NF1 Thesis Analysis

Marshall McQuillen

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Contents

Overview

The paper that this analysis was created for was submitted for publication in "The Journal Of Genetic Counseling." As the journal name implies, it's focus is on genetic counseling and not on the field of statistics, and therefore some of the nuances of the statistics had to be left out. This report will take a closer look at those nuances. The paper that was submitted is titled, "Assessment of the Impact of a Positive Family History and Genetic Counseling on Parental Knowledge of Neurofibromatosis Type 1 (NF1)."

Statistical Analysis

Study Aims

The study had two hypotheses, namely:

1. Parental knowledge of NF1 is influenced by having a personal diagnosis of NF1.
2. Parental knowledge of NF1 can be increased by exposure to genetic counseling.

Data Overview & Cleaning

Prior to performing any statistical tests, some minor data cleaning was needed. The below lists all alterations made to the data for the purposes of this and the submitted paper.

There were a total of 303 responses to the questionare:

- 11 respondents were "Not sure" if they had NF1. These respondents were dropped from the data set since both study aims are contingent upon knowing this information.
- One respondent skipped the question, "Does your child's other parent have NF1?" This respondent was kept since they answered the question, "Do you have NF1?" and their answer to "Does your child's other parent have NF1?" was imputed as "No."
- One respondent skipped the question, "Doe you have NF1?" This respondent was dropped from the data set since both study aims are contingent upon knowing this information.
- 14 respondents do not have NF1 and are "Not sure," if the childs other parent has NF1. These respondents were dropped from the data set since both aims of the thesis are dependent on this information.
- Three additional respondents were dropped from the study due to leaving other questions that will be discussed blank.

After the above adjustments, the final data set contains 274 respondents, with an age ranging from 25 to 82 and a mean age of 42.1135531. The standard deviation of the ages is 9.9638095.

Approach 1

Testing Methodology

In order to assess the first hypothesis of the study, to determine whether parental NF1 knowledge is influenced by the parent having a personal diagnosis of NF1, respondents were asked whether they or their affected child's other parent has NF1. This split the respondents into three groups:

- The **Unaffected** group - Respondents who do not have NF1 and the child's other parent also does not have NF1.
- The **Other Parent Affected** group - Respondents who do not have NF1 and the child's other parent does have NF1.
- The **Affected** group - Respondents who do have NF1 and the child's other parent does not have NF1.

Originally, since we (myself and the PI of the study) knew we wanted to compare each of the three groups to the other two, I decided to forgo an initial ANOVA and go straight to the pairwise comparisons. Therefore, the three tests, and their accompanying hypotheses are as follows:

1. Test 1 hypothesis: The "Unaffected" group and the "Other Parent Affected" group do not have the same mean NF1 knowledge score, as measured by the knowledge questionaire.
   - $H_0: \mu_{unaffected} = \mu_{other\ parent\ affected}$
   - $H_a: \mu_{unaffected} \neq \mu_{other\ parent\ affected}$
2. Test 2 hypothesis: The "Unaffected" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is greater than that of the "Affected" group.
   - $H_0: \mu_{unaffected} \leq \mu_{affected}$
   - $H_a: \mu_{unaffected} > \mu_{affected}$
3. Test 3 hypothesis: The "Other Parent Affected" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is greater than that of the "Affected" group.
   - $H_0: \mu_{other\ parent\ affected} \leq \mu_{affected}$
   - $H_a: \mu_{other\ parent\ affected} > \mu_{affected}$

Although the variance of test scores are very close to each other, the sample sizes are not (see *Table 1. Study Aim 1: Sample Statistics*), leading to the choice of Welch's t-test as the statistical test to be used.

The second hypothesis of the study, to determine whether parental knowledge of NF1 can be increased by exposure to genetic counseling, was also tested using Welch's t-test for the same reasons as mentioned above (see *Table 2. Study Aim 2: Sample Statistics*). The hypothesis for this test are below.

4. Test 4 hypothesis: The mean NF1 knowledge score, as measured by the knowledge questionaire, of those who have had exposure to genetic counseling is greater than that of those who have not had exposure to genetic counseling.
   - $H_0: \mu_{seen\ GC} \leq \mu_{not\ seen\ GC}$
   - $H_a: \mu_{seen\ GC} > \mu_{not\ seen\ GC}$

In order to control the Family-Wise Type I error rate, the original significance level ($\alpha = 0.05$) was adjusted using the Bonferroni Correction, reducing the level of statistical significance to $\alpha_{adj} = 0.0125$.

Table 1: Study Aim 1: Sample Statistics

	Mean	Variance	N
Unaffected	0.8276901	0.0084524	193
Other Parent Affected	0.8436247	0.0056361	29
Affected	0.7768336	0.0075135	52

Table 2: Study Aim 2: Sample Statistics

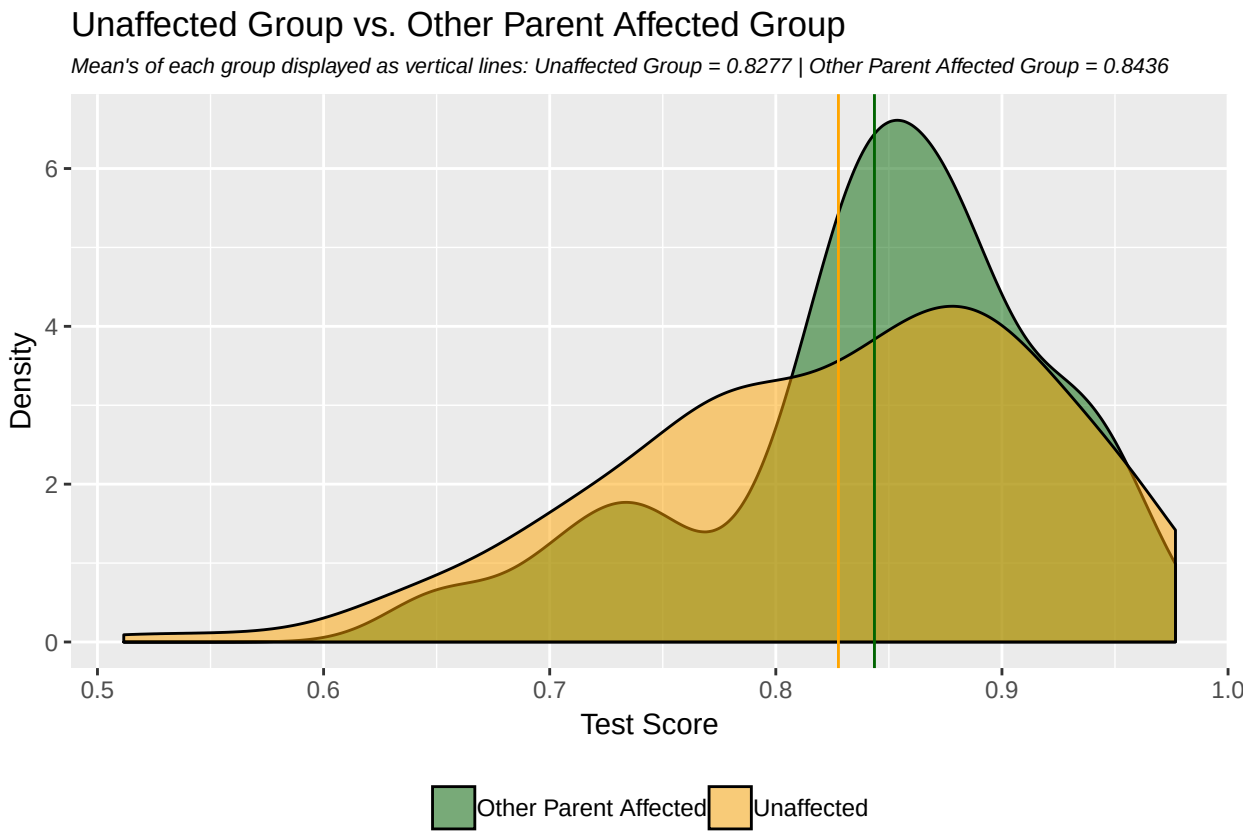
	Mean	Variance	N
Has seen a Genetic Counselor	0.8315752	0.0075986	194
Has not seen a Genetic Counselor	0.7909884	0.0092215	80

This brings us to the tests for the first study aim.

Study Aim 1 - Parental knowledge of NF1 is influenced by having a personal diagnosis of NF1.

Test 1

- H_0 : The "Unaffected" group has the same mean NF1 knowledge score, as measured by the knowledge questionaire, as the "Other Parent Affected" group.
- H_a : The "Unaffected" group does not have the same mean NF1 knowledge score, as measured by the knowledge questionaire, as the "Other Parent Affected" group.

