



# Familial Psychosis Associated With a Missense Mutation at *MACF1* Gene Combined With the Rare Duplications DUP3p26.3 and DUP16q23.3, Affecting the *CNTN6* and *CDH13* Genes

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## OPEN ACCESS

### Edited by:

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### Specialty section:

This article was submitted to  
Behavioral and Psychiatric Genetics,  
a section of the journal  
Frontiers in Genetics

Received: 29 October 2020

Accepted: 15 March 2021

Published: 07 April 2021

### Citation:

Pol-Fuster J, Cañellas F,  
Ruiz-Guerra L, Medina-Dols A,  
Bisbal-Carrió B, Asensio V,  
Ortega-Vila B, Marzese D, Vidal C,  
Santos C, Lladó J, Olmos G,  
Heine-Suñer D, Strauch K, Flaquer A  
and Vives-Bauzá C (2021) Familial  
Psychosis Associated With  
a Missense Mutation at *MACF1* Gene  
Combined With the Rare Duplications  
DUP3p26.3 and DUP16q23.3,  
Affecting the *CNTN6* and *CDH13*  
Genes. *Front. Genet.* 12:622886.  
doi: 10.3389/fgene.2021.622886

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Psychosis is a highly heritable and heterogeneous psychiatric condition. Its genetic architecture is thought to be the result of the joint effect of common and rare variants. Families with high prevalence are an interesting approach to shed light on the rare variant's contribution without the need of collecting large cohorts. To unravel the genomic architecture of a family enriched for psychosis, with four affected individuals, we applied a system genomic approach based on karyotyping, genotyping by whole-exome sequencing to search for rare single nucleotide variants (SNVs) and SNP array to search for copy-number variants (CNVs). We identified a rare non-synonymous variant, g.39914279 C > G, in the *MACF1* gene, segregating with psychosis. Rare variants in the *MACF1* gene have been previously detected in SCZ patients. Besides, two rare CNVs, DUP3p26.3 and DUP16q23.3, were also identified in the family affecting relevant genes (*CNTN6* and *CDH13*, respectively). We hypothesize that the co-segregation of these duplications with the rare variant g.39914279 C > G of *MACF1* gene precipitated with schizophrenia and schizoaffective disorder.

**Keywords:** copy number variant, whole exome sequencing, schizophrenia, *CDH13*, *CNTN6*, *MACF1*

## INTRODUCTION

Schizophrenia (SCZ), despite a relatively low prevalence, estimated to be around 0.75% (Moreno-Küstner et al., 2018), is ranked among the top 15 leading causes of disability worldwide (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). As much as 80% (Lichtenstein et al., 2009; Hilker et al., 2018) of SCZ risk may be explained by genetic factors, including both

common and rare genomic variants. Through genome-wide association studies (GWAS) hundreds of variants have been identified (Manolio et al., 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018), with an overall contribution of around 25% (Lee et al., 2012). Common SNPs have shown weak individual effects (odds ratios,  $<1.2$ ); however, copy number variants (CNVs), with a high impact on gene dosage, have been implicated in SCZ with larger effect size compared to SNPs (odds ratios, 2–57) (Rees et al., 2014). Moreover, increased SCZ susceptibility has been associated with increased frequencies of CNVs (The International Schizophrenia Consortium, 2008; Grozeva et al., 2012; Szatkiewicz et al., 2020) and large deletions (Bergen et al., 2012; Szatkiewicz et al., 2020). In addition to CNVs, rare *de novo* or recent single-nucleotide variants (SNVs) with large effects have emerged as highly penetrant contributors (Purcell et al., 2014; Genovese et al., 2016; Singh et al., 2016; Steinberg et al., 2017), thanks to the availability of next-generation whole genome or exome sequencing. Overall, psychotic disorders as SCZ or bipolar disorder (BD) are the consequence of inheriting a genomic architecture where rare variants with high impact precipitate together with common variants with lower impact. Family-based studies are a powerful strategy to identify rare SNVs or CNVs without the need of collecting large cohorts. The purpose of the current study was to conduct a systematic genomic approach to study a Majorcan family enriched for psychosis, having two subjects with SCZ, and two with schizoaffective disorder (SCA).

## MATERIALS AND METHODS

### Psychiatric and Cognitive Assessments

All family members underwent semi-structured interviews, using the Spanish version of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) Axis I (SCID-I), the Positive and Negative Syndrome Scale (PANNS), and the Diagnostic Interview for Genetic Studies (DIGS) (Supplementary Table 1). The cognitive assessment was performed using the Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery (MATRICS). It includes the assessment of 7 cognitive domains: speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition. The same trained psychologist performed all cognitive assessments and most of them were done domiciliary. Medication status was taken into account at the moment of the assessment.

### Subject Description

The proband of the Majorcan Family affected by SCA is a mother of 5 children (subject SCA-3, Figure 1A). She is characterized by alternating depressive and manic episodes accompanied by delusional ideation of paranoid type. The onset of symptoms was after the birth of her twin daughters. The proband has three sons (Figure 1A): SCZ-7 presents SCZ from the age of 19 with

