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Cell line specific alterations in genes associated with dopamine metabolism and signaling in midbrain dopaminergic neurons derived from 22q11.2 deletion carriers with elevated dopamine synthesis capacity.

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Abstract

Microdeletions at the 22q11.2 locus are associated with increased risk for schizophrenia. Recent work has demonstrated that antipsychotic naïve 22q11.2 carriers display elevated levels of dopamine synthesis capacity (DSC) as assessed by $^{18}$F-DOPA PET imaging. While this is consistent with a role for abnormal dopamine function in schizophrenia, it is unclear what molecular changes may be associated with this neuro-imaging endophenotype, and moreover, if these alterations occur independently of clinical presentation. We therefore conducted a pilot study in which we generated human induced pluripotent stem cells (hiPSCs) from two 22q11.2 deletion carriers with elevated DSC in vivo, but distinct clinical presentations. From these and neurotypical control lines we were able to robustly generate midbrain dopaminergic neurons (mDA-neurons). We then assessed whether genes associated with dopamine synthesis, metabolism or signaling show altered expression between genotypes and further between the 22q11.2 deletion lines. Our data showed alterations in expression of genes associated with dopamine metabolism and signaling that differed between the two 22q11.2 hiPSC lines with distinct clinical presentations genetic background. This reinforces the importance of considering clinical, genetic and molecular information, when possible, when choosing which donors to generate hiPSCs from, to carry out mechanistic studies.

Key Words:

Schizophrenia, human induced pluripotent stem cells, midbrain floor plate neural progenitor, [18F]-DOPA PET, reprogramming.
1.0. Introduction

The 22q11.2 deletion syndrome is caused by a hemizygous microdeletion in the 22q11.2 chromosome region and is associated with a highly variable clinical presentation (Morrow et al., 2018). Typical microdeletions are around 3Mb in size, encompassing ~45 coding genes as well as 7 microRNAs (Morrow et al., 2018). Individuals carrying this microdeletion are at 40-fold greater risk of developing psychosis (Schneider et al., 2014) making 22q11.2 the strongest genetic risk factor for schizophrenia. Using $^{18}$F-DOPA PET, we recently showed that dopamine synthesis capacity (DSC) is significantly increased in antipsychotic naïve 22q11.2 deletion carriers (Rogdaki et al., 2021). Increased presynaptic striatal DSC has also been observed in individuals with schizophrenia (McCutcheon et al., 2018), predates the onset of psychosis and is directly associated with symptom severity (Egerton et al., 2013; Howes et al., 2013). Therefore, 22q11.2 deletion represents a genetically homogenous model holding potential for studying the molecular underpinnings of dopaminergic dysregulation in schizophrenia.

Despite the insights that animal models of 22q11.2 deletion have provided about dopaminergic function at molecular level (Chun et al., 2014; Zakharenko et al., 2015), human molecular phenotypes are less well understood. Dysregulation of genes associated with dopamine synthesis, storage and release has been identified in the midbrain of schizophrenic individuals in post-mortem studies, but not consistently reported (Howes et al., 2013; Perez-Costas et al., 2012; Purves-Tyson et al., 2017). Thus, the mechanism underlying dopamine dysregulation remains unclear. Human induced pluripotent cells (hiPSCs) retain the genetic background of the donor offering the unique advantage of allowing the study of potential molecular mechanisms underlying distinct and disease-relevant endophenotypes observed in
subjects, in an *in vitro* system. In this pilot study, we aimed to bridge PET findings with *in vitro* disease models by generating midbrain dopaminergic neurons (mDA-neurons) from hiPSCs from two individuals carrying 22q11.2 deletions, both with high levels of DSC compared to control participants, but only one with a diagnosis of schizophrenia. Our study had two aims: a) to determine whether mDA-neurons from 22q11.2 carriers exhibit similar molecular changes as that seen in post-mortem studies, and b) whether mDA-neurons derived from 22q11.2 deletion carriers with distinct clinical presentations displayed similar or unique molecular changes in genes involved in dopamine synthesis, metabolism or signalling.

2.0. Materials and Methods

Additional information on Material and Methods can be found in *Supplemental Material.*

2.1. Human induced pluripotent stem cells (hiPSCs) generation.

Participants were recruited and methods carried out in accordance with the ‘Patient iPSCs for Neurodevelopmental Disorders (PiNDs) study’ (REC No 13/LO/1218). Informed consent was obtained from all subjects for participation in the PiNDs study. Ethical approval for the PiNDs study was provided by the NHS Research Ethics Committee at the South London and Maudsley (SLaM) NHS R&D Office. All hiPSC lines were generated from primary keratinocytes as described previously (Cocks et al., 2014). Keratinocytes were reprogrammed using the CytoTune-iPS 2.0 Sendai expressing Reprogramming Kit (ThermoFisher, A16517).

2.2. Generation of mDA-neurons
Generation of midbrain floor plate neural progenitor (mFPP) cells and dopamine (DA) neurons was based on (Fedele et al., 2017; Kriks et al., 2011) (Supplemental Figure 2A). Briefly, hiPSCs were differentiated into mFFPs for 10 days before being expanded. Expanded mFFPs were then terminally differentiated into mDA-neurons until day 50. A detailed description of mDA-neuron generation can be found in Supplementary Material.

2.3. RT² Profiler PCR array

We used the Human Dopamine and Serotonin Pathway RT² Profiler array (Qiagen) to assay the expression of 84 genes associated with dopamine and serotonin synthesis and signaling. RT-PCR was performed using the RT² SYBR green qPCR Master Mix (Qiagen) using RNA from day 50 mDA-neurons using a BioMark HD cycler (Fluidigm). Analysis of gene expression was carried out using the RT² profiler PCR array data analysis v3.5 software provided by Qiagen. All data was normalized to 5 separate housekeeping genes (ACTB, B2M, GAPDH, HPRT1, and RPLP0). The 2^ΔΔCT comparative method for relative quantification was used to quantify the genes expression. Three independent differentiations per hiPSC line were used in these experiments.

