Spatiotemporal 7q11.23 Protein Network Implicates the GTF2I-PRKDC-DDR Pathway During Early-Fetal Brain Development in Psychiatric Diseases

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1 **Article**

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3 **Spatiotemporal 7q11.23 protein network implicates the GTF2I-PRKDC-DDR**
4 **pathway during early-fetal brain development in psychiatric diseases**

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Abstract

Background: Recurrent deletions and duplications of chromosome 7q11.23 copy number variants (CNVs) are associated with several psychiatric disorders. Previous works showed GTF2I associated with Williams-Beuren syndrome, but pathways affected by GTF2I are poorly defined. Although phenotypic abnormalities have been observed in patients and animal models, the targeted human brain regions, developmental stages, protein networks, and signaling pathways, influenced by this CNV remain unclear.

Results: Topological changes were observed in protein-protein interaction (PPI) networks throughout different stages of brain development. Early and late fetal periods of development in the cortex, striatum, hippocampus, and amygdala were observed as the vital periods and regions for 7q11.23 CNV proteins. As a driver gene, GTF2I interacted with PRKDC and BRCA1 to involve in DNA Damaging Response (DDR) pathway. The physical interaction between GTF2I with PRKDC was confirmed experimentally by the liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Conclusion: We identified that striatum, hippocampus, and amygdala are crucial regions for establishing connectivity between 7q11.23 proteins and their partners in early and late fetal periods. Our results suggested that GTF2I-PRKDC-DDR and GTF2I-BRCA1-DDR pathway is crucial for the 7q11.23 CNV genes to contribute to the pathogenesis of psychiatric diseases.

Keywords: PPIs, spatiotemporal network, Chromosome 7q11.23, GTF2I

Background

Copy number variants (CNVs) are alterations in the number of copies of a genomic DNA region; these alterations can include duplications and deletions [1, 2]. CNVs have been shown to act as significant risk factors for complex disorders (e.g., neurodevelopmental and autoimmune) in humans [3-5]. Previous studies have shown that the duplication or deletion of 7q11.23 has been associated with several psychiatric disorders [6, 7]. For example, the duplication of 7q11.23 can lead to the development of autism spectrum disorder (ASD) or attention deficit hyperactivity disorder [8-10], and the deletion of 7q11.23 would cause a specific neurodevelopmental disorder, Williams–Beuren syndrome (WBS). In addition, the microcephaly has been observed recurrently in carriers of the 7q11.23 deletion [11], while, the macrocephaly clinical phenotype has been observed sometimes in duplication carriers [12].

The intricacy and highly polygenic characteristics of psychiatric disorders hamper our ability to identify the underlying mechanisms of action with regard to their genetic risk factors. One way of addressing this issue is by analyzing omics data to identify a diverse range of genetic causes. For example, a previous transcriptomic study of the

7q11.23 CNV in patient-derived induced pluripotent stem cell (iPSC) lines suggested alterations in gene expression, related signaling pathways, and molecular networks [13]. Furthermore, one gene with a 7q11.23 deletion/duplication, general transcription factor II-I (*GTF2I*), was found to play an important role in the differentiation of iPSCs into disease-relevant lineages [13]. Although a related molecular network was established in this previous study, the network was static and only one developmental period was investigated.

A different approach of addressing this issue is based on creating animal or cell models to help identify the related molecular and cellular mechanisms. For instance, mice with a heterozygous deletion of *GTF2I* or *GTF2IRD1* show defects in skeletal and craniofacial. [14]. In addition, the embryos of these mice present with a small head; this is consistent with the clinical phenotype of patients carrying a 7q11.23 deletion. Nevertheless, the signaling pathways affected by this CNV remain unknown. Replication factor C subunit 2 (*RFC2*), another 7q11.23 gene, encodes a subunit of the replication factor C (RFC) complex [15] and is known to play a role in ATR signaling [16, 17]. Haploinsufficiency for *RFC2* led to G2/M checkpoint arrest after DNA damage [18]. However, little is known about how genes with the 7q11.23 deletion/duplication may affect the occurrence of neurodevelopmental disorders because these genes are involved not only in multiple developmental stages but also within different tissues. Hence, genes exhibiting 7q11.23 deletion/duplication play

different roles in different developmental stages and different anatomic structures.

CNVs have been reported to modulate gene expression, which, ultimately, might affect disease predisposition or clinical phenotypes [19, 20]. Several researches have investigated CNV pathogenesis in psychiatric disorders by constructing a static topological network based on a single developmental stage [21]. Within different developmental periods, protein expression can change, as can protein-protein interactions (PPIs) [22]. Nevertheless, protein expression is a dynamic process that can occur in a different manner across different anatomical areas [23, 24]. Analyses of molecular networks can reveal biological modularity and complex signaling pathways [25, 26]. Previous studies discovered the pathogenesis of CNVs by constructing dynamic protein-protein interaction (PPI) networks according to alterations of protein expression in different anatomical areas and during different developmental periods [27, 28].

In addition, multiple studies mentioned above focused only on one or two genes and were unable to demonstrate how the 7q11.23 CNV is involved in brain development. Although phenotypic abnormalities have been observed in patients and animal models, the targeted brain regions, periods, protein networks, and signaling pathways, influenced by this CNV remain unclear. To overcome these knowledge gaps, we

constructed a dynamic spatiotemporal network of genes exhibiting the 7q11.23 deletion/duplication by integrating data from the human brain developmental transcriptome with physical interactions of 7q11.23 proteins.

Our study demonstrated that 7q11.23 proteins interact with their partners mainly in three spatiotemporal intervals, and that the interaction patterns change across these intervals. We identified that striatum, hippocampus, and amygdala are crucial regions for the interactions between 7q11.23 proteins and their partners in early and late fetal periods. In addition, our results indicated that GTF2I plays a key role in a dynamic network by interacting with DNA-dependent protein kinase catalytic subunit (PRKDC) and Breast cancer type 1 susceptibility protein (BRCA1). We undertook co-immunoprecipitation (Co-IP) experiments and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify the interactions between GTF2I and PRKDC.

