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ORIGINAL ARTICLE

Abnormal melatonin synthesis in autism spectrum disorders

J Melke[1], H Goubran Botros[1], P Chaste[1], C Betancur[2], G Nygren[3], H Anckarsäter[3,4], M Rastam[3], O Ståhlberg[3], IC Gillberg[3], R Delorme[1], N Chabane[5], M-C Mouren-Simeoni[5], F Fauchereau[1], CM Durand[1], F Chevalier[1], X Drouot[6], C Collet[7], J-M Launay[7], M Leboyer[2,8], C Gillberg[3,9], T Bourgeron[1,10] and the PARIS study[11]

[1]Human Genetics and Cognitive Functions, Institut Pasteur, Paris, France; [2]INSERM U513, Université Paris XII, Créteil, France; [3]Department of Child and Adolescent Psychiatry, Göteborg University, Göteborg, Sweden; [4]Institute of Clinical Sciences, Lund University, Malmö, Sweden; [5]Service de Psychopathologie de l'Enfant et de l'Adolescent, Hôpital Robert Debré, Assistance Publique-Hôpitaux de Paris, Paris, France; [6]Service de Physiologie-Explorations Fonctionnelles, Hôpital Henri Mondor, Créteil, France; [7]Service de Biochimie, IFR 139, Hôpital Lariboisiére, Assistance Publique-Hôpitaux de Paris, EA 3621, Faculté de Pharmacie, Paris, France; [8]Département de Psychiatrie, Hôpital Henri Mondor et Albert Chenevier, Assistance Publique-Hôpitaux de Paris, Créteil, France; [9]Saint George's Hospital Medical School, London, UK and [10]University Denis Diderot Paris 7, Paris, France

Melatonin is produced in the dark by the pineal gland and is a key regulator of circadian and seasonal rhythms. A low melatonin level has been reported in individuals with autism spectrum disorders (ASD), but the underlying cause of this deficit was unknown. The *ASMT* gene, encoding the last enzyme of melatonin synthesis, is located on the pseudo-autosomal region 1 of the sex chromosomes, deleted in several individuals with ASD. In this study, we sequenced all *ASMT* exons and promoters in individuals with ASD ($n=250$) and compared the allelic frequencies with controls ($n=255$). Non-conservative variations of *ASMT* were identified, including a splicing mutation present in two families with ASD, but not in controls. Two polymorphisms located in the promoter (rs4446909 and rs5989681) were more frequent in ASD compared to controls ($P=0.0006$) and were associated with a dramatic decrease in *ASMT* transcripts in blood cell lines ($P=2\times10^{-10}$). Biochemical analyses performed on blood platelets and/or cultured cells revealed a highly significant decrease in ASMT activity ($P=2\times10^{-12}$) and melatonin level ($P=3\times10^{-11}$) in individuals with ASD. These results indicate that a low melatonin level, caused by a primary deficit in ASMT activity, is a risk factor for ASD. They also support *ASMT* as a susceptibility gene for ASD and highlight the crucial role of melatonin in human cognition and behavior.
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Keywords: autism; melatonin; circadian rhythm; sleep; HIOMT; ASMT

Correspondence: Professor T Bourgeron, Human Genetics and Cognitive Functions, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France.
E-mail: thomasb@pasteur.fr

[11]Paris Autism Research International Sibpair Study: Sweden, Department of Child and Adolescent Psychiatry, Göteborg University, Göteborg; Christopher Gillberg, Maria Råstam, Carina Gillberg, Gudrun Nygren, Henrik Anckarsäter, Ola Ståhlberg, Catrin Håkansson. France, Department of Psychiatry, Hôpital Albert Chenevier et Henri Mondor, Créteil; Marion Leboyer, INSERM U513, Université Paris XII, Créteil: Catalina Betancur; Service de Psychopathologie de l'Enfant et l'Adolescent, Hôpital Robert Debré, Paris; Pauline Chaste, Richard Delorme, Nadia Chabane, Marie-Christine Mouren-Siméoni; INSERM U679, Hôpital Pitié-Salpêtriére, Paris; Alexis Brice. Norway, Center for Child and Adolescent Psychiatry, University of Oslo, Oslo; Eili Sponheim, Department of Pediatrics, Rikshospitalet, University of Oslo, Oslo; Ola H Skjeldal, USA, Department of Pediatrics, Georgetown University School of Medicine, Washington DC; Mary Coleman, Children's National Medical Center, George Washington University School of Medicine, Washington, DC; Philip L Pearl, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York; Ira L Cohen, John Tsiouris. Italy, Divisione di Neuropsichiatria Infantile, Azienda Ospedaliera Senese, Siena; Michele Zappella. Austria, Department of General Psychiatry, University Hospital, Vienna; Harald Aschauer. Belgium, Centre de Génétique Humaine, Institut de Pathologie et de Génétique, Gerpinnes, Loverval; Lionel Van Maldergem.

