Supplement

A smaller recurrent deletion within 15q13.3 associated with a range of neurodevelopmental phenotypes

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Supplementary Note

Data for *de novo* and inherited cases of deletion 15q13.3

Deletions of 15q13.3 (BP4-BP5) are frequently inherited in the index case and also sometimes transmitted to the offspring of index cases. Lack of penetrance is also reported frequently. These data are summarized in **Supplementary Table 1** online. For 37 cases where the index case was known to be *de novo* or inherited, 10 of 37 (27%) were *de novo* and 27 of 37 (73%) were inherited. In two of the *de novo* cases, the index person transmitted the deletion to an offspring, so that there was transmission of the deletion in 29 of these 37 families (78%). Taking deletion 16p11.2 as a comparison, only one study[1] showed a high frequency of inherited cases with 57% of deletion cases being inherited. We were able to identify 42 cases of 16p11.2 deletion from the literature[1-6] and from our local collection (manuscript under review) and found that the deletion was *de novo* in 71% of cases (including the series with 57% inherited) which is quite different from the 27% *de novo* observed for the 15q13.3 deletion. For penetrance (**Supplementary Table 1** online), excluding the 680-kb deletions in this report, there were 120 deletion individuals with 15q13.3 deletion from 76 families. Subtracting the index cases yields 44 individuals where nonpenetrance might have been detected. Of these 44, 15 showed lack of penetrance and five had a very mild phenotype or the phenotype was not specified as normal or abnormal. More in depth phenotypic analysis of nonpenetrant individuals might reveal very mild deficits in some, but they were regarded as normal by themselves and their families.

Nature Genetics: doi:10.1038/ng.481

Further discussion of pharmacology

The CHRNA7 protein is a target for rational drug design[7] and is likely to be very susceptible to alteration of function using agonists and antagonists. A naturally occurring agent, α-bungarotoxin, is a selective antagonist of the α7 nicotinic acetylcholine receptor in the brain and has long been used to study the nicotinic central nervous system. A "proof-of-concept" trial has been performed in patients with schizophrenia using 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A), a natural alkaloid derivative and a partial α7 nicotinic cholinergic agonist[8]. Eleven patients were studied. Significant neurocognitive improvement and improvement in P50 inhibition were found compared with placebo. The authors concluded that the nicotinic agonist appeared to have positive effects on neurocognition in persons with schizophrenia. A similar study of patients with deletion chromosome 15q13.3 and schizophrenia or other neurocognitive phenotypes would be of interest.

Supplementary Methods

Subject recruitment. We obtained DNA samples from probands and their family members after informed consent using protocols approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine.

DNA isolation. Patients' genomic DNA was extracted from peripheral blood via the Puregene DNA isolation kit (Gentra System, Minneapolis, MN, USA).

Nature Genetics: doi:10.1038/ng.481

Array CGH analysis. Initial array CGH analysis was performed using the Agilent 244K (with dye swap) in patient 1 in accordance with the manufacturer's instructions. Chromosomal Microarray Analysis (CMA) was performed using V6.4 OLIGO (patients 9 and 10) and V7.4 OLIGO (patient 7), designed by Baylor Medical Genetics laboratories (http://www.bcm.edu/geneticlabs/cma/) and manufactured by Agilent Technology (Santa Clara, CA) as previously described[18,19].

To fine map the sizes and extents of the deletions, oligonucleotide microarray CGH analyses were performed using whole-genome NimbleGen array with 2.1M oligonucleotides (in patient 7) as well as custom-designed 15q11.2q14 region-specific high-resolution oligonucleotide microarrays: 135K (NimbleGen Systems, Madison, WI) (in patients 1, 7, 9, and 10) and 44K (Agilent Technologies, Santa Clara, CA)[20] (in patients 1, 7, 9, 10, and in a patient with a similar-sized duplication involving *CHRNA7*) according to the manufacturers' instructions.

FISH analysis. Confirmatory and parental fluorescence *in situ* hybridization (FISH) analyses in patients 7, 9, and 10 were performed using BAC clone RP11-265I17 via standard procedures.