Results

Construction of a spatiotemporal interaction network for 7q11.23

In general, PPIs take place only if proteins are located in the same cell components simultaneously [29, 30]. A range of studies has demonstrated robust correlations between higher co-expression and protein interaction across most cellular conditions

[31]. Thus, the combination of data relating to gene expression and protein interaction could reveal protein interactions at different developmental stages and in different anatomical regions. To investigate the regulatory roles of the 7q11.23 CNV in the development of the human brain, we extracted 21 brain-expressed genes located on the chromosomal region of 7q11.23 encompassing ~1.4Mb (chromosome 7: 72.4–73.4 Mb) (**Supplementary Table S1**) and constructed dynamic networks by integrating spatiotemporal gene co-expressions of the developing human brain with the physical PPIs of 7q11.23 proteins (**Fig. 1**).

We obtained gene expression data in the brain from BrainSpan (www.brainspan.org) and partitioned different regions and periods, as described by Lin and colleagues (**Supplementary Table S2**) [27]. We defined 32 spatiotemporal intervals based on eight periods of brain development (P1–P8) and four brain regions (R1–R4) and excluded P3R4 (P3, early mid-fetal; R4, mediodorsal nucleus of the thalamus and cerebella cortex) due to insufficient data for analyses. Three control datasets were used for controlling biases in the analyses: (i) physically interacting partners interacting with proteins of common CNVs identified in the 1000 Genomes Project; (ii) all possible pairs between 7q11.23 CNV genes and brain expressed genes; and (iii) all brain-expressed proteins interacting with their physically interacting partners.

7q11.23 co-expressed interacting protein pairs are enriched in early and late fetal periods

To identify significant enrichment of connectivity for 7q11.23 CNV, fractions of co-expression interacting pairs were calculated for 7q11.23 proteins and three control datasets across 31 spatiotemporal intervals. After false-discovery rate (FDR) correction for multiple testing, we observed significantly more co-expressed interacting pairs in two spatiotemporal network intervals than in all three controls: P1R1 (P1: early fetal; R1: parietal, temporal, and occipital cortex; Fisher's exact test; $p = 1.05 \times 10^{-6}$, $p = 3.41 \times 10^{-15}$, $p = 4.82 \times 10^{-6}$) and P1R3 (P1: early fetal; R3: amygdala, hippocampus, and striatum; Fisher's exact test; $p = 1.41 \times 10^{-8}$, $p = 3.41 \times 10^{-15}$, $p = 2.19 \times 10^{-15}$ (**Fig. 2**). Another spatiotemporal interval, P4R3 (P4: late fetal; R3: amygdala, hippocampus, and striatum), showed a significantly more co-expressing interacting pairs in 7q11.23 CNV network than two of control networks: (i) co-expressing physical PPIs of common CNVs ($p = 4.34 \times 10^{-2}$) and (ii) possible pairs between proteins with the 7q11.23 CNV and all brain-expressed proteins ($p = 2.24 \times 10^{-7}$) (**Fig. 2**).

Similarities and differences between the 7q11.23 networks

Next, we investigated similarities between the three significant networks, by identifying their convergence by computing the fraction of shared proteins in these

networks. We observed that 13 of 23 (56%) 7q11.23-CNV proteins and 71 of 290 (24.5%) of their co-expressed interacting partners were shared by all three networks in three intervals: P1R1 (P1: early fetal; R1: parietal, temporal, and occipital cortex), P1R3 (P1: early fetal; R3: amygdala, hippocampus, and striatum) and P4R3 (P4: late fetal; R3: amygdala, hippocampus, and striatum) (**Fig. 3**). Next, we undertook analyses of functional enrichments on shared CNV genes and shared interacting partners using Metascape (<http://metascape.org>) [32]. The top-three significant categories for the “biological process” were “signal transduction by p53 class mediator”, “regulation of cell cycle process”, and “mRNA processing (**Fig. 3**).

Next, we compared connectivity of co-expressed interacting protein pairs either within the same developmental period (early fetal, P1) or within the same brain region (R3) to identify both topological and functional differences between the spatiotemporal 7q11.23 networks. As noted, we have identified three spatiotemporal networks with significantly enriched co-expressed PPI pairs across different brain regions (R1 and R3) within the same developmental period (early fetal, P1) and also across different developmental periods (early fetal (P1) and late fetal (P4)) within the same region (R3). Network changes were assessed by calculating the fractions of co-expressed PPI pairs that were unique to one network against the fractions of co-expressed PPI pairs that were shared among networks (**Fig. 4**). We noted a significant difference between identical region within different developmental periods

(P1R3 and P4R3; ANOVA; $p = 2.95 \times 10^{-16}$) (**Supplementary Table S3**). In contrast, no significant difference was observed between same period within different regions (P1R1 and P1R3, ANOVA; $p = 0.349$) (**Supplementary able S4**).

7q11.23 networks involved in the regulation of DNA repair, DNA replication, and the cell cycle

Next, we set to investigate the biological functions of 7q11.23 proteins and their partners within three dynamic 7q11.23 networks, P1R1, P1R3 and P4R3. Hence, we analyzed functional enrichment of the pathways of related genes using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). For 7q11.23 proteins and their partners from the P1R1 network, the top-three significant terms for the biological process were “DNA repair”, “regulation of cell cycle process”, and “double-strand break repair” (**Fig. 5**). The top-three terms for the biological processes involving 7q11.23 proteins and partners from the P1R3 network were “DNA-dependent DNA replication”, “RNA splicing via transesterification reactions with bulged adenosine as nucleophile”, and “regulation of cell cycle process” (**Fig. 5**). For 7q11.23 proteins and their partners from the P4R3 network, the top-three significant terms were “regulation of DNA metabolic process”, “RNA splicing”, and “regulation of cellular protein localization” (**Fig. 5**). Next, we observed that 71 co-expressed and interacting partners of CNV proteins were exclusively from the P4R3 network, not from P1R1 and P1R3 networks, and associated with “signaling

pathways regulating pluripotency of stem cells (PSCs)", "EGFR tyrosine kinase inhibitor resistance", and "cell cycle", and the "hippo signaling pathway". (Supplementary Fig. S1).

