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## Deletion of MAOA and MAOB in a male patient causes severe developmental delay, intermittent hypotonia and stereotyped hand movements

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1 **Deletion of MAOA and MAOB in a male patient causes severe developmental delay,**  
2 **intermittent hypotonia and stereotyped hand movements**

3

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22

23 **Running Title:** Dual monoamine oxidase deletion

24

25

26

27 **Abstract**

28 The monoamine oxidases (MAO-A and MAO-B) play a key role in the degradation of  
29 amine neurotransmitters, such as dopamine, norepinephrine, and serotonin. We  
30 identified an inherited 240kb deletion on Xp11.3-p11.4 which encompasses both  
31 monoamine oxidase genes but, unlike other published reports, does not affect the  
32 adjacent Norrie Disease gene (*NDP*). The brothers who inherited the deletion, and thus  
33 have no monoamine oxidase function, presented with severe developmental delay,  
34 intermittent hypotonia and stereotyped hand movements. The clinical features accord  
35 with published reports of larger microdeletions and selective MAO-A and MAO-B  
36 deficiencies in humans and mouse models and suggest considerable functional  
37 compensation between MAO-A and MAO-B under normal conditions.

38

39

40 **Keywords:** monoamine oxidase; MAOA; MAOB; deletion; CGH; X chromosome;  
41 abnormal hand movement

42

43

## 44 INTRODUCTION

45

46 The monoamine oxidases, MAO-A and MAO-B, catalyse the oxidative deamination of  
47 biogenic and dietary amines. Monoamine oxidase (MAO) substrates include the  
48 neurotransmitters serotonin, norepinephrine and dopamine, and the neuromodulator  
49 phenylethylamine (reviewed in Shih and Thompson<sup>1</sup>). The rapid degradation of  
50 bioactive monoamines by the MAO isoenzymes is essential for appropriate synaptic  
51 neurotransmission and monoaminergic signaling affects motor, perceptual, cognitive  
52 and emotional brain functions.<sup>2</sup> The *MAOA* and *MAOB* genes occur in tandem  
53 suggesting an ancestral duplication event, but lie in opposite orientations on Xp11.23  
54 where they share 70% identity at the amino acid level and exhibit identical organisation  
55 of their 15 exons.<sup>3,4</sup> Despite this sequence similarity, MAO-A and MAO-B have distinct  
56 but overlapping substrate specificities and spatial and temporal expression patterns in  
57 the brain and peripheral tissues, although most tissues express both isoenzymes.<sup>5-7</sup>

58

59 Previous reports of MAO gene deletions have always encompassed the adjacent *NDP*  
60 gene and are associated with an atypical presentation of Norrie disease (ND, OMIM  
61 #310600). ND is a neurodevelopmental disorder characterised by congenital blindness  
62 due to bilateral retinal malformation and lens opacity. To date, over 70 pathogenic *NDP*  
63 mutations have been identified.<sup>8-10</sup> Accessory phenotypes in 'classical' ND include  
64 progressive sensorineural deafness in approximately one third of cases and some  
65 degree of intellectual disability, autism or psychosis in over 50% of cases.<sup>11</sup> Atypical ND

66 patients with a contiguous deletion of *NDP* and both MAO genes present with a more  
67 severe neurological involvement, with profound psychomotor and verbal deficits.<sup>12-17</sup>  
68 Affected individuals have also been noted to have growth retardation, seizures and  
69 display manneristic, self-injurious behaviours and often have delayed sexual maturation.

70

71 The clinical presentation of rare individuals with selective loss of either *MAOA* or *MAOB*  
72 offers a marked contrast to the severe phenotype associated with deletion of both MAO  
73 genes and *NDP*. In a single reported ND family with a deletion spanning *NDP* and *MAOB*  
74 only, no intellectual impairment or behavioural disturbances were noted.<sup>18</sup> Selective  
75 MAO-A deficiency has been described in a large Dutch kindred with borderline mental  
76 retardation and abnormal behaviour which manifests specifically as impaired impulse  
77 control and increased aggression.<sup>19</sup> Affected individuals in this pedigree had a point  
78 mutation which prematurely truncated the MAO-A protein.<sup>20</sup> Lenders *et al*<sup>18</sup>  
79 demonstrated that patients with dual *MAOA/B* loss (with *NDP*), selective *MAOA* deficit  
80 and selective *MAOB* deficit (with *NDP*) have distinct biochemical profiles of  
81 catecholamines and their metabolites, and suggest that this likely explains the different  
82 clinical phenotypes of each mutation category.