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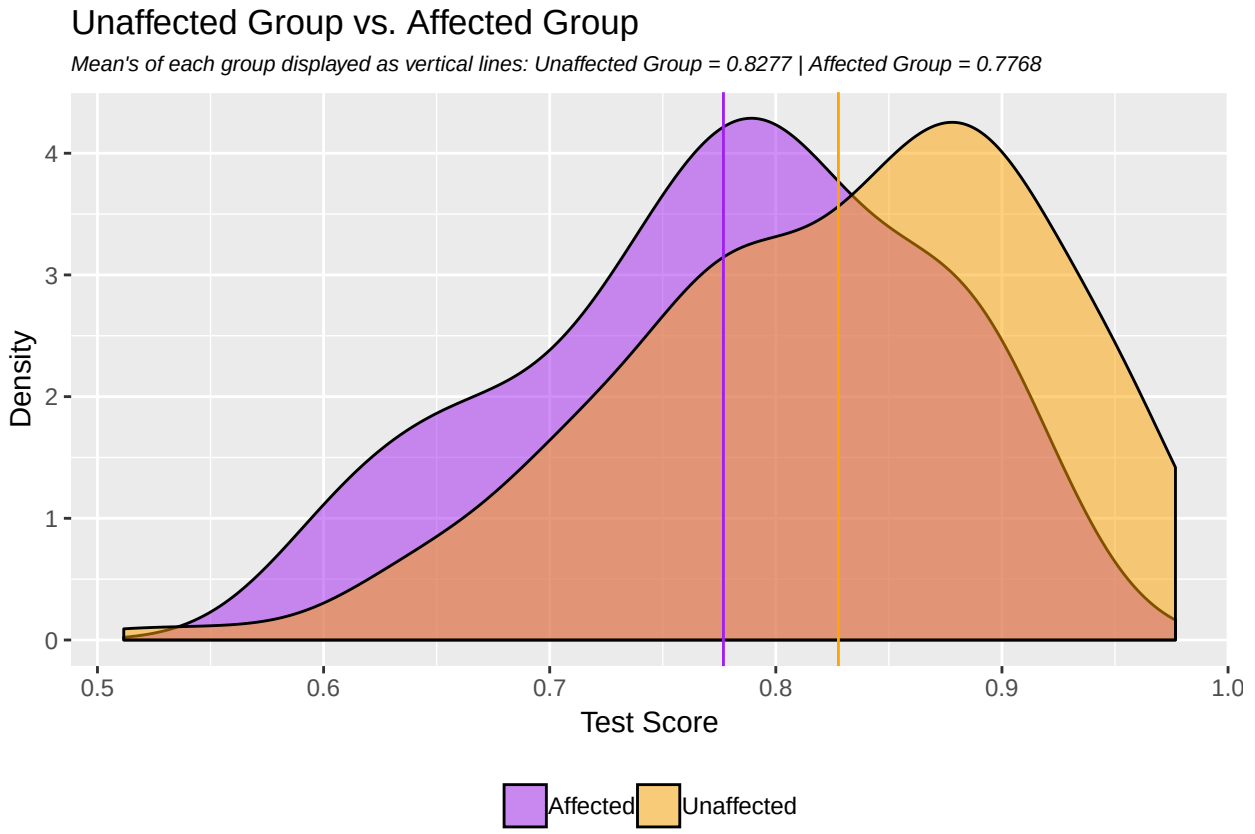
## [1] "Test 1 p value = 0.307749444337635"
```

Welch's two-tailed t-Test returns the above p value, with which we fail to reject the null hypothesis.

Test 2

- H_0 : The "Unaffected" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is less than or equal to that of the "Other Parent Affected" group.
- H_a : The "Unaffected" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is greater than that of the "Affected" group.

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## [1] "Test 2 p value = 0.000187363981785434"
```
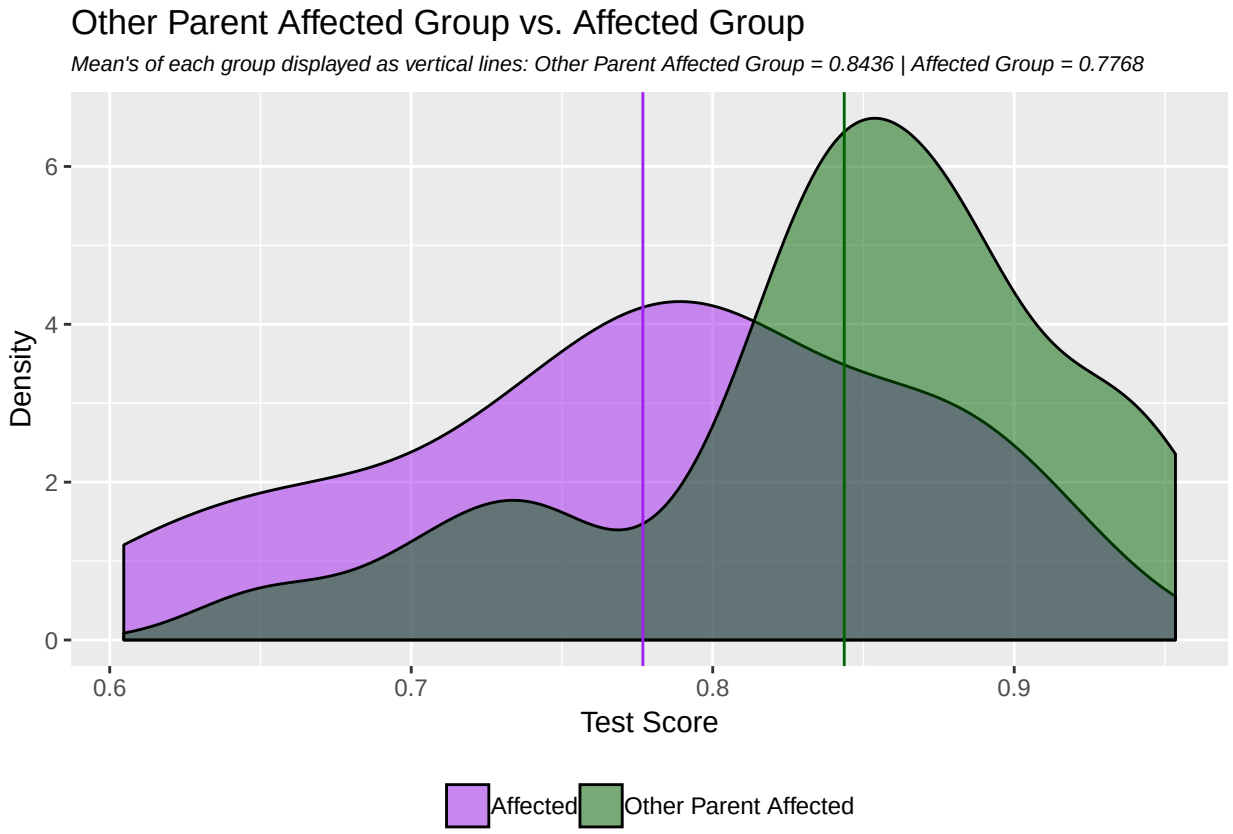

Welch's one-sided t-test returns the above p value. Being well below the Bonferroni corrected significance level of 0.0125, H_0 is rejected in favor H_a.

Test 3

- H_0 : The "Other Parent Affected" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is less than or equal to that of the "Affected" group.
- H_a : The "Other Parent Affected" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is greater than that of the "Affected" group.

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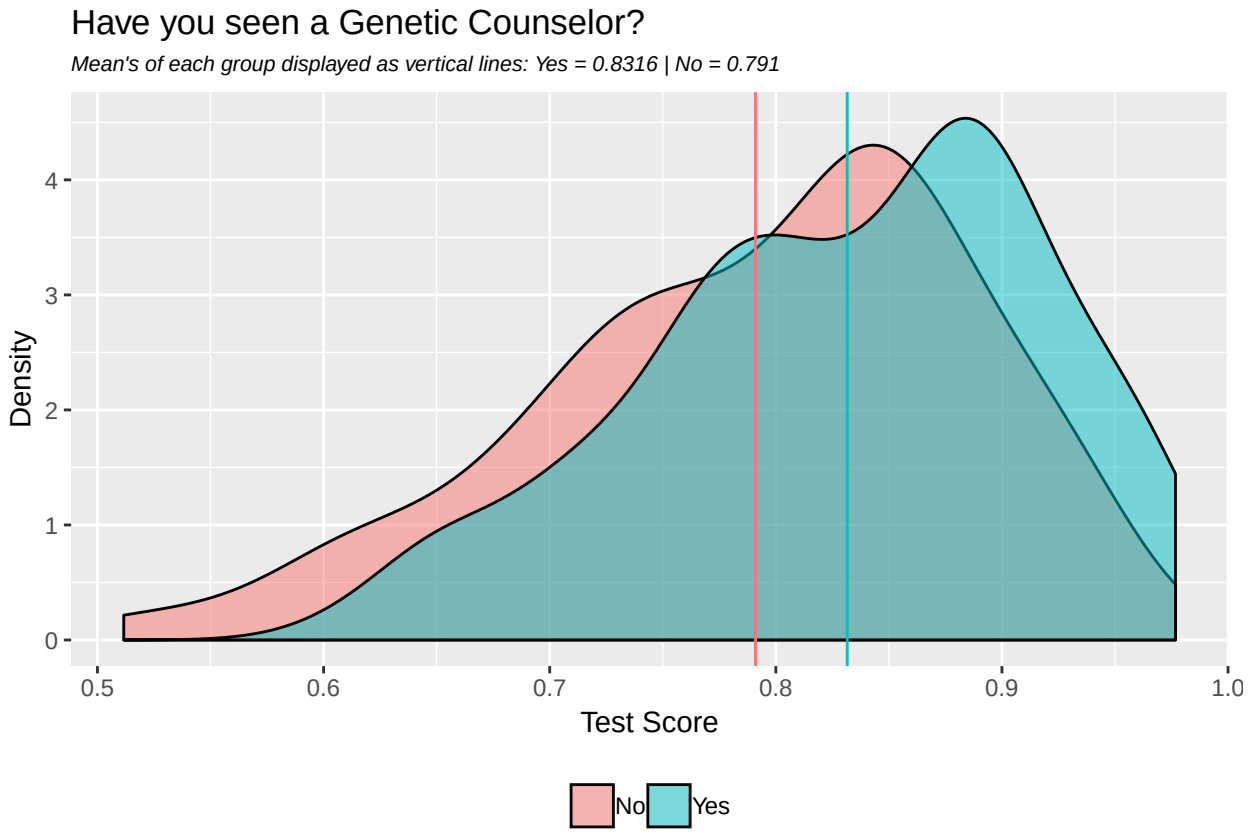
## [1] "Test 3 p value = 0.000280154187709805"
```

Welch's one-sided t-Test returns the above p value. Being well below the Bonferroni corrected significance level of 0.0125, H_0 is rejected in favor H_a.

Study Aim 2 - Parental knowledge of NF1 can be increased by exposure to genetic counseling.

Test 4

- H_0 : The "Seen GC" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is less than or equal to that of the "Not Seen GC" group.
- H_a : The "Seen GC" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is greater than that of the "Not Seen GC" group.

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## [1] "Test 4 p value = 0.000691029479878431"
```

Welch's one-sided t-Test returns the above p value. Being well below the Bonferroni corrected significance level of 0.0125, H_0 is rejected in favor H_a.

