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The longevity gene *Klotho* is differentially associated with cognition in subtypes of schizophrenia

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ABSTRACT

Cognitive impairment is a core feature of schizophrenia and impacts negatively the functioning of affected individuals. Cognitive decline correlates with aging, and is the primary cause of loss of independence and reduced quality of life. The *klotho* gene is a key modulator of aging, with expression deficiency resulting in premature aging, while overexpression extends lifespan and enhances cognition. A haplotype and functional human variant of the gene, KL-VS, increases expression and promotes longevity. KL-VS heterozygosity is associated with enhanced cognition and a larger volume of the right dorsolateral prefrontal cortex, a region involved in planning and decision-making, which is especially susceptible to shrinkage with age. We examined the effect of KL-VS heterozygosity on cognition in 497 schizophrenia patients and 316 healthy controls from the Western Australian Family Study of Schizophrenia (WAFSS) who had been comprehensively characterised by neurocognitive tests and classified into cognitively deficient (CD) and cognitively "spared" (CS) clusters. An older, cognitively normal population sample from the Health in Men Study (HIMS) was included to allow assessment of heterozygosity and memory in aged individuals. We show that heterozygosity is associated with better learning and memory in the younger WAFSS healthy controls but not in the aging HIMS sample. However, in schizophrenia patients, KL-VS has a selective effect on memory, with heterozygotes in CD and CS clusters performing worse than non-carriers. This effect was significant and more severe in the CD cluster, reinforcing the utility of subtyping patients into CD and CS clusters that may differ in their genetic underpinnings.

1. Introduction

Schizophrenia (SZ) is a complex, polygenic brain disorder that affects approximately 1% of the world's population (Jablensky, 2006). Cognitive impairment is a core feature of the disorder and has a significant negative effect on occupational, social, and economic functions (Keefe and Harvey, 2012). Some cognitive domains may improve with early intervention, while others decline as the illness progresses (Bartholomeusz and Allott, 2012). Cognitive decline is also strongly correlated with aging in the general population and is the primary cause of loss of independence and reduced quality of life (Deary et al., 2009). The extent of decline leading to memory impairment and executive dysfunction is influenced by genetic factors, age and environmental stress (Deary et al., 2009). Given the impact such dysfunction has on the quality of life of the aging population, a major effort has been undertaken to understand its biological mechanisms, but clarity is still lacking on the actual molecular machinery by which related biological processes/pathways are disrupted to result in cognitive decline.

The klotho gene in mice (*kl*), linked to both cognition and longevity, was serendipitously identified by Kuro-o et al. (1997) in transgenic mice during their search for premature-aging syndrome genes in animal models. Mice homozygous for the insertion of DNA into the 5′ promoter region of the α-klotho gene (*kl*/*kl*) are severe hypomorphs, with very low expression. They appear normal at birth, but at 3–4 weeks of age start to show multiple phenotypes, analogous to normal human aging and premature-aging syndromes: gait abnormality, infertility, arteriosclerosis, ectopic calcification, osteoporosis, growth retardation, skin atrophy, hypokinesia and premature death at around 8 weeks of age. The Klotho protein is also required for brain maturation and the *kl* mutant mouse suffers from memory retention deficits, most likely due to increased susceptibility to oxidative stress damage (Nagai et al., 2003; Park et al., 2013; Shin et al., 2015) which leads to brain pathology involving neuronal degeneration in the hippocampus (Shiozaki et al., 2008) and hypomyelination (Chen et al., 2013). Overexpression of *kl* in mice promotes longevity and specifically improves learning and memory without altering exploratory, avoidance- and anxiety-related

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behaviours (Dubal et al., 2014). This suggests that Klotho does not affect *all* cognitive domains to the same extent but rather influences specific domains of cognitive ability. Studies in mice (Dubal et al., 2014) suggest that the mechanism underlying the effects of *kl* overexpression involves synaptic enrichment of the NMDAR subunit GluN2B through posttranscriptional mechanisms in the hippocampus and frontal cortex, regions directly involved in cognitive function, resulting in enhanced long-term potentiation (Dubal et al., 2014). Raising protein levels of Klotho in a mouse model of Alzheimer's disease also improved synaptic and cognitive function in spite of the accumulation of toxins such as amyloid-beta and tau in the brain (Dubal et al., 2015).

