Supplementary Table 1. *ASMT* primers used in this study

Exon	Exon size (bp)	PCR fragment size (bp)	Primers	Annealing (°C)
Sequencing				
1A	78	875	ASMT1AF: GAGCGATTCTTCTGCCTCAGC ASMT1AR: TCTGCGCACACTCCCAGGTG	67
1B/C	136 (coding 69)	491	ASMT1BF: GAGGCAGGAGAATCGCTTGAA ASMT1BR: CAACAATGGAACGTGAGTGTG	65
2	175	552	ASMT2F: TGGTGCAATCTCATTTGACTCTG ASMT2R: GGGTTCATGCCATTCTCCTG	58
3	130	950	ASMT3F: CAGCTGTACAAGGCAAGAGGA ASMT3R: CTTTCACCTCCTCCACTGCCA	55
4	69	283	ASMT4F: GCCTGGGCTACAGAGCTGAAA ASMT4R: CTCCTGGGTTGTGCCATTTG	55
5	119	331	ASMT5F: CCTGTGGGGTATAGCTCCGTTC ASMT5R: CGCACATGTCAAAGCATCAGA	64
6	84	342	ASMT6F: AGCTTGCAGTGAGCGGAAATC ASMT6R: GCACCCATCGACTCGTCATTT	64
7	141	352	ASMT7F: TGGGTTGGACCCTTCATGAGT ASMT7R: GTGTTTCCGGGAGTGAGAGGA	64
8	123	338	ASMT8F: AGCCTGGAAGACCTGGGAAAG ASMT8R: CCTGTGGGATGATTTCAGTGC	64
9	279 (coding 212)	506	ASMT9F: GGTGCCCTGACTGTCCTCTGA ASMT9R: CCATCAGCGTGGTCCTCAGTA	64
Expression				
E 4-6		223	ASMTsplice4F: CCAGTACCTGGAGACGTTTGG ASMTexp6R1: TCCTTGCGATAGTTTGCTGA	55

All PCRs were performed with Qiagen HotStarTaq at the following temperatures: 15 min at 95°C, 35 cycles of: 30 s at 95°C, 30 s at annealing temperature, 0.5-1 min at 72°C, followed by a final extension step of 10 min at 72°C. One 28 bp insertion/deletion located 194 bp upstream from exon 1A was used for the association study. bp, base pairs.

Supplementary Table 2. Stratification of the ASD and control populations used for the case control study.

Variations	Allele	ASD	Control	Chi2	df	p value
SNPs						
RS2289311	A	0.66	0.66	0.06	1	0.8
RS4782053	G	0.67	0.69	0.4	1	0.5
RS1921361	C	0.64	0.62	0.1	1	0.7
Alu						
Ya5NBC27	Empty	0.71	0.76	3.4	1	0.07
Ya5NBC51	Empty	0.57	0.58	0.07	1	0.8
YaNBC102	Empty	0.64	0.64	0.1	1	0.8
YaNBC109	Full	0.60	0.56	1.7	1	0.2
YbNBC65	Empty	0.51	0.54	0.8	1	0.4
Microsatellite[1]						
Androgen receptor	Multiple alleles			15	19	0.7
MtDNA[2]						
HVR1	Multiple Haplotypes			1.6	3	0.6

[1]Mothers of children with ASD and affected girls were compared to control females. [2]The test concerns the haplotype matching the control reference sequence and those containing the variations 16126, 16223 located in the hypervariable region 1 (HVR1) of the mtDNA. df, degrees of freedom.

Supplementary Table 3. Clinical features of patients with rare variants of the *ASMT* gene