83

84 Here, we report the clinical and molecular characterisation of a sub-microscopic  
85 deletion of the *MAOA* and *MAOB* genes without the concomitant deletion of *NDP*. This  
86 mutation affords the opportunity to further dissect the phenotypic consequences of  
87 MAO deficiency.

88

89 **CLINICAL REPORT**

90 Figure 1a shows an X linked pedigree where two brothers (IV:2 and IV:3, pictured in  
91 Figure 1b and 1c respectively) presented with severe mental retardation, hand wringing,  
92 lip smacking and epilepsy. The brothers were born to a healthy non-consanguineous  
93 Caucasian couple; mother aged 30 years and father 31 years at the birth of IV:1; an  
94 older daughter was healthy. The maternal grandmother had a male sibling who died  
95 around 2 years of age and had slow development but no further medical records were  
96 available.

97

98 IV:2 was born by normal delivery with a birth weight of 2.38kg at about 36 weeks  
99 gestation after a normal pregnancy. He was noted to be hypotonic in the newborn  
100 period and fed poorly necessitating tube feeding. In the first 6 months he had several  
101 attacks of sudden profound hypotonia thought to represent seizures; EEG showed short  
102 wave activity in the occipital area and he was treated at 9 months with phenobarbital  
103 and then sodium valproate. These episodes continued in spite of the medication. CT  
104 scan showed widening of the ventricles and basal spaces. All investigations for  
105 metabolic disorders and viral infections were negative. His development was  
106 significantly delayed, he sat at 20 months and walked with support at 2 years 6 months;  
107 he never walked independently. At 4 years his OFC was between the 3<sup>rd</sup> and 10<sup>th</sup>  
108 centiles and his weight and length were just below the 3<sup>rd</sup> centile. He had recurrent  
109 screaming episodes with head banging and hand flapping; he scratched his face and

110 chewed his hands. He had no major dysmorphic features but had inner canthal folds,  
111 long eyelashes, an extra upper lateral incisor and an unusual hair whorl on his posterior  
112 hairline. He died unexpectedly at 5 years of age. Autopsy did not reveal any  
113 abnormalities of his internal organs except for his brain which was mildly underweight  
114 and on microscopy showed small foci of perivascular calcification, occasional  
115 haemosiderin granules in the Virchow-Robin spaces, loss of Purkinje cells in the  
116 cerebellum and some loss of neurons in the cortex.

117

118 IV:3 was born by vertex delivery at 38 weeks gestation after a normal pregnancy and  
119 weighed 2.5kg. In the newborn period he was noted to make jerky movements when  
120 falling asleep or waking up. Soon after he began to have episodes of hypotonia similar to  
121 his brother. He began to purse and smack his lips and had an intermittent convergent  
122 strabismus. At 13 weeks he had definite axial hypotonia and from 10 months he  
123 developed writhing movements of his hands. [48 hour EEG recording was normal.](#) He sat  
124 at 15 months, bottom shuffled from 2 years 8 months and walked independently at 4  
125 years. Facially he resembled his brother and also had inner canthal folds and long  
126 eyelashes. [He continued to have episodes of restlessness followed by hypotonia, eye](#)  
127 [flickering and loss of consciousness lasting around 30 seconds. They seemed more](#)  
128 [frequent when he awoke and when placed on the toilet. He appeared to be able to](#)  
129 [induce these himself by lying on the floor, staring at a light and shaking his head from](#)  
130 [side to side. Treatment with lamotrigine 100mg twice daily was tried between the ages](#)  
131 [of 6 years and 8 years and, although initially the episodes seemed less frequent, they](#)

132 then increased up to between 20 and 30 times per day and so the treatment was  
133 stopped. MRI scan of his brain did not reveal any abnormalities. At 15 years his OFC was  
134 between the 3<sup>rd</sup> and 10<sup>th</sup> centiles, his height was on the 0.4<sup>th</sup> centile and his weight was  
135 on the 25<sup>th</sup> centile. He walked independently and could run clumsily. He enjoys social  
136 events and company and communicates with many single words and British Sign  
137 Language. His episodes of hypotonia continued. During these he becomes pale and he  
138 may drop to the ground. Recently an EEG was performed during one of these episodes  
139 and showed no focal signs or paroxysmal features. Numerous previous investigations for  
140 cytogenetic abnormalities, for mutations in *MECP2*, *FRAXA* and *ARX* and isoelectric  
141 focussing screening for CDG deficiency were all normal.

