Molecular Psychiatry (2014) 19, 960–965

www.nature.com/mp

LETTERS TO THE EDITOR

Epigenetics and the war on mental illness

Molecular Psychiatry (2014) **19,** 960; doi:10.1038/mp.2014.16; published online 4 March 2014

J Peedicayil[1]
[1]*Department of Pharmacology and Clinical Pharmacology, Christian Medical College, Vellore, India*
E-mail: jpeedi@cmcvellore.ac.in

I found the editorial by Licinio and Wong[1] entitled 'Launching the war on mental illness' very interesting. The authors discussed the need for a coordinated and well planned assault on mental illness, and suggested that the present time for this 'war' to be launched is propitious. They suggested that this endeavour has to be done using the processes of translational science, and keeping this in mind, they discussed the modalities involved.

I would like to discuss the role of epigenetics in this endeavour. Epigenetics includes molecular mechanisms like DNA methylation, histone modifications, and RNA-mediated regulation of gene expression. Epigenetics is presently a very active area of research in biomedicine the world over and is proving to be pervasive in its relevance, and monumental in its importance, to mental illness. For example, there is increasing evidence that epigenetics plays a key role in various aspects of normal behaviour[2] and In the pathogenesis of virtually every type of mental illness.[2–4] Epigenetics could also play a key role in several ways in the clinical management of mental illness: (1) In the diagnosis of mental illness: epigenetic changes in the body may prove to be useful in the clinical diagnosis of mental illness by providing suitable biomarkers.[5] (2) In the prevention of mental illness since abnormal epigenetic mechanisms of gene expression are potentially reversible.[6] Prevention of mental illness could be attempted by one or more of the interventional modalities described below (items 3 to 7). (3) In the pharmacotherapy of mental illness: epigenetic therapy, the use of drugs to correct epigenetic defects, could be used for the treatment of mental illness. At present most work on epigenetic therapy is focussed on the design and development of drugs targeting DNA methylation and drugs inhibiting the enzyme histone deacetylase.[6] These drugs are being investigated for use in mental illness.[2] (4) In the nutritional management of mental illness.[7] For example, methyl donors like L-methylfolate and S-adenosylmethionine which donate a methyl group in the body and hence enhance DNA methylation, are being investigated for use in mental illness.[7] (5) In psychotherapy: there is evidence that psychotherapy exerts its beneficial effects on patients with mental illness by acting by epigenetic mechanisms.[7,8] (6) Electoconvulsive therapy (ECT): there is accumulating evidence that ECT exerts its beneficial effects on patients at least partially by changing epigenetic mechanisms of gene expression in the brain in treated patients.[2,9] (7) Physical exercise, which is known to benefit patients with mental illness: there is accumulating evidence that physical exercise exerts its beneficial effects on the mind and body at least partially by epigenetic mechanisms.[10]

From the preceding information, it appears that epigenetics has a prominent role in the pathogenesis and clinical management of mental illness. Hence, I suggest that epigenetics should be given due attention in the 'war' on mental illness. I suggest that epigenetics should be one of the spearheads of this endeavour.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1 Licinio J, Wong M-L. *Mol Psychiatry* 2014; **19**: 1–5.
2 Peedicayil J. *Clin Epigenetics* 2011; **2**: 249–256.
3 Peedicayil J. *Indian J Med Res* 2007; **126**: 105–111.
4 Peedicayil J. *Curr Pharm Des* 2014; **20**, in press.
5 Peedicayil J. *Br J Pharmacol* 2008; **155**: 795–796.
6 Peedicayil J. *Indian J Med Res* 2006; **123**: 17–24.
7 Peedicayil J. *J Clin Pharm Ther* 2012; **37**: 499–501.
8 Stahl SM. *J Clin Pharm Ther* 2012; **37**: 249–253.
9 McGowan PO, Kato T. *Environ Health Prev Med* 2008; **13**: 16–24.
10 Ehlert T, Simon P, Moser DA. *Sports Med* 2013; **43**: 93–110.