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Introduction

Melatonin is a powerful antioxidant molecule, involved in the regulation of circadian and seasonal rhythms and immune function.[1–4] It is released mainly by the pineal gland during the night and is produced by the conversion of serotonin to N-acetylserotonin by the rate-limiting enzyme AA-NAT (arylalkylamine N-acetyltransferase; EC 2.3.1.87), followed by the conversion of N-acetylserotonin to melatonin by ASMT (acetylserotonin methyltransferase; EC 2.1.1.4), also known as HIOMT (hydroxyindole O-methyltransferase).[5] Melatonin secretion is highly heritable in humans,[6] modulates neuronal plasticity[7–9] and regulates circadian gene expression.[10] It also plays a key role in communication behavior related to seasonal changes, such as song learning in birds.[11] Abnormal melatonin concentrations can have a dramatic effect on human behavior, as shown in patients with Smith–Magenis syndrome, who have an inverted melatonin circadian rhythm and display autistic behavior.[12] In autism spectrum disorders (ASD), low melatonin levels have been reported by three independent groups,[13–15] but the underlying cause of this deficit and its relationship to susceptibility to ASD was unknown. ASD affect at least 6/1000 individuals and are characterized by impairments in communication skills and social interaction, as well as restricted, repetitive and stereotyped patterns of behavior.[16–18] The genes responsible for ASD are largely unknown,[19,20] but cytogenetic abnormalities are observed in at least 3–5% of the affected individuals.[21] The PAR1 of the sex chromosomes, located at the tip of their short arms, has been found to be deleted in several individuals with ASD.[22] Among the 12 PAR1 genes referenced, *ASMT*[23] is an excellent candidate for susceptibility to ASD because it encodes the last enzyme in the melatonin biosynthesis pathway.[24] In this study, using a combination of genetics and functional experiments, we report evidence showing that a low melatonin concentration caused by a primary deficit in ASMT activity is a risk factor for ASD.

Materials and methods

Subjects

Families with ASD were recruited by the Paris Autism Research International Sibpair study at specialized clinical centers in seven countries (France, Sweden, Norway, Italy, Belgium, Austria and the United States). Diagnosis was based on clinical evaluation by experienced clinicians, DSM-IV criteria and the Autism Diagnostic Interview-Revised (ADI-R).[25] In Sweden, the Diagnostic Interview for Social and Communication Disorders (DISCO-10)[26] was used instead of the ADI-R in some cases. Patients with Asperger syndrome were evaluated with the Asperger Syndrome Diagnostic Interview.[27] Patients diagnosed with medical disorders, such as fragile X syndrome or chromosomal anomalies, were excluded from the study.

For mutation screening, the study sample ($n = 250$, 187 men and 63 women) was constituted of 250 independent families (71 subjects from multiplex families and 179 sporadic cases) and included 233 patients with autistic disorder and 11 with Asperger syndrome; six individuals narrowly missed the criteria for autistic disorder and were considered to have atypical autism (pervasive developmental disorder, PDD-NOS). There were 222 Caucasian, nine Black, three Asian, one Hispanic/Latin-American family and 15 families of mixed ethnicity. For association studies, the ASD sample consisted of 278 patients of Caucasian origin (201 men and 77 women) from 72 multiplex families and 206 sporadic cases. There were 258 patients with autism, 14 with Asperger syndrome and six with atypical autism. The control sample ($n = 255$) comprised 160 French and 95 Swedish individuals. An additional control group of 171 individuals from North Africa was screened for rare variants because one proband carrying the splice–site mutation (IVS5 + 2T > C) and one proband with the L326F variant had parents originating from this region.

Blood and platelet biochemical analyses were performed in ASD probands ($n = 43$; 14 female and 29 male patients, 14.8 ± 7 years old), their parents ($n = 34$; 18 female and 16 male patients: 44 ± 9 years old) and in controls matched for sex and age ($n = 75$; 30 female and 45 male patients; 27 ± 16 years old). The ASD patients were initially recruited for the analysis of serotonin levels and not chosen on the basis on their *ASMT* genotype. The controls were recruited at the Department of Orthopedics of the Lariboisière and Robert Debré hospitals in Paris. The control group for the biochemical analyses in B lymphoblastoid cell lines (BLCL) comprised 14 French individuals also used in the association study and 19 healthy relatives of patients with Hirschsprung syndrome or mitochondrial diseases. The local research ethics boards reviewed and approved the study. Informed consent was obtained from probands (if possible), parents and controls.

Cell culture, DNA and RNA isolation

BLCL were established from EBV-transformed lymphocytes and grown at 37°C in RPMI-1640 medium (Life Technologies Inc., Grand Island, NY, USA), supplemented with 10% undialyzed fetal calf serum, 2 mM glutamine, 2.5 mM sodium pyruvate, 100 mg/ml streptomycin and 100 IU/ml penicillin, under standard conditions. DNA was extracted by the phenol/chloroform method, and RNA was isolated using the NucleoSpin RNA II kit (Macherey–Nagel, Duren, Germany). DNA/RNA concentrations were determined by measuring absorbance at 260 nm on a biophotometer (Eppendorf, Hamburg, Germany). Human pineal gland cDNAs were obtained from the Incyte cDNA library # LHS1565 (BioCat, Heidelberg, Germany).

Mutation screening and genotyping
Genotyping for the association study and mutation screening were performed by direct sequencing or TaqMan technology. PCR products were sequenced with the BigDye Terminator Cycle Sequencing Kit (V3.1, Applied Biosystems, Foster City, CA, USA). Samples were then subjected to electrophoresis, using an ABI PRISM genetic analyzer (Applied Biosystems). Absence of genotyping errors was controlled by sequencing the PCR product with the opposite primer in a subset of patients. For primers and PCR conditions, see Supplementary Table 1.

