Original article

Prevalence of the 5-HTT Linked Polymorphic Region among Faculty of Science Students and Its Association with General Mental Well-Being

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Abstract
The short allele of the 5-HTTLPR, located within the promoter of the serotonin transporter gene, has been linked with increased anxiety-related personality traits and affective disorders after exposure to stress. We aim to investigate the relationship between the short allele and the general well-being of Science Faculty students at the University of Misurata who have lived in Libya their whole lives and experienced the unrest of the past eight years. 226 participants were selected randomly, and the Oxford Happiness Questionnaire (OHQ) was used to assess their state of well-being. The OHQ results were used to divide the participants into three groups: the highest-scoring individuals, the lowest-scoring individuals, and those within the confidence interval for the mean of the sample. Members of each group were genotyped using conventional PCR analysis. Frequency test analysis showed the presence of a significant association between 5-HTTLPR and well-being in this sample, where the SS genotype was strongly associated with membership of the lowest-scoring group, while LL and LS were equally associated with that of the highest -scoring group.
Keywords. Serotonins, Transporter Gene, Polymorphism, Wellbeing, PCR.

Introduction

The serotonin transporter polymorphism (5-HTTLPR) is a common genetic variation affecting the serotonin transporter protein, which plays a key role in regulating serotonin levels in the brain. This polymorphism has been extensively studied concerning its potential impact on mental health and well-being. There is evidence to suggest that individuals with the short allele variant of the 5-HTTLPR polymorphism may be more susceptible to developing mood disorders such as depression and anxiety, particularly in response to stressful life events. In contrast, individuals with the long allele variant may be more resilient to these conditions [1]. However, the relationship between 5-HTTLPR and well-being is still a topic of ongoing research and debate. While some studies have suggested that the short allele variant may be associated with lower levels of well-being and life satisfaction, other studies have found no such association or have reported mixed results. It is important to note that genetic factors such as 5-HTTLPR are only one of many factors that can influence mental health and well-being. Environmental factors such as social support, lifestyle factors, and access to healthcare can also play important roles in determining an individual's well-being [2].

In Libya, the political and social events of 2011, including the subsequent civil war, had a significant impact on the country's mental health. The conflict resulted in widespread violence and displacement, leading to increased rates of trauma, anxiety, and depression among the population. A lack of resources and infrastructure for mental health services in Libya compounded the impact of the conflict on mental health. According to the World Health Organization (WHO), there is a significant shortage of mental health professionals and services in the country, with only one psychiatrist per 100,000 people [3].

Misrata is one of the cities that was affected by the conflict in Libya in 2011, and the subsequent political and social instability had a significant impact on the mental health of women in Misrata, as well as in other parts of the country. Women faced a range of challenges related to the conflict, including displacement, insecurity, and exposure to violence and trauma.

According to a study published in 2012, the conflict in Libya had a significant impact on the mental health of women in Misrata. The study found that many women experienced symptoms of depression, anxiety, and post-traumatic stress disorder (PTSD) in the aftermath of the conflict. The study also found that women who had experienced direct exposure to violence or trauma were more likely to experience mental health problems [3]. The impact of the conflict on mental health in Misrata was compounded by a lack of resources and infrastructure for mental health services. According to the World Health Organization (WHO), there is a significant shortage of mental health professionals and services in Libya, particularly in areas affected by the conflict [4].

Despite these challenges, there have been efforts to address the mental health needs of women in Misrata. For example, the United Nations Population Fund (UNFPA) has supported tthe establishment of a women's center in Misrata that provides counseling and psychosocial support to women affected by the conflict [5]. The center also provides health services, vocational training, and other forms of support to help women rebuild their lives. Overall, the mental health of women in Misrata after 2011 is a complex issue that involves a range of factors, including exposure to violence and trauma, lack of resources and infrastructure for mental health services, and gender-based discrimination. While there have been efforts to address these

challenges, more needs to be done to ensure that women in Misrata and other parts of Libya have access to appropriate and effective mental health services and support [6]. In this research, we tried to investigate the genotyping effect of the conflict and the environment in Misrata women, which can be useful in evaluating the mental health condition in the city. Furthermore, the result of this research can serve as support for previous studies that relate life stress in the long term to people's genes, which leads to changes in the mental health of those people.

Methods
Questionnaire and Sample Selection
The questionnaire was derived from the Oxford Happiness Inventory (OHI) by Hills & Argyle (2002) [7]. The Measurement of Personal Happiness contains 29 items that ask about life satisfaction and various happy traits. The questionnaire uses a Likert-scale ranging from strongly disagree to strongly agree, with scores added up and divided by 29. The study included 226 university students in Libya, all single and without highly emotional experiences in the past two years. The questionnaire was distributed, and 1.1% of the data was missing. The results were converted from the original ordinal form to an interval measure and divided into three groups based on scores. Blood was collected from 40 participants for DNA extraction.

