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Influence of functional gene polymorphisms on human behaviour: the case of *CCR5*

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The idea that genetic changes can impact behaviour and lead to novel therapeutic approaches for mental illness has been an attractive, yet elusive hypothesis. A couple of recent papers in *JPN* build the case for the effectiveness of maraviroc in ameliorating depression[1] and reducing the addictive properties of opioids without preventing their analgesic effects.[2] Maraviroc is an antagonist of C-C motif chemokine receptor-5 (CCR5) and has been shown prevent HIV infection by blocking its coreceptor CCR5. These new studies build on the finding that people with a 32-bp deletion mutation that inactivates *CCR5* (*CCR5-Δ32*) and show resistance to HIV also have improved cognitive outcomes after a stroke.[3] The studies also extend a growing literature implicating *CCR5* in mental illness and suggest that further clinical studies using maraviroc as a novel treatment or adjuvant, particularly in patients with inflammatory depression involving immune dysregulation,[4] may be warranted. These findings suggest genetic polymorphisms with a strong functional impact (e.g., loss of function) may provide insight into mechanisms underlying certain forms of mental illness and lead to new treatments.

Much effort has been directed at studying genetic variants (such as polymorphisms) that can be used to identify risk of major depression or to improve its treatment. Improving diagnosis of depression may help overcome obstacles to treatment response. The first-line treatment for major depression remains the selective serotonin reuptake inhibitor (SSRI) class of antidepressants,[5] even though these medications are effective in only 50% of patients, take several weeks to yield a response, and are associated with many adverse effects.[6,7] Thus, alternative strategies, including augmentation or switching to alternative monoamine-targeting compounds, cognitive behavioural therapy (CBT) and various brain stimulation approaches, are indicated for patients with SSRI-resistant depression. Ultimately, about 30% of patients are treatment resistant,[6] failing to respond to at least 3 treatments; most often electroconvulsive therapy or another form of brain stimulation is indicated, which may help half of these patients.[8,9] Better alternatives are needed to treat major depression, and genetic approaches may reveal new drug targets that could be exploited for treatment-resistant depression.

Genes and major depression

Two main approaches have been used to identify genetic polymorphisms implicated in major depression or its treatment: candidate gene studies and genome-wide association studies (GWAS).[10,11] Using animal and human models, candidate gene studies focus on polymorphisms in genes implicated in depression — ideally functional polymorphisms shown to affect gene function. Genome-wide studies provide an unbiased method of associating genetic change with phenotype. However, for complex diseases like major depression involving small contribution of many genes, hundreds of thousands of participants are required for sufficient statistical power. Identification of genetic polymorphisms associated with major depression or treatment response[12] using genome-wide approaches has required enormous investment and resulted in few replicable polymorphisms across different studies and ethnicities,[13] and each polymorphism contributes only a very small increase in risk for depression or treatment response.[14–16] On the other hand, functional polymorphisms that have been shown to affect the function of a gene, either in vitro or in vivo, and to affect rodent behaviour (e.g., *BDNF* Val66Met rs6265),[17] have not always been replicated in clinical association studies, nor in GWAS.[10,11] Several factors could account for this variability, including the heterogeneity of the large GWAS sample sets, unreliability of diagnosis of depression, and changes in depression phenotype over time.[18,19] There is a need to demonstrate functionality of human polymorphisms in human cells and ultimately in human behaviour. Polymorphisms that lead to genetic loss of function (e.g., copy number variations) are extremely rare, but some, like the *CCR5-Δ32* polymorphism (allele frequency of 0.092 in Caucasian people), are abundant enough to be studied. This polymorphism was shown to confer resistance to HIV

infection since it disrupts the co-receptor that allows viral infection of the cell.[20] More recently, this mutation has become highly contentious as an example of unethical germline gene manipulation in humans. In an infamous experiment to confer HIV resistance, the *CCR5* mutation has been incorporated using CRISPR-Cas9 gene editing technology, resulting in 1 homozygous and 1 heterozygous mutation in a pair of twin girls.[21] Nevertheless, the hereditary mutation and its effect on the brain has become of increasing interest with the finding that *CCR5-Δ32* confers cognitive protection following stroke.[3]

CCR5 and depression

Recently, it was reported that in rodent models of stroke, Ccr5 expression is induced in cortical microglia and neurons within 12 hours post-stroke. Nine weeks of treatment with maraviroc (or gene knockdown of *Ccr5* in the premotor cortex) was effective to promote recovery of motor function after photothrombotic focal stroke and of cognitive function after traumatic brain injury in C57BL6 mice.[3] In the same study, human stroke patients from the TABASCO trial in Israel with the *CCR5-Δ32* were found to have better scores on the NIH stroke scale (NIHSS). These patients also displayed greater scores on a battery of cognitive tests. These results suggest that genetic inactivation or pharmacological inhibition of *CCR5* is associated with better sensorimotor and cognitive outcomes after stroke. In the July–August 2021 issue of *JPN*, Tene and colleagues examined depressive symptoms in the TABASCO cohort of stroke patients and showed that the *CCR5-Δ32* patients had a significant reduction compared with noncarriers.[1] While the severity of depression was mild in these patients, who had mild to moderate strokes, *CCR5-Δ32* was associated with a significant reduction in depressive symptoms, which was most pronounced in participants having *CCR5-Δ32* at both alleles. Interestingly, patients with the 5-HTTLPR-L allele and *CCR5-Δ32* showed the greatest difference compared with noncarriers with the 5-HTTLPR-S allele, suggesting a potential interaction with the serotonin system. Consistent with this interaction, a recent observational study reported that antidepressant responders (SSRI/serotonin-norepinephrine reuptake inhibitors [SNRI]) showed reduced leukocyte RNA levels of *CCR5* and its ligand *CCL5* compared with patients who did not respond after 5 weeks of treatment.[22] This effect could be driven by the action of SSRI treatment ex vivo to reduce macrophage *CCR5* expression[23] or by 5-HT$_{1A}$ receptor-mediated downregulation of *CCR5* in macrophages.[24] Furthermore, compared with healthy controls, like other cytokines (e.g., interleukin-6), levels of *CCR5* and *CCL5* are increased in individuals with depression, the latter correlating with increasing severity.[25] It remains to be seen whether patients with depression, particularly those with "inflammatory depression,"[4] in whom inflammatory cytokine and *CCR5* levels are elevated, might benefit from treatment with maraviroc, or the combination of an SSRI and maraviroc.

