Supplementary methods

Human subjects. Children with ASD were recruited and diagnosed by the Children's Hospital Developmental Medicine Center, using the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R) protocols, under IRB approval. Collection of control subjects was performed through an IRB approved protocol that enables recruitment through Children's Hospital Primary Care Center (CHPCC). Patients seen in the CHPCC for a well-child visit that involves a routine blood draw (for example to obtain lead levels) were offered enrollment. Post mortem cerebella were obtained from the University of Maryland Brain Bank, also under IRB approval. Sample ethnicity, age and gender distributions are summarized in supplementary figure S1. The subclassification of children with ASD is summarized in supplementary table S1, and the characteristics of samples found to express an exon 3 skipped isoform are detailed in supplementary table S2.

RNA extraction. Peripheral blood RNA was isolated with PAXgene Blood RNA Kit (QIAGEN) and cerebellar RNA was extracted using miRvana RNA Isolation Kit (Ambion), following the manufacturers' protocols.

RT-PCR. SuperScript III cDNA Synthesis Kit (Invitrogen) was used for reverse transcription, and the Sadakata PCR protocol was followed for exon 1-5 amplification (1). PCR products were visualized using 2% agarose gel electrophoresis. A 661bp band represented the full-length exon 1-5 product, and a 328bp band represented the exon 3-skipped isoform. We noticed that 4 out of 24 samples (about 15%) lacked reproducibility when different thermocyclers were used for PCR. We only included those samples that reproducibly yielded the same PCR products with the Sadakata protocols, and used an additional amplification, with nested primers 5'-gctgacgaagcattttgcaacgcag-3' (forward) and 5'-actggaagactttccaaattggcc-3' (reverse). The PCR conditions were 70 cycles of 94°C for 15 seconds, 63°C for 30 seconds, and 72°C for 45 seconds, followed by a final hold at 72°C for 5 minutes. PCR products: normal - 563bp; exon 3 skipped - 230bp.

Sequencing. 328bp bands were cut from agarose gels, purified with the QIAquick Gel Extraction Kit (QIAGEN), and sequenced on the Applied Biosystems 3730 DNA Analyzer.

Figure S1. (A) Ethnicity, (B) age, and (C) gender distributions of tested samples

