

## Comment on “Autistic-like phenotypes in *Cadps2*-knockout mice and aberrant CADPS2 splicing in autistic patients”

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### Letter

Sadakata et al. (1) reported that a CADPS2 isoform lacking exon 3 is aberrantly spliced in the peripheral blood of autistic patients. However, we found this splice isoform in the blood of normal subjects at a similar frequency to that of individuals with autism spectrum disorder (ASD) (95% CI of the difference,  $-0.06$  to  $0.1$ ). Moreover, this splice variant exists as a minor isoform in cerebellar RNA of both normal individuals and individuals with ASD. Thus, exon 3 skipping likely represents a minor isoform rather than aberrant splicing and is probably not an underlying mechanism of autism. Defects of CADPS2 function might contribute to autism susceptibility, but likely not through aberrant splicing. Sadakata et al. (1) reported that 4 of 16 patients with autism expressed an exon 3–skipped variant of CADPS2 mRNA in the blood, while the CADPS2 mRNA of all 24 normal subjects included exon 3. They thus concluded that CADPS2 is aberrantly spliced in autism, and they performed further experiments showing that the subcellular localization of exogenously expressed exon 3–skipped CADPS2 is disturbed in primary cultured neocortical and cerebellar neurons. We aimed to replicate the CADPS2 findings in an independent set of peripheral blood samples from 41 children with ASD and 39 control children, following the Sadakata et al. protocols (Figure 1A). Furthermore, we performed sequencing (Figure 1B) [...]

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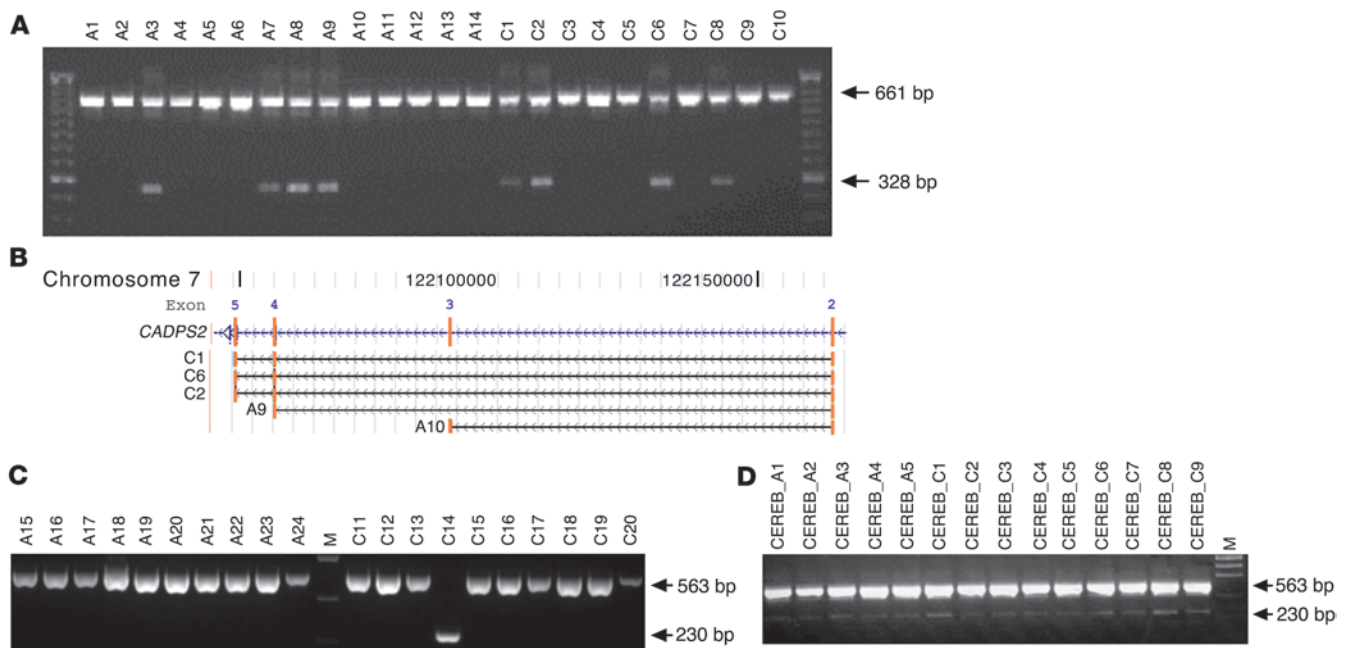
# Comment on “Autistic-like phenotypes in *Cadps2*-knockout mice and aberrant *CADPS2* splicing in autistic patients”