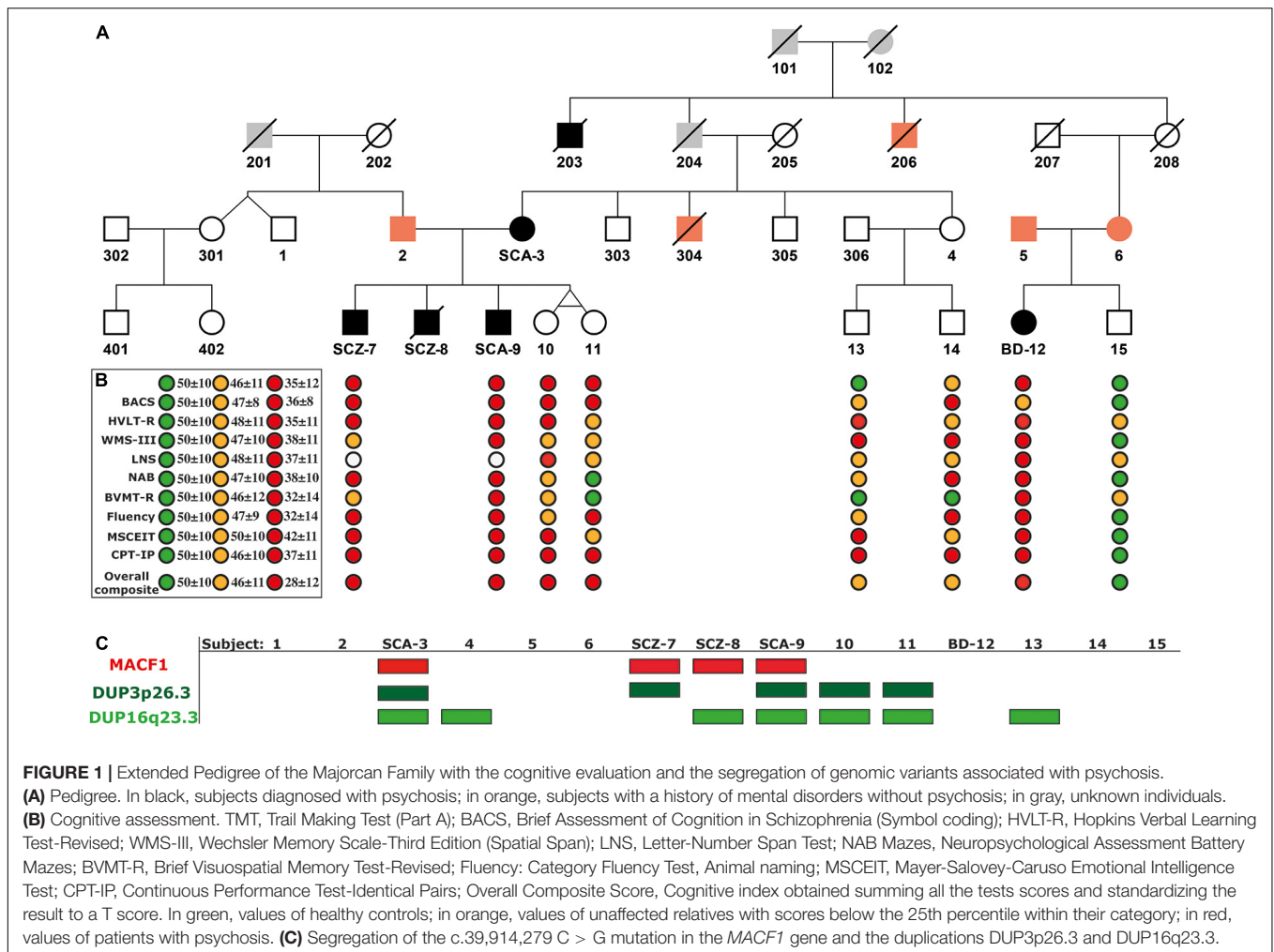
predominant negative symptoms. SCZ-8 onset with psychotic symptoms when he was 16 years old; he was diagnosed with paranoid SCZ and died by suicide in 2014. And SCA-9 started to evidence behavioral disorders, anxiety and somatic symptoms at the age of 14, diagnosed later as SCA. The twin daughters do not present psychiatric symptoms (subjects 10 and 11) at the age of 33 years old. The family history of the proband included a brother who died of cirrhosis due to alcoholism (subject 304, Figure 1A), an uncle with autism and psychosis (subject 203, Figure 1A), another uncle with mental illness, and epilepsy (subject 206, Figure 1A), and a cousin with anxiety (subject 6, Figure 1A). Subject 6 has a daughter affected by BD I (subject BD-12, Figure 1A). Her father (subject 5, Figure 1A) is diagnosed with major depressive disorder (MDD). The analysis presented in this article is focused on the nuclear family comprised of SCA-3, her husband, subject 2, and their children, SCZ-7, SCZ-8, SCA-9, and the twins, 10 and 11 (Figure 1A). Additionally, some extended family members of this nuclear family could be analyzed and were checked for the variants identified (Subjects 1, 4, 5, 6, 13, 14, BD-12, and 15; Figure 1). All studied family members signed the informed consent and the project was approved by the ethics committee of the Balearic Islands (CEI-IB).

### Blood Collection and DNA Extraction

Peripheral blood samples were obtained in EDTA tubes for the 7 members of the nuclear family and 8 additional subjects of the extended family. DNA extraction was performed using the DNA Blood Extraction Kit (5Prime®) according to the manufacturer's protocol.

### Genotyping by SNP Array

Genotyping was carried in the Genome Analysis Centre (GAC, Helmholtz Zentrum München) using the Infinium Global Screening Array-24 v1.0 (GSA) from Illumina (Illumina Inc., San Diego, CA, United States), which includes 642,824 SNPs. Besides, a pool of 57,254 SNPs previously related to neurological disorders was also genotyped. The genotype calling and CNV analysis were performed using the Genome Studio 2.0 (Illumina Inc. San Diego, CA, United States). Family samples genotyped by SNP array included all the samples of the nuclear family (subjects 2, SCA-3, SCZ-7, SCZ-8, SCA-9, 10, and 11), and extended family members (subjects 1, 4, 5, 6, 13, 14, BD-12 and 15). In total, 15 samples were genotyped by SNP array (Figure 1). For the CNV analysis, the *cnvPartition* algorithm, implemented in Genome Studio 2.0, was used (Illumina Inc. San Diego, CA, United States), taking as a reference GRCh37/hg19. This algorithm is based on two parameters: the B allele frequency (BAF) and the LogRRatio (LRR); both can be used to test the genotyping quality of the samples and to check the presence of CNVs across the genome. The BAF is a measure of allelic imbalance. In a normal well-genotyped sample, three genotypes are expected: homozygous AA, heterozygous AB, and homozygous BB. Once referred to the B allele, BAF is expected to have three discrete values: 0, 0.5, and 1 (representing AA, AB, and BB genotypes, respectively). R is defined as the sum of the probe intensities used to genotype the different markers. When R is normalized, it becomes the LRR which is a measure of relative intensity,



the logarithm (base 2) of the observed value of R (observed probe intensity) divided by the expected value (expected probe intensity) (Peiffer et al., 2006). Before running CNV analysis, array samples were quality controlled considering the missing call rate per subject, the number of null SNPs of a sample over the total number of genotyped SNPs. The missing call rate for all genotyped samples was below 1% (call rate over 99%) except for sample SCZ-9 which had a 10.92% missing call rate (call rate 89%). When the SCZ-9 array sample was visualized in Genome Studio 2.0, it could be observed that both the BAF and the LRR were altered for this sample. These results were consistent with a pattern of contamination, reason why this sample was excluded from the analysis. CNVs < 80,000bp were filtered. CNVs were visually inspected using Genome Studio 2.0, and CNVs carried by more than one subject of the nuclear family were validated by Digital Droplet PCR (ddPCR) (Supplementary Table 2).

