

CNTN6 mutations are risk factors for abnormal auditory sensory perception in autism spectrum disorders

Mercati O^{1,2,3,§}, Huguet G^{1,2,3,§}, Danckaert A⁴, André-Leroux G^{5,6}, Maruani A⁷, Bellinzoni M⁵, Rolland T^{1,2,3}, Gouder L^{1,2,3}, Mathieu A^{1,2,3}, Buratti J^{1,2,3}, Amsellem F⁷, Benabou M^{1,2,3}, Van-Gils J^{1,2,3}, Beggiano A⁷, Konyukh M^{1,2,3}, Bourgeois J-P^{1,2,3}, Gazzellone MJ⁸, Yuen RKC⁸, Walker S⁸, Delépine M⁹, Boland A⁹, Régnauld B¹⁰, Francois M¹¹, Van Den Abbeele T¹¹, Mosca-Boidron AL¹², Faivre L¹², Shimoda Y¹³, Watanabe K¹³, Bonneau D¹⁴, Rastam M^{15,16}, Leboyer M^{17,18,19,20}, Scherer SW^{8,21}, Gillberg C¹⁶, Delorme R^{1,2,3,7}, Cloëz-Tayarani I*^{1,2,3}, Bourgeron T*^{1,2,3,16,20}

Supplementary data

- Supplementary Table S1.** Description of the patients from the PARIS cohort used for the CNV screening
- Supplementary Table S2.** Description of the cohorts used for CNV screening
- Supplementary Table S3.** Description of the patients from the PARIS cohort used for the SNV screening
- Supplementary Table S4.** Primers used for mutation screening of *CNTN5* and *CNTN6*
- Supplementary Table S5.** Primers used for site-directed mutagenesis of *CNTN5* and *CNTN6* variants
- Supplementary Table S6.** *CNTN5* coding variants identified in this study
- Supplementary Table S7.** *CNTN6* coding variants identified in this study
- Supplementary Table S8.** Clinical characterization of the patients carrying *CNTN5* or *CNTN6* variants and evaluated for auditory brainstem responses
- Supplementary Table S9.** Auditory brainstem responses of the patients and the controls.
- Supplementary Figure S1.** Genetic and clinical characterizations of family AUDIJ001 and AUDIJ002 carrying *CNTN5* CNVs
- Supplementary Figure S2.** *CNTN5* and *CNTN6* CNVs identified in patients with ASD
- Supplementary Figure S3.** Localization of the exonic CNVs affecting *CNTN5* or *CNTN6* in patients and controls
- Supplementary Figure S4.** Families with *CNTN5* and *CNTN6* coding SNVs from the PARIS cohort
- Supplementary Figure S5.** Families with *CNTN5* and *CNTN6* coding SNVs from Canada
- Supplementary Figure S6.** Frequency of *CNTN5* and *CNTN6* private variants in this study, in Murdoch et al. PLOS Genetics (2015) and in the ExAC cohort
- Supplementary Figure S7.** Venn diagram of the ASD-risk genes from different databases.
- Supplementary Figure S8.** Impact of the *CNTN6* variants on neurite outgrowth.
- Supplementary Figure S9.** Effect of *CNTN6* variants on protein structure.

Supplementary Table S1. Description of the patients from the PARIS cohort used for CNV screening.

<i>Total</i>		<i>Sex</i>		<i>Age class</i>				<i>Array platform (Illumina)</i>			
		<i>Male</i>	<i>Female</i>	<i>< 12 y</i>	<i>12 – <18 y</i>	<i>≥ 18y</i>	<i>NA</i>	<i>660W Quad</i>	<i>1M Duo – 1M</i>	<i>Omni1</i>	<i>Omni2.5</i>
<i>ASD with ID</i>	<i>n = 361</i>	<i>294</i>	<i>67</i>	<i>4</i>	<i>68</i>	<i>203</i>	<i>86</i>	<i>0</i>	<i>250</i>	<i>108</i>	<i>3</i>
<i>ASD without ID</i>	<i>n = 195</i>	<i>162</i>	<i>33</i>	<i>3</i>	<i>43</i>	<i>113</i>	<i>36</i>	<i>27</i>	<i>151</i>	<i>14</i>	<i>3</i>
<i>NA</i>	<i>n = 77</i>	<i>66</i>	<i>11</i>	<i>4</i>	<i>8</i>	<i>1</i>	<i>64</i>	<i>0</i>	<i>36</i>	<i>41</i>	<i>0</i>
<i>Total</i>	<i>n = 633</i>	<i>522</i>	<i>111</i>	<i>11</i>	<i>119</i>	<i>317</i>	<i>186</i>	<i>27</i>	<i>437</i>	<i>163</i>	<i>6</i>

Supplementary Table S2. Description of the cohorts used for CNV screening.

Project	Technologies	N individuals	CNTN5		CNTN6	
			Loss	Gain	Loss	Gain
Patients with ASD						
The PARIS cohort	Illumina (1M, 1M duo, Omni 1, 2.5M, 5M)	633	1	0	4	0
Pinto et al. 2010	Illumina 1M	901	0	0	2	2
Total Patients with ASD	Illumina	1534	1	0	6	2
Controls ^a						
This study	Illumina (660Wq, 1M, 1M duo, Omni 1, 5M)	2126	0	0	0	1
SAGE+Hapmap3 (Pinto et al. 2010)	Illumina 1M	1287	0	1	0	5
HBAC	Illumina 1M duo	2566	1	1	0	4
KORA	Illumina 2.5M Quad	1775	0	1	1	2
COGEND	Illumina 2.5M	1182	0	0	0	0
Total Controls	Illumina	8936	1	3	1	12

^a Control datasets were obtained, along with permission for use, from the database of Genotypes and Phenotypes (dbGaP) found at <http://www.ncbi.nlm.nih.gov/myaccess.library.utoronto.ca/gap> through accession numbers phs000169.v1.p1 (Whole Genome Association Study of Visceral Adiposity in the HABC Study), phs000303.v1.p1 (Genetic Epidemiology of Refractive Error in the KORA Study), and phs000404.v1.p1 (COGEND; The Genetic Architecture of Smoking and Smoking Cessation).

Supplementary Table S3. Description of the patients from the PARIS cohort used for SNV screening.

<i>Total</i>		<i>Sex</i>		<i>Age class</i>				<i>Family status</i>		
		<i>Male</i>	<i>Female</i>	<i>< 12 y</i>	<i>12 – <18 y</i>	<i>≥ 18y</i>	<i>NA</i>	<i>simplex</i>	<i>multiplex</i>	<i>single</i>
<i>ASD with ID</i>	<i>n = 125</i>	95	30	1	22	93	9	89	36	0
<i>ASD without ID</i>	<i>n = 82</i>	66	16	2	14	60	6	46	20	16
<i>NA</i>	<i>n = 5</i>	5	0	1	0	4	0	3	2	0
<i>Total</i>	<i>n =212</i>	166	46	4	36	157	15	138	58	16

