2016 Data Analysis First Year Exam

Statistical Analysis of the Effects of Ornithine Decarboxylase Inhibitors for Chemoprevention of Colorectal Cancer

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Abstract

Colorectal cancer is one of the major cause of deaths due to cancer in the United States. Due to the impracticality of regular screening, supplementary practices are in need. Previous studies have pointed to inhibition of ornithine decarboxylase inhibitors (ODC) as a potentially effective preventative treatment. To assess the effectiveness of D-CARB, an ODC inhibitor, as a chemoprevention agent for colorectal cancer, 364 adult patients from across the United States were recruited, stratified by daily aspirin usage, and randomized 1:1 to D-CARB treatment (600 mg/d) or matched placebo and had colorectal adenoma development tracked for three years. D-CARB treatment produced a significant reduction in the 3-year rate of adenoma development adjusting for gender and age (adjusted incidence rate ratio (aIRR) 0.0848, $P < 0.0001$). The rate of adenoma development for patients using D-CARB with a daily aspirin regimen was not found to differ significantly from D-CARB patients with no aspirin use (aIRR 0.199, $P = 0.283$). To determine the effect of D-CARB treatment on hearing, a subset of 184 patients were given three hearing tests over the course of the study. D-CARB treatment was not found to have a significant impact on hearing degradation for either ear at any tested frequency.

1 Introduction

The National Cancer Institute estimates 134,000 new cases and 49,000 deaths related to colorectal cancer in 2016, making it the second most common cause of cancer deaths in the United States [4]. Many important risk factors have been identified, including diet, race, sex, inflammatory intestinal conditions, and age [2], but screening of high-risk individuals remains inconsistent, mainly due to expense. To supplement screening practices, previous research has considered the use of dietary supplements and chemoprevention agents to prevent the development of adenomas (benign precursor tumors in the colon).

A potential target for preventative therapies identified by animal models is the inhibition of the enzyme ornithine decarboxylase (ODC), which plays an important role in the synthesis of polyamines needed for cell growth. ODC has been shown to be significantly related to a variety of cancers. Deng et al. [3] found that ODC was overexpressed in breast cancer tissues relative to normal tissue, with the level of ODC expression positively correlated with tumor stage.

While ODC does appear to be a viable target for cancer treatment, some studies have raised concerns regarding potential side effects and toxicity risks of long-term and high-dose application of ODC inhibitors. A 1999 review by Meyskens and Gerner [6] on the use of the ODC inhibitor DFMO concluded that significant side effects, most notably hearing loss, occur when using DFMO in high-dose therapeutic trials, but that the severity of these side effects may be substantially lower for the doses used for chemoprevention. Meyskens and Gerner also recommend studying the efficacy of combining an ODC inhibitor with a non-steroidal anti-inflammatory drug (NSAID) in the suppression of tumorigenesis. A study by Lao et. al [5] on the use of DFMO for esophagus chemoprevention found significant hearing loss (15-dB decreasing in hearing in the right ear at frequencies 250, 2000, and 3000 Hz, and a ≥ 20 decrease in the left ear for frequencies 4000 to 6000 Hz) after taking 0.5 $g/m^2/d$ of DFMO for 13 weeks (4).

The present study considers the use of a particular ODC inhibitor, referred to as D-CARB. Interest is on the effect of D-CARB on the incidence rate of adenomas over a 3-year period, identifying any differential effects when using D-CARB in combination with aspirin, and determining if D-CARB chemoprevention therapy results in significant hearing loss over a 3-year period.

2 Materials and Methods

Patients

A total of $N = 364$ high-risk patients were recruited from 9 academic sites from across the United States and consented to participation in the study. High-risk patients were defined as those with $\geq 3mm$ resected adenomas; no other eligibility or exclusion criteria were explicitly specified. Patients were randomized in a 1:1 fashion to receive a daily 600 mg oral dose of D-CARB or a matched placebo for 36 months. Randomization occurred after stratifying patients by daily aspirin use, so that an equal number of aspiring users were assigned to each treatment group. Adenoma development was measured by annual sigmoidoscopies. Additional patient characteristics recorded include study site where patient was recruited, aspirin usage, age, sex, and self-identified ethnicity. Brief descriptions of the covariates and their codings in the raw data set are given in Table 5.