142

## 143 **METHODS**

144 Genomic DNA was extracted from peripheral blood and lymphoblastoid cell lines  
145 obtained from the proband (IV:3) and his mother using standard protocols. No  
146 additional family samples were available for analysis. Appropriate clinical research  
147 ethics review board (MREC) approval was obtained for the studies at the University of  
148 Cambridge and University of Manchester. Clinical data was obtained from the family  
149 with informed consent.

150

151 Array Comparative Genomic Hybridisation (aCGH) was performed using a custom-  
152 designed X chromosome-specific Nimblegen 385K oligonucleotide microarray (full  
153 details of array design available on request). Hybridisations were performed by the

154 Roche Nimblegen Inc. service laboratory (Reykjavik, Iceland) according to standard  
155 protocols. Hybridisation was carried out against a reference normal human male of  
156 Caucasian origin (NA10851; obtained from the Coriell Cell Repository). A single  
157 hybridisation experiment was performed, with patient DNA labelled with Cy5-dCTP and  
158 the reference individual with Cy3-dCTP. Following data normalisation, array analysis  
159 was performed using the ADM-1 calling algorithm (Agilent CGH Analytics version 3.4).  
160 250K Nsp GeneChip array (Affymetrix) whole genome analysis was also performed using  
161 standard published protocols.<sup>21</sup> Dual colour fluorescent in situ hybridisation (FISH) was  
162 carried out on metaphase chromosome spreads with PAC probes selected from the  
163 Sanger Institute whole genome tile path 30k clone set to confirm the deletion in the  
164 proband using standard techniques. Metaphases were examined using Olympus BX61  
165 fluorescent microscope and images captured using Hamamatsu ORCA camera and  
166 Smartcapture 3 software.

167

168 Long range PCR was used to amplify a PCR product spanning the deletion junction.  
169 Primers were designed to flank the deletion: JF\_F: 5'-  
170 GAGATAATGAAATCTACTCCAAGGGATGGTTG-3' and JF\_R: 5'-  
171 TATCAGGGCTTATATGATCAGTCACTGGAGAG-3'. PCR was performed with 150ng genomic  
172 DNA in a 50µl reaction containing 2.5U LaTaq (TaKaRa Bio Inc), 1x GC Buffer I, 400µM  
173 each dNTP and 400nM each primer. Thermal cycling, gel purification and direct Sanger  
174 sequencing of products were performed using standard protocols. Subsequent rounds  
175 of sequencing using nested primers (JF\_int1: 5'-TCTTGCTCTCTGGTGCCATTC-3' and

176 JF\_int2: 5'-TTCTTTGAGACGGAGTCTTGCTC-3') were performed to obtain sequencing  
177 reads spanning the deletion junction. The X inactivation pattern in peripheral blood  
178 leukocytes was assessed by PCR analysis of the polymorphic exon 1 CAG repeat of the  
179 AR locus, as described in Plenge *et al.*<sup>22</sup>

180

## 181 RESULTS

182 Copy number analyses using the custom X chromosome array and 250K *Nsp* GeneChip  
183 array were performed in parallel. The X chromosome array indicated a 240kb deletion  
184 in Xp11.3 which contained exons 2-15 of *MAOA* and all exons of *MAOB*. The deletion  
185 was reported by 847 probes, with an average log<sub>2</sub> ratio of -1.08 (Figure 2a). Using the  
186 SNP chip platform, the deletion was reported by 16 SNPS (Figure 2b). No significant  
187 deletions or duplications were identified elsewhere in the genome with the 250K SNP  
188 array. FISH analysis of a fresh lymphocyte culture from the proband using clones tiled  
189 across the region supported the array estimates of the deletion bounds (Figure 3). In  
190 addition, the gene content of the deletion was confirmed by PCR amplification of coding  
191 regions predicted to be at the flanks of the deletion (data not shown). FISH analysis of  
192 the proband's mother revealed that she was a carrier of the deletion (Figure 4). No  
193 significant skewing of X inactivation was noted in maternal leukocytes (41:59 ratio).

194

195 The high resolution of the X chromosome oligo array facilitated the design of primers  
196 flanking the deletion. Using a long range PCR strategy, an amplicon of approximately  
197 9kb was obtained for the proband sample but not from control individuals. Direct

198 sequence analysis of this PCR product was performed and sequence spanning the  
199 deletion breakpoint junction was obtained (Figure 5). The deletion mapped to the  
200 reference sequence nucleotides 43,426,230-43,666,584 (genome build hg18). The  
201 proximal breakpoint was contained within a *L1PA7* LINE element and the distal  
202 breakpoint within an *AluY* element. BLAST2 analysis of 2kb genomic reference sequence  
203 flanking both breakpoints revealed no significant sequence homologies. There was  
204 minimal sequence identity in the 30bp surrounding each breakpoint but there was 2bp  
205 of microhomology at the junction itself. These features are consistent with a deletion  
206 mechanism involving a non-homologous end-joining (NHEJ) event. No similar deletions  
207 are reported in the public access databases of genomic variants either for diseased or  
208 normal individuals.

