Supplementary Materials: A Novel Microduplication in the Neurodevelopmental Gene *SRGAP3* that Segregates With Psychotic Illness in the Family of a COS Proband

MATERIALS AND METHODS

Patient samples

Patient samples were collected as previously described [1]. The NIMH Institutional Review Board approved sample collection after written and informed consent was obtained from all study participants.

High density genotyping CNV analysis

The Illumina microarray analysis was carried out as previously described [2].

FISH analysis

Metaphase chromosomes from lymphoblastoid cell lines were prepared using standard methods. To determine the location of the duplicated material, we performed FISH using two fosmid probes from the duplicated region, G248P86704D9 and G248P8810B10. We hybridized fosmid probes to metaphase chromosomes and analyzed FISH signals in 10 metaphase spreads per cell line. In all cases, two overlapping fosmid signals were present on the two chromosomes 3, with no signals on other chromosomes. We identified the chromosomes 3 using inverted DAPI images. Although the small size of the duplication precluded interphase analysis of the duplication, our FISH experiments demonstrated that the duplicated sequence was not present on another chromosome, consistent with a local duplication.

High resolution targeted oligonucleotide array CGH analysis

We designed custom oligonucleotide arrays using the 8x15K platform, AMADID # 029459 (Agilent Technologies, Santa Clara, CA). The duplication region was targeted with probes from coordinates chr3: 8,000,000 - 10,000,000 (Build 37, hg19) with a mean probe spacing of 1 probe per 181 bp. We also added a backbone of probes outside of the duplication from coordinates 1-8,000,000 and 10,000,000 - 18,000,000 with a mean probe spacing of 1 probe per 200 kb. Array CGH was performed following the manufacturer's protocol. Genomic DNA was extracted from lymphoblastoid cell lines derived from family members and a control (GM10851, Coriell Cell Repositories, Camden, New Jersey). Patient and control DNA samples were cohybridized in seven separate hybridizations. Arrays were scanned using a GenePix® 4000B scanner (Molecular Devices, Sunnyvale, CA, USA) and signal intensities were evaluated using Feature Extraction Version 9.5.1.1 software (Agilent Technologies, Santa Clara, CA). We used DNA Analytics Version 4.0 software (Agilent Technologies, Santa Clara, CA) to analyze the array data and narrow the duplication breakpoints.

PCR and sequencing

Using the duplication breakpoints sized by array CGH, we designed PCR primers that would amplify the duplication junction either in tandem or inverted orientation [3]. The primer sequences are as follows: NIH_P1.5: GCTGTCAACTTCCAGATTCTGTTTG and NIH_P2.4: GAGCAGCAGCCAAATTAGCTCG. The PCR amplified a 900-bp product in DNA from cell lines derived from NSB499, NSB617, and NSB619, but not other family members. We TA-cloned the PCR product and sequenced 8 individual clones. All inserts had the same sequence and were consistent with a tandem duplication junction. Purified plasmid DNA and live culture stabs are available upon request.

mRNA transcript analysis

To predict the reading frame of the mRNA resulting from the tandem duplication, we used Sequencher® software version 3.0 to analyze the NM_014850.2 transcript of SRGAP3.

Sanger Sequencing

Sequencing was carried out as previously described [4].

Materials and Methods References

1. Addington, A.M. and J.L. Rapoport, *The genetics of childhood-onset schizophrenia: when madness strikes the prepubescent.* Curr Psychiatry Rep, 2009. **11**(2): p. 156-61.
2. Walsh, T., et al., *Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia.* Science, 2008. **320**(5875): p. 539-43.
3. Arlt, M.F., et al., *Replication stress induces genome-wide copy number changes in human cells that resemble polymorphic and pathogenic variants.* Am J Hum Genet, 2009. **84**(3): p. 339-50.
4. Hamdan, F.F., et al., *No association between SRGAP3/MEGAP haploinsufficiency and mental retardation.* Arch Neurol, 2009. **66**(5): p. 675-6.