Table S4. Primers used for mutation screening of *CNTN5* and *CNTN6*

Exon	Size (bp)	F primer	Forward primer sequence (5'-3')	R primer	Reverse primer sequence (5'-3')	T _m (°C)
3	418	CNTN5_1	TCTATCTCAAACAGGGCACTC	CNTN5_2	GCCTTCTGAAAGCTAGATGG	60
4	524	CNTN5_3	TGCAGCTCCAAAGAAGGTTT	CNTN5_4	TCTCCCCTAACATCCTCCAG	60
5	763	CNTN5_5	CACCCCAGAATTGTTAGCAC	CNTN5_6	TTCCATTTCGAAGCCATCTT	60
6	817	CNTN5_7	AGTTCGTGGCAATCCAGTTC	CNTN5_8	CATGAAAAGGACTTCAACCTG	60
7	731	CNTN5_9	GAGAGAATGGGCGTTATTGG	CNTN5_10	CGCTTCCTTTGTGCTCTAGG	60
8	527	CNTN5_11	GGCTTTGTGTTTGAGCTTT	CNTN5_12	TTCTCCCTTTCTGGCATTAG	60
9	396	CNTN5_13	TCCTCAGGGGAAGACTAGGA	CNTN5_14	GGGAAATGACACCTAGCAA	60
10	549	CNTN5_15	AACCCACGTGAAAAGTGAAA	CNTN5_16	CACCGATAATTCCTCCATCA	60
11	556	CNTN5_17	CCCCACTACATTGAAAGAGC	CNTN5_18	GCCTAGTTTGTCTGTGTGA	60
12	591	CNTN5_19	TACATTGCTAAGTGCCAAA	CNTN5_20	CTACCCCTCAAGGCATTCTTC	60
13	621	CNTN5_21	AGGTCACCTCCATCTTACCA	CNTN5_22	TACGTCTCACGGTACTGCAA	60
14	620	CNTN5_23	AAACTCAGGGTCACCCCTTC	CNTN5_24	TGCCAATAAGAGGTTCTCTCA	60
15	850	CNTN5_25	AGGAACAGAGGCTCTTAAATC	CNTN5_26	GGCTAATGTGCATTTATGACT	56
16	593	CNTN5_27	CCCACAAGTTGCTGTCCCTT	CNTN5_28	GATGTGCTCAAAGTCCATGC	60
17	636	CNTN5_29	TTCCCCCAGAAATCCTAGA	CNTN5_30	GAGTGGGTCCACAGGAACTC	60
18	766	CNTN5_31	GGAGGTGACACCATGACCAC	CNTN5_32	TAGGTCAAGGAAGGGCAATG	60
19	839	CNTN5_33	CATGGTTTGGTGAATGAAG	CNTN5_34	TCCTCCCCTGTTTTTAGCC	60
20	849	CNTN5_35	TTCCCCAACATGTTAATCC	CNTN5_36	CCAGAGAAAGGAATACACTGC	56
21	649	CNTN5_37	TTCCCCAGAAATGTCTTAGC	CNTN5_38	ACCTGTAGCTCCTGCTTTTG	60
22	509	CNTN5_39	CTGCTGTAGTGCTGCAATTT	CNTN5_40	TCAAACTCCCCTTTGACTC	60
23	515	CNTN5_47	TGCCAGCCAACACTAAGCA	CNTN5_48	GCCTAAATCTGCTGGTCCT	60
24	522	CNTN5_43	TGGCCCTTTGTGTACTTCT	CNTN5_44	TCTGCAATGTGAGCAGATT	60
25	702	CNTN5_45	TCTCATGATTGGATGTGTTTG	CNTN5_46	CTTAACAAATGGATGGCACA	60
2	646	CNTN6_1	ATTATGATGCAACCCCTTA	CNTN6_2	AAACTTTCCTGTCTGAGTCAT	60
3	460	CNTN6_3	GGGTAATAGGGGATTGGTTT	CNTN6_4	ATTGTACATGGCTGCATAC	60
4	469	CNTN6_5	CCAAGACAGCTACCTTTGGT	CNTN6_6	CATTGTCAAAGCCATAAAA	60
5	542	CNTN6_7	ACCTTCCTTGGACAGACAAA	CNTN6_8	GCCTTGACGAGAGTCACAA	60
6	625	CNTN6_9	AAAAGTCAATGATGGGGAAA	CNTN6_10	GAACCATGTCTACTGCACCA	60
7	602	CNTN6_11	TTAGCTGAAAACATCCCCTTG	CNTN6_12	GAATTGCAGCCCTGATCATT	60
8	486	CNTN6_13	GCATACGGCAGGCACTTAAT	CNTN6_14	TCCCGTGACACTTTTTTACA	60
9	591	CNTN6_15	ATTGAAGCTCCTGCATGTCC	CNTN6_16	TATCCGTGTCGATGATGACG	60
10	589	CNTN6_17	GAAAGCCAAGGCAGTGTCT	CNTN6_18	AAAATTGGGTTGGGATTATGC	60
11	591	CNTN6_19	GGTCCAAGAACACCTTGAGC	CNTN6_20	TTCAGGAGGAAGCCTTCAA	60
12	559	CNTN6_21	TGCTTTGAAATCGACAATGA	CNTN6_22	ACTGGCAAAGTGTGCATGTT	60
13	468	CNTN6_23	TGTAGAACTCCAGGTTGCAT	CNTN6_24	TCTACGGGAAGACCGAAACA	60
14	492	CNTN6_25	TTGTTTTCGTGTCTCCGTA	CNTN6_26	TACCCATACCATGGAGTCTCT	60
15	533	CNTN6_27	TGCAATCATCCTATGCCTGA	CNTN6_28	AAGGCACCTAGGTTGGGACT	60
16	587	CNTN6_29	CAGGCTGTGCTACAGGTGA	CNTN6_30	GATGGCATGCAAAACAGTT	60
17	496	CNTN6_31	GAGCCAGGGTGCTCATGTAT	CNTN6_32	TTGGGAGAAAATGTGTGTA	60
18+19	780	CNTN6_33	TGGCATTCCAAATAAAAAGTG	CNTN6_34	GCTCACCTACCCTGTCTCAA	60
20	602	CNTN6_35	CCTTGTGGTTGTGGTTGATA	CNTN6_36	TACCACCTAACGTTCCAAGG	60
21	590	CNTN6_37	CATTCCCAAATGACCAGATT	CNTN6_38	TGCACATAAGCCATCATCTT	60
22	590	CNTN6_39	CAGTGACTTTCCACAGCAAG	CNTN6_40	CTCATCACCTTTTGTCTACA	60
23	573	CNTN6_41	GACACGGTAAGGCACTTTCT	CNTN6_42	AGCATCATTGAGGGGAGTTA	60

PCR reactions were performed using BioTaq DNA polymerase (Gentaur) and the following protocol: 94°C for 4 min, followed by 35 cycles of: 94°C-initiation for 30 seconds; annealing for 40 seconds and 72°C-elongation for 30 seconds; and a final 72°C-elongation for 10 min.

Table S5. Primers used for site-directed mutagenesis of *CNTN6* variants

a.a. change	rat cDNA nt change(s)	Forward primer (5'-3')	Reverse primer (5'-3')
CNTN6_S57L	c170t + t171a	AGTTGTAAGTCAAGTGGATACCCTT TA CCCCACTACAGGT	ACCTGTAGTGGGG TA AAGGGTATCCACTTGCAGTACAAC
CNTN6_R303Q	g908a	CGTTGCAGGTAACCTT CA AGGAAGAAACCTTGCAA	TTGCAAGGTTTCTTCC T GAAGGTTACCTGCAACG
CNTN6_G310S	g928a	GAGGAAGAAACCTTGCAAAG AG TCAACTCATTTTCTATGCT	AGCATAGAAAATGAGTTGACT T CTTTGCAAGGTTTCTTCTC
CNTN6_N377S	a1130g	GACTCTTATCATCACCATGCTGAG CG TGTCAGACTC	GAGTCTGACACG C TCAGCATGGTGATGATAAGAGTC
CNTN6_S419C	c1256g + a1257t	TCCAGATTTCTCAAAAAATCCCATTAATAAAAAATTT GT GTTGTTCAAGTTGGTGGTG	CACCACCAACTTGAACAAC AC AAATTTTTTAATGGGATTTTTTGAGAAATCTGGA
CNTN6_G678S	g2032t + g2033c	AATATGAATTTTCGTGTTGTTGCT TC GAACAACATTGGAATTGGAGAGC	GCTCTCCAATTCCAATGTTGTT CGA AGCAACAACACGAAATTCATATT
CNTN6_I683S	t2048g	TGTTGCTGGGAACAACATTGGAA AG TGGAGAGCCAAG	CTTGGCTCTCC CA CTTCCAATGTTGTTCCAGCAACA
CNTN6_P770S	c2308t	ACAGAAATGAAAGCATCATG T CTTTGTCTCCCTTTGAAGTC	GACTTCAAAGGGAGACAA AG CATGATGCTTTCATTTCTGT
CNTN6_S858N	g2573a	AAAGAATCTATGATTGGGAAAATTAGAGTCA A TGGAAATGTCACAACC	GGTTGTGACATTTCC A TGACTCTAATTTTCCAATCATAGATTCTTT
CNTN6_T958I	c2873t	GTAAACTCATGTCTTGGAAACAATA TAT ATCAGCTGAGCTACTG	CAGTAGCTCAGCTGAT A TATTATTTGTTTCCAAGACATGAGTTTTAC

Mutated nucleotides are shown in blue.