From these patients, $N = 184$ individuals were randomly selected and asked to take three hearing tests over the course of the study, at baseline (time of randomization), after approximately 18 months, and again after approximately 36 months of treatment. Each hearing test presented subjects with tones at 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz, and 8000 Hz. For each tone the volume was increased until the subject indicated that they could hear the tone. The left and right ears were tested separately for all subjects. To model how hearing changes with D-CARB use, the baseline measurement for each patient, ear, and frequency was subtracted from the subsequent two measures.

Statistical analysis

There were three primary goals of analysis:

1. quantify the effect of D-CARB treatment on the rate of adenoma development;
2. determine if the efficacy of D-CARB is modified by concurrent daily aspirin use;
3. determine if there is significant evidence of hearing loss due to D-CARB treatment, and characterize any significant effects of treatment on hearing.

The effect of D-CARB treatment on colorectal adenoma incidence rates was modelled using Poisson regression, adjusting for age, sex, and aspirin usage. For reference, we compare the marginal effect of D-CARB treatment from an unadjusted model, with the conclusions from the adjusted model. These models are

$$\log(\mu_i) = \beta_0 + \beta_1 tx_i, \tag{1}$$

$$\log(\mu_i) = \beta_0 + \beta_1 tx_i + \beta_2 asa_i + \beta_3 age_i + \beta_4 sex_i, \tag{2}$$

where μ_i is the rate of adeonma development per three years for subject i with corresponding covariates $tx_i, asa_i, age_i, sex_i$. All patients were observed for an equal amount of time, so no offset terms were included in the model. The significance of the marginal effect of D-CARB treatment on the 3-year rate of adenoma development was tested from Model 1 with statistic

$$\frac{\hat{\beta}_1}{\hat{se}(\hat{\beta}_1)} \overset{H_0}{\dot{\sim}} N(0,1).$$

The significance of the effect of D-CARB conditional on the adjustment covariates was tested for using the estimate $\hat{\beta}_1$ from Model 2 and using the same test statistic. A significance level of $\alpha = 0.05$ was used for all tests.

Regarding the inclusion of adjustment variables, because of randomized assignment to treatment group there should be no significant confounding to consider. Age and sex are known to be strongly associated with colorectal cancer incidence rates (3, CDC), and included in the model as precision variables. Aspirin usage is included in the primary model since it was used as a stratification variable for randomization[1]. Ethnicity is also strongly associated with colorectal cancer incidence, but is not included in the model due 82% of the sample subjects identifying as White. There is a strong regional association with colorectal cancer incidence rates as well, with a contiguous block of states in the Midwest and Southeast showing the highest colorectal cancer death rates in the United States. This may be due to similar high-risk diets (high fat, low fiber) across these states. Ideally geographic region (or another proxy for diet) would also be included in the model, but the distribution of patients across the participating sites is imbalanced, making it difficult to incorporate the site information into the model without more precise information on the locations.

For patient i with Y_i observed adenomas, the Poisson regression model assumes $Y_i \sim Pois(\mu_i)$, implying the variance structure $Var(Y_i) = \mu_i$. To test for overdispersion of the data relative to the variance structure imposed by the Poisson model, we fit a negative binomial regression model and applied the likelihood ratio test. To account for overdispersion, inference for the effect of treatment was conducted using standard errors estimated by the Huber-White robust variance estimator, including all reported confidence intervals unless otherwise noted. We compared the results of the simple Poisson model with only treatment as covariate to a fuller model including some adjustment covariates. Overdisperion parameters were estimated for the models using

$$\hat{\phi} = \sum_{i=1}^{n} \frac{e_{i,P}^2}{N-p},$$

[1]Including stratification variables as adjustment variables is in accordance with the guidelines for clinical trial analysis established by the European Medicines Agency [1]. Model 2 without aspirin use included was also fit and yielded equivalent conclusions regarding the effects of D-CARB treatment.

where $e_{i,P}$ is the Pearson residual for subject i, and p is the number of coefficients in the relevant model. Validity of the resulting quasipoisson model with this estimated dispersion parameter was assessed using a goodness of fit test and through examination of diagnostic plots.

