Article

Exploring the Role of Contactins across Psychological, Psychiatric and Cardiometabolic Traits within UK Biobank

Julia Morris 1, Soddy Sau Yu Leung 1, Mark E.S. Bailey 2 @, Breda Cullen 1, Amy Ferguson 1, Nicholas Graham 1, Keira J. A. Johnston 1,2,3, Donald M. Lyall 1, Laura M. Lyall 1, Joey Ward 1, Daniel J. Smith 1 and Rona J. Strawbridge 1,4,5, *

1 Institute of Health and Wellbeing, University of Glasgow, Glasgow G12 8RZ, UK; 2113744M@student.gla.ac.uk (J.M.); soddy@connect.hku.hk (S.S.Y.L.); breda.cullen@glasgow.ac.uk (B.C.); afergus8@exseed.ed.ac.uk (A.F.); Nicholas.Graham@glasgow.ac.uk (N.G.); 2340814J@student.gla.ac.uk (K.J.A.J.); donald.lyall@glasgow.ac.uk (D.M.L.); Laura.Lyall@glasgow.ac.uk (L.M.L.); Joey.Ward@glasgow.ac.uk (J.W.); Daniel.Smith@glasgow.ac.uk (D.J.S.)

2 School of Life Sciences, College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK; mark.bailey@glasgow.ac.uk

3 Deanery of Molecular, Genetic and Population Health Sciences, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh EH8 9AG, UK

4 Health Data Research UK, Glasgow G12 8RZ, UK

5 Cardiovascular Medicine Unit, Department of Medicine Solna, Karolinska Institutet, 171 77 Stockholm, Sweden

* Correspondence: Rona.Strawbridge@glasgow.ac.uk

Received: 26 October 2020; Accepted: 7 November 2020; Published: 10 November 2020

Abstract: Individuals with severe mental illness have an increased risk of cardiometabolic diseases compared to the general population. Shared risk factors and medication effects explain part of this excess risk; however, there is growing evidence to suggest that shared biology (including genetic variation) is likely to contribute to comorbidity between mental and physical illness. Contactins are a family of genes involved in development of the nervous system and implicated, though genome-wide association studies, in a wide range of psychological, psychiatric and cardiometabolic conditions. Contactins are plausible candidates for shared pathology between mental and physical health. We used data from UK Biobank to systematically assess how genetic variation in contactin genes was associated with a wide range of psychological, psychiatric and cardiometabolic conditions. We also investigated whether associations for cardiometabolic and psychological traits represented the same or distinct signals and how the genetic variation might influence the measured traits. We identified: A novel genetic association between variation in CNTN1 and current smoking; two independent signals in CNTN4 for BMI; and demonstrated that associations between CNTN5 and neuroticism were distinct from those between CNTN5 and blood pressure/HbA1c. There was no evidence that the contactin genes contributed to shared aetiology between physical and mental illness

Keywords: contactins; psychiatric disorders; cardiometabolic diseases; hypertension; Type 2 diabetes; genetic variation; single nucleotide polymorphisms; UK Biobank; gene expression

1. Introduction

Individuals with severe mental illness (SMI, such as schizophrenia, bipolar disorder and major depressive disorder) have an increased risk of cardiovascular and metabolic diseases (CMD), compared to the general population [1–3]. Indeed CMD (including obesity, type 2 diabetes,
coronary artery disease and stroke) is a key factor in the reduced life expectancy observed for those with severe mental illness (typically 15–20 years for schizophrenia and 10–15 years for bipolar disorder [4]). Some risk factors for CMD are more common in severe mental illness, (such as physical inactivity, poor diet, high alcohol consumption and cigarette smoking), or worsen with duration of SMI (such as low socioeconomic status) and the side effects of psychotropic drugs (particularly atypical antipsychotics) further increase CMD risk. There is also growing evidence to support the existence of shared biological mechanisms [1,4–7], with observational studies proposing mechanisms such as disrupted circadian rhythms, hypothalamic–pituitary axis dysfunction and abnormal inflammation [8]. If specific components of shared mechanisms for CMD and SMI can be identified, there is potential for better prevention and treatment.

The family of contactin genes (CNTNs) represents a possible common mechanism between mental and physical illness because genome-wide association studies (GWAS) have implicated contactins in a wide variety of cardiometabolic and mental health conditions, summarized in Table 1. It has yet to be determined whether the same or distinct genetic signals influence the cardiometabolic and psychiatric/behavioural traits. Apart from these recent GWAS findings, the study of contactins has so far been predominantly from a neurobiology perspective. Expression of contactins has been observed in neurons, oligodendrocytes and oligodendrocyte precursors [9], and demonstrates involvement in cell contact formation, axonal growth control and pathfinding, axonal domain organisation, myelination, neuronal development and synaptogenesis [9]. Further, loss of contactin genes leads to malformed axons and impaired nerve conduction [9]. Contactin expression is not restricted to the brain, although understanding of functional effects of contactins outside of the brain is currently lacking.

