Gait Abnormalities and Aberrant D2 Receptor Expression and Signaling in Mice Carrying the Human Pathogenic Mutation DRD2I212F


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Running Title: Mouse Model of the DRD2I212F Mutation

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Supplemental Table S1 – Primers for genomic analysis of Drd2<sup>I212F</sup> mice

<table>
<thead>
<tr>
<th>Primer for analyzing knock-in allele</th>
<th>Primer Sequence</th>
<th>PCR product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon5-F1</td>
<td>5’-TCTTCTGGTGCTGCTATAGCC-3’</td>
<td>376 bp</td>
</tr>
<tr>
<td>Exon5-RC1</td>
<td>5’-CTATTTCCAGCCTAGACTTCCAC-3’</td>
<td></td>
</tr>
<tr>
<td>Exon5-F2</td>
<td>5’-GAGTGTATCATCATTGCAACCC-3’</td>
<td>181 bp</td>
</tr>
<tr>
<td>Exon5-RC2</td>
<td>5’-TGAGTGGTGTCCTCAGGTTG-3’</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primer for analyzing off-targets</th>
<th>Primer Sequence</th>
<th>PCR product</th>
</tr>
</thead>
<tbody>
<tr>
<td>KI-OT1-Forward (left)</td>
<td>5’-TCCCGGTGTGGTTCAGAAGGC-3’</td>
<td>281 bp</td>
</tr>
<tr>
<td>KI-OT1-Reverse (right)</td>
<td>5’-TTCAGCCTCTTTCAGCCGCTG-3’</td>
<td></td>
</tr>
<tr>
<td>KI-OT2-Forward</td>
<td>5’-TGCTGCAGTGAAGCCTGTC-3’</td>
<td>359 bp</td>
</tr>
<tr>
<td>KI-OT2-Reverse</td>
<td>5’-AGTTGGAGTTACAGGACGTGG-3’</td>
<td></td>
</tr>
<tr>
<td>KI-OT3-Forward</td>
<td>5’-ATGTGGTGCGCCCACTGCAG-3’</td>
<td>419 bp</td>
</tr>
<tr>
<td>KI-OT3-Reverse</td>
<td>5’-CTTGGGTGAGATTGTCAGTG-3’</td>
<td></td>
</tr>
<tr>
<td>KI-OT4-Forward</td>
<td>5’-CTTCGTGTCCACACAGAGAGC-3’</td>
<td>381 bp</td>
</tr>
<tr>
<td>KI-OT4-Reverse</td>
<td>5’-AGACTAGCCTGGACTGCTAGG-3’</td>
<td></td>
</tr>
</tbody>
</table>

Custom primers were synthesized by Integrated DNA Technologies (IDT).
Supplemental Table S2 – List of studied off-target loci for the gRNA.

<table>
<thead>
<tr>
<th>Name</th>
<th>Off-target sequence</th>
<th>CFD Score</th>
<th>Chr</th>
<th>Start&lt;sup&gt;1&lt;/sup&gt;</th>
<th>End&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Strand</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT1</td>
<td>acACCaGCTTCaGACGCTTGAGG</td>
<td>0.45918</td>
<td>8</td>
<td>31482093</td>
<td>31482115</td>
<td>-</td>
<td>Nrg1</td>
</tr>
<tr>
<td>OT2</td>
<td>TGACCatCTTCTgACGtTTTGGG</td>
<td>0.13333</td>
<td>1</td>
<td>13902388</td>
<td>13902410</td>
<td>+</td>
<td>Intergenic</td>
</tr>
<tr>
<td>OT3</td>
<td>TggCCTgCTTCTgAgGCTTGAGG</td>
<td>0.01528</td>
<td>11</td>
<td>9564789</td>
<td>9564811</td>
<td>-</td>
<td>Grb10</td>
</tr>
<tr>
<td>OT4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>TggCCCcCTTCaAGGCTTGAGG</td>
<td>0.01500</td>
<td>9</td>
<td>29867081</td>
<td>29867103</td>
<td>-</td>
<td>Intergenic</td>
</tr>
<tr>
<td>OT5&lt;sup&gt;3&lt;/sup&gt;</td>
<td>TGACCGCcTCaGAcGCTgGCgG</td>
<td>0.00824</td>
<td>8</td>
<td>93387983</td>
<td>93388005</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Guide sequence: TGACCGCTTCCGACGCTTGAGG. Potential off-target (OT) sites were predicted using CRISPOR (www.crispor.tefor.net; Concordet and Haeussler, 2018).

Capital letters in these off-target candidate sites indicates matches with the target sequence and lower-case letters indicate mismatches with the target sequence. All off-targets have 4-nt mismatches.

<sup>1</sup>Reference *Mus musculus* genome, strain C57BL/6NJ (GeneBank assembly accession: GCA_001632555.1).