Supplementary Table S6. CNTN5 coding variants identified in this study

Exon ^a	Detected variants			Frequency ^c			Domain	PolyPhen2 (Hum_Div) ^d	Inheritance		
	Nucleotide change ^b (chr. 11)	dbSNP (build 137)	a. a. change	ASD PARIS ^c (n=212)	ASD Canada (n=289)	Controls (n=217)			PARIS	Canada	
	8	g. 99827588 G>T [#]	unknown	p. D242Y	1	0	0	Ig2	deleterious	M	-
	8	g. 99827624 C>T [#]	unknown	p. L254F	1	0	0	Ig2	deleterious	P	-
	10	g. 99931978 G>A	rs200767659	p. G339S	0	1	0	Ig3	deleterious	-	P
	10	g. 99932054 C>T	rs200797524	p. A364V	1	0	0	Ig3	deleterious	M	-
ASD only	14	g. 100061860 T>C	unknown	p. I528T	1	0	0	Ig5	neutral	M	-
	20	g. 100169923 A>C	rs200512456	p. E805D	0	2	0	FN2	deleterious	-	2 M
	20	g. 100170018 G>A	rs199937129	p. R837Q	0	1	0	FN2	deleterious	-	M
	21	g. 100179121 C>T	rs200726875	p. A884V	0	1	0	FN3	deleterious	-	P
	21	g. 100179166 T>C	rs201978001	p. I899T	1	0	0	FN3	neutral	M	-
	21	g. 100211262 C>T	rs201747311	p. S933F	0	1	0	FN3	deleterious	-	1 M
	21	g. 100211267 G>A	unknown	p. V935I	0	1	0	FN3	neutral	-	M
	6	g. 99715891 T>G	rs61749255	p. I158M	1	6	2	Ig1	deleterious	-	2 P, 4 M
ASD and controls	14	g. 100061865 A>G	rs11223168	p. I530V	32 htz	27 htz (3 hmz)	40 htz (1 hmz)	Ig5	neutral	-	-
	19	g. 100168410 T>A	rs141228828	p. L790I	6	2	4	FN2	deleterious	4 P, 1 M	1 M
	20	g. 100170119 G>C	rs200130506	p. A871P	1	0	1	-	deleterious	-	-
	25	g. 100226883 T>A	rs1216183	p. S1079T	50 htz (2 hmz)	54 htz (4 hmz)	46 htz (6 hmz)	-	neutral	-	-
	24	g. 100221596 A>T	rs1944169	p. Y1065F	0	0 ^e	1	-	neutral	M	-
Controls only	8	g. 99827639 G>A	unknown	p. V259I	0	0	1	Ig2	neutral	-	-
	10	g. 99931945 C>G [#]	rs201910584	p. P328A	0	0	1	Ig3	deleterious	-	-
	10	g. 99932033 C>T [#]	rs56122118	p. P357L	0	0	1	Ig3	deleterious	-	-
	18	g. 100141888 delA	unknown	p. E743Dfs*5	0	0	1	FN1	-	-	-

^a Variants in Exon 4 of *CNTN5* were not reported since there were discrepancies between results obtained through next generation sequencing and Sanger sequencing. ^bNucleotide positions correspond to the NCBI37/hg19 build. ^c In order to have geographically matched patients and controls, among the 226 patients with ASD, 14 individuals were non from European descent and were not included in the statistical analysis. Yet, one coding-sequence variant, CNTN5^{Y1065F}, was identified in a patient from African origin. ^d PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>), which classifies variants into benign, possibly damaging and probably damaging. htz: Heterozygote; hmz: homozygote. P : Paternal; M: Maternal

Supplementary Table S7. CNTN6 coding variants identified in this study

Exon	Detected variants			Frequency			Domain	PolyPhen2 (Hum_Div) ^b	Inheritance		
	Nucleotide change ^a (chr. 11)	dbSNP (build 137)	a. a. change	ASD PARIS (n=212)	ASD Canada (n=289)	Controls (n=217)			PARIS	Canada	
	3	g. 1262427 C>G	unknown	p. P38A	0	1	0	Ig1	deleterious	-	P
	3	g. 1262485 C>T	unknown	p. S57L	1	0	0	Ig1	neutral	P	-
	4	g. 1269644 A>G	unknown	p. I109V	0	1	0	Ig1	neutral	-	M
	5	g. 1320187 T>C	rs6808056	p. F150S	1	0	0	Ig2	neutral	P	-
	8	g. 1363500 G>A	rs149799168	p. G310S	1	1	0	-	deleterious	P	1 M
	9	g. 1367553 A>C	unknown	p. N334T	0	1	0	Ig4	neutral	-	M
	11	g. 1371511 C>G	unknown	p. S419C	1	0	0	Ig5	deleterious	P	-
ASD only	13	g. 1414075 A>C	rs139645898	p. I529L	0	2	0	Ig6	neutral	-	1 P, 1 M
	16	g. 1415710 T>G	unknown	p. I683S	1	0	0	FN1	deleterious	P	-
	18	g. 1424767 C>T	unknown	p. P770S	1	0	0	FN2	deleterious	P	-
	18	g. 1424768 C>T	unknown	p. P770L	1	0	0	FN2	deleterious	de novo	-
	19	g. 1425055 C>T	rs115667338	p. A827V	0	1	0	FN3	deleterious	-	P
	20	g. 1427350 G>A	unknown	p. S858N	1	0	0	FN3	neutral	P	-
	21	g. 1443180 G>A	unknown	p. W923X	0	1	0	FN4	-	-	1 M
	22	g. 1444057 C>T	unknown	p. T958I	1	0	0	FN4	deleterious	M	-
	22	g. 1444167 T>A	rs139391275	p. S995T	0	1	0	FN4	deleterious	-	P
ASD and Controls	8	g. 1363480 G>A	rs41293401	p. R303Q	3 htz (1 hmz)	5	6	Ig3	deleterious	1 M, 1 P	4 P, 7 M
Controls	10	g. 1369187 A>G	unknown	p. N377S	0	0	1	Ig4	deleterious	-	-
only	16	g. 1415694 G>A	rs138682306	p. G678S	0	0	1	FN1	neutral	-	-

^a Nucleotide positions correspond to the NCBI37/hg19 build. ^b PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>), which classifies variants into benign, possibly damaging and probably damaging. htz: Heterozygote; hmz: homozygote.

Supplementary Table S8. Clinical characterization of patients carrying *CNTN5* or *CNTN6* variants and evaluated for auditory brainstem responses