The effect of FTO rs9939609 on major depression differs across MDD subtypes

Molecular Psychiatry (2014) **19,** 960–962; doi:10.1038/mp.2014.4; published online 4 February 2014

Recently, Samaan *et al.*[1] reported in this journal that the fat mass and obesity-associated protein (FTO) rs9939609 A variant was associated with a lower risk of major depressive disorder (MDD) independently from BMI. However, clinical heterogeneity may contribute to variability of results in association studies investigating the biologic and genetic underpinnings of MDD. Using data from the Netherlands Study of Depression and Anxiety (NESDA) we showed[2] in a previous issue of the journal that depressed patients can be divided using data-driven techniques into a 'typical' (termed also 'melancholic') and an 'atypical' subtype differentiated mainly by the direction of change in vegetative symptoms (that is, appetite, weight and sleep) and associated with distinct biological correlates, pointing to a partially distinct pathophysiological and genetic liability. Therefore, considering our prior observations that obesity and increased appetite are more prevalent in atypical depression, and obesity rates are not different or even less prevalent as compared with controls in typical depression,[2,3] it can be expected that the FTO variant shows different associations across MDD subtypes. As an exemplification of our hypothesis, we performed the same association test between rs9939609 and MDD as in the paper of Samaan *et al.*[1] In addition, we explored whether this association differed across clinical subtypes.

Our sample consisted of 4350 unrelated participants (42.0 ± 14.1 years of age, 63.6% females) of European ancestry from the NESDA[4] (1544 cases and 336 controls) and from the Netherlands Twin Registry[5] (2470 controls). Genotyping and imputation methods and QC checks have been extensively detailed elsewhere.[6] Briefly, genotyping has

Table 1. Characteristics of the study sample and adjusted association between rs9939609 with major depression and subtypes

		MDD cases								
Characteristics	*Controls* (n = *2806*)	*All MDD* (n = *1544*)	P*	*Severe typical* (n = *235*)		*Moderate severity* (n = *687*)		*Severe atypical* (n = *256*)		P*
Sex(%)			5.14E-06							2.01E – 04
Males	38.9	31.9		32.8		36.8		25.8		
Females	61.1	68.1		67.2		63.2		74.2		
					post hoc P		*post hoc P*		*post hoc P*	
Age (years) (mean ± s.d.)	43.0 ± 15.0	42.5 ± 12.3	0.29	40.6 ± 12.5		42.5 ± 12.6		43.6 ± 11.1		0.07
BMI (kg m^{-2}) (mean ±s.d.)	24.5 ± 4.0	25.8 ± 5.1	2.35E – 20	24.6 ± 4.4	a	25.1 ± 4.7	a,b	28.7 ± 5.8	b,c,d	< 1.0E–30
FTO rs9939609 (A)		*OR (95 % CI)*	P**	*OR (95 % CI)*	P***	*OR (95 % CI)*	P***	*OR (95 % CI)*	P***	
Model 1	Ref.	1.11 (*1.02–1.22*)	0.023	1.02 (*0.84–1.23*)	0.88	1.11 (*0.98–1.25*)	0.09	1.42 (*1.18–1.71*)	1.84E – 04	
Model 2	Ref.	1.07 (*0.98–1.18*)	0.107	1.01 (*0.83–1.23*)	0.93	1.10 (*0.97–1.24*)	0.14	1.34 (*1.11–1.61*)	0.003	

Abbreviations: BMI, body mass index; CI, confidence interval; FTO, fat mass and obesity-associated protein; MDD, major depressive disorder; OR, odds ratio.
post hoc P from Tukey's test: a, significantly different vs severe-atypical; b, significantly different vs controls; c, significantly different vs severe-typical; d, significantly different vs moderate severity.
Model 1: adjusted for age, sex and principal components 1–3.
Model 2: Model 1+BMI.
*From χ^2 test or general linear model.
**From binomial logistic regression.
***From multinomial logistic regression.

been subsequently performed on multiple chip platforms in (partially overlapping) different subsets of the total sample (Affymetrix-Perlegen 5.0, Illumina 370 K, Illumina 660 K, Illumina Omni 1 M and Affymetrix 6.0) and data were imputed using the 1000 Genomes phase 1 INTEGRATED RELEASE version 3 ALL panel.[7] rs9939609 was typed in some of the panels used for imputation and all post-imputation QC criteria were met; r2hat value was 0.99, indicating an almost perfect correlation between the imputed genotype and its true underlying genotype. Minor allele frequency was 0.38. The diagnosis of lifetime and/or current MDD according to DSM-IV was ascertained using the Composite Interview Diagnostic Instrument.[8] Among 1178 cases with available symptom-level data, depressive symptoms were used as indicator variables in a latent class analyses approach as previously described;[2] we identified two classes characterized by high severity, typical (20%) and atypical (21.7%), and a class of moderate severity (58.3%). Height and weight were measured to calculate body mass index (BMI) in kg m^{-2}. The association between rs9939609 (additive model) and different outcomes were tested using linear and (multinomial) logistic regressions adjusting for age, sex and population stratification (three principal components). Analyses were performed in SAS (v. 9.2, SAS Institute, Cary, NC, USA) and R (v. 3.0.1, R Project for Statistical Computing).