Sadakata et al. (1) reported that a *CADPS2* isoform lacking exon 3 is aberrantly spliced in the peripheral blood of autistic patients. However, we found this splice isoform in the blood of normal subjects at a similar frequency to that of individuals with autism spectrum disorder (ASD) (95% CI of the difference, -0.06 to 0.1). Moreover, this splice variant exists as a minor isoform in cerebellar RNA of both normal individuals and individuals with ASD. Thus, exon 3 skipping likely represents a minor isoform rather than aberrant splicing and is probably not an underlying mechanism of autism. Defects

of *CADPS2* function might contribute to autism susceptibility, but likely not through aberrant splicing.

Sadakata et al. (1) reported that 4 of 16 patients with autism expressed an exon 3-skipped variant of *CADPS2* mRNA in the blood, while the *CADPS2* mRNA of all 24 normal subjects included exon 3. They thus concluded that *CADPS2* is aberrantly spliced in autism, and they performed further experiments showing that the subcellular localization of exogenously expressed exon 3-skipped *CADPS2* is disturbed in primary cultured neocortical and cerebellar neurons.

We aimed to replicate the *CADPS2* findings in an independent set of peripheral blood samples from 41 children with ASD and 39 control children, following the Sadakata et al. protocols (Figure 1A). Furthermore, we performed sequencing (Figure 1B) and nested priming (Figure 1C) to validate the presence or absence of exon 3. Our results showed that, of 39 control samples, 1 was apparently homozygous for the exon 3-skipped allele in peripheral blood, 5 were heterozygous, and 33 were wild type. Of the 41 ASD samples, 5 were heterozygous for the exon 3-skipped isoform, while the rest were wild type.



**Figure 1**

Exon 3 skipping in *CADPS2* mRNA from 41 children with ASD and 39 control children. (A) RT-PCR of *CADPS2* mRNA in blood from subsets of patients with ASD (A1–A14) and control patients (C1–C10) following the Sadakata et al. protocols (1). The 661-bp band represents the full-length exon 1–5 fragment of *CADPS2* mRNA, while the 328-bp band is a result of exon 3 skipping. Four control samples (C1, C2, C6, and C8) and 4 ASD samples (A3, A7, A8, and A9) were heterozygous for the exon 3-skipped isoform. The flanking marker is a 50-bp ladder. The remaining samples showed only the 661-bp band (data not shown). (B) Alignment of sequences obtained from the 328-bp bands of samples C1, C2, C6, and A9 to human chromosome 7 showed that all sequences lacked exon 3. Sequencing the 661-bp band of A10 (which was representative of other samples not showing the 328-bp band) demonstrated that this fragment does include exon 3, as expected. (C) RT-PCR of blood *CADPS2* mRNA using a nested amplification. A single major band (563 bp), indicating the presence of exons 2–5, is shown in all autistic samples. Control sample C14 was apparently homozygous for a 230-bp band that resulted from skipping of exon 3. (D) RT-PCR of cerebellar *CADPS2* mRNA from individuals with ASD and control individuals showed that all cerebella contained the exon 3-skipped splice variant as a minor isoform (230-bp fragment). M, low-DNA-mass ladder (Invitrogen).