Spatiotemporal networks identified driver gene and the GTF2I-PRKDC-DDR pathway

Of the three significant networks that we identified above, GTF2I possesses the highest radiality value, thus indicating that GTF2I is a driver gene and adopts a central position within these networks (Supplementary table S5). It has been reported that *GTF2I* heterozygotes exhibit microcephaly and retarded growth [14]. Recent studies have also demonstrated that *GTF2I* is involved in neurodevelopment [33, 34]. Importantly, the phenotype observed in mice mirrors that observed in humans [35]. As a driver gene within networks, *GTF2I* is a crucial contributor to neuropsychiatric disorders [36-38]. Hence, we investigated the interaction pattern of GTF2I across three significant spatiotemporal networks.

Seventy-three proteins interacted physically and were co-expressed with GTF2I across three spatiotemporal intervals (Fig. 4). Several of these partners were hub proteins which interacted with several CNV proteins. These hub proteins also interacted physically and were co-expressed with each other, thereby forming one

functional module (**Fig. 6A**). These hub partners were PRKDC, BRCA1, ZMYM2, ZMYM3, HDAC3, RPA1, RPA2, and RPA3. Of these partners, PRKDC possesses the highest radiality value in the functional module (**Supplementary Table S6**). GTF2I interacts with PRKDC, which acts as a “sensor” for double-strand DNA breaks [39, 40]. PRKDC lies within the 8q11.21 locus and promotes DNA repair *via* nonhomologous end-joining (NHEJ) [41].

GTF2I also interacts with BRCA1, which is a nuclear phosphoprotein required for the repair of double-strand DNA breaks and homologous recombination [42, 43]. Tanikawa M and colleagues showed that GTF2I proteins bind directly to BRCT (the carboxyl-terminal domain of BRCA1) [44], thus indicating that GTF2I plays an important role in DNA repair. ZMYM2 acts as transcription factor and is involved in DDR [45]. ZMYM3 is a component of histone deacetylase (HDAC)-containing multiprotein complexes. ZMYM3 and HDAC3 involve in a DNA-damage pathway and facilitates DNA repair [46, 47]. Furthermore, RPA1, RPA2, and RPA3, are subunits of the heterotrimeric replication protein A (RPA) complex, which is involved in DNA repair and DNA replication.

PRKDC and BRCA1 exhibited higher levels of connectivity in the early-fetal period than in the late-fetal period, thus suggesting that these two hub proteins play a more

important role during the early-fetal period. PRKDC and BRCA1 interact with two CNV proteins (GTF2I and RFC2) in P1R1 network. PRKDC interacts with GTF2I and RFC2 within P1R3 network. BRCA1 interacts with three CNV proteins (GTF2I, RFC2 and GTF2IRD1) in P1R3 network. PRKDC interacts with GTF2I in P4R3 network. BRCA1 interacts with GTF2I and GTF2IRD in P4R3 network. Within P4R3 network, the interactions between PRKDC and BRCA1 with RFC2 were not observed. PRKDC and BRCA1 exhibit a similar interaction pattern during early-fetal and late-fetal periods. The interactions between GTF2I and RFC2 with RPA1 exhibit no change during early-fetal and late-fetal stages.

Validation of the interaction between GTF2I and PRKDC by immunoprecipitation and LC-MS/MS

Next, we attempted to identify the proteins that interact with GTF2I. Hence, the immunocomplex arising from the immunoprecipitation of the control immunoglobulin G (IgG) antibody or Myc antibody was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by staining with Coomassie brilliant blue (CBB). Several unique protein bands were observed consistently in complexes that were pulled-down by the Myc antibody, but not in the control IgG (**supplementary Fig. S2**). Then, the interaction between GTF2I and PRKDC was detected by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (**Fig. 6B**).

De novo mutations are significantly enriched in spatiotemporal network

De novo mutations have been implicated in various psychiatric disorders as potential disease risks [48, 49]. Thus, we compared all of the interacting partners and 7q11.23 proteins with the *de novo* mutations observed in psychiatric diseases. Proteins from the spatiotemporal 7q11.23 network were enriched significantly in ASD genes (FDR-corrected $p = 7.21 \times 10^{-4}$). These proteins were also significantly enriched in genes associated with developmental delay (FDR-corrected $p = 0.0045$) and the target genes for fragile X mental retardation protein (FMRP) (FDR-corrected $p = 0.0017$). There was no significant difference between the entire 7q11.23 network and genes for genes associated with neurodegenerative disease (**supplementary table S7**).

Discussion

We constructed a dynamic network for the 7q11.23 CNV, which is a crucial risk factor for psychiatric disorders. Importantly, the spatiotemporal network indicated that 7q11.23 CNV genes played a crucial role in three intervals: P1R1 (early fetal, frontal lobe), P1R3 (early fetal, striatum, hippocampus and amygdale) and P4R3 (late fetal, striatum, hippocampus and amygdale). The early-fetal and late-fetal periods were the vital periods for 7q11.23 CNV proteins to affect human brain development. These results are in agreement with studies showing that *GTF2I* haplo-insufficient mice exhibit a small brain and neural defects during embryonic development [14, 50].

