Microarray gene expression and cerebral cortical grey matter changes in treatment naive schizophrenia patients in Sri Lanka

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MBBS

This thesis is submitted in partial fulfilment of the requirement for the Degree of PhD in Behavioural Sciences in Relation to Medicine
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Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1966.

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Acknowledgement of Collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

Nishantha Kumarasinghe
Acknowledgement of Authorship

I hereby certify that this thesis is in the form of a series of published papers of which I am the principal author. References for publications are included as an appendix.

Nishantha Kumarasinghe

I certify the contribution of the candidate (Nishantha Kumarasinghe) as the First Author of the above-mentioned publications.

Professor Ulrich Schall (Principal Supervisor)

Dr Paul A Tooney (Co-Supervisor)
Acknowledgement of Contribution

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Summary

With an estimated heritability of 80%, molecular genetic research into schizophrenia has remained inconclusive. Recent large-scale genome-wide association studies only identified a small number of susceptibility genes with individually very small effect sizes. However, the variable expression of the phenotype is not well captured in diagnosis-based research as well as when assuming a “heterogenic risk model” (as apposed to a monogenic or polygenic model). Hence, the expression of susceptibility genes in response to environmental factors in concert with other disease promoting or protecting genes has increasingly attracted attention. Over the past decade, microarray gene expression research has been applied to post mortem brain tissue, peripheral tissues, and animal models of schizophrenia. Altered gene expression has been linked to presynaptic function, signalling, myelination, neural migration, cellular immune mechanisms, and response to oxidative stress consistent with multiple small effects of many individual genes. However, the majority of results are difficult to interpret due to small sample sizes (i.e. potential type-2 errors), confounding factors (i.e. medication effects) or lack of plausible neurobiological theory.

The current thesis investigated gene expression in peripheral blood mononuclear cells in a Sri Lankan cohort of drug treatment-naïve schizophrenia patients prior to introducing antipsychotic pharmacotherapy and again 6 to 8 weeks into treatment. Prior to introducing medication, 624 out of a total of 10,207 genes were found to be differently expressed (208 up- and 416 down-regulated) when compared to closely match healthy control subjects from the same communities. Differently expressed genes included new candidate genes of the disorder, such as AKT1, DISC1 and DGCR6. Patients significantly improved with antipsychotic pharmacotherapy of 200 mg/day chlorpromazine equivalents of risperidone or risperidone/haloperidol and abnormal expression was only confirmed for 106 genes (i.e. 6 up- and 100 down-regulated with 67 genes continued to show the same directional change in expression after treatment). These findings suggest a normalisation of the majority of altered gene expression
with treatment when compared to the more acute phase of illness at study entry. A pathway analysis of differentially expressed genes implicated dysregulation of biological functions, which are related to infectious diseases, inflammation and the immune system in patients with schizophrenia. Particularly AKT1 up-regulation prior to treatment was related to significant overrepresentation of altered genes in pathways that are triggered by growth factors and neurotrophic factors, but also respond to infections, including the EIF2 pathway, the mTOR and eIF4/p70S6K pathways.

The association of altered gene expression with cerebral grey matter pathology was then investigated with cortical pattern matching in high-resolution magnetic resonance imaging brain scans. The findings confirm widespread cerebral grey matter deficits in schizophrenia with grey matter deficits in the right dorsolateral prefrontal cortex as the strongest predictor of diagnosis. Symptom severity and treatment response were associated with regional grey matter deficits in older patients with a longer history of untreated illness, while significant structure/function associations with cognitive impairment in prefrontal and temporal cortices were found across all ages.

The expression of some of the candidate genes correlated with grey matter abnormalities. For instance, a higher expression of DGCR6 was associated with reduced grey matter in prefrontal, orbitofrontal, frontal, temporal, parietal and occipital areas. Moreover, DISC1 was found to be over-expressed in treatment-naïve patients while its expression normalised in the course of pharmacotherapy along with improving symptoms. DISC1 expression in patients also predicted grey matter deficits in right anterior cingulate cortex – a brain area strongly implicated in schizophrenia – along with grey matter deficits in various other associated brain regions.

While the results are promising and demonstrating the feasibility of linking in vivo peripheral schizophrenia candidate gene expression to in vivo measures of cerebral grey matter brain pathology, the findings should be interpreted with caution given the small sample size and when assuming the heterogeneous phenotype of the disorder.