Association and statistical analyses
The linkage disequilibrium (LD) map for *ASMT* was calculated using pairwise LD (D′) between the 13 *ASMT* variations in 533 individuals (278 ASD and 255 controls). The LD calculation and the case–control study were performed with Haploview software.[28] To detect population stratification bias, individuals with ASD and controls were screened for three single nucleotide polymorphisms (SNPs) (rs2289311, rs4782053, rs1921361), five ALU insertions (Ya5NBC27, Ya5NBC51, YaNBC102, YaNBC109, YbNBC65), and the mtDNA hypervariable region 1 (HVR1). In addition, all mothers from ASD patients were screened for the androgen receptor microsatellite. No significant genotype difference was observed for any of the markers tested (Supplementary Table 2). The transmission disequilibrium test (TDT) was performed using the family-based association test (FBAT)[29] and haplotype-based association test (HBAT)[30] using the empirical variance ('-e' option). For the TDT, only the four SNPs in promoter B were tested in 278 ASD families in which both parents had been genotyped. All SNPs were at Hardy–Weinberg equilibrium. We evaluated the distribution of the quantitative variables by the Kolmogorov–Smirnov test for Gaussian normality. Because the values for most of the samples were not normally distributed, we used the two-tailed non-parametric Mann–Whitney *U*-test to compare two groups and Spearman's ρ test to evaluate the correlation between ASMT activity and melatonin concentration. We used SPSS version 13 for these tests.

RT-PCR and quantitative RT-PCR
Oligo(dT)-primed cDNA was prepared from 5 μg of BLCL RNA, using Superscript II (Invitrogen, Grand Island, NY, USA), according to the manufacturer's instructions. The cDNAs were used directly in TaqMan assays, using the ABI PRISM 7500 Sequence Detection System (Applied Biosystems). Samples were run in duplicate or triplicate on 96-well optical PCR plates (ABgene, Surrey, UK). *ASMT* mRNA was quantified using commercially available assays. Two different assays were used, one covering the boundary between exon 1B and exon 2 (Hs00946625_m1), and the other covering the boundary between exon 8 and exon 9 (Hs00187839_m1). The two assays gave similar results and only the data obtained with Hs00946625_m1 are presented. Relative values of expression were determined for each sample, using the standard curve method (ABI user's manual), and these values were normalized to the threshold cycle (C_t) values of *GAPDH*, using the Hs99999905_m1 assay. For *ASMT* and *GAPDH*, the thresholds were set at 0.2 and 0.25, respectively, within the linear region of the semi-logarithmic plot in all assays (data not shown). The consequence of the splice–site mutation was investigated by sequencing the abnormal transcript after cloning the RT-PCR product.

Biochemical analyses
Blood samples were collected in the morning, between 0900 and 1100 h. The procedures for collecting and processing blood samples were designed to prevent release reactions. The anticoagulant was ACD-A (1 vol to 9 vol of whole blood). For the melatonin profile of family ASD 1, blood samples were collected every 2 h, from 1800 h to 1600 h the following day. Overnight, blood samples were collected in dimly lit conditions (<20 lux). Samples were drawn from an indwelling forearm catheter into Becton-Dickinson plastic tubes, centrifuged and frozen at 20°C. Platelets were counted with a Colter ZBI electronic counter. ASMT enzymatic activities were determined, at least in duplicate, by radioenzymology,[31] on the platelet pellet obtained by centrifugation of the platelet-rich plasma at 800 *g* for 20 min at room temperature and lysis with 100 hemolytic units of a purified SH-activated toxin (streptolysin O or alveolysin, generously provided by Professor J Alouf, Pasteur Institute, Paris). Melatonin content was measured in the resulting supernatant (that is, platelet-poor plasma or plasma) by HPLC, with random controls by mass spectrometry.[32]

Sleep analysis
The three individuals from family ASD 1 were taking no medication that could interfere with melatonin secretion. Sleep analysis was performed by standard polysomnography (two EEG, two electro-oculograms, one submental electromyogram, two anterior tibialis muscle electromyograms and respiratory signals, Embla N7000, Flaga, Iceland), 1 week before blood was collected for the melatonin assay.

Results

Rare ASMT variations in ASD
We investigated whether variations in *ASMT* were associated with ASD by directly sequencing all *ASMT* exons and the two promoters, A and B, in 250 affected individuals. Several *ASMT* variants were identified (Figure 1), including a splice–site mutation (IVS5 + 2T > C), four non-synonymous variations (N17K, K81E, G306A, L326F) and two synonymous variations (N167N, Q205Q). Two of these variations, N17K (rs17149149) and L326F, were also observed in the general population. N17K was found in one family with ASD from China (ASD 3) and is present