FAMILY	AU-DIJ-001	AU-DIJ-002	AU-RD-124	AU-FRA-035	AU-RD-183	AU-RD-192	AU-FRA-033	AU-RD-237	AU-RD-068
GENETICS									
Gene	<i>CNTN5</i>	<i>CNTN5</i>	<i>CNTN5</i>	<i>CNTN6</i>	<i>CNTN6</i>	<i>CNTN6</i>	<i>CNTN6</i>	<i>CNTN6</i>	<i>CNTN6</i>
Mutation	CNV/deletion	CNV/X5	L254F	CNV/deletion	CNV/deletion	CNV/deletion	I683S	S858N	G310S
Transmission	unknown	maternal	paternal	paternal	paternal	maternal	paternal	paternal	paternal
SEX	f	f	m	f	m	m	m	m	m
AGE	32	6	21	13	13	13	13	24	39
DIAGNOSIS ASSESSMENT	ASD	ASD	ASD	ASD	ASD	ASD	ASD	ASD	ASD
ADI-R									
Age at the time of evaluation (years)	32	5	10	6	3	3	7		28
Age of first symptoms (months)		24	2	18		18	30		10
Social domain (cut-off: 10)	13	9	28	23	13	28	17		21
Communication domain: verbal (cut-off: 8) & non verbal (cut-off: 7)	13 - 7	13	11 (nv)	18 - 12	11 (nv)	11 (nv)	10 - 6		18 - 11
Repetitive domain (cut-off: 3)	4	1	5	4	4	4	8		9
ADOS									
Communication domain (cut-off: 5)		2	6	9		7	4		10
Social interaction domain (cut-off: 6)		4	12	14		7	15		13
Total		6	18	23		14	19		23
Interest and Behaviors			2	1			2		1
DSM-5 criteria	yes	yes	yes	yes	yes	yes	yes		yes
IQ MEASUREMENT									
Scales	RPM	Wechsler	RPM	RPM	Wechsler	RPM	Wechsler	RPM	RPM
Full IQ scores	70	82	75	84	96	93	85	84	66
MEDICAL HISTORY									
Pregnancy and delivery									
Course of pregnancy	normal	normal	normal	normal	normal	normal	normal	twin	normal
Delivery course	eutocic	dystocic	dystocic	dystocic	eutocic	eutocic	dystocic	eutocic	eutocic
APGAR at 1 et 10 min (/10)		10 - 10	9 - 10	9 - 10	10 - 10	10 - 10	8 - 10	10 - 10	10 - 10

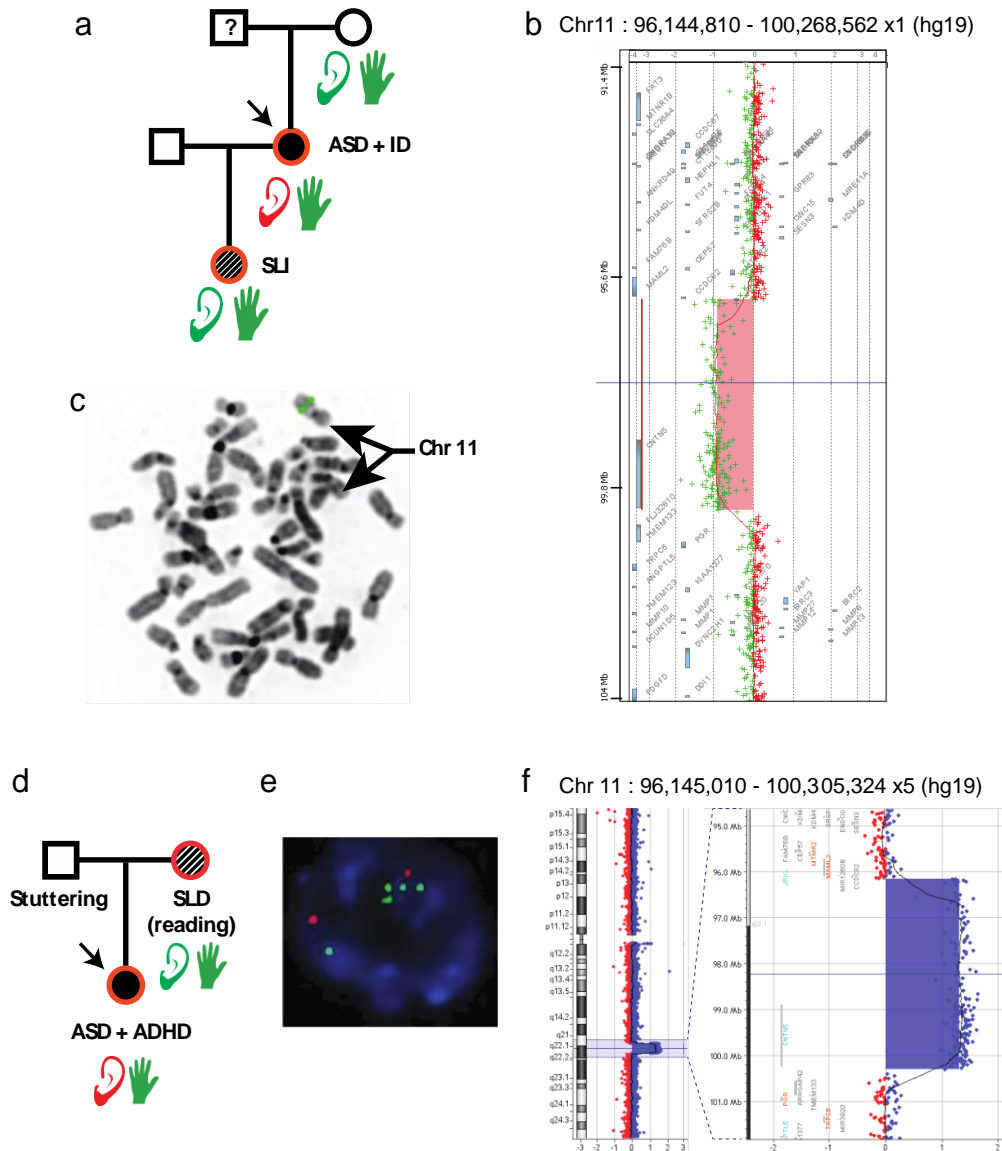
Birth height (cm) / weight (g) / head circumference (cm)		46/2790/32	54/3780/36	49/2600/ --	51/2960/33,5	51/3500/36	48 /3300/33	--/--/--	48/2950/33
Milestones									
walking (m)	18	12	10	14	14	13	13	18	24
first words (m)		18	18	14		24	13		48
first sentence (m)	48	30	no functional language	40	no functional language	54	26	36	no language
Main somatic comorbidities	Alopecia areata Hair loss	no	Febrile seizures at day 2 post birth	no	no	no	no		
Main psychiatric comorbidities		ADHD	no	Single phobia	ADHD	ADHD	Sleep-onset insomnia, ADHD	Panic disorder with agoraphobia, cannabis dependence	
CLINICAL EXAMINATION									
Developmental Coordination Disorder *	yes	yes	yes	yes	yes	yes	yes	NA	yes
Ear Nose Throat examination									
Subjective features	painful hyperacusis	painful hyperacusis	painful hyperacusis	painful hyperacusis	painful hyperacusis	painful hyperacusis	painful hyperacusis	auditory hallucinations	painful hyperacusis
Auditory acuity tests	normal range		normal range	normal range	normal range	normal range	normal range	normal range	non compliant
Auditory brainstem response test	normal range		shortened wave I-V interpeak latency	shortened wave I-V interpeak latency	shortened wave I-V interpeak latency	normal range	shortened wave I-V interpeak latency	wave I-V interpeak latency shortened	non compliant
FAMILY HISTORY									
Father		sluttering		ASD without ID			chronic motor tic disorder	GAD	
Mother		dyslexia/ dysgraphia			MDD, anxiety disorder		MDD	GAD	
Siblings, other members	One daughter with ID carrying the deletion	Cousin with epilepsy		One brother with ASD but without ID not carrying the deletion			One brother with ASD and ID not carrying the variant	Two brothers, both with ASD without ID all carrying the variant	

GAD: generalized anxiety disorder; IQ: intellectual quotient; MDD: mood disorder; RPM: Raven's Progressive matrices, m: male; f: female; nv: non verbal;