| Locus | DISEASE/TRAIT                                                                 | PMID          |
|-------|------------------------------------------------------------------------------|---------------|
| CNTN1 | Antidepressant treatment resistance (>2 drugs prescribed)                    | 30700811      |
|       | Bipolar disorder                                                             | 22925353      |
|       | Blood protein levels                                                         | 30072576      |
|       | Cerebral amyloid deposition in APOEe4 non-carriers (PET imaging)              | 26252872      |
|       | Cognitive ability in schizophrenia                                           | 3120164      |
|       | Dementia and core Alzheimer’s disease neuropathologic changes                 | 25188341      |
|       | IgG glycosylation                                                            | 23382691      |
|       | Metabolite levels (X-11787)                                                   | 29344736      |
|       | Parkinson’s disease                                                          | 24842889      |
|       | Reaction time                                                                | 29844566      |
|       | Adventurousness                                                              | 30643258      |
|       | Blood protein levels                                                         | 29875488, 28240269 |
|       | Cerebrospinal fluid biomarker levels                                          | 28031287      |
|       | Highest math class taken                                                      | 30038396, 30038396 |
|       | Red blood cell count                                                         | 27863252      |
|       | Schizophrenia                                                                | 30285260, 26198764, 28991256 |
|       | High density lipoprotein cholesterol levels                                   | 32203549      |
|       | Type 2 Diabetes                                                              | 32541925      |
|       | Waist–hip ratio                                                              | 30239722      |
| CNTN2 | Age at first sexual intercourse                                               | 27899180      |
|       | Economic and political preferences                                           | 22566364      |
|       | Educational attainment (years of education)                                  | 30038396      |
|       | Frontal fibrosing alopecia                                                   | 30850646      |
| CNTN3 | Heart rate response to recovery post exercise                                 | 29766521, 29490742 |
|       | Paediatric bone mineral content (hip)                                         | 28181694      |
|       | Peripheral arterial disease (traffic-related air pollution interaction)       | 27082954      |
|       | Smoking initiation and quantity                                               | 30643251, 24665060 |
|       | Systolic blood pressure                                                       | 30224653      |
| Locus | DISEASE/TRAIT | PMID |
|-------|--------------|------|
| CNTN4 | Blood cell phenotypes | 32888493, 27863252, 30595370, 28957414 |
|       | Protein levels in obesity | 29234017 |
|       | Bipolar and/or Depressive disorders | 31926635, 31969693, 27089181, 29942085, 30643256, 29292387 |
|       | Carotid intima media thickness interaction with smoking | 32117412 |
|       | Adverse idiopathic scoliosis | 30019117 |
|       | Alcohol dependence | 26365420 |
|       | Atrial fibrillation | 26634245 |
|       | Blood protein levels | 29875488, 30072576, 28240269 |
|       | Bone mineral density (femoral neck) | 29611590 |
|       | Chronotype and sleep phenotypes | 30646823, 30804565, 30804657 |
|       | Core binding factor acute myeloid leukaemia | 27903959 |
|       | Educational attainment (MTAG) | 30038396 |
|       | Factor VII activity | 30642921 |
|       | Feeling miserable | 29500382 |
|       | Glycated haemoglobin levels | 29889252 |
|       | Gout vs. Hyperuricemia | 31289104 |
|       | Immune response to smallpox (secreted IL-2) | 22610502 |
|       | Interleukin-2 receptor antagonist levels | 29898323 |
|       | Life satisfaction | 30643256 |
|       | Loneliness (multivariate analysis) | 27629369 |
|       | Lung function (FEV1/FVC) | 30595370 |
|       | Menarche (age at onset) | 25667675 |
|       | Metastasis in stage I-III microsatellite instability low/stable colorectal cancer | 30738427 |
|       | Myopia (pathological) | 23049088 |
|       | Neurological blood protein biomarker levels | 31320639 |
|       | Neuroticism | 30595370, 29942085, 29292387 |
|       | Objective response to lithium treatment in bipolar disorder | 26503763 |

Table 1. Cont.
In this study we used data from ~400,000 individuals from UK Biobank study to determine whether contactin genes contribute to a shared biology between CMD and SMI. Specifically, we (a) defined the impact of genetic variation in the contactin loci on psychiatric, psychological and cardiometabolic traits; (b) assessed in whether the genetic effects for CMD were distinct from, or shared with, those for SMI; and (c) we investigated how the genetic variants impacted on the measured phenotypes.

2. Materials and Methods

2.1. Genes

Genes encoding members of the contactin family were identified using NCBI Gene and chromosomal locations were defined by the UCSC genome browser (build 37). Regions of 400 kb up and downstream of each gene were analysed (Table 2).

2.2. Cohort

The UK Biobank has been previously described in detail [10]. In short, 502,000 individuals were recruited between 2006 and 2010 from 22 centres across the UK. All participants donated a blood sample for DNA analysis as well as completing a physical assessment and extensive online questionnaires detailing medical history (personal and family), lifestyle, education, and economic status. An online “Thoughts and feelings” questionnaire was sent to a subset of individuals (those who had consented to email contact) between 2016–2017. Only white British ancestry individuals were included in this study. This project was completed under UK Biobank applications 6533 (PI. DJS) and 1755 (PI. JPP).

2.3. Genotyping

UK Biobank participants were genotyped using either the Affymetrix UK Biobank Axiom or the Affymetrix BiLEVE Axiom array. Complete information regarding the genotyping, quality control, and imputation, which were conducted centrally by UK Biobank, has been published [10,11]. This study
used the fully imputed genetic data, which was released in March 2018. SNPs were excluded for minor allele frequency (MAF) < 1%, imputation quality score < 0.4, deviation from the Hardy–Weinberg equilibrium \((p < 5 \times 10^{-6})\) or low call rate (<95%). Individuals were excluded for sex mismatch (reported vs. genetic), relatedness (one each pair of individuals with a KING-estimate kinship coefficient > 0.0442 was randomly removed), low call rate (<95%), non-white British ancestry (self-reported and based on principal component analysis) and quality control failure.

Table 2. Genes encoding the contactin family and the regions studied.

| Gene  | Chr | Start   | End     | −400 kb | +400 kb |
|-------|-----|---------|---------|---------|---------|
| CNTN1 | 12  | 40,692,442 | 41,072,412 | 40,292,442 | 41,472,412 |
| CNTN2 | 1   | 205,042,937 | 205,078,272 | 204,642,937 | 205,478,272 |
| CNTN3 | 3   | 74,262,568  | 74,521,140  | 73,862,568  | 74,921,140  |
| CNTN4 | 3   | 2,098,803   | 3,057,961   | 1,698,803   | 3,457,961   |
| CNTN5 | 11  | 99,021,190  | 100,358,885 | 98,621,190  | 100,758,885 |
| CNTN6 | 3   | 1,092,661   | 1,403,594   | 692,661     | 1,803,594   |

Where: Positions refer to build 37.

2.4. Phenotypes

The baseline mental health questionnaire in UK Biobank included questions to assess mood instability (“Does your mood often go up and down?” variable #1920) and risk-taking behaviour (“Would you describe yourself as someone who takes risks” variable #2040). Single item questions are an imperfect measure of complex psychological traits; however, their validity has been demonstrated relative to the expected associations with psychiatric disorders [12,13]. Additionally, the validity of the risk-taking question has been demonstrated relative to more detailed phenotyping [14]. Neuroticism was assessed using the Eysenck Personality Questionnaire (Revised Short Form) involving 12 yes/no questions (one of which was variable #1920 regarding mood instability). A yes response was coded 1, and these were added to give a score of between one and 12 for each individual. Of note, the mood instability question is a component of the neuroticism score.

Psychiatric disorder phenotyping was based on the online mental health (“Thoughts and Feelings”) questionnaire, completed between 2016 and 2017 (so between 6–9 years after baseline assessment) [15]. This questionnaire gathered information on history of major depressive disorder (MDD), bipolar disorder (BD), generalised anxiety disorder (GAD) and addiction. This enabled classification of likely lifetime experience of these disorders [15].