To test for a synergistic effect of aspirin use concurrent with D-CARB treatment, Models 1 & 2 were expanded to include an interaction term between aspirin use and treatment group. We again compared the results of the unadjusted model with the full model:

$$\log(\mu_i) = \beta_0 + \beta_1 tx_i + \beta_2 asa_i + \beta_3 tx_i \times asa_i, \tag{3}$$

$$\log(\mu_i) = \beta_0 + \beta_1 tx_i + \beta_2 asa_i + \beta_3 age_i + \beta_4 sex_i + \beta_5 tx_i \times asa_i. \tag{4}$$

From Model 4, the significance of the interaction of aspirin and D-CARB treatment was tested for by comparing the rates of adenoma development between aspirin users and non-aspirin users in the treatment group. The formal null hypothesis for this contrast is $H_0 : \beta_2 + \beta_5 = 0$, with test statistic

$$\frac{\hat{\beta}_2 + \hat{\beta}_5}{\hat{se}(\hat{\beta}_2 + \hat{\beta}_5)} \overset{H_0}{\dot{\sim}} N(0,1),$$

where $\hat{\beta}_j$ are the estimated regression coefficients from Model 4. As with Model 2, Model 4 was tested for significance of overdispersion with respect to a Poisson model was tested via a likelihood ratio test comparison with a negative binomial fits. All inference procedures and reported confidence intervals use standard errors from the Huber-White robust variance estimator.

To determine the effects of D-CARB on hearing loss, we modelled the change in hearing with respect to baseline over time for were modelled using the following linear mixed effects model:

$$v_i^{f,k}(t) = (\beta_1 + b_{1,i})t + \beta_2 t \times tx_i + \varepsilon_i(t), \tag{5}$$

where $v_i^{f,k}(t)$ is the hearing change in dB with respect to baseline for subject i, frequency f, ear k, at time t. The subject-specific random effects b_i are assumed to be distributed $b_i \overset{iid}{\sim} N(0, \sigma_b^2)$, and the error terms are assumed to be independent across subjects and distributed as $\varepsilon_i \sim N(0, \Sigma_{\varepsilon_i})$. Note that the intercept and treatment main effect terms are excluded since the response is measured with respect to the baseline test, necessitating $v_i^{f,k}(0) = 0$. The random slope terms b_i are included here to account for within-subject correlation. In this model β_2 is the difference in rate of change in hearing between the treatment group and placebo group. Noting that larger slopes with respect to time indicate worse degradation of hearing, we test for a significant effect of D-CARB treatment on hearing by testing $H_0 : \beta_2 = 0$ against $H_1 : \beta_2 > 0$ with the Wald test

$$\frac{\hat{\beta}_2}{\hat{se}(\hat{\beta}_2)} \overset{H_0}{\dot{\sim}} N(0,1).$$

Since our primary interest is the long-term effect of D-CARB therapy on hearing, we also compare the results from fitting a simpler model on only the change in hearing with respect to baseline from the last hearing test taken by each patient. Although it ignores the data from the second hearing test, this model is fit on uncorrelated data, allowing us to avoid the problem of estimating within-subject correlation from very short time series (only 2 time points per subject). The model can be written

$$v_i^{f,k}(t) = \beta_1 t + \beta_2 t \times tx_i + \varepsilon_i(t). \tag{6}$$

3 Results

3.1 Descriptive Statistics

A summary of recorded patient characteristics by treatment group and overall is given in Table 1. Patients were stratified by aspirin use (daily use or no use), and then randomly assigned a treatment group (D-CARB or placebo). We observe for each level of each covariate patients are approximately equally distributed between treatment groups, as expected from random assignment.

The mean 3-year rate of adenoma development in the D-CARB treatment group was 0.055 with sample variance 0.130; the mean 3-year rate of adenoma development in the placebo group was 0.681 with sample variance 1.467. Figure 1 illustrates the empirical distribution of adenoma counts overall in the sample and by treatment group. These coarse measures certainly suggest that D-CARB may be effective in reducing the rate of adenoma development. It also appears that the data are likely overdispersed relative to a Poisson distribution, with variances roughly double the mean rates.

Patient ages in the sample are approximately normally distributed (Figure 2), with a mean of 61 years and standard deviation of 8.4 years.

