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Genetic risk scores associated with temperament clusters in Finnish depression patients

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Original Article

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Abstract

Objective: Cloninger's temperament dimensions have been studied widely in relation to genetics. In this study, we examined Cloninger's temperament dimensions grouped with cluster analyses and their association with single nucleotide polymorphisms (SNPs). This study included 212 genotyped Finnish patients from the Ostrobothnia Depression Study. *Methods:* The temperament clusters were analysed at baseline and at six weeks from the beginning of the depression intervention study. We selected depression-related catecholamine and serotonin genes based on a literature search, and 59 SNPs from ten different genes were analysed. The associations of single SNPs with temperament clusters were studied. Using the selected genes, genetic risk score (GRS) analyses were conducted considering appropriate confounding factors. *Results:* No single SNP had a significant association with the temperament clusters. Associations between GRSs and temperament clusters were observed in multivariate models that were significant after permutation analyses. Two SNPs from the DRD3 gene, two SNPs from the SLC6A2 gene, one SNP from the SLC6A4 gene, and one SNP from the HTR2A gene associated with the HHA/LRD/LP (high harm avoidance, low reward dependence, low persistence) cluster at baseline. Two SNPs from the HTR2A gene were associated with the HHA/LRD/LP cluster at six weeks. Two SNPs from the HTR2A gene and two SNPs from the COMT gene were associated with the HP (high persistence) cluster at six weeks. *Conclusion:* GRSs seem to associate with an individual's temperament profile, which can be observed in the clusters used. Further research needs to be conducted on these types of clusters and their clinical applicability.

Significant outcomes

- Genetic risk scores in a multivariate model associated with a clustered model of Cloninger's temperament dimensions. These clusters have been shown to be associated with the comorbidity of anxiety disorders in depressed patients and treatment response.

Limitations

- No predetermined widespread criteria for clustering multiple Cloninger's temperament dimensions exist.
- This study had a limited number of participants, which might have resulted in the inability to detect subtle clinical differences.

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Introduction

In previous research, Cloninger has proposed seven dimensions, all with several subscales, which could be used to describe and study the temperament and character of an individual (Cloninger, 1986; Cloninger *et al.*, 1993). He proposed that especially four of these dimensions, novelty seeking (NS; initiation or activation of appetitive behaviour in response to novelty), harm avoidance (HA; inhibition of behaviour in response to signs of punishment), reward

dependence (RD; maintenance of behaviour in response to cues of social reward), and persistence (P; maintenance of behaviour despite frustration) are mainly genetically defined, and are linked to specific neurotransmitters: NS to the dopaminergic system, HA to the serotonergic system, and RD to the noradrenergic system. Cloninger also created a questionnaire, which could be used to grade an individual's temperament and character traits on these dimensions (Cloninger *et al.*, 1994). This questionnaire is called the Temperament and Character Inventory (TCI), which was later revised to create the Temperament and Character Inventory-Revised (TCI-R), which has 240 items, each with a scale of 5 possible answers. This questionnaire is the primary tool used when assigning an individual's temperament and character traits to Cloninger's model.

An association between Cloninger's temperament and character traits and depression (Kampman & Poutanen, 2011; Zaninotto *et al.*, 2016) and anxiety (Kampman *et al.*, 2014) has been established in meta-analyses. Research has also been conducted about the relationship between the genes defining the catecholamine and serotonin systems and Cloninger's temperament dimensions (Suzuki *et al.*, 2007; Lee *et al.*, 2008; Alfimova *et al.*, 2010; Tsuchimine *et al.*, 2012; Tsuchimine *et al.*, 2013; Glavina Jelas *et al.*, 2018). At this point in time, the results of these studies are inconclusive, and further studies need to be conducted.

