

ARTICLE

20 ans après: a second mutation in MAOA identified by targeted high-throughput sequencing in a family with altered behavior and cognition

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Intellectual disability (ID) is characterized by an extraordinary genetic heterogeneity, with > 250 genes that have been implicated in monogenic forms of ID. Because this complexity precluded systematic testing for mutations and because clinical features are often non-specific, for some of these genes only few cases or families have been unambiguously documented. It is the case of the X-linked gene encoding monoamine oxidase A (*MAOA*), for which only one nonsense mutation has been identified in Brunner syndrome, characterized in a single family by mild non-dysmorphic ID and impulsive, violent and aggressive behaviors. We have performed targeted high-throughput sequencing of 220 genes, including *MAOA*, in patients with undiagnosed ID. We identified a c.797_798delinsTT (p.C266F) missense mutation in *MAOA* in a boy with autism spectrum disorder, attention deficit and autoaggressive behavior. Two maternal uncles carry the mutation and have severe ID, with a history of maltreatment in early childhood. This novel missense mutation decreases *MAOA* enzymatic activity, leading to abnormal levels of urinary monoamines. The identification of this new point mutation confirms, for the first time since 1993, the monogenic implication of the *MAOA* gene in ID of various degrees, autism and behavioral disturbances. The variable expressivity of the mutation observed in male patients of this family may involve gene–environment interactions, and the identification of a perturbation in monoamine metabolism should be taken into account when prescribing psychoactive drugs in such patients.

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INTRODUCTION

The *monoamine oxidase A* (*MAOA*) gene and its close homolog *MAOB* are located at Xp11.3 and encode enzymes crucial for the metabolic degradation of biogenic amines, and particularly neurotransmitters such as norepinephrine, dopamine and serotonin. The two enzymes share 70% of amino-acid sequence identity but differ by their expression, substrate affinities and inhibitor specificities. *MAOA* is mainly involved in endogenous bioamine (metanephrine (MN), normetanephrine (NMN) and serotonin (5-hydroxytryptamine or 5-HT)) degradation, whereas *MAOB* preferentially metabolizes exogenous bioamines such as phenylethylamine, and both enzymes are active on dopamine. Combined loss of *MAOA* and *MAOB* genes has been described in some patients with a continuous syndrome also including a deletion of the Norrie disease gene. These patients present, in addition to Norrie disease symptoms, with severe intellectual disability (ID), autistic-like behavior and seizures.¹ Severe developmental delay and hypotonia was more recently observed in

a few patients with only *MAOA* and *MAOB* deletion.^{2–4} In 1993, Brunner *et al*⁵ described a large Dutch family with X-linked borderline ID and prominent behavioral abnormalities. The linkage study and the biochemical analyses suggested that *MAOA* could be responsible for this syndrome. This was confirmed by sequencing which revealed a nonsense c.886C>T (p.Q296*) mutation.⁶ All the affected male patients in this family carried the mutation and showed, in addition to borderline ID, very characteristic abnormal behavior, in particular impaired impulse control and stress-induced aggressive and violent behavior. Shortly thereafter, aggressive behavior was described in a mouse line with an inactivated *Maoa* gene,⁷ and this was later confirmed in a different mouse line carrying a spontaneous mutation mimicking the human mutation.^{7–9} *Maoa*-deficient mice also present autistic-like features.^{7–9} Despite the extensive attention given to these early reports, and early attempts at replication, by screening for *MAOA* deficiency in cohorts of patients with ID and/or abnormal behavior,¹⁰ no other clearly pathogenic mutation in *MAOA*

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was reported to our knowledge in other patients in the past 20 years,¹¹ with the possible exception of a missense variant predicted to be damaging reported in a single patient with autism spectrum disorder (ASD).¹² Many association studies investigated the potential role of MAOA in risk of abnormal behaviors, focusing on a 'variable number of tandem repeats' (VNTR) polymorphism¹³ located in the MAOA promoter region, whose alleles are associated with variations of transcriptional activity, with a 'low' and a 'high' activity frequent alleles. Notably, Caspi *et al*¹⁴ reported that maltreated children with a genotype conferring high levels of MAOA expression were less likely to develop antisocial problems. This VNTR has also been reported to be a modifier of ASD severity, with lower intelligence quotient (IQ) and more severe behavioral problems observed in patients with the 'low activity allele'.^{15,16}

Since the original publication,⁶ MAOA is considered as an ID gene^{17,18} and is included in the diagnostic panels of genes screened for X-linked ID mutations. Here we report, for the first time since 1993, a novel pathogenic mutation of the MAOA gene segregating in a small family with three affected male patients showing various degrees of cognitive impairment and behavioral disturbances evocative of Brunner syndrome.

