Figures and figure supplements

NUDT21-spanning CNVs lead to neuropsychiatric disease and altered MeCP2 abundance via alternative polyadenylation

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Figure 1. Subjects with NUDT21-spanning copy-number variations (CNVs). (A) Five intrachromosomal rearrangements of chromosome 16q including the NUDT21 gene, identified by clinical array comparative genomic hybridization. Duplications shown in blue, deletion in red. Del, deletion; Dup, duplication; Mb, megabases. The striped bars indicate that the copy variant is not drawn to scale. (B) Array plots of oligonucleotide arrays on subjects 1, 2, and 4. The array plots of subject 3 and 5 were unavailable due to the closure of the Signature Genomics microarray laboratory in June 2014. Black dots indicate probes with normal copy-number, green dots indicate copy-number gain, and red dots indicate copy-number loss. Solid and dotted lines respectively define the minimum and maximum expected boundaries of the CNVs.

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Figure 2. CFIm25 regulates MeCP2 protein levels in patient-derived lymphoblastoid cells with NUDT21 CNVs. (A) Representative western blot picture for three duplication patients compared to four age-matched controls showing the increase of CFIm25 and decrease of MeCP2 protein levels. (B) Representative western blot picture for one deletion patient compared to four age-matched controls showing the decrease of CFIm25 and increase of MeCP2 protein levels. Quantification of protein levels for both CFIm25 and MeCP2 from three duplication patients and one deletion patient compared to a total of 13 age-matched controls are shown below the corresponding western blot. Data represent mean ± SEM from a total of six technical replicates. Data were normalized to GAPDH protein levels. (C) Western blot and its relative quantification showing that knockdown of NUDT21 by siRNA-NUDT21 nucleofection increases MeCP2 in control and duplication subjects, and normalizing CFIm25 in duplication patients rescue MeCP2 to control levels. Data represent mean ± SEM.

Figure 2. continued on next page.
Figure 2. Continued

from four age-matched control and three duplication cases. Data were normalized to GAPDH protein levels. **p < 0.01, ***p < 0.001. M, mosaic patient.

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Figure 2—figure supplement 1. siGLO nucleofection showing patient-derived lymphoblastoid cells can be transfected with small RNA. (A) Representative histograms of control (i) and siGLO-nucleofected lymphoblastoid cells showing nearly 100% efficiency using both the functionality (ii) and efficiency (iii) protocols. (B) Time series showing the presence of small RNA in nearly 100% of nucleofected lymphoblastoid cells up to 48 hr after nucleofection. (C) Time series showing cell survival following nucleofection using either the functionality or efficiency nucleofection protocol. DOI: 10.7554/eLife.10782.006
**Figure 3.** *NUDT21* mRNA levels correlate with inefficiently translated long *MECP2* mRNA. (A) RNA quantification by quantitative RT-polymerase chain reaction (qRT-PCR) from lymphoblastoid cells of *NUDT21* duplication and deletion patients. The bar graph shows the total mRNA fold change of *NUDT21*, total *MECP2*, and long *MECP2* for the three duplication patients and one deletion patient compared to 13 age-matched controls. Data represent mean ± SEM from five independent experiments. Data were normalized to GAPDH mRNA levels. (B) Relative polyribosomal and non-polysomal enrichment of total and long *MECP2* mRNA isoforms of *NUDT21* duplication patients compared to age-matched controls. Data represent mean ± SEM from a total of three control and duplication cases. Data were normalized to ACTB mRNA levels. (C) Proposed model showing that duplication and deletion patients have more or less CFIm25, respectively leading to a relative increase in long and short *MECP2* 3' UTR isoforms. In both cases, there is an accumulation of mRNA: in the deletion patient, this leads to more MeCP2 protein, but in the duplication patients, it results in less MeCP2 protein due to a translational block from the CFIm25-mediated increase in long *MECP2* isoforms and putative binding of miRNAs or RNA-binding proteins to the 3' UTR. *p < 0.05, **p < 0.01, ***p < 0.001.

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Figure 3—figure supplement 1. Northern blot assay from patient-derived lymphoblastoid cells. Northern blot assay of lymphoblastoid cells showing that duplication (left panel) and deletion (right panel) patients respectively have more long or short MECP2 3’ UTR isoforms. Relative northern blot quantification of three duplication patients and one deletion patient compared to 13 age-matched controls (bottom panel). Data represent mean ± SEM from four independent experiments. Data were normalized to GAPDH mRNA levels. For all the experiments, p values were calculated by Student’s t-test comparing controls with duplication patients. *p < 0.05, **p < 0.01. M, mosaic patient.
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Figure 3—figure supplement 2. Polyribosome fractionation traces of control and duplication subjects. Representative polyribosome traces from control (A-C) and duplication (D-F) subjects. UV absorption at 254 nm was plotted vs time depicting the successful resolution of the different ribosomal fractions.

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