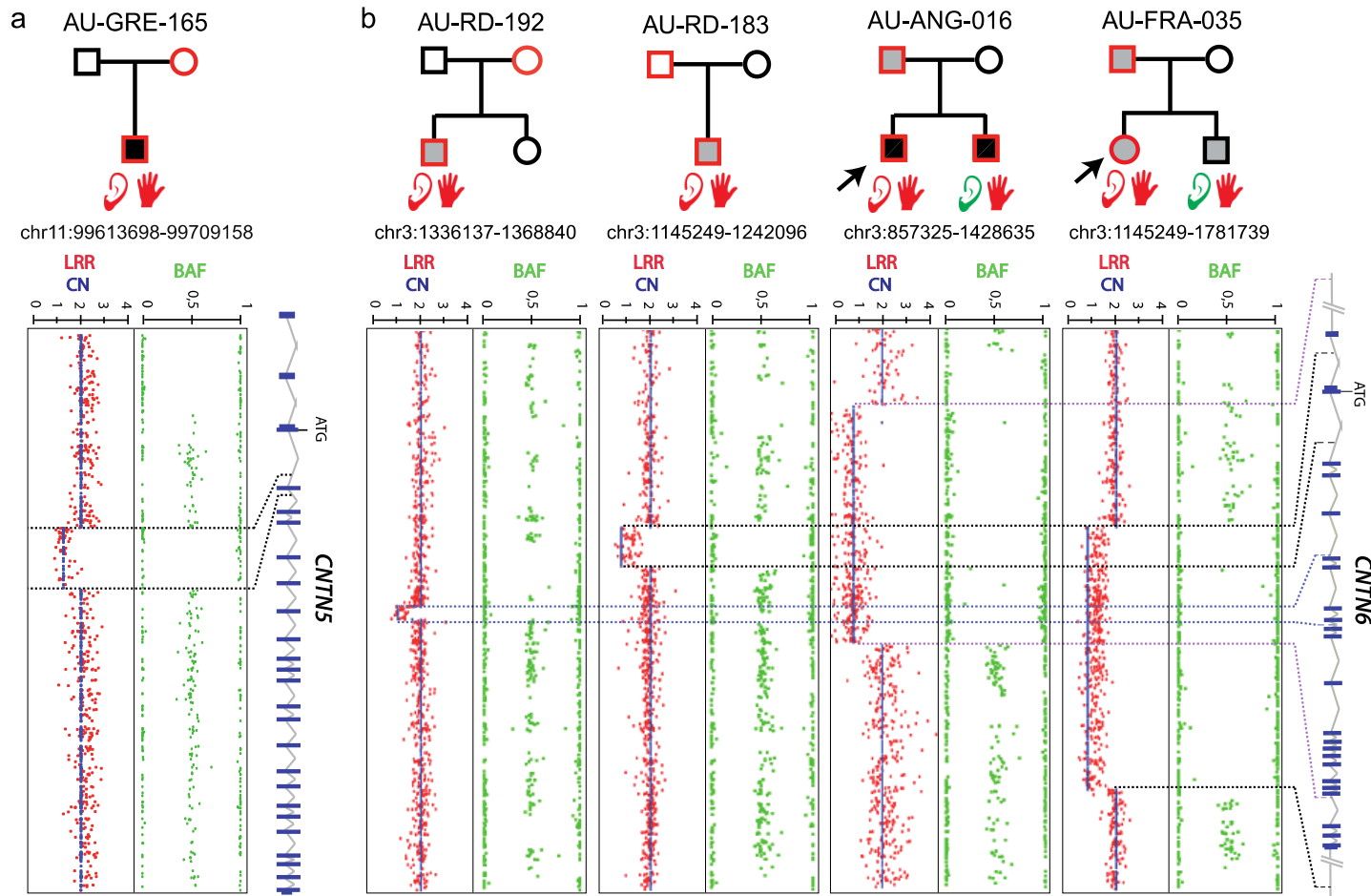
* based on the Neurological exam, the ADI-R and the Developmental Coordination Disorder Questionnaire

Supplementary Table S9. Auditory brainstem responses in patients and their relatives.

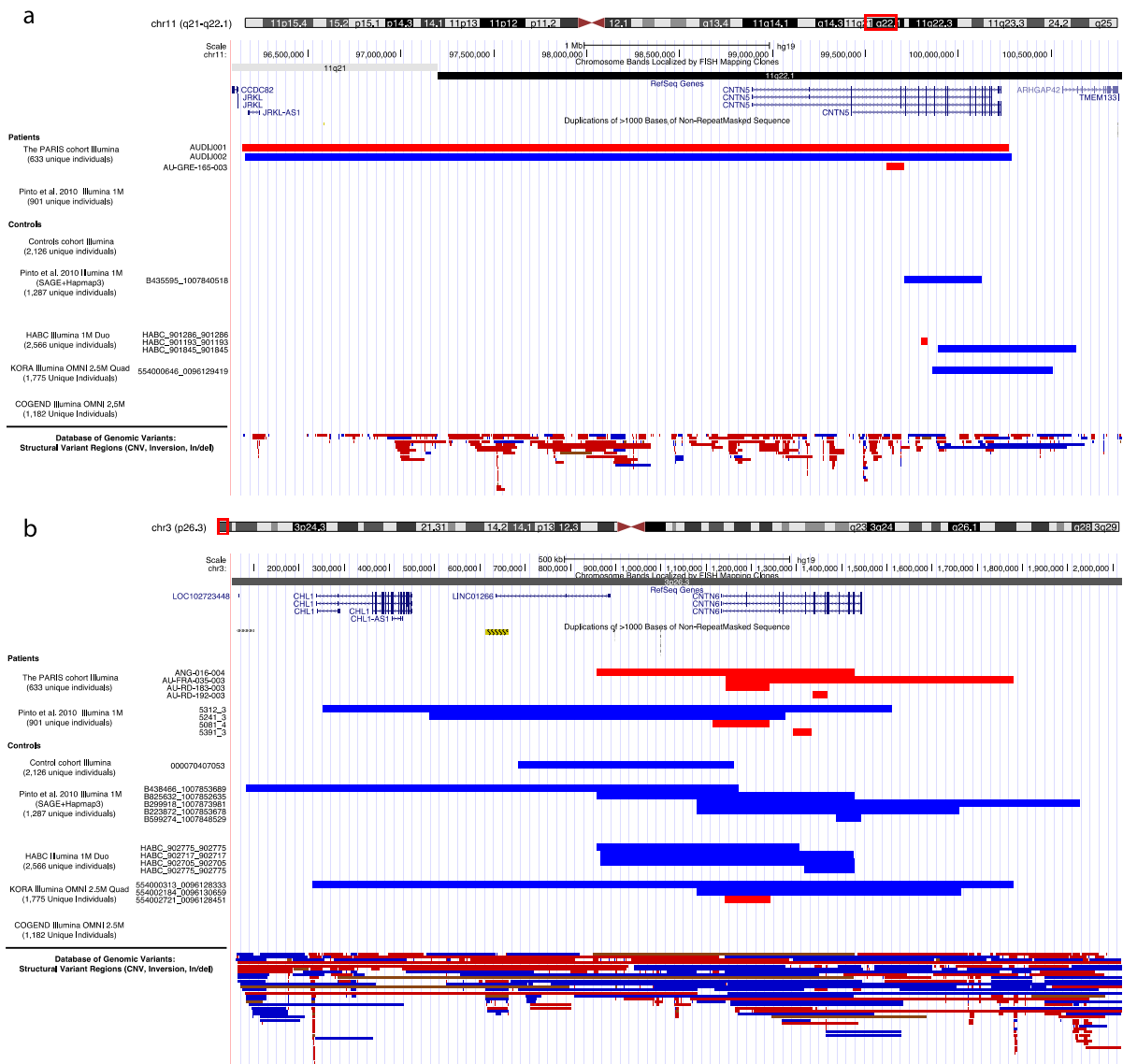
	LEFT EAR					RIGHT EAR				
	<i>Frequency: 29 clicks / sec</i>					<i>Frequency: 29 clicks / sec</i>				
	Affected Carriers (mean/SD/N)	Unaffected carriers (mean/SD/N)	Unaffected non carriers (mean/SD/N)	KW(2df) χ^2	p	Affected Carriers (mean/SD/N)	Unaffected carriers (mean/SD/N)	Unaffected non carriers (mean/SD/N)	KW(2df) χ^2	p
<i>Intensity: 80 dB</i>										
latency V	5.50/0.38/6	5.55/0.45/7	5.41/0.21/5	0.51	0.78	5.47/0.39/7	5.75/0.46/7	5.44/0.38/8	1.89	0.39
latency III-V	1.88/0.22/5	1.90/0.24/6	1.78/0.18/5	1.56	0.46	2.03/0.36/5	2.02/0.63/7	1.65/0.15/7	4.26	0.12
latency I-V	3.96/0.43/4	4.14/0.33/6	3.86/0.14/5	2.22	0.33	4.23/0.21/5	4.11/0.48/7	3.72/0.49/7	4.63	0.09
latency I-III	2.06/0.22/4	2.23/0.19/6	2.07/0.15/5	2.17	0.34	2.30/0.21/5	2.09/0.40/7	2.17/0.10/6	2.19	0.33
latency III	3.41/0.52/5	3.78/0.2/6	3.75/0.19/5	2.55	0.28	3.48/0.46/4	3.62/0.37/7	3.65/0.28/6	0.33	0.84
latency I	1.25/0.33/4	1.54/0.12/6	1.65/0.32/5	4.74	0.09	1.30/0.30/5	1.53/0.13/7	1.61/0.15/7	4.21	0.12
<i>Intensity: 60 dB</i>										
latency V	5.94/0.61/9	6.61/0.74/5	6.12/0.44/8	4.18	0.12	5.89/0.32/9	6.33/0.29/5	5.95/0.45/8	4.72	0.09
latency III-V	1.90/0.30/7	2.05/0.07/2	1.79/0.27/5	2.67	0.26	2.00/0.25/7	1.93/0.21/4	1.71/0.16/6	5.18	0.08
latency I-V	3.88/0.24/6	4.60/0.32/2	4.20/0.32/6	5.87	0.05	3.94/0.48/4	3.10/0.25/3	3.77/0.32/8	1.63	0.44
latency I-III	2.13/0.18/6	2.27/0.00/1	2.16/0.33/5	0.21	0.90	2.10/0.41/4	2.21/2.3/3	0.81/2.90/6	2.51	0.29
latency III	4.03/0.70/7	3.95/0.11/2	4.04/0.52/4	0.17	0.92	3.91/0.14/7	4.55/0.43/4	4.10/0.45/6	4.76	0.09
latency I	1.80/0.14/6	1.72/0.07/2	1.83/0.32/6	0.99	0.61	1.90/0.18/4	2.52/0.20/3	2.04/0.35/8	5.62	0.06