PATIENTS AND METHODS

Targeted HTS

DNAs from a cohort of 50 patients with ID patients (with normal karyotype and negative results from CGH array, Fragile X and ARX expansions testing) were prepared as described elsewhere.¹⁹ They were enriched in coding sequence of 220 genes known to cause ID, including MAOA, by a target custom capture (SureSelect, Agilent, Santa Clara, CA, USA). These enriched libraries were tagged and pooled by 12 in one lane of a new generation sequencer (HiSeq2000, Illumina, San Diego, CA, USA) for a 100 bp paired-end run. Read mapping and variant calling were performed following standard procedures, and variants were filtered using VaRank, an in-house software which collects variant-specific information to rank them according to their predicted pathogenicity.^{19,20}

Bioinformatic analyses

The potential functional effects of the amino-acid change on the protein has been assessed using several bioinformatic programs including SIFT,²¹ PolyPhen2,²² Mutation Taster²³ and KD4v.²⁴ Possible effects on splicing were determined by MaxEnt,²⁵ NNSplice,²⁶ GeneSplicer²⁷ or Human Splicing Finder²⁸ programs via Alamut version 2.2 (Interactive Biosoftware, Rouen, France). On the basis of the published 3D structure (2z5y), a 3D model structure of the mutated C266F MAOA was computed using the KD4v webserver. The Exome Variant Server (EVS) and dbSNP have been used to test the presence of the variation in the general population.

Sanger sequencing, RT-PCR analysis, VNTR genotyping and X-inactivation assay

Sanger sequencing was used to confirm the presence of the mutation in the proband and in the different family members and to sequence coding regions of MAOB (primers available on request). Patient III-1 mRNA was extracted from blood (PAXgene Blood RNA System, Preanalytix, Hombrechtikon, Switzerland) and was studied by RT-PCR using specific primers (5'-GTGGCCAGGAACGGAAGTTTGTA-3' and 5'-CGGGCAAGAAATGAAGCC-CATGAT-3'). VNTR genotyping of patient III-1 was performed on genomic DNA using standard primers as previously described.²⁹ The X chromosome inactivation assay was performed on proband's mother genomic DNA extracted from peripheral blood, as described elsewhere.³⁰

Bioamine and MAOA assays

Levels of catecholamine catabolites (MN, NMN, dihydroxyphenylglycol (DHPG), homovanillic acid (HVA) and vanillylmandelic acid (VMA))

were measured by HPLC with electrochemical detection.³¹ Plasma 5-hydroxyindoleacetic acid (5-HIAA) was measured by HPLC with fluorimetric detection.³² MAO (EC.1.4.3.4.)-A enzymatic activity was determined on human fibroblasts whole cell homogenates by a radioenzymatic assay using [¹⁴C]-5-HT creatinine sulfate (1.96 GBq/mmol, Amersham GE Healthcare, Little Chalfont, UK, final concentration 20 μM) as substrate according to Denney *et al*.³³ MAOA protein concentration was assessed by measuring the binding of [³H]-Ro 41-1049 (0.31 TBq/mmol, Amersham GE Healthcare), a reversible inhibitor of MAO-A, to fibroblast membranes, as described by Cesura *et al*.³⁴

Patient's evaluations

Patient 1, III-1 was evaluated using WISC IV (Wechsler Intelligence Scale for Children) and ADOS³⁵ scales. Considering the severity of their clinical manifestations, patients 2 and 3 (II-3 and II-4) could only be evaluated using Vineland scales.³⁶

