-value = 0.911). Haplotype analysis of all the markers within the *TGIF* gene with this novel SNP showed that only one informative haplotype (H3 in Table 5) was present in our population containing the novel SNP (D18S63(10)–rs47988096(A)–rs3866990(C)–rs4468717(T)). The association analysis using FBAT of this haplotype with the phenotype psychosis has a bi-allelic *P*-value = 0.0773.

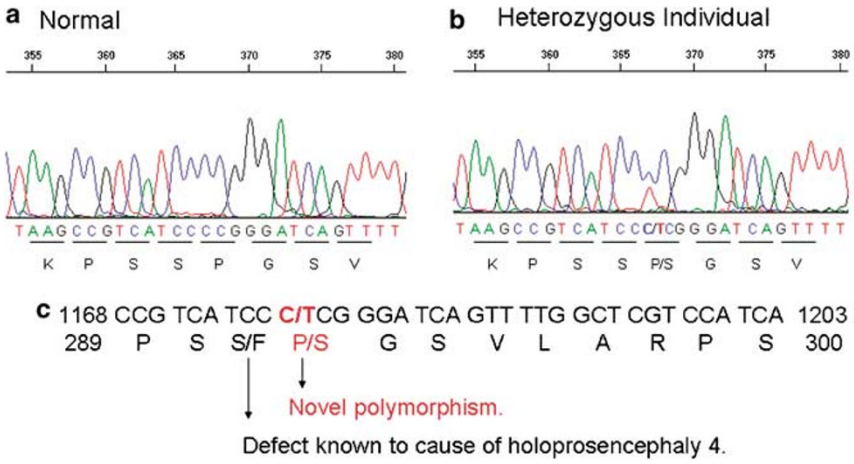

Figure 2 Novel nonsynonymous polymorphism in exon 5 of TGIF. (**a**) An individual with the normal C/C genotype at position 1177 of the gene (GenBank accession no. BC031268). (**b**) An individual heterozygous for the C/T polymorphism in position 1177. This novel polymorphism changes the residue proline for a serine in position 292 (P292S) of the protein (**c**), a change in position 291 is known to cause holoprosencephaly 4.

Discussion

Several association studies have reported association of different regions of chromosome 18 to the phenotype of BP and SC.[10,16,18–25] Furthermore, there have been several reports of persons with karyotypic abnormalities on chromosome 18 who have psychotic spectrum disorders.[26–31] Specifically the region of 18p11 has been shown to be associated with psychotic-related disorders in different populations[10,17,32,33] The present study provides additional evidence that a gene related to psychotic disorders lies in this region, and furthermore presents evidence that variation in a specific gene in this region (*TGIF*) is associated with psychosis in the CVCR population.

We previously found association of a specific allele on marker D18S63, in the 18p11.3 region,[10] with psychosis in the CVCR population. In the present study we replicated this finding in an independent sample from the same CVCR population (Table 1). D18S63 actually lies within the gene that codes for TGIF. In a search of the NCBI databases (http://www.ncbi.nlm.nih.gov/), we found that there are four additional genes in this region in close proximity to D18S63: Lipin-2 (*LPIN2*), Myomesin-1 (*MYOM1*), Myosin regulatory light chain 2 (*MRLC2*) and Disks large-associated protein (*DAP1*) (Figure 1). MYOM1 and MRLC2 both form part of the cytoskeleton and their best-known function is in the contractile activity of muscle cells. Even though they are slightly expressed in the brain, their function in the brain is not well understood. LPIN2 is expressed in the brain and it is involved in lipid metabolism; TGIF is expressed in the brain and regulates transcription of several genes by binding to the retinoid X receptor (RXR)-responsive element. DAP1 forms part of the postsynaptic scaffold in neuronal cells. All five genes could potentially be involved in the pathogenesis of psychotic disorders.

