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The effect of the DISC1 Ser704Cys polymorphism on striatal dopamine synthesis capacity: an [¹⁸F]-DOPA PET study

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Abstract

Whilst the role of the Disrupted-in-Schizophrenia 1 (*DISC1*) gene in the aetiology of major mental illnesses is debated, the characterisation of its function lends it credibility as a candidate. A key aspect of this functional characterisation is the determination of the role of common non-synonymous polymorphisms on normal variation within these functions. The common allele (A) of the *DISC1* SNP rs821616 encodes a serine at the Ser704Cys polymorphism, and has been shown to increase the phosphorylation of extracellular signal-regulated protein Kinases 1 and 2 (ERK1/2) which stimulate the phosphorylation of tyrosine hydroxylase, the rate-limiting enzyme for dopamine biosynthesis. We therefore set out to test the hypothesis that human A (serine) homozygotes would show elevated dopamine synthesis capacity compared to individuals cysteine hetero/homozygotes (AT or TT genotype) for rs821616. [¹⁸F]-DOPA PET was used to index striatal dopamine synthesis capacity as the influx rate constant K_i^{cer} in healthy volunteers *DISC1* rs821616 serine homozygotes (N=46) and healthy volunteers *DISC1* rs821616 ~~eysteine carriers~~cysteine hetero/homozygotes (N=56), matched for age, gender, ethnicity and using three scanners. We found *DISC1* rs821616 serine homozygotes exhibited a significantly higher striatal K_i^{cer} compared to ~~eysteine carriers~~cysteine hetero/homozygotes (p-value=0.012) explaining 6.4% of the variance (partial eta squared=0.064). Our finding is consistent with its previous association with heightened activation of ERK1/2, which stimulates tyrosine hydroxylase activity for dopamine synthesis. This could be a potential mechanism mediating risk for psychosis, lending further credibility to the fact that *DISC1* is of functional interest in the aetiology of major mental illness.

Introduction

The dopamine hypothesis has been a leading theory underlying the neurobiology of schizophrenia for the last four decades (1, 2). The hypothesis was initially based on evidence showing that antipsychotic medications block dopamine receptors (3-5) and that drugs increasing dopamine levels elicit psychotic symptoms in healthy people (6-8) and people with schizophrenia (9, 10). Using [¹⁸F] fluoro-3,4-dihydroxyphenyl-L-alanine (F-DOPA) Positron Emission Tomography (PET), increased presynaptic dopamine synthesis capacity has been found in schizophrenia (11), people with prodromal psychotic symptoms (12, 13) and those with clinical progression to psychosis (14). Whilst a substantial body of evidence supports the role of increased presynaptic dopamine synthesis capacity in the pathoetiology of psychosis, little is known about how genetic factors affect the implicated dopamine system(s) (15).

The *Disrupted-in-Schizophrenia 1 (DISC1)* gene was originally discovered at the breakpoint of a balanced t(1;11)(q42;q14.3) translocation in a Scottish family with a high-prevalence of psychiatric disorders including schizophrenia (16-18). Further evidence for a link between *DISC1* and psychotic and affective disorders emerged from the follow-up of families displaying rare *DISC1* mutations (19, 20) and large family-based studies in the population isolate of Finland (21-23) although a large meta-analysis of families did not observe linkage at this region (24). Furthermore, evidence from individual population-based cohorts has been inconsistent (25, 26) leading to ongoing debate on its involvement in schizophrenia (27, 28). Whilst this controversy remains unresolved, there is value in seeking convergent evidence via studies elucidating the functional impact of the gene and its variations (29-32). *DISC1* is a scaffold protein involved in a wide range of neuronal functions including neuro-signalling (30, 33). Preclinical studies show that *DISC1* variant models exhibit increased amphetamine-induced dopamine release in the ventral striatum (see (34-37) reviewed in (38), indicating that *DISC1* variations might affect presynaptic dopamine synthesis capacity.