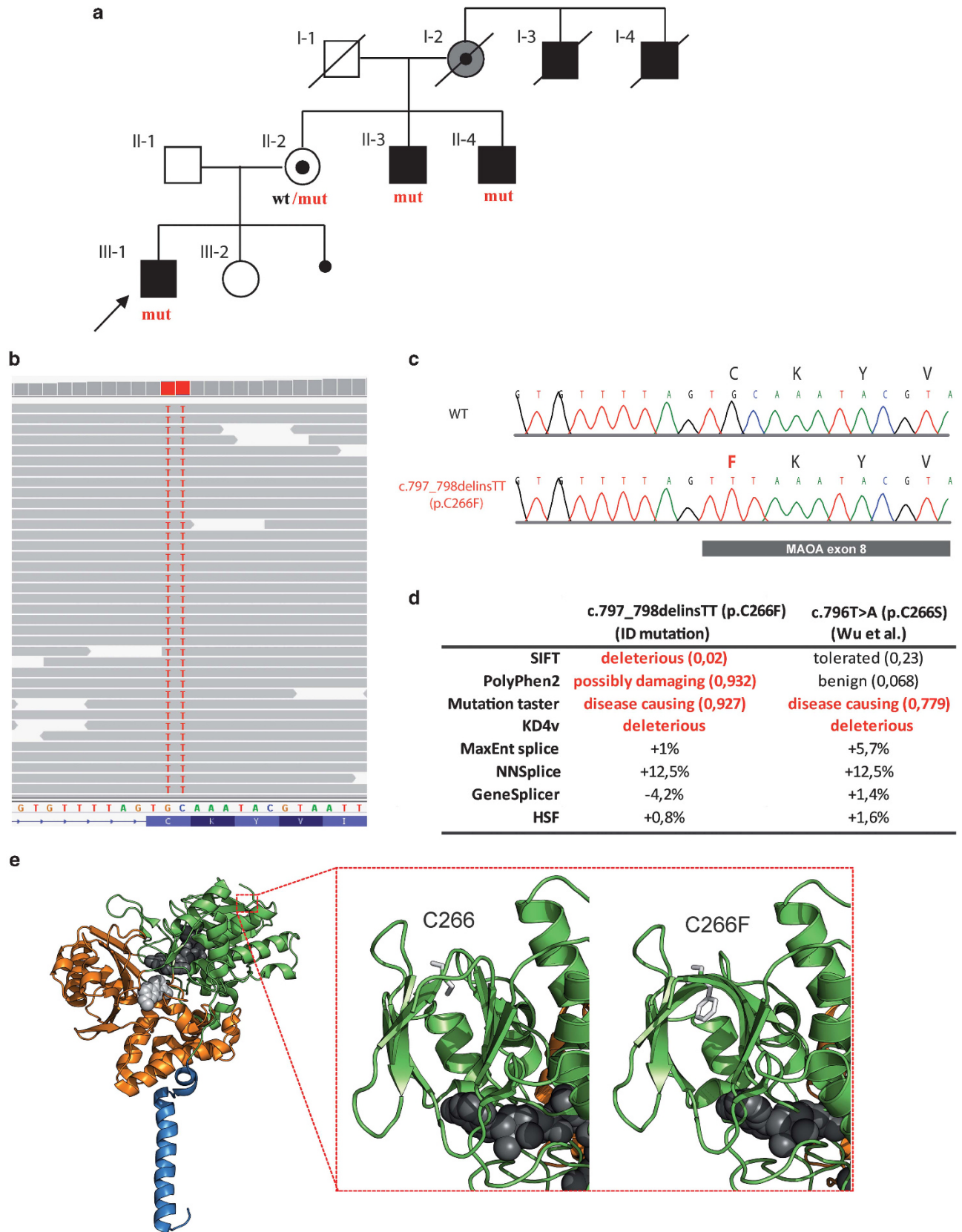
RESULTS

Clinical description of the family

The index case III-1 was referred at the age of 7 years for investigation of suggested ID with prominent behavioral disturbances. Family history suggested a possible X-linked inheritance, although the phenotype of two maternal uncles appeared much more severe (Figure 1). Later investigations indicated that the phenotype of II-3 and II-4 was complicated by familial neglect, maltreatment and sexual abuse during childhood with parental psychiatric disturbances and substance abuse, leading to their placement at the respective ages of 7 and 5 years.

Patient III-1. The index case is the first child of non-consanguineous healthy young parents. The patient has a healthy younger sister. Labor was induced at 35+3 weeks of gestation because of maternal diabetes. Apgar was 10–10 and birth measurements were normal: weight 3010 g, length 49 cm, occipital frontal circumference (OFC) 34 cm (50th percentile). Feeding difficulties were noticed at the time of food diversification with high selection of food. Sleep disorder was reported in the first year of life including difficulty in falling asleep, night terrors and frequent awakenings. Gross psychomotor acquisitions were in normal range but the infant was described as passive with low interactive skills. Sensory deficits were ruled out. Walk was acquired at 18 months and speech at 15 months. He was toilet trained at 3 years of age. He went to standard school but attended at the same time twice-weekly a treatment center for individual and group therapy, psychomotricity and school remediation. At the age of 6 years, he entered primary school with a classroom assistant. He appreciated activities such as music listening or memorizing tasks. The parents reported that he did not appreciate danger, experimenting notably with fire and autoaggressive actions (shaving his head and manipulation of household products). No treatment was introduced.

When referred to the genetics clinic at the age of 7 years, reading and writing were in a learning process. Global clinical examination was normal except few hand stereotypies and behavioral abnormalities. No specific cranio-facial dysmorphism was noticed. Both gross psychomotor skills (climb stairs and bicycle) and fine psychomotor skills (dressing and putting on shoes) were delayed. Behavioral troubles during the interview included an amimic facial expression leading to a bizarre contact, oral and motor perseverations, ideomotor slowness, auto-mutilation and angers when frustrated or failing an exercise. He had restricted patterns of interests such as recurrent questions about water cycle or electrical circuit. The IQ was not calculable but a diagnosis of ASD was confirmed using ADOS scale (Table 1 and Supplementary data).