The characteristics of the study sample and the main results for the association tests are shown in Table 1. As compared with controls, cases were more likely to be female and had higher BMI. Consistently with the results of Samaan *et al.*,[1] the rs9939609 A variant was confirmed to be positively associated with BMI (β = 0.40, s.e. = 0.09, *P* = 1.56E – 05) and BMI was positively associated with presence of MDD (odds ratio (OR) = 1.08; 95% confidence interval (CI) = 1.07–1.10; *P* = 5.47E – 24). However, in contrast to the study by Samaan *et al.*,[1] the FTO variant was associated with a higher probability of having MDD (*P* = 0.023) but the association was no longer significant after additional adjustment for BMI (Table 1). When considering MDD subtypes, results from a multinomial logistic regression showed that, as compared with controls, the rs9939609 A variant was associated with a 1.42-fold increased odd of having atypical MDD (*P* = 1.84E – 04). Typical and moderate subtypes were not associated with rs9939609. After additional adjustment for BMI, the association between the FTO variant and atypical subtype, despite a 19% reduction in effect size, remained statistically significant (*P* = 0.003). We confirmed the significant association of rs9939609 with atypical subtype via permutation analyses. After comparing the actual atypical MDD cases with controls in binary logistic regression, we sampled from the overall cases 256 patients (N of atypical) 10 000 times and compared them again against controls. The results indicated that obtaining a lower *P*-value was unlikely to have occurred by chance (empirical-*P* = 0.002).

Taken together, these findings suggest that MDD subtypes contribute to variability of results in genetic association studies. In contrast to the study by Samaan *et al.*[1] we found a positive association between the FTO rs9939609 A variant and MDD, but this was completely driven by the atypical MDD subtype, independently from BMI. The profile of MDD subtypes in the studies included in the paper by Samaan *et al.*[1] is unknown, but we could hypothesize that the conclusions about the relationship between FTO and depression may have differed when considering the specific clinical subtypes as compared with the overall MDD diagnosis.

Not considering the heterogeneity of depression in genetic associations studies may contribute to discrepant or blurred effect sizes. Recent simulations studies confirmed that phenotypic heterogeneity may have negatively impacted the results of large collaborative genetic studies.[9] Disentangling MDD heterogeneity by characterizing subtypes with different clinical and pathophysiological profiles may benefit the research on genetic determinants of depression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Netherland Twin Register and Netherlands Study of Depression and Anxiety (NESDA): Funding was obtained from the Netherlands Organization for Scientific Research (NWO) and MagW/ZonMW grants Middelgroot-911-09-032, Spinozapremie 56-464-14192, Geestkracht program of the Netherlands Organization for Health Research and Development (ZonMW 10-000-1002), Center for Medical Systems Biology (CSMB, NWO Genomics), Genetic influences on stability and change in psychopathology from childhood to young adulthood (ZonMW 912-10-020), NBIC/BioAssist/RK(2008.024), Biobanking and Biomolecular Resources Research Infrastructure (BBMRI–NL, 184.021.007), VU University's Institute for Health and Care Research (EMGO+) and Neuroscience Campus Amsterdam (NCA); the European Science Council (ERC Advanced, 230374). Part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health, Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota (USA) and the National Institutes of Health (NIH R01 HD042157-01A1, MH081802, Grand Opportunity grants 1RC2 MH089951 and 1RC2 MH089995).

Y Milaneschi[1], F Lamers[1], H Mbarek[2], J-J Hottenga[2], DI Boomsma[2] and BWJH Penninx[1]

[1]*Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Center/GGZ inGeest, Amsterdam, The Netherlands and*

[2]*Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands*

E-mail: y.milaneschi@ggzingeest.nl

REFERENCES

1 Samaan Z, Anand S, Zhang X, Desai D, Rivera M, Pare G, Thabane L *et al. Mol Psychiatry* 2013; **18**: 1281–1286.
2 Lamers F, Vogelzangs N, Merikangas KR, de Jonge P, Beekman AT, Penninx BW. *Mol Psychiatry* 2013; **18**: 692–699.
3 Penninx BW, Milaneschi Y, Lamers F, Vogelzangs N. *BMC Med* 2013; **11**: 129.
4 Penninx BW, Beekman AT, Smit JH, Zitman FG, Nolen WA, Spinhoven P *et al. Int J Methods Psychiatr Res* 2008; **17**: 121–140.
5 Boomsma DI, de Geus EJC, Vink JM, Stubbe JH, Distel MA, Hottenga JJ *et al. Twin Res Hum Genet* 2006; **9**: 849–857.
6 Nivard MG, Mbarek H, Hottenga JJ, Smit JH, Jansen R, Penninx BW *et al. Genes Brains Behav* advance online publication, 13 November 2013; doi:10.1111/gbb.12095 (e-pub ahead of print).
7 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM *et al. Nature* 2012; **491**: 56–65.
8 World Health Organization WHO: Geneva, Switzerland, 1997.
9 Manchia M, Cullis J, Turecki G, Rouleau GA, Uher R, Alda M. *PLoS One* 2013; **8**: e76295.