Sanders SJ and colleagues showed that the 7q11.23 CNV is involved in the pathogenesis of autism [28]. We observed that mutations in the proteins within the spatiotemporal 7q11.23 network were significantly enriched in ASD genes and the genes associated with developmental delay. Our results are in agreement with previous studies that discovered the fetal period was crucial for the pathogenesis of ASD [28, 51]. Our study suggests that the hippocampus, amygdala, striatum, and frontal lobe, are crucial regions affected by CNV genes. This result is in accordance with a previous report that showed the amygdala, cortex, and hippocampus to be abnormal in mice exhibiting a heterozygous deletion of 7q11.23 [52]. These results indicate the 7q11.23 CNV plays a significant role in development of the amygdala, cortex, and hippocampus of the human brain.

Our data also suggest that *GTF2I* is a candidate driver gene within the significant networks. Deurloo MHS and colleagues previously showed that *GTF2I* plays a pathological role in WBS [34]. Microcephaly and retarded growth were observed in mice that were heterozygous for *GTF2I* [14]. Importantly, one of the pathways our study suggests as being most likely impacted by the 7q11.23 CNV is DNA repair pathway. We observed that *GTF2I* and *RFC2* interacted with *PRKDC*, a vital hub partner which possesses the highest radiality value. Through proteomic analyses, we identified that *GTF2I* interacted with *PRKDC*. As a significant hub partner, *PRKDC* encodes the catalytic subunit of DNA-dependent protein kinase and is associated with

the repair of DNA double-strand breaks by NHEJ [53, 54]. Mice exhibiting the homozygous deletion of PRKDC show increased levels of apoptosis in the neocortex [55]. PRKDC maintains the integrity of the genome and plays a neuroprotective role in the nervous system following DNA damage [55]. A common pathogenic mechanism of microcephalic disorders is defective DNA repair [56]. O'Driscoll M and co-workers previously showed that PRKDC mutations can lead to microcephaly [57]. Our present data further demonstrated that GTF2I interacts with PRKDC to involve in a pathway of DNA-damage repair. Dysregulation of DDR pathway has been shown to be a pivotal cause of neurodevelopmental disease [58]. Based on the assessment of spatiotemporal networks, the interaction between GTF2I and PRKDC was observed within the frontal lobe (R1) and striatum, hippocampus, amygdale (R3) during early-fetal (P1) and late-fetal (P4) periods.

We found that several hub partners involved in DNA-repair pathway. BRCA1 interacts with three CNV proteins, GTF2I, GTF2IRD1, and RFC2. GTF2I interacts with BRCA1 in vivo and improves the transcriptional activation of BRCT [44]. The BRCA1-associated genome surveillance complex is related to the recognition and repair of DNA damage. BRCA1 affects the embryonic development of mouse brains and postnatal mouse brain size [59, 60]. The deletion of BRCA1 in neural progenitors leads to the disruption of normal differentiation [60]. Pyramidal neurons originating from BRCA1-knockout mice lack the typical radial orientation of apical dendrites

[59]. GTF2I and RFC2 interacts with RPA, which consists of three subunits: RPA1, RPA2, and RPA3. RPA activates ATR-mediated pathways and is involved in ATR-dependent DDR and cell-cycle arrest [61, 62]. O'Driscoll M and colleagues suggested a causal relationship between dysfunction in ATR signaling and developmental delay. GTF2I and GTF2IRD1 heterozygotes exhibit microcephaly and neural defects [14, 50]. GTF2I and GTF2IRD1 can interact with HDAC3, ZMYM2, and ZMYM3. HDAC3 mediates the deacetylation of histones and plays an important role in cell survival and cell-cycle progression. ZMYM2 and ZMYM3 are members of the MYB transcription factor family. ZMYM2 involves in DDR pathway and transition of the G1/S phase of the cell cycle [63]. ZMYM3 facilitates DNA repair by regulating BRCA1 localization at damaged chromatin [46]. Our results suggest that GTF2I interacts with these hub proteins and involves in DNA-repair pathway to affect brain development.

Conclusions

We identified that striatum, hippocampus, and amygdala are crucial regions for establishing connectivity between 7q11.23 proteins and their partners in early and late fetal periods. Our results showed that GTF2I interacted with PRKDC, BRCA1 and other partners to involve in DDR pathway, and demonstrated its crucial role in the early and late fetal stages of brain development.

Methods

Identification of 7q11.23 genes and the human brain transcriptome data collection

Twenty-three genes located on the regions encompassing ~1.4Mb (chromosome 7: 72.4–73.4 Mb) [9, 64]. *FKBP6* and *WBCRS28*, were excluded because these two genes are not expressed ($\log_2$ -intensity <0.4) in the human brain.(supplementary table 1). This study utilized region- and time-specific transcriptomic data from the developing human brain; these data were acquired from BrainSpan (www.brainspan.org/) and linked to Gencode v3c genes.RNA-sequencing data from 16 cortical and subcortical structures of 13 developmental stages. To reduce noise, we removed genes with a $\log_2$ -intensity <0.4 in all samples and with a coefficient of variation <0.07 . Consequently, 15,095 genes were regarded to be expressed in the brain. Biogrid 3.4.161 (www.Biogrid.com) was downloaded in May 2018. Only physical PPIs were reserved. Following the removal of redundant and self-interacting data, 241123 pairs were retained. Physical PPIs were combined with the human brain transcriptome to construct a brain-expressed human interactome (HI_{BE}).