Patient II-3. Patient II-3 was born premature (1650 g) leading to hospitalization during his first 8 months, but no complication was reported. Psychomotor development was delayed with sitting acquired at 16 months and walking at the age of 2 years. A diagnosis of autism was proposed at the age of 3 years. He had invasive behavioral troubles that included auto and hetero aggressive bursts and very low interactive skills. Speech remained restricted to simple sentences with limited vocabulary. He started attending a school for special needs at the age of 5. Behavior worsened at the age of 6 years. Psychotropic and sedative drugs were early introduced, and stabilized the behavioral aggravation. Intellect was not tested but was severely impaired. He was evaluated at 38 years of age and measurements were as follows: weight, 72 kg; height, 1.58 m (body mass index (BMI) = 28.8); and OFC, 54 cm (-2 SD). Clinical examination revealed no extrapyramidal sign but dystonic movements of the head and hands that were attributed to secondary effect of the treatment. The patient could neither read nor write, and he was not autonomous for daily living. Vineland Adaptative Behaviors Scale (VABS) for social worker aid was evaluated by semi-structured interview and indicated very impaired autonomy, socialization, communication and locomotion, with equivalent developmental ages between 2 and 5 years (Table 2). The clinical observation was in favor of a neurodevelopmental psychomotor and socio-emotional very early stagnation and/or regression.

Patient II-4. Patient II-4 was born premature after 8 months of a normal pregnancy (weight: 2150 g). His mother quickly worried about encephalopathy and abnormal behaviors. No detailed data were available regarding early psychomotor development. He was placed in institution at the age of 5 years and followed a school for special needs. Behavioral troubles were invasive with very low interactive

skills and auto-aggressive behavior. He was expressing with poor speech, echolalia and frequent perseverations. He developed stereotypies, interests for repetitive tasks and became very intolerant to changes. Autism was diagnosed during childhood. The evolution was complicated with auto-mutilation, frequent hetero aggressive bursts and tantrum. Cognitive performances had not been evaluated but intellectual deficiency was considered as severe. He was neither able to read nor write. Psychotropic drug treatment was introduced early with anti-psychotic and sedatives drugs.

He was evaluated at 36 years of age and measurements were as follows: weight, 80 kg; height, 1.62 m (BMI = 30.8); and OFC, 54 cm (-2 SD). Clinical examination was normal except for a marked extrapyramidal syndrome probably secondary to the high neuroleptics posology. Speech was poorly understandable. He could only execute simple orders. Daily life was evaluated in a semi-structured interview using VABS for social worker, revealing very impaired autonomy, communication, socialization and locomotion, with developmental age equivalents between about 18 months and 4 years (Table 2). This clinical observation was also in favor of a neurodevelopmental psychomotor and socio-emotional with very early stagnation and/or regression.

Other family members. Two maternal great uncles of III-1, I-3 and I-4, were reported to present encephalopathy. They were institutionalized all their life. No other detail could be available. The mother of III-1 (sister of II-3 and II-4) had normal scholarship, normal behavior and obtained a high school diploma. The maternal grandmother of the index case presented with a depression and psychotic disturbances.

Table 1 Neuropsychological assessments of patient III-1 using WISC IV

Wechsler Intelligence Scale For Children Fourth Edition (WISC IV)		
Factorial index	Total score	Subtest's results
Verbal comprehension index	112	Vocabulary: 11/19 Comprehension: 13/19 Similarities: 12/19
Perceptual reasoning index	Invalid calculation because of significant difference between two subtests	Cubes: 6/19 ^a Concepts identification: 14/19 Matrices: 6/19 ^a
Working memory index	Invalid calculation because of significant difference between two subtests	Memory of numbers: 7/19 ^a Arithmetic: 2/19 ^b
Processing speed index	76 ^b	Code: 4/19 ^b Dams: 4/19 ^b Symbols: 1/19 ^b

^aLow, average low, cut-off score.

^bSignificantly lower than standard score.