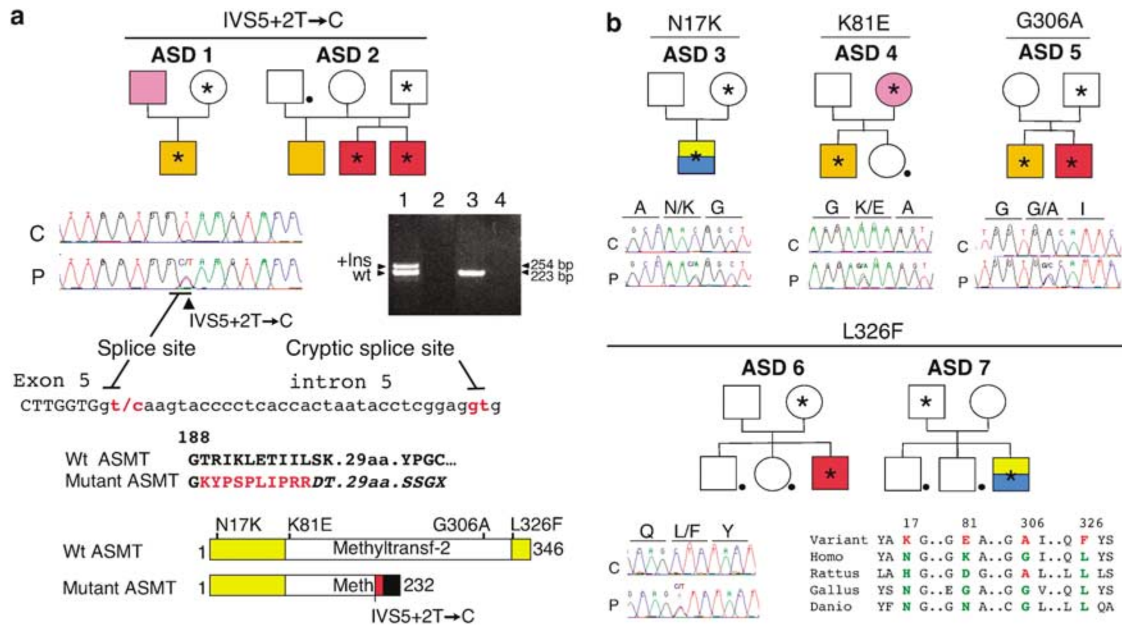

Figure 1 Non-synonymous *ASMT* variations in autism spectrum disorders (ASD) families. (**a**) Pedigree structure of the families carrying the splice–site mutation IVS5 + 2T > C; reverse transcriptase-polymerase chain reaction (RT-PCR) amplifying exons 4 to 6 of the *ASMT* cDNA from B lymphoblastoid cell lines of the ASD 1 proband carrying the splice–site mutation IVS5 + 2T > C (lane 1 +RT, lane 2 −RT) and a control (lane 3 +RT, lane 4 −RT). The insertion (+Ins) of 31 bp in the *ASMT* transcript originates from a cryptic donor splice–site downstream from exon 5. This insertion should lead to the additional sequence indicated in red and to a frame shift (characters in italics), causing premature truncation of the protein, lacking the methyl-transferase domain. Wt, wild-type. (**b**) Pedigree structure of the families carrying rare non-synonymous *ASMT* variations and conservation of the variant amino-acid in different species. Color codes in the pedigrees: autism with mild (orange) or severe (red) mental retardation, Asperger syndrome or high-functioning autism (yellow), attention-deficit/ hyperactivity disorder ADHD (light blue) and depression (pink). The proband ASD 3 fulfilled diagnostic criteria for both high functioning autism and ADHD. The proband ASD 7 fulfilled diagnostic criteria for both Asperger syndrome and ADHD. The asterisk and the dot indicate the presence of the mutation and the absence of a DNA sample for analysis, respectively.

in the SNP database at a frequency of 0.4–0.7% in the Han Chinese population. L326F was found in two ASD families (ASD 6 and ASD 7) and in 3 out of 426 controls (two from Sweden and one from North Africa). The splice–site mutation (IVS5 + 2T > C) was present in two ASD families (ASD 1 and ASD 2) (Figure 1a), but not in controls ($n = 426$). Using BLCL RNA from the ASD 1 proband, we detected abnormal *ASMT* transcripts, encoding a putative truncated ASMT protein lacking the methyl-transferase domain (Figure 1a). Biochemical analysis of the IVS5 + 2T > C and L326F variations indicated that these mutations were associated with very low levels of ASMT activity and melatonin (Figure 2a, b). We could not analyze the functional consequences of the remaining mutations due to a lack of blood samples or cell lines. Interestingly, the nine individuals with ASD carrying rare mutations were also hyperactive and several had sleep problems (see clinical description of the patients in Supplementary Table 3).

ASMT polymorphisms in ASD

We investigated whether frequent polymorphisms of the *ASMT* gene were associated with ASD by studying one insertion/deletion located in promoter A and 12 SNPs with a minor allele frequency greater than 5%, capturing most of the haplotype diversity (Figure 3a). ASD patients ($n = 278$) differed significantly from controls ($n = 255$) in terms of the allelic frequency of two SNPs – rs4446909 ($P = 0.006$) and rs5989681 ($P = 0.007$), located in promoter B (Figure 3b, Table 1 and Supplementary Figure 1). The H1 GGGC haplotype in promoter B was more frequent in ASD ($P = 0.002$) than in controls, whereas the haplotype H3 ACGC was more frequent in controls than in ASD ($P = 0.005$). We carried out a TDT using FBAT and HBAT on 278 families (Supplementary Table 4), and observed an overtransmission of haplotype H1 GGGC to probands (additive model $P = 0.05$; dominant model $P = 0.02$) and overtransmission of alleles G and C of the SNPs P1BC (dominant model $P = 0.02$) and rs6644635 (dominant model $P = 0.04$), respectively.