Next we completed fine mapping analyses in the combined sample of the CVCR population to identify which candidate genes in this region are in strong LD with D18S63 in the CVCR. Analysis of LD between the SNPs and D18S63 showed that only the SNPs within the *TGIF* gene were in complete LD with the microsatellite marker (Table 3). None of the other SNPs spanning the other genes in this region had significant LD with D18S63. Although this does not rule out variants in the other genes in the region as having a potential role in predisposing to psychosis, our evidence most strongly supports variation in the TGIF gene as being responsible for the association of D18S63 to psychosis in the CVCR population.

Our analysis of five markers within the *TGIF* gene suggests two distinct haplotypes (H2 and H3, Table 5) that are over-represented on subjects with psychosis in this population. However, identification of the specific variants in (or near) *TGIF*, which are causally responsible for the increased risk for psychosis in our sample, remains a challenge. Of the five polymorphisms (one microsatellite and four SNPs) we tested within the *TGIF* gene, only allele 11 of D18S63 was individually associated with psychosis ($P = 0.0005$). Although recent studies have shown that the number of copies in an intronic di-nucleotide repeat can be associated with functional changes in splicing of exons,[34] it is unlikely that this one specific variant within a microsatellite is causally associated with the disease, as none of the other allelic variants for this marker show evidence of association to psychosis in Table 2 (i.e. there is no clear pattern showing that higher or lower copy numbers are more associated with psychosis). Further sequencing of the *TGIF* gene in this sample, including intronic and promoter regions of the gene, may help to identify a clearer causal variant that is in strong LD with allele 11 of D18S63 and the associated high-risk haplotypes (H1 and H2) which contain this allele.

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Homo sapiens       272 SCTAGPNPTLGRPLSPKPSSPGSVLARPSVICHTTVTALKD 312
Macaca mulatta     127 SCTAGPNPTLGRPLSPKPSSPGSgLARPSVICHTTVTALKD 167
Bos Taurus         234 SCTAGPNPaLGRPLSPKPSSPGSILARPSVICHTTVTALKD 274
Canis familiaris   143 SCTAGPNPaLGRPLSPKPSSPGSILARPSVICHTTVTALKD 183
Mus musculus       143 SCvvGPNPTLGRPVSPKPSSPGSILARPSVICHTTVTALKD 183
Rattus novergicus  158 SCvvGPNPTLGRPVSPKPSSPGpILARPSVICHTTVTALKD 198
Gallus gallus      145 ------saTLsktVSsKPvSPGSVLARPSVICHTTVTALKD 180
Xenopus laevis     145 --sAGaNqssGkllasKlSpPqSlLARPSVICHTTVTsLsD 183
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Figure 3 Amino-acid sequence homology of the TGIF protein in different species. Proline (P) in position 292 of the protein TGIF is conserved through different species. The conserved proline (P) is highlighted in bold.

A third haplotype (H3) within the *TGIF* gene, distinct from the haplotypes containing the 11 allele of D18S63, also showed a trend towards significant association with psychosis in our sample. Distinct haplotypes associated with increased risk for a disorder can occur when two ancestral variants within the same gene are introduced into a population. This H3 haplotype (Table 5) is of special interest, as it contains a novel nonsynonymous SNP that produces a change of a conserved residue in position 292 (P292S) of the protein (Figure 2). This region of the protein is highly conserved throughout evolution, remaining unchanged in several species (Figure 3). TGIF regulates normal neuronal development and mutations within the gene have been found to cause holoprosencephaly (HPE), the most common defect of the developing forebrain in humans.[35–38] A change in position 291 of this protein from Ser to Phe is known to cause holoprosencephaly type 4 (HPE4) (UniProtKB/Swiss-Prot: Q15583; variant: VAR_009964) (Figure 2). Our current finding of a novel mutation immediately contiguous to the site of the HPE4 mutation makes this novel SNP very attractive for further functional studies of this polymorphism and its potential relation to a spectrum of psychotic disorders. However, it is important to note that, in our sample, this variant only shows a trend to association with psychosis, and this is only seen when it occurs on a specific haplotypic background (H3). Haplotypic backgrounds, including variations in introns, have been known to influence the severity of an associated phenotype caused by an exonic variant.[39]