2.4. Statistical analysis

All statistical analysis was performed in GraphPad. Differences in 2^ΔΔCT, relative expression (Fold change) and cell number parameters were identified by comparisons between multiple conditions: main effects were probed by one-way-ANOVAs with Tukey or Bonferroni correction for multiple comparisons. Differences were considered significant if P was lower than 0.05 (p<0.05). Error bars represent standard errors of the mean unless stated otherwise.
3.0. RESULTS

3.1. Characterisation of 22q11.2 hiPSCs.

HiPSCs were generated from two 22q11.2 deletion carriers: 22DM_287(+) and DF_191(-). Detailed clinical information can be found in Supplemental Information. Both 22q11.2 carriers had elevated DSC in vivo, as measured by \(^{18}\text{F}-\text{DOPA}\) PET (Supplemental Table 1 and 2) (Rogdaki et al., 2021). Participant 22DM_287(+) had a diagnosis of autism spectrum disorder, mild intellectual disability and schizophrenia. By contrast, participant 22DF_191(-) had cardiac defects but no diagnosis of psychiatric illness. For clarity, we use (+) following the cell line name to denote a diagnosis of schizophrenia, and (-) to indicate a lack of diagnosis. HiPSCs were confirmed as karyotypically normal; to express pluripotency markers and showed three germ layer differentiation (Supplemental Figure 1A-D, Supplemental Figure 2B, and Supplemental table 1). For comparison we used HiPSCs from three (neurotypical) individuals with no history of psychiatric illness (CTR_M3; CTM_127; CTF_007) that have been previously characterized (Adhya et al., 2021).

Single polymorphism (SNP) array (Infinium PsychArray) was used to determine genomic integrity, and to confirm the 22q11.2 deletion (Figure 1A and Supplemental Figure 3). The 22DM_287(+) line had a single hemizygous ~2.5Mb deletion affecting 46 coding genes and 7 microRNAs (Supplemental Figure 2). Cell line 22DF_191(-) had a total of eight copy number variants (CNVs) at the 22q11.2 locus. Of these, 4 were hemizygous deletions (~3.4 Mb in total); 1 was a homozygous deletion (~0.5Mb), and 3 were duplications (~0.62Mb in total). The deletions encompassed 41 coding genes and 6 microRNAs within the 22q11.2 locus, and 13 coding genes outside of this region. The duplicated regions included ARVCF.
and TANGO2 genes, as well as the microRNA, MIR185 (Supplemental Figure 3). Genome-wide SNP genotype data were also used to derive schizophrenia polygenic risk score (PRS) using Psychiatric Genomics Consortium 3 genome wide association study (GWAS) summary statistics (Trubetskoy et al., 2022) for all hiPSC lines. This revealed that 22DM_287(+) had a higher adjusted PRS compared to 22DF_191(-) and all control lines, except for CTM_007, which also showed a high PRS (Supplemental Figure 1E).

3.2. Generation of midbrain dopaminergic neurons from patient hiPSCs

After 50 days of differentiation, all hiPSCs, irrespective of genotype, generated mDA-neurons positive for MAP2 (>70%), LMX1A (>75%), a maker of midbrain dopamine ontogeny; and tyrosine hydroxylase (TH; >75%) (Figure 1B-D). mDA-neurons from all genotypes also expressed mFPP specific transcription factors NURR1, ASCL1 (Figure 1E) as well as GRIK2 and PITX3 (Figure 1E). Intriguingly, control mDA-neurons highly expressed FOXA2, whilst 22q11.2 mDA-neurons did not have detectable levels of FOXA2 expression, irrespective of clinical presentation (Figure 1E). Taken together, these data indicate that control and 22q11.2 hiPSC lines successfully differentiate into a midbrain dopaminergic neuronal fate.

3.3. mDA-neurons generated from 22q11.2 carriers with distinct clinical presentations, display differential expression of genes involved in dopamine synthesis and signaling.
We next utilized a PCR array to profile the expression levels of genes involved in dopamine and serotonin synthesis and signaling from day 50 mDA-neurons. A total of 86 genes were assessed across all hiPSC lines. A summary of the main findings for each gene can be found in Table 1. We observed no difference in the expression of TH across all DA-neurons (Figure 2A). As expected, 22q11.2 mDA-neurons expressed COMT at ~50% (Figure 2A) given the hemizygosity for COMT gene in the 22q11.2 genomic region. These data indicated the reliability of the PCR array.

Overall, 23 genes were differentially expressed in 22q11.2 mDA-neurons (Figure 2A-F; Table 1). The majority of differentially expressed genes were those involved in dopamine synthesis/metabolism, receptor signaling and gene targets (Table 1) consistent with previous post-mortem studies (Purves-Tyson et al., 2017). Sixteen of these genes showed differential expression in only one 22q11.2 hiPSC line, which was of potential interest in light of the different clinical presentations. For example, mDA-neurons from 22DM_287(+) displayed reduced expression of MAOB, whereas mDA-neurons from 22DF_191(-) had elevated expression of DBH (Figure 2B). Both genes are involved in conversion of dopamine into either DOPAC or noradrenaline; no difference in the expression of MAOA was observed (Figure 2C). The expression of DDC (AADC) was not significantly different from control hiPSCs but trended towards elevated expression in 22DF_191(-) and lower expression in 22DM_287(+) mDA-neurons (Figure 2C).

Of the genes involved in DA signaling, ADCY2, CREB1 and AKT3 were of note as these genes were only differentially expressed in the 22DM_287(+) mDA-neurons (Figure 2D). All of these genes have been associated with increased risk for SCZ. Conversely, MAPK1 was reduced expression in 22DF_191(-) DA-neurons,
consistent with its location within a deleted region of the 22q11 locus in this cell line (Supplemental Figure 2).