The human klotho gene (*KL*) maps to chromosome 13q12 (Kuro-o et al., 1997) and encodes a single-pass type I transmembrane protein which shows 86% amino acid identity with the mouse protein (Matsumura et al., 1998). It is expressed in a wide variety of human tissues (Lim et al., 2015), specifically in the distal convoluted tubule in the kidney, parathyroid glands, cerebellar Purkinje cells and choroid plexus in the brain from where it is secreted into the CSF (German et al., 2012; Imura et al., 2004; Kuro-o et al., 1997). The protein exists in membrane-bound and secreted forms, each having distinct functions. The secreted form is generated both by alternative splicing and by shedding of the extracellular domain of the transmembrane form by ADAM10 and ADAM17 into blood and cerebral spinal fluid, respectively. It is detectable therein throughout life (Imura et al., 2004), declines with age (Semba et al., 2011) and is lower in people with Alzheimer's disease (Semba et al., 2014). Membrane-bound Klotho acts as an obligate co-receptor for FGF23 in the kidney (Urakawa et al., 2006) and regulates calcium and phosphate homeostasis in conjunction with active Vitamin D (Imura et al., 2007; Kuro-o, 2010), while circulating Klotho functions as a humoral factor and a novel beta-glucuronidase (Tohyama et al., 2004), suppressing insulin/IGF1 (Kurosu et al., 2005) and *wnt* signalling (Liu et al., 2007); regulating ion channels and transporters (Chang et al., 2005), oligodendrocyte maturation (Chen et al., 2013); and antioxidant enzyme expression (Shin et al., 2015).

Genetic studies to date have not implicated the *KL* locus in SZ pathology. A few have identified suggestive linkage to chromosome 13q (Brzustowicz et al., 2000; Maziade et al., 2005, 2009; Bureau et al., 2013) but these findings have been in chromosomal bands further telomeric to the *KL* locus. Furthermore, a large multi-stage SZ GWAS study did not identify any genome-wide significant signals from chromosome 13 (Schizophrenia Working Group of the Psychiatric Genomics, C, 2014).The complex and pleiotropic role of Klotho in mice has however led to numerous studies on the association of genetic variation in *KL* with aging-related diseases in humans (reviewed in Xu and Sun, 2015), including neurocognitive deterioration (Deary et al., 2005; Dubal et al., 2014; Hao et al., 2016; Mengel-From et al., 2016; Yokoyama et al., 2015). Predictably, almost all of these studies have been focused on aging populations, as age is an independent risk factor for cognitive impairment. The findings across different studies are inconsistent in terms of the variant showing association and the direction of the effect. Interestingly, significantly elevated klotho promoter methylation has also been reported in individuals with mild cognitive impairment (Luo et al., 2015). A haplotype and common functional variant of the human *KL* gene, KL-VS, is common in Caucasian populations and, in the heterozygous state, has been reported to lead to increased levels of Klotho in serum (Dubal et al., 2014), promoting longevity (Arking et al., 2002, 2005; Dubal et al., 2014; Invidia et al., 2010) and better cognitive function in healthy older adults (Dubal et al., 2014). On the other hand, homozygosity is associated with decreased lifespan and detrimental effects on cognition (Arking et al., 2002, 2005; Deary et al., 2005). Heterozygosity is also associated with a larger volume of the right dorsolateral prefrontal cortex, which in turn correlates with better executive function in normal aging populations (Dubal et al., 2014; Yokoyama et al., 2015). As this brain region is especially susceptible to shrinkage with age, and vulnerable in several psychiatric and neurologic diseases, it would be important to see if *KL* variation plays a role in cognitive functioning in these disorders. KL-VS is composed of six SNPs and spans exon 2 and its flanking sequence. Two of these SNPs result in amino acid substitutions: F352V (rs9536314) and C370S (rs9527025) (Arking et al., 2002), and KL-VS refers to the V and S alleles of these SNPs respectively. Since all six SNPs occur in perfect linkage disequilibrium, a single variant, F352V, can be used to tag the haplotype. The conservation of F352 among homologous proteins in eukaryotes suggests that it is functionally important (Arking et al., 2002) with enzymatic studies pointing to both altered secretion and a loss of catalytic activity for the KL-VS allele.