Long range PCR and DNA sequencing. Long range PCR (LR-PCR) with primers designed to harbor CHRNA7-LCR copy-specific *cis*-morphisms were performed in accordance with the manufacturer's instructions (Takara Bio, Japan). PCR products were purified with the PCR Purification Kit (QIAGEN, Valencia, CA, USA) and sequenced by Sanger di-deoxynucleotide sequencing using the initial primers and primers specific for

Nature Genetics: doi:10.1038/ng.481

both CHRNA7-LCR copies to map the NAHR sites (Lone Star Labs, Houston, TX, USA) (**Supplementary Table 2** online).

Bioinformatics and *in silico* sequence analysis. Genomic sequence based on the oligonucleotide coordinates from the array CGH experiment was downloaded from the UCSC genome browser (build 36, March 2006) and assembled with the Sequencher v4.2 software. Interspersed repeat sequences were analyzed by RepeatMasker.

Supplementary Table 1. Inherited versus *de novo* occurrence of a deletion 15q13.3 (BP4-BP5).

Report	Focus	# Fam.	Index *de novo*	Index inherited	Index unknown	Trans-mission	Nonpenetrant/ total
Sharp *et al.*[9]	General	6	2	2[ab]	2	2[a]	0/9
International Schizophrenia Consortium[10]	Schizophrenia	9	0	0	9	n/a	0/9
Stefansson *et al.*[11]	Schizophrenia	7	0	0	7	n/a	0/7
Miller *et al*.[12]	Gen. & autism	5	0	2	3	2	0/7
Ben-Shachar *et al.*[13]	Gen. & autism	12	1	6[a]	5	6[a]	3/20
Helbig *et al.*[14]	Epilepsy	12	1	4	7[b]	4	3/19
van Bon *et al*.[15]	General	18	3[b]	11	4[b]	11	5/5//37[c]
Dibbens *et al.*[16]	Epilepsy	7	3	2	2	4	4/12
Total 15q13.3		76	10	27	39	29	15/5/120
This report	General	4	0	2	2	2	0/10

[a] Includes one family with affected sibs but parents were unavailable.
[b]Includes one family deleted BP3-BP5; others BP4-BP5.
[c] Five nonpenetrant, five very mild phenotype or not specified of 37 total.
Total families with known origin: 10/37 *de novo* and 27/37 inherited.

Nature Genetics: doi:10.1038/ng.481

Supplementary Table 2. Long range PCR primers used in patients 1, 7, and 9 to map the NAHR sites.

Pt	Initial amplification and DNA sequencing	DNA sequencing of LR PCR products
1	F2: CATGGGTATGTGGTTCTGAGAGAGAAAACAC R2: TTATTGTACCCTCAGAACAGTGTGCAGCCTA	TTCCATTTCTTAGTCATCGAGTCTTGAAGTCC
7, 9	F1: ACATAACCTCCACAACACCAGCTAGAGATAAA R1: GGCCTTACAAAGTTCACTTTCGTGATTAAG	AAAGAGGCTTCTCACCCAGTTCAAATTGTTAC

Supplementary Figure legends

Supplementary Figure 1. Summary of the results in patients with a recurrent 680-kb deletion. (a) Genomic region chr15:25,800,000-31,000,000 (UCSC March 2006) with low-copy repeat clusters BP3, BP4, and BP5. The 680-kb deletion (b,c) and a similar-sized duplication involving *CHRNA7* (d) are shown by the dotted vertical lines. These deletions and duplications were detected through screening the clinical lab database of array CGH studies performed in 8882 patients using CMA Versions 6 OLIGO and 7 OLIGO. The *CHRNA7* rearrangements were subsequently characterized via a catalogue 2.1 M whole-genome array CGH (NimbleGen Systems, Madison, WI, USA) (*CHRNA7* deletions) (b) and the custom designed 15q13.3-specific high-resolution 8x15K array CGH (Agilent Technologies, Santa Clara, CA, USA) (*CHRNA7* deletions and duplications) (c,d) and 12x135K (NimbleGen) (*CHRNA7* deletions) (**Fig. 1**). BP3, BP4, and BP5 were not covered with oligonucleotide probes in the Agilent array. (e) Pedigree for patient 1 (arrow). Shaded boxes and circles indicate males and females, respectively, who have the 680-kb deletion, whereas "-" indicates that the respective individual was tested negative for the 680-kb deletion. The proband's mother has mental retardation