Preliminary evidence that early reduction in p11 levels in natural killer cells and monocytes predicts the likelihood of antidepressant response to chronic citalopram

Molecular Psychiatry (2014) **19,** 962–964; doi:10.1038/mp.2014.13; published online 11 March 2014

The prediction of treatment response in many neuropsychiatric disorders would be facilitated by easily accessible biomarkers. Using flow cytometry, we herein demonstrate correlations between early reduction in p11 levels in natural killer (NK) cells and monocytes, and antidepressant response to citalopram in patients with major depressive disorder (MDD).

The multifunctional protein p11 amplifies serotonin receptor-mediated signaling[1,2] and regulates gene transcription.[3,4] P11 levels are reduced in neurons of the frontal cortex, nucleus accumbens and hippocampus from depressed individuals and suicide victims.[1,5] Neuronal p11 levels are also reduced in animal models of depression,[1] but are upregulated by various antidepressant treatments, including selective serotonin reuptake inhibitors.[1,6] P11 knockout mice show a depression-like phenotype and reduced behavioral improvements and neurogenesis in response to antidepressant regimens.[6,7] Conversely, overexpression of p11 in mice mimics the behavioral phenotype seen after antidepressant treatment.[1] Here, we examined the possibility that p11 in white blood cells could serve as a biomarker of antidepressant response using the selective serotonin reuptake inhibitor, citalopram.

After giving informed consent, 26 patients with MDD in a current major depressive episode were recruited at the National Institute of Mental Health (for details see Supplementary Information). Their average (s.d.) age was 36.9 ± 10.4, and there were 11 females and 15 males. The patients were screened and diagnosed using Diagnostic and Statistical Manual of Mental Disorders, 4th Edition and Structured Clinical Interview for DSM-IV. Severity of depression was assessed using the Montgomery–-Åsberg Depression Rating Scale (MADRS) and the Quick Inventory of Depressive Symptomatology-C16 (QIDS). Subjects were given a daily dosage of citalopram, doses were increased over time (see Supplementary Information), but were held constant for the week before blood was drawn. Subjects were evaluated on a weekly basis for 8 weeks. Peripheral blood mononuclear cells (PBMCs) were prepared at baseline and following 2 and 8 weeks of citalopram treatment; 14 patients had all three sets of blood collected. A separate group of nine patients with MDD was recruited at UT Southwestern in Dallas and assessed using the Inventory of Depressive Symptomatology (IDS) (for details see Supplementary Information). They were treated with citalopram and had blood collected at baseline and following 1 and 12 weeks; six patients had all three sets of blood collected. The protocols were approved by local IRBs. The PBMC samples were coded at the NIMH and UT Southwestern, respectively. All subsequent analysis and quantifications of p11 were done blind at Karolinska Institutet. PBMCs were isolated and stored at − 80 °C in 90% FCS/10% DMSO. For analysis, PBMCs were thawed, washed in PBS, permeabilised and incubated for 30 min at +4 °C with anti-human p11 (148; 2.5 μg ml^{-1}; BD Biosciences, Stockholm, Sweden) or isotype control mouse IgG1 monoclonal antibodies. The bound antibody was detected by PE-conjugated antimouse antibody (Dako, Glostrup, Denmark). To distinguish between NK cells, monocytes and T cells, surface staining (CD3, CD14 and CD56; BD Biosciences) was performed after blocking of an unbound antimouse antibody using 1% normal mouse serum (Sigma, Stockholm, Sweden). After labeling, cells were subjected to flow cytometry analysis to detect p11 levels in gated populations of white blood cells (Supplementary Figure S1). Analyses were done using FlowJo software (Tree Star, Ashland, OR, USA). Statistical analyses used Pearson's correlation test and determined 95% confidence intervals (GraphPad Prism5, La Jolla, CA, USA).

Flow cytometry analysis of PBMCs showed that p11 was present in essentially all CD3-CD56+ NK cells (Figure 1a), all CD14+ monocytes (Figure 1b) and most CD3+ T cells (Figure 1c), with monocytes having an order of magnitude higher levels. Clinical evaluations showed that most patients in the NIMH cohort responded to citalopram (see Supplementary Information). P11 was reduced in NK cells and monocytes after 2 weeks of