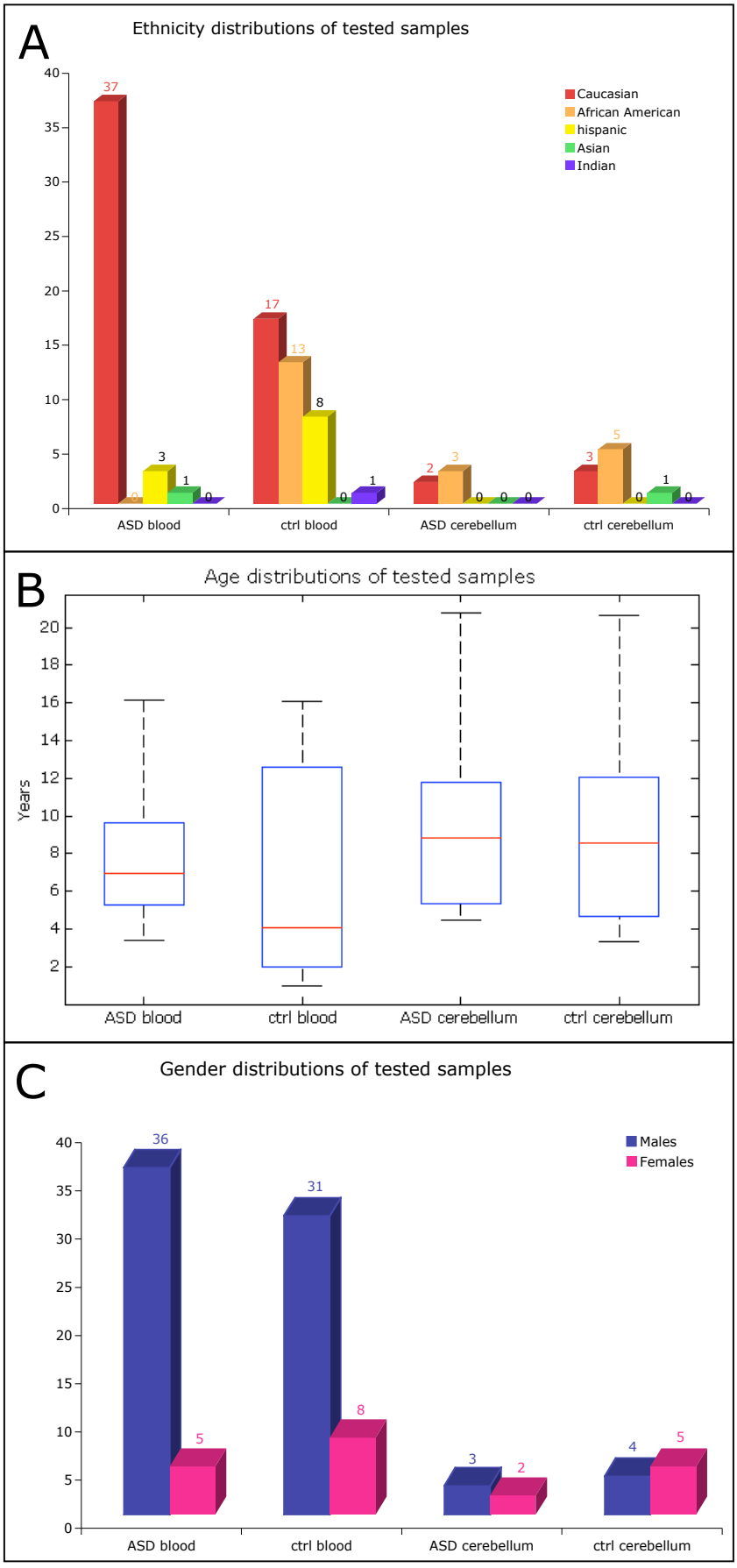

Table S1. Subclassification of individuals with ASD tested for CADPS2 exon 3 skipping

Disorder	Samples tested
Autistic disorder	19
PDD-NOS	18
Asperger Syndrome	4

Table S2. Characteristics of individuals found to express the CADPS2 exon 3 skipped isoform.
No covariates could be identified in the studied cohort. Thus exon 3 skipping is likely not associated with autism, rather represents a normal minor isoform of CADPS2.

Tissue	Sample ID	Dx	Exon 3 skipped alleles	Gender	Age	Ethnicity
Blood	A0009P2	PDD-NOS	1	Female	10.94	Caucasian
	A0070P1	Autism	1	Male	10.67	Caucasian
	A0043P2	Autism	1	Female	5.27	Caucasian
	A0033A1	PDD-NOS	1	Male	11.47	Caucasian
	A0034P1	PDD-NOS	1	Male	9.66	Hispanic
	F08033	Control	1	Female	8.58	Caucasian
	F16020	Control	1	Female	16.08	Caucasian
	M15023	Control	1	Male	15.25	Caucasian
	F13029	Control	1	Female	13.00	Caucasian
	M12052	Control	1	Male	12.83	African American
	M01140	Control	1	Male	1.00	Indian
Cerebellum	1407	Control	1	Female	9.13	African American
	1499	Control	1	Female	4.47	Asian
	1541	Control	1	Female	20.62	Caucasian
	1706	Control	1	Female	8.59	African American
	1793	Control	1	Male	11.74	African American
	1860	Control	1	Male	8.01	Caucasian
	4787	Control	1	Male	12.87	African American
	1185	Control	1	Male	4.71	Caucasian
	1284	Control	1	Female	3.34	African American
	1349	Autism	1	Male	5.60	Caucasian
	1638	Autism	1	Female	20.76	Caucasian
	4231	Autism	1	Male	8.82	African American
	4671	Autism	1	Female	4.45	African American
	4721	Autism	1	Male	8.83	African American