Supplementary Figure S1. Genetic and clinical characterization of the family AUDIJ001 and AUDIJ002 carrying *CNTN5* CNVs. **Family AUDIJ001** (a) The *CNTN5* deletion was transmitted from the proband with ASD to her daughter with specific language impairment (SLI). (b) Agilent array-CGH 180K profile of the daughter showing the 11q21q22.1 deletion (4.1 Mb) encompassing all the exons of *CNTN5* (chr11: 96,144,810-100,268,562). *JRKL antisense RNA 1* is deleted, but not *MAML2*. (c) Inverse DAPI FISH image: the control probe RP11-176D23 (green) spanning *CNTN5* confirmed the heterozygous 11q21q22.1 deletion. Arrows indicate both chromosomes 11. **Family AUDIJ002** (d) The proband with ASD and ADHD carried 5 copies of *CNTN5* inherited from her mother diagnosed with specific learning disorder (SLD, reading). (e) Interphase FISH (Fluorescent In Situ Hybridization) image of the 5 copies of *CNTN5* in the proband (probe RP11-418D05 in green) on chromosome 11 (the probe RP11-583A17 in 11p15.5 is labeled in red). (f) Agilent array-CGH (Comparative Genomic Hybridization) 180K profile of the daughter showing the 5 copies of *CNTN5* (chr11:96,145,010-100,305,324).



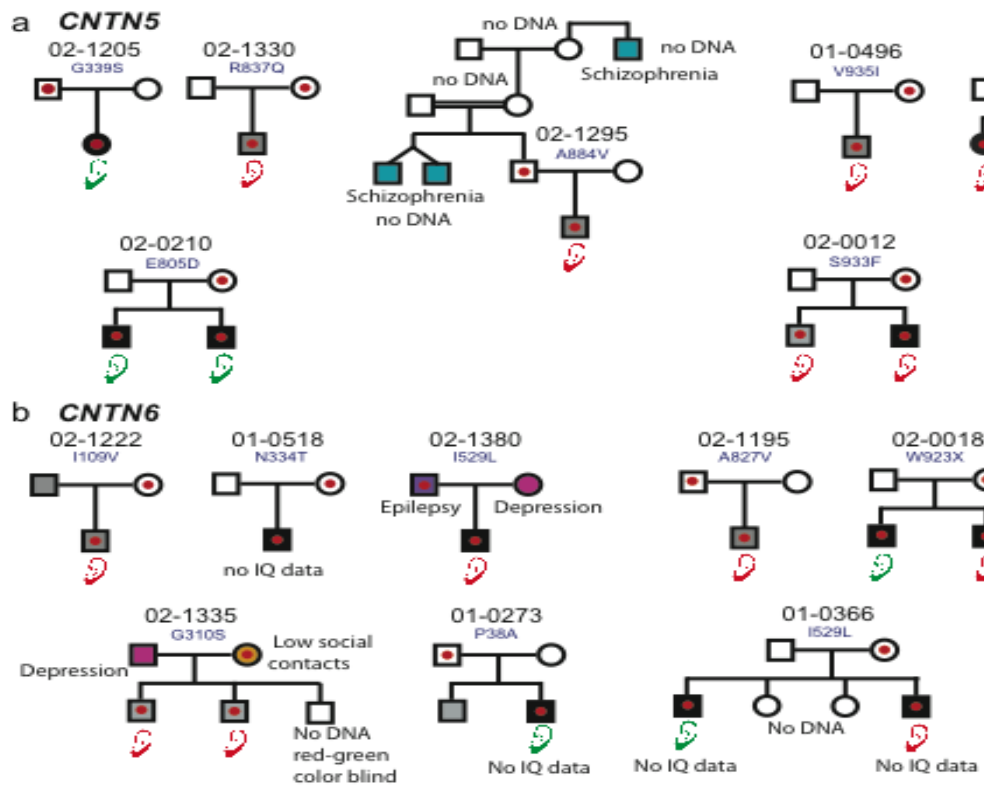
Supplementary Figure S2. *CNTN5* and *CNTN6* copy-number variants identified in patients with ASD. *CNTN5* and *CNTN6* CNVs were detected using the HumanOmni1 Illumina array. For each SNP, a green dot represents the B Allele Frequency (BAF) and a red dot, the log R ratio (LRR) of hybridization intensity. The blue line shows the predicted number of copies (CN). Black-filled symbols indicate a diagnosis of ASD with intellectual disability, while grey-filled symbols indicate a diagnosis of ASD without intellectual disability. In multiplex families, an arrow indicates the proband. Based on the ADI-R and clinical examination, normal (green) or abnormal (red) hypersensitivity to sounds and motor coordination ability are indicated by a schematic ear and hand, respectively. The absence of ear/hand means that information was not available. All exonic CNVs of *CNTN5* or *CNTN6* identified in patients and controls are shown in Supplementary Figure S3.