Paavonen *et al.* (2018) have proposed that instead of describing an individual's risk for specific psychiatric disorders based on single TCI-R dimensions, it would be more sensible to create a cluster defined by multiple TCI dimensions to assess the risk of these conditions. Paavonen et al. proposed the following clusters: HNS, HHA/LRD/LP, LNS, and LHA/HP, where H stands for high level of a dimension, L stands for low level of a dimension, and non-specified dimensions being at an intermediate level. Based on their findings, these clusters have an association with specific forms of anxiety in depressed patients: the HNS cluster with panic disorder and agoraphobia, the HHA/LRD/LP cluster with social anxiety disorder (SAD), and the LNS cluster with generalised anxiety disorder. The LHA/HP cluster was associated with a lower risk of anxiety in general. In an earlier study, different temperament clusters have been associated with the severity of depression, and together with vegetative symptoms, predicted how well individuals responded to antidepressants (Paavonen *et al.*, 2014).

The aim of this study is to investigate the association between single nucleotide polymorphisms in the catecholamine and serotonin systems and the temperament clusters in Finnish population who were depressed. In this study, we investigated if specific single nucleotide polymorphisms (SNPs) related to the catecholamine and serotonin systems that had a connection with dimensions in the TCI-R questionnaire based on previous studies are associated with the temperament clusters proposed by Paavonen *et al.* (2018).

Material and methods

Patients

The study included 242 patients from the Finnish region of Southern Ostrobothnia, who were referred to psychiatric specialised healthcare units for symptoms of depression, anxiety, suicidality, insomnia, and substance abuse during 2009–2013. The participants of the study were screened before inclusion in regards to alcohol use with Alcohol Use Disorders Identification test (AUDIT) (Bohn *et al.*, 1995), and depressive symptoms using Beck Depression Inventory (BDI) version 1A (Beck *et al.*, 1997). The inclusion criterion was a score of at least 17 on the BDI scale, which is an indication of moderate depression. Patients whose symptoms were present due to organic conditions of the brain or had psychotic symptoms (ICD-10 F2* diagnoses) were excluded.

At baseline, the participants were assessed with the Mini International Neuropsychiatric Interview 5.0 (MINI) (Sheehan *et al.*, 1998) and the Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979), and the TCI-R. There were some dropouts after screening, a total of 228 patients (94% of all recruited patients) were examined with MADRS at baseline. A psychiatric assessment was also conducted on the participants at baseline, where their medication was assessed and optimised. The fluoxetine equivalent dose was calculated (Hansen *et al.*, 2009) to make comparing the participants' medication possible. At 6 weeks, the MADRS score and TCI-R questionnaire were repeated.

Demographic and clinical assessments were conducted at the beginning of the study. In this study, depressive disorders were considered as primary, anxiety disorders as secondary, and other diagnoses as tertiary, and all diagnoses were assigned based on clinical standards. For their main diagnoses, 181 (88.7%) patients had MDD, eight (3.9%) had dysthymic disorder, eleven (5.4%) had an anxiety disorder), three (1.5%) had self-destructiveness, and one (0.5%) had alcohol use disorder. 67% of patients with a mood disorder as their primary diagnosis had comorbid anxiety disorders. 65.7% of patients reported having at least one episode of MDD in their history. The patients were also divided into two groups based on their AUDIT questionnaire scores. Patients with a score of <11 were considered to have no alcohol use problems (non-AUP), and patients with a score of ≥ 11 were considered to have alcohol use problems (AUP). After some patient dropouts, these criteria created two groups: 127 non-AUP patients (mean age 38.5 ± 12.8 years), and 89 AUP patients (mean age 38.7 ± 11.4 years). The MINI was conducted on 82 AUP patients (data missing in 7 cases), and 61 (74.4%) of them were diagnosed with current alcohol use disorder based on the DSM-IV criteria, and eight (9.8%) of them with other current substance use disorders. The specific demographics of each temperament cluster are presented in Table 1.

Further specifics of the study protocol and patient details can be found in previous publications of the Ostrobothnia Depression Study (ClinicalTrials.gov identifier NCT02520271), Paavonen *et al.* (2016). The local Human Subjects Review Committee approved gathering the data used in this study, and it was carried out in accordance with the latest version of the Declaration of Helsinki. Since individuals with psychosis or organic brain diseases were excluded, all participants were able to give their informed written consent. For further information on the ethical aspects of this study, visit ClinicalTrials.org, identifier NCT02520271.