5-HTT Linked Polymorphism PCR
DNA Extraction and 5-HTTLPR Genotyping
200µl of whole blood from each sample was placed in a 1.5ml microcentrifuge tube containing 10µl of proteinase K, followed by the addition of 200µl of the AL buffer. The contents of each tube were mixed by pulse-vortexing and incubated at 56°C for 30 minutes for cell lysis. After the cells were completely digested, 200µl of ethanol was added to each tube and mixed thoroughly by pulse-vortexing, which was followed by a brief spin down. The total contents of each tube were transferred to a QIAamp Mini spin column placed in a 2ml collection tube. The columns were then centrifuged for 1 minute at 8000 r.p.m to remove contaminants. The collection tubes containing the flow-through were then discarded and replaced with a new one. The spin column membrane was washed twice using two different buffers, first by using 500µl of the AW1 buffer, then using 500 µl of the AW2 buffer. The spin column was centrifuged for 1 minute at 8000 r.p.m after the first wash, and 3 minutes at full speed after the second. After each wash, the collection tubes containing the flow-through were discarded and replaced with new ones. Finally, each DNA sample was eluted by the addition of 40µl AE buffer directly to the center of the column, which had been placed in a sterile 1.5 ml microcentrifuge tube. The column was incubated with the elution buffer at room temperature for 5 minutes, then centrifuged for 1 minute at 8000 r.p.m. The extracted DNA was stored at -20°C.

Amplification of ß-globin as a Reference Gene
The ß-globin gene was used as a reference gene to eliminate false negative results by checking the quality of DNA. To detect the ß-globin gene, DNA from each sample was amplified by PCR.

Amplification of 5-HTT Linked Polymorphism
PCR of this area using primers positioned before and after the deletion/insertion site allows the detection of the participants' genotypes by producing two different product sizes. The primer pair shown in (Table 1) was used here for this detection.

Table 1. Primers were used in this study.

Primers	Nucleotide sequence	Function
PC03	5'-ACACAACTGTGTTCACTAGC-3'	Detection of ß-globin
PC04	5'-CAACTTCATCCACGTTCACC-3'	[8]
5-HTTLPR-for	5'-GGCGTTGCCGCTCTGAATGC-3'	Amplification of
5-HTTLPR-rev	5'-GAGGGACTGAGCTGGACAACCAC-3'	5-HTTLPR gene [1]

Results and Discussion
226 randomly chosen science faculty students filled out the OHQ, and their results were calculated. 135 did not fill out their questionnaires completely and were excluded. The results were then converted to a more accurate interval score as described by Medvedev O. et al (2017) to improve their accuracy and allow them to be applied in biological research [9]. The scores ranged from 28.98 to 52.57, with an average of 35.02. 53.11% of the students were relatively happy, which is a slightly lower result than the 60.8% found by Kamthan S. et al. in saudi second year medical students [10]. These results were then divided into three groups based on their scores to represent different levels of well-being. The first group, group A, contained the participants who had gotten the 34 lowest interval scores. The second group, group B, contained all the individuals with average scores compared to the total sample, in addition to any participants within the confidence interval for this average. This group had four different interval scores. The last one, group C, was chosen similarly to group A, but with the participants who had the highest 28 scores instead. As well as, participants whose score values fell exactly on the defined cutoff threshold were assigned the symbol "X,"

with a total of 70 individuals in this category. The score ranges, averages, and standard deviations of each group and for the total sample can be seen in (Table 2). Participants located at the extremes of Groups A and C, as well as those within the intermediate Group B, were contacted to obtain informed consent for participation in the genetic polymorphism analysis. A total of 40 participants were ultimately included. This sample size was determined based on the limited capacity of the DNA extraction kit, which accommodated a maximum of 50 samples; accordingly, 40 samples were selected to represent the overall population of Faculty of Science students at that time, with an estimated confidence level of 90%.

Table 2. Questionnaire results

Group	N	Maximum	Minimum	Average	Standard Deviation
A	34	32.37	28.98	31.25	1.13
B	47	37.02	34.83	35.77	0.72
C	28	52.57	41.12	43.97	2.69
X	70	-	-	-	-
Total Sample	179	52.57	28.98	35.02	5.83

Amplification of ß-globin as a Reference Gene
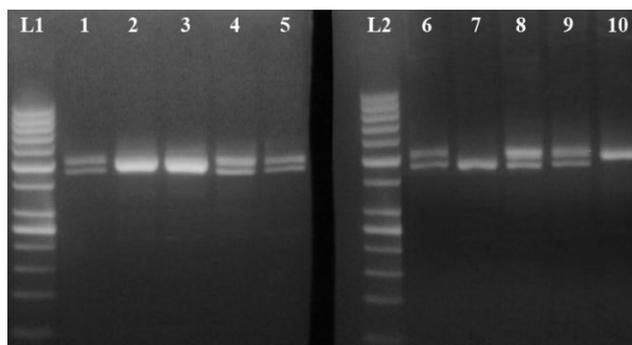
Gel Electrophoresis of the PCR product demonstrated the amplification of the β-globin gene fragment, which had occurred accurately and at the expected size based on the primer pair used. (Figure 1) This is in agreement with the observations of Mills et al., (2013), who found that the β-globin gene was amplified in all specimens [11]. This demonstrated the adequate quality of the extracted DNA and the absence of PCR inhibitors [11]. This is proof that the DNA sample can be used reliably to test for the presence and size of other areas in the genome.