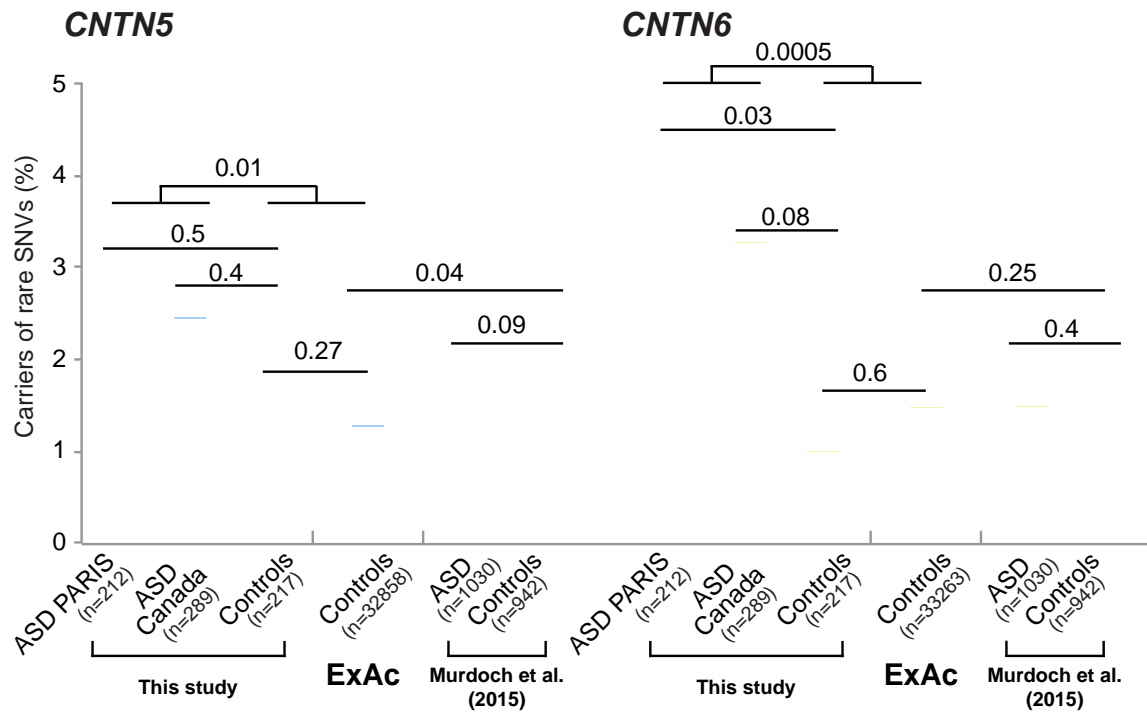
Supplementary Figure S3. Localization of the exonic CNVs (> 30kb) affecting *CNTN5* (a) or *CNTN6* (b) in patients and controls. HBAC: Health Aging and Body Composition cohort; KORA: KORA ("Kooperative Gesundheitsforschung in der Region Augsburg" which translates as "Cooperative Health Research in the Region of Augsburg"); COGEND: Collaborative Genetic Study of Nicotine Dependence



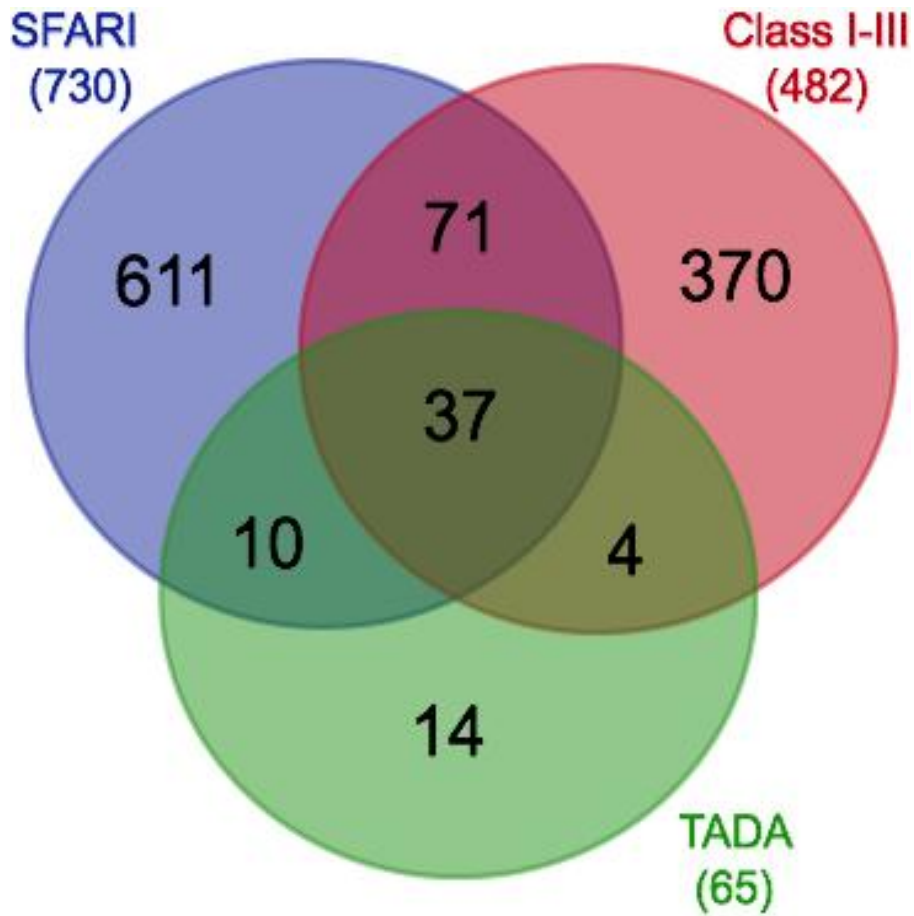
Supplementary Figure S4. Families with *CNTN5* and *CNTN6* coding SNVs from the PARIS cohort. The patients with ASD from the PARIS cohort (N=212) were investigated using Sanger sequencing. Coding-sequence variants are represented by dots (two dots = homozygous state). Black-filled symbols indicate a diagnosis of ASD with intellectual disability, while grey-filled symbols indicate a diagnosis of ASD without intellectual disability. In multiplex families, an arrow indicates the proband. Based on the ADI-R and clinical examination, normal (green) or abnormal (red) hypersensitivity to sounds and motor coordination ability are indicated by a schematic ear and hand, respectively. The absence of ear/hand means that information was not available. The scores for Social Responsiveness Scale are indicated when available. A total score of 76 or higher (red) is considered severe and strongly associated with clinical diagnosis of Autistic Disorder. Scores of 60 through 75 (Orange) are interpreted as indicating mild to moderate deficiencies in reciprocal social behavior. Scores of 59 and below (green) are considered to be within typical limits and generally not associated with clinically significant ASD. A question mark means that DNA was unavailable. The family AU-SWE-162 carried the *CNTN5*^{L790I} and the *CNTN6*^{R303Q}.



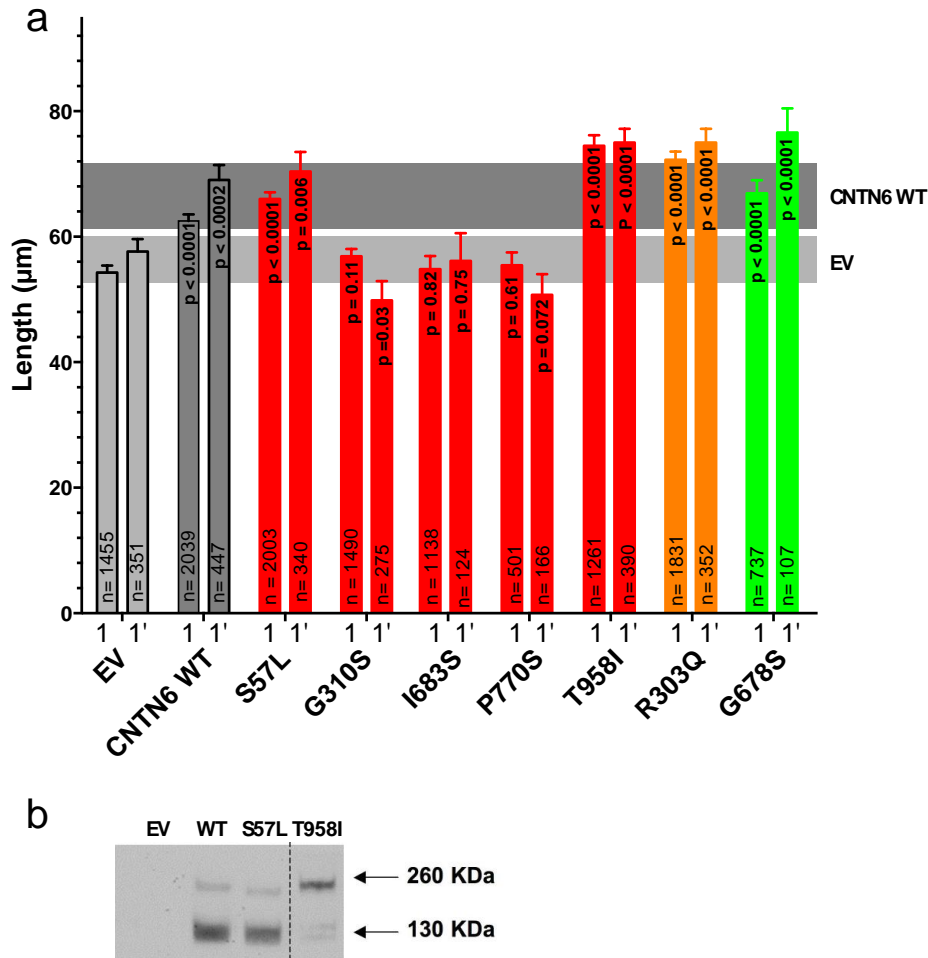
Supplementary Figure S5. Families with *CNTN5* and *CNTN6* coding SNVs from Canada. The cohort of patients with ASD from Canada (N=289 families) was investigated using whole genome sequencing. Coding-sequence variants are represented by dots. Black-filled symbols indicate a diagnosis of ASD with intellectual disability, while grey-filled symbols indicate a diagnosis of ASD without intellectual disability. For some individuals there was no information on IQ. Based on the ADI-R and clinical examination, normal (green) or abnormal (red) hypersensitivity to sounds is indicated by a schematic ear. The absence of ear means that information was not available.