### CNV Analysis by ddPCR

To confirm the CNVs identified by SNP array, Droplet Digital PCR (ddPCR) was performed. Before ddPCR, the genomic DNA of each family member was digested for 1 h

at 37°C with the restriction enzyme Hind III. ddPCR was performed using the following set of primers: for DUP16q23.3 5'-TTGGTGTGGACCCTGTGAA-3' (Forward) and 5'-TGA GCTAGGGCTCCCACTTA-3' (Reverse); for DUP3p26.3 5'-TCAGTGAAGTGCCTGGTTTG-3' (Forward) and 5'-GGCT GTTCCATGAGGAATGT-3' (Reverse). And the following 5'-FAM probes with a 3'-BHQ1 quencher: for DUP16q23.3 5'-FAM-TTTGGATTGCTTTGCCTACC-BHQ1-3', and DUP3p26.3 5'-FAM-CTAGGCTGGGCTCACTTGTC-BHQ1-3'. RPP30 was used as a reference control: 5'-GATTTGGACCTG CGAGCG-3' (Forward), 5'-GCGGCTGTCTCCACAAGT-3' (Reverse), and the probe VIC-CTGACCTGAAGGCTCT-BHQ1 (Supplementary Table 3). The PCR was performed in a C1000 touch thermocycler (Bio-Rad, Hercules, CA, United States) with the following protocol: Initial denaturalization at 95°C for 10 min, followed by 39 cycles at 94°C for 30 s and an extension at 57°C for 1 min, with a final denaturing at 98°C for 10 min. Droplet analysis was performed using the Q200 Droplet Reader (Bio-Rad). CNVs were calculated using the QuantaSoft software (Bio-Rad)<sup>1</sup>.

<sup>1</sup><http://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system>

## Whole Exome Sequencing (WES)

WES included three affected members (SCA-3, SCZ7, and SCZ-8) and one of the unaffected twin sisters (subject 10). Additionally, subject BD-12 from the extended family was also whole-exome sequenced. The SureSelectXT Human All Exon V6 capture library from Agilent Technologies (Santa Clara, CA, United States) was used for target enrichment. Exome sequencing was performed on an Illumina HiSeq4000 sequencer (Illumina Inc. San Diego, CA, United States), using 100 base paired-end sequencing, by BGI Tech Solutions. The workflow to obtain variant call format (VCF) files from raw data (FASTQ) provided by Macrogen was based on GATK Best Practices (Deprieto et al., 2011). FASTQ files, containing raw unmapped reads and Phred scores, were quality controlled using the FastQC v0.11.2<sup>2</sup>. Low-quality sequences (Phred score < 20) and adaptors were removed using Cutadapt (v1.4)<sup>3</sup>. QC sequences were aligned against the reference human genome (GRCh37/hg19) using the BWA-MEM algorithm implemented in the Burrows-Wheeler Alignment tool (BWA v0.7.12)<sup>4</sup>. Aligned data in SAM (Sequence Alignment/Map) format were then sorted and converted into BAM files using SAMtools (v1.1)<sup>5</sup>. To generate new BAM files, PCR duplicates were removed using Picard Tools v1.118<sup>6</sup>, and realignment around INDELS and base recalibration was performed (BQRS) using Genome Analysis Toolkit (GATK). SNV and INDEL calling was carried from the cleaned BAM files using GATK (v3.3.0)<sup>7</sup> producing unfiltered primary VCF files; which were then hard filtered to generate the definitive VCF files. To verify the exome sequencing results, Sanger sequencing was performed in the Genetic Analyzer AbiPrism 3700 (Applied Biosystems, Foster City, CA, United States) using standard polymerase chain reaction conditions. Results were visualized using Sequencing Analysis 5.1.1. (Applied Biosystems).

## Analysis of Rare SNV and CNV Variants

VCF files were annotated using variant annotation and effect prediction tool (SnpEff and SnpSift; Version 4.3)<sup>8</sup> (Cingolani et al., 2012). It allows annotating variants using the dbNSFP database, an integrated database of functional predictions from multiple algorithms. Variants were annotated, based on predictions comprised in the 2.1 version of the dbNSFP database, for protein impact using SIFT (Kumar et al., 2009), PolyPhen2 (Carter et al., 2013), LRT (Chun and Fay, 2009), MutationAssessor (Reva et al., 2007), Meta SVM and LR (Dong et al., 2015), VEST3 (Carter et al., 2013), FATHMM (Shihab et al., 2013), and MutationTaster (Schwarz et al., 2010); and for conservation using SiPhy\_29way\_logOdds (Garber et al., 2009), PhyloP100way\_vertebrate (Pollard et al., 2010), PhastCons100way\_vertebrate (Felsenstein and Churchill, 1996) and GERP++ (Davydov et al., 2010). The resulting files were

managed with R 3.6.1. Alternatively, VCF files were managed using ENLIS Genome Research version 1.9 (Berkeley, CA, United States). ENLIS uses its annotation pipeline. The RVI Score (Residual Variation Intolerance Score)<sup>9</sup> (Petrovski et al., 2013) was also used to assess the tolerance to variation of the genes affected by a variant in the family. RVIS is a ranked score of genes according to the number of common functional genetic variants they carry compared to the genome-wide expectation. RVIS is based on 6,503 WES samples from the NHLBI Exome Sequencing Project and it is the result of regressing the number of common functional variants on the total number of protein-coding variants. The loss-of-function observed/expected upper bound fraction (LOEUF) (Karczewski et al., 2019) was also considered as an indicator of gene tolerability to predicted loss of function variants. It is based on the continuous metric of the observed/expected (o/e) ratio and its confidence interval<sup>10</sup>.

Shared variation among affected individuals was filtered for Read Depth >10 and Minor Allele Frequency (MAF) <0.01. All genomic data for molecular variants in this study were compatible with Genome build GRCh37. Allele frequencies were checked in 1000G<sup>11</sup> (Birney and Soranzo, 2015) and in ExAC and its successor, the Genome Aggregation Database (gnomAD) (see text footnote 10) (Lek et al., 2016). Before validation, SNV variants were checked directly from BAM files using Integrative Genomics Viewer (IGV)<sup>12</sup> (Thorvaldsdottir et al., 2013). The identified SNV variants and the genes affected by them were checked on VarElect<sup>13</sup> (Stelzer et al., 2016), DisGeNET<sup>14</sup> (Piñero et al., 2020), and Schizophrenia Exome Sequencing Genebook (Purcell et al., 2014) to identify potential previous reports of these variants or genes in psychosis. Regarding CNVs, we checked different databases to find previous reports of the CNVs identified in the family and to study their pathogenicity and conservation: DGV (Database of genomic variants)<sup>15</sup> (MacDonald et al., 2014) and DECIPHER (Database of genomic variation and Phenotype in Humans using Ensembl Resources)<sup>16</sup> (Firth et al., 2009). VarSome<sup>17</sup> (Kopanos et al., 2019) and UniProt<sup>18</sup> (Bateman, 2019) were used to predict the levels of variant penetrance.