Approach 2

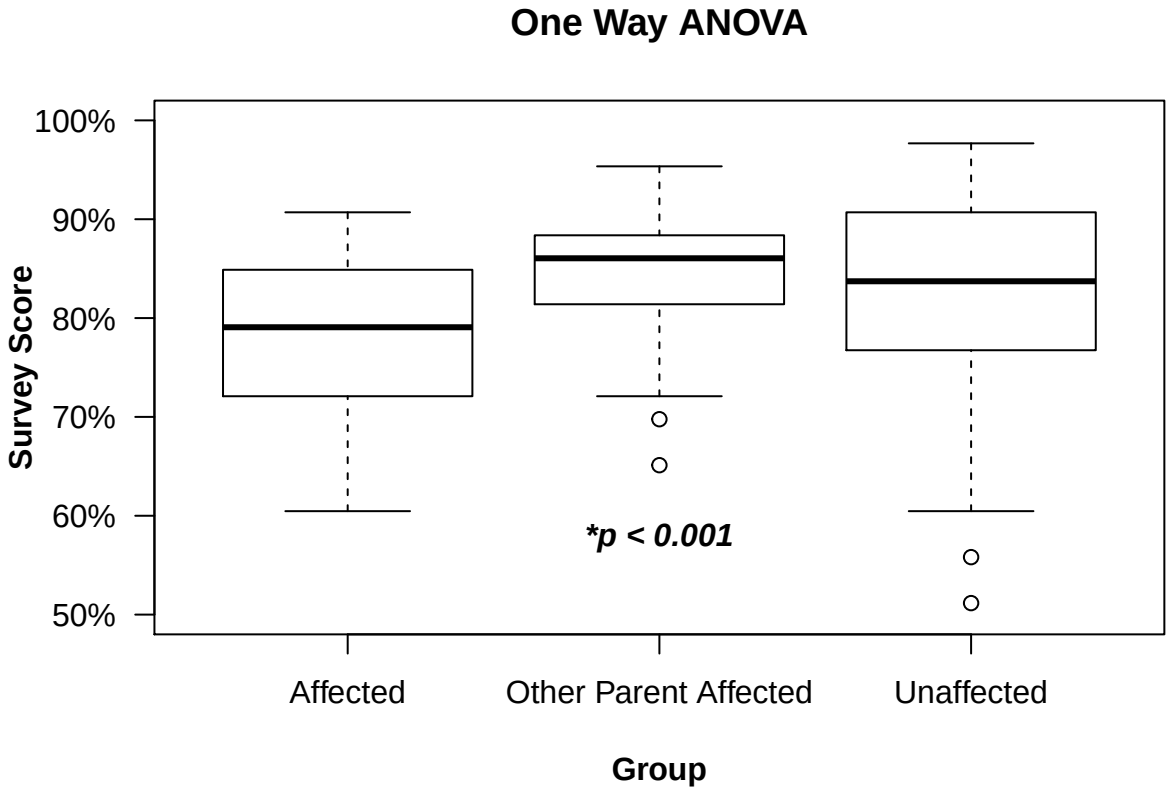
Upon initial submission of the paper, the editor of the journal noted that the "statistical norms" of studies in the Journal of Genetic Counseling (JOGC) are to use an ANOVA followed by post-hoc pairwise comparisons should the ANOVA prove significant. In addition, should the paper be published in black and white, the above images wouldn't be as clear as they could be. With these suggestions in mind, the analysis and images were redone to fit the guidelines of the journal.

Study Aim 1 - Parental knowledge of NF1 is influenced by having a personal diagnosis of NF1.

ANOVA

Since there are three groups that need to be tested ("Affected", "Other Parent Affected" and "Unaffected"), an ANOVA test will determine whether there is evidence that at least one group mean is different from the others.

