The role of IGF2, DRD4 and 5-HTTLPR genes in the development of eating disorders

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Introduction

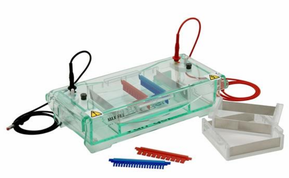
Many risk factors have been suggested for the development of certain conditions such as eating disorders; and genetic factors are believed to play an essential role among other factors. However, individual contributions by these factors including the genetics have yet to be characterized and described; although three genes such as insulin like growth factor (IGF2), serotonin-transporter-linked polymorphic region (5-HTTLPR) and dopamine receptor D4 (DRD4), are believed to play a central role.

Purpose

The purpose of this study is to determine the expression and the cause of the alteration in expression in IGF2, 5-HTTLPR and DRD4 genes. We postulate that the alterations in the levels of expression are caused by methylation of the promoter region on these genes. We are currently determining the expression of these genes in blood drawn from patients with eating disorder conditions. We extract RNA and DNA from the blood and measure the expression of the selected genes. Following this we will measure the methylation status of the promoter region of the gene. Primers for expression have been designed and will be used in a technique using real-time quantitative polymerase chain reaction (RT-qPCR). Currently we are working on the expression levels of 5 HTTLPR and having perfected the technique will analyze the expression levels of other genes.

Materials

1. Primer design software
2. Primers
3. Blood samples from patients with eating disorders
4. DNA extraction materials
5. Electrophoresis bath
6. Thermal cycler



5. Electrophoresis bath

6. Thermal Cycler

Methods

1 Primer Design

a. Acquire FASTA sequences for the genes of interest

b. Locate CpG islands

c. Design primers within CgP islands using PrimerQuest and analyse them using OligoAanalyser

a.

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ATGTTGAATCTGTGTCATGCTCTTCGAGGCGTACGTCAGTTTTCCTGTTCTGTGATTGTG
AAAGTGAAATGTGCGTCATGTTCCATAAAACTACAGGATCAGGATCCTAGTAAACCAGGA
TATTATACGAAGCCAAAAAGTCTACCCGACTCAAACTAAATCCTGACCTCCAAGATCTG
AAGTATTGCTATTCAGTCAAGACATACAGCTCTCGAAACAAGCCAACTCAAATGATCCT
GACCTCAAACCAAAAAGATTACTATTGAAGTGATTTGCAAAGTGCAGTAATGCC
CTTCATCATAACAATTATAACCCGGAAGGTTCCCGAAAGCACATTAAATGATATTCTG
AACTACGTCCCCAGAGCTCAAATGTTATGCATATTGTCCCTTTGTAAATTTCCCTTA
CATTTAGACCCCAATGTTTGAAACGAAATGATTAGATACTACATTAGTGTTAACGAAA
AGCGATCAAGTTTTAAAGATAAAAATGCTGTCAGCAAAAAGGTCCTATATTCATGAAG
CAATTTTAAAAGACTTTAAAAATTAACTCAAATAAAACATTCGCCATATCTGCCTG
AAAACTGGAACATCCATGTTTACAACTACTTTAAAACTACACATATTTGCTAGGC
AACCTAATGTTGATAACTAACTTTACAACTTACAATCTACTACAGAAATATCTTGGATAT
AAGGTTAAAATTGATTCTACTGGGAAAATTAACTCCCCCTCTGAAGAAGTGATGCAAGAA
GCTTTACAACCCGAAGAATTTTTCAAGATTCAAGTCTGGTGTATCACATATCCC
AATTAACTAGATCCGTACAAGCCTACCAAGTTGGAGGTAAAATACTTTTGATCTTCCA
GGCTATTCAACTTCCACCTCCAGATTACAGCTGAAAACTAATCAGCGAGCTTGGCTT
CAAAGGCTGCGAAAAACCGATTTATTCAATCGAAGCATATAAACACAGAAGCTTATGAG
TCTATGAAGGGCACATCACAGGGAGGCTGTTATACCGTGGGAGGGATTTTCTACCTGGTA
CCTCCGAAAGGATCCATAAATCAGATTGTGAAATACACATACAGCACATCAAAACAGTTC
AAGAACATTGAAAAAGGTATTGATGTATTCAACTCGTGCAATTCATCATCTGGCACACAT
CCGTTATCACGGTATTTGTAATTAAAAGCGTTATATGCAAAAAGCCCAGTACAAAAGG
TACGCAATACCTCCATTTATTGGAAGTATAGAAGTCGTTCTGAAAGATATAGGATATATT
TTATTAAGGACCACTGGAGGTATGATTCAAAGACTACATGAGATATGGATACCTAGG
GGTATCCAAGTAGGTATAAGGGAACCGCTAGAAACCTGATTGAGTCTGGCTACCAGCGT
TACATTGAAACAAATGGTAAAGAGTCAAGCTGTCCCAGAGATAAGCCGATCATCAGTTCC
CTATATAAGAGCGCCCCGGTGAAGCTGACTTGAACTTGAACGCAGTGAAGAAGTCGTATTTA
GAAAAACCGAGAAAGATTTATCTGCTAGAAGATTTGTCGACGATGACCTTATGATTTG
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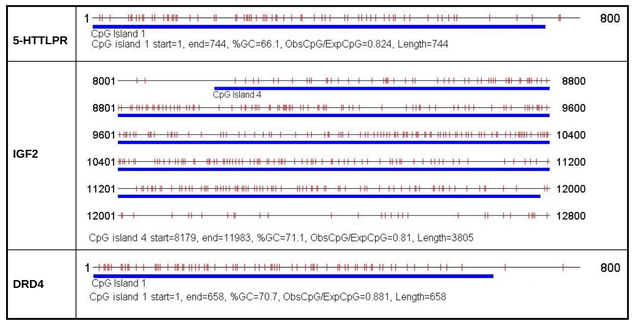
b.



c.

