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Variation H452Y in HTR2A Gene Affects Immediate Visual Memory

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Abstract

Serotonin and its receptors, including the 5-Hydroxytryptamine Receptor 2A encoded by the HTR2A gene, are important for learning and memory in animals and humans. Polymorphic variation in the HTR2A gene, which encodes the 5-HT2A serotonin receptor, has previously been shown to associate with some memory traits, in particular affecting delayed verbal memory. In the current study ie we have examined the HTR2A His452Tyr (H452Y) substitution for association in a cohort of healthy individuals whose memory traits were assessed using a comprehensive battery of memory tests including, but not limited to, measures of prospective and retrospective memory.Although we failed to replicate previous findings of an affect of the polymorphism on delayed verbal memory, we found a significant association between the HTR2A H452Y polymorphism and immediate visual memory, showing that the heterozygous genotype is associated with poorer immediate visual memory, with delayed visual memoryunaffected, although, with correction for multiple testing, this no longer passed significance thresholds. No HTR2A Tyr/Tyr individuals were detected in this cohort due to the low minor allele frequency. This study suggests this variant of HTR2A may have implications on memory consolidation and immediate memory of healthy individuals with further examination of this marker warranted in other cohorts.

Keywords

Visual memory, Verbal memory, HTR2A, Genetics of memory, H452Y polymorphism.

Abbreviations

AD: Alzheimer's Disease, BADL: Basic Activities in Daily Living, CAPM: Comprehensive Assessment of Prospective Memory, DRI: Delayed Recall Index, FDR: False Discovery Rate, H452Y: Histidine452Tyrosine substitution, HTR2A: 5-Hydroxytryptamine (Serotonin) Receptor 2A, HVLT-R: Hopkins Verbal Learning Test Revised, IADL: Instrumental Activities in Daily Living, IQ: Intelligence Quotient, LI: Learning Index, LNST: Letter-Number Sequencing Test, MIST: Memory for Intentions Screening Test, OLS: Overall Learning Score, PM: Prospective Memory, PRMQ: Prospective Retrospective Memory Questionnaire, RII: Retention after Interference Index, RM: Retrospective Memory, SVLT: Shum Visual Learning Test, SNP: Single Nucleotide Polymorphism, WAIS: Weschler Adult Intelligence Scale, WASI: Wechsler Abbreviated Scale of Intelligence

Introduction

Human memory concerns the development and mental representation of information and is a continuing major theme in behavioural and cognitive neuroscience. One of the most important goals in the study of memory is to understand how humans are able to encode, retain, and retrieve past occurrences in terms of memory systems-specific neural networks that support specific mnemonic processes [1]. As memory is a polygenic trait, different memory systems are responsible for the encoding, retaining and retrieving abilities of memory [2]. Knowledge of the pathways that regulate memory along with the genes and associated molecules playing roles in formation-storage-retrieval process is still limited, however, at least half of the genes identified to date are known to play a role in neural development [3-5].

Memory is not a unitary concept, with different memory systems and pathways involved in the formation, storage and retrieval processes. Retrospective memory (RM) is the ability to remember things that have happened in the past, and is comprised of a range of memory systems, including short-term memory, working memory and long-term memory. It includes remembering facts and words (semantic information) as well as remembering people and past events (episodic information) and requires access to the major memory systems during the consolidation and retrieval of such information [6]. In contrast, prospective memory (PM) is the ability to remember to perform a particular action in the future, and it often requires retrospective memory to retain the basic information about the intended action and its context. PM is a fairly new concept in the study of memory systems; its basic definition is remembering to remember [7]. PM encompasses event-based actions (e.g.carrying out a task after lunch) or time-based actions (e.g. performing a task, such as taking medication, every few hours) and sometimes it can be triggered by a cue (e.g. remembering to send an email after having seen a computer) [8].

It is conceivable that memory performances may vary between individuals due to a genetic influence such as the result of single

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nucleotide polymorphisms (SNPs) and de novo mutations. The 5-Hydroxytryptamine (Serotonin) Receptor 2A (HTR2A) gene has been implicated in a number of neuropsychiatric disorders including schizophrenia, attention deficit hyperactivity disorder and Alzheimer's disease (AD), all of which have affects on cognitive ability [9]. A number of studies have also shown that serotonin and its receptors are important for learning and memory in both animals and healthy human subjects [10]. The SNP rs6314 is a functional variant in the HTR2A gene, with a minor allele frequency of approximately 9%, which results in a histidine to tyrosine substitution at amino acid 452 (H452Y) in the cytoplasmic tail of the receptor; the tyrosine allele results in a blunted receptor response upon serotonin stimulation [11], and also in impaired downstream signaling [12]. In 2003 de Quervain et al. (2003) reported that the H452Y substitution is associated with decreased delayed verbal recall in His/Tyr heterozygotes when compared to those with a His/His genotype [13]. A number of subsequent studies found similar results strengthening the idea that polymorphisms in the HTR2A gene may influence aspects of episodic memory, particularly delayed verbal, but also delayed visual memory [14-16].