Figure 1 Identification of a c.797_798delinsTT (p.C266F) mutation in MAOA in a family with Brunner syndrome-like behavioral disturbances. (a) Pedigree of the family: the mutation (mut) in the proband (arrow) is inherited from his heterozygote mother and the two uncles with ID, autistic traits and aggressive behavior also carry the mutation. (b) Integrative Genome Viewer (IGV, Broad Institute, Cambridge, MA, USA) view of the HTS results in the MAOA exon 8 region: identification of a c.797_798delinsTT (p.C266F) mutation. (c) Sanger sequencing validation of the c.797_798delinsTT (p.C266F) mutation. (d) Prediction for the c.796T>A (p.C266S) mutagenesis performed by Wu *et al*⁴⁰ and for the c.797_798delinsTT (p.C266F) ID mutation identified in this paper (see Methods). The predicted effects at the protein level were calculated using SIFT, PolyPhen2, Mutation Taster and KD4v. Potential effects on splicing were determined by MaxEnt, NNSplice, GeneSplicer or Human Splicing Finder (HSF): percentage of increase or decrease of the score for the normal acceptor splice site is indicated for each mutation according to each program (e) MAOA structure (PDB:2Z5Y Pymol view) according to Se-Young Son *et al*: the membrane-binding domain (colored in blue) and the extra membrane domain, divided into the substrate/inhibitor subdomain (orange) and the FAD-binding subdomain (green). The MAOA cofactor FAD (dark gray) and the MAOA inhibitor Harmine (light gray) are displayed using the sphere model. A detailed view of the cysteine 266 is shown as well as the modeled structure (using KD4v) of the mutated p.C266F MAOA.

Identification of a pathogenic missense mutation p.C266F in MAOA

Targeted high-throughput sequencing (HTS) of the coding exons of 220 'ID genes' in a cohort of 50 patients with ID (unpublished data) led to the identification of a non-synonymous mutation in the MAOA coding sequence in the proband III-1 (Figure 1a). This replacement of two nucleotides c.797_798delinsTT (NM_000240.3), at the very beginning of exon 8 (Figure 1b), leads to the change of a cysteine at position 266 to a phenylalanine (p.C266F). This mutation, confirmed by Sanger sequencing (Figure 1c), was never previously described, neither in dbSNP database nor in the 10 562 X chromosomes of the NHLBI Exome Sequencing project. As the mutation affects the very first bases of exon 8, we tested a possible effect on splicing but neither the different prediction programs (Figure 1d) nor RT-PCR analysis on blood mRNA (data not shown) revealed any effect. The p.C266F amino-acid change that affects drastically both the size and the chemical characteristics (hydrophobic versus polar) of the side chain of a highly conserved residue is predicted to be damaging according to all four *in silico* methods used (Figure 1d). MAOA is composed of three functional domains namely a membrane-binding domain, a substrate/inhibitor domain and the flavin adenine dinucleotide (FAD)-cofactor-binding domain (Figure 1e). Interestingly, a full-length crystal structure of the human MAOA is available (Protein Data Bank 2Z5Y, from positions 12–524 out of 527 aa).³⁷ The analysis of the modeled 3D structure by the prediction program KD4v indicates that the missense affects an aminoacid in a beta sheet close to the FAD-binding pocket, and the bulky aromatic ring of phenylalanine is oriented toward the FAD-binding pocket (Figure 1e), which may explain the observed effect on enzymatic activity (see below). We also genotyped the VNTR located in the MAOA promoter and observed that the proband III-1 carries the 3R (three repeats) allele, associated with 'low expression' of MAOA,¹³ which may thus potentiate the effect of the missense mutation on the level of MAOA activity.

Sanger sequencing revealed that the missense c.797_798delinsTT (p.C266F) mutation is inherited from the proband's mother, who presents a skewed X inactivation profile (92:8), and is present in the two severely affected maternal uncles II-3 and II-4.

Reduction of MAOA level and activity in patient III-1

A reduction of MAOA activity in the patient was firstly assessed by the measurement of urinary catecholamine catabolites. The levels of urinary MN and NMN, two specific MAOA substrates, exceeded normal range, strikingly so for NMN, whereas the product VMA was at the lower threshold (Figure 2). An *in vitro* assay of MAOA activity using 5-HT, specific MAOA substrate, showed a significant reduction (0.15 ± 0.04 vs 0.68 ± 0.16 nmoles/mg prot/h, $P < 0.0001$) in patient's III-1 fibroblasts when compared with other boys of the same age range (Figure 3a, left panel). A quantitative radioligand binding assay in fibroblast revealed a three-fold reduction of the binding to

MAOA protein (0.11 ± 0.02 vs 0.36 ± 0.08 pmoles/mg prot, $P < 0.0001$) in patient III-1 (Figure 3a, right panel), reflecting a reduction of MAOA protein level confirmed by western blot analysis (data not shown). Moreover, the plasma concentrations of 5-HIAA and 3,4-DHPG, two products of the degradation of serotonin and norepinephrine, respectively, reflecting MAOA activity *in vivo*,^{38,39} were found to be 40 (4.57 ± 0.3 vs 177 ± 28 nM, $P < 0.0001$) or 10 times (0.67 ± 0.07 vs 7.7 ± 0.6 nM, $P < 0.0001$) reduced in the proband III-1 compared with control age-matched boys (Figure 3b).

