This data article tested whether polymorphisms within the dopamine D4 receptor (DRD4) gene promoter can lead to differences in the promoter activity. The variants, a 120-bp variable number tandem repeat (VNTR), −906 T/C, −809 G/A, −616G/C, and −521C/T, were introduced into the DRD4 promoter and the promoter activity was measured in a neural cell line using the luciferase assay. However, no differences were detected among the haplotypes investigated, and the in vitro data obtained from our protocol could not support the involvement of DRD4 promoter polymorphisms in heritable human traits.

© 2016 Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Experimental factors | Polymorphisms (120-bp VNTR, rs3758653 for −906 T/C, rs936461 for −809 G/A, rs747302 for −616 G/C, and rs1800955 for −521 C/T) were introduced into the promoter sequence of the DRD4 gene
Experimental features | DRD4 expression was detected by RT-PCR using cDNA from SH-SY5Y cells. Firefly luciferase gene downstream of DRD4 promoter was expressed in SH-SY5Y cells, and the luciferase activity of each construct was measured 48 h after transfection
Data source location | University of Tokyo, Japan
Data accessibility | Data supplied with this article

Value of the data

- We examined the effect of DRD4 promoter polymorphisms on gene expression in an in vitro reporter gene experiment.
- This data is useful for characterising the link between heritable mental traits and the polymorphisms.
- Our data can provide insight into methodology and considerations for investigation of polymorphisms in non-coding regions.

1. Data

Endogenous dopamine D4 receptor (DRD4) gene expression in SH-SY5Y cells was detected by RT-PCR using cDNA derived from the cell line (Fig. 1).

To test whether the polymorphisms within the promoter change the promoter activity, luciferase activity was measured under the influence of the DRD4 promoter into which polymorphisms were introduced (Fig. 2 and Table 1). All of the reporter plasmids containing the DRD4 fragment exhibited significantly higher luciferase activity than the control pGL3-Basic, and although every possible combination of haplotypes was investigated, there were no activity differences among the introduced mutations in SH-SY5Y cells (Figs. 3 and 4).

2. Experimental design, materials and methods

2.1. Construction of reporter plasmid

A DNA fragment spanning −1576 to −1 of the DRD4 promoter region was amplified from human genomic DNA with TaKaRa LA Taq (TaKaRa) and inserted into pCR-Blunt (Life Technology). The cloned sequence was confirmed by Sanger sequencing and shown in Supplementary Fig. 1. Mutations were introduced using PCR-based site-directed mutagenesis for the four SNPs, and with NotI treatment for

![Fig. 1. RT-PCR analysis of DRD4 gene expression. DRD4 expression in SH-SY5Y cells was detected using RT-PCR. The housekeeping gene GAPDH was amplified as an internal control.](image-url)
the VNTR; DNA ligation after NotI treatment converts a 2-repeat allele into a 1-repeat allele because one NotI recognition site is present within the repeat. The mutated insertion was subcloned into the XhoI and HindIII sites of pGL3-Promoter Vector (Promega) replacing the original SV40 promoter with the DRD4 promoter. The primer sequences used for construction are shown in Table 2.

2.2. Cell culture and transfection

Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco’s Modified Eagle’s Medium (Sigma-Aldrich) supplemented with 10% foetal bovine serum (Invitrogen) at 37 °C in a humidiﬁed atmosphere containing 5% CO2. Twenty-four hours before transfection, cells were plated at 4 x 10^5 cells/well in 96-well plates.

The reporter plasmid (0.2 ng/well) and pRL-TK (0.01 ng/well) were transfected into SH-SY5Y cells with 0.06 μL/well FuGENE 6 Transfection Reagent (Promega), according to the manufacturer’s protocol.

2.3. Luciferase assay

Forty-eight hours after transfection, the luciferase activity was measured in quadruplicate with the Dual-Glo Luciferase assay System (Promega) using Centro LB960 (Berthold), following the manufacturer’s instructions. Relative luciferase activity was calculated as the ratio of ﬁrefly to Renilla luciferase activity.

2.4. Total RNA isolation and RT-PCR

Total RNA of SH-SY5Y was extracted with GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich). First-strand cDNA was synthesized from extracted RNA using Prime Script RT Reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa). DRD4 mRNA expression was detected using TaKaRa LA Taq, as described [1]. To verify the procedure, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was ampliﬁed as an internal control. The primer sequences used for RT-PCR are shown in Table 2.
Table 1
The constructed haplotypes consisted of 120-bp VNTR and four SNPs of the DRD4 gene.