5-HTTLPR Polymorphism

Gel electrophoretic analysis of the PCR products clearly visualized the 5-HTTLPR genotype for all the participants. (Figure 2) The distribution of 5-HTTLPR genotypes among participants is shown in (Figure 3). Our data shows that the LS genotype is the most frequent in this sample (42.51%). This is in agreement with the findings of Caspi et al (2003), who studied 5-HTTLPR genotypes in Caucasian populations and found that the LS genotype frequency was 51% [12].
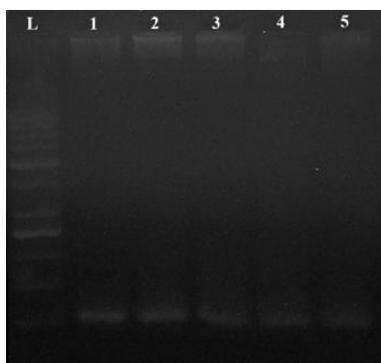

Figure 1. Amplification of β-globin gene in DNA samples. The PCR products were subjected to 3% Ethidium bromide stained agarose gel electrophoresis. Lane L: Ladder starting from 1000bp and decreasing by 100bp each band, Lanes 1-5 indicate the products from different DNA samples

Figure 2. Amplification of the 5-HTTLPR. The PCR products were subjected to 3% Ethidium bromide stained agarose gel electrophoresis. Lanes L1 and L2 contain the ladders, each starting from 1000bp and decreasing by 100bp each band. Lane 10 indicates the LL genotype, lanes 1, 4, 5, 6, 8, and 9 indicate the LS genotype, and lanes 2, 3, and 7 indicate the SS genotype.

Since Libya has been undergoing severe unrest over the past 8 years, young adults who have lived here for their entire lives have all been affected by the stress it has caused. We investigated the relationship between students' 5-HTTLPR genotypes, along with such stress exposure, and their happiness and general well-being. Our hypothesis was that, as the entire sample had been exposed to relatively high levels of stress, there is a very strong association between the SS genotype and having very low OHQ scores, the LS genotype and having average OHQ scores, as well as the LL genotype and scoring higher on the OHQ.

Frequency test analysis using SPSS (Table 3) shows that this is only partly true. First, the association between SS genotypes and group A was very clear, where 62.5% of participants carrying the S allele belonged to group A, as expected. On the other hand, individuals carrying the LS and LL genotypes were both equally associated with membership of group C, with 41.2% and 40% frequency of the groups in each, respectively. These results indicate that the S allele is associated with the well-being of our sample, but only in a recessive manner.
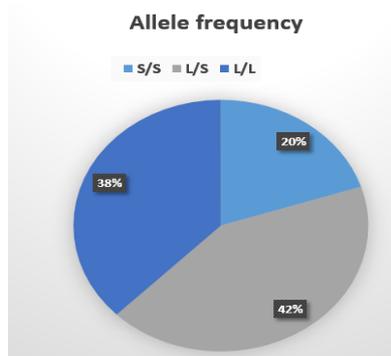
Figure 3. 5-HTTLPR genotypes distribution among Faculty of Science students. The sample consists of 40 students, 8 of them having the SS genotype, 17 the heterozygous genotype, and 15 the LL genotype.

Our results on the association of the SS genotype with lower levels of well-being correspond with the findings of the four previous studies described in section 2.1: Caspi A. et al (2003) [12], Goldman N. et al (2010) [13], De Neve J (2011) [1] and Artero S. et al (2011) [14]. All these findings were on individuals who had undergone copious amounts of stress. Several studies have attempted to uncover the reason behind this association. Hariri A. et al (2005) [15] found that the response of the amygdala is prolonged and amplified in individuals carrying the S allele. This points to the possible cause of the association between the SS genotype and having very low OHQ scores. The allele makes the amygdala more reactive during stressful life experiences.

Table 3. Frequency test analysis using SPSS.

Genotype	% of Group A	% of Group B	% of Group C
SS	62.5%	25.0%	12.5%
LS	29.4%	29.4%	41.2%
LL	33.3%	60.0%	40.0%

Conclusion

This Research demonstrates the impact of the 5-HTTLPR polymorphism on an individual's subjective well-being or happiness. This study specifically focuses on the aspect of subjective well-being that previous research has identified as highly significant. Our findings indicate a strong regulating influence of the 5-HTTLPR on happiness and overall well-being when exposed to environmental threats. The effects of the S alleles on an individual's happiness in the presence of stressful life events appear to manifest in a recessive manner. It is important to note that this genetically determined effect is observed in healthy individuals and does not directly predict mood or temperament characteristics. Consequently, the 5-HTTLPR may serve as a fundamental susceptibility factor for affective disorders by altering the functional response of the human amygdala in the context of stressful life experiences.

Conflicts of Interest
The authors declare no conflicts of interest.

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