26 One of the most studied *DISC1* single nucleotide polymorphisms (SNPs) is rs821616 which is a non-
27 synonymous mutation leading to the translation of a serine (A allele) or a cysteine (T allele) at codon
28 704 in exon 11 (39). Importantly, this polymorphism represents therefore not only a variation at the
29 genetic sequence level but also at the protein sequence level of *DISC1*. At a molecular level,
30 Hashimoto et al. (2006) found that overexpression of the serine variant of codon 704 by viral
31 transduction resulted in a significant increase in phosphorylated ERK1/2, the more biologically active
32 form (40). ERK1/2 in turn regulates the state of phosphorylation of tyrosine hydroxylase, the rate-
33 limiting enzyme for dopamine biosynthesis, to increase its activity and subsequent dopamine synthesis
34 by up to two-fold (41-44). Dopamine is synthesized by converting first tyrosine into dihydroxyphenyl-
35 L-alanine (L-DOPA) by tyrosine hydroxylase, and second dihydroxyphenyl-L-alanine (L-DOPA) into
36 dopamine by aromatic acid decarboxylase (45). [¹⁸F]-DOPA PET signal reflects aromatic acid
37 decarboxylase function and dopamine storage capacity (45), but not directly tyrosine hydroxylase
38 function. However, it should be noted that 1) tyrosine hydroxylase is the rate limiting step for
39 dopamine synthesis capacity (43) and 2) the topological distribution of the [¹⁸F]-DOPA signal
40 correlates highly with tyrosine hydroxylase immunostaining in unilaterally 6- hydroxydopamine (6-
41 OHDA)-lesioned rats, thus indicating that the [¹⁸F]-DOPA signal is strongly influenced by
42 endogenous dopamine formed by tyrosine hydroxylase (46).

43
44 In summary, preclinical findings suggest that the Ser704Cys variation affects dopamine synthesis by
45 regulating ERK1/2 and its control over tyrosine hydroxylase activity. However, it remains unknown
46 whether the Ser704Cys variation is associated with altered dopamine synthesis in humans. The aim of
47 this study was therefore to test the hypothesis that serine homozygotes would exhibit increased striatal
48 dopamine synthesis capacity relative to ~~eysteine~~ cysteine hetero/homozygotes.

Results

Demographics, scan parameters including the injected dose and substance use characteristics are shown in table 1. A total of 46 serine homozygotes and 56 ~~eysteine-carriers~~cysteine hetero/homozygotes (which encompass 45 heterozygotes and 11 cysteine homozygotes) were included in the study. The genotype frequencies (shown in table 1) did not significantly deviate from Hardy-Weinberg equilibrium ($\chi^2 = 1.422$ with $p=0.233$), with a Minor Allele Frequency (T allele) of 0.335. Age (year) and K_i^{ser} (1/min) in the whole striatum were normally distributed across the two groups whereas injected dose (MBq) was not. There was no significant difference in age between groups $t(100)=1.588$, $p=0.115$ (independent t test) and no significant difference in injected dose $p=0.408$ (Mann Whitney test). Levene's test indicated no difference between the variances in the two groups, $F=0.398$, $p=0.529$. The univariate ANCOVA showed that the main effect of the *DISC1* SNP rs821616 on the dopamine synthesis capacity in the whole striatum was significant, $F(1,96) = 6.555$, $p=0.012$, partial eta squared =0.064. The effects of the covariates were: for scanner, $F(1,96)=16.573$, $p<0.01$, partial eta squared =0.147, age, $F(1,96)=1.056$, $p=0.307$, partial eta squared =0.011, gender, $F(1,96)=0.114$, $p=0.736$, partial eta squared=0.001, ethnicity, $F(1,96)=0.061$, $p=0.805$, partial eta squared=0.001.

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Discussion

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In line with our hypothesis, we found that participants ~~with the AA genotype~~ (serine homozygotes ~~(AA genotype)~~) ~~for~~ the Ser704Cys functional DISC1 polymorphism exhibited a significantly greater K_i^{cer} value in the whole striatum, indicating greater dopamine synthesis capacity compared to ~~cysteine hetero/homozygotes (AT or TT genotype)~~ ~~(cysteine carriers)~~. This result is in accordance with preclinical evidence showing that the serine 704 DISC1 variant increases the activity of ERK1/2, which in turn enhances the phosphorylation of tyrosine hydroxylase, the rate limiting step in dopamine synthesis (41, 47).