Construction of a spatiotemporal protein network

Human-brain transcription data were divided by 13 dissection stages from 16 anatomic structures [23].We defined eight non-overlapping periods by merging the developmental stages ranging from 8 post-conception weeks to 39 years-of-age, and

by eliminating samples from those that were 40 years-of-age due to their limited size [27]. According to anatomical and functional similarities, anatomical structures were divided into four areas (Supplementary Material). Therefore, 31 spatiotemporal protein networks were constructed following the removal of one region from P3 (P3R4) due to a lack of RNA-sequencing data. Genes within the 7q11.23 CNV were mapped to the HI_{BE} network to establish a static network. Data exhibiting spatiotemporal expression were incorporated with static PPI networks and the Spearman correlation coefficient was calculated. Interactions were corroborated only if the Spearman correlation coefficient was >0.5 . Consequently, 31 networks were constructed. Cytoscape software (version 3.7.2; <http://www.cytoscape.org/>) was used to visualize the network or specific module, and to calculate topological parameters.

Enrichment analyses in three spatiotemporal networks

One-way analysis of variance (ANOVA) was performed to assess differences between 7q11.23 networks from the same developmental period (P1R1 and P1R3) or from the same anatomical area (P1R3 and P4R3). Topological features were defined for each gene with the 7q11.23 CNV: the ratio of interacting partners unique to one network and the ratio of interacting partners shared by two networks. Significant differences were identified by ANOVA. Genes within specific networks were analyzed by online tools in Metascape. Functional enrichment was investigated in three GO categories: biological process, molecular function, and cellular component. Terms with $P < 0.01$, a minimum count of 3, and an enrichment factor >1.5 (the enrichment factor was

defined as the ratio of the observed count to the count expected by chance) were collected and grouped into clusters based on their membership similarities. More specifically, P-values were calculated based on the cumulative hypergeometric distribution. The Q-value was calculated using the Benjamini–Hochberg correction to account for multiple testing.

De novo mutations were associated with psychiatry disorders according to 19 publications. FMRP genes, ASD risk genes, and developmental-delay genes were obtained as described previously [65]. Differences between the mutations of interacting proteins from 7q11.23 spatiotemporal networks and mutations from 20240 genes were analyzed by Fisher’s exact test. P-values were corrected using the Benjamini–Hochberg method.

Cell culture and transfection

HEK293T cells were cultured in Dulbecco’s modified Eagle’s supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, and maintained in a humidified incubator at 37°C in an atmosphere containing 5% CO₂. For cell transfection, 1.5×10⁶ cells were seeded into a 10-cm dish until they reached 80–90% confluency. Transfections were undertaken using the jetPRIME Transfection Reagent with pCMV6-entry-myc-GTF2I. After 48 h, cells were washed with phosphate-buffered saline, collected, and resuspended in lysis buffer (20 mM Tris-Cl, 5 mM EDTA, pH

406 7.4, 150 mM NaCl, 1% Triton X-100, and 10% (v/v) glycerol), supplemented with
407 phenylmethylsulfonyl fluoride (1 mM) and complete protease inhibitor cocktail.
408 Proteins in the supernatant were collected by centrifugation at 13,000 rpm for 15 min
409 at 4°C; 5% of the supernatant was saved so that it could act as an input control. The
410 remaining cell lysates were immunoprecipitated with anti-Myc antibody
411 (Sigma–Aldrich, Saint Louis, MO, USA) or normal mouse IgG (Sigma–Aldrich)
412 rotated for 12 h at 4°C. Subsequently, cell lysates were added to 40 µL of protein G
413 beads and rotated overnight at 4°C. Immunocomplexes were washed three times in
414 lysis buffer and boiled with 5× SDS sample buffer; the supernatant was then collected
415 by centrifugation at 12000 rpm for 1 min at 4°C. Supernatants were resolved by 4-20%
416 polyacrylamide Tris-glycine SDS gels and stained by Coomassie Brilliant Blue.
417 Protein bands were excised (120 kDa and above 270 kDa)

418 **Peptide preparation and LC-MS/MS**

419 First, gels were de-stained with 50% (v/v) methanol and vortexed vigorously for 30
420 min. After de-staining, gel pieces were washed in water for 15 min. Gel pieces were
421 then dehydrated in 100% acetonitrile for 10 min and dried in a vacuum centrifuge.
422 The disulfide bonds of proteins were then reduced with dithiothreitol (10 mM) and
423 alkylated with iodoacetamide (55 mM). Next, gel pieces were washed with 50% (v/v)
424 acetonitrile, NH_4HCO_3 (25 mM) and dehydrated with 100% acetonitrile. Gel pieces
425 were digested with trypsin in NH_4HCO_3 (25 mM). Peptides were extracted with 50%
426 (v/v) acetonitrile and 1% (v/v) trifluoroacetic acid. Free peptides were dried using a

vacuum centrifuge and separated using a liquid chromatograph (Easy-nLC 1000; Thermo Fisher, Waltham, MA, USA) and introduced into a Q Exactive mass spectrometer (Thermo Fisher). Finally, peptides were analyzed by MASCOT (www.matrixscience.com).

Proteome analyses

Data analyses were undertaken using Proteome Discoverer 1.4 (Thermo Scientific) which incorporates the MASCOT search engine. The *Homo sapiens* database from Uniprot was downloaded on 15 August 2019 and human protein sequences were searched. Carbamidomethyl was used as the fixed modification, with oxidation as the dynamical modification. The maximum number of missed cleavages considered was two. Immunoprecipitation samples were prepared in three independent experiments. Analyses involved only proteins that were detected by MS at least twice.