TGIF is a member of the TALE superclass of homeobox proteins. The genes encoding these proteins are highly conserved in different species. TGIF acts as an inhibitory transcription factor by repressing transcription of several genes and this activity plays an important role in the brain, where a large number of genes are expressed with unique regional distributions.[40] Human TGIF (5′-TG-3′ interacting factor) is known to bind to the RXR-response element in the cellular retinol-binding protein II promoter, which contains an unusual DNA target for homeobox proteins. TGIF inhibits the 9-*cis*-retinoic acid-dependent RXRα transcription activation of the retinoic acid responsive element. TGIF is also an active transcriptional co-repressor of the SMAD2/SMAD4 complex in the transforming growth factor beta (TGF-beta) signaling pathway. This repressive action of TGIF is produced by an indirect competition with transcriptional activators that also bind the SMAD2/SMAD4 complex and recruit histone acetylases to activate transcription. The competition between repressors such as TGIF and activators determines the magnitude of the response to TGF-beta signals.[37] The TGF-beta signaling pathway in the brain is involved in neuroprotection against glutamate cytotoxity, cell migration of the cerebral cortex, control of neuronal death and microgliosis, induction of midbrain dopaminergic phenotype and influences neuronal survival.[41–49] This TGF-beta pathway is indirectly linked to activation by proteins encoded by the *NRG1* gene,[50] another strong candidate gene for psychotic-related disorders in the CVCR.[50–57] The genes encoding for the effectors of this pathway (SMAD2 and SMAD4) are both in chromosome 18 and *SMAD4* is located in a region for which we have also found association to psychosis in the CVCR population.[58]

The interplay of a variety of transcription factors like homeoproteins plays an important role in the regulated, tissue-specific and developmental expression of eukaryotic genes.[59] For example, MEIS2, another homeobox protein which activates the Dopamine 1A (*D1A*) gene promoter in most cells, is in direct competition with TGIF for binding to DNA *cis*-acting factors. TGIF represses MEIS2-induced transcription activation of the D1A receptor; the delicate ratio between MEIS2 and TGIF expression in a given cell type determines the cell-specific expression of the *D1A* gene.[60]

The present study has several limitations. First, we did not analyze all possible SNPs in the region surrounding D18S63, and therefore cannot rule out the possibility of other genes in the region being associated with psychosis in this sample. Similarly, although we tested SNPs from several genes in this region, to determine if they were in strong LD with D18S63, there may be rare variants that are associated to the 11 allele of D18S63 within those other genes that cannot be determined without exhaustive sequencing of the region. For testing the LD between D18S63 and the other SNPs, we used the D' value as calculated by FBAT, which does not test for r^2 between the markers. Compared to r^2, LD as defined by D' may be inflated. Since we were performing LD in order to help narrow our focus (i.e. to rule out those genes not showing high LD with D18S63), and D'

overestimates LD compared to r^2 (a more accurate measure of LD), this approach is conservative in terms of not excluding genes. Despite these limitations, the present results do offer confirmatory evidence that a variant within the *TGIF* gene (the 11 allele of D18S63) is associated with psychotic disorders in the Costa Rican population, and suggest that the *TGIF* gene is the most likely candidate for further analysis in this region of chromosome 18. Specific variants can now be studied in additional samples from the CVCR and other populations to confirm the potential role of this gene in contributing to the development of psychotic disorders.

Altogether, our results point to the possible involvement of *TGIF* in the pathophysiology of psychotic-related disorders. To our knowledge this is the first report of association between *TGIF* and the phenotype of psychosis. Since TGIF is necessary for development of the brain, survival of neurons and the expression of dopamine receptors, functional alterations in this gene may contribute to one or more of the pathways currently hypothesized to underlie the development of schizophrenia, including neurodevelopmental,[61] neurodegenerative[62] and dopaminergic[63] etiologic models. Replication analyses analyzing *TGIF* variants in different populations, comparison to control samples in the CVCR, more intensive sequencing of intronic and promoter regions in this gene in affected subjects, as well as functional studies of the newly defined *TGIF* variant (rs4468717) should now be carried out to determine the possible involvement of this gene in schizophrenia and other psychotic disorders.

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