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Limitations

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The main limitation of this study was that we used data from three different PET scanners, which could add error variance. However, scanner was included as a covariate to adjust for this. Furthermore, the effect of the Ser704Cys polymorphism remained significant when we only included subjects from PET scanner 2 ($F(1,28) = 5.273$, $p=0.029$ (N=16 ~~eysteine carriers~~cysteine hetero/homozygotes, N=17 serine homozygotes)), but not PET scanner 1 only ($F(1,30) = 0.766$, $p=0.388$, (N=19 ~~eysteine carriers~~cysteine hetero/homozygotes, N=16 serine homozygotes)) and PET scanner 3 only ($F(1,29) = 0.426$, $p=0.519$, (N=21 ~~eysteine carriers~~cysteine hetero/homozygotes, N=13 serine homozygotes)). It is important to recognise that we measured the final step in the synthesis of dopamine, the conversion of L-DOPA into dopamine via aromatic acid decarboxylase (AADC). However, the parameter measured could be affected by other variables including the uptake of L-DOPA into the brain, although this should be controlled for by the reference region and there is no *a priori* reason to consider that this should be affected by the DISC1 protein. Importantly, this polymorphism was chosen based on a specific prior hypothesis. Although there was evidence to reject the null hypothesis, the p-value would not survive genome-wide correction and therefore the result requires replication.

Implications for mental disorders

The Ser704Cys polymorphism has been associated with schizophrenia with an odds ratio in the range of 1.3 – 4.18 in various populations including European (48), mixed European/African-American (49), and Chinese Han (50-52). Inconsistencies have been found, with some studies indicating increased risk associated with the A allele (serine) (48, 51), whilst others the T (cysteine) allele (50, 52) and no association found (25) mainly in the Japanese population (53-55). A recent meta-analysis has also reported association of the A (serine) allele with schizophrenia in Chinese (OR=1.338) and Japanese populations (OR=1.524), as well as in the overall mixed race sample (56). The inconsistencies in these results might be due to different ethnic populations. It should be noted that ever expanding studies of European ancestry population level genetic variants in schizophrenia continually demonstrate no significant associations at the entire DISC1 locus (57, 58), although there is evidence implicating the DISC1 interactor phosphodiesterase 4B (PDE4B) as a genome-wide significant single gene locus in a recent large schizophrenia genome-wide association study (GWAS) (58). Whilst GWAS have made crucial advances in the understanding of the genetic of schizophrenia, the biological mechanisms directly underlying the disorder remain yet poorly elucidated (59-61). In this context, the DISC1 protein has been suggested as a biological candidate of interest for investigating molecular mechanisms of mental illnesses at the protein levels (33, 62). Beyond studies of dichotomous diagnoses, the serine allele has also been associated with increased risk for poor concentration among Korean patients with schizophrenia (63), increased severity of positive symptoms and hallucinations in European patients with First-Episode Psychosis (64) and increased lifetime severity of delusions in European patients with schizophrenia (65). A potential mechanism for the increased risk could be by dysregulating the control of dopamine to lead to increased dopamine synthesis. Findings in prodromal populations show that increased dopamine synthesis is associated with increased risk for psychosis (12, 13). The difference in dopamine synthesis capacity we observe here between serine homozygotes and carriers of the alternative allele is much smaller than the differences seen in at risk subjects (14,

114 66). It is therefore likely that the Ser704Cys variant interacts with other genetic changes to mediate
115 risk, potentially by affecting dopamine synthesis.

116
117 The fact that the common serine allele has been described as the risk allele is compatible with
118 schizophrenia GWAS, in which approximately 50% of the implicated index SNPs are the more
119 common alleles (67). At the population level, the genetic susceptibility to schizophrenia is caused by a
120 few rare variants of high penetrance (mainly copy number variants and translocations) and many
121 common variants of small penetrance (SNPs and variable number of tandem repeats) (68). As each
122 SNP very minimally impacts schizophrenia risk and is compatible with modern models of natural
123 selection (67), it is expected that other genetic factors are needed, in the same individual, to increase
124 the liability to a point of schizophrenia onset. For example, the Ser704Cys site affects interaction with
125 nuclear distribution element-like 1 (NDEL1) and its homolog Nuclear Distribution Element 1 (NDE1,
126 also known as NudE) (69, 70), and there is evidence for an interaction between NDEL1 rs1391768
127 and the Ser704 allele and the NDE1 rs3784859 and the Cys704 allele on the risk for schizophrenia in
128 European participants (71). Ser704Cys is also the binding site for proteins such as kendrin (also
129 known as pericentrin PCNT) and Pericentriolar material 1 (PCM1) (72), which have been both
130 described as risk factor genes for schizophrenia (73). Furthermore, environmental factors such as
131 exposure to psychosocial stress may also interact with the Ser704Cys polymorphism to affect
132 dopamine function and mediate risk for schizophrenia (15). Interestingly, using a transgenic
133 expression of truncated human Disc1 protein with dominant-negative effect, Niwa et al. have shown
134 that an interaction between *DISC1* and stress exposure, as a 3 week social isolation paradigm,
135 increased dopamine release after amphetamine challenge (34) and induced alterations in DNA
136 methylation of the tyrosine hydroxylase gene (74).