DNA extraction and genotyping

Blood samples for genotyping were gathered from a total of 212 participants. DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Mini kit and an automated biorobot M48 extraction (versus, Hilden, Germany). Genotyping was performed using Illumina Infinium HumanCoreExome-12 DNA Analysis Beadchip version 1.0, according to the manufacturer's recommendation at Helmholtz Zentrum, München, Germany. The following quality control filters were applied: GenCall score <0.15, GenTrain score <0.20, sample and SNP call rate <0.95, Hardy–Weinberg equation p-value $<10^{-6}$, excess heterozygosity,

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Table 1. Demographics of each temperament cluster including age, sex, smoking status, MADRS and AUDIT scores at the beginning of the study and antidepressant doses in fluoxetine equivalents

	Percentage of whole (*n*)	Age ± sd	Male gender (*n*)	Smoking (*n*)	MADRS score ± sd (a)	AUDIT score ± sd (b)	Antidepressant dose (converted to fluoxetine equivalent) ± sd mg
HNS, 0 weeks	25.9% (56)	36.7 ± 12.5	51.8% (29)	80.4% (45)	22,4 ± 6,55	14.4 ± 11.1	27.2 ± 21.4
LHA/HP, 0 weeks	16.7% (36)	42.5 ± 12.2	33.3% (12)	55.6% (20)	23.1 ± 7.67	8.81 ± 8.50	25.2 ± 18.7
LNS, 0 weeks	30.6% (66)	41.4 ± 11.6	33.3% (22)	53.0% (35)	22.6 ± 6.09	8.98 ± 9.00	25.0 ± 21.0
HHA/LRD/LP, 0 weeks	26.5% (58)	34.8 ± 11.4	43.1% (25)	62.1% (36)	24.5 ± 5.39	10.9 ± 9.27	32.4 ± 19.2
HNS/HP, 6 weeks	15.8% (28)	34.8 ± 13.0	50.0% (14)	89.3% (25)	17.6 ± 9.48	8.29 ± 6.92	27.0 ± 20.6
HP, 6 weeks	31.6% (56)	41.5 ± 12.6	42.9% (24)	48.2% (27)	14.3 ± 7.75	8.95 ± 9.03	25.0 ± 19.5
LNS/HHA/HRD, 6 weeks	24.3% (43)	38.8 ± 11.8	30.2% (13)	62.8% (27)	17.8 ± 7.35	11.2 ± 10.1	28.2 ± 22.6
HHA/LRD/LP, 6 weeks	28.2% (50)	38.2 ± 11.8	38.0% (19)	60.0% (30)	19.0 ± 7.23	9.58 ± 9.53	28.4 ± 18.0

- MADRS score at the time of creating the corresponding cluster (either at the beginning of the study or 6 weeks).
- AUDIT score at the beginning of the study.

cryptic relatedness (pi-hat >0.2), gender check and multidimensional scaling.

Literature search and selection of genes

A search of PubMed was carried out on the 18th of December 2018, to explore which SNPs had been studied in relation to Cloninger's temperament dimensions. A search that included the keywords 'temperament', 'harm avoidance' or 'temperament and character inventory', and had a mention of 'genetics', as well as '5-HT', 'norepinephrine', 'noradrenaline', or 'dopamine' were included in the search. With this search, 217 studies were included. All abstracts from these studies from 1997 onwards were read, from which specific SNPs and genes were recorded. SNPs and genes with only one research paper written on them were excluded. From this list, all the genes that were related to the dopaminergic, noradrenergic, or serotonergic synaptic cleft or the immediate metabolism of the neurotransmitters from the synaptic cleft were included in the next step. From these genes, and from a 5000 base pair distance backwards from the beginning of these genes, all SNPs included in the HumanCoreExome-12 beadchip were included in the study. Uncommon SNPs with a mean allele frequency (MAF) of <5% in the population of this study were excluded.