Cardiometabolic phenotypes (body mass index (BMI), waist and hip circumferences, systolic and diastolic blood pressure (SBP and DBP respectively)) were assessed in a standard manner. Waist-to-hip circumference adjusted for BMI (WHRadjBMI) was calculated as per Shungin et al. [16]. Average SBP and DBP were adjusted to reflect treatment-naive levels, with addition of 15 mmHg and 10 mmHg respectively, for those on anti-hypertensive medication prior to analysis [17]. Type 2 diabetes (T2D) was defined as per Eastwood et al. [18]. Current smoking was assessed by questionnaire (variable #20116) and analysed as a binary trait (Current smokers vs. never and former smokers). Cardiovascular disease (CVD) was defined as of heart attack/myocardial infarction or angina (variable # 6150, assessed with the question “has your doctor told you that you have/have had a heart attack/myocardial infarction or angina”).

2.5. Genetic Analyses

All continuous phenotypes (except WHRadjBMI) were normally distributed and none required transformation prior to analysis. The calculation of WHRadjBMI is normalised, sex-specific, and includes adjustment for population stratification and age.

Genetic variants (specifically single nucleotide polymorphisms, SNPs) in the contactin loci were selected after genetic quality control. In the six contactin loci, 23,225 SNPs were available for analysis.
Pairwise analysis and pruning (PLINK 1.07 [19], -indep-pairwise command with default settings (50 bp, 5 bp shift, LD r2 0.5)) resulted in 8008 independent SNPs. Genotype–phenotype analyses were conducted in Plink v1.90 [20] using linear or logistic regression as appropriate (for continuous and binary traits respectively), assuming additive allelic effects. With the exception of WHRadjBMI, all analyses were adjusted for age, sex, population structure (8 principal components), and genotyping chip. BMI and T2D were also adjusted for CVD case-control status. WHRadjBMI was only adjusted for CVD case-control status. We defined Bonferroni-corrected significant associations as those with \( p < 6.24 \times 10^{-6} \) (8008 independent SNPs) and suggestive evidence of association as \( p < 1 \times 10^{-5} \). Conditional analyses, where the lead SNP was included as a covariate, was conducted to determine whether there were any additional independent signals in the locus.

2.6. Data Mining

Linkage disequilibrium (LD) calculations were conducted using a randomly selected sample of 5000 unrelated white British ancestry individuals from UK Biobank, using Haploview [21]. Due to substantial computing requirements, 5000 individuals was considered sufficient. The Genotype Tissue Expression project (GTEx portal) was used to explore tissue expression patterns and genotype-specific effects on tissue expression of contactin genes [22]. The GWAS catalogue was used to identify previously reported associations with members of the CNTN family (20201008). All SNPs within the CNTN family with suggestive (\( p < 1 \times 10^{-5} \)) or genome-wide evidence (\( p < 5 \times 10^{-8} \)) of association with at least one phenotype were assessed for predicted functional effect using Variant Effect Predictor software (VEP) [23].

3. Results

The cohort characteristics are presented in Table 3.

|                  | Men        | Women      | All        |
|------------------|------------|------------|------------|
| N (% men)        | 185,228    | 217,635    | 402,863 (46.0) |
| Age (years)      | 57.1 (8.1) | 56.7 (7.9) | 56.9 (8.0) |
| WHR              | 0.94 (0.06)| 0.82 (0.07)| 0.87 (0.09) |
| BMI (kg/m²)      | 27.8 (4.2) | 27.0 (5.1) | 27.4 (4.8) |
| SBP (mmHg)       | 141 (17)   | 136 (19)   | 138 (19)   |
| DBP (mmHg)       | 84 (10)    | 81 (10)    | 82 (10)    |
| SBP * (mmHg)     | 145 (19)   | 138 (21)   | 141 (21)   |
| DBP * (mmHg)     | 87 (11)    | 82 (11)    | 84 (11)    |
| HbA1C (mmol/mol) | 36.3 (7.3) | 35.7 (5.7) | 36.0 (6.5) |
| Neuroticism score| 3.6 (3.2)  | 4.6 (3.2)  | 4.1 (3.3)  |
| CVD              | 5886 (3.2) | 2294 (1.1) | 8180 (2.03) |
| T2D              | 11,189 (6.0)| 6232 (2.9) | 17421 (4.3) |
| BD               | 875 (1.5)  | 999 (1.4)  | 1874 (1.5) |
| GAD              | 3069 (7.2) | 6029 (12.8)| 9098 (10.1) |
| MDD              | 9684 (19.8)| 21,199 (35.0)| 30,883 (28.2) |
| Addiction        | 3895 (6.9) | 3580 (4.9) | 7475 (5.8) |
| Mood instability | 77,173 (41.7)| 100,841 (46.3)| 178,014 (44.2) |
| Risk-taking      | 18,519 (33.2)| 13,702 (19.4)| 32,221 (25.5) |
| Current Smoking  | 21,821 (11.8)| 18,803 (8.7) | 40,624 (10.1) |

Where *, adjusted to reflect treatment-naïve levels; CVD, cardiovascular disease; BD, bipolar disorder; GAD, generalised anxiety disorder; MDD, major depressive disorder. Continuous variables are presented as means (standard deviation), binary variables are presented as N (%).

The significant associations between SNPs in the contactin loci and CMD- or SMI-related phenotypes are summarised in Table 4.
Table 4. Lead SNP by gene and phenotype.

| Gene   | Trait             | Lead SNP | A1    | A1F   | N      | β/OR   | SE     | P        |
|--------|-------------------|----------|-------|-------|--------|--------|--------|----------|
| CNTN1  | Smoking           | rs11174809 | T | 0.35  | 398,675 | 1.025 | 0.005 | 9.52 × 10^{-7} |
| CNTN2  | Risks            | rs35068223 | T | 0.19  | 377,622 | 1.036 | 0.007 | 1.77 × 10^{-7} |
| CNTN2  | WHRadjBMI         | rs6593925 | G | 0.09  | 401,149 | -0.001| 0.000 | 2.38 × 10^{-11}|
| CNTN2  | * WHRadjBMI       | rs11240349 | A | 0.46  | 400,770 | -0.001| 0.000 | 3.98 × 10^{-8} |
| CNTN4  | BMI               | rs3856837 | C | 0.48  | 394,050 | -0.054| 0.011 | 4.28 × 10^{-7} |
| CNTN4  | * BMI             | rs4685542 | C | 0.14  | 391,199 | -0.075| 0.016 | 1.66 × 10^{-6} |
| CNTN4  | Risks            | rs62232818 | T | 0.10  | 383,065 | 1.042 | 0.009 | 3.09 × 10^{-6} |
| CNTN5  | Neuroticism       | rs10790767 | T | 0.40  | 324,775 | 0.042 | 0.008 | 2.43 × 10^{-7} |
| CNTN5  | HbA1C            | rs11606890 | C | 0.10  | 384,094 | -0.007| 0.001 | 1.14 × 10^{-37}|
| CNTN5  | DBP              | rs633185  | G | 0.29  | 370,841 | -0.459| 0.028 | 1.94 × 10^{-59}|
| CNTN5  | SBP              | rs633185  | G | 0.29  | 370,770 | -0.787| 0.049 | 2.85 × 10^{-58}|

Where A1, effect allele; A1F, effect allele frequency; risks, risk-taking behaviour, 1:204945338 = rs35068223; *, conditional analysis.