5-HTTLPR	Sequence	Tm (°C)	Hairpin Tm (°C)	Self-Dimer Deltat G (kcal/mole)	Hetero-Dimer Delta G (kcal/mole)	Cut sites	Amplicon length
Forward	TCTAGAGATCAGACCATGT GAGG	55.2	24.6 (2) 21.9 (2) 15.3 (3) 6 (1)	-7.31 to -1.34 (14)	-3.17 to – 1.57 (15)	7	177
Reverse	TTCGAGGCGGAGAGGAAA	55.9	21.2 (2) 5 (3)	-6.76 to – 1.57 (7)	-3.17 to – 1.57 (15)		

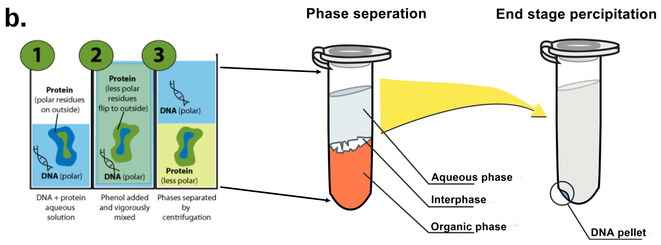
2 DNA Extraction

a. Acquire blood sample

b. DNA isolation through repeated steps of phenol / chloroform application, centrifugation and aspiration of the organic phase

c. Determine DNA concentration using Nano drop

b.



c.

Blood Samples	KR	GT	LL
Concentration (ng/µl)	1558.1	728.5	158.9

3 Gel Electrophoresis

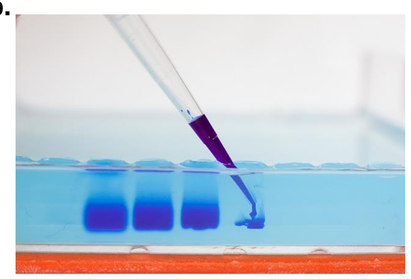
a. Fill the electrophoresis bath with agrose gel (loaded with ethidium bromide)

b. Load samples and ladders into the wells

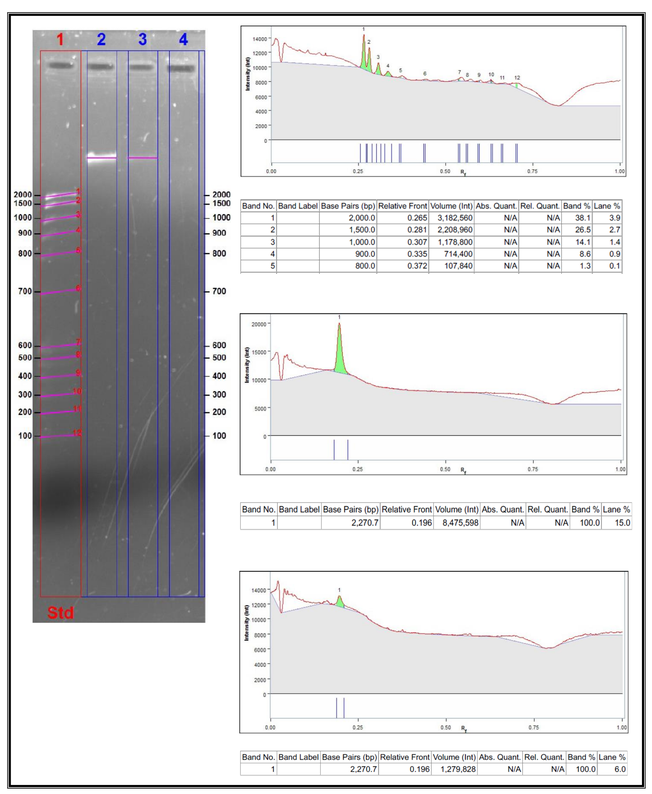
c. Run the gel

d. Analyse the gel under UV light

b.



d.



Band No.	Band Label	Base Pairs (bp)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		2,000.0	0.265	3,182,560	N/A	N/A	38.1	3.9
2		1,500.0	0.281	2,208,960	N/A	N/A	26.5	2.7
3		1,000.0	0.307	1,178,800	N/A	N/A	14.1	1.4
4		900.0	0.335	714,400	N/A	N/A	8.6	0.9
5		800.0	0.372	107,840	N/A	N/A	1.3	0.1

Band No.	Band Label	Base Pairs (bp)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		2,270.7	0.196	8,475,598	N/A	N/A	100.0	15.0

Band No.	Band Label	Base Pairs (bp)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		2,270.7	0.196	1,279,828	N/A	N/A	100.0	6.0

Research in Progress

RNA extraction from blood samples

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Run RT-qPCR for methylation on DNA

↓

Run RT-qPCR for expression on RNA

↓

Analyse results

Summary

We hypothesized that the results that we will acquire from the RT-qPCR will demonstrate that there is a evident change in the methylation status on the promoter regions of the selected genes. A hypomethylation or hypermethylation status will translate into an increase or decrease in the expression of the genes. Thus, a resulting disequilibrium in the products of the genes will demonstrate that the methylation status of the promoter region could be a factor in the development in conditions such as eating disorders.

Future Applications

Epigenetic therapy is techniques or drugs that are used to treat medical conditions. Epigenome- influencing techniques and drugs that target specific enzymes involved in the epigenetic regulation of gene expression are often used. We aspire that our research will have future applications for the treatment of eating disorders in the field of epigenetic therapy.

Acknowledgements

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Sources

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