## RESULTS

### The Majorcan Family Is Characterized by Poor Cognitive Profiles

Psychotic subjects obtained lower overall composite scores compared to healthy family controls (**Supplementary Table 4**).

<sup>2</sup><https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

<sup>3</sup><https://cutadapt.readthedocs.io/en/stable/>

<sup>4</sup><https://github.com/lh3/bwa/releases/tag/0.7.12>

<sup>5</sup><https://github.com/samtools/samtools/releases/tag/1.1>

<sup>6</sup><https://github.com/broadinstitute/picard/releases/tag/1.118>

<sup>7</sup><https://github.com/broadinstitute/gatk/releases>

<sup>8</sup><http://snpeff.sourceforge.net/>

<sup>9</sup><http://genic-intolerance.org/>

<sup>10</sup><https://gnomad.broadinstitute.org/>

<sup>11</sup>[www.1000genomes.org](http://www.1000genomes.org)

<sup>12</sup><http://software.broadinstitute.org/software/igv/>

<sup>13</sup><http://varelect.genecards.org/>

<sup>14</sup><https://www.disgenet.org>

<sup>15</sup><http://dgv.tcag.ca/dgv/app/home>

<sup>16</sup><https://decipher.sanger.ac.uk/>

<sup>17</sup><http://varsome.com>

<sup>18</sup><https://www.uniprot.org>



Once scores were compared with normal population values obtained from Mucci et al. (2017) (Figure 1B), all family members performed below average. Processing Speed was especially low in subjects SCZ-7 and SCA-9, obtaining both of them the lowest possible scores in TMT and BACS tests (percentiles <0.1 considering their reference group matched for age and sex; Supplementary Table 4). Yet, these subjects performed better in fluency evaluation. Their sisters (subjects 10 and 11) and subject BD-12 also had low scores in processing speed (percentiles <5 considering their reference group matched for age and sex; Supplementary Table 4). Subjects 13 and 14 had scores in the range of healthy individuals. Working memory was especially low in the affected subject BD-12. LNS was not tested on affected subjects SCZ-7 and SCA-9 because they could not remember the alphabet.

## Identification of Two Inherited CNVs Associated With Psychosis

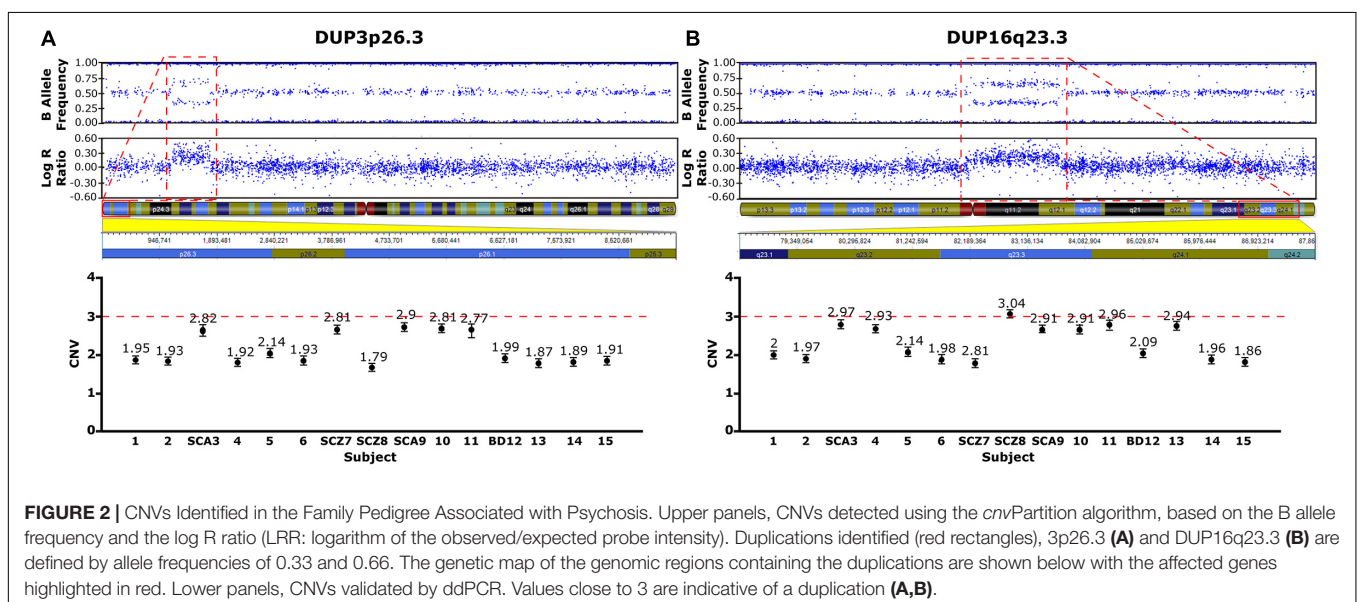
Two rare duplications (DUP3p26.3 and DUP16q23.3) were identified in the nuclear family (Figures 2A,B and Supplementary Table 2). These CNVs were validated by ddPCR in all studied family members (Figures 2A,B, lower panels). DUP3p26.3 is located on chromosome 3 (from 1,159,787 to 1,781,739 bp) and contains the contactin 6 (CNTN6) gene. Within these coordinates, 48 CNVs of similar size (under 1 Mb), containing the CNTN6 gene, have been reported (DECIPHER) (Firth et al., 2009). The gain of dose of this gene has been associated with intellectual disability (ID), delayed speech and language development, cognitive impairment, micro and macrocephaly, and autism (DECIPHER). The mother (SCA-3) carries DUP3p26.3 and transmitted it to her affected sons SCZ-7 and SCA-9 (Figure 2A). The healthy twin daughters also carry this duplication (subjects

10 and 11, Figure 2A). None of the extended relatives of the family carries it.