4.0. DISCUSSION

In this pilot study, we aimed to identify molecular changes present in the mDA-neurons, which may be relevant for this imaging endophenotype. We observed cell line specific dysregulation of genes involved in dopamine metabolism and signaling in mDA-neurons, with a greater number of genes showing differential expression in the 22q11.2 line generated from the individual with a diagnosis of schizophrenia compared to those from individuals with no psychiatric illness and a similar microdeletion.

The finding that TH expression is unaltered is consistent with previous studies in COMT deficient mice (Gogos et al., 1998; Huotari et al., 2002), and a post-mortem study of 22q11.2 deletion carriers (Butcher et al., 2013). Similarly, the expression of COMT at ~50% is consistent with this gene being within deletion CNVs within the 22q11.2 region of 22DM_287(+) and 22DF_191(-). Four genes involved in DA metabolism and signaling (MAOB, ADCY2, CREB1, AKT3) showed altered expression in 22DM_287(+) mDA-neurons only. Furthermore, expression levels of DCC (AADC) and DBH, two dopaminergic catabolic enzymes, displayed differential expression between 22DM_287(+) and 22DF_191(-). The observed decrease in expression of dopamine catabolic enzymes is in agreement with a previous report indicating a reduction of peripheral HVA in 22q11.2 deletion carriers (Boot et al., 2008). Thus, one possible explanation for these findings is that disruption of genes involved in dopamine metabolism and signaling may segregate between 22q11.2
deletion carriers with distinct clinical presentations. It is interesting to note that mDA-neurons generated from 22DF_191(-) had elevated expression levels of DBH, an enzyme that could degrade dopamine, and thus speculatively we suggest, may reflect a compensatory mechanisms in this hiPSC line to offset the increase in DA due to COMT heterozygosity. Another possibility is that differences between 22q11.2 lines may be due to additional genetic variants within the genome. Consistent with this, 22DM_287(+) hiPSCs had a higher PRS compared to 22DF_191(-) and all control hiPSC lines, with the exception of CTF_007, which has a similar PRS for schizophrenia to 22DM_287(+). It also important to note that 22DF_191(-) has 4 duplication CNVs in addition to deletions within the 22q11.2 region. Notably, duplications within the 22q11.2 region has been associated with lower risk for psychosis compared to the general population, suggesting a possible protective role (Rees et al., 2014). Therefore, it is possible that both molecular and clinical differences may be influenced by the presence of duplications at the 22q11.2 locus. Another area of importance that requires further investigation is the influence of biological sex in these findings. For example, sex-specific transcriptional differences have been observed in hiPSCs from schizophrenic individuals (Tiihonen et al., 2019), which may influence pathophysiology. In addition, MAOB and MAOA are located on the X chromosome, and thus may be subject to x-inactivation in females. Whether this is the case or not would impact the interpretation of gene expression data in the context of comparison between male and female 22q11.2 deletion carriers. Moreover, it will be important to consider whether sex also drives the increased DSC levels seen in 22DF_191(-): recent work indicates that women showing greater DSC capacity than men (Nordio et al., 2022).
Previous hiPSC studies using neurons derived from 22q11.2 deletion carriers have demonstrated robust alterations in microRNAs expression, mitochondrial deficits and altered functional properties in forebrain-like neurons (Khan et al., 2020; Li et al., 2021; Zhao et al., 2015), as well as PERK dysfunction in mDA-neurons (Arioka et al., 2021). Clinical phenotypes associated with 22q11.2 deletion, however, are highly variable and not all of the aforementioned studies have used hiPSCs from 22q11.2 deletion subjects with the same clinical presentation. Cellular phenotypes associated with 22q11.2 deletion show variable penetrance, segregating with clinical presentation (Li et al., 2021). It is, therefore, important to consider if any observed cellular and molecular phenotypes relate to the 22q11.2 deletion and/or the associated clinical phenotype (Khan et al., 2020) or other variants within the genome. We have explored this further by generating hiPSCs from 22q11.2 deletion individuals, who have distinct clinical presentations, but a common neuro-imaging endophenotype in elevated DSC. We have subsequently looked for common or distinct molecular phenotypes associated with dopamine synthesis or signaling. Of note, our study used two 22q11.2 deletion hiPSCs lines, and therefore future studies using larger number of lines are required to confirm our findings as well as addressing the issue of sex as a biological variable, given the reported sexual dimorphism of DSC data. Nevertheless, our preliminary results reinforce the importance of considering clinical as well as genetic and molecular information, where possible, when choosing which donors to generate hiPSCs for mechanistic studies relevant understanding genotype-phenotype associations in 22q11.2 deletion carriers.

References
Adhya, D., Swarup, V., Nagy, R., Dutan, L., Shum, C., Valencia-Alarcon, E.P.,
Jozwik, K.M., Mendez, M.A., Horder, J., Loth, E., Nowosiad, P., Lee, I., Skuse, D.,
Flinter, F.A., Murphy, D., McAlonan, G., Geschwind, D.H., Price, J., Carroll, J.,
Srivastava, D.P., Baron-Cohen, S., 2021. Atypical Neurogenesis in Induced
Pluripotent Stem Cells From Autistic Individuals. Biol Psychiatry 89(5), 486-496.
Arioka, Y., Shishido, E., Kushima, I., Suzuki, T., Saito, R., Aiba, A., Mori, D., Ozaki,
N., 2021. Chromosome 22q11.2 deletion causes PERK-dependent vulnerability in
dopaminergic neurons. EBioMedicine 63, 103138.
Boot, E., Booij, J., Zinkstok, J., Abeling, N., de Haan, L., Baas, F., Linszen, D., van
Amelsvoort, T., 2008. Disrupted Dopaminergic Neurotransmission in 22q11 Deletion
Syndrome. Neuropsychopharmacology 33(6), 1252-1258.
Butcher, N.J., Kiehl, T.R., Hazrati, L.N., Chow, E.W., Rogaeva, E., Lang, A.E.,
Bassett, A.S., 2013. Association between early-onset Parkinson disease and
22q11.2 deletion syndrome: identification of a novel genetic form of Parkinson
disease and its clinical implications. JAMA Neurol 70(11), 1359-1366.
Chun, S., Westmoreland, J.J., Bayazitov, I.T., Eddins, D., Pani, A.K., Smeyne, R.J.,
to the auditory cortex in schizophrenia models. Science 344(6188), 1178-1182.
Cocks, G., Curran, S., Gami, P., Uwanogho, D., Jeffries, A.R., Kathuria, A.,
utility of patient specific induced pluripotent stem cells for the modelling of Autistic
Spectrum Disorders. Psychopharmacology (Berl) 231(6), 1079-1088.
Egerton, A., Chaddock, C.A., Winton-Brown, T.T., Bloomfield, M.A., Bhattacharyya,
dysfunction in people at ultra-high risk for psychosis: findings in a second cohort. Biol Psychiatry 74(2), 106-112.