We then explored the relationship between these frequent variations of the *ASMT* gene and *ASMT* expression. Promoter A activity is restricted to the retina,[24] whereas promoter B is active in BLCL and in the pineal gland. In view of the results of quantitative RT-PCR, *ASMT* transcript level was found to be significantly associated with the two SNPs linked to

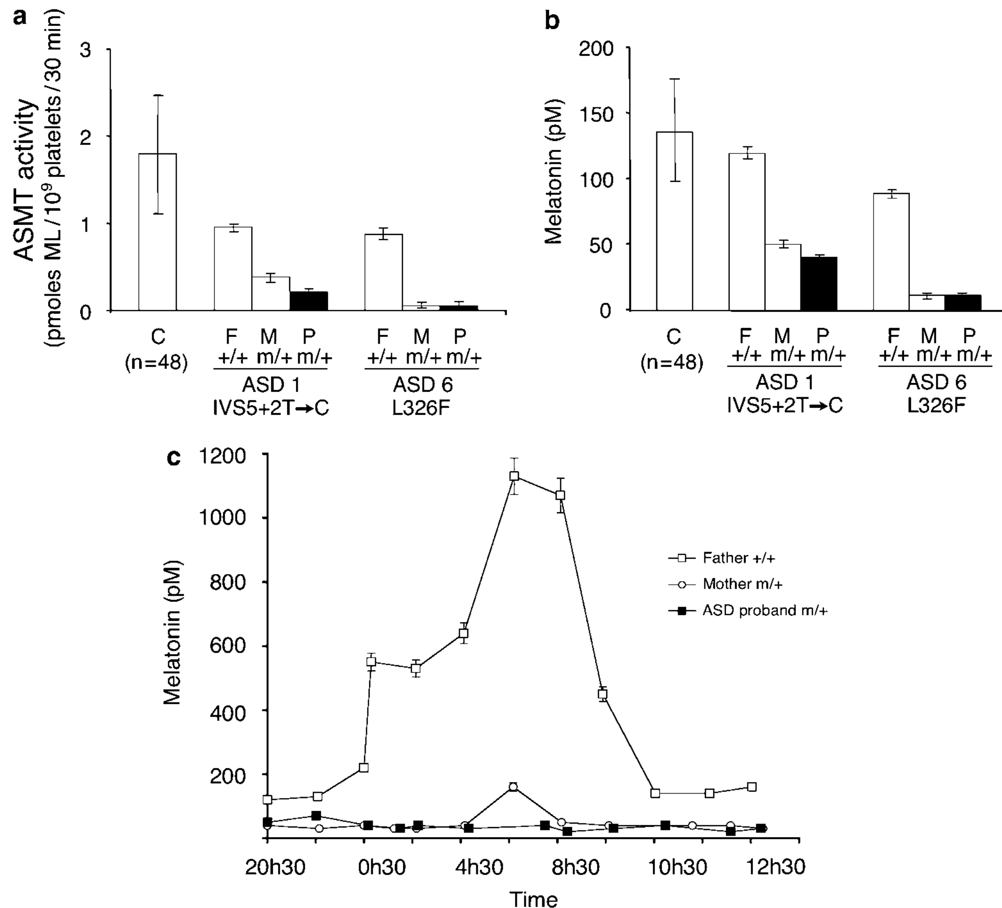

Figure 2 Impact of the ASMT mutations on enzyme activity and melatonin concentration. (**a**) ASMT activity, measured in platelets of the members of families autism spectrum disorder (ASD) 1 and ASD 6 carrying the splice–site IVS5 + 2T > C and the L326F *ASMT* mutations, respectively. (**b**) Blood melatonin concentration in the same individuals. (**c**) Nocturnal melatonin profile of family ASD 1. The proband (male, 24 years old) and his mother (53 years old) are heterozygous (m/ +) for the splice–site mutation IVS5 + 2T > C. The proband's father (55 years old) has no *ASMT* mutation (+ / +). Error bars represent s.d. C: controls; F: father; M: mother; P: proband.

ASD – rs4446909 and rs5989681 (Figure 3c). Interestingly, the G alleles of both SNPs were more frequent in ASD patients and were associated with a decrease in *ASMT* transcript levels by a factor of 4 to 20, respectively (rs4446909, $P = 2 \times 10^{-8}$ and rs5989681, $P = 2 \times 10^{-10}$).

Serotonin, ASMT activity and melatonin concentration in ASD

We then measured the serotonin concentration, the ASMT activity and melatonin concentration in blood platelets from 43 ASD patients, 34 parents of ASD patients and 48 control individuals. Consistent with previous studies,[33] the serotonin level was significantly higher in individuals with ASD ($P = 2 \times 10^{-11}$) and their parents ($P = 10^{-8}$) than in controls (Figure 4a). In contrast, the ASMT activity levels were significantly lower in individuals with ASD ($P = 2 \times 10^{-12}$) and their parents ($P = 10^{-5}$) than in controls (Figure 4b). This deficit in ASMT activity was accompanied by a lower plasma melatonin concentration in patients with ASD ($P = 3 \times 10^{-11}$) and their parents ($P = 9 \times 10^{-5}$) than in controls (Figure 4c). Platelet ASMT activity and plasma melatonin levels were not correlated in controls (Figure 4d), whereas they were strongly correlated in patients with ASD ($P = 0.83$; $P = 10^{-6}$). Thus, the decreased ASMT activity in ASD patients acted as a limiting factor for the production of melatonin. We investigated this ASMT deficiency further by analyzing BLCLs from 53 individuals with ASD (for 15 of whom blood samples had already been tested) and 33 new independent controls (Figure 4e). We found that ASD patients had lower levels of ASMT activity than controls ($P = 7 \times 10^{-8}$), as shown previously with platelets. These results obtained with cultured cells replicate our previous finding and exclude possible effects of environmental factors or regulation acting at a higher physiological level. We found no significant correlation between the severity of the deficit and clinical phenotype (IQ, language level or ADI-R scores in the three major domains of impairment: Reciprocal