The genes that were included in the study were the catechol-O-methyltransferase (COMT), the dopamine receptor D2 (DRD2), the dopamine receptor D3 (DRD3), the dopamine receptor D4 (DRD4), the 5-hydroxytryptamine receptor 2A (HTR2A), the 5-hydroxytryptamine receptor 2B (HTR2B), the 5-hydroxytryptamine receptor 2C (HTR2C), the monoamine oxidase A, the solute carrier family 6 member 2 (SLC6A2), the solute carrier family 6 member 3 (SLC6A3), and the solute carrier family 6 member 4 (SLC6A4). The chip used did not have any SNPs with a MAF of over 5% in our sample in the DRD4 gene; thus, the DRD4 gene was excluded from the study. A total of 59 SNPs from the selected genes were available for the analyses from the used genotyping array. A combination of three models was created from the SNPs: the recessive, additive, and dominant model, as described in a study by Solismaa *et al.* (2017). Other types of gene polymorphisms, such as insertions and deletions, were not available with the genotyping methods used in this study.

Statistical methods

Confounding factors were controlled in the analyses. Sex, age, and smoking were controlled for all the analyses. The MADRS score at the beginning of the study was also considered for all the clusters created at baseline, since the TCI dimensions used to create the temperament clusters may be subject to confoundment from major depressive disorder (MDD) or the other psychiatric conditions the participants had. At six weeks after re-assessing the optimal medication, the TCI dimensions likely reflect the individual's temperament and character with less confoundment as the baseline TCI dimensions (Hansenne & Bianchi, 2009; Kampman & Poutanen, 2011). Thus, taking the MADRS score at six weeks into account was not done automatically for the analyses.

Other possible confounding factors that were explored were the fluoxetine equivalent dose of antidepressants, the MADRS score at the sixth week of the study, the AUDIT score at baseline, if the participant's MDD is recurrent or not and BMI. The correlation of each temperament cluster and these possible confounding factors were analysed with the Welch two-sample *t*-test (continuous confounding factors) or Pearson's chi-square test (dichotomic confounding factors). All the confounding factors with an association with the temperament clusters with a *p*-value of <0.05 were used as explanatory variables in the analyses. Different models with different explanatory factors were explored. In these explorations, confounding factors with a *p*-value of <0.05 were included in all analyses, while other possible factors were explored in different combinations. Before performing multivariate analyses, interactions between confounding factors were explored with Spearman's rank-order correlation. Correlations of $r > 0.5$ were considered significant.

The SNPs were analysed in relation to the temperament clusters, which were based on the studies of Paavonen *et al.* (2014, 2018). In these studies, only clusters at baseline were used. In our study, we also included clusters which were formed at six weeks from the beginning of the study, created with similar methods as the clusters at baseline. At six weeks, the clustering algorithm formed slightly different clusters: HNS/HP (corresponding with HNS at baseline), HHA/LRD/LP (corresponding with HHA/LRD/LP at baseline), LNS/HHA/HRD (corresponding with LNS at baseline), and HP (corresponding with LHA/HP at baseline).

The clusters at both baseline and at six weeks were analysed, for a total of eight different cluster analyses. Eight different dichotomised variables were formed: the cluster of interest was compared against the other clusters at the same timepoint. The association of SNPs with the temperament clusters was analysed with logistic regression models. Appropriate confounding factors were used in the logistic regression analyses as explanatory factors. The p-values of this analysis were adjusted with the false discovery rate (FDR) method to counteract the multiple comparison problem when analysing a large number of SNPs with their different modes of inheritance. Genetic risk scores (GRS) were calculated for each temperament cluster separately. The final GRS consists of the weighted sum of risk alleles related to each cluster.

To form the GRSs, SNPs with a non-adjusted *p*-value of ≤0.05 were inserted into the same model as the confounding factors, and analysed with a stepwise logistic regression analysis, using Akaike information criteria (Akaike, 1981). This model was used to select SNPs that together best explain the response variable. This model also takes into account the problem of multiple SNPs having a high linkage disequilibrium (LD) by only including the single best SNP of these for the model. The score for each SNP was calculated by taking the beta estimate values obtained from the initial logistic regression analysis and using it to weight the individual SNPs in the GRS. The GRS was used in the final logistic regression analysis, which again included the confounding factors as explanatory variables.