137
138 Evidence also suggests that the Ser704Cys polymorphism is a risk factor for affective disorders. The
139 cysteine allele has been associated with major depression in Japanese population (47), and shown to
140 form a protective haplotype for bipolar spectrum disorder with two others *DISC1* SNPs (rs1411771

141 and rs980989) in Finnish population (75), whereas a higher serine allele rate has been found in South
142 Indian population with bipolar disorder (76). Interestingly, increased dopamine synthesis capacity is
143 seen in both mania (77) and bipolar psychosis (78), whilst major depression with affective flattening
144 is characterized by a decreased synthesis capacity (79, 80).

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146 The Ser704Cys SNP has also been shown to have a functional impact at the brain level (39).
147 Compared to healthy ~~eysteine carriers~~cysteine hetero/homozygotes, serine homozygotes display
148 increased (for the same level of performance, thus putatively inefficient) prefrontal cortex activation in
149 the left middle and left superior frontal gyri and in the homologous right superior frontal gyrus, the left
150 inferior frontal and cingulate cortex, the thalamus and the caudate nucleus in a verbal fluency task
151 (81), as well as an effect on thalamic-prefrontal connectivity (82). Ser704Cys SNP has also been
152 shown to affect activation during declarative memory task with inconsistent findings. Callicott et al
153 (48) found decreased activation bilaterally in the hippocampal formation during a declarative memory
154 task and increased activation bilaterally in the hippocampal formation in an N-back task in Ser704
155 homozygotes controls compared to ~~eysteine carriers~~cysteine hetero/homozygotes, whereas Di Giorgio
156 et al (83) found increased hippocampal formation/dorsolateral prefrontal cortex coupling during
157 memory encoding in a declarative memory task in serine homozygotes compared to healthy ~~eysteine~~
158 ~~carriers~~cysteine hetero/homozygotes.

159
160 In summary, our results provide unprecedented preliminary evidence that DISC1 Ser704Cys has an
161 impact on the dopamine synthesis capacity, in a large sample of 102 healthy volunteers. Further
162 studies should aim at 1) replicating this result in different cohorts; 2) investigating potential epistatic
163 interactions with *DISC1* and other risk genes. Genetic studies based on molecular evidence could help
164 identify the molecular mechanism that underlies the pathoetiology of dopamine-related disorders
165 such as psychotic disorders, and help identify novel potential treatment targets (15).

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Conclusion

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We found that the serine allele of DISC1 Ser704Cys (rs821616) was associated with significantly higher striatal dopamine synthesis capacity, consistently with its previous association with heightened activation of ERK1/2 which stimulates tyrosine hydroxylase activity for dopamine synthesis. This implicates the DISC1 polymorphism in altering a psychosis relevant mechanism in the brain i.e. the facilitation of greater dopamine synthesis capacity. Although, this effect of rs821616 may be of too small effect to be identified in population-based studies of end state diagnoses at their current large size, it continues to implicate the functional role of DISC1. Firstly by highlighting the role of this polymorphism at this gene in creating variation within the normal functioning of the brain, but also by indicating this function as a potential mechanism through which other rare or familial mutations for major mental illnesses could disrupt functioning and increase risk to these devastating disorders.

177

178 **Material and Methods**

179 **Overview**

180 All participants gave informed written consent to take part after full description of the study. All
181 studies were approved by the institutional review board and the local research ethics committee.