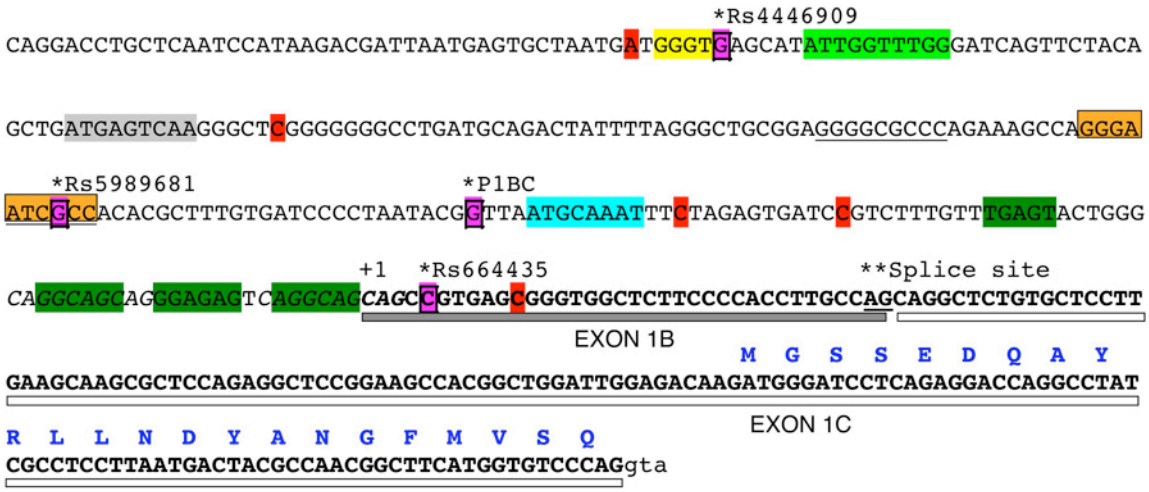
Family and patient	Location and nucleotide change	Protein change	ASMT activity deficit	Inheritance	Sex	Age at last evaluation (years)	Ethnic origin	Diagnosis	Cognitive level	Language	Attention/ Hyperactivity/ Sleep	Seizures	Family history
ASD 1													
Patient 1	IVS5+2T>C	Frameshift mutation	yes	Maternal	M	25	European	autism	mild MR	language delay (started to speak at 5y)	ADHD symptoms present until the age of 12 y. Abnormal sleep pattern, falls asleep very late, nocturnal awakenings.	—	The father has depression, the mother is healthy but she has a particular sleep organization, sleeping only when she's very tired, at any time, otherwise sleeping few hours.
ASD 2													
Patient 1	—	—	nd	—	M	12	North Africa	autism	mild MR	no language delay	Hyperactivity during childhood; no longer present at 12 y	—	Three affected children from two fathers; the mother and the father of the two younger sibs are healthy, no information about the father of the first child
Patient 2	IVS5+2T>C	Frameshift mutation	nd	Paternal	M	7	North Africa	autism	severe MR	non verbal	Severe hyperactivity during childhood; still pesent at 7 y	+	
Patient 3	IVS5+2T>C	Frameshift mutation	nd	Paternal	M	4	North Africa	autism	severe MR	non verbal	Hyperactive in the evening	+	
ASD 3													
Patient 1	exon 1B, C51A	N17K	nd	Maternal	M	9	China	autism + ADHD combined type	low normal	first words 14 m sentences 36 m	ADHD; no sleep problems	—	Only child. Negative family history.
ASD 4													
Patient 1	exon 2, A241G	K81E	nd	Maternal	M	8	African-American	autism	mild MR	first words 19 m , sentences 40 m	Slept very little during the first years but his sleep is now normal. Marked hyperactivity and difficulty maintaining attention	—	Father: negative medical and psychiatric history; mother: depressive episode during first pregancy
ASD 5													
Patient 1	exon 9, G917C	G306A	nd	Maternal	M	12	European	autism	mild MR	first words 14 m, sentences at 36 m, followed by language regression	Motor hyperactivity; very active during night	—	The parents have no medical or psychiatric history.
Patient 2	exon 9, G917C	G306A	nd	Maternal	M	9	European	autism	severe MR	language development stopped at 15 m; very limited language at 9 y	Motor hyperactivity	—	
ASD 6													
Patient1	exon 9, C976T	L326F	yes	Maternal	M	11	North Africa	autism	severe MR	first words 18 m, never acquired any sentences	Motor hyperactivity; normal sleep.	—	The parents have no medical or psychiatric history
ASD 7													
Patient 1	exon 9, C976T	L326F	nd	Maternal	M	6	European	Asperger + ADHD combined type	normal	first words 24 m, sentences 36 m	ADHD, no sleep problems.	—	The parents have no medical or psychiatric history

MR, mental retardation; nd, not determined

Supplementary Table 4. TDT of the polymorphisms located in *ASMT* promoter B in 278 families with ASD using FBAT and HBAT.

		Additive model						Dominant model					
	freq	fam#	S	E(S)	Var(S)	Z	P	fam#	S	E(S)	Var(S)	Z	P
SNPs													
rs4446909													
A	0.25	104	81	84	55	-0.4	0.68	104	66	68	35	-0.4	0.7
G	0.75	104	163	160	55	0.4	0.68	39	31	30	13	0.2	0.8
rs5989681													
C	0.3	110	93	96	62	-0.4	0.66	114	78	79	40	-0.12	0.9
G	0.7	110	171	167	62	0.4	0.66	55	46	43	15	0.7	0.48
P1BC													
A	0.12	67	38	45	29	-1.29	0.2	73	44	48	28	-0.8	0.42
G	0.88	67	128	121	29	1.29	0.2	12	13	10	1.3	2.4	**0.02**
rs6644635													
C	0.64	119	206	193	73	1.54	0.12	68	66	57	18	2	**0.04**
T	0.36	119	104	117	73	-1.54	0.12	128	90	95	50	-0.7	0.51
Haplotypes													
H1 GGGC	0.34	116	197	183	52	1.97	**0.05**	115	164	150	34	2.3	**0.02**
H2 GGGT	0.24	105	133	137	52	-0.47	0.63	105	121	120	44	0.2	0.9
H3 ACGC	0.23	102	114	115	50	-0.21	0.83	92	101	100	33	0.07	0.9
H4 GGAT	0.11	63	55	57	25	-0.42	0.67	62	54	56	23	-0.3	0.7
H5 GCGC	0.05	38	26	28	12	-0.69	0.49	38	26	28	12	-0.7	0.5

Freq, frequency; fam#, number of informative families; S, observed transmission; E(S), expected transmission; Var(S) Variance of the transmission observed. P values below 0.05 are indicated in bold.



Melke *et al.* Supplementary Figure 1

Supplementary Figure 1. Sequence and variations of promoter B of the *ASMT* gene. The SNPs used in the association analysis are indicated in pink. The two SNPs associated with low transcript levels are rs4446909 and rs5989681, 109 bp apart, in strong linkage disequilibrium (D'= 0.94). The SNP rs4446909 is located –207 bp from the transcription site, in a CCCAC box (yellow). Six nucleotides downstream, there is a CAAT/10 mer box (bright green) also present in promoter A. SNP rs5989681 is located 97 bp upstream from the transcription site (+1), in a putative binding site for the transcription factor NF-kappaB (orange). A putative binding site for AP1-TRE and a sequence homologous to that of *ASMT* promoter A are indicated in gray and dark green, respectively. A GC-rich region resembling an Sp1 site is underlined. Repeat sequences overlapping the transcription initiation site are shown in italics. The five variants indicated in red (-213 A/T, -160 C/T, -56 C/A, -45 C/T, +11 C/G) were observed in five patients, but not in controls (n=426). Two patients had the same combination of variations, –45 T and +11 G.