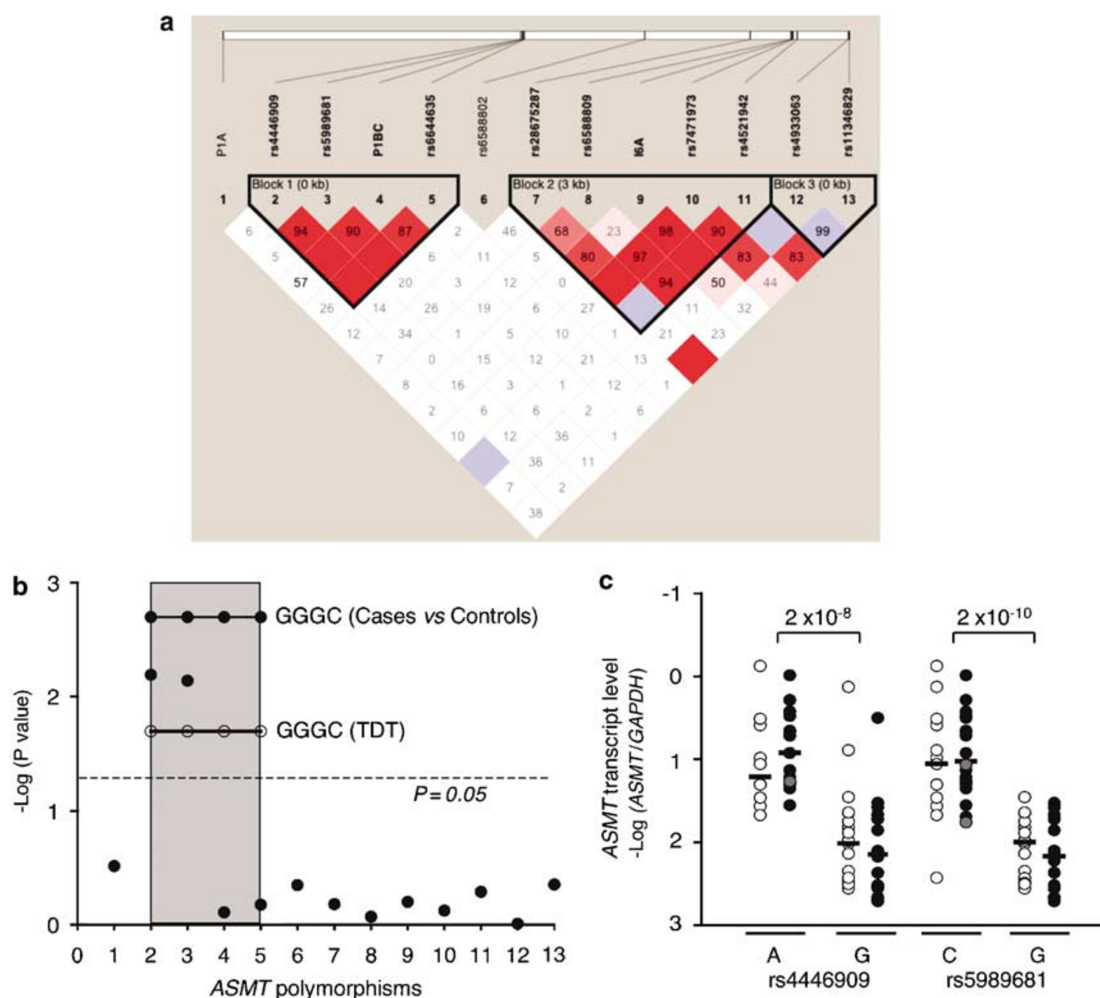

Figure 3 Association studies and transcript analyses of the *ASMT* gene. (**a**) Haplotype block structure of the *ASMT* gene. The relative physical position of each single nucleotide polymorphism (SNP) is given in the upper diagram, and the pairwise LD (D′) between all SNPs is given below each SNP combination. (**b**) Plot of the case–control *P*-values (−log10) for all variations studied within *ASMT*. 1: E1A; 2: rs4446909; 3: rs5989681; 4: P1BC; 5:rs6644635; 6: rs6588802; 7: rs28675287; 8: rs6588809; 9: I6A; 10: rs7471973; 11: rs5431942; 12: rs4933063; 13: rs11346829. SNPs located in promoter B are included in the shaded box. *P*-values for the risk haplotype GGGC are indicated as straight lines with close (cases vs controls) or open circles (transmission disequilibrium test (TDT)). (**c**) Quantification of *ASMT* transcripts relative to rs4446909 and rs5989681 genotypes (A represents the individuals with an A/A or A/G genotype; C represents the individuals with C/C or C/G genotype; G represents the individuals homozygous G/G). Black and white circles indicate individuals with autism spectrum disorders (ASD) and controls, respectively. The gray symbols indicate individuals homozygous A/A and C/C for rs4446909 and rs5989681, respectively. Real-time reverse transcriptase (RT)-PCR was performed with B lymphoblastoid cell lines from 38 ASD probands and 29 controls. Horizontal bars indicate medians. No statistical difference was observed between ASD and controls (Mann–Whitney *U*-test).

