

Correlation between the rs53576 SNP and stress
levels in high school students

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Personal Statement

I've always had a huge interest in science. I've taken classes in everything from biology to psychology, competed in the Chemistry Olympiad and LIPTA Physics Olympics, and been a member of my school's science honor society since the tenth grade. But most importantly, I've been able to do research. I'm lucky enough that my high school has a three-year Advanced Science Research program, meant to give students a chance to design and conduct their own studies. I did my project as part of this program, and it was one of the most rewarding experiences I had in high school.

When I first joined the program, I was really excited about researching, but I had no idea what exactly I wanted to do my research on. I went through a lot of ideas, did a lot of background research, and sent out a lot of emails before getting anywhere. But eventually I connected with a faculty researcher at LIU Post. I started designing my project, trying to combine aspects of biology, psychology, and economics. Originally, I wanted to analyze how high school art classes affected students' stress levels, but my hypothesis changed to looking at the effect of the rs53576 SNP, a gene variation, on stress management.

I ran trials at my high school with students who volunteered to be part of the experiment. I worked at LIU Post over the summer and during the school year with two faculty researchers and a graduate student. I analyzed data and organized my findings at school with some help from my research teacher, and continued working on that after school and at home. I wrote my paper, revised my paper, and presented my research in competitions and symposia. Being involved in research gave me so many amazing experiences, and such varied experiences; and it allowed me to work with incredible mentors in the field and other students doing research in the program.

I learned a lot because of research. I'd had prior knowledge in genetics because of AP Biology, but through my research, I learned more about single nucleotide polymorphisms and protein receptors and polymerase chain reactions. I was introduced to statistics for pretty much the first time, besides some cursory knowledge from math class. That meant working with t-tests and Pearson's tests and p-values and R-values. I've never really liked math, and I still don't, but I got to actually apply those skills for the first time. I finally got to use a lot of the knowledge and skills I've learned in school but never used.

Going into college, I want to major in molecular and cellular biology on the pre-med track, and, of course, continue with research. I'm really excited to do research in college and to work with professors and other students. I've become more and more interested in psychology and neuroscience in the past year, so I might try to perform research regarding that. But regardless of subject, I can't wait for the opportunity.

Being part of research is such an amazing experience. You only need to be passionate about something and excited to learn more and willing to do the work. Research can be difficult sometimes – after all, experiments are learning experiences, and things don't always go as planned – but it's definitely worth it. You get to learn from a new perspective and actually contribute to what you're learning about; you can find new interests or further ones you already have. Either way, it's really interesting. Whether you want to join a lab or do field work, focus on STEM or a humanity, there are tons of ways to get involved in research. So, if you get the chance, take it.

Abstract

The purpose of this study was to determine the relationship between the rs53576 single nucleotide polymorphism (SNP) and stress management in high school students. I'd hypothesized that individuals with the G variation of the SNP would be more capable of managing stress than those with the A variation. Saliva samples were collected from 22 participants and used to analyze cortisol levels and DNA. Participants also completed surveys and Perceived Stress Scales in order to determine the various factors contributing to their stress. There was no relationship between the variation of the rs53576 SNP and cortisol levels, but there was a negative correlation between the G variation and feeling in lack of control. There were also several positive correlations between being unable to handle external circumstances and feelings of internal stress.

Introduction

My primary hypothesis was that external stressors would correlate with internal levels of anxiety. There are many factors which cause high school students stress, including schoolwork, course rigor, and extracurricular activities. Other factors include part-time employment and worry about future plans. In two studies, high school students self-reported the stressors which they viewed as most significant: examination results, future educational and occupational plans, and balancing schoolwork with extracurricular commitments were among the most pressing (Kouzma & Kennedy, 2004) (Armacost, 1989).

Secondly, I hypothesized that a student's stress management would correlate with their variation of the rs53576 single nucleotide polymorphism (SNP). Polymorphisms are differences in the genetic sequences between individuals; when they occur in a single nucleotide, they are called single nucleotide polymorphisms. The rs53576 variation is part of the OXTR gene, responsible for the oxytocin receptor. Oxytocin is a hormone with some involvement in stress management. Having a guanine (G) nitrogenous base as opposed to an adenine (A) nitrogenous base has been linked to better stress management (Rodrigues, Saslow, Garcia, John, & Keltner, 2009) (Chen et al., 2011).