- H_0: There is no difference in mean NF1 knowledge score, as measured by the knowledge questionaire, between the three groups ("Affected", "Other Parent Affected" and "Unaffected").
- H_a: At least one group has a different mean NF1 knowledge score, as measured by the knowledge questionaire, than the other groups.




```
##              Df Sum Sq Mean Sq F value   Pr(>F)
## group_id      2 0.1245 0.06224   7.794 0.000511 ***
## Residuals   271 2.1639 0.00798
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

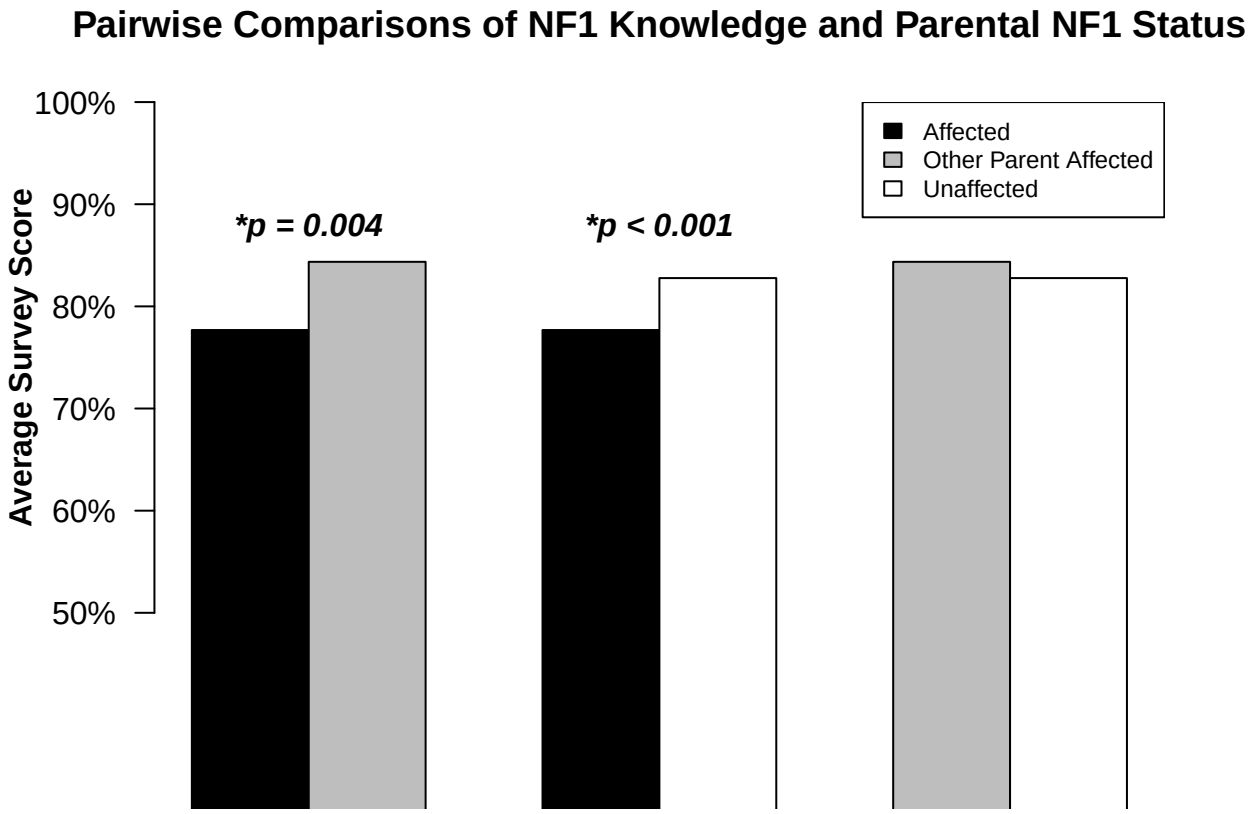
The one way ANOVA, $F(2, 271) = 7.794$, $p = 0.000511$, shows that there is ample evidence for the alternate hypothesis, which is accepted and followed up with the pairwise comparisons.

Tukey's HSD

Following the ANOVA result with Tukey's Honestly Significant Difference (HSD) test, it is shown that the "Affected" group has a significantly lower NF1 knowledge score, as measure by the knowledge questionaire, than the other two groups. A significant difference between the "Unaffected" and "Other Parent Affected" groups was not found.

Table 3: Tukey HSD Results

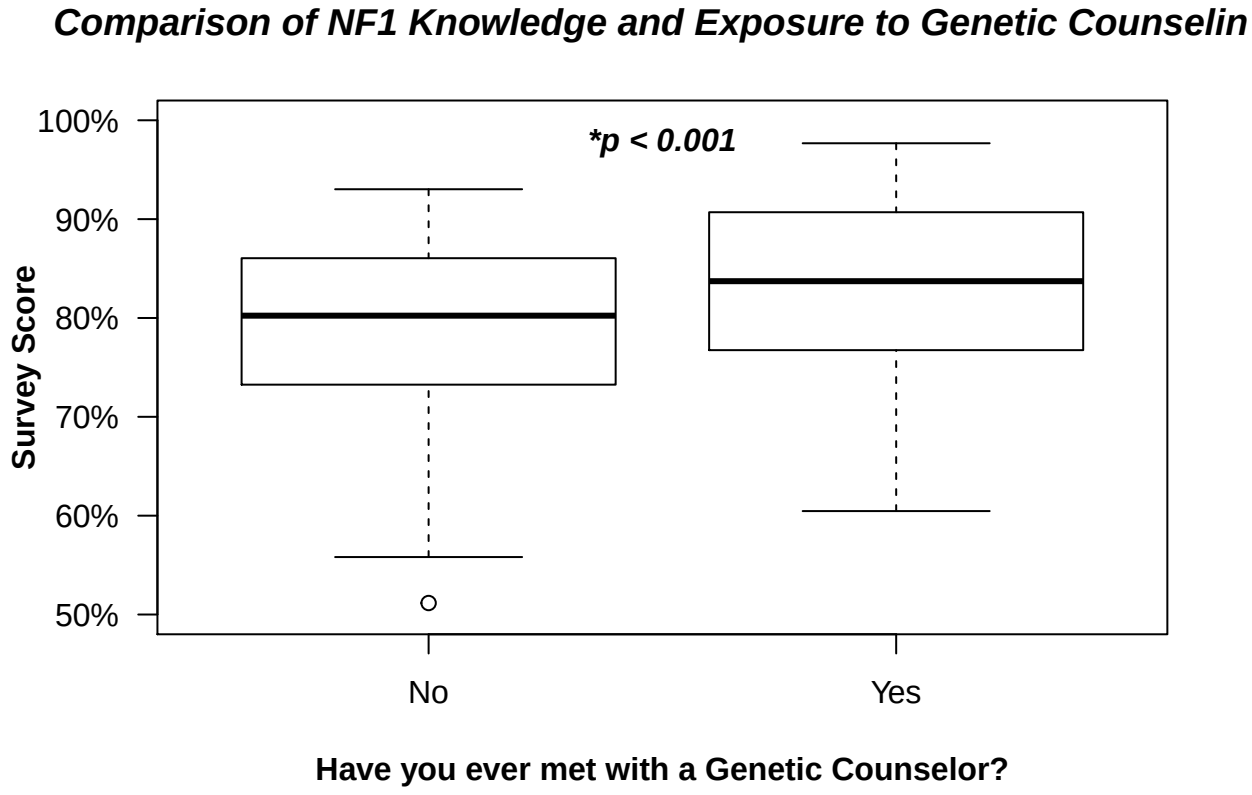
	(Adj.) P value
Other Parent Affected-Affected	0.0040266
Unaffected-Affected	0.0009406
Unaffected-Other Parent Affected	0.6436802



Study Aim 2 - Parental knowledge of NF1 can be increased by exposure to genetic counseling.

The testing methodology for the second study aim remained the same for both approaches, however the plot was changed to display the findings in a more traditional manner (hypotheses reproduced below)

- H_0 : The "Seen GC" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is less than or equal to that of the "Not Seen GC" group.