DUP16q23.3 is located on chromosome 16 (from 82,180,075 to 83,664,582 bp) and contains the cadherin-13 (CDH13) gene. 28 CNVs of similar size (under 2 Mb) have been reported within these coordinates, 18 of which are duplications affecting the CDH13 gene and are associated with ID, delayed speech, language development, and global developmental delay (DECIPHER). The mother (SCA-3) carries this duplication and she transmitted it to her affected sons SCZ-8 and SCA-9 (Figure 2B). Interestingly, the healthy twin daughters also carry this duplication (subjects 10 and 11, Figure 2B). Among the extended relatives of the family, DUP16q23.3 is also carried by an unaffected sister of the mother (subject 4), who transmitted it to one of her two unaffected sons, subject 13 (Figure 2B and Supplementary Table 2).

## Contributions of Rare SNVs Identified by WES

To identify rare variants of potentially higher penetrance, WES analysis was restricted to MAF < 1% according to ExAC, 1000G, and gnomAD. The psychotic patients shared 19 rare missense SNVs in conserved regions according to different conservation algorithms that were absent from the healthy twins (Tables 1, 2). Nonsense variants were not identified (Tables 1, 2). Regarding the 19 shared missense variants, all except two (rs146468598 and rs112913396) affect genes that are expressed in the cerebral cortex (GTEx database; Table 1). Out of these 17 variants, 9 are predicted “VUS” and 8 “benign” or “likely benign” by VarSome (Table 1). Among the 9 variants predicted “VUS,” 3 variants stood out because they were predicted “deleterious” by almost all protein prediction algorithms; p. P-56-L in MTMR11, p. P-241-S in TINF2, and p. Y-161-C in ZRANB3 (Table 3). Nevertheless, to our knowledge, the genes affected by these variants have no described functions in the central nervous



**TABLE 1 |** Rare non-synonymous variants identified in conserved regions by WES shared only among affected subjects.

Chr	bp	Ref	Alt	rs	Gene	Protein Impact	aa Change	GTEX	VarSome	DisGeNET	ExAC	gnomAD	1000G
1	39914279	C	G	rs376728302	MACF1	MISSENSE	T-4642-S	Yes	VUS	Yes	0.000058	0.000092	NA
1	149907574	G	A	rs202234090	MTMR11	MISSENSE	P-56-L	Yes	VUS		0.000115	0.000159	NA
2	136107663	T	C	rs183525970	ZRANB3	MISSENSE	Y-161-C	Yes (low)	VUS		0.001391	0.001672	0.001374
2	219528732	C	T	rs138321594	RNF25	MISSENSE	R-443-Q	Yes	VUS		0.000069	0.000089	NA
3	102187900	A	G	rs145497598	ZPLD1	MISSENSE	K-301-R	Yes (Very low)	VUS		0.000025	0.000021	NA
5	54721089	G	A	rs201703457	PPAP2A	MISSENSE	T-268-I	Yes	VUS		0.000016	0.000011	NA
5	175812257	G	A	rs35916027	NOP16	MISSENSE	R-120-C	Yes	VUS		0.004062	0.004068	0.005037
14	24709965	G	A	rs17102311	TINF2	MISSENSE	P-241-S	Yes	VUS		0.002158	0.002167	0.004121
22	38061637	A	T	rs142028345	PDXP	MISSENSE	Y-217-F	Yes	VUS		0.007042	0.000004	0.005952
1	23743859	T	C	rs192746462	TCEA3	MISSENSE	E-88-G	Yes (low)	Likely Benign		0.004760	0.004642	0.002289
2	109382936	T	A	rs61748150	RANBP2	MISSENSE	S-1981-T	Yes	Benign		0.008895	0.008229	0.006410
2	121748118	G	A	rs138987487	GLI2	MISSENSE	R-1543-H	Yes (low)	Benign	Yes	0.000502	0.000534	0.000916
2	230723775	G	A	NA	TRIP12	MISSENSE	S-205-L	Yes	Benign		0.000025	0.000028	NA
4	39450295	G	A	rs143809363	KLB	MISSENSE	V-1042-I	Yes (low)	Likely Benign		0.003534	0.003275	0.000458
9	79323853	C	T	rs200875180	PRUNE2	MISSENSE	D-1113-N	Yes	Likely Benign		0.003781	0.003776	0.000916
10	81925866	T	C	rs72807973	ANXA11	MISSENSE	I-278-V	Yes	Benign		0.002990	0.003189	0.003205
15	94841610	G	A	rs149237812	MCTP2	MISSENSE	R-39-Q	Yes (low)	Likely Benign	Yes	0.001400	0.001199	0.000916
2	238289767	T	C	rs112913396	COL6A3	MISSENSE	D-563-G		VUS		0.002314	0.002079	0.000916
5	56777915	C	T	rs146468598	ACTBL2	MISSENSE	R-207-Q		VUS		0.001820	0.002011	0.003205

19 variants with a MAF < 1% were shared by the 3 psychotic patients.

Chr, chromosome; bp, base pair; Ref, reference Allele; Alt, alternate allele; GTEX, GTEX expression; DisGeNET, previous relationship to SCZ; VUS, variant of uncertain significance.

\*Low median tpm below 2, \*\*Very low median tpm below 0.05, \*\*\*Genetic coordinates are given taking as a reference GRCh37/hg19, \*\*\*\*Variants were annotated based on the 2.1 version of the dbNSFP database, \*\*\*\*\*Allele frequencies from gnomAD were obtained from the 2.1.1 gnomAD version.

**TABLE 2 |** Conservation algorithms.

Chr	bp	Ref	Alt	rs	Gene	SiPhy	GERP_RS	GERP_NR	phyloP100	phastCons100
5	56777915	C	T	rs146468598	ACTBL2	15.631	4.91	4.91	7.645	0.989
10	81925866	T	C	rs72807973	ANXA11	9.0566	1.76	5.6	3.668	0.879
2	238289767	T	C	rs112913396	COL6A3	15.793	5.6	5.6	6.22	1
2	121748118	G	A	rs138987487	GLI2	18.456	4.98	4.98	9.657	1
4	39450295	G	A	rs143809363	KLB	12.583	4.83	5.68	1.477	0.998
1	39914279	C	G	rs376728302	MACF1	20.393	5.95	5.95	7.818	1
15	94841610	G	A	rs149237812	MCTP2	9.3437	3.19	5.13	1.658	0.044
1	149907574	G	A	rs202234090	MTMR11	12.986	4.68	4.68	3.916	1
5	175812257	G	A	rs35916027	NOP16	11.072	3.46	6.17	3.367	0.423
22	38061637	A	T	rs142028345	PDXP	10.036	4.54	5.58	4.666	1
5	54721089	G	A	rs201703457	PPAP2A	14.027	4.8	5.66	5.819	1
9	79323853	C	T	rs200875180	PRUNE2	14.954	5.94	5.94	4.165	0.984
2	109382936	T	A	rs61748150	RANBP2	16.063	5.75	5.75	5.214	1
2	219528732	C	T	rs138321594	RNF25	8.8576	3.74	5.55	1.989	0.99
1	23743859	T	C	rs192746462	TCEA3	11.332	5.02	5.02	1.69	1
14	24709965	G	A	rs17102311	TINF2	8.383	3.46	4.36	1.43	0.942
2	230723775	G	A	NA	TRIP12	19.489	5.72	5.72	9.434	1
3	102187900	A	G	rs145497598	ZPLD1	15.196	5.28	5.28	5.352	1
2	136107663	T	C	rs183525970	ZRANB3	15.459	5.42	5.42	6.253	1