Methods

Data Collection

Student volunteers from four classes participated in the study. Each participant filled out a questionnaire meant to obtain general information about them and their perceived stress levels; the questionnaire included a Perceived Stress Scale and questions about their age, overall grade averages, course rigor, caffeine consumption, etc.

Each participant gave two saliva samples during the in-class trials: one would be used to analyze cortisol levels as an indicator of stress, and the second to analyze DNA for the rs53576 SNP. Participants were supervised by teachers and qualified scientists and instructed on the proper procedure for saliva collection. To obtain the samples, participants held cotton swabs in their mouths for two minutes to absorb saliva, after which the swabs were placed into vials and stored in portable coolers.

Cortisol Analysis

Salimetrics' salivary cortisol enzyme immunoassay kit was used to analyze the cortisol samples. The reagents, microtitre plate, and assay diluent were prepared first. Standards, controls, and saliva samples were pipetted into the appropriate wells of the microtitre plate; diluted conjugate solution was added to each well. The plate was mixed, incubated, and then washed before Substrate Solution was added; this was repeated for the addition of Stop Solution. The plate was read in a plate reader, and the concentration of cortisol in each saliva sample was determined using data reduction software.

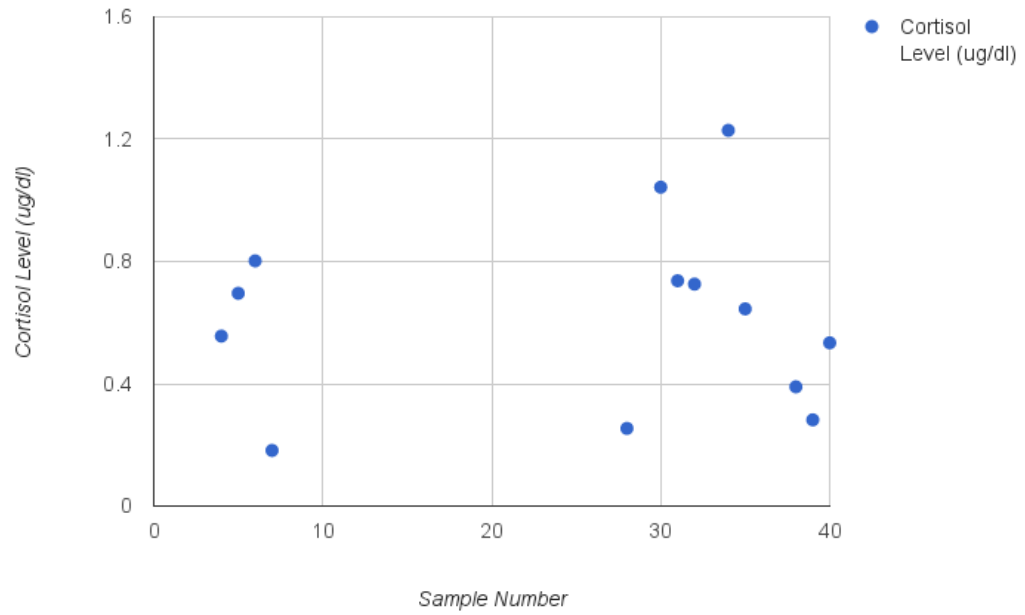
Single Nucleotide Polymorphism (SNP) Detection

The DNA was first extracted from the tubes. Polymerase Chain Reaction (PCR) was then used to replicate the DNA samples so that they could be analyzed for the rs53576 SNP using Typhoon.