J., Therman, S., Suvisaari, J., Kaprio, J., Cheng, L., Hill, A.F., Lahteenvuoto, M.,
Tohka, J., Giniatullin, R., Lehtonen, S., Koistinaho, J., 2019. Sex-specific
transcriptional and proteomic signatures in schizophrenia. Nat Commun 10(1), 3933.
Trubetskoy, V., Pardinas, A.F., Qi, T., Panagiotaropoulou, G., Awasthi, S., Bigdeli,
T.B., Bryois, J., Chen, C.Y., Dennison, C.A., Hall, L.S., Lam, M., Watanabe, K., Frei,
O., Ge, T., Harwood, J.C., Koopmans, F., Magnusson, S., Richards, A.L., Sidorenko,
J., Wu, Y., Zeng, J., Grove, J., Kim, M., Li, Z., Voloudakis, G., Zhang, W., Adams,
M., Agartz, I., Atkinson, E.G., Agerbo, E., Al Eissa, M., Albus, M., Alexander, M.,
Alizadeh, B.Z., Alptekin, K., Als, T.D., Amin, F., Arolt, V., Arrojo, M., Athanasiu, L,
Azevedo, M.H., Bacanu, S.A., Bass, N.J., Begemann, M., Belliveau, R.A., Bene, J,
Benyamin, B., Bergen, S.E., Blasi, G., Bobes, J., Bonassi, S., Braun, A., Bressan,
R.A., Bromet, E.J., Bruggeman, R., Buckley, P.F., Buckner, R.L., Bybjerg-Grauholm,
J., Cahn, W., Cairns, M.J., Calkins, M.E., Carr, V.J., Castle, D., Catts, S.V.,
Chambert, K.D., Chan, R.C.K., Chaumette, B., Cheng, W., Cheung, E.F.C., Chong,
S.A., Cohen, D., Consoli, A., Cordeiro, Q., Costas, J., Curtis, C., Davidson, M.,
Davis, K.L., de Haan, L., Degenhardt, F., DeLisi, L.E., Demontis, D., Dickerson, F.,
Dikeos, D., Dinan, T., Djurovic, S., Duan, J., Ducci, G., Dudbridge, F., Eriksson, J.G.,
Fananas, L., Faraone, S.V., Fiorentino, A., Forstner, A., Frank, J., Freimer, N.B.,
Fromer, M., Frustaci, A., Gadelha, A., Genovese, G., Gershon, E.S., Giannitelli, M.,
Giegling, I., Giusti-Rodriguez, P., Godard, S., Goldstein, J.I., Gonzalez Penas, J.,
Gonzalez-Pinto, A., Gopal, S., Gratten, J., Green, M.F., Greenwood, T.A., Guillin, O.,
Guloksuz, S., Gur, R.E., Gur, R.C., Gutierrez, B., Hahn, E., Hakonarson, H.,
Haroutunian, V., Hartmann, A.M., Harvey, C., Hayward, C., Henskens, F.A., Herms,
S., Hoffmann, P., Howrigan, D.P., Ikeda, M., Iyegbe, C., Joa, I., Julia, A., Kahler,
A.K., Kam-Thong, T., Kamatani, Y., Karachanak-Yankova, S., Kebir, O., Keller, M.C.,


Contributions


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Conflict of Interest Statement
The authors declare no competing interests.

Figure Legends

Figure 1. Generation of midbrain dopamine neurons from 22q11.2 deletion hiPSCs. (A) Schematic of the 22q11.2 CNVs and total number of coding gene affected in the hiPSC lines generated in this study. (B) Representative images of day
50 mDA-neurons generated from control and 22q11.2 deletion hiPSCs. After 50 days of differentiation mDA-neurons are positive for the pan-neuronal marker MAP2. In addition, mDA-neurons robustly express markers of dopamine identity, including LMX1A and tyrosine hydroxylase (TH). (C) Box and whisker blots represent quantification of DAPI cells positive MAP2; and MAP2 cells positive for LMX1A and TH in DA-neurons generated from control hiPSC lines (CTR_M3, CTM_127 and CTF_007) and 22q11 deletion hiPSC lines (22DF_191(-) and 22DM_287(+)). Error bars are represented as minimum and maximum values; each data point represents technical repeats from 3 independent experiments for each hiPSC line. (D & E) Quantitative PCR (qPCR) analysis of day 50 DA-neurons demonstrates expression of key genes inferring generation of midbrain dopaminergic neurons (TH, LMX1A, NURR1, ASCL1, GRIK2, PITX3 and FOXA2). Comparisons between groups (hiPSC lines) was made using ANOVA with Bonferroni post-hoc analysis; n = 3 independent experiments per hiPSC line; error bars are represented as ±sem.