Patients were recruited from 9 academic sites located across the United States, with as many as 67 patients and as few as 4 coming from each site. Within each site, aspirin users and treatment groups were approximately evenly distributed. Although geographic region is an important risk factor for colorectal cancer, this covariate was not included in the present analysis due to imprecise information and imbalance across sites.

There is also imbalance in the racial distribution of the sample, possibly related to the imbalance across recruitment sites, with Whites making up 82%, and hispanics the next largest group at 8% of the sample. Ethnicity is known to be an important risk factor for colorectal cancer, with African-Americans exhibiting the highest incidence and death rates, and Asians exhibiting the lowest incidence and death rates [4]. Due to the sparsity of non-white ethnicities in the sample and the widely differing incidence rates across the other ethnicities, ethnicity was excluded from the analysis.

There are a total of 12 patients (3.3% of the sample) with missing ethnicity values, 8 (2.2%) of whom are also missing gender and age values (Table 6). Those patients with missing gender and age were excluded from modelling. Ethnicity was not used in the analysis, and so those patients with only missing ethnicity were included. The hierarchy of missing values is illustrated in Figure 3.

Because of the imbalance across the demographic covariates, care should be taken when generalizing the results of the following analysis to the broader population.

	D-CARB (N = 182)	**Placebo** (N = 182)	**Total** (N=364)
Covariate	N (%) or Mean (SD)	N (%) or Mean (SD)	N (%) or Mean (SD)
Adenomas	0.055 (0.360)	0.681 (1.211)	0.368 (0.946)
Age Group†			
40-49	11 (0.060)	21 (0.115)	32 (0.0879)
50-59	66 (0.363)	62 (0.341)	128 (0.352)
60-69	71 (0.390)	69 (0.379)	140 (0.385)
> 70	30 (0.165)	26 (0.143)	56 (0.154)
NA	4 (0.022)	4 (0.022)	8 (0.022)
Site			
1	33 (0.181)	34 (0.187)	67 (0.184)
2	20 (0.110)	19 (0.104)	39 (0.107)
3	28 (0.154)	29 (0.159)	57 (0.157)
4	13 (0.071)	16 (0.088)	29 (0.080)
5	24 (0.132)	24 (0.132)	48 (0.132)
6	37 (0.203)	35 (0.192)	72 (0.198)
8	4 (0.022)	5 (0.027)	9 (0.025)
9	2 (0.011)	2 (0.011)	4 (0.011)
11	17 (0.093)	14 (0.077)	31 (0.085)
Sex			
Male	133 (0.731)	135 (0.742)	268 (0.736)
Female	45 (0.247)	43 (0.236)	88 (0.242)
NA	4 (0.022)	4 (0.022)	8 (0.022)
Ethnic			
AI/AN	3 (0.016)	2 (0.011)	5 (0.014)
A/PI	7 (0.038)	10 (0.055)	17 (0.047)
Black	8 (0.044)	10 (0.055)	18 (0.049)
GIHS	2 (0.011)	3 (0.016)	5 (0.014)
Hispanic	15 (0.082)	14 (0.077)	29 (0.080)
Other	3 (0.016)	4 (0.22)	7 (0.019)
Spanish	3 (0.016)	2 (0.011)	5 (0.014)
White	151 (0.830)	147 (0.808)	298 (0.819)
NA	6 (0.033)	6 (0.033)	12 (0.033)
Aspirin Use			
Yes	65 (0.379)	69 (0.401)	134 (0.390)
No	113 (0.643)	113 (0.643)	222 (0.632)

† Ages recorded in years in data set, grouped here for summarization.

Table 1: Recorded patient characteristics by treatment group and overall.

3.2 Effect of D-CARB Treatment on Adenoma Development

As an initial point of reference, fitting Model 1 estimates the incidence rate ratio (IRR) due to D-CARB treatment at 0.0833 (95% CI 0.0317, 0.219, $P < 0.001$). That is, the three-year rate of adenoma development for the D-CARB treatment group, marginal across all other covariates, is estimated to be 8.33% the rate of development in the control group.