- H_a : The "Seen GC" group has a mean NF1 knowledge score, as measured by the knowledge questionaire, that is greater than that of the "Not Seen GC" group.



Cronbach's Alpha

Cronbach's alpha is a statistic used to measure the reliability of a set of test items, or more specifically, how well a set of test items (questions) measures the underlying concept which the test is attempting to assess. In the context of this thesis, Cronbach's Alpha can be interpreted as a numeric summary of how well the knowledge based questions assess a respondents true knowledge level of NF1.

Using 43 quiz questions returned a Cronbach's Alpha value of 0.7010484.

Statistical Theory: Approach 1 vs. Approach 2

As with any statistical test, there are limitations imposed by the methods I have chosen to use. In contrast to Student's t-test, Welch's t-test does not assume that the variances of the two samples are equal. However, the assumption that the data is Normally distributed is maintained.

The Shapiro-Wilk test is a statistical test used to determine whether that particular sample came from a population that is normally distributed. The null hypothesis of this test is that the sample came from a Normally distributed population, the alternative therefore being that the sample did not originate from a Normally distributed population. As shown in the table below, all samples have a "low" p value from the Shapiro-Wilk test, groups B and C being the only ones that are above the Bonferroni adjusted significance threshold.

Table 4: Shapiro-Wilk Test P-Values

	PValue
Group A	0.0000616
Group B	0.0623626
Group C	0.0390724