We have tested a cohort of healthy volunteers with a battery of memory tests to measure performance of these different memory systems and simultaneously collected saliva samples from study participants for DNA to investigate genes involved in memory [17,18]. These tests included specific measures for immediate and delayed verbal and visual memory in our battery of tests for RM. The aim of this study was to investigate the affect of the HTR2A H452Y polymorphism in our cohort of healthy individuals, for whom we have conducted extensive memory phenotype profiling, to investigate potential associations with aspects of PM and RM. This will increase our knowledge on the role of serotonin receptor son memory in a healthy population and may provide insight into the influence of genetic factors on memory function.

Material and Methods

Participants

Participants were recruited by advertisements around Griffith University and Queensland University of Technology (QUT) campuses and were comprised predominantly of staff and students. Volunteers were also invited to participate by posters and advertisements displayed around local shopping centers and health clinics. Selection criteria required that the subjects were adults who were non-pathological with no serious head injury or psychiatric disorders. We also excluded individuals with knowledge of Chinese or Japanese language because one of the memory tests (SVLT) involves using Chinese symbols. This study was approved by the Griffith University (MSC/01/09/HREC) and QUT Human Research and Ethics (1300000486) Committees.172 healthy individuals participated in the study. The samples were comprised of 117 females (68%) and 55 males (32%) whose ages ranged from 16 to 51 years (M=22.30, SD=6.056). Participants largely reported English to be their first language (91.5%), and mostly (76.9%) identified as Caucasian in ethnicity with varying levels of education. The Wechsler Abbreviated Scale of Intelligence (WASI) two subtest form (vocabulary and matrix reasoning) was used to estimate each participant's intelligence quotient (IQ) and was found to be normally distributed in the cohort, ranging from 84to 137. Mean IQ was 111.54 (SD=11.066).

Phenotyping

For assessing the memory phenotypes of the participants, a comprehensive battery of memory tests and self-answered questionnaires were completed by individuals to score their memory status.

Prospective memory: The Prospective Retrospective Memory Questionnaire (PRMQ) was administeredfor PM memory evaluation. Eight questions in the questionnaire reflect PM performance on a five point scale (ranging from never to very often) concerning everyday life basic memory tasks [19,20]. For measuring PM ability, in addition to PRMQ, the Comprehensive Assessment of Prospective Memory (CAPM) questionnaire was also administered. This questionnaire uses the same five point scale asPRMQ. It defines the frequency of PM failures in everyday life regarding instrumental activities (IADL) and basic activities (BADL) [21]. Finally, the Memory for Intentions Screening Test (MIST) was performed to evaluate time based and event based PM with and without cues. Examples from MIST for examining PM are 'In 15 minutes please tell me it is time to take a break' for time based task, and 'When I hand you a red pen, please sign your name on your paper' for an event based task with a cue. The MIST also involved a delay task whereby participants were asked to send an email to the tester at a certain time the following day, answering a specific question which was asked during the test (i.e. 'how many hours sleep did you get last night?') [22].

Retrospective memory: PRMQ was also performed for RM memory evaluation, with 8 questions in the questionnaire reflecting the performance of RM on a five point scale ranging from never to very often, concerning everyday life basic memory tasks [19,20]. A Hopkins Verbal Learning Test Revised (HVLT-R) was administered to evaluate RM according to verbal learning capacity. Participants were asked to memorize a word list of 12 items that they have listened to and free-recall them across three trials. The HVLT-R recall score is the sum of all three trials. The HVLT-R learning score is the trial with the highest score. The HVLT-R utilizes a delay trial whereby the participant is asked to recall the world list a fourth time, 20 minutes after the third trial to produce a delayed verbal memory score. The HVLT-R retain score was obtained by dividing trial 4 with the highest score of trial 2 or 3. Recognition was also evaluated from a semantically categorized list of 24 words with yes/no for identification. Words were semantically categorized by adding six related and six unrelated words to the word list. HVLT-R discrimination score was calculated by subtracting false positive answers from correct answers [23]. The Shum Visual Learning Test (SVLT) was also completed by the participants, which measures visual memory learning and capacity. Chinese characters were shown to individuals and they were asked to memorize each target and distinguish the target from a similar, but slightly different Chinese character (a distractor). The learning index (LI) was obtained by summing the number of correctly recognized items across the first 3 trials, to evaluate the learning ability. The retention after interference index (RII) was obtained after the subject was distracted by a different set of target Chinese characters. The delayed recall index (DRI) is the number of correctly identified targets from the initial set of Chinese characters after a 20 minute delay. And finally, an overall learning score is calculated (OLS), which is the sum of LI, RII and DRI trials [24]. A Letter-Number Sequencing Test (LNST), a subtest of Weschler Adult Intelligence Scale (WAIS) III was administered to evaluate working memory and an information subtest was completed for evaluating semantic memory [25]. Wechshler's Visual Reproduction test was also undertaken to obtain a measure of immediate visual memory and delayed visual memory [26].