Analysis of these results showed no significant difference in the frequency of the exon 3-skipped allele in ASD versus control samples ( $P = 0.6$ , two-proportion  $z$  test). Although the samples tested here might differ from those tested by Sadakata et al. in their ethnicity, gender, or age distributions (Supplemental Figure 1 and Supplemental Tables 1 and 2; supplemental materials available online with this article; doi:10.1172/JCI38620DS1), the finding of exon 3 skipping in healthy controls at a high frequency suggests that this isoform does not represent aberrant splicing and likely is not a mechanism underlying autism.

Since Sadakata et al. extrapolate function of the exon 3-skipped isoform within the cerebellum, we additionally tested the presence of exon 3 in mRNA extracted from the cerebella of 9 control children and 5 children with ASD. All ASD and control samples were found to contain the exon 3-skipped splice variant as a minor isoform (Figure 1D).

Thus, our experiments suggest that exon 3 skipping represents a normal, minor isoform of CADPS2 in the cerebellum. As we observed no difference in prevalence of this allele between ASD and control samples, we conclude that exon 3 skipping is

likely not a mechanism underlying autism susceptibility or pathogenesis.

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1. Sadakata, T., et al. 2007. Autistic-like phenotypes in *Cadps2*-knockout mice and aberrant CADPS2 splicing in autistic patients. *J. Clin. Invest.* **117**:931–943.

## Response to the letter by Eran et al.

**W**e read with great interest the comment by Eran et al. (1) regarding our recently published CADPS2 article in the *JCI* (2). We appreciate their comment that “exon 3 skipping likely represents a minor isoform rather than aberrant splicing” in the blood and postmortem cerebella of both healthy and autistic individuals. However, we are concerned about the sensitivity of detection of exon 3 skipping in their experiments and have a few replies to their letter.

First, the signal intensity of the exon 3-skipped CADPS2 band was considerably weaker than that of the normal band in some ASD and control blood samples (Figure 1A in ref. 1), in contrast to our results using samples from autism, but not pervasive development disorder — not otherwise specified (PDD-NOS), samples. Moreover, only a trace amount of skipped band was detected in all postmortem cerebella they analyzed (Figure 1D in ref. 1).

Second, they utilized RT-PCR with a nested amplification (at 70 cycles) to detect a control sample C14 with a skipped, but no normal, band (which was called “homozygous” in their comment; Figure 1C in ref. 1) and claimed that only one sample (control sample C14) was homozygous in their study. Little quantitative gain is generally noticed when increasing the number of cycles to such an extraordinary number. We are left wondering if only the skipped band would also be detected in case C14 using an ordinary RT-PCR method (similar to our method with 48 cycles), such as that used to generate the data shown in Figure 1A (1).

Third, their argument regarding one sample “homozygous for the exon 3-skipped allele” and “heterozygous” samples may not be appropriate, since the terms are usually used for genomic DNA, not mRNA. Also, it is yet unknown whether exon 3 skipping is of a *cis*- or *trans*-acting genetic origin or some other origin such as epigenetic.

Fourth, considering the results of Eran et al., we assume that a balance between exon 3-skipped and normal CADPS2 is important for the local secretion property (somato-dendritic, axonal, and synaptic secretion) of CADPS2. Our *JCI* article indicated that exon 3-skipped CADPS2 is not transported into the axons of cultured neurons and suggested that disturbance of this balance may cause a defect in local secretion. Impaired synaptic secretion should be more serious in neurons that dominantly express exon 3-skipped CADPS2 than in those that weakly express it. Thus, excessive exon 3 skipping, together with a combination of other genetic mutations, might contribute to susceptibility to autism.

Finally, we have recently succeeded in generating a mouse line expressing exon 3-skipped *Cadps2* and have confirmed that exon 3 is critical for the subcellular localization of *Cadps2* in neurons (our unpublished observations). Further studies will



shed light on the association of exon 3 skipping with disturbed brain development and behavioral traits.

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2. Sadakata, T., et al. 2007. Autistic-like phenotypes in *Cadps2*-knockout mice and aberrant CADPS2 splicing in autistic patients. *J. Clin. Invest.* **117**:931–943.