Abbreviations:

CNVs: Copy number variants; PPI: Protein-protein interaction; DDR: DNA Damaging Response; iPSC: Induced pluripotent stem cell; Co-IP: Co-immunoprecipitation; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; FDR: False-discovery rate; ANOVA: Analysis of variance; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; ASD: Autism spectrum disorder; WBS: Williams–Beuren syndrome; FMRP: fragile X mental retardation protein; IgG: Immunoglobulin G; CBB: Coomassie brilliant blue;

Acknowledgments

Not applicable

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

G.-N.L designed the experiments, interpreted the results, revised and approved the final version of the manuscript. Liang Chen designed the experiments, performed data analysis, conducted the major experiments, interpreted the results and drafted the manuscript. W.-X.C conducts part of experiments. W.-C.S and W.Q participated in data collection. W.-D.W revised the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

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659

660 **Fig. 1** A flow-chart shows the plan of work involved in this research study. (A)
661 Twenty-three 7q11.23 CNV genes expressed in the brain were identified. (B) Physical
662 protein-protein interaction dataset combined with 7q11.23 CNV genes to construct
663 CNV protein-protein interactions (PPIs). (C) 7q11.23 CNV PPIs combined with the
664 Human brain transcriptome dataset. (D) 7q11.23 spatiotemporal co-expression PPIs
665 network was established. (E) Functional enrichment analysis and functional module
666 analysis were performed.

Fig. 2 The 7q11.23 co-expressed interacting protein pairs are significantly enriched in three spatiotemporal intervals. The fractions of protein pairs from 7q11.23 CNV co-expressed and interacting with HIBE proteins (red line), all co-expressed and interacting HIBE proteins (black line), proteins from 1000 Genome Project CNVs co-expressed and interacting with HIBE proteins (dark gray line), and 7q11.23 CNV proteins co-expressed with all brain-expressed human genes (aquamarine line). Thirty-one spatiotemporal intervals of brain development are shown on the x-axis. 7q11.23 co-expressed interacting protein pairs are significantly enriched in spatiotemporal intervals (indicated by star symbol) compared with control networks. The statistical enrichment was calculated using Fisher's exact test, and p values were FDR-corrected for multiple comparisons.

Fig. 3 Functional convergence of the 7q11.23 spatiotemporal networks. The overlap of 7q11.23 genes (left Venn diagram) and their co-expressed interacting partners (right Venn diagram) across three significant spatiotemporal intervals. Top 20 significant enriched biological process GO terms of shared proteins are showed.

Fig. 5 Functional analyses of proteins within three significant intervals, P1R1, P1R3 and P4R3. Dot plot shows top 10 enriched GO terms of biological process for CNV proteins and their partners within three significant intervals.

corresponding networks, and the PPIs between co-expressed genes at a particular developmental period are showed as colored edges (P1R1, blueviolet; P1R3, green; P4R3, turquoises). (B) GTF2I and PRKDC were identified and the amino acids highlighted are peptides identified by immunoprecipitation (IP) and LC-MS/MS.

Supplementary

Table S1. Genes of 7q11.23 Copy number variation (CNV) region

Gene ID	Gene symbole	Official full name	position (hg38)	size
8468	FKBP6	FKBP prolyl isomerase 6	chr7:73,328,161-73,358,625	30,465
8326	FZD9	frizzled class receptor 9	chr7:73,433,778-73,436,120	2,343
9031	BAZ1B	bromodomain adjacent to Zinc finger domain 1B	chr7:73,440,406-73,522,293	81,888
9275	BCL7B	B-cell lymphoma 7	chr7:73,536,356-73,557,690	21,335
26608	TBL2	transducin-beta like 2	chr7:73,567,537-73,578,579	11,043
51085	MLXIPL	MLX interacting protein like	chr7:73,593,194-73,624,540	31,347
155382	VPS37D	VPS37D subunit of ESCRT-I	chr7:73,667,831-73,672,110	4,280
84277	DNAJC30	DnaJ heat shock protein family member C30	chr7:73,680,918-73,683,453	2,536
114049	WBSCR22	Williams-Beuren syndrome chromosome region 22	chr7:73,683,568-73,698,212	14,645
6804	STX1A	syntaxin 1A	chr7:73,700,827-73,719,631	18,805
83451	ABHD11	abhydrolase domain containing 11	chr7:73,736,094-73,738,867	2,774
1365	CLDN3	claudin 3	chr7:73,768,997-73,770,270	1,274
1364	CLDN4	claudin 4	chr7:73,827,744-73,832,684	4,941
155368	WBSCR27	methyltransferase like 27	chr7:73,834,590-73,842,516	7,927
135886	WBSCR28	transmembrane protein 270	chr7:73,861,159-73,865,890	4,732
2006	ELN	elastin	chr7:74,027,789-74,069,907	42,119
3984	LIMK1	LIM domain kinase 1	chr7:74,082,933-74,122,525	39,593
7458	EIF4H	eukaryotic translation initiation factor 4H	chr7:74,174,245-74,197,101	22,857
7462	LAT2	linker for activation of T cells family member 2	chr7:74,209,396-74,229,834	20,439
5982	RFC2	replication factor C, subunit 2	chr7:74,231,499-74,254,389	22,891
7461	CLIP2	CAP-Gly domain containing linker protein 2	chr7:74,289,475-74,405,943	116,469
9569	GTF2IRD1	GTF2I repeat domain containing 1	chr7:74,453,970-74,602,604	148,635
2969	GTF2I	general transcription factor II-I	chr7:74,650,231-74,760,692	110,462

Table S2. Developmental brain period from the BrainSpan related to Figure 1

stage	Description	Age	Developmental period
1	Early fetal	8-9 PCW	P1
2	Early fetal	10-12 PCW	
3	Early mid-fetal	13-15 PCW	P2
4	Early mid-fetal	16-18 PCW	
5	Late mid-fetal	19-23 PCW	P3
6	Late fetal	24-37 PCW	P4
7	Early infancy	0-5 M	P5
8	Late infancy	6-11 M	
9	Early childhood	1-5 Yr	P6
10	late childhood	6-11 Yr	
11	Adolescence	12-19 Yr	P7
12	Young adulthood	20-40 Yr	P8

Table S3. Results of ANOVA test for interaction patterns of proteins from P1R3 and P4R3 networks.