CCR5 and opioid addiction

With regard to the role of *CCR5* in modulating opioid actions, based on the heterodimerization and cross-desensitization of CCR5 and μ-opioid receptors,[26] Iriah and colleagues addressed the effect of maraviroc on oxycodone actions and addiction in rats, while monitoring changes in functional brain connectivity.[2] Interestingly, maraviroc reduced conditioned place preference for oxycodone and oxycodone-induced functional connectivity of the reward circuitry. Maraviroc also selectively reduced oxycodone self-administration over 10 days compared with inactive lever and reduced drug-seeking after abstinence. However, maraviroc did not affect locomotion or oxycodone-induced analgesia in the tail flick test. These studies suggest that maraviroc preserves the anti-nociceptive actions of oxycodone while reducing its actions on brain circuitry associated with addiction. In mice, it was found that maraviroc or other CCR5 antagonists actually increased the potency of morphine-induced antinociceptive activity in 3 different tests.[27] Similarly, maraviroc enhanced the potency of morphine to reduce incisional or cold-water pain in rats without affecting the dose for respiratory depression.[28,29] Interestingly, a bivalent CCR5 antagonist–μ-opioid agonist (MCC22) was several thousand times more potent an analgesic than the opioid agonist alone or combined with CCR5 antagonist, implicating receptor heterodimerization.[30,31] Unlike with morphine, rats did not acquire tolerance or conditioned place preference to this bivalent ligand, suggesting that MCC22 may be useful as an analgesic in humans.[32] It would be interesting to test whether opioid analgesia, tolerance or addiction has been reported in *CCR5-Δ32* carriers.

But how is maraviroc-induced inhibition of morphine's addictive action induced? A clue comes from a study of cocaine-induced addiction in rats, which induces *Ccr5* RNA expression in the mesolimbic reward centre.[33] Treatment with maraviroc reduced conditioned place preference for cocaine and cocaine-induced locomotion, both actions mediated via the mesolimbic dopamine reward circuitry. This suggests that blockade of CCR5 may reduce dopamine activity, as seen in *Ccr5*-knockout mice,[34] thus reducing the addictive properties of both cocaine and opioids. It would be important to test whether maraviroc could also affect stress-induced drug seeking and reinstatement.

Conclusion

The evidence from preclinical pharmacological studies and clinical studies of carriers of the *CCR5-Δ32* polymorphism, while still preliminary, favours a potentially important role of *CCR5* inhibition in improving a variety of neuro-psychiatric conditions, including major depression, opioid addiction, and stroke recovery. These studies highlight the importance of investigating established functional polymorphisms that have a large effect on gene expression

(*CCR5* loss of function) and demonstrated effects of human physiology (HIV resistance). We have previously emphasized the importance of considering the functional status of genetic polymorphisms,[17] namely, a functional ranking of class 0 (no experimental evidence); 1 (in vitro evidence); 2 (cellular evidence) and 3 (in vivo evidence). Perhaps a class 4 (in vivo evidence in humans) is needed for polymorphisms that have clear evidence of a functional effect. Typically, the polymorphisms identified in GWAS remain completely in class 0, with only bioinformatic predictions of what their function might be.[14,35,36] Thus, little insight into specific mechanisms of disease have been provided to date. Perhaps using new leads like the *CCR5-Δ32* polymorphism, it is time to refocus more on functional polymorphisms that affect behaviour to gain insights into disease mechanisms.

The case of the *CCR5-Δ32* polymorphism illustrates how a single mutation in a single gene can impact human physiology, endowing resistance to HIV infection. Whether it also leads to benefits in mental and cognitive health remains to be further verified. Whole exome or genome sequencing may reveal rare polymorphisms with high functional impact,[37] but thus far functional validation often remains unaddressed. Few mutations have been unequivocally linked to depression, but brain physiology can be highly impacted by single gene mutations. An example is narcolepsy induced by rare mutation of the hypocretin/orexin receptor gene, first found in dogs then in humans.[38] Subsequently, human narcolepsy was shown to involve loss of hypocretin neurons or reduced cerebrospinal fluid hypocretin levels in some, but not all, patients. This understanding has led to the development of orexin ligands to treat sleep disorders, including agonists for hypersomnia and antagonists for daytime sleepiness.[39] Thus, understanding how specific genes contribute to brain function remains a largely under-explored and under-funded goal that needs greater focus if we are to understand what processes lead to alterations in human behaviour. As seen for the *CCR5-Δ32* variant, it may be that gene variants associated with specific alterations in immune response could also impact behaviour and susceptibility to psychiatric disorders. On the other hand, patients with elevated levels of CCR5 or its ligand may be responsive to maraviroc. Identification of these functional variants and their mechanisms could inform treatment approaches to provide more targeted and effective personalized medicine.[4,40]

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