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Prevalence of the long-allele genotype of the serotonin transporter-linked gene in female centenarians

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ABSTRACT

OBJECTIVE: To determine the distribution of the long and short allele of the serotonin transporter (5-HTT) gene-linked polymorphism region (*5-HTTLPR*) in centenarians.

DESIGN: Descriptive study using convenience sampling.

SETTING: Residential care facilities and private dwellings in Australia.

PARTICIPANTS: A convenience sample of 68 centenarians.

MEASUREMENTS: Buccal DNA from 68 centenarians was genotyped for the presence of either the long (*l*) or the short (*s*) allele of the 5-HTT gene.

RESULTS: Female centenarians had a significantly higher *l/l* genotype frequency compared to either form of the *s* genotype ($p = 0.003$) and a 2-fold greater *l* allele frequency ($p < 0.01$) compared to male centenarians.

CONCLUSIONS: We report a significantly higher *l*/*l* genotype frequency compared to either form of the *s* genotype in female centenarians. This finding may be suggestive of higher serotonin efficiency in the brain of female centenarians that enable them to cope with everyday living and adapt to aging and its limitations.

Key words: adaptation, gene-environment interaction, coping mechanism, successful aging.

INTRODUCTION

Discovering genetic variations that explain the variability in survival to extreme old age could yield important clues regarding cellular and biochemical mechanisms that impact basic mechanisms of aging and susceptibility to age-associated diseases (Perls and Terry, 2003).

Serotonin plays a critical role in neurotransmission. Many vital physiological functions including sleep, appetite, pain and emotion are regulated via serotonin. The duration and magnitude of the serotonin response is regulated by the serotonin transporter (5-HTT) - system (encoded by the *SLC6A4* (solute carrier family 6 [neurotransmitter transporter, serotonin], member 4) gene) and hence this system has faced immense scrutiny. A large body of literature has focussed on the interlude between the 5-HTT-system and affect-related behaviours. A polymorphic variation (5-HTTLPR) within the promoter region of the *SLC6A4* gene, approximately 1 kb upstream of the transcription initiation site, results in the insertion or deletion of a 43 bp coding region (1). The longer variant (*l*) has been associated with increased transcriptional activity (2) and hence, more efficient transport of serotonin.

Recent research in genome studies has indicated that genetic polymorphisms may contribute to human longevity. Gene-environment (G X E) interactions play a critical role in determining health and disease. While certain genetic contributions have been shown to be beneficial to health and/or prevent disease, allelic variations and mutations can and regularly do prove detrimental (e.g., Apo E in Alzheimer's disease). G X E interactions play a larger role the longer the lifespan of an individual due to the longer period of exposure to environmental factors. Therefore, the ability to overcome maladies associated

with G X E interactions may be associated with successful ageing or may be explained by adaptive mechanisms that are controlled by genetic variability. We have recently embarked on a project to verify the presence of a cohort of genes that have been associated with longevity. Using a convenience sample of Australian centenarians of Caucasian ancestry, the aim of the current investigation was to assess the distribution of the *l* and *s* allele of the 5-HTT gene.

METHODS

Participants: A total of 68 Australians of Caucasian ancestry (15 males and 53 females) was recruited for the current study from geographically varied locations across Australia including New South Wales, Queensland, Victoria, Australian Capital Territory and South Australia. A convenience sampling approach, including telephoning residential aged care facilities in Sydney, New South Wales listed in the Aged Care Directory Online; identifying centenarians in local and national news items; liaising with general practitioners who provided centenarians from their patient database and engaging aged care support groups was used to identify participants. The average age of the participants was 101.03 ± 0.2 years (100.73 ± 0.38 for males and 101.13 ± 0.24 for females). Informed consent was obtained from each individual or their next-of-kin prior to any procedure being performed. This study was conducted under ethical approval from The University of New South Wales as well as the Sydney South West Area Health Services (SSWAHS) Human Ethics Committees.

DNA extraction and 5HTT-LPR analysis: Buccal DNA was extracted from sterile cotton-tipped mouth swabs using the ChargeSwitch® gDNA Normalized Buccal Cell Kit

following manufacturer's instructions (Invitrogen, NSW). The serotonin transporter gene promoter polymorphism was typed by PCR using the flanking primer pair 5′-TCCTCCG CTTTGGCGCCTCTTCC-3′ (forward) and 5′-TGGGGGTTGCAGGGGAGATCCTG-3′ (reverse). PCR products were separated on agarose gels and allelic variation recorded based on DNA migration patterns.

Statistical analysis: Analysis was performed using the SAS software package. We tested for differences in the distribution of genotype and allele frequencies in our combined sample and for an association between gender and the *l/s* and the *s/s* genotypes using a Chi-square test. Since our data for the *l*/*l* genotype contained a cell with a value of zero (males, Table 1), we employed a test of proportions using a conditional binomial to account for this observation and tested for an association between the *l*/*l* genotype and either short form. Significant differences were declared if $P < 0.05$.