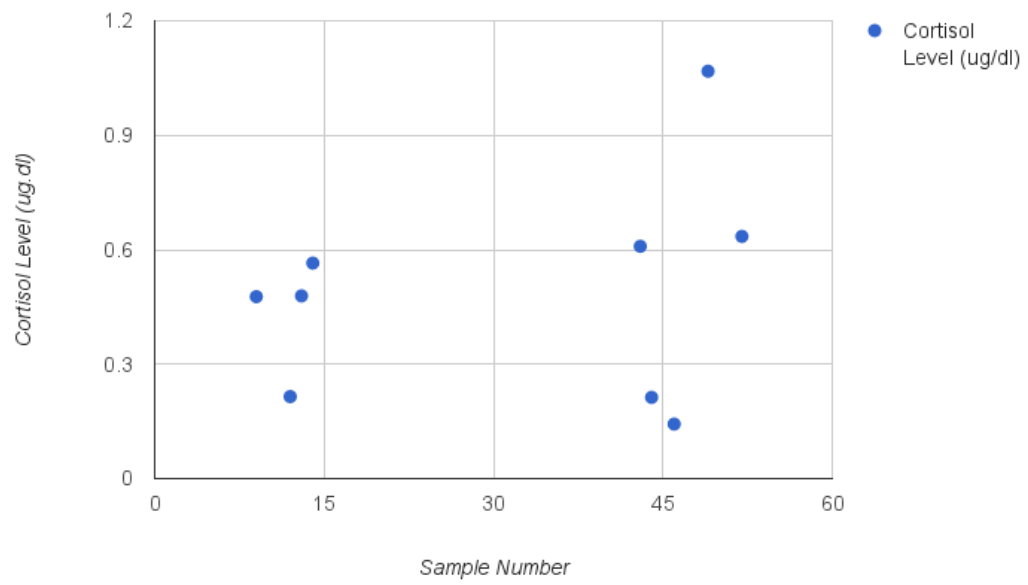
Results

Twenty two subjects participated in this study with ages ranging from 16 to 18 years old (M=17.3). The overall mean cortisol level was 0.537 ug/dl with a standard deviation of 0.063 ug/dl. The average cortisol level of participants in the earlier two trials was higher than those in the later two trials; however, t-tests determined that the difference in averages was not statistically significant.

Morning Trials



Afternoon Trials



Overall averages of the participants were based on a one hundred point scale and weighted for Honors and AP courses. Of the 22 students, over half had an overall grade average ranging from 90-100; the remaining subjects were evenly split in the seventies and eighties. One subject chose not to answer.

Approximately half of the participants were enrolled in one or more honors classes; similarly, half were enrolled in one or more AP classes. The average cortisol level for the former was 0.452 ug/dl, and for the latter was 0.523 ug/dl. When these averages were compared to those of students not enrolled in honors and AP classes, the differences were found to be statistically insignificant according to t-tests. The p-values were 0.294 and 0.254 respectively. Pearson's tests were run regarding honors and AP courses. In both cases, there was a positive correlation between enrollment in the course and a feeling of being overwhelmed.

Pearson's correlation tests were also run to analyze the relationships between different stress factors. These factors were all derived from a standard Perceived Stress Scale and describe an individual's degree of stress, ability to cope with stress, and emotions related to stress. Several relationships were positively correlated and statistically significant. Correlations were described by r-values ranging from -1 (perfect negative correlation) to +1 (perfect positive correlation).

Thirteen of the 22 participants (59%) had the G variation of the rs53576 SNP, and nine (41%) had the A variation. The differences in average, minimum and maximum cortisol levels between the two groups were not statistically significant, although in each case the values were slightly lower for those with the G variation than for those with the A variation. There was a statistically significant negative correlation between feeling out of control and having the G variation of rs53576 ($r=-0.462$) ($p=0.027$).

| Factor 1 | Factor 2 | R value | P value |
|-----------------|---------------------------|---------|---------|
| Lack of control | Nervous | 0.584 | 0.003 |
| Lack of control | Unable to cope | 0.471 | 0.027 |
| Confident | Things going my way | 0.529 | 0.009 |
| Confident | In control of irritations | 0.482 | 0.020 |
| Confident | On top of things | 0.727 | 0.000 |
| Honors | Too much to overcome | 0.435 | 0.038 |
| AP | Too much to overcome | 0.512 | 0.013 |
| Age | Confident | 0.451 | 0.031 |
| Age | In control of irritations | 0.473 | 0.023 |

Conclusion

Stressors

Based on the questionnaire and self-reports, there were several statistically significant correlations between stress conditions. Participants who reported feeling very stressed and nervous had also reported feeling as though they could not control important things nor irritations in their lives. Alternatively, participants who reported feeling confident had also reported feeling in control and as though things were going their way. In either case, the subjects' emotions were positively correlated with their ability to cope with and manage the tasks and difficulties in their lives. Failure to control external circumstances correlated with internal stress, and success in external circumstances correlated with internal confidence.

Rs53576 SNP and Stress Management

I had hypothesized that individuals with the G variation of the rs53576 SNP would be better capable of managing stress. Pearson's test did indicate that there was a negative correlation between the G variation and lack of control. However, the rest of the data did not support this. Additionally, the hypothesis was not supported by biological evidence, as there was not a statistically significant difference in the cortisol levels of participants with the G variation and those with the A variation.

References

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