DISCUSSION

Targeted HTS of the protein-coding sequences of 220 genes involved in ID identified (after variant filtration using variant databases such as dbSNP or EVS) a non-synonymous c.797_798delinsTT (p.C266F) missense mutation in MAOA in a family including male patients presenting with a phenotype overlapping with the one described by Brunner *et al* in 1993^{5,6} (data submitted to ClinVar database). Two other nonsynonymous variants were identified in this patient in genes associated with dominant forms of ID (c.1954A>G; p.I652V in *DOCK8* and c.2260G>A; p.V754I in *HDAC4*). However, they were both predicted to be benign and found to be inherited from one of the unaffected parents, excluding a potential pathogenic role. The missense mutation found in MAOA was regarded as pathogenic by all the prediction programs. In a systematic site-directed mutagenesis

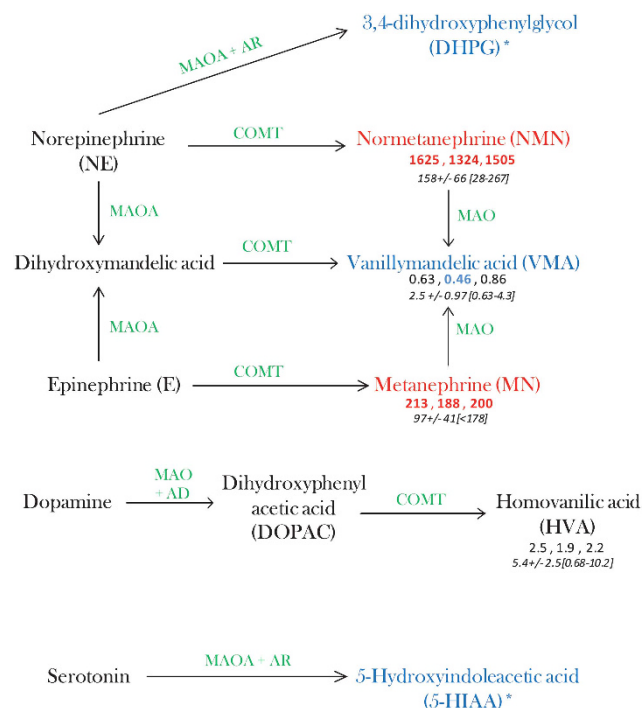


Figure 2 Schematic representation of catecholamine and serotonin metabolism and urinary metabolite dosages in patient III-1. Urinary catecholamine metabolite dosages for patient III-1 have been performed at three different days and are expressed in $\mu\text{mol}/\text{mmol}$ of creatinine for VMA and HVA and in nmol/mmol of creatinin for MN and NMN. Reference values from Pussard E, 2009 are indicated in italic (into bracket the 2.5 and 97.5th percentile). In red /blue are indicated abnormal elevated/reduced values (above two standard deviations). Abbreviations: MAO, monoamine oxidase; COMT, catechol-O-methyl transferase; AD, aldehyde dehydrogenase; and AR, aldehyde reductase. *DHPG and 5-HIAA are found reduced in patient III-1 plasma (see Figure 3).

Table 2 Vineland scales of patients II-3 and II-4

	Patient II-3		Patient II-4	
	Raw score	Age equivalent score	Raw score	Age equivalent score
Sub-domains				
Communication	47	2–1	38	1–8
Autonomy	96	5–3	76	3–11
Socialization	67	4–1	39	1–7
Locomotion	51	2–11	51	2–11

