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Supplemental Text

Human brain procedures

Brains were collected through the Offices of the Chief Medical Examiner of the District of Columbia and of Northern Virginia, after autopsy, and through tissue donations via funeral homes mostly from DC, Northern Virginia area. Informed consent to obtain and study brain tissue was obtained from the surviving next-of-kin for all cases, according to Protocol #90-M-0142 approved by the NIMH/National Institutes of Health Institutional Review Board. Interviews with the next-of-kin to gather basic demographic information and medical, substance use, and psychiatric history was conducted, and followed by detailed toxicological analysis. Brains were removed from the skull, wrapped in plastic, and transported on wet ice. The brains were hemisected, cut into 1.5 cm coronal slabs, rapidly frozen in a prechilled dry-ice/isopentane slurry bath (–40°C), and stored at -80°C. A block of lateral superior cerebellar hemisphere was cut transversely to the folia. A portion of cerebellum was pulverized for pH measurement. For the dorsolateral prefrontal cortex dissections, gray matter tissue from the crown of the middle frontal gyrus was obtained from the coronal slab corresponding to the middle one-third immediately anterior to the genu of the corpus callosum. Subcortical white matter was carefully trimmed from the area immediately below the middle frontal gyrus.

RNA extraction

Total RNA was extracted from ∼100 mg of tissue using the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The yield of total RNA was determined by spectophotometry by measuring the absorbance at 260 nm. The RNA quality was assessed by high-resolution capillary electrophoresis on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA), samples with RNA integrity number<5 were excluded. cDNA was created from 4 μg total RNA using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol.

RNA sequencing and 2bp-deletion results

We used RNA sequencing (RNAseq) to identify 2bp deletion (30). Briefly, after fragmentation of dorsolateral prefrontal cortex RNA, reverse transcriptase and random primers copied the cleaved fragments into first-strand cDNA. Second-strand cDNA was synthesized using T4 DNA polymerase, T4 polynucleotide kinase, and Klenow DNA polymerase, adding a single adenine base using a 3′ to 5′ exo$^-$ Klenow fragment, and ligating the paired-end adapters using T4 DNA ligase. An index (up to 12 nucleotides) was inserted into Illumina adapters so multiple samples could be sequenced in 1 lane of an 8-lane flow cell. Products were purified and enriched with polymerase chain reaction (PCR) to create the final cDNA library for high-throughput sequencing using the HiSEquation 2000 system (Illumina). Results were mapped to GRCh37/hg19 using the TopHat2 splice-aware alignment software.

We obtained the data for the two-base-pair deletion in exon-6 through RNA sequencing of a subset of samples (controls and schizophrenia, N=169). The 2 bp deletion was found in 74 subjects (44 controls, 30 schizophrenia), absent in 95 subjects (42 controls, 53 schizophrenia), and was marginally less common in patients with schizophrenia than controls (36% versus 51%, p=0.05), although again this study is underpowered to conduct genetic associations. There was no significant difference in the frequency of 2-bp deletion between Caucasians and African

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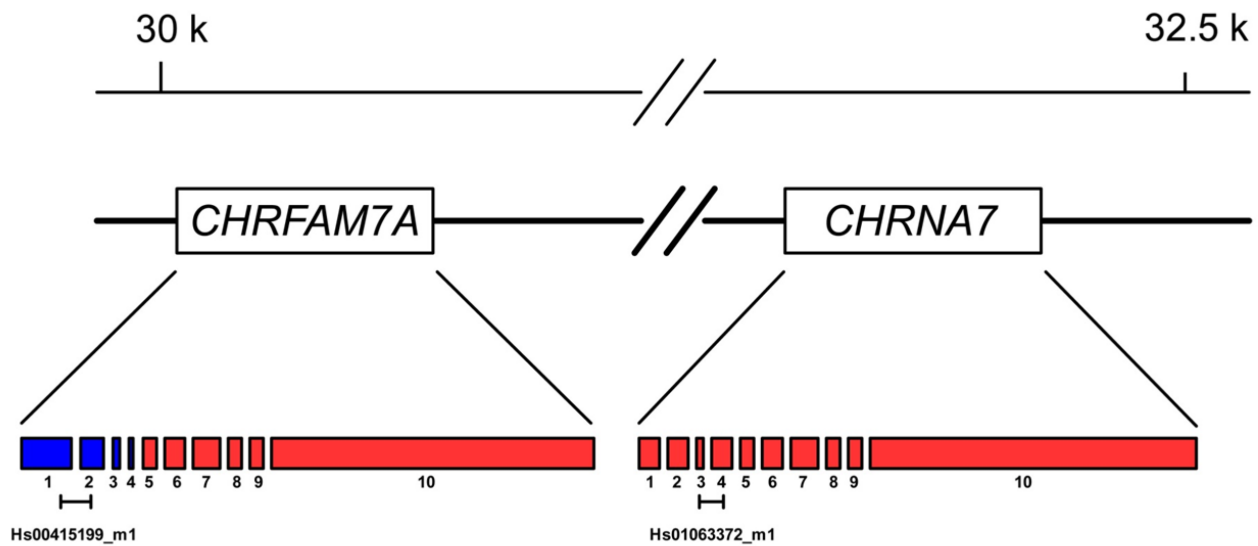
American subjects. Interestingly, this 2-bp deletion was associated with decreased *CHRFAM7A* mRNA expression (2.4 versus 3.1, by ~26%, p=0.0009) and had no association with the expression of *CHRNA7* mRNA, p=0.6. This deletion had a similar effect in both diagnostic groups (deletion by diagnosis interaction F (1,164)=0.95, p=0.33, n.s.) and in both races (n.s.), and did not account for the observation of increased expression of *CHRFAM7A* or decreased expression of *CHRNA7* in schizophrenia.