209

210

## 211 **DISCUSSION**

212 We present a family with an inherited partial deletion of *MAOA* and complete deletion  
213 of *MAOB* resulting in loss of function of both genes. Although there are no previous  
214 reports of a genomic microdeletion affecting only *MAOA* and *MAOB*, the consequences  
215 of dual MAO loss of function can be predicted by comparisons to reports of probands  
216 with Norrie disease resulting from larger contiguous gene deletions or with selective  
217 *MAOA* loss of function.<sup>12-19</sup> These reports reveal that the presence of either a functional  
218 *MAOA* or *MAOB* gene product is sufficient to preserve intellectual processing and  
219 development to within mild to borderline mental retardation levels. However, as

220 described in this report and in atypical Norrie disease patients with dual MAO loss,  
221 deletion of both genes results in severe to profound mental retardation apparent  
222 shortly after birth indicating that these genes are critical for early brain development  
223 and function.

224

225 In males, the loss of *MAOA* and *MAOB* is associated with severe mental retardation and  
226 unusual stereotypic behaviours of hand wringing and lip smacking. The stereotyped  
227 hand movements and lip smacking were also noted in families with loss of function  
228 mutations or deletions of *MAOA*.<sup>13,19</sup> Whilst these stereotyped behaviours are similar to  
229 those seen in Rett syndrome and Angelman syndrome, they have distinctive features  
230 which can provide a useful diagnostic aid. The absence of clinical manifestations in the  
231 female deletion carrier, coupled with the assumption that the random X chromosome  
232 inactivation pattern in lymphocytes is echoed in the central nervous system itself,  
233 suggests that mosaic expression of these genes in brain is sufficient to sustain normal  
234 neural development and function.

235

236

237 In a study of an atypical Norrie disease patient, Rodriguez-Revena *et al*<sup>17</sup> suggested  
238 that the co-deletion of *EFHC2* underlies the seizures observed in their proband as the  
239 paralogous *EFHC1* is associated with autosomal juvenile myoclonic epilepsy.<sup>23,24</sup> Whilst  
240 seizures are uncommon in classical Norrie disease, there are examples of seizures in  
241 cases with *NDP* point mutations.<sup>25,26</sup> The occurrence of seizures in the family described

242 here further supports a role for the disruption of several genes in this region being  
243 sufficient to cause epilepsy.

244

245 MAO inhibitors have been widely used in the past to treat severe depression and,  
246 although significant side effects are reported, long term use is not associated with  
247 cognitive decline or stereotypic hand movements seen in this family. This suggests that  
248 the clinical features we observed are likely to be due to disturbances in the  
249 development of serotonin, norepinephrine and dopamine neuronal populations during  
250 early embryogenesis and brain formation rather than a reflection of altered neuronal  
251 function once normal neuronal connections have been established.

252

253 There are interesting parallels to the clinical presentations in MAO-deficient mouse  
254 models. *MAOA* knockout mice exhibit abnormal behaviours, including trembling and  
255 increased fearfulness as pups and increased aggression in adult males.<sup>27</sup> *MAOB*  
256 knockout mice display increased reactivity to stress but, as with humans, this phenotype  
257 is relatively mild compared to *MAOA* loss.<sup>28</sup> The double *MAOA* and *MAOB* knockout has  
258 an extreme behavioural hyper-reactivity compared to the single knockouts, again  
259 recapitulating the more severe phenotype seen in humans and indicating partial  
260 functional redundancy between *MAOA* and *MAOB*.<sup>29</sup>

261

262 In summary, the likely clinical features of patients with dual MAO deficiency have  
263 previously been inferred from patients with selective loss of genes within the region of

264 Xp11.2 and this case confirms the predictions. The distinct clinical phenotype of  
265 patients without MAO function should be included in the list of conditions associated  
266 with abnormal hand postures and movements.

267

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272 to elucidate the genetic basis of inherited learning disability. This work was supported  
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275

## 276 **Conflict of interest**

277 None declared.

278

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388  
389  
390

391 Titles and Legends to Figures

392

393 **Figure 1:** (a) Pedigree of the family; IV-2(b) and IV-3 (c). Both individuals

394 illustrate the typical hand posturing observed.