3.1. Variation in CNTN Genes

At the CNTN1 locus, 9 SNPs demonstrated significant associations with current smoking (lead SNP rs11174809, p = 9.52 × 10^{-7}, Figure 1A, and Table S1). Analysis of linkage disequilibrium between these SNPs demonstrate that they represent the same signal (Figure S1A).
Figure 1. Regional plots demonstrating genetic associations between CNTN1 and (A) current smoking, CNTN2 and (B) risk-taking behaviour, (C) WHRadjBMI and (D) WHRadjBMI conditioned on rs6593925, CNTN4 and (E) BMI, (F) BMI conditioned on rs3856837, (G) risk-taking behaviour and CNTN5 and (H) neuroticism score, (I) DBP, (J) SBP and (K) HbA1c.

3.2. Comparison with Previous Findings

Previous findings are summarised in Table S5. Where it is possible to compare with previous reports, the effect directions observed in this study are, for the most part, consistent. Where inconsistency is observed, it is highly likely that differences in phenotyping and/or sample size are...
We identified consistent eWHRadjBMI signals are independent, and that the region has many small blocks of LD (Figure S1B). Analysis of LD in this region suggests that the signals for HbA1C and blood pressure are independent (LD R² = 0.68, Figure S1D). In contrast, there was a great deal of overlap between the SNPs associated with SBP and DBP. Whilst the signals associated with HbA1C overlap with those for SBP and DBP, the SNPs are significantly associated either with blood pressure, or with HbA1c, but not with both (Table S4).

At the CNTN2 locus, significant evidence of association was demonstrated for seven SNPs with risk-taking behaviour (lead rs35068223, \( p = 1.77 \times 10^{-7} \), Figure 1B and Table S2) and 133 SNPs with WHRadjBMI (rs6593925, \( p = 2.38 \times 10^{-11} \), Figure 1C). Further analysis of WHRadjBMI, conditioning on rs6593925, demonstrated a second signal with 70 significant associations (rs11240349, \( p = 3.98 \times 10^{-8} \), Figure 1D). This SNP was only nominally significant (\( p = 0.0014 \)) in the initial analysis; however, there are five SNPs which were significant in both the unconditional and conditional analyses (rs12048743, \( p = 2.86 \times 10^{-10} \) and 2.90 \( \times 10^{-6} \), respectively). Analysis of LD demonstrates that the risk-taking and WHRadjBMI signals are independent, and that the region has many small blocks of LD (Figure S1B).

For variants in the CNTN4 locus, 58 significant associations were evident for BMI (lead rs3856837, \( p = 4.28 \times 10^{-7} \), Figure 1E and Table S3). Further analysis of BMI, specifically conditioning on rs3856837, demonstrated a second significant signal -360Kb downstream (rs4685542, \( p = 1.66 \times 10^{-6} \), Figure 1F). One SNP was significantly associated with risk-taking behaviour (lead rs62232818, \( p = 3.09 \times 10^{-6} \), Figure 1G). Analysis of LD structure of this region indicates that the two BMI signals and the risk-taking signal are distinct from each other (Figure S1C).

The CNTN5 locus (Table S4) demonstrated significant associations for 64 variants with the neuroticism score (rs10790767, \( p = 2.43 \times 10^{-7} \), Figure 1H), 409 variants with SBP (rs633185, \( p = 2.25 \times 10^{-58} \), Figure 1I), 395 variants with DBP (rs633185, \( p = 1.94 \times 10^{-59} \), Figure 1J) and 301 with HbA1C (rs11606890, \( p = 1.14 \times 10^{-37} \), Figure 1K). As shown in Figure 1, the SNPs associated with neuroticism score did not overlap with those for SBP/DBP or HbA1c. Indeed, these signals are ~1Mb apart and analysis of LD across this locus suggests that they are independent signals (Figure S1D). In contrast, there was a great deal of overlap between the SNPs associated with SBP and DBP. Whilst the signals associated with HbA1C overlap with those for SBP and DBP, the SNPs are significantly associated either with blood pressure, or with HbA1c, but not with both (Table S4). Analysis of LD in this region suggests that the signals for HbA1C and blood pressure are independent (LD R² = 0.16). No significant associations were observed for SNPs in the CNTN3 or CNTN6 loci.

3.2. Comparison with Previous Findings

Previous findings are summarised in Table S5. Where it is possible to compare with previous reports, the effect directions observed in this study are, for the most part, consistent. Where inconsistency is observed, it is highly likely that differences in phenotyping and/or sample size are the reason. We identified consistent effect direction for SNPs in CNTN5 and neuroticism [24], smoking [25], WHRadjBMI [16], and HbA1C [26], CNTN4 and smoking [25], and CNTN2 and T2D [27]. In the CNTN2
locus, rs3903399-T allele has previously been associated with increased WHRadjBMI [28], whereas the WHRadjBMI-increasing allele in this study was rs3903399-C (Table S5). The rs3903399-T has also been associated with increased high density lipoprotein (HDL) cholesterol levels [29], which would be consistent with our findings for WHRadjBMI.

3.3. Effect Prediction in VEP

In order to identify any SNPs with predicted functional effects (that is those expected to change the protein sequence or quantity), all SNPs within the CNTN loci with significant evidence of association with at least one phenotype, were assessed for predicted functional effect using the Variant Effect Predictor [23] (Table S6). Only one missense variant was identified (rs3851294), but this is predicted to be benign/tolerated. The remaining variants had no clear functional effect.