Seen Genetic Counselor	0.0000620
Not Seen Genetic Counselor	0.0034579

This, however, leads to another important topic, the problem of multiple comparisons. When one decides to correct for multiple comparisons, as I have done here using the Bonferroni Correction, the goal is to reduce the probability of a false positive. This practice *necessarily* increases the probability of a false negative. I adjusted the original significance level of 0.05 by accounting for the 4 statistical tests that addressed the study aims of this thesis. However, had I included the above 5 statistical tests that might fall under the heading of *Assumption Testing*, the adjusted significance level would fall to 0.0055556. Clearly, deciding where the bounds of a "family" of statistical tests begin and end has a large impact on the whether a particular test is deemed "significant," and, "there is no firm rule on this."[1]

The problem of multiple comparisons is an area of active research and, seeing as, "There is no universally accepted approach for dealing with the problem of multiple comparisons,"[1] deciding on the Bonferroni Correction was one of many possible solutions to the problem.

A total of 5 statistical tests were performed, broken into two "families" of tests, one family for the first study aim and one family for the second study aim. The first family of tests consisted of a one way ANOVA test followed by 3 post hoc pairwise comparisons. The second "family" consisted of one test - Welch's one-sided t-test. An important note is that the number of tests in a family can increase the Family-Wise Type I error rate. Tukey's HSD, the method used in this study to control the Family-Wise Type I error rate, is one of many possible solutions to the problem of multiple comparisons and, "there is no universally accepted approach for dealing with the problem of multiple comparisons" (McDonald, 2014)