When combining the genetic effects of individual SNPs that were not statistically significant after FDR adjustment, there is a risk of overfitting the data and causing type I errors. To avoid this, it is important to create an estimate of the null distribution of the GRS test statistics (Hu *et al.*, 2013). Thus, a permutation test was conducted. Patient ID numbers were sampled from the phenotype data, mixing the genotype and phenotype data. This data was then analysed with identical methods as the original data. This sampling was repeated at least 999 times for each cluster and each combination of confounding factors, and up to 9999 times for each cluster with possible significant findings. The p-values from all these permutations were collected. If the *p*-value from the actual data ended up being among the smallest 5% of all the collected p-values, the results were deemed statistically significant. Lastly, LD analyses were conducted on all the SNPs in the study, with a window of 10,000 kbp and a threshold of $r^2 = 0.8$.

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Plink version v1.90b5.4 (updated on the 10th of April 2018) and version 2.00a3 (updated on the 23rd of September 2020) were used to extract the SNPs from the gene data and to calculate the SNPs' LD. Statistical analyses were performed with R (version 1.2.5042, R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

Results

Exploration and correlation of confounding factors in significant clusters

At baseline, patients in the HHA/LRD/LP cluster had a higher MADRS score (mean 24.5 ± 5.39 (HHA/LRD/LP) versus mean 22.6 ± 6.60 (rest of the sample), $p = 0.033$) and had higher antidepressant doses (fluoxetine equivalent doses: mean 32.4 ± 19.2 SD (HHA/LRD/LP) versus mean 25.8 ± 20.5 SD (rest of the sample), $p = 0.031$) compared to the rest of the sample. Patients in the HHA/LRD/LP cluster at baseline were also younger than in the rest of the sample (mean 34.8 ± 11.4 SD (HHA/LRD/LP) versus mean 40.0 ± 12.2 SD (rest of the sample), $p = 0.005$).

At six weeks, patients in the HP cluster had lower MADRS scores (mean 14.3 ± 7.75 SD (HP) versus mean 18.2 ± 7.81 SD (rest of the sample), $p = 0.0027$), and tended to be older (mean 41.5 ± 12.6 SD (HP) versus mean 37.6 ± 12.1 SD (rest of the sample), $p = 0.055$) than the rest of the sample. At six weeks, there were less smokers in the HP cluster when compared to the rest of the sample (40.8% (HP) versus 59.8% (rest of the sample), $\chi^2 = 3.98$, $p = 0.046$). At six weeks, the HHA/LRD/LP cluster had a slightly higher MADRS score than the rest of the sample (mean 19.0 ± 7.23 SD (HHA/LRD/LP) versus mean 16.2 ± 8.15 SD (rest of the sample), $p = 0.034$). The other analysed clusters also had their own combination of significant confounding factors, but as they had no significant results in SNP analyses, so the group differences aren't reported here.

No two confounding factors had an association of 0.5 or higher with each other in Spearman's rank-order correlation analyses, thus none of the factors were dropped from multivariate models based on this calculation. The HHA/LRD/LP cluster at six weeks was found to have both the MADRS score at baseline and at six weeks as significant confounding factors based on the exploration of these factors. To avoid bias, only the MADRS score at six weeks was chosen as an explanatory variable out of these two variables. The confounding factors considered in the clusters with significant results can be found in Table 2.

Results of SNP analyses

No single SNP had a significant adjusted p-value in the models where appropriate confounding factors were included as explanatory variables. In the GRS analyses, significant associations were observed in the HHA/LRD/LP cluster at baseline, in the HP cluster at six weeks, and in the HHA/LRD/LP cluster at six weeks. In the model used, when explanatory factors were considered, six SNPs had an association with the HHA/LRD/LP cluster at baseline, four SNPs had an association with the HP cluster at six weeks, and two SNPs had an association with the HHA/LRD/LP cluster at six weeks. All significant findings are summed up in Table 2.

