
Lab 7: Science of Opioid Dependence

Homework:

Type your answers to the following questions into this document and submit it through Turnitin links on eCampus by the due date. Note: the fill-in boxes should expand as you type, so you do not need to limit your answers to the space. Use complete sentences and consider your writing style. Well written answers will convey your message better.

1. What was the question you wrote for this research study? (3 pts.)

Since ANKK1 is associated with opioid dependence, would it be more prevalent in the case population?

2. What particular region of DNA was isolated and amplified for this study? Why was it selected? What was unique about this region? (4 pts.)

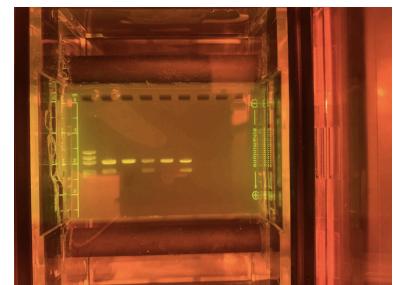
In this study, rs1800497 was targeted. This is because it contains both a C allele and a T allele. The genotype can be found by a restriction site in the C allele but not the T allele.

3. Briefly describe the steps or methods for studying genes. Start with targeting a gene region and end with gel electrophoresis. (4 pts.)

The target region is flanked by forward PCR primers and reverse PCR primers. It is then amplified by PCR. A 532 bp product is then generated. The restriction enzyme TaqI digests the PCR product. Digestion then causes DNA fragments, which are then analyzed by agarose gel electrophoresis.

4. How did using gel electrophoresis help you determine the genotypes of individuals? Incorporate and explanation of how gel electrophoresis works in your answer. Include a picture of your gel to help you explain. (4 pts.)

In gel electrophoresis, the smaller DNA fragments will move farther than the large ones. TaqI will cut the PCR products of the C allele but not the T allele meaning the T allele won't move as far as the C allele. In our gel electrophoresis, the products with the C allele were closer to the positive electrode.



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5. Your TA will provide you with numbers collected across lab sections in the course. Compare those numbers with the ones collected by your lab section.
- Calculate genotype and allele frequencies for your lab section and across lab sections in the course. (3 pts.)

Your lab section:

Genotypes of Individuals

<u>Genotype</u>	<u>Number in Control Population</u>	<u>Number in Case Population</u>
CC	60	55
CT	56	67
TT	22	31
Total:	138	157

Genotype Frequencies

<u>Genotype</u>	<u>Frequency in Control Population</u>	<u>Frequency in Case Population</u>
CC	43%	35%
CT	41%	43%
TT	16%	20%

Numbers of Alleles

<u>Alleles</u>	<u># in Control Population</u>	<u># in Case Population</u>
C	176	177
T	100	129

Allele Frequencies

<u>Alleles</u>	<u>Frequency in Control Population</u>	<u>Frequency in Case Population</u>
C	63.8%	56%
T	36%	41%

Combined Lab sections:

Genotypes of Individuals

<u>Genotype</u>	<u>Number in Control Population</u>	<u>Number in Case Population</u>
CC	125	90
CT	110	160
TT	35	60
Total:	270	310

Genotype Frequencies

<u>Genotype</u>	<u>Frequency in Control Population</u>	<u>Frequency in Case Population</u>
CC	46%	29%
CT	41%	52%
TT	13%	19%

Numbers of Alleles

<u>Alleles</u>	<u># in Control Population</u>	<u># in Case Population</u>
C	470	500
T	290	440

Allele Frequencies

<u>Alleles</u>	<u>Frequency in Control Population</u>	<u>Frequency in Case Population</u>
C	62%	53%
T	38%	47%

- b. Explain how your lab section compares to combined lab sections. (3 pts.)

My lab sections results is similar to the combined lab sections. In my lab section, the genotype frequency was 43% (CC), 16% (TT), and 41% (CT) for the control population and 35% (CC), 43% (CT) 20%(TT) for the case population. For the combined lab sections, the control population was 46%(CC), 41%(CT) 13% (TT) and the case population was 29% (CC), 52% (CT) and 19% (TT). In my lab section, the frequency for allele in control population was 63.8% (C) and 36% (T). In case population, it was 56% (C) and 41% (T). In the combined lab sections, the control population was 62% (C) and 38%(T). In case population it was 53%(C) and 47% (T).

- c. Do chi square analyses to determine if there are significant differences between control and case populations with regard to genotype and allele frequencies for your lab section and combined lab sections. (3 pts.)

Your lab section:

p-values From Chi Square Analyses

	p-value from chi square analysis	Significant difference?
Genotype frequencies	<.00001	Yes
Allele frequencies	0.000261	Yes

Combined Lab sections:

p-values From Chi Square Analyses

	p-value from chi square analysis	Significant difference?
Genotype frequencies	0.000114	Yes
Allele frequencies	1	No

- d. Explain how your lab section compares to combined lab sections in the course. (3 pts.)

In both my lab section and the combined lab sections, there was a significant difference in genotype frequencies. The p values for the genotype frequencies was similar. My lab sections p value was slightly higher. In the allele frequencies, my lab section found a significant difference. The allele value for the combined lab sections did not find a significant difference.

- e. What could account for differences between your lab section and across lab sections in course? Do you think one data set is more reliable than the other? Explain. (3 pts.)

Some lab groups could have made errors in the study such as reading the data incorrectly. This could have lead to outliers in the data, skewing the results. I believe the data across lab sections is more accurate because it is a bigger population.

6. What conclusions can you make about the case and control populations. Is there a genetic association to opioid dependence? (4 pts.)

Yes there is a genetic association with opioid dependence. There are higher number of opioid dependence associated with the T allele. Although the number is higher, it is not significantly higher in the T allele.

7. Why is it inaccurate to say that if someone has a T or C allele for the rs1800497 SNP will become dependent on opioids? (4 pts.)

Although it can be more common to become opioid dependent by carrying this allele, it is not a definite case that they will become opioid dependent. It is just more likely that this could occur.

8. Current published research on the ANKK1 gene and the SNP rs1800497 showed that the frequency of the T allele was 38% in a control group and 44% in a group of opioid dependent participants in a study (Zhang et al. 2018). Chi square analysis resulted in a p-value of 0.04. Do your results align with published data? Explain. (4 pts.)

Yes those were the exact numbers we found when studying opioid dependence and doing this lab. The p-value was different but there was still a significant difference in both our research and the previous research.

9. How do you think the methods for this lab (i.e. studying genes and DNA) could be used to address the opioid epidemic? (4 pts.)

By seeing if patients have these alleles, Doctors could be more cautious when prescribing opioids to these patients. Other uses of PCR could be used as well to further stop opioid dependence.

10. Thinking more broadly than in the last question, what do you think could be done to address the opioid crisis? List at least two ideas you have. Explain why you think your suggestions would be effective. (4 pts.)

I believe the use of medical marijuana instead of opioid after a painful procedure is done could cut down the number of opioid dependent individuals. Medical marijuana still gives pain relief affects, but is not addictive. There are also many health benefits in this as well.

Another solution to cut down on the opioid crisis is to more strictly regulate how many pills are being given when they are prescribed. A lot of times, patients continue to take the rest of their prescription when it isn't even needed anymore. Giving less of the drug at a time could help the crisis.

This is the hardest homework assignment I have ever done. I'm sorry if this makes no sense, I'm trying my very best. This took me 4 hours to complete I'm just trying to pass. :((- Sincerely, a student going through it.