Interaction patterns of 7q11.23 CNV proteins across networks from different development periods.(P1:early fetal; P4: early mid-fetal) of the same brain region (R3: hippocampus, amygdala and striatum) and the results of the ANOVA test related to Fig 4. Bold font indicates the p-value from the ANOVA test (p-value < 0.05)

CNV genes		Interacting partners in P1R3 and P4R3				
Entrez gene ID	Official symbol	Total partners	unique to 1 count	network Freq	shared by 2 count	networks Freq
8326	FZD9	1	1	1.0000	0	0.0000
9031	BAZ1B	27	14	0.5185	13	0.4815
9275	BCL7B	5	5	1.0000	0	0.0000
26608	TBL2	16	14	0.8750	2	0.1250
155382	VPS37D	2	2	1.0000	0	0.0000
84277	DNAJC30	8	5	0.6250	3	0.3750
114049	WBSCR22	5	4	0.8000	1	0.2000
6804	STX1A	19	13	0.6842	6	0.3158
83451	ABHD11	2	2	1.0000	0	0.0000
1365	CLDN3	1	1	1.0000	0	0.0000
1364	CLDN4	1	1	1.0000	0	0.0000
2006	ELN	9	6	0.6667	3	0.3333
3984	LIMK1	18	15	0.8333	3	0.1667
7458	EIF4H	34	21	0.6176	13	0.3824
7462	LAT2	1	1	1.0000	0	0.0000
5982	RFC2	45	30	0.6667	15	0.3333
7461	CLIP2	3	3	1.0000	0	0.0000
9569	GTF2IRD1	22	13	0.5909	9	0.4091
2969	GTF2I	63	34	0.5397	29	0.4603

ANOVA Test

Summary

Groups Frequency of interactors	Count	Sum	Average	Variance
Unique to 1 network	19	15.418	0.811	0.0349
Shared by 2 network	19	3.582	0.189	0.0349

Result

Source of variation	SS	df	MS	F	p-value
Between Groups	3.686	1	3.686	105.7	$2.95 \times 10^{-12}***$
Within Groups	1.256	36	0.035		
Total	4.942	37			

Table S4. Results of ANVOVA test for interaction patterns of proteins from P1R1 and P1R3 networks.

Interaction patterns of 16p11.2 CNV proteins across networks from different brain regions (R1: parietal, temporal and occipital cortex; R3:hippocampus, amygdala and striatum) of the same developmental period (P1:early fetal;) and the results of the ANOVA test related to Fig 4. Bold font indicates the p-value from the ANOVA test (Not significant).

CNV genes		Interacting partners in P1R1 and P1R3				
Entrez	Official	Total partners	unique to network 1		shared by 2 networks	
gene ID	symbol		count	Freq	count	Freq
8326	FZD9	1	0	1.000	1	4.000
9031	BAZ1B	22	3	0.136	19	0.864
9275	BCL7B	5	4	0.800	1	0.200
26608	TBL2	16	12	0.750	4	0.250
84277	DNAJC30	8	3	0.375	5	0.625
114049	WBSCR22	3	1	0.333	2	0.667
6804	STX1A	16	10	0.625	6	0.375
83451	ABHD11	4	4	1.000	0	0.000
1365	CLDN3	1	1	1.000	0	0.000
1364	CLDN4	1	1	1.000	0	0.000
2006	ELN	10	8	0.800	2	0.200
3984	LIMK1	16	13	0.813	3	0.188
7458	EIF4H	29	10	0.345	19	0.655
5982	RFC2	42	12	0.286	30	0.714
7461	CLIP2	2	0	0.000	2	1.000
9569	GTF2IRD1	15	13	0.867	2	0.133
2969	GTF2I	50	17	0.340	33	0.660

ANOVA Test

Summary

Groups Frequency of interactors	Count	Sum	Average	Variance
Unique to 1 network	17	10.469	0.616	0.122
Shared by 2 network	17	10.531	0.619	0.122

Result

Source of variation	SS	df	MS	F	p-value
Between Groups	0.111	1	0.1106	0.904	0.349
Within Groups	3.912	32	0.1223		
Total	4.023	33			

Figure S1. Significant enriched KEGG pathways of seventy-one partners which exclusively from P4R3 network.

Table S5. Parameters of spatiotemporal networks

A. Parameters of P1R1 network

Entrez gene ID	Gene symbole	Radiality	Degree	Eccentricity
9031	BAZ1B	0.7509	21	5
9275	BCL7B	0.5077	2	7
26608	TBL2	0.6505	13	7
2006	ELN	0.5723	8	7
7458	EIF4H	0.5791	23	7
5982	RFC2	0.7645	38	5
9569	GTF2IRD1	0.6386	6	7
2969	GTF2I	0.7951	48	5

Note: FZD9, DNAJC30, WBSCR22, STX1A, ABHD11, LIMK1, CLIP2 were excluded because of isolating from network.

B. Parameters of P1R3 network

Entrez gene ID	Gene symbole	Radiality	Degree	Eccentricity
9031	BAZ1B	0.6125	20	5
7458	EIF4H	0.6667	25	5
5982	RFC2	0.7009	34	5
9569	GTF2IRD1	0.6581	11	5
2969	GTF2I	0.7265	35	5

Note: FZD9, BCL7B, TBL2, DNAJC30, WBSCR22, STX1A, CLDN3, CLDN4, ELN, LIMK1, CLIP2 were excluded because of isolating from network.

C. Parameters of P4R3 network

Entrez gene ID	Gene symbole	Radiality	Degree	Eccentricity
9031	BAZ1B	0.6115	20	5
26608	TBL2	0.5687	11	5
7458	EIF4H	0.6025	22	5
5982	RFC2	0.6453	26	5
9569	GTF2IRD1	0.5980	20	5
2969	GTF2I	0.8007	57	3

Note: BCL7B, VPS37D, DNAJC30, WBSCR22, STX1A, ABHD11, ELN, LIMK1, LAT2, CLIP2 were excluded because of isolating from network.