When exploring the combination of explanatory factors in the analyses, the combination of significant SNPs in the model remained constant in the HHA/LRD/LP and HP clusters at six weeks. In the HHA/LRD/LP cluster at baseline, taking out sex as an explanatory factor affected the combination of these SNPs. Compared to the analysis with sex as an explanatory variable, this latter set of SNPs did not include rs582385 in the HTR2A gene and did include rs582854, rs4942582 and rs7330636 in the HTR2A gene. We conducted a post hoc exploration about the linkage of these SNPs. The largest LD was between rs582385 and rs582854 in the HTR2A gene, with an LD of 0.28, and rs7330636 had no linkages in the analysis. In the other clusters analysed, we observed no significant results based on the p-values from the permutations conducted. In the LD analyses, rs6280 in the DRD3 gene was linked to rs324029 in the same gene (LD $r^2 = 0.84$), and rs223939 in the COMT gene was linked to rs4818 in the same gene (LD $r^2 = 0.96$). Detailed results about the SNPs and LDs can be found in Table 3.

Discussion

The main result of this study is that significant associations were observed between the combination model of SNPs from the DRD3, SLC6A2, SLC6A4 and HTR2A genes and the HHA/LRD/LP cluster at baseline, the combination models of the SNPs from the HTR2A gene and the HHA/LRD/LP cluster at six weeks, and the combination models of the SNPs from the HTR2A and COMT genes and the HP clusters at six weeks. Based on this finding, it seems that polymorphisms in the DRD3, SL6A2, SLC6A3, SLC6A4, HTR2A, and COMT genes may alter the catecholaminergic and serotonergic transmission processes significantly, which influences an individual's temperament. In the study by Paavonen *et al.* (2018), the HHA/LRD/LP cluster at baseline was associated with SAD in depressed patients. In the same study, the LHA/HP cluster at baseline, which corresponds with the HP cluster at six weeks, was associated with the lowest prevalence of anxiety disorders in general. Paavonen *et al.* (2014) also observed these temperament clusters to predict treatment response in depression when including high vegetative symptoms in the analysis, though this effect was only observed in post-treatment. Based on these findings, the risk of anxiety disorders during depressive episodes associated with these temperament profiles could be mediated by certain genotype clusters. By genotyping the SNPs associated with these clusters in depressed patients, we could estimate the risk of specific anxiety disorders in these patients. Further research needs to be conducted to repeat these results and estimates the clinical importance of these types of findings before widespread genotyping could be considered in MDD patients. However, having a genetic model to help with diagnostics and treatment would be beneficial in clinical practice.

The results of the HHA/LRD/LP cluster at baseline changed depending on whether sex was controlled as a confounding factor. In the initial exploration of confounding factors, sex did not associate with the cluster. The SNPs in question varied only in the HTR2A gene. A possible explanation for this is that there is a considerable difference in the how the SNPs in the HTR2A gene behave between the sexes. We speculated that this finding might be due to a linkage between these SNPs, but the post hoc analysis only revealed a minor linkage between some of these SNPs, thus not explaining the result. The reason for the difference in these analyses remains unclear. One speculation would be that these genes are expressed differently between the two sexes. In a meta-analysis by Miettunen *et al.* (2007), sex was observed to have an effect on the HA and RD dimensions, which could also at least partially explain this finding, as the HHA/LRD/LP cluster includes the extremities of both of these dimensions.

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Table 2. Logistic regression models with different temperament clusters as dependent variables, explanatory variables used in the model and results of the permutation analysis