Figure 2. Expression of genes associated with dopamine synthesis, metabolism and signalling in mDA-neurons generated from control and 22q11.2 deletion hiPSCs. (A) Heatmap of expression values of genes assessed in PCR array clustered based on $2^{\Delta\Delta CT}$ values. Data for all three control lines (CTR_M3, CTM_127 and CTF_007) were combined, whereas data for each 22q11.2 deletion line (22DF_191(-) and 22DM_287(+) were assessed separately. Gene expression values were normalised to 5 housekeeping genes. (B) Normalised expression of TH and COMT in day 50 DA-neurons generated from control hiPSC lines and 22q11 deletion hiPSC lines. (C) Normalised expression of gene associated with dopamine synthesis and metabolism in day 50 DA-neurons generated from
control hiPSC lines and 22q11 deletion hiPSC lines. (D) Normalised expression of gene associated with dopamine signalling in day 50 DA-neurons generated from control hiPSC lines and 22q11 deletion hiPSC lines. (E) Normalised expression of gene associated with dopamine targets in day 50 DA-neurons generated from control hiPSC lines and 22q11 deletion hiPSC lines. (F) Normalised expression of dopamine receptor expression in day 50 DA-neurons generated from control hiPSC lines and 22q11 deletion hiPSC lines. Comparisons between groups (hiPSC lines) was made using ANOVA with Bonferroni post-hoc analysis; n = 3 independent experiments per hiPSC line; error bars are represented as ±sem.
Table 1. Summary of RT² Dopamine and Serotonin PCR array Profiler. All 86 gene assessed (and housekeeper genes) are listed in day 50 DA-neurons from control (CTR) or 22q11.2 deletion (22DF_191(-); 22DM_287(+)) hiPSC lines. Columns named “CTR vs 22DF_191(-)” and “CTR vs 22DM_287(+)” are colour red or green to indicate expression of gene is either significantly upregulated or downregulated respectively, compared to control mDA-neurons.

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<tr>
<th>Class</th>
<th>Gene</th>
<th>ANOVA P&lt;0.05</th>
<th>CTR vs 22DF_191(-)</th>
<th>CTR vs 22DM_287(+)</th>
<th>22DM_287 vs. 2DF_191</th>
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<tr>
<td>Dopamine / catecholamine metabolism</td>
<td>COMT</td>
<td>F (2, 8) = 8.288 P=0.0143</td>
<td>P=0.191</td>
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<td>DDC (AADC)</td>
<td>MAOA</td>
<td>F (2, 8) = 5.265; P=0.0347</td>
<td>P=0.0440</td>
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<td>MAOB</td>
<td>F (2, 8) = 8.661; P=0.0100</td>
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<td>DRD1</td>
<td>F (2,8) = 4.746; P=0.0498</td>
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<td>DRD3</td>
<td>F (2, 8) = 7.481; P=0.0147</td>
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**Dopamine / serotonin gene targets**

**Signal transduction – cAMP & Protein A Kinase signalling**
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<td><strong>PRKACA</strong></td>
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<td><strong>Signal transduction - AKT &amp; PI3 Kinase signalling</strong></td>
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<td><strong>Signal transduction - Phospholipase C signalling</strong></td>
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<td><strong>PLCB3</strong></td>
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<td>F (2, 8) = 7.885</td>
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<td><strong>RPLP0</strong></td>
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</table>
**A**

22DM_287(+)  
CNVs: 1  
#Genes within deleted region: 126

22DF_191(-)  
CNVs: 8  
#Genes within deleted region: 132  
#Genes within duplicated region: 6

**C**

% DAPI cells +ve for MAP2  
% MAP2 cells +ve for LMX1A  
% MAP2 cells +ve for TH

**D**

Normalised TH expression 2^(-ΔΔCt)  
Normalised LMX1A expression 2^(-ΔΔCt)

**E**

Normalised NUR1 expression 2^(-ΔΔCt)  
Normalised ASCL1 expression 2^(-ΔΔCt)  
Normalised FOXA2 expression 2^(-ΔΔCt)  
Normalised PITX3 expression 2^(-ΔΔCt)  
Normalised GIRK2 expression 2^(-ΔΔCt)
Matthew J. Reid$^{1,2}$, Maria Rogdaki$^{1,3,4}$, Lucia Dutan$^{1,2}$, Bjørn Hanger$^{1,2}$, Kaarin Sabad$^{1,2}$, Roland Nagy$^{1,2}$, Dwaipayan Adhya$^{1,6}$, Simon Baron-Cohen$^{6}$, Grainne McAlonan$^{2,5}$, Jack Price$^{1,2}$, Anthony C. Vernon$^{1,2}$, Oliver D. Howes$^{2,4}$, Deepak P. Srivastava$^{1,2}$.

$^\*$ = equal contribution

$^\#$ = joint senior authors

* = corresponding author: deepak.srivastava@kcl.ac.uk

**Supplemental Material:**

Supplemental Materials and Methods

Supplemental Results

Supplemental Tables
Supplemental Materials and Methods

Clinical information on the individuals with 22q11.2 deletion

Participants were recruited and methods carried out in accordance with the ‘Patient iPSCs for Neurodevelopmental Disorders (PiNDs) study’ (REC No 13/LO/1218). Informed consent was obtained from all subjects for participation in the PiNDs study. Ethical approval for the PiNDs study was provided by the NHS Research Ethics Committee at the South London and Maudsley (SLaM) NHS R&D Office.

Participant 22DM_287(+) was a male individual (30 years old) carrying a 22q11.2 deletion. This individual had a diagnosis of autism spectrum disorder and mild intellectual disability. During the clinical assessment, he scored highly in prodromal psychotic and anxiety symptoms, as measured with the Comprehensive Assessment of at-Risk Mental state and Beck’s Anxiety scale respectively (Beck et al., 1988; Yung et al., 2005). In addition, this individual displayed elevated levels of dopamine synthesis capacity as determined by [18F]-DOPA PET, compared to healthy controls (Rogdaki et al., 2021). Six months following the PET scan, this individual developed psychosis and later on received the diagnosis of schizophrenia. He was not on any medication at the time of the scanning.