Data Visualizations

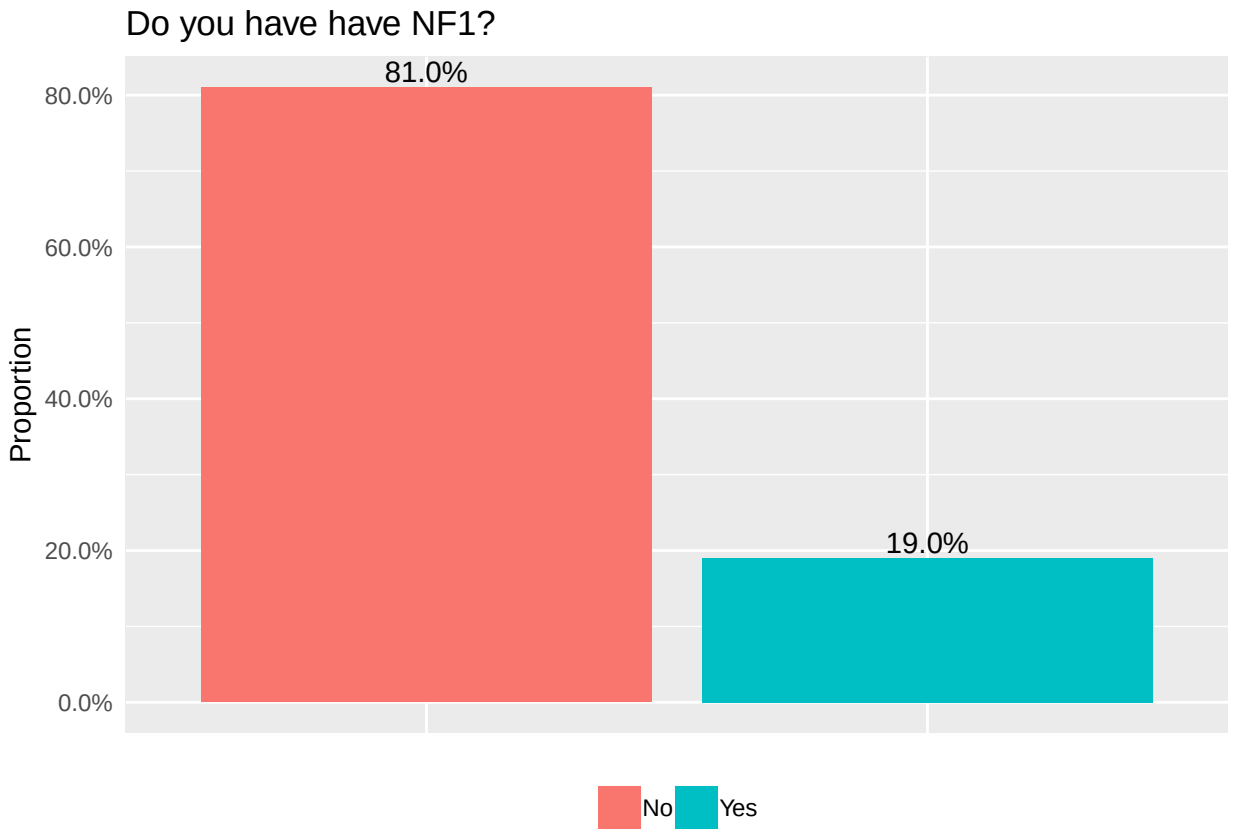


Table 5: Do you have NF1?

Response	Count
No	222
Yes	52

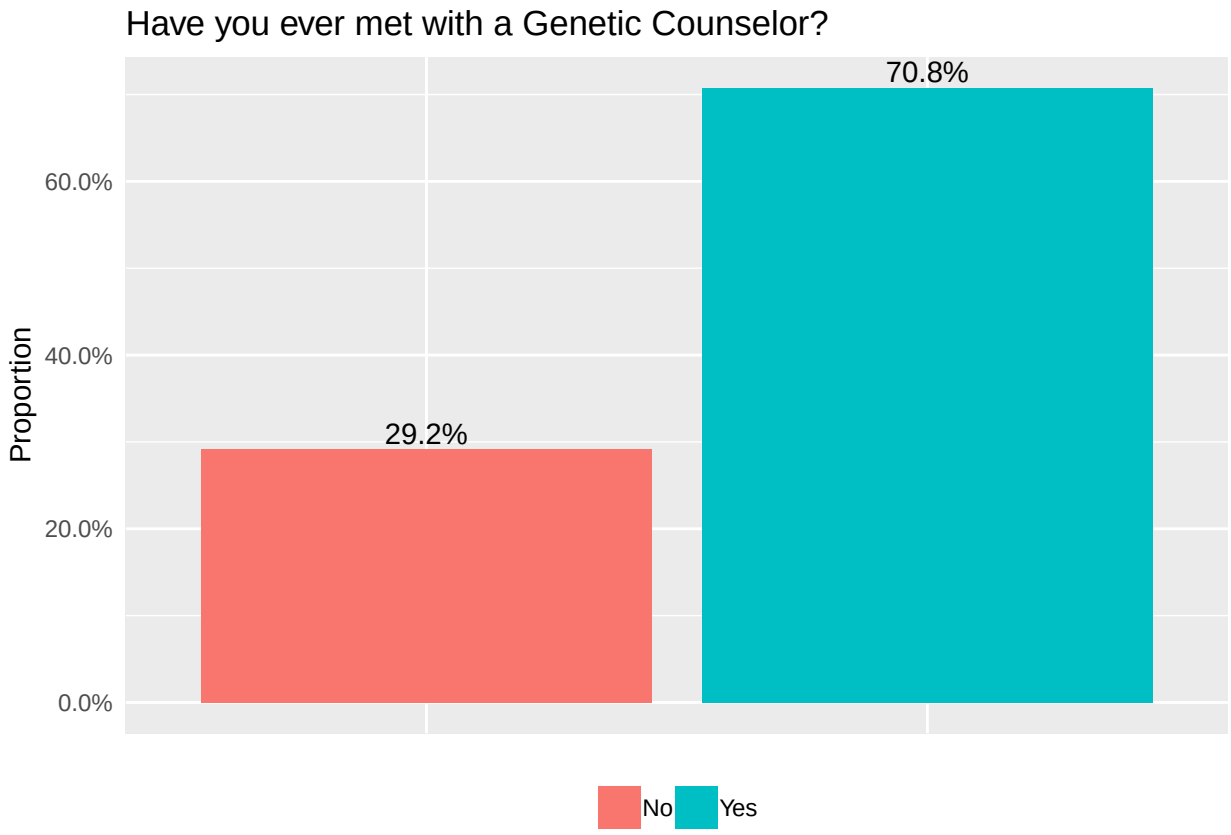


Table 6: Have you ever met with a Genetic Counselor?

Response	Count
No	80
Yes	194

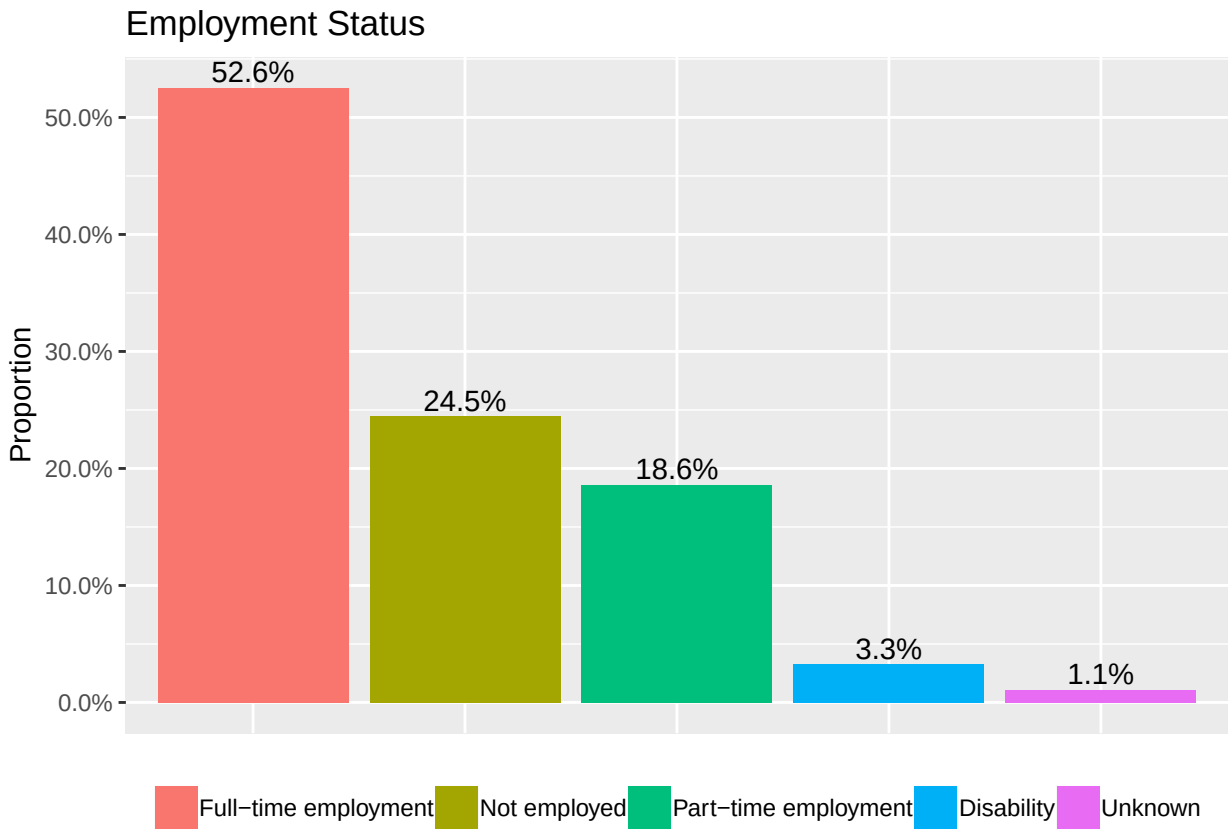


Table 7: What is your employment status?

Response	Count
Disability	9
Full-time employment	144
Not employed	67
Part-time employment	51
Unknown	3

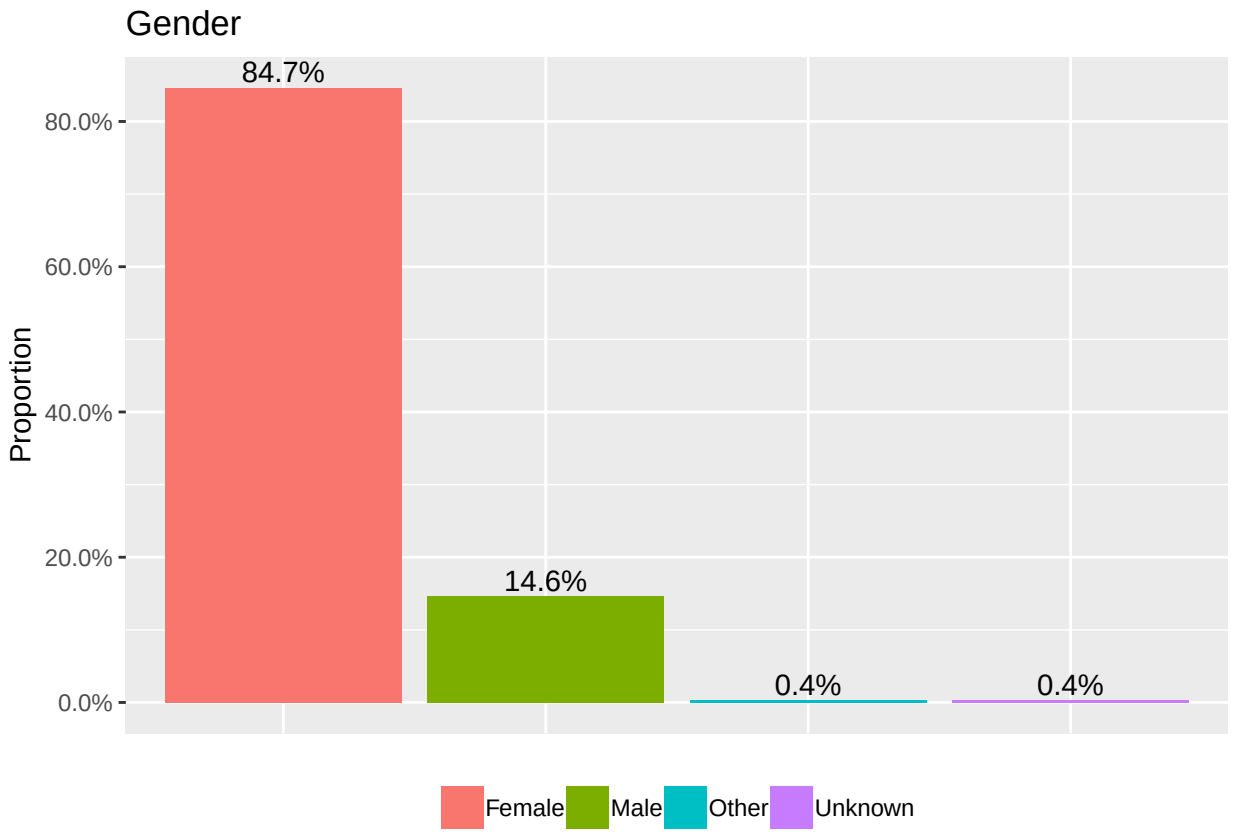


Table 8: Gender

Response	Count
Female	232
Male	40
Other	1
Unknown	1

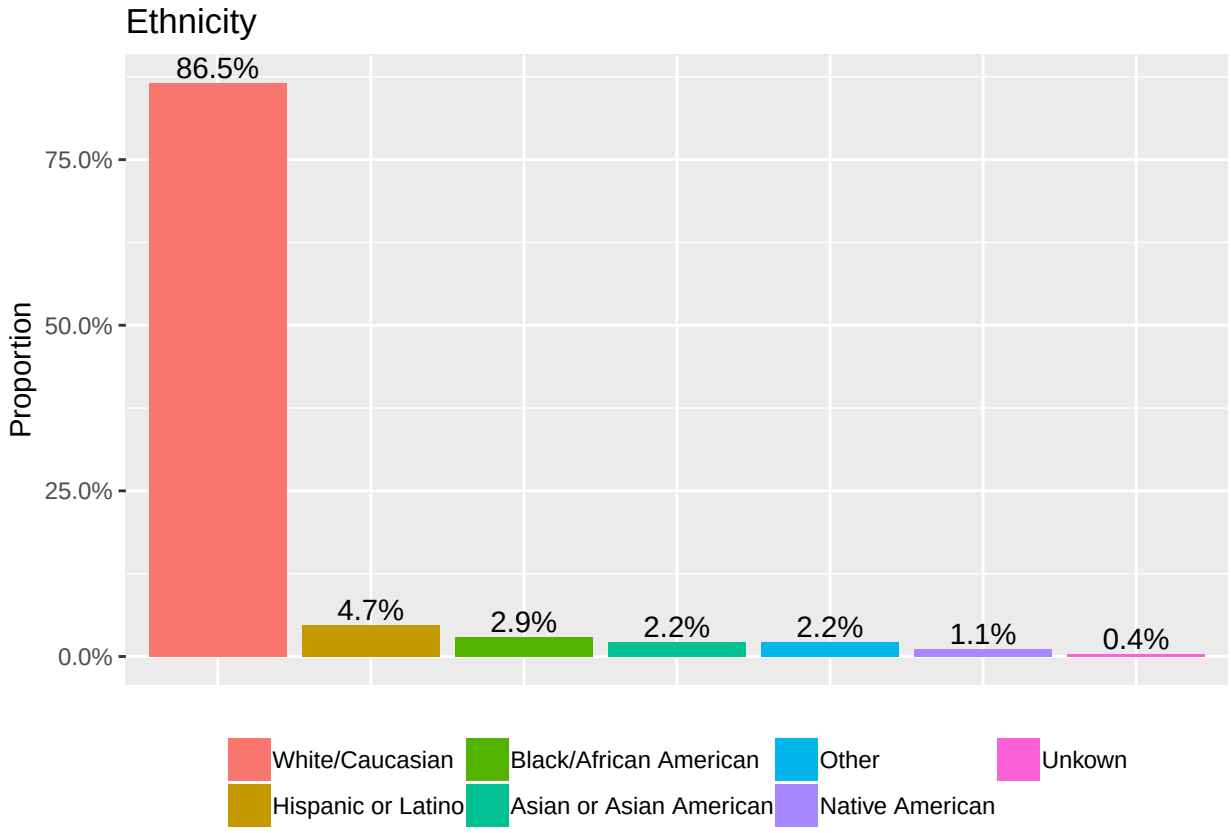


Table 9: Ethnicity

Response	Count
Asian or Asian American	6
Black/African American	8
Hispanic or Latino	13
Native American	3
Other	6
Unkown	1
White/Caucasian	237

One person did not answer the question regarding their educational background, and therefore was excluded from the below plot.

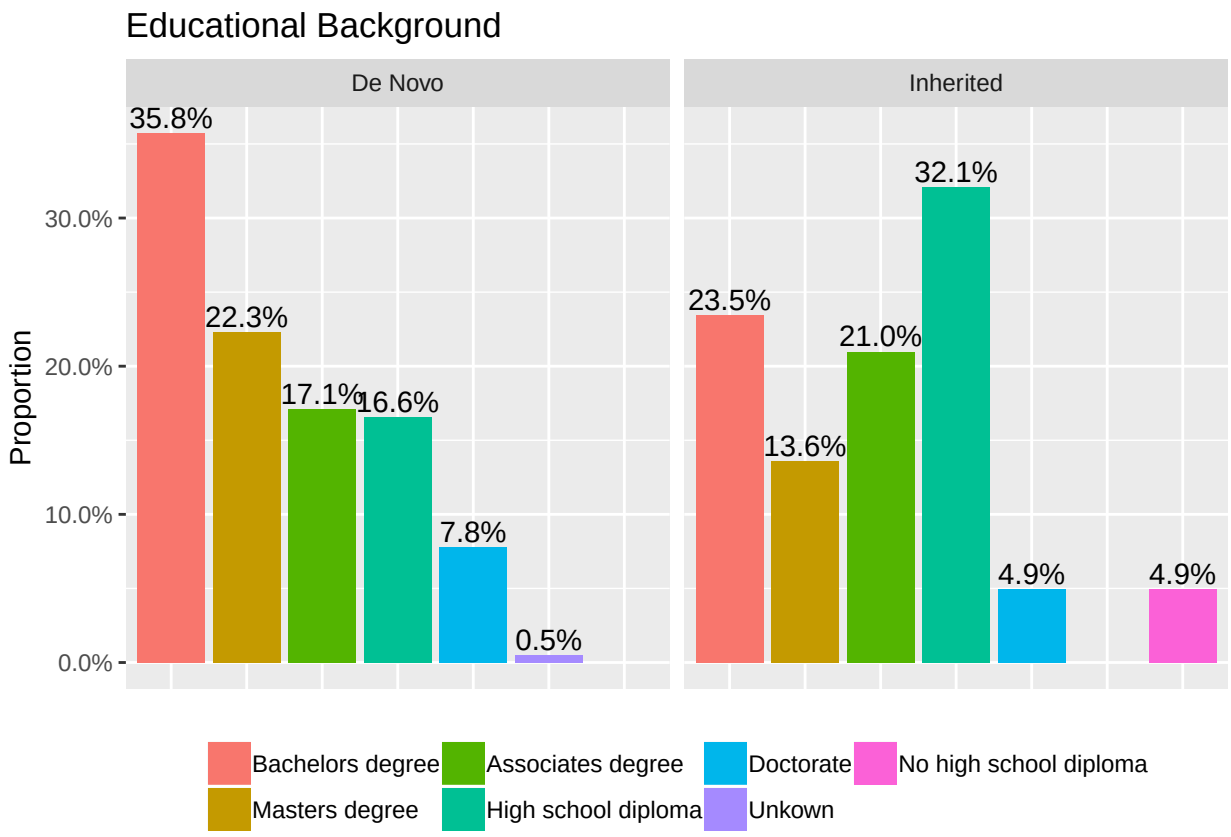
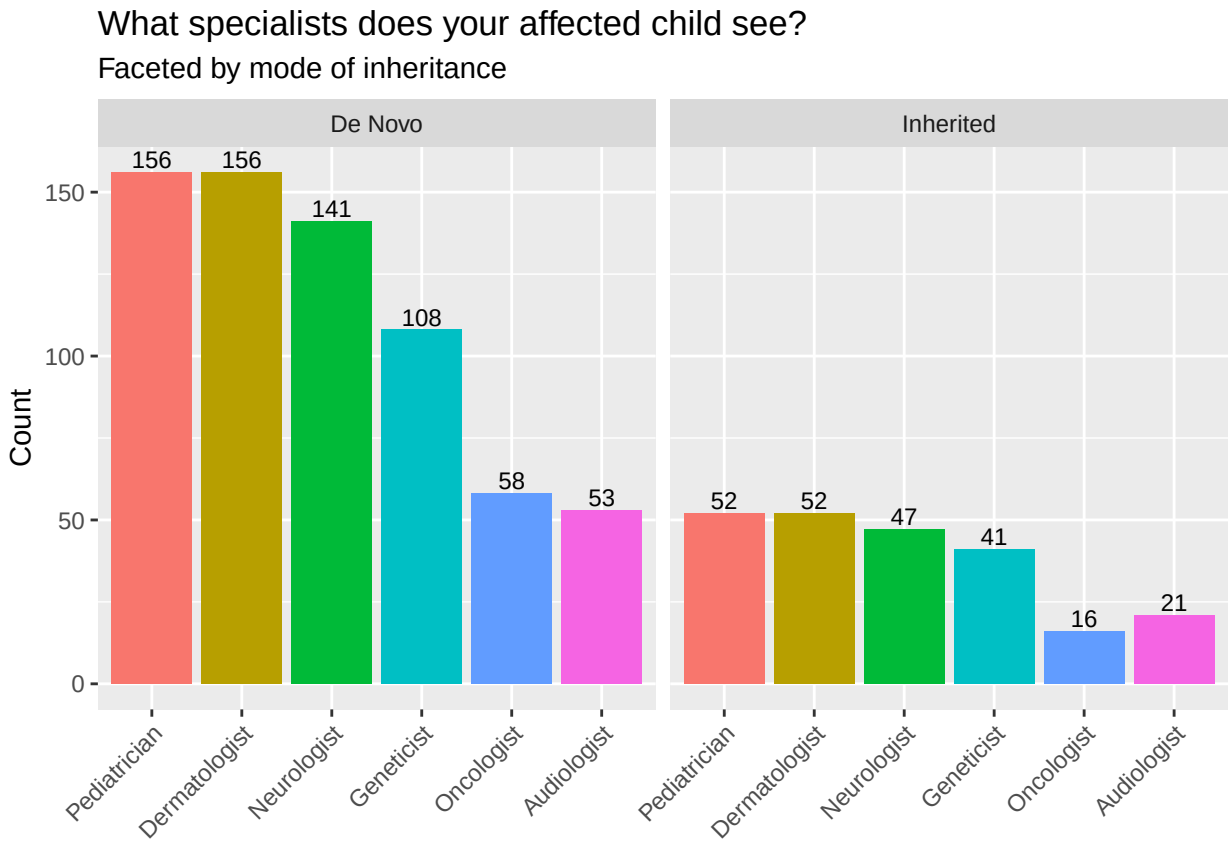


Table 10: Educational Background

Response	Count
Associates degree	50
Bachelors degree	88
Doctorate	19
High school diploma	58
Masters degree	54
No high school diploma	4

It is important to note that the answers to the below question, "What specialists does your affected child see?" are *not* mutually exclusive - children who see a Neurologist may have seen an Oncologist and a Geneticist as well.



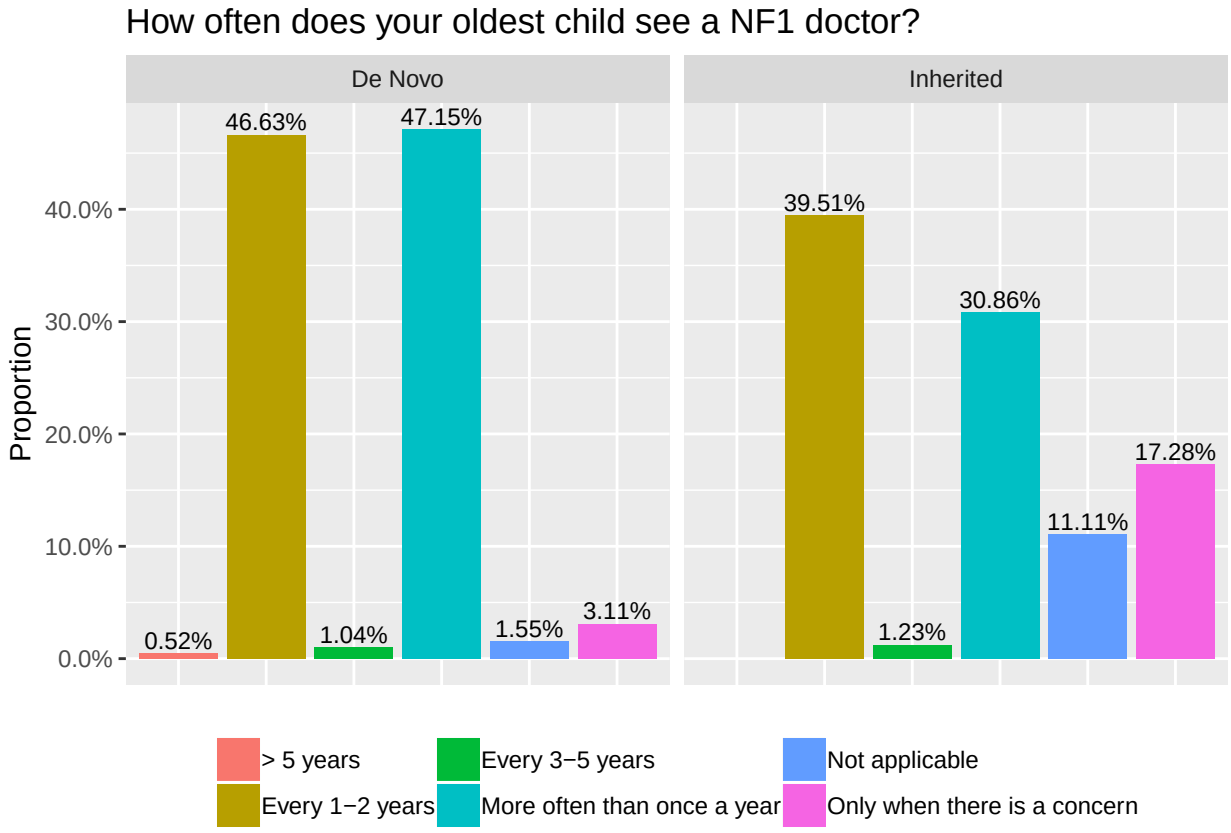
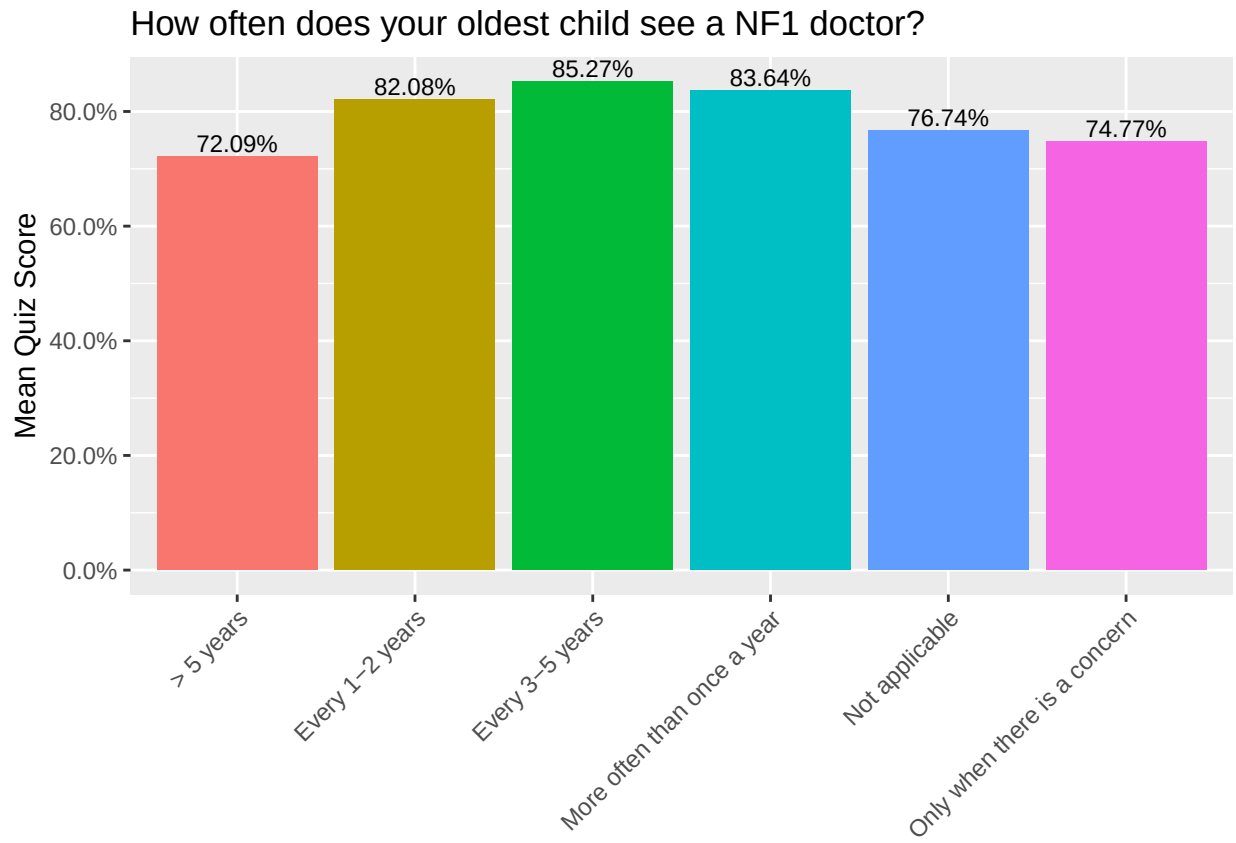