Dependent variable	Explanatory variables	Estimate of effect in model	*p*-value	Permutations *p*-value	Genes in GRS	SNPs in GRS	Allele coding	Nucleotide
HHA/LRD/LP, 0 weeks	Fluoxetine equivalent dose	0.022	0.050	<0.001	DRD3	rs6280	REC**	C
	Sex	−0.22	0.65		DRD3	rs3773678	DOM***	A
	Smoking	−0.71	0.14		SLC6A2	rs7194256	REC**	T
	Age	−0.066	<0.001		SLC6A2	rs3785157	REC**	T
	MADRS score at baseline	0.13	0.0031		SLC6A4	rs2020936	ADD*	G
	GRS	0.93	<0.001		HTR2A	rs582385	ADD*	G
HHA/LRD/LP, 0 weeks	Fluoxetine equivalent dose	0.023	0.035	<0.001	DRD3	rs6280	REC**	C
	Smoking	−1.0	0.040		HTR2A	rs582854	REC**	A
	Age	−0.079	<0.001		DRD3	rs3773678	DOM***	A
	MADRS score at baseline	0.12	0.0034		SLC6A2	rs7194256	REC**	T
	GRS	1.2	<0.001		HTR2A	rs4942582	REC**	A
					HTR2A	rs7330636	REC**	T
					SLC6A4	rs2020936	ADD*	G
					SLC6A2	rs3785157	REC**	T
HHA/LRD/LP, 6 weeks	MADRS score at 6 weeks	0.060	0.031	0.0041	HTR2A	rs582385	ADD*	G
	Age	−0.0058	0.74		HTR2A	rs9567737	DOM***	T
	Sex	−0.14	0.75					
	Smoking	−0.34	0.45					
	GRS	1.1	<0.001					
HP, 6 weeks	Sex	0.86	0.058	<0.001	HTR2A	rs9567746	DOM***	G
	Age	0.016	0.38		HTR2A	rs9316232	REC**	A
	MADRS score at 6 weeks	−0.067	0.016		COMT	rs5993883	REC**	G
	Smoking	−0.68	0.12		COMT	rs2239393	REC**	G
	GRS	1.4	<0.001					

*= Additive coding, ** = Recessive coding, *** = Dominant coding.

Table 3. Linkage disequilibrium (LD) with other SNPs in the sample and annotations of SNPs in the GRSs found to be statistically significant after permutations

	Chromosome	Gene in score	SNPs in score (a)	Annotation	LD
HHA/LRD/LP, 0 weeks	3	DRD3	rs6280	Missense	rs324029, LD $r^2 = 0.839826$
	3	DRD3	rs3773678	Intron	None
	16	SLC6A2	rs7194256	Intron, UTR-3	None
	16	SLC6A2	rs3785157	Intron	None
	17	SLC6A4	rs2020936	Intron	None
	13	HTR2A	rs582385	Intron	None
HHA/LRD/LP, 0 weeks (b)	13	HTR2A	rs582854	Intron	None
	13	HTR2A	rs4942582	Intron	None
	13	HTR2A	rs7330636	Intron	None
HHA/LRD/LP, 6 weeks	13	HTR2A	rs582385	Intron	None
	13	HTR2A	rs9567737	Intron	None
HP, 6 weeks	13	HTR2A	rs9567746	Intron	None
	13	HTR2A	rs9316232	Intron (c)	None
	22	COMT	rs5993883	Intron, nearGene-5	None
	22	COMT	rs2239393	Intron (d)	rs4818, LD $r^2 = 0.95644$
SNPs found in LD	3	DRD3	rs324029	Intron	
	22	COMT	rs4818	Cds-synon (d)	

(a) A literature search on the significantly associated SNPs in this study can be found in the supplementary material.
(b) Significant SNPs in the analysis without sex as a confounding factor.
(c) Also as an Intron in gene HTR2A-AS1.
(d) Also nearGene-5 in gene MIR4761.

The participants in this study were depressed, which can affect the dimensions in the TCI-R questionnaire. Previous studies have shown that HA and P seem to change during the acute phase of MDD compared to the remission phase (Hansenne & Bianchi, 2009; Kampman & Poutanen, 2011). In this study, we assumed that each individual has a 'trait' temperament, as well as a 'state' temperament profile. The trait temperament is what the individual's genetics define based on Cloninger's theory, and the state temperament is the temperament that can be quantified but is influenced by psychiatric and external conditions, such as acute depression. Since this study focused on genetics, the aim was to get as close to the trait temperament profiles as possible. Especially during the acute phase of MDD, which corresponds to the baseline of this study, the state temperament recorded by the TCI-R questionnaire is probably different from individual's genetic trait temperament. Therefore, the MADRS score was used as an explanatory variable to try to diminish the effect of MDD symptoms on the temperament dimensions. Also, a depressive episode has shown to leave a permanent 'scar' on an individual's TCI profile (Kampman & Poutanen, 2011). Striving to study the trait temperament of individuals with psychiatric disorders is somewhat difficult, since the exact effect of psychiatric disorders on an individual's temperament dimensions is difficult to establish without prior data about the individual's temperament. However, despite the variety of known risk factors for MDD, we cannot reliably predict future episodes in individuals who end up with MDD. In a clinical setting, patients are assessed after symptoms of psychiatric disorders begin, thus the temperament dimensions collected would probably be affected by these disorders. Thus, either the state temperament or trait temperament altered by MDD might be more relevant in a clinical setting than the pure trait temperaments of healthy individuals.