Participant 22DF_191(-) was a female individual (19 years old) carrying a 22q11.2 deletion, and who had cardiac defects. She had unremarkable past psychiatric history and did not present with any significant psychopathology during the assessment. This individual also displayed elevated levels of dopamine synthesis capacity as determined by [18F]-DOPA PET, compared to healthy controls (Rogdaki et al., 2021).

For further details, please Supplementary Table 1.

Reagents
The following antibodies were used: anti-MAP2 chicken polyclonal (Abcam; AB104896); anti-LMX1A rabbit polyclonal (Abcam; AB139726); anti-tyrosine hydroxylase (TH) rabbit polyclonal (Millipore; AB152); anti-COMT rabbit monoclonal (Abcam; AB126618); anti-SMA rabbit polyclonal (Abcam; AB5694); anti-AFP mouse monoclonal (Millipore; AB3980); anti-Nanog rabbit polyclonal (Abcam; AB80892); anti-OCT4 rabbit polyclonal (Life Technologies; 701756); anti-SSEA4 mouse monoclonal (Life Technologies; MA0121); anti-TRA-1-81 mouse monoclonal (Life Technologies; MA0124); anti-Tuj1 (TUBB3) mouse monoclonal (BioLegend; 801201).

**Generation of human induced pluripotent stem cells (hiPSCs)**

All hiPSC lines were generated from primary keratinocytes as described previously (Cocks et al., 2014). Briefly, 1 x 10^5 primary hair root keratinocytes were reprogrammed by introducing OCT4, SOX2, KLF4 and C-MYC factors with a CytoTune-iPS 2.0 Sendai expressing Reprogramming Kit (ThermoFisher, A16517). Transformed keratinocytes were plated onto an irradiated MEF feeder layer (Millipore) supplemented Epilife medium for ten days before switching to 'hES media', which consisted of KO-DMEM/F12 supplemented with 20% Knock-out serum replacement, Non-essential amino acids, Glutamax, β-mercaptoethanol (all from Life Technologies) and bFGF (10 ng/ml; Peprotech) (KOSR media). After a further two weeks, reprogrammed colonies were selected and plated on Geltrex (Life technologies) coated Nunc treated multidishes (Thermo Scientific) into E8 media (Life Technologies). hiPSCs reprogramming was validated by genome-wide expression profiling using Illumina Beadchip v4 and the bioinformatics tool 'Pluritest' (Supplemental Figure 1A). Additionally, the tri-lineage differentiation potential was established by embryoid body formation; immunocytochemistry (ICC) to validate the
expression of different pluripotency markers including Nanog, OCT4, SSEA4 and TRA1-81 and the alkaline phosphatase activity by Alkaline phosphatase expression kit (Milipore) (Supplemental Figure 1B-D). Genome integrity was assessed by an Illumina Human CytoSNP-12v2.1 beadchip array (~300,000 markers) and analyzed using KaryoStudio software (Illumina). hiPSCs were incubated in hypoxic conditions at 37°C and maintained in E8 media replaced every 24 hours until the cells monolayer reach ~95% confluence. A summary of hiPSC lines used in this study can be found in Supplementary Table 2.

**Neuronal differentiation**

Generation of midbrain floor plate neural progenitor (mFPP) cells from hiPSC lines and subsequent generation of dopamine (DA) neurons was performed as outlined in (Fedele et al., 2017; Kriks et al., 2011) (Supplemental Figure 2A). Briefly, neuronal differentiation of 30% confluent iPSCs was initiated by replacing Essential 8 medium (Gibco) with ‘neuralization media’ composed of KnockOut Serum Replacement (KOSR) medium (Gibco) supplemented with various small molecules to induce neuralization (Fedele et al., 2017; Kriks et al., 2011). Cells were maintained at 37°C in normoxic conditions in neuralization medium for 10 days. During this period, media was replaced every 24 hours whilst transitioning from base KOSR into N2 medium (Gibco) and altering small molecules. Media was supplemented with 10 μM SB431542 (Sigma-Aldrich) and 250 ng/ml LDN193189 (Sigma-Aldrich) on day 1. Subsequent media on days 2-4 contained additional 2 μM purmorphamine (Sigma-Aldrich), 50 μg/ml smoothened agonist (SAG; Sigma-Aldrich) 100 ng/ml Fibroblast growth factor 8 (FGF8) and 3 μM CHIR99021 (Sigma-Aldrich). SB43 was removed from day 5. SAG and FGF8 were removed from day 8. Purmorphamine was removed from day 9. On
day 10, mFPPs were passaged using Accutase (Life Technologies) and re-plated in
N2 media supplemented with 250 ng/ml LDN193189 (Sigma-Aldrich) and 3 μM
CHIR99021 (Sigma-Aldrich) only (mFPP expansion-media). Cells were passaged and
expanded a further three times at reduced ratios. Cells were cryopreserved in mFPP
expansion-media with 10% DMSO for future use. For terminal plating of mFFPs into
dopamine neurons (DA-neurons), cells were passaged or thawed and plated as a
single cell suspension at low density onto 0.6 μg/cm² poly-D-lysine (Gibco), 1 μg/cm²
fibronectin (Sigma-Aldrich) and 2 μg/cm² laminin (Sigma-Aldrich) coated Nunc Cell-
Culture treated multidishes (Thermo Scientific) in B27 media (Gibco) supplemented
with 20 ng/ML BDNF (Peprotech), 20 ng/ml GDNF (Perpotech), 200 μM AA2P (Sigma-
Aldrich), 0.5 mM cAMP (Sigma-Aldrich), 1 ng/ml TGFβ (Miltenyi Biotec) and 10 μM
DAPT (Santa Cruz Biotechnology). Media was then changed every other day until day
50 when they were used for experimentation. During all passaging and terminal plating
steps 10 μM Rock Inhibitor Y-27632 (Sigma Aldrich) was added to aid cell survival.