Genotyping

Saliva samples were collected from each subject immediately after the memory tests were performed using Oragene® DNA Self-Collection kits. DNA was extracted according to the manufacturer's protocol. Participants were genotyped using mass spectrometry on a 96-well Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA) to detect polymorphism of rs6314 C/T. Amplification and extension primers used to detect the variation were designed with the Sequenom Assay Designer Software.

Data analysis: Descriptive statistics were calculated using SPSS v20.0 (SPSS Inc., Chicago, IL, USA). Quantitative genetic analyses were performed using PLINK v1.07. The SNP rs6314 was tested for deviation from Hardy-Weinberg equilibrium. A linear regression (ANOVA) model was carried out for each memory phenotype using age, sex and IQ as covariates. p-values of ≤ 0.05 were considered significant.

Table 1: Association results of H452Y with memory tests.

Test	Memory assessed	t	p
Wechsler Memory Scale III:	**Visual memory**		
Visual reproduction I	Immediate visual memory	-2.19	**0.03**
Visual reproduction 2	Delayed visual memory	-1.35	0.18
MIST	**Prospective memory**		
	Prospective Total	-0.16	0.87
	Delay task	-0.33	0.74
HVLT-R	**Verbal Memory**		
	Immediate verbal memory	-0.05	0.96
	Delayed verbal memory	-0.03	0.98
Shum Visual Learning Test	**Visual Learning**		
	Overall learning score	-0.39	0.70
	Learning index	1.21	0.23
	Retention after interference index	-0.73	0.47
	Delay retention index	-0.88	0.38
WAIS III	**Semantic memory and Working memory**		
	Information	-0.09	0.93
	Letter number sequencing task	-0.64	0.52
PRMQ	**Prospective memory**	0.61	0.55
	Retrospective memory	-0.88	0.38
CAPM	**Prospective memory**		
	BADL	0.99	0.33
	IADL	-0.66	0.51
	Total score	-0.22	0.83

Results with p ≤ 0.05 are considered significant and shown in bold.

Table 2: Visual Reproduction memory test linear regression analysis with covariate.

Covariates	β	t	p
Age, Sex and IQ	-0.1585	-2.188	**0.03009**
Age and IQ	-0.1594	-2.204	**0.02894**

Results with p ≤ 0.05 are considered significant and shown in bold.

Results

Of 172 participants, 4 were discarded due to weak genotype data confidence and subsequent analysis performed on the remaining 168 samples. The SNP rs6314 was found to be in Hardy-Weinberg equilibrium (p=0.277). We detected 142 His/His individuals (84.5%) and 26 His/Tyr individuals (15.5%), but no individuals with the rare Tyr/Tyr genotype. The frequency of the minor allele in our population was 0.077 which is similar to the reported value of 0.069 in the 1000 genomes project and 0.09 in the literature [27]. Linear regression analysis (ANOVA) in PLINK was performed with age, gender and WASI IQ set as covariates, memory tests results as dependent variables and SNP genotypes as fixed factors. Lacking the rare genotype for H452Y meant that we could not perform analysis with a recessive model so analysis was completed with both a dominant model and an additive model. Similar results were obtained for both analyses and results for the latter are presented. The linear regression analyses to determine the affect of HTR2A genotype on the various aspects of memory tested are shown in Table 1. The only observed significant association for the H452Y variant was with the Visual Reproduction I memory test of the Wechsler Memory Scale III (p=0.03). The Visual Reproduction I memory test measures the immediate visual memory of subjects, whereas the Visual Reproduction II memory test measures delayed visual memory by obtaining a score after a 30-40 minutes delay.We did not find significant association for delayed visual memory, nor for immediate or delayed verbal memory measured by the HVLT.

We then examined age, sex and IQ as covariates in linear regression analysis. Age showed a negative correlation, and IQ showed a positive correlation, with Visual Reproduction I memory test scores. Sex failed to show any correlations and the p-value was slightly decreased after reanalysis with age and IQ covariates only (Table 2). However, to take into account the implications of multiple testing considering that genotype was correlated with 17 different measures of memory, Bonferroni correction adjusts the p-value to an alpha of 0.0029 and renders the result non-significant. Similarly, false discovery rate (FDR) correction was performed on all test results and we found that, once the significance threshold was corrected, none of the associations were significant. We next tested for correlations between the various memory tests using Spearman's correlation. While the majority of tests were not correlated, we did find some to be positively correlated, e.g. the immediate and delayed scores of visual reproduction, CAPM IADL and BADL scores, and WASI IQ with WAISIII scores and visual memory test scores.