Clustering the TCI dimensions has not been done widely in previous studies, and no predetermined criteria for these clusters have been established. Despite this, creating a more complex model of an individual's temperament might be a sensible approach to studying the behaviour, trajectories, and genetic influences in general, as the nature of human temperament is complex. A problem with the clustering used in this study is the applicability of these findings in a clinical environment. SNPs have been widely studied in previous literature separately, as well as in combinations. A strength of this study is using the SNPs as combination, since finding the psychiatric relevance of a single SNP would need a large pool of high-quality data, and even then, the effect and clinical relevance of individual SNPs would most likely be low.

Based on the preliminary literature search, a considerable number of studies were found which have investigated the influence of genetics on temperament, though many of the studies focused on other genetic polymorphisms than SNPs, and some used other methods than the TCI-R to quantify an individual's temperament. For example, a substantial amount of research has been conducted on the effects of the short and long alleles of SLC6A4 in regard to temperament and psychiatric conditions (Ma *et al.*, 2014; Borkowska *et al.*, 2015). Some SNPs, for example rs2020936 in the SLC6A4 combined with another SNP in the same gene, have been found to have an association with the short allele of SLC6A4 (Wray *et al.*, 2009), though this seems to be more of an exception than a rule. As another example, studies have been

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conducted on the variable number of tandem repeat mutations in the DRD4 gene (Kang *et al.*, 2008; Oniszczenko & Dragan, 2012). Due to the available data, this study only included SNPs, so validating and studying other types of gene polymorphisms was not possible.

A limitation of this study is the small number of participants. This may result in the inability to detect subtle clinical differences between individuals with different SNPs. A strength of this study is the inclusion of both inpatients and outpatients, thus including a wide range and severity of symptoms. Even though the participants' medication was optimised, compliance was only assessed with patient reports.

In general, the possibility of using genetic tests in clinical applications, for example in the treatment of depression, currently has its limitations. In a recent review by Lewis & Vassos (2020), the authors estimated an individual's risk of depression to be 2% from genetics and 98% from unaccounted variation from unmodelled genetic and environmental factors. When comparing polygenic risk scores, they observed a 2.5-fold increase in risk of depression in the highest decile compared to the lowest decile. At this point in time, the genetic impact on an individuals' risk of depression seems to be small, but measurable. The clinical significance of measuring an individual's genome in the context of depression is most likely small but might be an additional tool in the future if genotyping of patients becomes more widespread in a clinical setting, especially if an individual's genome data would be available before their psychiatric symptoms arise.

In conclusion, associations between combinations of SNPs and HHA/LRD/LP and HP clusters were observed in the multivariate models used in this study. Further research needs to be conducted to validate these findings and to explore the possibility of using these temperament clusters more widely. In addition, researching other types of gene polymorphisms in relation to a cluster model would be beneficial, as well as studying the temperament clusters of individuals with no history of psychiatric disorders.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/neu.2023.33.

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Author contribution. Simo-Pekko Salminen, Anssi Solismaa, and Olli Kampman were responsible for planning the research. Olli Kampman and Esa Leinonen participated in organising and designing the ODS study and collecting the data. Leo-Pekka Lyytikäinen, Nina Mononen, and Terho Lehtimäki performed the laboratory analyses and genotyping. Simo-Pekko Salminen, Anssi Solismaa, and Leo-Pekka Lyytikäinen conducted the statistical analyses. Simo-Pekko Salminen, Anssi Solismaa, Vesa Paavonen, Terho Lehtimäki, and Olli Kampman contributed to the interpretation of the data. Simo-Pekko Salminen and Anssi Solismaa drafted the manuscript. Vesa Paavonen, Terho Lehtimäki, Leo-Pekka Lyytikäinen, and Olli Kampman edited the manuscript. All authors have reviewed and approved the manuscript.

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