| Reporter construct | 120 bp VNTR | −906 C/T | −809 G/A | −616 G/C | −521 C/T |
|--------------------|-------------|----------|----------|----------|----------|
| 1R-WT              | 1           | T        | G        | G        | C        |
| 1R-521             | 1           | T        | G        | G        | T        |
| 1R-616             | 1           | T        | G        | C        | C        |
| 1R-809             | 1           | T        | A        | G        | C        |
| 1R-906             | 1           | C        | G        | G        | C        |
| 2R-WT              | 2           | T        | G        | G        | T        |
| 2R-521             | 2           | T        | G        | C        | C        |
| 2R-616             | 2           | T        | A        | G        | C        |
| 2R-809             | 2           | T        | A        | G        | C        |
| 2R-906             | 2           | C        | G        | G        | C        |
| 1R-521-616         | 1           | T        | G        | C        | T        |
| 1R-521-809         | 1           | T        | A        | G        | T        |
| 1R-521-906         | 1           | C        | G        | G        | T        |
| 1R-616-809         | 1           | T        | A        | C        | T        |
| 1R-616-906         | 1           | C        | G        | C        | C        |
| 1R-809-906         | 1           | C        | A        | G        | C        |
| 1R-521-616-809     | 1           | T        | A        | C        | T        |
| 1R-521-616-906     | 1           | C        | G        | C        | T        |
| 1R-521-809-906     | 1           | C        | A        | G        | T        |
| 1R-616-809-906     | 1           | C        | A        | C        | C        |
| 1R-521-616-809-906 | 1           | C        | A        | C        | T        |

Fig. 3. The effect of the polymorphisms on the DRD4 promoter activity. DRD4 promoter activity was measured as the luciferase activity in SH-SY5Y cells. The relative luciferase activity of pGL3-Basic was defined as 1 and pGL3 promoter was used as positive control. The assay failed to detect any significant differences between haplotypes. Data are expressed as means ± SD (n=5) (Tukey-Kramer test, **p < 0.01).
Acknowledgements

This work was supported in part by an Intramural Research Grant (26-8) for Neurological and Psychiatric Disorders from NCNP, a Research Grant for Comprehensive Research on Disability Health and Welfare from the Ministry of Health, Labour and Welfare (H26-sinkeikinn-ippan-004) of Japan, and a Grant-in-Aid from the MHLW of Japan (to S.I.).

Table 2
Primer sequences and application.

| Application                  | Forward                                | Reverse                                |
|------------------------------|----------------------------------------|----------------------------------------|
| Amplification of DRD4 promoter| ACCActcgaGAGGCTGGGCTGGACTCGCCGTTT      | AAGGagttGGCGCGCCGGCCGGCCGG            |
| Nucleotide substitution –916 T>C| GAAAGTTCTCATAAGACGCTCTTCTGGCGGTTTGC  | GCAAGGGCAGCAAGA-                      |
| Nucleotide substitution –809 G>A| GAGGCAGACCTACTGTGCGGTCCCG            | CGGGACGGACAGTGTTTCCGGGCTCG            |
| Nucleotide substitution –616 G>C| GGCGGGGTGAGACGAGGCTTC              | GCACCTCGCTGTCAGCCCGCAG              |
| Nucleotide substitution –521 T>C| GCTGGAGGGCGCAGCGCAGCG              | CCTGTCGCCGCCTTCACAGGC               |
| RT-PCR of DRD4              | GCCACCCCTCACCTTCACC                  | CGGAACCTGGGGCACTAGAGG                 |
| RT-PCR of GAPDH             | AAGGCTGAGAAGGGAGCTGTGCCATCAAT        | TCCCCGCTAGCTAGGGAT-GACCTGCC           |

Fig. 4. The effect of the combined polymorphisms on the DRD4 promoter activity. DRD4 promoter activity was measured as luciferase activity in SH-SYSY cells. The relative luciferase activity of pGL3-Basic was defined as 1 and pGL3 promoter was used as positive control. The assay failed to detect any significant differences between haplotypes. Data are expressed as means ± SD (n=4) (Tukey–Kramer test, **p < 0.01).
Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.03.084.

References

[1] S. Kamakura, A. Iwaki, M. Matsumoto, Y. Fukumaki. Cloning and characterization of the 5'-flanking region of the human dopamine D4 receptor gene, Biochem. Biophys. Res. Commun. 235 (1997) 321–326.

[2] Y. Okuyama, H. Ishiguro, M. Nankai, H. Shibuya, A. Watanabe, T. Arinami. Identification of a polymorphism in the promoter region of DRD4 associated with the human novelty seeking personality trait, Mol. Psychiatry 5 (2000) 64–69.

[3] U.M. D'Souza, C. Russ, E. Tahir, J. Mill, P. McGuffin, P.J. Asherson, I.W. Craig. Functional effects of a tandem duplication polymorphism in the 5'flanking region of the DRD4 gene, Biol. Psychiatry 56 (2004) 691–697.

[4] E. Kereszturi, O. Kiraly, C. Barta, N. Molnar, M. Sasvari-Szekely, Z. Csapo. No direct effect of the −521 C/T polymorphism in the human dopamine D4 receptor gene promoter on transcriptional activity, BMC Mol. Biol. 7 (2006) 18.

[5] E. Kereszturi, O. Kiraly, Z. Csapo, Z. Tarnok, J. Gadoros, M. Sasvari-Szekely, Z. Nemoda. Association between the 120-bp duplication of the dopamine D4 receptor gene and attention deficit hyperactivity disorder: genetic and molecular analyses, Am. J. Med. Genet. B: Neuropsychiatr. Genet. 144B (2007) 231–236.