3.4. Gene Expression Patterns

Tissue expression patterns of the CNTN genes are presented in Figure 2. Expression of CNTN1 (Figure 2A) is highest in the brain, therefore the association of SNPs in this gene with a behavioural trait such as smoking is plausible. CNTN2 is predominantly expressed in the brain (Figure 2B), which is consistent with effects on behavioural traits such as risk-taking. The effect on WHRadjBMI is less clear but could be indirectly through influencing eating preferences and behaviours. Whilst there is evidence to suggest that genetic variants associated with WHRadjBMI act through expression of genes in adipose tissue, [16], there is significant [16,30] genetic overlap of this trait with BMI, whereby genetic variants are through to act via gene expression in the brain [30]. In contrast, CNTN4 is widely expressed (Figure 2D), with brain (consistent with risk-taking behaviour) and adipose (consistent with BMI) expression being observed. The expression of CNTN5 is predominantly observed (Figure 2E) in the brain (consistent with neuroticism). Whilst CNTN5 expression was also observed in arterial tissue (consistent with SBP and DBP) and the pituitary gland (consistent with HbA1c).
Figure 2. Cont.
Figure 2. Tissue expression profiles of members of the CNTN family in the GTex dataset (A) CNTN1, (B) CNTN2, (C) CNTN3, (D) CNTN4, (E) CNTN5 and (F) CNTN6.

3.5. Genotype-Specific Expression Patterns for Associated SNPs (eQTLs)

Using the GTEx data, genetic variants with genotype-specific effects on CNTN gene expression were identified (Table S7). Of the eQTLs identified for the contactin family, only those for CNTN2 expression levels overlapped with significant associations in these analyses. The missense SNP rs3851294 (CNTN2, associated with WHRadjBMI) shows effects on CNTN2 expression in the thyroid and oesophageal muscle. Additional eQTL SNPs for CNTN2 which overlap with those significantly associated with risk-taking behaviour or WHRadjBMI demonstrated effects in a variety of tissues, which is curious given that CNTN2 expression is predominantly in the brain (Table S7). How expression of CNTN2 levels in the oesophagus, nerve, skin or thyroid influence risk-taking behaviour or WHRadjBMI is unclear. Irrespective of the tissue analysed, the WHRadjBMI-lowering allele was associated with reduced levels of CNTN2 expression. Of the SNPs in CNTN5, those associated with blood pressure and HbA1c are downstream of CNTN5 and closer to ARHGAP42 and TMEM133 than to CNTN5. The lead SNPs for these signals have genotype-specific effects on expression of ARHGAP42 and TMEM133 in arterial and heart tissues (Table S8), suggesting that the effect of these signals are via ARHGAP42 or TMEM133 rather than CNTN5.
4. Discussion

This study, of multiple phenotypes in a single-protocol, very large population-based cohort, identified genomic loci for current smoking at CNTN1, risk-taking and WHRadjBMI at CNTN2, risk-taking and BMI at CNTN4 and neuroticism, DBP/SBP and HbA1c at CNTN5. Loci for psychological traits were independent from those for cardio-metabolic traits, therefore these results do not support contactins as a putative biological link between mental and physical illness.

The association between CNTN1 and current smoking is novel. This locus has previously been associated with neuro-psychiatric (Parkinson’s disease [31], Alzheimer’s disease [32], bipolar disorder [33] and antidepressant response [34]), but not behavioural traits. Whilst CNTN1 gene expression is not limited to the brain, this study provides no evidence for a role of CNTN1 in cardio-metabolic disease.

We provide replication of the association between CNTN2 and risk-taking behaviour/adventurousness [35], however the association with a metabolic phenotype, specifically WHRadjBMI, is novel. CNTN2 has previously been associated with WHR and schizophrenia [36–38]. Whilst it has been established that patients with schizophrenia have a tendency towards abdominal obesity [3,4], our results suggest that the signals for psychiatric and metabolic phenotypes are independent signals, with the schizophrenia-associated SNPs showing only nominal associations with BMI (lowest \( p = 0.0053 \)).

Whilst an association between the CNTN4 locus and longitudinal BMI has previously been reported [39], our demonstration of two independent signals for BMI is novel. In addition, the association of this region with risk-taking is novel. Although it has previously been demonstrated that genetic regulation of risk-taking overlaps with that for schizophrenia, and CNTN4 has previously been associated with schizophrenia [12,40], the null association between schizophrenia-associated SNPs and risk-taking in this study suggests that the signals for these traits are independent.

The associations observed between CNTN5 and neuroticism, blood pressure and HbA1c are not novel, however the demonstration that these signals are independent of each other is an advancement in understanding the impact of this locus on biology. It is worth noting there is a ctn5-knockout mouse model, which reported no behavioural phenotype but effects on obesity and blood pressure variables [41]. According to the NCBI genome data viewer (mus musculus), arhgap42 overlaps the ctn5 gene, therefore it is conceivable that the ctn5-knockout model also results in arhgap42 loss of function and thus a phenotype consistent with that in humans.

This study did not find evidence for the effects of the contactin family on MDD; this is unlikely to be due to the number of cases present. One possibility is due to imprecise phenotyping: We used a measure of probable lifetime MDD; a confirmed diagnosis of MDD as per DSM or ICD criteria may have yielded different results. Investigating the locus in the Psychiatric Genetics Consortium (PGC) data was considered; however, the conclusions drawn would be less clear: The PGC data also using highly heterogeneous phenotype definitions, depending upon where the samples were collected, and covariates differ between cohorts and analyses. In addition, assessing the independence of signals (multiple signals for one trait or between multiple traits) is more difficult from summary statistics, and could be biased by the population structure/ancestry and/or how the population structure was accounted for in the analysis. Hence restricting the analysis to only UK Biobank meant that the assessment of genetic architecture of the contactin genes was robust, even if the lack of clinical diagnosis for psychiatric illness is a weakness. Additionally, due to the nature of individuals recruited to UK Biobank, there are unlikely to be many cases of severe MDD. Having a preponderance of mild/single episode depression cases could dilute the phenotype, therefore differences between MDD cases and controls (which potentially include subclinical or undiagnosed MDD) are relatively small.

MDD is a heterogenous phenotype [42–44]. Attempts to subdivide MDD [45] have included by clinical presentation (e.g., atypical, melancholic, psychotic), by timing of onset (age of onset, seasonality, postpartum), course (single episode, recurrent, chronic) and severity. Subtypes of MDD may have different genetic risk factors, for example, childhood-onset MDD is genetically more similar to schizophrenia and bipolar disorder than to adult-onset MDD [46]. However, large-scale studies
assessing whether different clinical presentations of MDD have different genetic risk factors are in the early stages [47], despite increasing evidence for different biological pathways being implicated (HPA-axis dysregulation for melancholic depression and inflammation in atypical depression) [8,46,48]. It remains to be seen whether the CNTN family influences subtypes of depression specifically.