qRT-PCR

Total RNA harvested and lysed with Trizol reagent (Life technologies) and isolated by
centrifugation with 100% Chloroform, following by 100% isopropanol and lastly by 75%
ethanol. The RNA was purified by precipitation with 100% ethanol and Sodium acetate
(Life technologies) and quantify with the NanoDrop 1000 Spectrophotometer (Thermo
scientific). Residual genomic DNA was removed by addition of TURBO DNA-free (Life
technologies) and incubation at 37°C for 30 minutes. Complementary DNA (cDNA)
was synthesized from 1 μg of total RNA from each extraction using random hexamer
primers and SuperScript III (Life Technologies) following the manufacturer's
recommendations. qPCR was performed with HOT FIREPol EvaGreen qPCR Mix Plus
ROX (Solis Biodyne) carried out according to the manufacturer’s instructions in a total volume of 20 µl, containing 1:5 diluted cDNA, qPCR mix and primers at to a final concentration of 0.3 μM. PCR reaction conditions: 95°C for 15 minutes for the initial denaturation followed by 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds during 33 cycles. The melting curve analyses was preformed from 60°C to 95°C with readings every 1°C. The 2^ΔΔCT comparative method for relative quantification was used to quantify the genes expression. Three independent differentiations per hiPSC line were used in these experiments. The data CT values were normalized to GAPDH, RPL27 and SDHA housekeeping genes. Primer sequences can be found in Supplementary Table 3.

**RT² Profiler PCR array**

To assess the expression profile of dopamine-related genes in DA-neurons derived from hiPSC lines, we used the Human Dopamine and Serotonin Pathway RT² Profiler array (Qiagen) on the BioMark HD (Fluidigm). This assay profiles the expression of 84 genes associated with dopamine and serotonin systems. Generation of cDNA and subsequent preamplification of RNA isolated from day 50 DA-hiPSC-neurons, was performed using the RT² PreAMP cDNA Synthesis Kit and RT² PreAMP Pathway Primer Mixes kits (Qiagen). Following preamplification, samples were diluted with nuclease-free water and added to the RT² SYBR green qPCR Master Mix (Qiagen); loaded onto reaction plates and the real-time amplification data (Ct values) were determined using a BioMark HD cycler (Fluidigm). Analysis of gene expression from real-time results was carried out using the RT² profiler PCR array data analysis v3.5 software provided by Qiagen. Each array contained 5 separate housekeeping genes (ACTB, B2M, GAPDH, HPRT1, and RPLP0) that were used for normalization of the
sample data. Normalization to the house keeping genes (HKG) was performed by calculating the $\Delta$Ct for each gene of interest (GOI) in the plate (Ct value of GOI - Ct value of HKG). Any Ct value $>35$ was considered to be a negative call. The $2^{\Delta\Delta\text{CT}}$ comparative method for relative quantification was used to quantify the genes expression. Three independent differentiations per hiPSC line were used in these experiments. The differential expression analyses were performed by using a one-way ANOVA with Bonferroni correction and 95% confidence interval with the Prism package of GraphPad software. The heatmap was generated by using the log2-transformed $2^{\Delta\Delta\text{CT}}$ values using hierarchical clustering methods with the packages pheatmap and rcolourbrewer in RStudio.

**Immunocytochemistry (ICC)**

Treated DA-neurons were fixed with 4% formaldehyde plus 4% sucrose in PBS. Fixed neurons were permeabilized in 0.1% Triton-X-100 in PBS for 15 minutes and blocked in 4% normal goat serum in PBS for 1 hour at room temperature. Primary antibodies were added to the block solution in an antibody dependent concentration and incubated overnight at 4°C. Immunoreactivity was achieved by incubating the cells with 1:500 concentration of Alexa Fluor 594 conjugated anti-mouse IgG, Alexa Fluor 594 conjugated anti-goat IgG and Alexa Fluor 488 conjugated anti-rabbit IgG in block buffer. For nuclei staining a 1:2000 concentration of DAPI (Thermo Fisher) was used.

**Imaging of immunofluorescence by high content image screening**

mFPs were plated at a density of $1\times10^4$ cells/well on poly-D-lysine and laminin-coated optical-bottom 96 well plates with polymer base (ThermoScientific). mFPs were differentiated into DA-neurons, grown until day 50, fixed and processed for ICC as
described above. Image acquisition was performed using an Opera Phenix High Content screening platform (Perkin Elmer): images were acquired using a 20 x (NA 0.4) objective. The Harmony High Content Imaging and Analysis Software was used to determine the number of DAPI or MAP2 cells positive for specific markers. For each hiPSC line, 2 biological replicates with 3 technical replicates per condition were imaged and analyzed: 15 randomly selected fields from each technical replicate was examined. Data from each technical replicate was used as a single data point and compared between each biological replicate and each hiPSC line. The means of percentages of positive cells were compared by an ANOVA.

**Statistical analysis**

All statistical analysis was performed in GraphPad. Differences in $2^{\Delta\Delta CT}$, relative expression (Fold change) and cell number parameters were identified by comparisons between multiple conditions: the main effects and simple effects were probed by one-way ANOVAs with Tukey or Bonferroni correction for multiple comparisons. Differences were considered significant if $P$ was lower than 0.05 ($p < 0.05$). Error bars represent standard errors of the mean unless stated otherwise.
Supplemental Results

**Supplemental Figure 1. Characterisation of hiPSC lines generated from patient hair keratinocytes.** (A) PluriTest analysis of Illumina HT12v4 transcriptome array data was used to determine that hiPSC generated cluster with pluripotent stem cells (red cloud) and not with partly- or differentiated cells (blue clouds). (B) Representative example of positive Alkaline phosphatase activity of hiPSCs. (C) Example of ICC validation of pluripotency of hiPSC lines using antibodies against Nanog, OCT4, SSEA4 and TRA1-81. (D) Representative example of tri-lineage differentiation potential by embryoid body formation. (E) Predicted polygenic risk score for schizophrenia based on Psychiatric Genomics Consortium (PGC) 3 GWAS summary.
statistics, using PRSice (Euesden et al., 2015) and genome-wide SNP genotype data of control and 22q11.2 deletion lines.