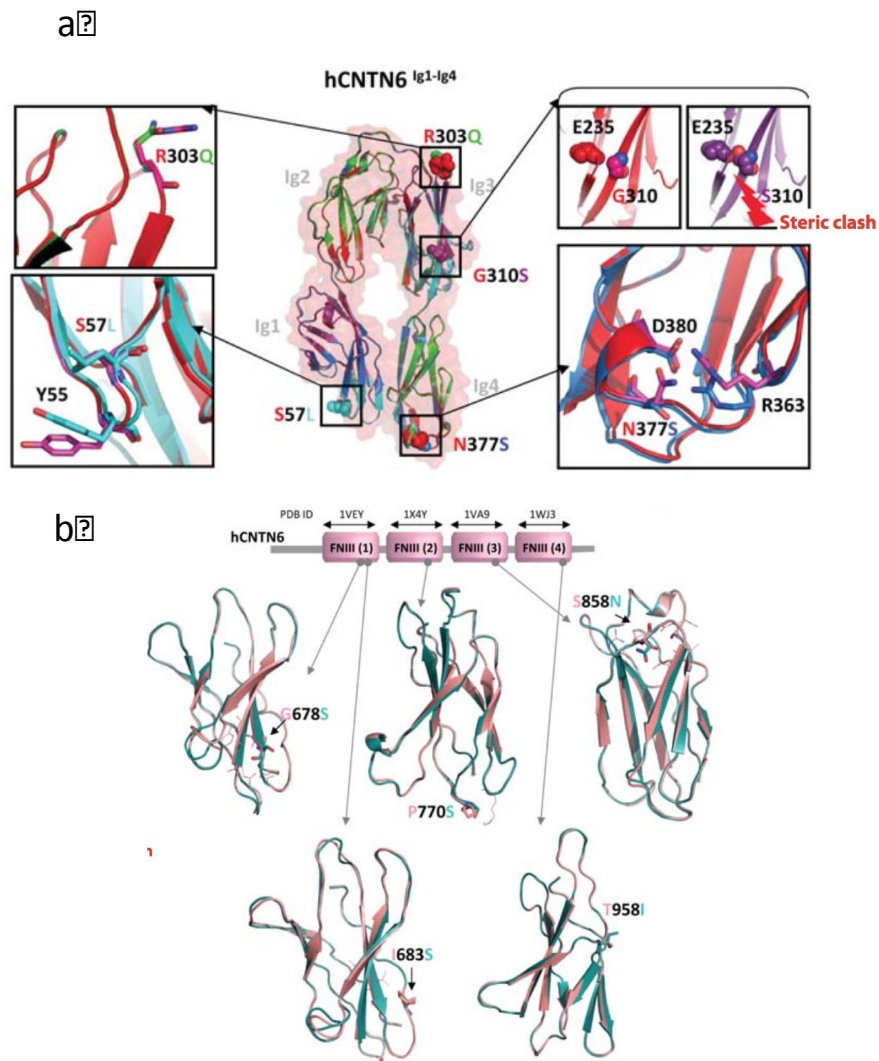
Supplementary Figure S6. Frequency of *CNTN6* private SNVs in this study, in Murdoch et al. PLOS Genetics (2015) and in the ExAC cohort. The SNVs with a single allele count in the ExAC cohort can be found at <http://exac.broadinstitute.org/>. Private mutations in this study and in Murdoch et al. (2015) met the following criteria: seen only once in either cases or controls exclusively, missense, nonsense, splice site, or start or stop codon disruptions with a frequency of less than 1% in the ExAC database.



Supplementary Figure S7. Venn diagram of the ASD-risk genes from different databases. The ASD genes were taken from the Class I-III genes of Yuen et al. (2015)¹, the TADA genes from Sanders et al. (2015)² or from the SFARI database (<https://gene.sfari.org/autdb/Welcome.do>). The complete list of genes is available as an excel file in the supplementary Table S10. The 37 genes present in the 3 databases are: *NRXN1*, *SHANK3*, *KDM5B*, *WDFY3*, *KDM6B*, *NCKAP1*, *ASH1L*, *PTEN*, *TCF7L2*, *DYRK1A*, *BCL11A*, *GRIN2B*, *SYNGAP1*, *ADNP*, *ANK2*, *ETFB*, *APH1A*, *CTTNBP2*, *CUL3*, *NAA15*, *KATNAL2*, *NLGN3*, *MIB1*, *SUV420H1*, *TBR1*, *SHANK2*, *MBD5*, *CHD8*, *ARID1B*, *POGZ*, *SETD5*, *TRIO*, *KMT2C*, *MYT1L*, *GABRB3*, *SCN2A*, *FOXP1*.



Supplementary Figure S8. Impact of the CNTN6 variants on neurite outgrowth. Cultures of cortical neurons were prepared using newborn rats (P0-P1) as described in Materials and Methods. **a.** The length of the longest neurite (mean +/- SEM) was measured in two independent experiments (1 and 1'). Neurons were co-cultured with HEK cells expressing the empty vector (EV), the wild-type CNTN6 (WT) or the CNTN6 carrying the variants identified in patients only (red), in patients and controls (orange) or in controls only (green). The intervals for the length of the longest neurite for experiments performed with the EV or the CNTN6 WT are indicated by the light and dark grey bars, respectively. The numbers of neurons analyzed are indicated in the graph. Statistical analyses were performed by using a F test to compare variances, followed by an impaired t-test with Welch correction. **b.** Representative western blots of selected co-culture supernatants for the detection of CNTN6 protein. No CNTN was detected with the empty vector (EV). Western blot of CNTN6 WT, CNTN6^{S57L} show a major band at the expected molecular weight of 130 KDa. For CNTN6^{T958I}, we observed a major band at 260KDa suggesting a dimeric form of the protein.



Supplementary Figure S9. Effect of CNTN6 variants on protein structure. (a) Structure of the immunoglobulin domains. Models of wild-type and mutated human CNTN6 Ig1-4 are superimposed, the variants are mapped in spheres. Close views highlight the impact of CNTN6^{G310S} on the structure. **(b)** Structure of the fibronectin domains. Each Protein Data Bank (PDB) ID used for the 3D-BLAST is indicated.

References

1. Yuen RK, Thiruvahindrapuram B, Merico D, Walker S, Tammimies K, Hoang N *et al.* Whole-genome sequencing of quartet families with autism spectrum disorder. *Nat Med* 2015; 21: 185-191.FORMAT
2. Sanders SJ, He X, Willsey AJ, Ercan-Sencicek AG, Samocha KE, Cicek AE *et al.* Insights into Autism Spectrum Disorder Genomic Architecture and Biology from 71 Risk Loci. *Neuron* 2015; 87: 1215-1233.FORMAT