In view of these findings, we hypothesize that variation in *KL* will also influence cognition in SZ. We examined the association of KL-VS heterozygosity with general cognitive ability and episodic verbal memory in a case-control sample from the Western Australian Family Study of Schizophrenia (WAFSS), with the hypothesis that heterozygosity would be associated with better cognition. The case–control sample had been richly phenotyped with a battery of 12 neurocognitive tests which delineate two main endophenotype case clusters: a cognitively deficient (CD) and a cognitively "spared" (CS) cluster (Hallmayer et al., 2005). To assess if the effects of KL-VS heterozygosity on memory are age-dependent, we included in our analysis an older, cognitively normal sample from The Health in Men Study (HIMS) cohort (Norman et al., 2009) to compare with the much younger WAFSS normal controls as this allowed us to follow a possible link between KL-VS heterozygosity and cognition across the lifespan.

2. Materials and methods

2.1. Study samples and neurocognitive testing

The WAFSS case-control subsample comprises 497 SZ patients (75% male, age range 20–68 years) and 316 healthy unrelated controls (58% male, age range 21–64 years) of European descent with predominantly Anglo-Irish ancestry. The patients were recruited from consecutive admissions to a major psychiatric hospital and community-based mental health centres within the same area. The controls were recruited from the Red Cross blood donor registry or by random sampling from local telephone directories, and screened for psychopathology to exclude those with a personal or family history of psychotic illness. Diagnostic assessment included a standardized interview, the Diagnostic Interview for Psychosis, DIP (Castle et al., 2006), which combines items from the Schedules for Clinical Assessment in Neuropsychiatry [SCAN (Wing et al., 1990)] and is scored using the OPCRIT algorithm (Mcguffin et al., 1991). Videorecorded interviews and clinical charts were independently reviewed by two senior clinicians who assigned consensus research diagnoses in terms of ICD-10 and DSM-IV. All participants were assessed for cognitive performance using a battery of neurocognitive tests that target multiple cognitive domains (Hallmayer et al., 2005): general cognitive ability (prior and current IQ), verbal memory, sustained attention, working memory, executive function and speed of information processing. General cognitive ability was assessed using the National Adult Reading Test (NART-IQ, which evaluates premorbid IQ), and the Shipley Institute of Living Scale (SILS-IQ; which evaluates current IQ). Learning and episodic verbal memory were assessed by the Rey Auditory-Verbal Learning Test, RAVLT (RAVLTi measures the immediate recall of a 15-word list and RAVLTd measures the delayed recall after distraction). In the HIMS sample memory was assesses by the California Verbal Learning Test (CVLT), which had been modelled on the RAVLT, differing mainly in the semantic organization of the list words. It similarly provides information on immediate and delayed recall in addition to verbal learning and recognition.

Homogeneous cognitive subtypes of SZ have successfully been used in genetic association studies (Green et al., 2013; Hallmayer et al., 2005; Jablensky, 2006; Morar et al., 2011; Verbrugghe et al., 2012), and this approach was adopted in the present study. Neurocognitive test data were integrated into composite continuous traits using a latent

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