Social Interaction, Communication and Repetitive Behaviors and Stereotyped Patterns) (data not shown).

Melatonin cycle and sleep pattern in family ASD 1

Finally, we investigated circadian melatonin synthesis *in vivo* and sleep patterns in family ASD 1, in which the unaffected mother and the son with ASD carry the splice–site mutation. Neither of the individuals carrying the *ASMT* mutation showed the normal increase in melatonin during the night (Figure 2c). They displayed modest sleep abnormalities, with poor sleep efficiency (proband 70 and mother 82%; controls >85%) and a moderately high arousal index (22 and 17/h; controls <10/h), but had normal proportions of rapid eye movement sleep (26 and 24%; control range 15–30%) and a normal amount of slow wave sleep (61 and 104 min; control range: 60–120 min).

Discussion

Abnormal melatonin concentration was previously observed in individuals with ASD by three indepen-

Table 1 Frequencies of the polymorphisms located in *ASMT* promoter B in ASD patients and controls

	ASD (n = *278*)	*Controls* (n = *255*)
SNPs		
rs4446909		
f(G)	0.77	0.70
P-value (P_c-value[a])	**0.006** (0.10)	
OR (95% CI)[b]	**1.5 (1.1–2)**	
rs5989681		
f(G)	0.73	0.65
P-value (P_c-value)	**0.007** (0.12)	
OR (95% CI)	**1.4 (1.1–2)**	
P1BC		
f(G)	0.90	0.90
P-value	0.78	
rs6644635		
f(C)	0.65	0.63
P-value	0.66	
Haplotypes[c]		
H1 GGGC	0.36	0.27
P-value (P_c-value)	**0.002 (0.04)**	
H2 GGGT	0.26	0.28
P-value	0.54	
H3 ACGC	0.21	0.29
P-value (P_c-value)	**0.005** (0.08)	
H4 GGAT	0.097	0.09
P-value	0.78	
H5 GCGC	0.055	0.06
P-value	0.88	

Abbreviations: ASD, autism spectrum disorders; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.
[a]P_c-value: Significance levels corrected for multiple comparisons using a stepdown permutation procedure (comprising 100 000 permutations).
[b]Odds ratio: major allele vs minor allele.
[c]Haplotype using rs4446909, rs5989681, P1BC and rs6644635.
Results with significance <0.05 are indicated in bold.

dent groups using different methodological approaches.[13–15] Our results confirm that low plasma melatonin concentration (half the mean of the control values) is a frequent trait in ASD patients, as observed in 65% of the patients tested, a proportion very similar (63%) to that previously reported by Tordjman *et al.*[15] We show for the first time that abnormal melatonin levels are also present in the unaffected parents of ASD patients, suggesting a genetic origin. Indeed, the melatonin deficit observed in the patients was associated with low ASMT activity, suggesting that variations in the *ASMT* gene could be the cause of this deficit. This hypothesis was supported by the identification of genetic variations, which probably contribute to the enzymatic deficit by decreasing transcript levels, or altering the sequence of the ASMT protein. However, other unidentified genetic or epigenetic factors are contributing to the ASMT deficit since non-conservative mutations were observed only in a limited number of patients and the genetic association with the polymorphisms located in the *ASMT* promoter does not solely contribute to the enzymatic deficiency. Furthermore, we found unaffected relatives and controls with *ASMT* mutations and/or low melatonin concentration in the blood. Therefore, low ASMT activity cannot be considered as a direct cause of ASD, but as a susceptibility factor for this condition (less than 100 pmol melatonin/10^9 platelets/30 min; odds ratio: 77; 95% confidence interval: $19 < OR < 320$).

Individuals with ASD frequently show irregularities in the circadian sleep–wake cycle[34–36] and some show a free-running pattern, which is suppressed by melatonin treatment.[37] In view of these clinical observations, it was postulated that one alteration at the origin of autism may occur as the child is entering into the day–night cycle.[38] In agreement with this hypothesis, the deficit in melatonin may cause abnormal sleep–wake cycle in affected individuals. Additionally, since melatonin influences synaptic plasticity,[5,7–9,11,39,40] a deficit in this molecule may also weaken neuronal networks, thereby increasing the effect of other pathological processes, such as abnormal synaptogenesis.[41–44]

Taken together, these findings indicate that a subgroup of individuals with ASD and low melatonin levels could benefit from the use of melatonin as a therapeutic compound. Melatonin treatment seems to help patients with ASD to fall asleep and to sleep through the night,[37,45–48] but it remains unknown if melatonin could have a more beneficial effect if given before 3 years of age. Further studies are required to determine the role of the melatonin deficit in the affected individuals, and more generally of circadian and seasonal rhythms, in the susceptibility to neuropsychiatric disorders.