Chr, chromosome; bp, base pair; Ref, reference Allele; Alt, alternate allele; SiPhy, SiPhy\_29way\_logOdds; phyloP100, phyloP100way\_vertebrate; phastCons, phastCons100way\_vertebrate.

\*Genetic coordinates are given taking as a reference GRCh37/hg19, \*\*Conservation scores produced by the different algorithms were obtained from the 2.1 version of the dbNSFP database.

**TABLE 3** | Protein prediction algorithms.

Chr	bp	Ref	Alt	rs	Gene	SIFT	Polyphen2	MutAssessor	SVM	LR	VEST3	FATHMM	LRT	MutTaster
5	56777915	C	T	rs146468598	ACTBL2	NA	0.461 (P)	3.37 (M)	0.7735 (D)	0.8058 (D)	0.785	-3.44 (D)	0.00001 (D)	0.999 (D)
10	81925866	T	C	rs72807973	ANXA11	0.174 (T)	0.005 (B)	-0.98 (N)	-0.915 (T)	0.0034 (T)	0.171	4.06 (T)	0.011694 (N)	1 (N)
2	238289767	T	C	rs112913396	COL6A3	0.005 (D)	1.0 (D)	2.82 (M)	-0.202 (T)	0.3558 (T)	0.932	0.75 (T)	0.000042 (D)	1 (D)
2	121748118	G	A	rs138987487	GLI2	0.054 (T)	1.0 (D)	1.89 (L)	-0.8456 (T)	0.1776 (T)	0.415	1.59 (T)	0.000001 (D)	1 (D)
4	39450295	G	A	rs143809363	KLB	0.035 (D)	0.18 (B)	0.695 (N)	-1.0599 (T)	0.0642 (T)	0.125	1.67 (T)	0.442178 (N)	0.994 (N)
1	39914279	C	G	rs376728302	MACF1	1 (T)	0.589 (P)	0.345 (N)	-1.0558 (T)	0.0883 (T)	0.546	0.78 (T)	0.000008 (D)	1 (D)
15	94841610	G	A	rs149237812	MCTP2	0.166 (T)	0.024 (B)	0.46 (N)	-0.8817 (T)	0.1776 (T)	0.161	-0.66 (T)	0.22127 (N)	0.999 (N)
1	149907574	G	A	rs202234090	MTMR11	0.02 (D)	0.932 (P)	2.045 (M)	0.2042 (D)	0.6049 (D)	0.568	-1.67 (D)	NA	1 (D)
5	175812257	G	A	rs35916027	NOP16	0 (D)	0.297 (B)	1.04 (L)	-1.0548 (T)	0.054 (T)	0.355	NA	0.000101 (N)	0.999 (N)
22	38061637	A	T	rs142028345	PDXP	1 (T)	0.087 (B)	-0.715 (N)	-1.0489 (T)	0.0258 (T)	0.25	1.59 (T)	NA	1 (D)
5	54721089	G	A	rs201703457	PPAP2A	0.077 (T)	0.263 (B)	1.905 (M)	-1.0907 (T)	0.2078 (T)	0.406	1.5 (T)	0.018731 (N)	1 (D)
9	79323853	C	T	rs200875180	PRUNE2	0.0 (D)	1.0 (D)	0.975 (L)	-0.8847 (T)	0.2006 (T)	0.202	0.31 (T)	0.000385 (D)	1 (D)
2	109382936	T	A	rs61748150	RANBP2	0.33 (T)	0.39 (B)	2.045 (M)	-1.0432 (T)	0.0603 (T)	0.16	1.56 (T)	NA	0.964 (N)
2	219528732	C	T	rs138321594	RNF25	0.005 (D)	0.223 (B)	0.895 (L)	-0.8015 (T)	0.1606 (T)	0.135	-0.21 (T)	0.020699 (N)	0.725 (N)
1	23743859	T	C	rs192746462	TCEA3	0.25 (T)	0 (B)	0.695 (N)	-1.0688 (T)	0.0598 (T)	0.379	NA	0.806532 (N)	0.992 (N)
14	24709965	G	A	rs17102311	TINF2	0.013 (D)	1.0 (D)	2.045 (M)	0.1966 (D)	0.7489 (D)	0.172	-3.19 (D)	0.000159 (N)	1 (D)
2	230723775	G	A	NA	TRIP12	0.017 (D)	0.994 (D)	0.695 (N)	-0.833 (T)	0.1914 (T)	0.684	0.8 (T)	0 (D)	1 (D)
3	102187900	A	G	rs145497598	ZPLD1	0.358 (T)	0.004 (B)	0.345 (N)	-0.6888 (T)	0.2761 (T)	0.5	-1.34 (T)	0 (D)	1 (D)
2	136107663	T	C	rs183525970	ZRANB3	0.001 (D)	1.0 (D)	3.255 (M)	1.0465 (D)	0.9121 (D)	0.857	-3.21 (D)	0 (D)	1 (D)