The estimated adjusted incidence rate ratios (aIRR) and corresponding robust 95% confidence intervals from Model 2 are given in Table 2. The estimated aIRR for D-CARB is 0.0848 (95% CI 0.033, 0.220, $P < 0.0001$). Thus there is strong evidence that D-CARB is very effective in reducing the rate of development of adenomas relative to no treatment even when adjusting for gender, age, and aspirin use regimen. We observe that for the other adjustment variables, including aspirin use, there is not significant evidence of an association with rate of adenoma development.

	aIRR Est.	95% CI Low	95% CI High	P-value
D-CARB	0.0848	0.0327	0.2201	< 0.0001
Daily Aspirin Use	1.4409	0.8300	2.5013	0.1943
Age	0.9860	0.9571	1.0157	0.3520
Sex: Male	1.1504	0.6228	2.1249	0.6545

Table 2: Results from Model 2, adjusted Poisson regression for treatment main effect. Confidence intervals calculated using robust variance estimates.

The overdispersion parameter for the data is estimated to be $\hat{\phi} = 2.364$. To verify the presence of overdispersion, the likelihood ratio test comparing a negative binomial model to a standard Poisson model for the data gives $T_{LR} = 199.107, P < 0.0001$, thus we conclude that there is significant evidence of overdispersion. The plot of the squared Pearson residuals against the fitted values from Model 2 verifies the estimate of the overdispersion parameter (Figure 4). A goodness of fit test on the Model 2, adjusting the likelihood to account for the estimated overdispersion parameter, gives a P-value close to 1, verifying that the quasipoisson adjustment to Model 2 results gives a satisfactory fit of the data.

A plot of Cook's Distance vs Leverage from Model 2 is given in Figure 5, with five potential influential or high-leverage observations identified. The covariates for these subjects are given in Table 9. The subjects with large Cook's distance all have high adenoma counts and are near or below the mean age. The high leverage subject (Study Number 11011) does not appear to have any erroneous measurements, and may have high leverage due to being a female with age near the sample minimum. These observations are likely not problematic for the validity of the model results.

3.3 Modification of D-CARB Treatment Effect by Daily Aspirin Use

Our main interest is in comparing the effect of D-CARB between aspirin users to non-users. The fit from Model 4 gives an estimated aIRR of 0.199 (95% CI 0.01, 3.798, $P = 0.283$), thus there is insufficient evidence to conclude that concurrent use of aspirin with D-CARB treatment produces a statistically significant change in the rate of adenoma development, adjusting for age and sex. Table 3 provides aIRR estimates from Model 4 for the four subpopulations of treatment group by aspirin use, taking the placebo/no aspirin group as referent. We see that the D-CARB treatment group

shows significantly reduced rates of adenoma development, with the D-CARB/daily aspirin group exhibiting the lowest aIRR at 0.029 (95% 0.002, 0.492). The placebo/daily aspirin group has an estimated aIRR of 1.617, indicating an estimated increase in adenoma development in this group, although this effect was not found to be significant. Estimates for unadjusted IRR from Model 3 are similar for the four subpopulations as those given by Model 4 (Table 10). Testing for significance of the interaction term by comparing likelihoods with Model 2 gives $T_{LR} = 2.475, P = 0.1157$, thus there is insufficient evidence to conclude that the D-CARB and daily aspirin use interaction effect is significantly different from 0.

The estimated overdispersion parameter for Model 4 is $\hat{\phi} = 2.031$. Again testing for significance of overdispersion with the likelihood ratio test gives Testing for significance of the interaction term with the likelihood ratio test gives $P < 0.0001$, thus the data exhibits significant overdispersion relative to the Poisson model.

Treatment	Aspirin Use	
	None	Daily
	aIRR Est. (95% CI)	aIRR Est. (95% CI)
Placebo	1.0 (—)	1.617 (0.953, 2.745)
D-CARB	0.147 (0.054, 0.400)	0.029 (0.002, 0.492)

Table 3: Adjusted incidence rate ratio estimates from Model 4, adjusted Poisson regression for treatment and aspirin interaction effect. Confidence intervals calculated using robust variance estimates. Estimates given are with respect to the referent group Placebo/No Aspirin.