We did not find evidence of association between the contactin family and addiction, mood instability, bipolar disorder, ischaemic heart disease and type 2 diabetes. CNTN5 has been considered a candidate gene for bipolar disorder [9], however we did not find evidence of an association, potentially because of the relatively small number of BD cases in this cohort.

5. Conclusions

In summary, our systematic analysis of genetic variation in the CNTN family of genes identified roles for CNTN1, CNTN2, CNTN4 and CNTN5 in a variety of psychological and cardiometabolic traits. The evidence presented here suggests that the effects of these genes on psychological and cardiometabolic traits are likely distinct, with no evidence of shared mechanisms.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/11/11/1326/s1, Figure S1: Linkage disequilibrium within the contactin genes: (A) CNTN1, (B) CNTN2, (C) CNTN4, (D) CNTN5. Red dots indicate lead SNPs. Table S1: Genetic variants in CNTN1 significantly associated with current smoking. Table S2: Genetic variants in CNTN2 significantly associated with WHRadjBMI or risk-taking. Table S3: Genetic variants in CNTN4 with significant associations with BMI or risk-taking. Table S4: Genetic variants in CNTN5 significantly associated with neuroticism, systolic and diastolic blood pressure, HbA1c. Table S5: Previously reported associations with the CNTN family of genes. Table S6: Predicted effects of SNPs significantly associated with one or more trait. Table S7: Genetic variants in CNTN1 CNTN2, CNTN4 and CNTN5 with genotype-specific effects on gene expression. Table S8: Genotype-specific expression of ARHGAP42 and TMEM133.

Author Contributions: Conceptualization, R.J.S.; Data curation, B.C., N.G. and J.W.; Formal analysis, J.M. and S.S.Y.L.; Project administration, D.J.S.; Writing—original draft, J.M., S.S.Y.L. and R.J.S.; Writing—review and editing, M.E.S.B., B.C., A.F., N.G., K.J.A.J., D.M.L., L.M.L., J.W. and D.J.S. All authors have read and agreed to the published version of the manuscript.

Funding: The UK Biobank was established by the Wellcome Trust, Medical Research Council, Department of Health, Scottish Government and Northwest Regional Development Agency. UK Biobank has also had funding from the Welsh Assembly Government and the British Heart Foundation. Data collection was funded by UK Biobank. This project was completed under UK Biobank applications 6533 (PI. D.J.S.) and 1755 (PI. J.P.P.). J.M. was supported by the Royal College of Psychiatrists’ Psych Star scheme. J.W. and L.M.L. were supported by the JMAS Sim Fellowships for depression research from the Royal College of Physicians of Edinburgh (173558). K.J.A.J. is supported by an MRC Doctoral Training Programme Studentship at the Universities of Glasgow and Edinburgh. D.J.S. acknowledges the support of the Brain and Behaviour Research Foundation (Independent Investigator Award 1930) and a Lister Prize Fellowship (173096). R.J.S. is supported by a UKRI Innovation-HDR-UK Fellowship (MR/S003061/1).

Acknowledgments: We thank all participants and staff of the UK Biobank study. This work uses data provided by patients and collected by the NHS as part of their care and support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Amare, A.T.; Schubert, K.O.; Klingler-Hoffmann, M.; Cohen-Woods, S.; Baune, B.T. The genetic overlap between mood disorders and cardiometabolic diseases: A systematic review of genome wide and candidate gene studies. *Transl. Psychiatry* 2017, 7, e1007. [CrossRef] [PubMed]
2. Bradshaw, T.; Mairs, H. Obesity and Serious Mental Ill Health: A Critical Review of the Literature. *Healthcare* 2014, 2, 166–182. [CrossRef] [PubMed]
3. De Hert, M.; Correll, C.U.; Bobes, J.; Cetkovich-Bakmas, M.; Cohen, D.; Asai, I.; Detraux, J.; Gautam, S.; Möller, H.-J.; Ndetei, D.M.; et al. Physical illness in patients with severe mental disorders. I. Prevalence, impact of medications and disparities in health care. *World Psychiatry* 2011, 10, 52–77. [CrossRef] [PubMed]
4. So, H.-C.; Chau, K.-L.; Ao, F.-K.; Mo, C.-H.; Sham, P.C. Exploring shared genetic bases and causal relationships of schizophrenia and bipolar disorder with 28 cardiovascular and metabolic traits. *Psychol. Med.* 2019, 49, 1286–1298. [CrossRef] [PubMed]

5. Mulugeta, A.; Zhou, A.; King, C.; Hyppönen, E. Association between major depressive disorder and multiple disease outcomes: A phenome-wide Mendelian randomisation study in the UK Biobank. *Mol. Psychiatry* 2020, 25, 1469–1476. [CrossRef] [PubMed]

6. Tang, B.; Yuan, S.; Xiong, Y.; He, Q.; Larsson, S.C. Major depressive disorder and cardiometabolic diseases: A bidirectional Mendelian randomisation study. *Diabetologia* 2020, 63, 1305–1311. [CrossRef] [PubMed]

7. Morris, J.; Bailey, M.E.S.; Baldassarre, D.; Cullen, B.; De Faire, U.; Ferguson, A.; Gigante, B.; Giral, P.; Goel, A.; Graham, N.; et al. Genetic variation in CADM2 as a link between psychological traits and obesity. *Sci. Rep.* 2019, 9, 7339. [CrossRef]

8. Lamers, F.; Vogelzangs, N.; Merikangas, K.R.; De Jonge, P.; Breekman, A.T.F.; Penninx, B.W.J.H. Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. *Mol. Psychiatry* 2013, 18, 692–699. [CrossRef]

9. Teunissen, C.E.; Chatterjee, M.; Schild, D. Contactins in the central nervous system: Role in health and disease. *Neural Regen. Res.* 2019, 14, 206–216. [CrossRef]

10. Matthews, P.M.; Sudlow, C.L. The UK Biobank. *Brain* 2015, 138 Pt 12, 3463–3465. [CrossRef]

11. Bycroft, C.; Freeman, C.; Petkova, D.; Band, G.; Elliott, L.T.; Sharp, K.; Motyer, A.; Vukcevic, D.; Delaneau, O.; O’Connell, J.; et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018, 562, 203–209. [CrossRef] [PubMed]