182 **Participants**

183 Participants were recruited via advertisement in local media based in London. One hundred and
184 twenty-three participants underwent a [¹⁸F]-DOPA PET scan. For all participants the inclusion criteria
185 were 1) age above 18 years; 2) capacity to give written informed consent. The exclusion criteria were
186 1) any current medical conditions or history of medical condition (past minor self-limiting conditions
187 were permitted); 2) history of a psychiatric disorder as determined by the Structured Clinical Interview
188 for DSM-IV Axis 1 Disorders, Clinician Version (SCID-CV) (84); 3) history of substance
189 abuse/dependence as determined by the Structured Clinical Interview for DSM-IV Axis 1 Disorders,
190 Clinician Version (SCID-CV) (84); 4) history of head injury with a loss of consciousness; 5) a family
191 history of any psychotic disorder in first- or second-degree relatives; 6) contraindications to positron
192 emission tomography (PET) scanning (significant prior exposure to radiation, pregnancy or breast
193 feeding). All participants provided urine samples prior to the scan to screen for drug use and
194 pregnancy test in women. Six participants were excluded due to positive urine THC screening, 12
195 participants were excluded to contamination of samples and 3 participants were excluded due to
196 current psychotropic medication use. This resulted in the final inclusion of 102 participants (46
197 females/56 males, age: 30.2±9.3 years (mean±Standard Deviation SD)). Both scanning and imaging
198 analysis were done blind to the genotype status.

[¹⁸F]-FDOPA PET

PET data were acquired using three different PET scanners. PET scanner 1 was an ECAT HR+ 962 PET scanner (CTI/Siemens, Knoxville, Tennessee). The dynamic images were acquired in 3D mode with an axial field of view of 15.5 cm and reconstructed using filterback projection. PET scanners 2 and 3 were two Siemens Biograph HiRez XVI PET-CT scanner (Siemens Healthcare, Erlangen, Germany) at Imanova, Centre for Imaging Sciences. PET scanner 1 and PET scanner 2-3 were identical with the only exception of the axial field of view: 16.2 cm vs 21.6 cm respectively. The dynamic images were also reconstructed using a 3D filtered back-projection algorithm (discrete inverse Fourier transform, DIFT) with a 128 matrix, a zoom of 2.6 and a 5mm isotropic Gaussian smoothing. Participants were scanned at various times of the day. Some of the imaging data has been included in prior reports but not for genetic analysis (85-88). For attenuation and model-based scatter correction, a 10 min transmission scan was performed using a 150-MBq cesium-137 rotating point source for the ECAT HR+ 962 PET scanner and a computed tomography scan (effective dose=0.36 mSv) for the Siemens Biograph HiRez XVI PET-CT scanners were acquired prior to each PET scan. Experimental protocol was consistent for all the participants (85). Participants were asked to fast and abstain from smoking from midnight on the day of the scan as tobacco use has been associated with increased striatal dopamine synthesis capacity (89) although this has not been replicated (85). Oral doses of carbidopa (150mg) and entacapone (400mg) were administered 1hour before scanning. While the first reduces the peripheral metabolism of the tracer (90), the latter minimizes the formation of radiolabeled [¹⁸F]-FDOPA metabolites, which can cross the blood-brain barrier (91). Head movement was monitored and minimized with a light head strap. If participants moved extensively during the acquisition or got out of the scanner a second attenuation correction image was acquired at the end of the acquisition. PET data were acquired dynamically during 95 minutes after bolus injection of the radioactive tracer [¹⁸F]-DOPA through a cannula inserted into a vein. Dynamic data were binned into 26 frames (PET scanner 1) and 32 frames (PET scanner 2 and 3).