Acknowledgments

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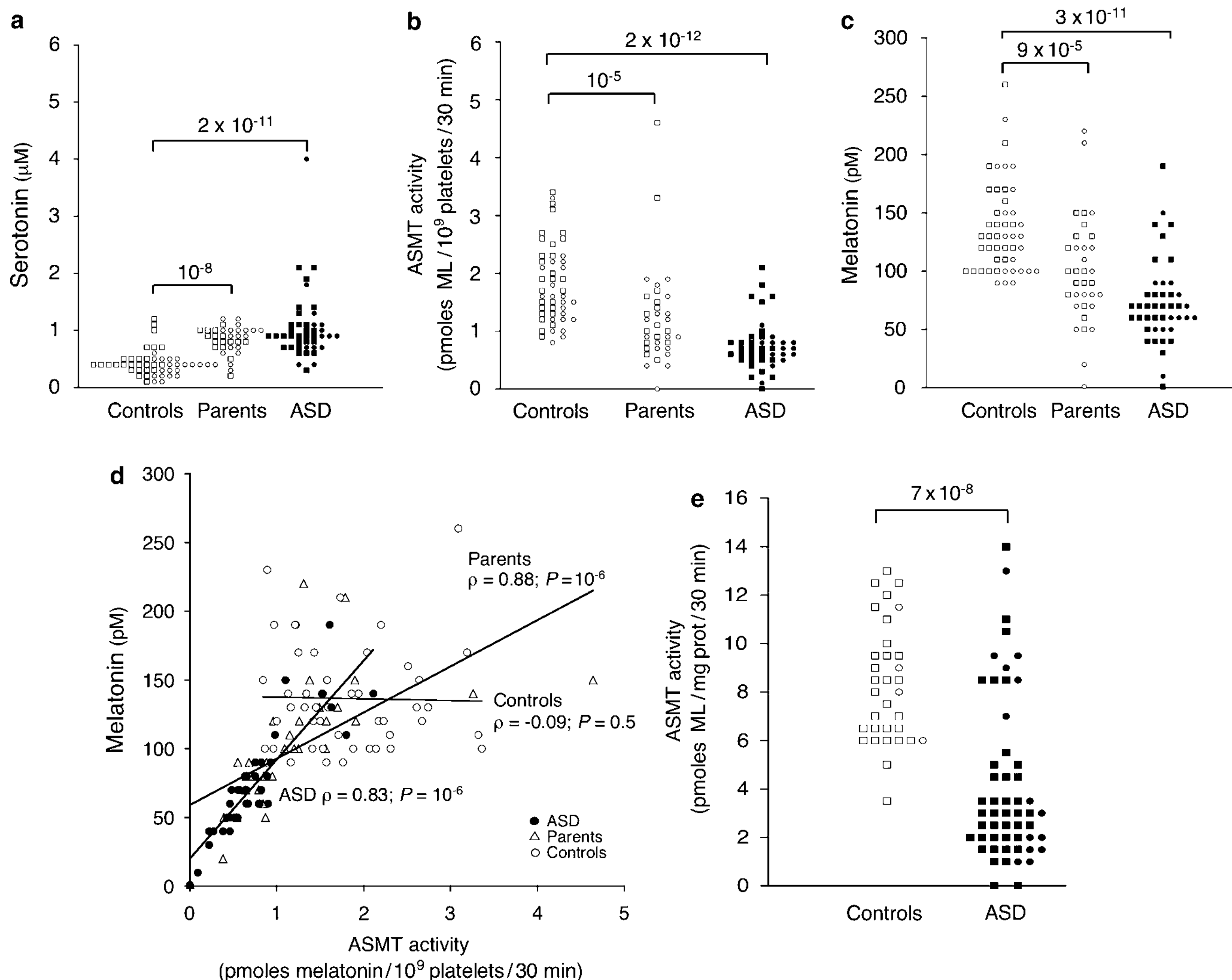

Figure 4 ASMT activity and melatonin concentration in patients, parents and controls. (**a**) Serotonin levels, measured in the platelets of 43 ASD patients (mean ± s.d.; $1 \pm 0.6\,\mu$M), 34 parents ($0.8 \pm 0.2\,\mu$M) and 48 controls ($0.4 \pm 0.2\,\mu$M). (**b**) ASMT activity, measured in the platelets of 43 ASD patients (0.73 ± 0.43 pmol/10^9 platelets/30 min), 34 parents (1.20 ± 0.85 pmol/10^9 platelets/30 min) and 48 controls (1.81 ± 0.68 pmol/10^9 platelets/30 min). (**c**) Plasma melatonin concentration in 43 autism spectrum disorders (ASD) patients (73 ± 36 pmol), 34 parents (99 ± 46 pmol) and 48 controls (136 ± 39 pmol). (**d**) Blood melatonin concentration expressed with respect to ASMT activity in platelets. ASMT activity and melatonin concentration were not correlated in controls (white circles). In contrast, in ASD patients (black circles), and their parents (white triangles), a significant correlation was observed. Spearman's rho test was used to evaluate the correlation between ASMT activity and melatonin concentration. (**e**) ASMT activity in B lymphoblastoid cell lines from 53 ASD patients (3.9 ± 3.4) and 33 controls (8.3 ± 2.4). Circles and squares indicate female and male subjects, respectively. Statistical significance was assessed with the Mann–Whitney *U*-test.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)