12. Strawbridge, R.J.; Ward, J.; Cullen, B.; Tunbridge, E.M.; Hartz, S.; Bierut, L.; Horton, A.; Bailey, M.E.S.; Graham, N.; Ferguson, A.; et al. Genome-wide analysis of self-reported risk-taking behaviour and cross-disorder genetic correlations in the UK Biobank cohort. *Transl. Psychiatry* 2018, 8, 39. [CrossRef] [PubMed]

13. Ward, J.; Strawbridge, R.J.; Bailey, M.E.S.; Graham, N.; Ferguson, A.; Lyall, D.M.; Cullen, B.; Pidgeon, L.M.; Cavanagh, J.; Mackay, D.F.; et al. Genome-wide analysis in UK Biobank identifies four loci associated with mood instability and genetic correlation with major depressive disorder, anxiety disorder and schizophrenia. *Transl. Psychiatry* 2017, 7, 1264. [CrossRef] [PubMed]

14. Frey, R.; Pedroni, A.; Mata, R.; Rieskamp, J.; Hertwig, R. Risk preference shares the psychometric structure of major psychological traits. *Sci. Adv.* 2017, 3, e1701381. [CrossRef]

15. Davis, K.A.S.; Coleman, J.R.I.; Adams, M.; Allen, N.; Breen, G.; Cullen, B.; Dickens, C.; Fox, E.; Graham, N.; Holliday, J.; et al. Erratum: Mental health in UK Biobank: Development, implementation and results from an online questionnaire completed by 157,366 participants-CORRIGENDUM. *BJPsych Open* 2018, 4, 136. [CrossRef]

16. Shungin, D.; Winkler, T.W.; Croteau-Chonka, D.C.; Ferreira, T.; Locke, A.E.; Mägi, R.; Strawbridge, R.J.; Pers, T.H.; Fischer, K.; Justice, A.E.; et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nat. Cell Biol.* 2015, 518, 187–196. [CrossRef]

17. Ehret, G.B.; CHARGe-EchoGen Consortium; Ferreira, T.; Chasman, D.I.; Jackson, A.U.; Schmidt, E.M.; Johnson, T.; Thorleifsson, G.; Luan, J.; Donnelly, L.A.; et al. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nat. Genet.* 2016, 48, 1171–1184. [CrossRef]

18. Eastwood, S.V.; Mathur, R.; Atkinson, M.; Brophy, S.; Sudlow, C.; Flaug, R.; De Lusignan, S.; Allen, N.; Chaturvedi, N. Algorithms for the Capture and Adjudication of Prevalent and Incident Diabetes in UK Biobank. *PLoS ONE* 2016, 11, e0162388. [CrossRef] [PubMed]

19. Purcell, S.; Neale, B.; Todd-Brown, K.; Thomas, L.; Ferreira, M.A.R.; Bender, D.; Maller, J.; Sklar, P.; De Bakker, P.I.W.; Daly, M.J.; et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am. J. Hum. Genet.* 2007, 81, 559–575. [CrossRef]

20. Chang, C.C.; Chow, C.C.; Tellier, L.C.A.M.; Vattikuti, S.; Purcell, S.M.; Lee, J.J. Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience* 2015, 4, 7. [CrossRef]

21. Barrett, J.C.; Fry, B.; Maller, J.D.M.J.; Daly, M.J. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005, 21, 263–2655. [CrossRef] [PubMed]

22. Lonsdale, J.; Thomas, J.; Salvatore, M.; Phillips, R.; Lo, E.; Shad, S.; Hasz, R.; Walters, G.; Garcia, F.; Young, N.; et al. The Genotype-Tissue Expression (GTEx) project. *Nat. Genet.* 2013, 45, 580–585. [CrossRef] [PubMed]
23. McLaren, W.; Gil, L.; Hunt, S.E.; Riat, H.S.; Ritchie, G.R.S.; Thormann, A.; Flicek, P.; Cunningham, F. The Ensembl Variant Effect Predictor. *Genome Biol.* 2016, 17, 122. [CrossRef] [PubMed]

24. Nagel, M.; Jansen, P.R.; Stringer, S.; Watanabe, K.; De Leeuw, C.A.; Bryois, J.; Savage, J.E.; Hammerschlag, A.R.; Skene, N.G.; Muñoz-Manchado, A.B.; et al. Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways. *Nat. Genet.* 2018, 50, 920–927. [CrossRef]

25. Liu, M.; Jiang, Y.; Wedow, R.; Li, Y.; Brazel, D.M.; Chen, F.; Datta, G.; Davila-Velderrain, J.; McGuire, D.; Tian, C.; et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat. Genet.* 2019, 51, 237–244. [CrossRef]

26. Wheeler, E.; Leong, A.; Liu, C.-T.; Hivert, M.-F.; Strawbridge, R.J.; Podmore, C.; Li, M.; Yao, J.; Sim, X.; Hong, J.; et al. Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis. *PLoS Med.* 2017, 14, e1002383. [CrossRef]

27. Vujkovic, M.; The HPAP Consortium; Keaton, J.M.; Lynch, J.A.; Miller, D.R.; Zhou, J.; Tcheandjieu, C.; Huffman, J.E.; Assimes, T.L.; Lorenz, K.; et al. Discovery of 318 new risk loci for type 2 diabetes and related vascular outcomes among 1.4 million participants in a multi-ancestry meta-analysis. *Nat. Genet.* 2020, 52, 680–691. [CrossRef]

28. Pulit, S.L.; Stoneman, C.; Morris, A.P.; Wood, A.R.; Glastonbury, C.A.; Tyrrell, J.; Yengo, L.; Ferreira, T.; Marouli, E.; Ji, Y.; et al. Meta-analysis of genome-wide association studies for body fat distribution in 694,649 individuals of European ancestry. *Hum. Mol. Genet.* 2019, 28, 166–174. [CrossRef]

29. Richardson, T.G.; Sanderson, E.; Palmer, T.M.; Ala-Korpela, M.; Ference, B.A.; Smith, G.D.; Holmes, M.V. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. *PLoS Med.* 2020, 17, e1003062. [CrossRef]

30. Locke, A.E.; Kahali, B.; Berndt, S.I.; Justice, A.E.; Pers, T.H.; Day, F.R.; Powell, C.; Vedantam, S.; Buchkovich, M.L.; Yang, J.; et al. Genetic studies of body mass index yield new insights for obesity biology. *Nat. Cell Biol.* 2015, 17, 198–206. [CrossRef]