Nature Genetics: doi:10.1038/ng.481

(MR) and absence seizure (sz). The siblings of the proband have global developmental delay (GDD). The 52-year-old maternal grandmother, who also has the deletion, was diagnosed with multiple sclerosis (MS) and had MR. (f) Schematic representation of the proposed mechanism for formation of the described 680-kb deletion and reciprocal duplication. Inverted chromosome region 15q13.3, present in high frequency of population, likely results from NAHR between BP4 and BP5. The second NAHR between ~ 103 kb proximal (chr15:28,757,712-28,860,892) and ~ 109 kb distal (chr15:29,697,286-29,806,912) CHRNA7-LCRs (of 97.7% DNA sequence identity) on the inverted and normal region of chromosome 15q13.3, respectively, likely leads to the ~ 680 kb deletion and its reciprocal duplication. The yellow rectangle represents the ~ 90 kb segment in BP4 (arrows in a-c). Note that NAHR can also be mediated by the other pair of CHRNA7-LCR copies, resulting in the opposite junction fragments (dist/prox vs. prox/dist CHRNA7-LCR).

Supplementary Figure 2. Genomic structure of the proximal and distal CHRNA7-LCRs. The NAHR site regions narrowed to 217 bp, 49 bp, and 67 bp in patients 1, 7, and 9, respectively, were identified using long range PCR with primers harboring *cis*-morphisms specific for each CHRNA7-LCR copy (**Supplementary Table 2** on line). Interestingly, the NAHR sites in patients 7 and 9 were mapped to the retrotranposable elements LINE1 in the vicinity (~ 1.3 kb) of the CCTCCCT sequence motif associated with recombination hot spots[17]. The black box depicts the LINE1 elements. Genomic coordinates are according to hg18.