395

396 **Figure 2:**(a) X chromosome array hybridisation Log<sub>2</sub> ratios for the X

397 chromosome region 43-44Mb. Each datapoint represents the Cy3/Cy5 log<sub>2</sub> ratio

398 of a single probe, following data normalisation. (b) Copy number analysis of

399 250K *Nsp* GeneChip for the X chromosome region 43-44Mb. Copy number state

400 and Log<sub>2</sub>ratios generated using the Affymetrix CN4 algorithm.

401

402 **Figure 3:** Scheme showing the deletion location. The numbers are deletion co-

403 ordinates (hg18). (a) Actual deletion extent. (b) X chromosome array estimate of

404 deletion extent. (c) Maximum and minimum SNP chip estimates of deletion

405 extent. The minimum extent corresponds to the position of the first and last SNPs

406 reporting the deletion, the maximum extent is defined by the location of the

407 flanking wild type copy number SNP calls. (d) FISH analysis of the deleted

408 region. Green bars indicate a BAC clone that was detected by FISH, orange bars

409 indicate BAC clones which failed to give a signal in metaphases from the

410 proband.

411

412 **Figure 4:** Representative dual colour FISH in metaphases from the proband (a)

413 and his mother (b) and a control female (c). Probe RP1-262B17 (red) is within

414 the deletion and probe RP11-265D20 (green) is adjacent to the deletion (see  
415 Figure 3).

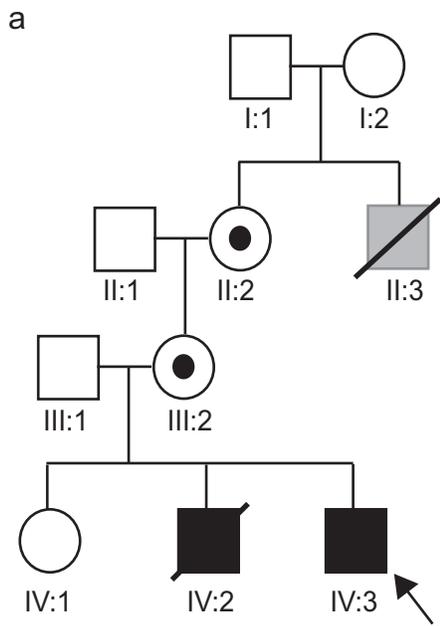
416

417

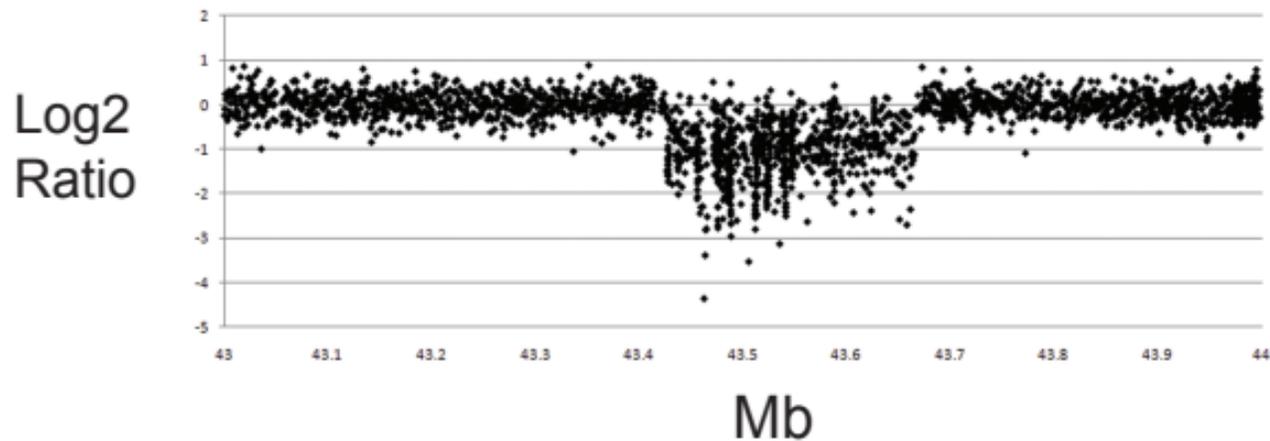
418 **Figure 5:** DNA sequence of the deletion junction fragment. Sequence from the  
419 proband (a) is aligned to the wild type reference sequence from the distal (b) and  
420 proximal (c) breakpoints. The 2bp of microhomology common to the proximal and  
421 distal breakpoint sequences is outlined by the box.

422

423



a



b