Table S6. Parameters of interacting partners from functional module within P1R1, P1R3, and P4R3 networks.

A. Parameters of interacting partners of functional module within P1R1

Entrez gene ID	Gene symbol	Radiality	Degree	Eccentricity
5591	PRKDC	0.8611	7	2
672	BRCA1	0.7500	5	3
6117	RPA1	0.7778	6	3
6118	RPA2	0.7778	6	3
6119	RPA3	0.7500	5	3
8452	CUL3	0.6667	2	3
8841	HDAC3	0.7778	4	2
7706	TRIM25	0.7222	3	3
3191	HNRNPL	0.6389	2	3
9203	ZMYM3	0.5278	2	3

B. Parameters of interacting partners of functional module within P1R3

Entrez gene ID	Gene symbol	Radiality	Degree	Eccentricity
5591	PRKDC	0.8864	7	3
672	BRCA1	0.8636	5	2
6117	RPA1	0.8864	7	3
8452	CUL3	0.7500	3	4
3191	HNRNPL	0.8409	4	2
6119	RPA3	0.7273	3	4
6118	RPA2	0.8182	4	3
8841	HDAC3	0.7045	2	4
9203	ZMYM3	0.5227	1	4

C. Parameters of interacting partners of functional module within P4R3

Entrez gene ID	Gene symbol	Radiality	Degree	Eccentricity
6117	RPA1	0.8750	6	2
8452	CUL3	0.7708	3	3
672	BRCA1	0.8125	4	3
7750	ZMYM2	0.7292	3	4
9203	ZMYM3	0.7500	4	4
3191	HNRNPL	0.7917	3	3
6118	RPA2	0.5000	1	4

5591	PRKDC	0.8333	5	3
8841	HDAC3	0.7292	3	4
6119	RPA3	0.6458	1	3

Figure S2. SDS-PAGE separation of the immunoprecipitated proteins.

One-dimensional SDS-PAGE separation of the immunoprecipitated proteins using control IgG or Myc antibody from HEK293 cells. Several protein bands that are unique which identified by LC-MS/MS were listed on the right side of the panel.

Table S7. Enrichment analysis of de novo mutations from 7q11.23 spatiotemporal networks

Number of genes within 7q11.23 spatiotemporal networks	Number of autism risk genes	Number of autism risk genes in spatiotemporal networks	Fisher exact p-value	Benjamini-Hochberg adj p-value
635	239	20	0.00012	0.00072

Number of genes within 7q11.23 spatiotemporal networks	Number of developmental delay genes	Number of developmental delay genes in spatiotemporal networks	Fisher exact p-value	Benjamini-Hochberg adj p-value
635	1302	60	0.00302	p=0.00450

Number of genes within 7q11.23 spatiotemporal networks	Number of fragile X mental retardation protein (FMRP)	Number of FMRP genes in spatiotemporal networks	Fisher exact p-value	Benjamini-Hochberg adj p-value
635	839	45	0.00057	p= 0.00170

Number of genes within 7q11.23 spatiotemporal networks	Number of neurodegenerative disease genes	Number of neurodegenerative disease genes in spatiotemporal networks	Fisher exact p-value	Benjamini-Hochberg adj p-value
635	93	7	0.03124	0.03749

Figures

Figure 1

A flow-chart shows the plan of work involved in this research study. (A) Twenty-three 7q11.23 CNV genes expressed in the brain were identified. (B) Physical protein-protein interaction dataset combined with 7q11.23 CNV genes to construct CNV protein-protein interactions (PPIs). (C) 7q11.23 CNV PPIs combined

with the Human brain transcriptome dataset. (D) 7q11.23 spatiotemporal co-expression PPIs network was established. (E) Functional enrichment analysis and functional module analysis were performed.

Figure 2

The 7q11.23 co-expressed interacting protein pairs are significantly enriched in three spatiotemporal intervals. The fractions of protein pairs from 7q11.23 CNV co-expressed and interacting with HIBE proteins (red line), all co-expressed and interacting HIBE proteins (black line), proteins from 1000 Genome Project CNVs co-expressed and interacting with HIBE proteins (dark gray line), and 7q11.23 CNV proteins co-expressed with all brain-expressed human genes (aquamarine line). Thirty-one spatiotemporal intervals of brain development are shown on the x-axis. 7q11.23 co-expressed interacting protein pairs are significantly enriched in spatiotemporal intervals (indicated by star symbol) compared with control networks. The statistical enrichment was calculated using Fisher's exact test, and p values were FDR-corrected for multiple comparisons.

Figure 3

Functional convergence of the 7q11.23 spatiotemporal networks. The overlap of 7q11.23 genes (left Venn diagram) and their co-expressed interacting partners (right Venn diagram) across three significant spatiotemporal intervals. Top 20 significant enriched biological process GO terms of shared proteins are showed.

ANOVA tests	sum of squares	df	mean square	F	P-values
P1R1 and P1R3	0.111	1	0.1106	0.904	0.349
P1R3 and P4R3	3.686	1	3.686	105.7	$2.95 \times 10^{-12}***$

Figure 4

Difference between the 7q11.23 spatiotemporal networks. Comparison of spatiotemporal networks across different brain regions within the same developmental period (P1R1 and P1R3) and cross different development periods within the same brain region (P1R3 and P4R3). 7q11.23 genes are shown as red nodes, their co-expressed interacting partners as gray node, and the PPIs between co-expressed genes at a particular developmental period are shown as gray edges. The nodes that lost all edges were removed from the corresponding networks. Significant differences are observed across developmental periods but not across brain regions (ANOVA statistics shown below the graphs).

Figure 5

Functional analyses of proteins within three significant intervals, P1R1, P1R3 and P4R3. Dot plot shows top 10 enriched GO terms of biological process for CNV proteins and their partners within three significant intervals.