<sup>2</sup>Off-target locus in chromosome 9, the chromosome on which Drd2 is located, is in bold font.

Nrg1 is Neuregulin 1; Grb10 is growth factor receptor bound protein 10.

<sup>3</sup>Chr, chromosome

<sup>4</sup>OT5, shown in grey, was not studied due to the low CFD score
**Supplemental Fig. S1.** Strategy for producing Drd2[^I212F] mice. **A**, A 1.5% agarose gel stained with ethidium bromide showed examples of PCR products from Drd2-exon 5 amplified using Exon5-F1/Exon5-RC1 (376 bp, left side) and Exon5-F2/Exon5-RC2 primer pairs (181 bp, right side). **B**, A representative gel is displayed to show Drd2^+/+^ (WT), Drd2^+/-^[^I212F^] and Drd2[^I212F]/[^I212F^] (+/+) genotypes. Representative 376-bp PCR amplicons were digested with ApoI-HF and DNA samples were run in a 1.5% agarose gel stained with SYBR Safe DNA Gel Stain (APExBIO; Houston, TX). GeneRuler 1 Kb DNA ladder (Thermo Scientific, Inc; Waltham, MA) was used as molecular weight DNA marker.
Supplemental Fig. S2. Generation of Drd2<sup>+/<sup>1212F</sup> mice. Molecular characterization of the Drd2-exon 5 target region in Lineage B. A. Chromatograms showing the Sanger sequencing confirmation of the c.634A>T; p.Ile212Phe in the F0-421 mouse. Top, the 181bp product obtained by PCR amplification of a gDNA sample using Exon5-F2 and Exon5-RC2 primers pair (Supplemental Table S1, Figure 1) was purified and sent for sequencing. Bottom, 376-bp product amplified by PCR using Exon5-F1 and Exon5-
RC1 primers pair (Fig. 1) was cloned into TOPO-TA vector (Invitrogen). Plasmids containing WT and mutated strands were sent for sequencing. For the WT strand, the reverse-complemented sequence is shown. Expected nucleotide changes are present in mutated but not in WT strands; however, a deletion of nine nucleotides (underlined sequence in the mutated strand) was observed in the WT strand, explaining the mixed sequence obtained by direct sequencing of the PCR product (top chromatogram, sequence shown in an oval). B, Representative chromatograms showing DNA sequence of both WT and mutated strands corresponding to an F1 mouse from lineage B. Reverse-complemented sequences are shown.

Founder F0-421 (male) was crossed with inbred control (WT) mice, and F1 offspring (eight females and eight males) were genotyped by PCR and restriction analysis. Positive F1 mice (two females and five males) were evaluated for integrity of their WT and mutated strands by cloning their 376-bp amplicon into TOPO-TA vector (Invitrogen) and sequencing both DNA strands. Wide-red arrow points to the A-T change to generate the Drd2<sup>I212F</sup> variant; blue arrows show synonymous nucleotide changes introduced downstream of the c.634A>T position. Black arrows show that no nucleotide changes are present in the WT strand. Moreover, the deleted 9-nt sequence in the founder-WT strand (A, middle chromatogram), is present in both WT and mutated strands from F1 offspring (underlined sequence, both strands).

Downstream of the c.634A>T position, a specific nucleotide change (uppercase) introduced to generate an RsaI site is identified by a dashed-line arrow and the site (GTAC) is shown in a box, whereas the nucleotide change showed in lowercase was introduced to remove a Cas9 PAM sequence.
Supplemental Fig. S3. Basal and cocaine-stimulated locomotor activity in \textit{Drd2}^{I212F} mice. Thirty-two mice (22 male and 10 female) tested on the DigiGait apparatus were subsequently assessed for basal and cocaine-stimulated locomotor activity using a 3-day procedure previously described in detail (Kamens \textit{et al}., 2005; Neve \textit{et al}., 2013). Briefly, mice were tested in Accuscan (AccuScan Instruments, Inc., Columbus, OH) automated activity monitors on 3 consecutive days, in which photocell beam interruptions were translated to distance traveled. On days 1 and 2, each animal received an i.p. injection of saline and activity was recorded for 30 min; day 1 provides the level of activity in a novel environment and day 2 provides baseline activity data in a now–familiar environment. On day 3, each animal received an injection of 10 mg/kg cocaine (i.p.) before being placed in the activity monitor for 30 min. An initial 3-way repeated measures ANOVA (day, genotype, sex) found no significant effects of sex, so data were analyzed by 2-way repeated measures ANOVA (day, genotype) and a significant effect of day was found (\(F = 19.5, P < 0.0001\)). Although cocaine significantly elevated activity compared to either saline day, there was no significant effect of genotype. *** \(P = 0.0001\), Newman-Keuls.
References for Supplemental Information