system and have not been associated with SCZ or any other mental disorder. Only three variants among the 17 expressed in the cerebral cortex affect genes previously associated with SCZ according to DisGeNET (Piñero et al., 2020): p. R-1543-H in *GLI2*, p. T-4642-S in *MACF1*, and p. R-39-Q in *MCTP2* (Table 1). Out of these three, only the *MACF1* variant is predicted “VUS” (Table 1). Moreover, the expression of *MCTP2* [median Transcripts Per Million (TPM) below 1] and *GLI2* (median TPM below 1.5) is low in the cerebral cortex (GTEx database). The variant located in the *MACF1* gene is located in the most conserved region among the 19 variants identified (Table 2). The variant in *GLI2* is also located in a very conserved region while the variant located in *MCTP2* is located in a less conserved region (Table 2). Moreover, among the 19 genes affected by the variants shared by psychotic individuals, *MACF1* is the most intolerant to variation (RVIS = -3.92 (0.21%); LOEUF = 0.084; Supplementary Table 5). Protein prediction algorithms show mixed results for the variants in *MACF1* and *GLI2* while the variant in *MCTP2* is predicted to be benign by all protein prediction algorithms used (Table 3). Given these results, we decided to analyze the segregation of the variants in *MACF1* and *GLI2*. The mother (SCA-3) transmitted the g.39914279 C > G (p. T4642S) mutation in *MACF1* to her three affected sons (SCZ-7, SCZ-8, and SCA-9), but not to her healthy twin daughters (subjects 10 and 11) (Figure 1C). Besides, this variant was not detected in any of the extended family members that were analyzed. The variant g.121748118 G > A (p. R1543H) in the *GLI2* gene was transmitted by the mother to her affected sons SCZ-7 and SCZ-8. The other affected individual, SCA-9, did not carry this variant which was neither detected in any of the extended family members analyzed. The presence of the duplications DUP3p26.3 and DUP16q23.3 *per se* is not sufficient to precipitate with psychosis,

since non-affected subjects carry both duplications (i.e., the twin sisters, subjects 10 and 11). The psychotic phenotype may have precipitated once patients inherited one of these two duplications together with the rare variant c.39,914,279 C > G in the *MACF1* gene.

## DISCUSSION

Despite their high heritability, the genetic architecture of psychotic disorders such as SCZ and BD has proven to be complex. Family-based studies, focused mainly on identifying rare inherited or rare *de novo* variants, have allowed identifying rare variants with very high penetrance, including CNVs (Malhotra and Sebat, 2012) and SNVs (Xu et al., 2011). Considering that psychosis is a complex phenotype and that the rare variants contribution is modulated by the genetic background (Nadeau, 2001; Koike et al., 2006; Kearney, 2011; Mitchell, 2011; Kerr et al., 2013) of the individuals carrying them, the more homogeneous the family and the closest the relationships included in the analysis the better.

### DUP3p26.3 and DUP16q23.3 Associated With Neurodevelopmental Disorders

The two CNVs identified in this pedigree are highly suggestive to play a significant role in the pathogenesis of psychosis. The duplication DUP3p26.3 has been extensively associated with neuropsychiatric disorders, including SCZ, autism spectrum disorder (ASD), BD, attention-deficit hyperactivity disorder (ADHD), ID, and Tourette syndrome (Kashevarova et al., 2014; Te Weehi et al., 2014; Hu et al., 2015; Huang et al., 2017; Mercati et al., 2017; Juan-Perez et al., 2018). This duplication alters the expression of the *CNTN6* gene, which encodes

the protein contactin-6, also termed NB-3, a member of the contactin family of immunoglobulin domain-containing cell adhesion molecules (IgCAMs). IgCAMs are important signal molecules that mediate cell-cell adhesion and cell-extracellular matrix (ECM) interactions in multiple neural developmental processes, including neuronal migration, neurite outgrowth, axon guidance, and synaptogenesis (Sethi and Zaia, 2017). In mice, *Cntn6* is exclusively expressed in the CNS, where it is needed for proper orientation of dendrite growth in cortical pyramidal neurons (Ye et al., 2008) and synapse formation in the cerebellum (Sakurai et al., 2009). The presence of the duplication in the family may result in a decrease of the *CNTN6* expression. Supporting this, a recent study comparing the *CNTN6* expression in neurons generated from inducible pluripotent stem cells (iPSCs) derived from fibroblasts of an ID patient carrying a *CNTN6* duplication and two healthy controls found that neurons carrying the duplication had significantly lower expression of *CNTN6* compared to wild-type neurons (Gridina et al., 2018). Although the crucial roles of *CNTN6* during brain development, the penetrance of this duplication in our pedigree and others previously reported (Kashevarova et al., 2014; Hu et al., 2015) may be low since there are healthy family carriers in different generations.

The second duplication identified in the pedigree is the DUP16q23.3, also very suggestive of playing a major role in psychosis. This duplication alters the expression of *CDH13*, a gene that encodes another adhesion molecule, Cadherin-13, a calcium-dependent class of transmembrane protein that forms adherent junctions and participates in cell-cell recognition and signal transduction, among other functions. Cadherin-13 is highly expressed in the brain, where it may function as a negative regulator of neurite outgrowth and axon guidance (reviewed in Rivero et al., 2013). *CDH13* involvement in neuropsychiatric disorders is well documented, associated with the five major psychiatric disorders: ADHD (Neale et al., 2008), ASD (Sanders et al., 2011), MDD (Terracciano et al., 2010), BD (Xu et al., 2014), and SCZ (Børglum et al., 2014; Otsuka et al., 2015). Despite none of these associations have been significant at a genome-wide level, as pointed by Prata et al. (2019), in their review of GWAS studies from 2012 to 2019, *CDH13* is a highly relevant candidate for psychosis because the GWAS evidence involving this gene with psychosis comes from at least two independent samples. As observed for the DUP3p26.3, the penetrance of DUP16q23.3 in this pedigree also seems low. Both duplications need to co-segregate with other genetic factors to precipitate with psychosis, and the rare variant g.39914279 C > G of *MACF1* is a good candidate. Interestingly, all the carriers of the duplications DUP3p26.3 and DUP16q23.3 evidenced very low cognitive performance; therefore, it seems obvious that both duplications have also an impact not only in psychosis but also in other dimensions related to the disease, like cognition. In fact, other authors found that CNVs conferring risk for SCZ and autism affected cognition not only in patients but also in carrier controls (Stefansson et al., 2014). This supports our hypothesis that the combination of these CNVs with the rare

variant in *MACF1* is precipitating with SCZ and SCA in the analyzed pedigree.