RESULTS

To evaluate the prevalence of the 5-HTT polymorphism in a population of centenarians of Caucasian ancestry in Australia, we performed a genotype analysis of 68 individuals from various geographical regions in Australia. DNA was extracted from buccal samples and typed for the presence or absence of the 43 base-pair polymorphic region within the promoter of the 5-HTT gene. Genotype frequencies (*l*/*l*, n = 21, 30.9%; *s*/*l*, n = 25, 36.8% and *s*/*s*, n = 22, 32.3%) were not in Hardy-Weinberg equilibrium ($\chi^2 = 4.76$ (1), $P = 0.03$). The frequencies of the genotypes for males were (*l*/*l*, n = 0; *s*/*l*, n = 8, 53.3% and *s*/*s*, n = 7, 46.7%; $\chi^2 = 1.98$ (1), $P = 0.16$) and females were (*l*/*l*, n = 21, 39.6%; *s*/*l*, n =

17, 32.1% and *s*/*s*, n = 15, 28.3%; χ^2 = 6.5 (1), P = 0.01). This result is similar to a study investigating the 5-HTT gene in Japanese centenarians (3). We found a significantly higher *l*/*l* genotype frequency compared to either form of the *s* genotype in females compared to males (χ^2 = 8.60 (1), P = 0.003). We also report a 2-fold higher *l* allele frequency in our female population (55.7%) compared to males (26.7%) (χ^2 = 7.86 (1), P < 0.01). There was no evidence (P = 0.99) of an association between gender and the *l*/*s* and the *s*/*s* genotypes or between the frequency of the s/s genotype and either form of the *l* genotype (χ^2 = 1.80 (1), P = 0.18). There was a very small probability of the absence of the *l*/*l* genotype in the sample of 15 males (P = 0.004) indicating that the lack of association between males and this genotype may be due to a small male sample size and warrants further investigation.

DISCUSSION

In our cohort of centenarians of Caucasian ancestry we observed a significantly higher *l*/*l* genotype frequency of the 5-HTT gene compared to either form of the *s* genotype in female centenarians. We also observed a 2-fold higher *l* allele frequency in our female population (55.7%) compared to males (26.7%).

Our findings are in agreement with a study of Japanese centenarians, which to the best of knowledge is the only other study investigating the prevalence of the 5-HTT gene variance in centenarians. The study by Gondo and colleagues (3) revealed that the prevalence of the *l*/*l* genotype and the *l* allele was highest in female centenarians. Although the proportion of the *l*/*l* genotype in females was much lower in the Japanese study (9.2%) compared to the present investigation (39.6%), the frequency of occurrence

of the *l*/*l* genotype and *l* allele is far less common in Japanese compared to Caucasians (4-6).

Numerous genetic studies have suggested a role for genes in determining the ability of individuals to age successfully. However, it is well documented that personality traits, behaviour and environmental factors play an important role in successful ageing. Understanding gene-environment interactions will be critical in determining factors that contribute to stress and disease as well as those that play a role in resilience and longevity. Centenarians are a unique group of individuals who have successfully navigated through stress and disease while demonstrating impressive resilience by delaying functional decline and compressing morbidity to later years of life. Identifying genetic variations in this cohort that may provide an advantage to living longer and healthier lives will aid in the understanding of factors that may contribute to early onset aging or disease. A gene that may influence aging is one that codes for 5-HTT.

Serotonin and the serotonin transporter modulate many brain and body processes involved in health and disease (reviewed in (7)). Central serotonergic neurons are found in and around the midline raphe nuclei of the brain stem, project widely throughout the brain and spinal cord and have been associated with critical functions of the CNS. The single serotonin transporter is responsible for the duration and magnitude of the serotonergic response as it regulates the clearance of 5HT from the synaptic cleft.

Considering that life-long adaptations to physiological stressors require an immediate response followed by a return to a homeostatic phase, it is conceivable that adaptations to such stressors will build a system of resilience but would be reliant on G X E interactions that mediate homeostasis. Since serotonin is intimately involved in numerous

physiological processes associated with health and disease via its single transporter, it is not surprising that the *l* allele, which is associated with 3-fold higher basal transcriptional activity compared to the *s* allele (1), is found with higher frequency in our female centenarian population. Although the reasoning for this gender-biased observation is unknown, previous studies of coping mechanisms have demonstrated that, in general, females use a more emotion-focussed strategy that involves anger and tension release compared to males, who use more of a problem-solving strategy when faced with adversities (8). These emotion-focussed strategies would involve serotinergic circuitries within the limbic region of the brain and the facilitative nature of the serotonin transporter would play a crucial role in adaptation to life-long adversities. We speculate that in our current cohort of centenarians, females carrying the *l* allele are likely to have better “negotiated” lifes’ stressors due to the increased transport capacity of serotonin. Although 28.3% of our female cohort is homozygous for the *s* genotype, our results suggest that the presence of the transcriptionally more active *l* allele may have contributed to resilience and longevity in our female centenarians. The limitation of our study is the relatively small number of subjects and the use of a sample of convenience. Future studies should be designed to include larger samples (especially males) and an assessment component whereby coping strategies and emotional status could be investigated. However, when performing these investigations on an aged population it is important to consider the subjects’ current cognitive and emotional status as these factors will affect recollection of events.

FINANCIAL DISCLOSURES

All authors report no financial interests or potential conflicts of interest.

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AUTHOR CONTRIBUTIONS

Damian Holsinger: wrote manuscript, performed experiments and performed statistical analysis of the data. Rebecca Brown: performed experiments. Robyn Richmond: study concept and design. Jenaleen Law: acquisition of subjects and sample collection. Frances Kay-Lambkin: study design. Adrienne Kirby: statistical analysis. Daniel Chan: study concept and design.

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Table 1. Comparison of serotonin transporter genotype and allele frequency between centenarians.

	Males	**Females**	**Combined**
N	15	53	68
Mean age (years ± SD)	100.73 ± 1.49	101.13 ± 1.72	101.03 ± .1.66
Genotype frequency			
l/l	0 (0%)	21 (39.6%)	21 (30.9%)
l/s	8 (53.3%)	17 (32.1%)	25 (36.8%)
s/s	7 (46.7%)	15 (28.3%)	22 (32.3%)
Allele frequency			
L allele	8 (26.7%)	59 (55.7%)	67 (49.3%)
S allele	22 (73.3%)	47 (44.3%)	69 (50.7%)