Supplemental Figure 2. Generation of mDA-neurons from control and 22q11.2 deletion hiPSC lines. (A) Schematic overview of differentiation protocol used in study. (B) QPCR assessment of OCT4 expression levels in undifferentiated hiPSCs, mFPP and day 50 mDA-neurons.
22DM_287(+) – Genes within deleted region

- FAM230F
- CLTCL1
- TBX1
- MIR1306
- USP41
- AIFM3
- DGR6
- HIRA
- GNB1L
- TRMT2A
- ZNF74
- LTR1
- PRODH
- MRPL40
- TXNRD2
- MIR6818
- SCARF2
- THAP7
- DGCR5
- C22orf39
- COMT
- RANBP1
- KLHL22
- P2RX6
- DGCR9
- UFD1
- MIR4761
- ZDHHC8
- LOC101928824
- SLC7A4
- DGCR2
- CDC45
- ARVCF
- CCDC188
- MED15
- MIR649
- DGCR11
- CLDN5
- TANGO2
- LINC00896
- PI4KA
- POM121L7P
- TSSK2
- LINC00895
- MIR185
- RTN4R
- SERPIND1
- RIMBP3B
- LINC01311
- SEPTINS
- DGCR8
- MIR1286
- SNAP29
- HIC2
- SLC25A1
- GP1BB
- MIR3618
- DGCR6L
- CRKL

46 coding genes
7 microRNA
5 IncRNAs

22DF_191(−) – Genes within deleted regions

- TSSK2
- CLDN5
- DGCR8
- USP41
- SNAP29
- TMEM191C
- PPM1F
- GSC2
- LINC00895
- MIR3618
- ZNF74
- CRKL
- RIMBP3C
- LINC01311
- CCDC188
- MIR1306
- SCARF2
- AIFM3
- UBE2L3
- TOP3B
- SLC25A1
- SEPTINS
- TRMT2A
- KLHL22
- P2RX6
- YDJC
- CLTCL1
- GP1BB
- MIR6818
- LTR1
- MIR649
- CCDC116
- HIRA
- RTN4R
- RANBP1
- THAP7
- LRRC74B
- SDF2L1
- MRPL40
- TBX1
- ZDHHC8
- MED15
- POM121L7P
- MIR301B
- C22orf39
- GNB1L
- LINC00896
- SLC7A4
- YPEL1
- MIR1308
- UFD1
- COMT
- MIR1286
- PI4KA
- RIMBP3B
- PPIL2
- CDC45
- MIR4761
- DGCR6L
- SERPIND1
- HIC2
- MAPK1

41 coding genes
6 microRNAs
2 IncRNAs
13 Coding genes outside of classic deletion

22DF_191(−) – Genes within duplicated regions

- ARVCF
- MIR185
- 2 coding genes
- 1 microRNA
Supplemental Figure 3. Characterisation of CNVs in 22q11.2 locus in 22q11.2 deletion hiPSC lines. Using genome-wide SNP genotype data from 22q11.2 deletion hiPSC lines, CNVs within the 22q11.2 chromosomal regions were calculated.

Supplementary Table 1. $K^{icer}$ estimates for the whole striatum and for the striatal subdivisions for a) the carriers of 22q11.2 deletion from whom hiPSCs were derived, b) 22q11.2 deletion carriers, c) healthy control group, as provided by Rogdaki et al, 2021. $K^{icer}$ estimates for groups b and c are provided in mean (SD).

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<th>$K^{icer}$ estimates</th>
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<tr>
<td></td>
<td>22DM_287(+)</td>
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<tr>
<td>Whole striatum</td>
<td>0.01599</td>
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<tr>
<td>Functional subdivisions</td>
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<tr>
<td>associative</td>
<td>0.01628</td>
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<tr>
<td>sensorimotor</td>
<td>0.015</td>
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<tr>
<td>limbic</td>
<td>0.0168</td>
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### Supplemental Table 2. Summary of hiPSC lines used in study.

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<th>iPSC line</th>
<th>Clone</th>
<th>Age range</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>CytoSNP-12v2.1</th>
<th>EB differentiation</th>
<th>‘PluriTest’</th>
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<tr>
<td>CTF_007</td>
<td>10</td>
<td>30-50</td>
<td>Apparently Healthy</td>
<td>Female</td>
<td>Pass</td>
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<td>CTM_M3</td>
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<td>22DF_191(-)</td>
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<td>Female</td>
<td>Pass</td>
<td>Yes</td>
<td>Pass</td>
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<tr>
<td>22DM_287(+)</td>
<td>01</td>
<td>30-50</td>
<td>22q11.2 DS - autism spectrum disorder; mild intellectual disability; schizophrenia</td>
<td>Male</td>
<td>Pass</td>
<td>Yes</td>
<td>Pass</td>
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Supplemental Table 3. Summary of qPCR primers used in study.

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<th>Primer sequence (5' to 3')</th>
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<td>FOXA2 F</td>
<td>GAGCCCGAGGGCTACTCC</td>
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<tr>
<td>FOXA2 R</td>
<td>GCCCACGTACGACGACAT</td>
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<tr>
<td>LMX1A F</td>
<td>AGAGCTCGCCTACCAGGTG</td>
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<tr>
<td>LMX1A R</td>
<td>AGAAGGAGGCGAGGTGT</td>
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<td>POU5F1 F</td>
<td>ATCCAGTCCCAGGACATCAA</td>
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<tr>
<td>POU5F1 R</td>
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<td>ASCL1 F</td>
<td>GGACGAGGGCTTTACGAC</td>
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<td>GIRK2 R</td>
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<td>TH R</td>
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<td>PITX3 R</td>
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<td>CGACATTTCGCTTTCCTCC</td>
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<tr>
<td>NURR1 R</td>
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References