Nature Genetics: doi:10.1038/ng.481

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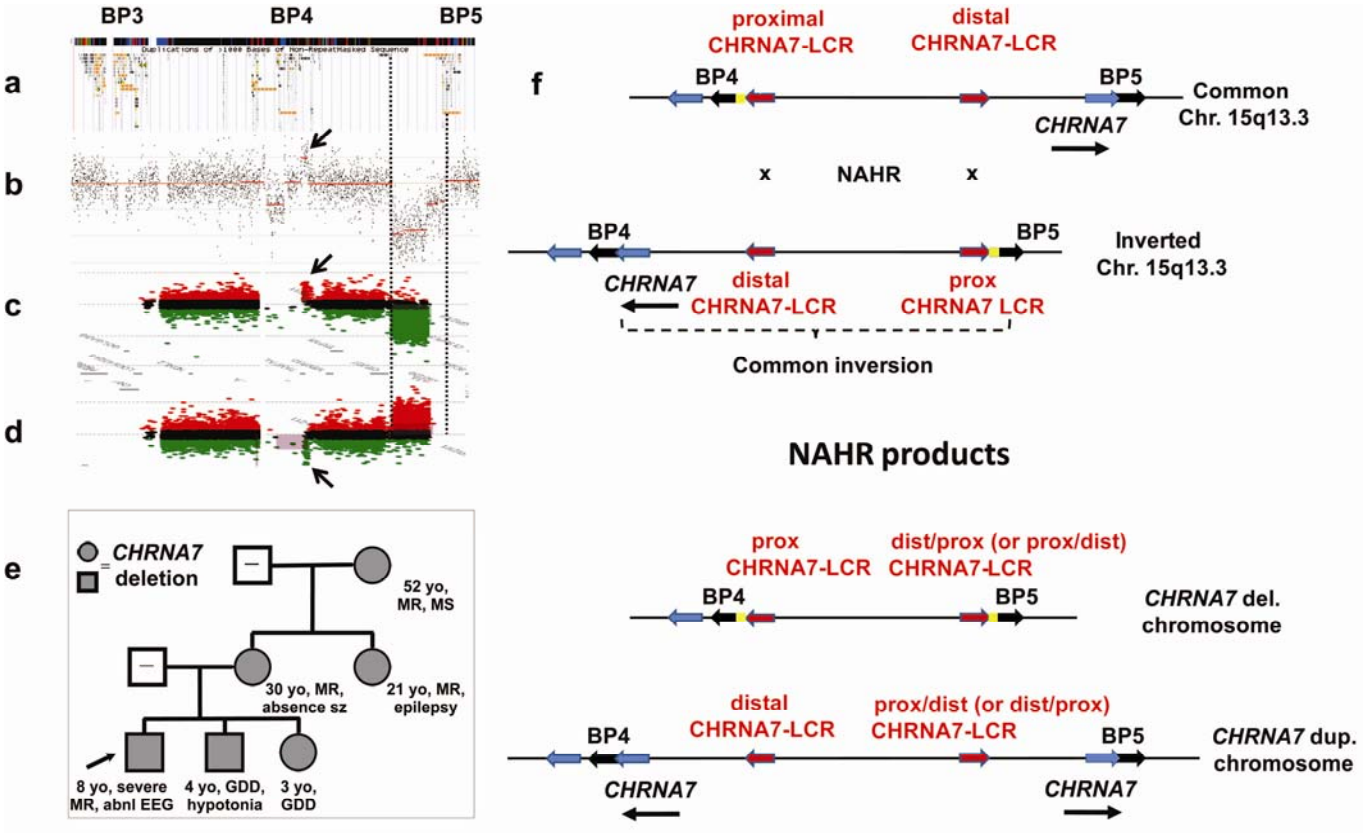
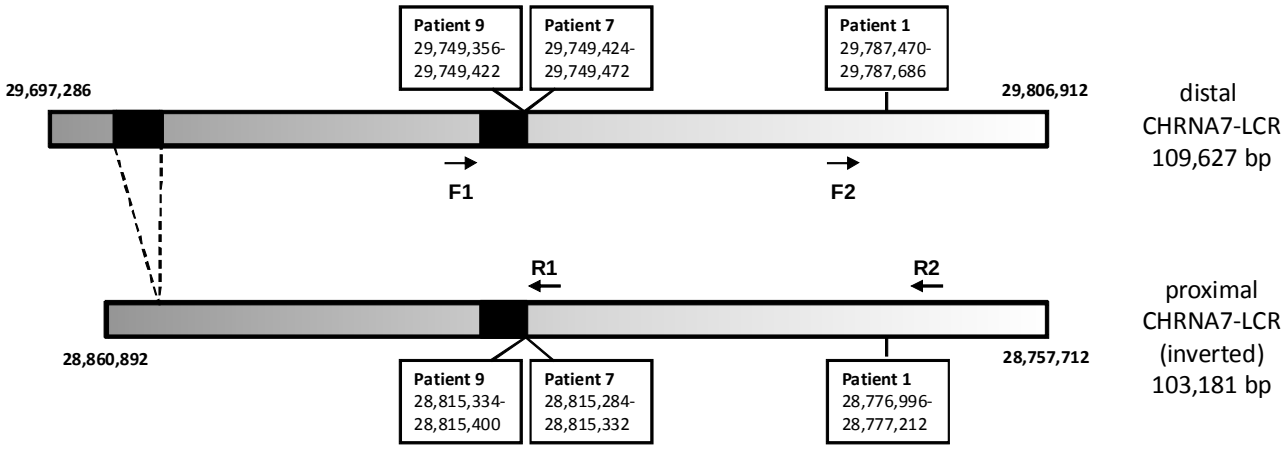
Supplementary Figure 1. Summary of the results in patients with a recurrent 680-kb deletion.



Nature Genetics: doi:10.1038/ng.481

Supplementary Figure 2. Genomic structure of the proximal and distal CHRNA7-LCRs.



Nature Genetics: doi:10.1038/ng.481