Image Analysis

Head movement was corrected using a frame-by-frame realignment and denoising algorithm (92) with a level 2 order 64 Battle-Lemarie wavelet filter applied on the non-attenuation-corrected dynamic images. These images were used because they include a significant scalp signal compared to attenuation-corrected images (93). Frames were realigned to a reference frame corresponding to the frame with the highest number of counts, i.e. obtained 7 minutes (for the ECAT HR+ 962 PET scanner-CTI/Siemens, Knoxville, Tennessee) and 17 minutes (for the Siemens Biograph HiRez XVI PET-CT scanners-Siemens Healthcare, Erlangen, Germany) after the radiotracer injection using a mutual information algorithm (94). The transformation parameters were then applied to the corresponding attenuation-corrected dynamic images. These realigned frames were summated, creating a movement-corrected dynamic image from which to extract the Time Activity Curves (TAC) for graphical analysis quantification. Standardized regions in Montreal Neurologic Institute (MNI) space were defined in the whole striatum delineated as previously described to create a Region of Interest (ROI) map (95) and in the cerebellum using the probabilistic Martinez atlas (95, 96). The cerebellum was used as a reference region as it is largely devoid of dopaminergic neurons or projections (45). A nonlinear transformation procedure on SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>) was used to normalize the ROI map together with the [¹⁸F]-DOPA template to each individual PET summation image, in order to place the ROI automatically on individual [¹⁸F]-DOPA PET dynamic images. Influx constant K_i^{eff} value, (min^{-1}) for the whole striatum was calculated relative to uptake in the reference region using a graphical approach (97), a method which has been shown to have good reliability (95).

Genetic analysis

DNA was extracted from blood or cheek swabs using standard methods (98). Genotyping of the rs821616 A>T SNP, was performed by KBioscience (Herts, UK, <http://www.kbioscience.co.uk>) using

248 a competitive allele specific Polymerase Chain Reaction system (CASP). Quality control procedures
249 included negative control (water) wells and duplicate wells.

250 **Statistical analysis**

251 The normality of the distribution for all variables was examined using the Shapiro Wilk test,
252 inspection of Q-Q plots and skewness and kurtosis values within range of ± 2 . Homogeneity of
253 variance was assessed with Levene's Test for Equality of Variances. An alpha threshold was set at
254 0.05 (two-tailed) for significance for all statistical comparisons. Statistical Package for the Social
255 Sciences (SPSS) version 24 was used for all statistical analysis (IBM, Armonk, N.Y.). All data are
256 shown as mean \pm SD. An univariate analysis of covariance (ANCOVA) was performed on 102 healthy
257 controls, with the DISC1 SNP Ser704Cys variation (serine homozygotes versus ~~eysteine~~
258 ~~carriers~~cysteine hetero/homozygotes) as the independent variable, K_i^{ser} in the whole striatum as the
259 dependent variable and age, gender, ethnicity (table 1) and the three PET scanners separately as
260 covariates as these variables have been previously found to influence dopamine synthesis capacity (99,
261 100). Effect sizes are reported as partial eta squared. Independent t test and Mann-Whitney test were
262 used to compare age and injected dose.

263

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281

282

Conflicts of interest

283

D.P. is a co-founder of the neuroimaging services company NeuroPsyAI, Ltd. O.D.H. has received

284

investigator-initiated research funding from and/or participated in advisory/ speaker meetings

285

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288

expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department

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Legend to Figure

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Figure 1: Mean (SEM) striatal dopamine synthesis capacity (K_{eff} value, min^{-1}) in *DISC1* rs821616 ~~eysteine-carriers~~cysteine

611

hetero/homozygotes (TT and TA, N=56) and *DISC1* rs821616 serine homozygotes (AA, N=46). Dopamine synthesis

612

capacity was significantly increased in serine homozygotes compared with ~~eysteine-carriers~~cysteine hetero/homozygotes (F

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(1,96)=6.555, p=0.012).

614

Table

Table 1		DISC1 SNP rs821616		
	Total	AT and TFCysteine hetero/homozygotes carriers	serine AA homozygotes carriers	P value
Total genotype counts	102	45 (AT) and 11 (TT)	46 (AA)	
Females	46	21	25	
PET scanner 1	35	19	16	0.549 ⁱⁱⁱ
PET scanner 2	33	16	17	
PET scanner 3	34	21	13	
Age	30.2 (9.3)	31.5 (9.9)	28.6 (8.4)	0.115 ⁱ
Tobacco smoking status (nonsmoker)	75	43	32	0.411 ⁱⁱ
Tobacco smoking status (smoker)	27	13	14	
Radioactivity injected (MBq)	157.7 (16.2)	156.6 (16.2)	159.2 (16.4)	0.529 ⁱⁱ
White European	70	35	35	0.503 ⁱⁱⁱ
Black British/other	22	15	7	
Asian British/other	5	3	2	
Mixed ethnicity	5	3	2	
All data ± SD. ⁱ Independent t test ⁱⁱ Mann-Whitney U test ⁱⁱⁱ Pearson Chi-Square				

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Abbreviations

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