3.4 Effect on D-CARB Treatment on Hearing

Table 4 gives the results of fitting Model 5 for all ears and frequency levels, with estimates for rate of change of hearing with respect to time (in years) and for the effect of hearing loss due to D-CARB treatment. From these estimates, D-CARB treatment was not found to significantly effect hearing performance for any ear or frequency level. The effect for the left ear at 3000 Hz was marginally significant with $P = 0.059$, but the effect estimate is -0.775 (95% CI -1.571, 0.022), which indicates less hearing degradation among the treatment group compared to the placebo group. Specifically, the model estimates that for the placebo group, hearing in the left ear at 3000 Hz degrades at a rate of 1.493 dB/y (95% CI 0.628, 2.358, $P = 0.001$), while in treatment group for the same ear and frequency, hearing degradation is estimated to be 0.718 dB/y (95% CI -0.150, 1.587, $P = 0.0525$).

Analogous results from fitting Model 6 are given in Table 11. This model again finds a significant difference in hearing degradation for the left ear at 3000 Hz, with a similar effect size of -0.649 (95% CI -1.12, -0.177), but with greater significance with $P = 0.007$. With this model, D-CARB was also found to have a significant effect on hearing in the the left ear at 250 Hz, again with a beneficial effect estimate of -0.635 (95% CI -1.189, -0.082, $P = 0.025$).

Figure 7 plots the changes in hearing with respect to baseline by treatment group for the left ear, 3000 Hz data. A clear pattern is difficult to discern, but slopes for the placebo group appear to be larger overall compared to the D-CARB group (indicating worse degradation of hearing). The data appears to be somewhat noisy, and a further study with more repeated measures on pa-

	Est. for Time Effect				Est. for Time-DCARB Interaction			
Ear, Freq. (Hz)	Est. Coeff.	95% CI Low	95% CI High	P-value	Est. Coeff.	95% CI Low	95% CI High	P-value
Left 250	1.064	-0.017	2.145	0.055	-0.637	-1.439	0.165	0.121
Left 500	0.969	0.033	1.906	0.044	-0.395	-1.139	0.349	0.299
Left 1000	1.219	0.460	1.978	0.002	-0.370	-1.017	0.277	0.264
Left 2000	1.230	0.372	2.088	0.005	-0.314	-1.118	0.491	0.446
Left 3000	1.493	0.628	2.358	0.001	-0.775	-1.571	0.022	0.059
Left 4000	1.935	0.960	2.910	< 0.001	-0.696	-1.548	0.156	0.111
Left 6000	0.761	-0.313	1.836	0.167	-0.388	-1.300	0.524	0.406
Left 8000	0.329	-0.815	1.474	0.573	-0.687	-1.683	0.308	0.178
Right 250	0.823	-0.101	1.748	0.083	0.021	-0.739	0.780	0.957
Right 500	0.851	0.014	1.689	0.048	-0.112	-0.837	0.613	0.763
Right 1000	1.053	0.315	1.790	0.006	-0.264	-0.903	0.375	0.419
Right 2000	1.775	0.939	2.610	0.001	-0.025	-0.763	0.714	0.948
Right 3000	1.172	0.252	2.093	0.014	0.273	-0.538	1.084	0.510
Right 4000	1.427	0.440	2.414	0.005	-0.223	-1.059	0.613	0.602
Right 6000	1.293	0.248	2.338	0.016	0.574	-0.424	1.572	0.261
Right 8000	1.686	0.404	2.968	0.011	0.507	-0.464	1.478	0.307

Table 4: Estimated coefficients from Model 5 for all ears and frequencies. Effect estimates are with respect to time measured in years.

tients over time may be advised to better understand what effect D-CARB may have on hearing loss.

We note that both models find, unsurprisingly, evidence of significant loss of hearing over time for both ears at many frequencies.

4 Discussion

The preceding analysis largely confirms previous findings regarding the effectiveness of potential side effects of using an ODC inhibitor as a chemoprevention agent for colorectal cancer. At a dosage of 600 mg/d, the rate of adenoma development was significantly reduced with respect to the control group with an estimated aIRR of 0.084. This effect was consistent for the unadjusted model and model adjusting for age, gender, and daily aspirin use. With such a substantial reduction in adenoma development, D-CARB treatment may be a viable method for reducing the risk of colorectal cancer in some patient populations.