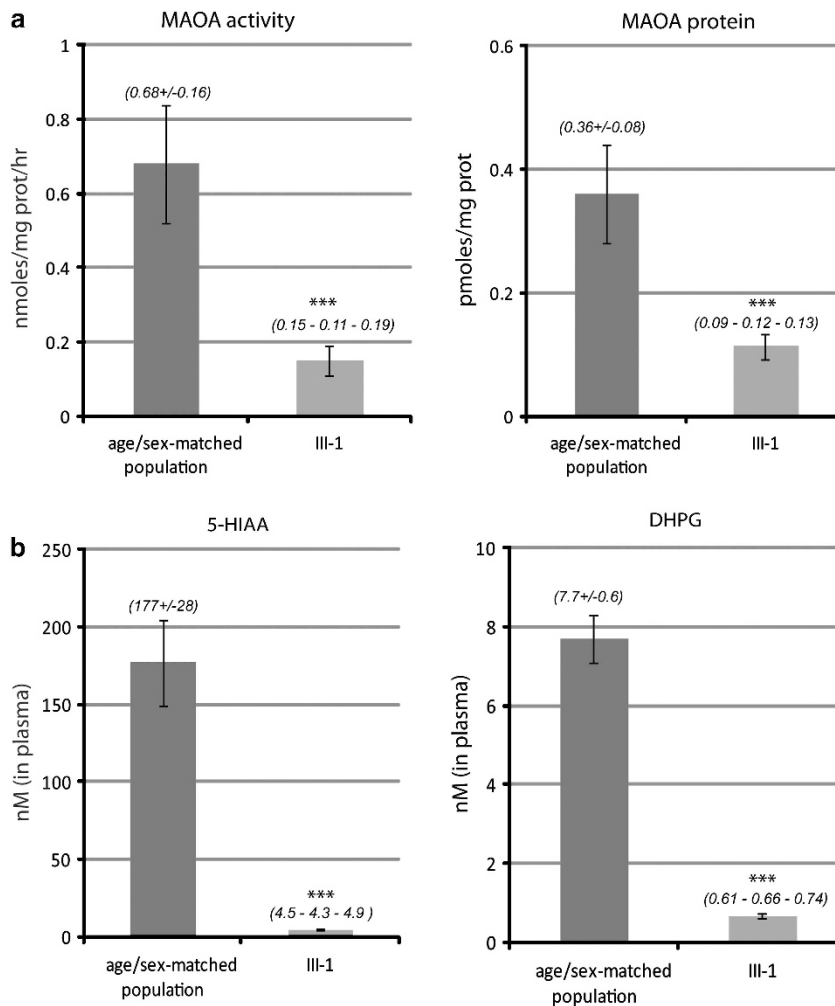


Figure 3 MAOA activity is decreased in the patient III-1 with c.797_798delinsTT (p.C266F) mutation (a) MAOA activity (left) and protein level (right) measures *in vitro* using fibroblasts of proband III-1 ($n=3$ measures) and normal 7-year-old boys ($n=13$ measures). The enzymatic activity assay was performed using [14C]-5-HT creatinine sulfate as MAOA substrate and the protein level was estimated with binding of a radioactive MAOA inhibitor [3H]Ro 41-1049. (b) Measure of the plasma 5-HT and norepinephrine metabolites 5-HIAA and 3,4-DHPG in the proband III-1 ($n=3$ measures) and in normal 7-year-old boys ($n=13$ measures) as a reflection of *in vivo* MAOA activity. *** $P<0.0001$ with Student's *t*-test.

study of the role of cysteines in the catalytic activity of human MAOA and MAOB, the change of cysteine 266 into a serine did not affect the binding of the substrate, but reduced the catalytic activity by 50%.⁴⁰ However, the change to a phenylalanine residue is predicted to be much more damaging, affecting drastically both the size and the chemical nature of the side chain, and was thus likely to have a bigger effect on MAOA activity (Figure 1d). It should be pointed out that missense changes affecting MAOA appear rare in the population, as in the EVS database, none exceeds 0.5% of minor allele frequency, and only two, c.515G>A (p.R172Q) and c.788A>C (p.H263L), predicted benign by the different programs, are present in hemizygous state in male patients (c.515G>A in five male patients; c.788A>C in only one). All the other rare missense variants are described in heterozygous female patients only. This suggests that most variants causing an amino-acid change in MAOA sequence have a phenotypic effect and are negatively selected.

The mutation c.797_798delinsTT (p.C266F) we reported here is in cis with a 'low-activity VNTR allele' in the MAOA promoter, and this might worsen its effect. Indeed, biochemical investigations of substrates and metabolites of MAOA in urine and plasma, as well as

measurement of MAOA activity in fibroblasts of the proband all indicated an important perturbation in the catabolism of catecholamines (notably norepinephrine) and serotonin. In fact, *in vivo* evaluation of MAOA activity showed a more drastic effect, with a 10–40-times decrease in MAOA product (5-HIAA and DHPG) levels in the proband's serum compared with what is observed in control age-matched boys, whereas *in vitro* reduction in enzymatic activity showed only a 3–4-fold reduction. Such a difference might be explained by the fact that *in vitro* assays use optimal substrate conditions for enzyme function that could alleviate in part enzymatic dysfunction manifest under *in vivo* conditions.