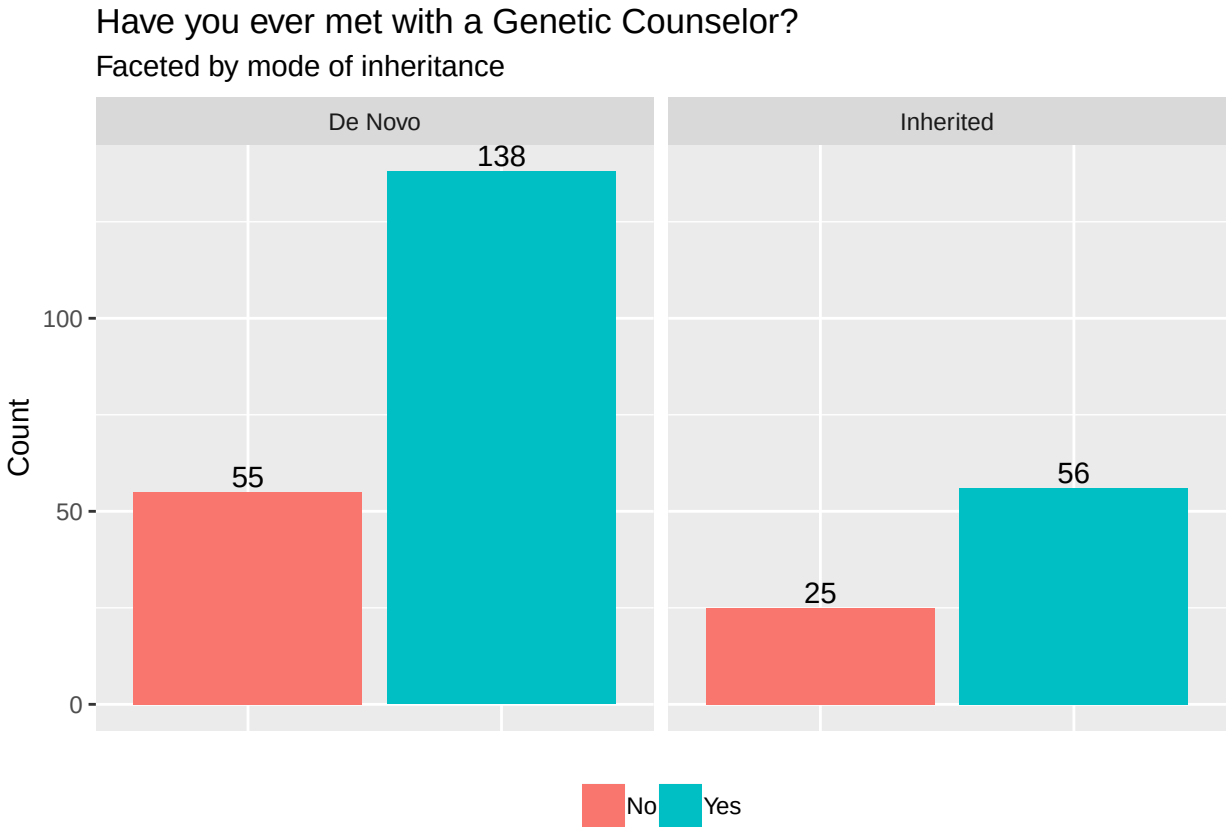


Table 11: How often does your oldest child see a NF1 doctor?

	De Novo	Inherited
> 5 years	1	0
Every 1-2 years	90	32
Every 3-5 years	2	1
More often than once a year	91	25
Not applicable	3	9
Only when there is a concern	6	14


How often does your oldest child see a NF1 doctor?
Mean Quiz Score
80.0%
60.0%
40.0%
20.0%
0.0%
72.09%
82.08%
85.27%
83.64%
76.74%
74.77%
> 5 years
Every 1–2 years
Every 3–5 years
More often than once a year
Not applicable
Only when there is a concern


Have you ever met with a Genetic Counselor?
Faceted by mode of inheritance
De Novo
Inherited
Count
100
50
0
55
138
25
56
No
Yes

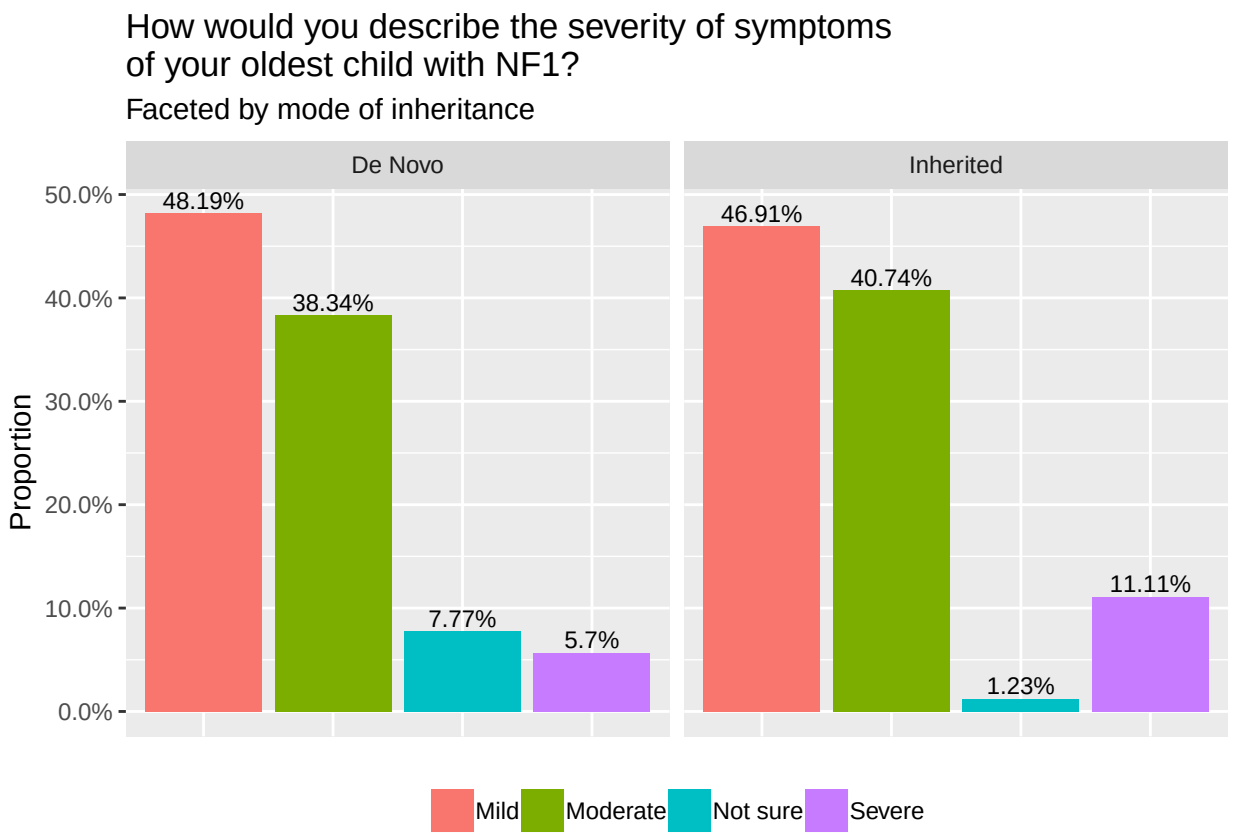
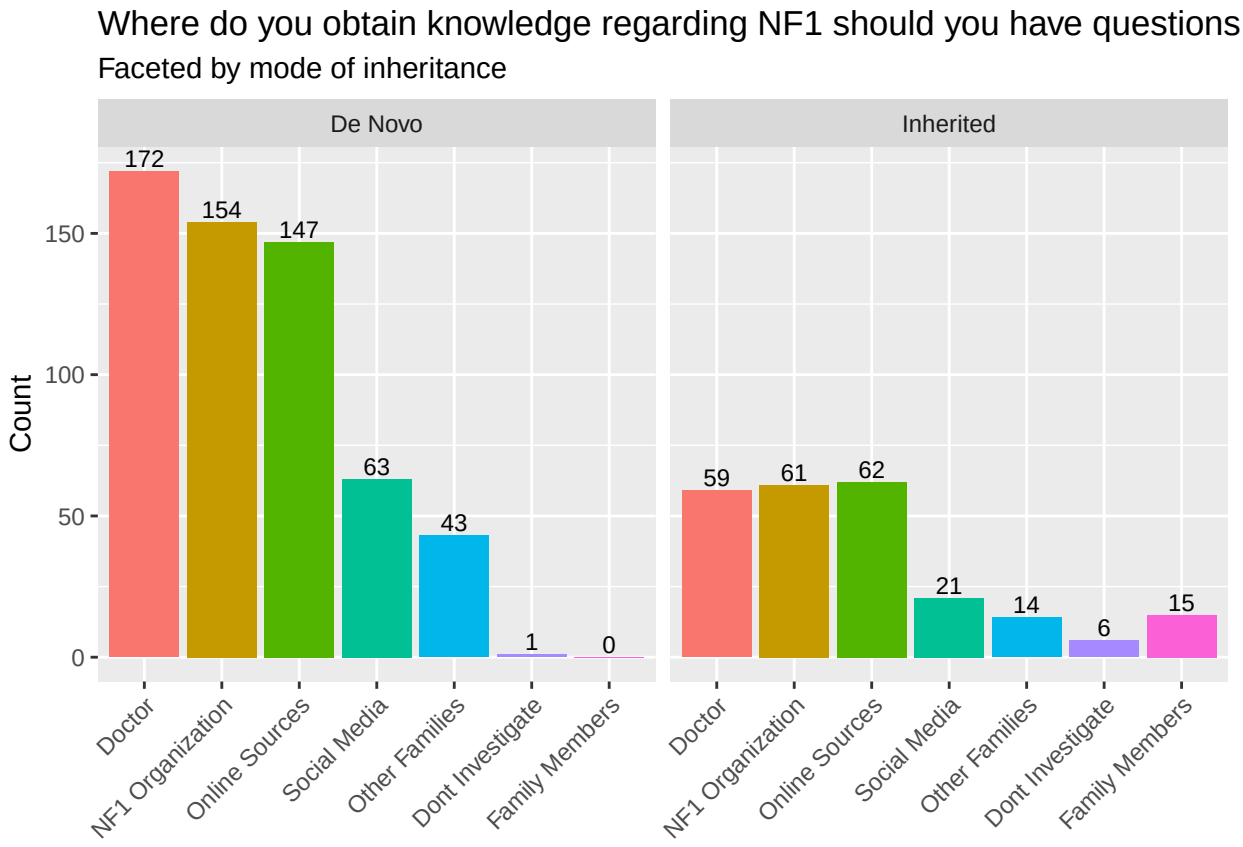
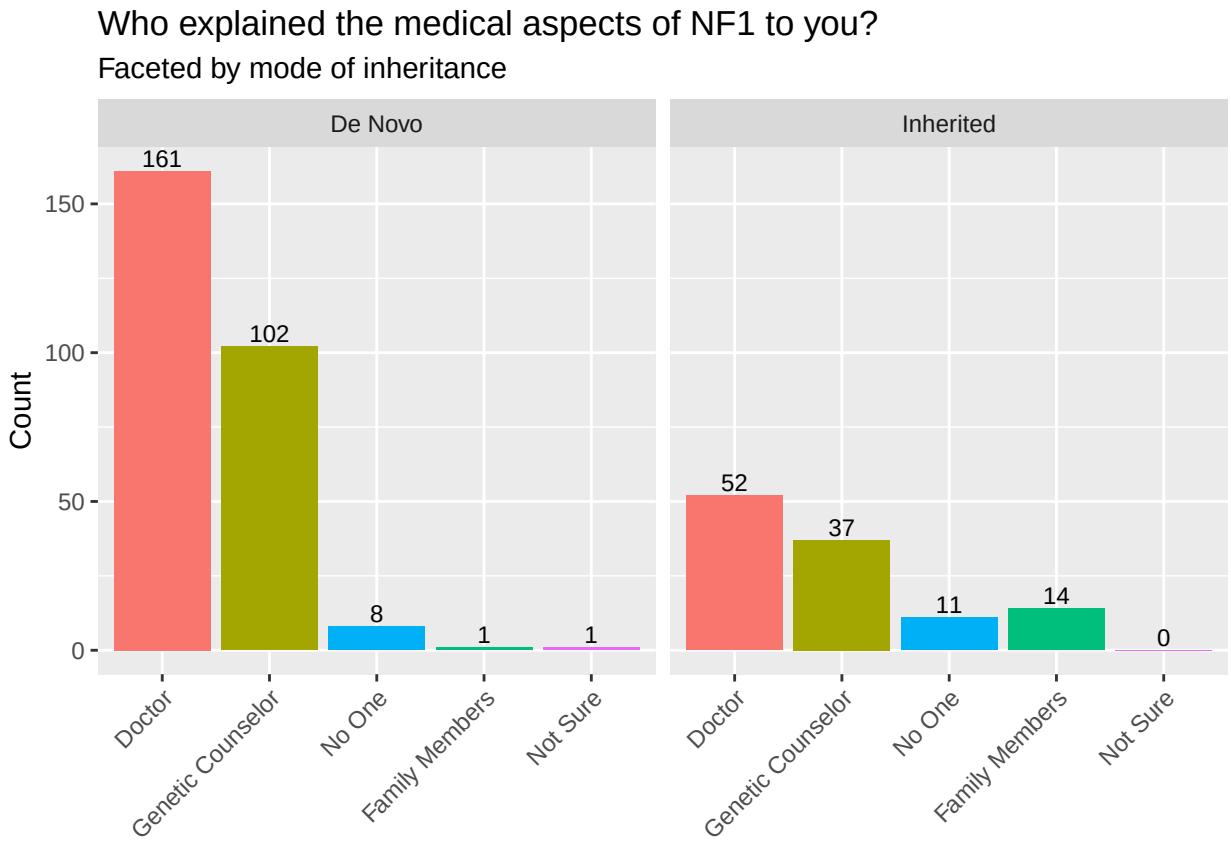


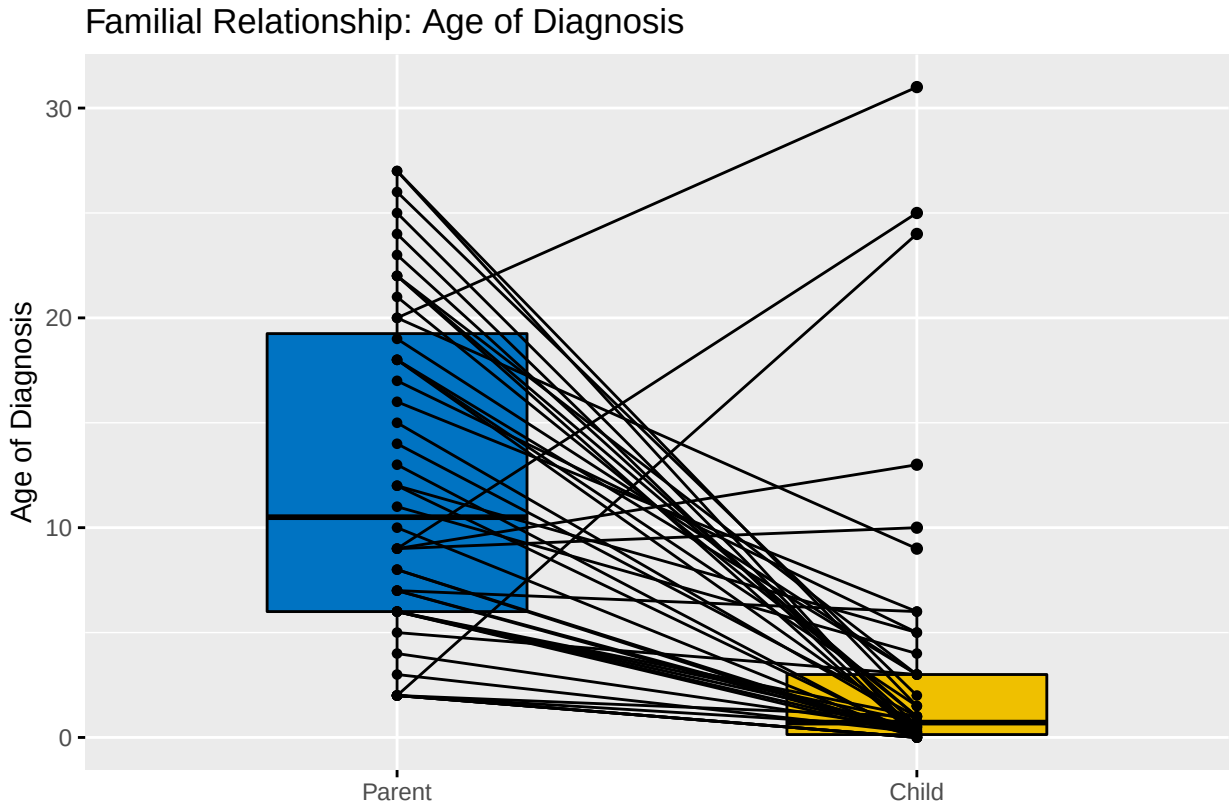
Table 12: How would you describe the severity of symptoms of your oldest child with NF1?

	De Novo	Inherited
Mild	93	38
Moderate	74	33
Not sure	15	1
Severe	11	9

Similar to the "What specialists does your affected child see?" plot, it is important to note that the answers to the below question, "Where do you obtain knowledge regarding NF1 should you have questions?" are not mutually exclusive - parents who ask their doctor might also reference online sources.




Who explained the medical aspects of NF1 to you?
Faceted by mode of inheritance
De Novo
Inherited
Count
150
100
50
0
161
102
8
1
1
52
37
11
14
0
Doctor
Genetic Counselor
No One
Family Members
Not Sure


Familial Relationship: Age of Diagnosis
Age of Diagnosis
30
20
10
0
Parent
Child

References

[1] McDonald, J.H. 2014. Handbook of Biological Statistics (3rd ed.). Sparky House Publishing, Baltimore, Maryland.