31. Vacic, V.; Ozelius, L.J.; Clark, L.N.; Bar-Shira, A.; Gana-Weisz, M.; Gurevich, T.; Gusev, A.; Kedmi, M.; Kenny, E.E.; Liu, X.; et al. Genome-wide mapping of IBD segments in an Ashkenazi PD cohort identifies associated haplotypes. *Hum. Mol. Genet.* 2014, 23, 4693–4702. [CrossRef] [PubMed]

32. Beecham, G.W.; Hamilton, K.; Naj, A.C.; Martin, E.R.; Huentelman, M.; Myers, A.J.; Corneveaux, J.J.; Hardy, J.; Vonsattel, J.P.; Younkin, S.G.; et al. Genome-wide association meta-analysis of neuropathologic features of Alzheimer’s disease and related dementias. *PLoS Genet.* 2014, 10, e1004606. [CrossRef] [PubMed]

33. Lee, H.-J.; Woo, H.G.; Greenwood, T.A.; Kripke, D.F.; Kelsoe, J. A genome-wide association study of seasonal pattern mania identifies NF1A as a possible susceptibility gene for bipolar disorder. *J. Affect. Disord.* 2013, 145, 200–207. [CrossRef] [PubMed]

34. Wiggmore, E.M.; Hafferty, J.D.; Hall, L.S.; Howard, D.M.; Clarke, T.-K.; Fabbri, C.; Lewis, C.M.; Uher, R.; Navrady, L.B.; Adams, M.J.; et al. Genome-wide association study of antidepressant treatment resistance in a population-based cohort using health service prescription data and meta-analysis with GENDEP. *Pharm. J.* 2020, 20, 329–341. [CrossRef] [PubMed]

35. Linnér, R.K.; Biroli, P.; Kong, E.; Meddens, S.F.W.; Wedow, R.; Fontana, M.A.; Lebreton, M.; Tino, S.P.; Abdellauoui, A.; Hammerschlag, A.R.; et al. Genome-wide association analyses of risk tolerance and risky behaviors in over 1 million individuals identify hundreds of loci and shared genetic influences. *Nat. Genet.* 2019, 51, 245–257. [CrossRef]

36. Goes, F.S.; McGrath, J.; Avramopoulos, D.; Wolyniec, P.S.; Pirooznia, M.; Ruczinski, I.; Nestadt, G.; Kenny, E.E.; Vacic, V.; Peters, I.; et al. Genome-wide association study of schizophrenia in Ashkenazi Jews. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* 2015, 168, 649–659. [CrossRef]

37. Ikeda, M.; Takahashi, A.; Kamatani, Y.; Momozawa, Y.; Saito, T.; Kondo, K.; Shimasaki, A.; Kawase, K.; Sakusabe, T.; Iwayama, Y.; et al. Genome-Wide Association Study Detected Novel Susceptibility Genes for Schizophrenia and Shared Trans-Populations/Diseases Genetic Effect. *Schizophr. Bull.* 2019, 45, 824–834. [CrossRef]

38. Li, Z.; Chen, J.; Yu, H.; He, L.; Xu, Y.; Zhang, D.; Yi, Q.; Li, C.; Li, X.; Shen, J.; et al. Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. *Nat. Genet.* 2017, 49, 1576–1583. [CrossRef]
39. McDonald, M.-L.N.; Won, S.; Mattheisen, M.; Castaldi, P.J.; Cho, M.H.; Rutten, E.; Hardin, M.; Yip, W.; Rennard, S.I.; Lomas, D.A.; et al. Body mass index change in gastrointestinal cancer and chronic obstructive pulmonary disease is associated with Dedicator of Cytokinesis 1. *J. Cachex Sarcopenia Muscle* **2017**, *8*, 428–436. [CrossRef]

40. Strawbridge, R.J.; Ward, J.; Lyall, L.M.; Tunbridge, E.M.; Cullen, B.; Graham, N.; Ferguson, A.; Johnston, K.J.A.; Lyall, D.M.; Mackay, D.; et al. Genetics of self-reported risk-taking behaviour, trans-ethnic consistency and relevance to brain gene expression. *Transl. Psychiatry* **2018**, *8*, 178. [CrossRef]

41. Smirnov, A.; Kontsevaya, G.V.; Feofanova, N.A.; Anisimova, M.V.; Serova, I.A.; Gerlinskaya, I.A.; Battulin, N.R.; Moshkin, M.P.; Serov, O.L. Unexpected phenotypic effects of a transgene integration causing a knockout of the endogenous Contactin-5 gene in mice. *Transgenic Res.* **2017**, *27*, 1–13. [CrossRef] [PubMed]

42. Fried, E.I. Moving forward: How depression heterogeneity hinders progress in treatment and research. *Expert Rev. Neurother.* **2017**, *17*, 423–425. [CrossRef] [PubMed]

43. Zimmerman, M.; Ellison, W.; Young, D.; Chelminski, I.; Dalrymple, K. How many different ways do patients meet the diagnostic criteria for major depressive disorder? *Compr. Psychiatry* **2015**, *56*, 29–34. [CrossRef] [PubMed]

44. Olbert, C.M.; Gala, G.J.; Tupler, L.A. Quantifying heterogeneity attributable to polythetic diagnostic criteria: Theoretical framework and empirical application. *J. Abnorm. Psychol.* **2014**, *123*, 452–462. [CrossRef]

45. Van Loo, H.M.; De Jonge, P.; Romeijn, J.-W.; Kessler, R.C.; Schoevers, R.A. Data-driven subtypes of major depressive disorder: A systematic review. *BMC Med.* **2012**, *10*, 156. [CrossRef]

46. Power, R.A.; Tansey, K.E.; Buttenschön, H.N.; Cohen-Woods, S.; Bigdeli, T.; Hall, L.S.; Kutalik, Z.; Lee, S.H.; Ripke, S.; Steinberg, S.; et al. Genome-wide Association for Major Depression Through Age at Onset Stratification: Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium. *Biol. Psychiatry* **2017**, *81*, 325–335. [CrossRef]

47. Cai, N.; Choi, K.W.; Fried, E.I. Reviewing the genetics of heterogeneity in depression: Operationalizations, manifestations and etiologies. *Hum. Mol. Genet.* **2020**, *29*, R10–R18. [CrossRef]

48. Bosaipo, N.B.; Foss, M.P.; Young, A.H.; Juruena, M.F. Neuropsychological changes in melancholic and atypical depression: A systematic review. *Neurosci. Biobehav. Rev.* **2017**, *73*, 309–325. [CrossRef]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).