The family displays variable expressivity in male patients, with systematic ASD and prominent behavioral disturbances, but variable ID. Indeed, ID could not be unambiguously diagnosed in the index case, despite some cognitive defects, while his maternal uncles had severe ID and behavioral disturbances necessitating a high level of psychotropic treatment. The history of maltreatment and sexual abuse during infancy of the two uncles in opposition to the protective familial environment of proband III-1 could explain at least in part this intrafamilial variable expression. Large-scale studies of behavioral

consequences of high vs low activity associated to short versus long VNTR MAOA alleles converged toward a gene/environment interaction theory.⁴¹ It was suggested that maltreated children with the MAOA genotype conferring low levels of MAOA enzyme developed more often antisocial behavior in adulthood than maltreated children with a high-activity MAOA genotype.⁴² The description of this family overlaps with the so-called Brunner syndrome, a neuropsychiatric disorder associating ASD, borderline cognitive abilities and behavioral abnormalities, caused by truncating mutation in MAOA segregating in a large kindred. Severe aggressiveness was a distinctive feature in this family, and was less obvious in the present family, although all three male carriers of the mutations showed auto-aggressive behavior, and the two uncles had also a history of hetero-aggressive behavior.

The absence of finding of another mutation in the past 20 years is puzzling, especially given the attention given to the initial 1993 reports. This could be attributable to a very low frequency of MAOA mutations. An alternative is that the low specificity of the associated clinical phenotype has precluded systematic testing for mutations in this gene in patients with borderline cognitive deficiency and mainly behavioral disorder. Indeed, apart the systematic sequencing of XLID families by Tarpey *et al*⁴³, there is no published report of systematic screening of mutation in patients with ID or behavioral impairments, and a few other published XLID genes similarly lack such confirmatory reports.⁴⁴ Lack of replication finding in these 20 years might also be explained by the necessity of another genetic or environmental event for the expression of the disease. An obvious candidate as modifier gene is the MAOB gene that is adjacent to the MAOA gene and carries overlapping function. We thus tested whether the present family, as well as the original Brunner-syndrome family carried some missense variants in MAOB that could affect enzymatic activity, and would have cosegregated with the MAOA nonsense mutation in the latter family. We did not identify such variant in MAOB coding regions in patient III-1 and in one member from the Brunner-syndrome family. This does not exclude a potential effect of some non-coding variants.

This study showed the manifest benefit of targeted HTS for genetic diagnosis of non-specific heterogeneous conditions such as ASD or ID associated with behavioral troubles. Of note, the presence of abnormal neurotransmitters in urine could be considered as a diagnostic test in patients with such phenotype, but it is likely that the use for HTS would considerably develop in the next years, and will be economically more appropriate than biochemical testing covering a wide range of potential dysfunctions.

Besides the benefit of genetic diagnosis for appropriate genetic counseling, it might also help in the patient's management. Pharmacogenetics studies on *Maoa*-deficient mice evidenced the counter indication of serotonergic drugs in these animals because of the risk of serotonergic syndrome complication.⁴⁵ MAOA agonists may be a good indication for reversing the behavioral symptoms (irritability, social withdrawal, stereotypy and repetitive speech) in the affected members, but such medication is not yet available. The role of atomoxetine, used in some countries for treatment of pervasive developmental disorders by targeting norepinephrine synaptic recapture,⁴⁶ could be questionable. Also, agonists of upstream/downstream enzyme such as the catechol-O-methyl transferase enzyme might optimize the remaining activity of the MAO enzyme, whereas use of inhibitors of MAOA activity should not be used.

To conclude, we are reporting on the second family with a non-ambiguous mutation in MAOA. This family presents with ASD, behavioral troubles, variable cognitive impairment and accumulation

of NMN in urine associated with a phenotype overlapping with the Brunner family. This HTS result could lead to improve management by giving clues for possible therapeutic options.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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WEB RESOURCES

The URLs for online tools and data presented herein are:

OMIM: <http://www.omim.org/>, UCSC: <http://genome.ucsc.edu/>, dbSNP: <http://www.ncbi.nlm.nih.gov/projects/SNP/>, ClinVar: <http://www.ncbi.nlm.nih.gov/clinvar/>, Mutation Nomenclature: <http://www.hgvs.org/mutnomen/recs.html>, Exome Variant Server, NHLBI Exome Sequencing Project (ESP): <http://evs.gs.washington.edu/EVS/>, Integrative Genomics Viewer (IGV): <http://www.broadinstitute.org/igv/>, PolyPhen 2: <http://genetics.bwh.harvard.edu/pph2/>, Sift Human Protein: http://sift.jcvi.org/www/SIFT_enst_submit.html, MutationTaster: <http://www.mutationtaster.org/>, KD4v: <http://decrpython.igbmc.fr/kd4v/cgi-bin/prediction>, NNsplice: http://www.fruitfly.org/seq_tools/splice.html, Max Ent: MES; http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html, Human Splicing Finder: (<http://www.umd.be/HSF/>), GeneSplicer: <http://cbcb.umd.edu/software/GeneSplicer/>.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)