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Response to the letter by Eran et al.

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Letter

We 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 […]

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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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Conflict of interest: The authors have declared that no conflict of interest exists.

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