Supplementary Figure S1. Genomic structure of *CHRNA7* and *CHRFAM7A*.

The *CHRNA7* (red) has 10 exons and exons 5–10 are duplicated. The chimeric gene *CHRFAM7A* is complex and maps centromeric to *CHRNA7*. Horizonal lines depict positions of Taqman assays (Hs01063372_m1, which measures *CHRNA7* as it spans exons 3–4, and Hs00415199_m1, which is specific for the hybrid gene *CHRFAM7A* as it spans exons 1–2).



Supplementary Table S1. A list of SNPs tested for association with expression of *CHRNA7* and *CHRFAM7A*. None of the SNPs showed significant association after correction for multiple testing (p<0.0.05) [a]

Chr	SNP Name	Position	Alleles
15	rs8039109	30012769	[T/C]
15	rs4435224	30016600	[A/G]
15	rs2137856	30016646	[T/C]
15	rs6494074	30025001	[A/G]
15	rs12442954	30029658	[A/C]
15	rs11636570	30044127	[A/C]
15	rs8035113	30044699	[A/G]
15	rs12442622	30045195	[A/G]
15	rs4779948	30046352	[T/C]
15	rs952284	30054781	[A/C]
15	rs10152238	30057610	[T/C]

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15	rs4779561	30057740	[A/G]
15	rs12442631	30058418	[T/C]
15	rs1567883	30060176	[A/C]
15	rs1001555	30060958	[A/G]
15	rs11636101	30061449	[T/G]
15	rs11636810	30067518	[A/G]
15	rs12437782	30071854	[A/C]
15	rs12440180	30072148	[T/C]
15	rs8038654	30072156	[T/C]
15	rs1399195	30077139	[A/G]
15	rs2063722	30083665	[T/G]
15	rs8036290	30083979	[T/C]
15	rs1514260	30086242	[T/C]
15	rs7168113	30087000	[A/G]
15	rs1567887	30087245	[A/G]
15	rs1567885	30088094	[T/C]
15	rs7180085	30090949	[T/C]
15	rs2337233	30094507	[A/G]
15	rs12439621	30096476	[A/G]
15	rs4779955	30097303	[A/G]
15	rs8026970	30098960	[T/C]
15	rs965434	30104934	[T/C]
15	rs6494165	30108578	[T/G]
15	rs3826029	30108777	[A/G]
15	rs883473	30112968	[A/G]
15	rs1606659	30119745	[T/C]
15	rs11635209	30121289	[T/C]
15	rs11071503	30122248	[A/G]
15	rs9672321	30131481	[T/C]
15	rs1913457	30133278	[A/G]
15	rs4779969	30136223	[A/C]
15	rs11071515	30136799	[A/G]
15	rs11637923	30138067	[T/C]
15	rs1355920	30145020	[A/G]
15	rs2337507	30146417	[T/C]
15	rs8027035	30149996	[A/G]
15	rs7179733	30160985	[A/G]
15	rs8033518	30168901	[A/G]
15	rs7175581	30172759	[A/G]

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15	rs4779565	30177362	[T/G]
15	rs8035668	30178638	[T/C]
15	rs6494223	30183749	[T/C]
15	rs8028396	30184013	[A/G]
15	rs10438342	30189338	[A/G]
15	rs11858834	30190213	[A/G]
15	rs11852956	30190622	[A/G]
15	rs13329490	30195523	[T/G]
15	rs12915265	30196358	[T/C]
15	rs1392808	30198807	[A/C]
15	rs904951	30205330	[T/C]
15	rs12904458	30207080	[T/C]
15	rs4779978	30207700	[T/C]
15	rs7175359	30212239	[A/G]
15	rs2651418	30226573	[T/C]
15	rs1909884	30226590	[A/G]
15	rs2611603	30228824	[T/G]
15	rs2611605	30228925	[A/G]
15	rs7179008	30231215	[T/C]
15	rs2337980	30231488	[T/C]
15	rs2926504	30297184	[A/G]
15	rs4072398	30297802	[A/G]
15	rs9672615	30298847	[A/C]
15	rs2946542	30300468	[A/G]
15	rs2946543	30300525	[A/G]
15	rs9672198	30301189	[A/G]
15	rs9672221	30301442	[T/G]
15	rs2611583	30301633	[A/G]
15	rs4779984	30302218	[A/G]
15	rs11637116	30302973	[A/G]
15	rs2946544	30303265	[T/C]

[a] Genotyping of 81 *CHRNA7* SNPs was performed according to the manufacturer's instructions, using Illumina Human 1M-Duo BeadChips (Illumina Inc., San Diego, CA). Two SNPs previously examined but not associated with schizophrenia (16) were highlighted in fluorescent yellow.