FUNDING

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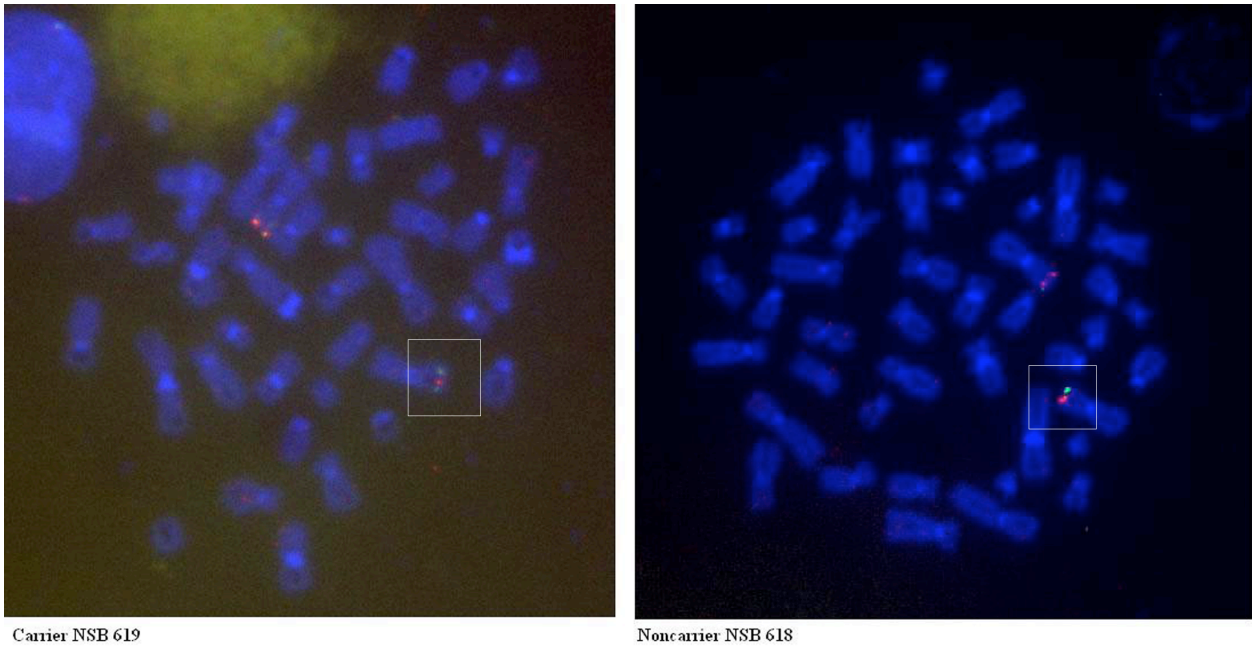
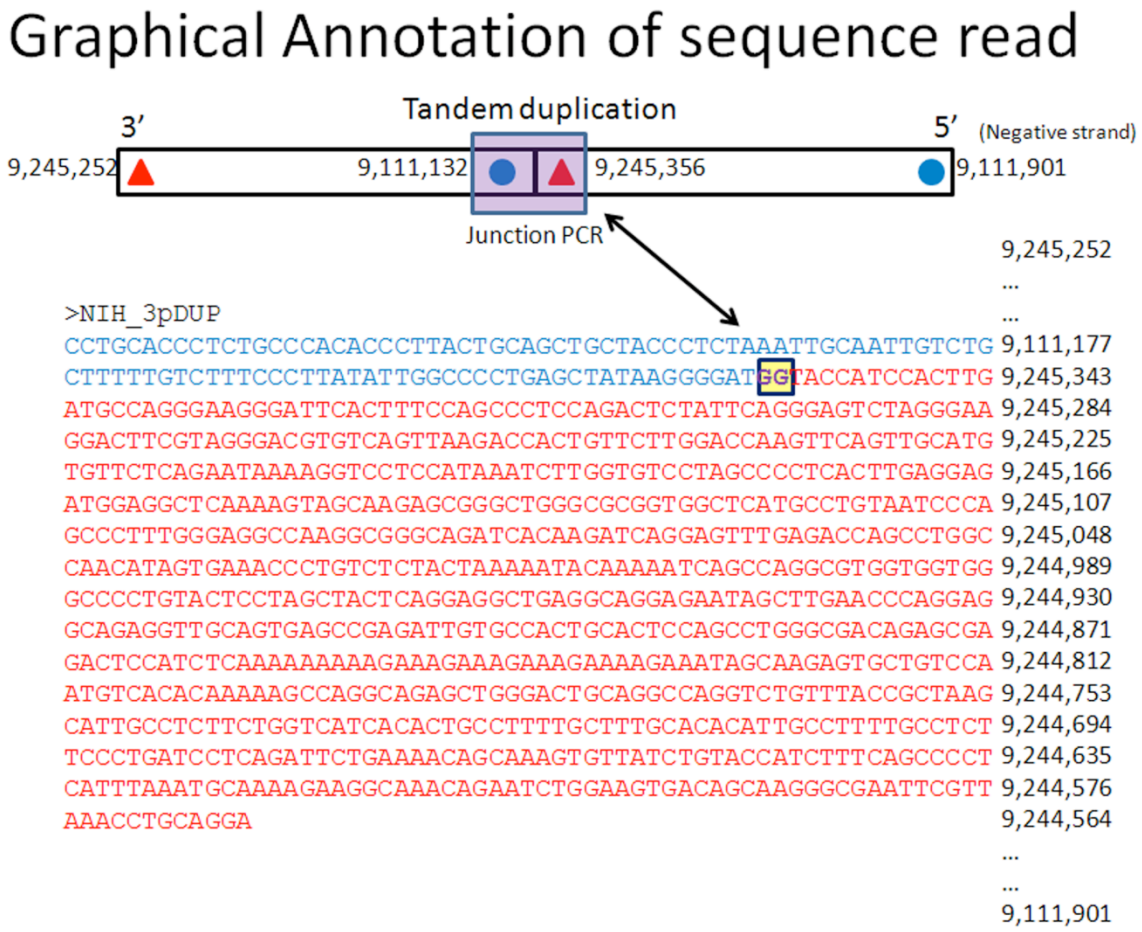
Supplemental Figures

Supplemental Figure 1



Supplemental Figure 1: Metaphase FISH signals of a carrier and noncarrier alike were displayed at the end of chromosome 3 and not on any other chromosomes, consistent with a local duplication.

Supplemental Figure 2



Supplemental Figure 2: Nucleotide sequence at the tandem duplication breakpoint.

Supplemental Figure 3

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browser position chr3:8,821,666-9,400,000
track name=coords description="SRGAP3 CNVs Anderson et al" visibility=2, itemRgb=on
chr3    9111132    9245356    NSB499 500    -    9111132    9245356    0,0,255
chr3    9111132    9245356    NSB617 500    -    9111132    9245356    0,0,255
chr3    9111132    9245356    NSB619 500    -    9111132    9245356    0,0,255
chr3    8895281    9025800    NeedPt1 500    -    8895281    9025800    255,0,0
chr3    9073423    9371030    NeedPt2 500    -    9073423    9371030    255,0,0
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Supplemental Figure 3: Text file in BED format uploaded into UCSC genome browser custom tracks to produce Figure 3.