## Facts to Consider *MACF1* a Genetic Risk Factor for Psychosis

The function of the Microtubule-actin crosslinking factor 1 (*MACF1*), also known as actin crosslinking factor 7 (*ACF7*), is to connect the actin and microtubule cytoskeletons with the sites of cell-matrix and cell-cell adhesions (Hu et al., 2016), being an important modulator of cytoskeletal networks (Hu et al., 2016; Moffat et al., 2017). Defects in these cross-talks may have a profound impact on cell proliferation and differentiation, neuronal migration, neurite development, and axon guidance (Sanchez-Soriano et al., 2009; Goryunov et al., 2010; Ka et al., 2014, 2017; Ka and Kim, 2016; Dobyns et al., 2018). Such defects are hypothesized as a pathogenic mechanism for psychosis and several genes involved in them have been associated with SCZ or BD (Lewis et al., 2005; Rossignol, 2011; Muraki and Tanigaki, 2015; Ka et al., 2017). The role of *MACF1* in cytoskeletal networks makes it an interesting candidate for psychosis (Ka et al., 2017). Besides, *MACF1* also interacts with *GSK3β*, participating in the Wnt/ $\beta$ -catenin and *GSK-3* signaling pathway, during the migration of pyramidal neurons or neurite differentiation (Chen et al., 2006; Ka et al., 2014; Ka and Kim, 2016). Moreover, *MACF1* also interacts with *DISC1* and *DTNBP1* (Camargo et al., 2007; Costas et al., 2013). Interestingly, rare *de novo* variants have been previously described in BD patients (Kataoka et al., 2016; Han et al., 2019) and SCZ patients (Xu et al., 2012; Kenny et al., 2014; Wang et al., 2015) affecting this gene, which is highly intolerant to protein-altering variants. Intriguingly, both the variant identified in the family, g.39914279 C > G (p. T4642S), and other variants previously reported in SCZ patients, g.39827053 C > T (p. R2097W), and g. 39904999 C > T (p. R4033W), are located in the spectrin repeats domain of *MACF1* which interacts with *GSK3β* and other proteins that regulate neuronal migration (Chen et al., 2006).

The rare variant identified in *GLI2* may also contribute to the psychosis phenotype. The transcription factor Gli Family Zinc Finger 2 (*GLI2*) is a downstream modulator of the Sonic Hedgehog (*Shh*) signaling pathway. Disruption of the *Shh* signaling has been associated with several neurodevelopmental disorders (Chen et al., 2018), including SCZ (Betcheva et al., 2013). Using GWAS, the *GLI2* gene has been associated with the manifestation of tardive dyskinesia in SCZ (Greenbaum et al., 2010).

Here we have demonstrated that by combining wide-genomic approaches it is currently feasible to disclose the genomic architecture associated with psychosis in highly penetrant and homogeneous pedigrees. Our results also show that the genomics of psychosis is highly heterogeneous, which makes sense given the involvement of rare variants in its etiology. In this sense, psychosis in the family can be explained by the presence of the variant g.39914279 C > G in *MACF1* in combination with the two rare duplications, but this variant does not explain the psychosis phenotype of their distant relative BD-12. The presence of two highly suggestive duplications, and the cognitive performance



of the individuals carrying them, also suggests that the variant in *MACF1* is modulated by the genetic background of the family. Besides, our results confirm previous results suggesting the involvement of *MACF1*, *CNTN6*, and *CDH13* in the etiology of psychosis. More research is needed to further elucidate the role of *MACF1* in psychosis as well as its interaction with *CNTN6* and *CDH13*.

## DATA AVAILABILITY STATEMENT

The raw data will be available under the accession numbers: EGAS0001004791 and EGAD00010002030 Array\_data.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Balearic Islands (CEI-IB). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JP-F performed most of the analyses, including WES and CNV analysis, and wrote the manuscript. FC recruited the patients and performed their clinical characterization. LR-G, AM-D, and BB-C performed INDELS validations and digital droplet PCR. VA and DM performed SNV prioritization analyses. BO-V performed the DNA extraction and the DNA quality control. CV and CS performed Sanger sequencing validations. JL, GO, DH-S, KS, and AF contributed to NGS data analyses and experimental design. CV-B conceived and designed the analysis, supervised the study, contributed to analyze the NGS data and gene ontology, and wrote the manuscript. All authors read and approved the final manuscript.

## FUNDING

This work was supported by the Carlos III Institute of Health (ISCIII), Ministry of Economy and Competitiveness (Spain) (grants PI15/00809 and PI18/00608), co-funded with European Union ERDF (European Regional Development Fund). JP-F

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FPU fellowship (FPU/2014-03876) was funded by the FPU fellowship granted by the Ministry of Education, Culture, and Sport (Spain). LR-G, AM-D, and BB-C were funded by the FPI fellowship granted by the General Direction of Innovation and Research, Ministry of Innovation, Research, and Tourism, Balearic Government, co-funded by European Union ERDF. DM was funded by ISCIII (CP17/00188).

## ACKNOWLEDGMENTS

The authors thank all the members of the family for their participation. This article is dedicated to subject SCZ-8 who bravely fought the disease and lost his battle.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fgene.2021.622886/full#supplementary-material>

**Supplementary Table 1** | Clinical characterization. PANSS, Positive and Negative Syndrome Scale.

**Supplementary Table 2** | CNVs identified. Chr, Chromosome; bp, base pair; Start (bp), Starting position of the CNV; End (bp), Ending position of the CNV; Size (bp), Size of the CNV in bp; \*Genetic coordinates are given taking as a reference GRCh37/hg19.

**Supplementary Table 3** | Validation by Digital PCR (ddPCR). CNV name, Copy Number Name; Chr, Chromosome; bp, base pair; \*Genetic coordinates are given taking as a reference GRCh37/hg19; \*\*Amplicon, Size of the amplicon in bp and its coordinates.

**Supplementary Table 4** | Cognitive assessment. TMT, Trail Making Test (Part A); BACS, Brief Assessment of Cognition in Schizophrenia (Symbol coding); HVL-T-R, Hopkins Verbal Learning Test-Revised; WMS-III, Wechsler Memory Scale-Third Edition (Spatial Span); LNS, Letter-Number Span Test; NAB Mazes, Neuropsychological Assessment Battery Mazes; BVM-T-R, Brief Visuospatial Memory Test-Revised; Fluency, Category Fluency Test, Animal naming; MSCEIT, Mayer-Salovey-Caruso Emotional Intelligence Test; CPT-IP, Continuous Performance Test-Identical Pairs; Pi, Percentile; Overall Composite Score: It is a cognitive index which is obtained summing all the tests scores and standardizing the result to a T score. \*Low scores in comparison to reference group according to Mucci et al. (2017).

**Supplementary Table 5** | Residual variation intolerance score (RVIS) and loss-of-function observed/expected upper bound fraction (LOEUF). RVIS, Residual Variation Intolerance Score; LOEUF, Loss-of-function Observed/Expected Upper bound Fraction; \*Percentiles are provided in parenthesis.

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