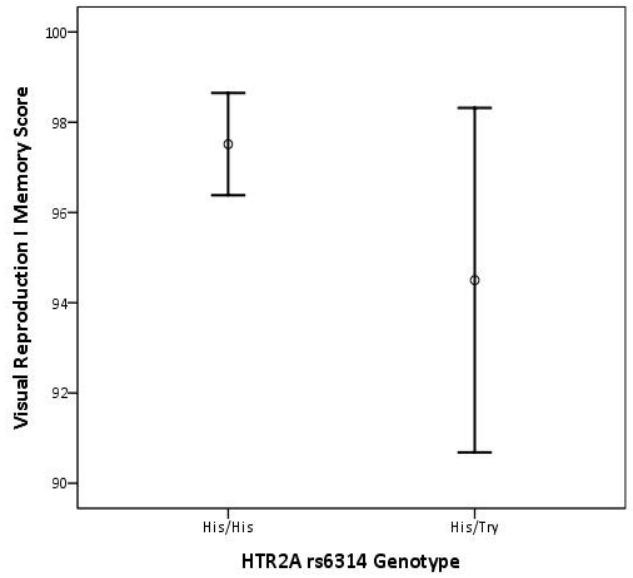

Figure 1: Affect of H452Y variant of HTR2A gene on immediate visual memory in healthy individuals.

Average score (open circles) and range (bars) for Visual reproduction I score of the Wechsler Memory Scale III test for the His/His or His/Tyr groups.

The HTR2A H452Y polymorphism appeared to show some affect on immediate visual memory with His/His subjects performing better when compared to His/Try subjects. Figure 1 shows the mean immediate visual memory score and range for His/His and His/Try individuals, demonstrating that subjects with the His/His genotype obtain on average higher memory scores than subjects with the His/Try genotype. Although there is little difference in the highest scores between the two groups the range of scores in the His/Tyr group demonstrates a much larger distribution with more low scores.

Discussion

Serotonin and its receptors are important to learning and memory in animals and humans. Therefore the H452Y polymorphism of HTR2A was selected as a good candidate to test for association with various aspects of retrospective and prospective memory, including immediate and delayed verbal and visual memory, in our cohort of healthy volunteers. Despite using a broad memory evaluation, we found that only immediate visual memory, as measured by the Visual Reproduction I memory test of the Wechsler Memory Scale III, showed a significant association with the H452Y polymorphism before correction for multiple testing. Age and IQ were correlated with immediate visual memory, but sex did not. Reanalysis of the dataset without sex as a covariate moderately increased the significance of HTR2A and immediate visual memory. However, this association failed to remain significant after correction for multiple testing using Bonferroni or FDR correction. We also found no significant associations for the HTR2A H452Y variant in any of the tests evaluating PM performance.

Although a number of previous studies found that individuals with the His/Tyr genotype had poorer delayed verbal memory, but that immediate verbal memory was unaffected, our study failed to replicate that result [13,14]. The effect of Tyr allele has been reported to be only important in younger people, whereas it did not affect memory performance in a group of older (46 to 90 years) cognitively healthy participants [28]. However, as the average age of participants in our study is 22, with the oldest being 55, this does not explain the lack of replication.

Rigbi et al. (2009) also failed to find an effect of H452Y on delayed verbal memory scores, but did find that the His/Tyr individuals performed the task significantly slower than those with the His/His genotype [15]. However, that study and others have pointed to a role of the HTR2A in visual memory. For example, the H452Y polymorphism was shown to affect delayed, but not immediate, visual recall [29]; with some finding significance only in the male subgroup [13,28]. Gong et al. (2011) found that another SNP in HTR2A, rs4941573, was associated with visuo-spatial working memory in Han Chinese [30], which similar to our Visual Repoduction I test, is a measure of short term memory.

This study is limited by the number of participants, which may also have contributed to non-significance for the H452Y variant with respect to measures of verbal memory. A future direction of the project would be to extend the study in a larger cohort, which will give more power to analyses. Fine-mapping of the HTR2A locus has revealed that there are multiple SNPs that can impact on human episodic memory performance [16], so other variants may be important to investigate.

Although our findings did not confirm previous reports of association between H452Y polymorphism and delayed verbal and visual learning and memory, it does indicate that genetic variation in HTR2A may affect immediate visual memory and thus has consequences on working memory, a key aspect of RM in healthy individuals. Our study and others suggest that variation in HTR2A has a role in some aspects of memory performance in healthy individuals warranting further dissection of the HTR2A locus with memory phenotypes.

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