The aIRR in patients who used a combination of D-CARB and daily aspirin was estimated at 0.029 (95% CI 0.002, 0.492), compared to an estimated aIRR of 0.147 (95% CI 0.054, 0.4000) for D-CARB patients with no aspirin use, adjusting for age and gender. However, this difference in effect was not found to be significant ($P = 0.283$), and so there is insufficient evidence to recommend a combination treatment of D-CARB with daily aspirin use. Similar results on the interaction of D-CARB and daily aspirin use were obtained from an unadjusted model as well.

A major concern with the use of ODC inhibitors is the possibility of irreversible hearing loss from long-term or high-dose treatments. For the present data, there was no evidence of hearing loss due to D-CARB treatment for either ear at any tested frequency. However, the data considered here consists of only three hearing test results for each patient, and so the strength of this conclusion is limited. There are some potentially conflicting results regarding the effect of another ODC inhibitor, DFMO, on hearing. Work by Meyskens and Gerner [6] concluded that the effect of DFMO on hearing is not significant at doses used for chemoprevention. A previous study by Lao [5] on the toxicity of DFMO found evidence of hearing loss, but the study was conducted with higher doses than what is needed for chemoprevention. Further work is needed to determine how the risk of hearing damage changes with daily and cumulative treatment dose before ODC inhibitors can be broadly recommended as a preventative treatment.

Although D-CARB shows promise as a preventative treatment for colorectal cancer, further studies are needed to determine how effective D-CARB is for different classes of patients. The patients for the present study were primarily white males, so analysis of D-CARB treatment in a more diverse patient population is needed before widespread adoption of D-CARB treatment, especially considering the differences in colorectal incidence rates across ethnicities.

Appendix A: Tables

Variable Name	Description
`study_no`	Patient ID in study.
`site`	Patient recruitment location, factor coded as 1, 2, 3, 4, 5, 6, 8, 9, 11.
`tx`	Treatment group, coded as 1 for D-CARB, 0 for Placebo.
`asa_usage`	Daily aspirin usage, coded as 1 for daily aspirin users, 0 otherwise.
`sex`	Patient gender, 1 for male.
`ethnic`	Patient race/ethnicity, with 8 identified levels. AI/AN: American Indian, Alaskan Native; A/PI: Asian, Pacific Islander; Black; GISH: German, Indian, Hispanic; Other; Spanish; White.
`adenomas`	Number of observed adenomas for time on study.

Table 5: Description of variables in original `demo_outcome_data` data set.

	study_no	site	tx	asa_use	age	sex	ethnic	adenomas
431	11064	5	0	0	NA	NA	NA	4
751	22043	4	1	1	NA	NA	NA	0
1231	33213	2	0	0	NA	NA	NA	0
1551	55027	4	1	1	NA	NA	NA	0
1711	55047	4	0	0	NA	NA	NA	0
2031	66029	1	1	1	NA	NA	NA	0
2191	66049	11	0	0	NA	NA	NA	0
2511	82005	11	1	1	65.44	Male	NA	0
2671	83007	9	0	0	72.44	Male	NA	0
3151	86019	6	1	1	69.28	Male	NA	0
3471	87022	4	0	0	71.75	Female	NA	1
363	88013	11	1	1	NA	NA	NA	0

Table 6: A total of 12 patients have missing demographic covariate values. No patients were missing values for adeonma count or aspirin use.

	IRR Est.	95% CI Low	95% CI High	P-value
D-CARB	0.0833	0.0317	0.2188	< 0.0001

Table 7: Results from Model 1, unadjusted Poisson regression with treatment covariate only. Confidence intervals calculated using robust variance estimates.

	Resid. Df	Resid. Dev	Df	Deviance	pValue
Poisson	351.00	373.30			
Negative Binomial	351.00	174.19	1	199.107	< 0.0001

Table 8: Likelihood ratio test for significance of overdispersion in Model 2. We conclude that there is strong evidence of overdispersion in the data.

Study No.	Site	Trt. Group	Aspirin Use	Age	Sex	Adenomas
11011	1	Placebo	Daily	43.62	Female	0.00
22014	2	D-CARB	None	59.06	Male	4.00
33207	3	Placebo	None	48.38	Female	5.00
33215	3	Placebo	None	47.52	Male	6.00
81022	1	Placebo	None	65.06	Female	5.00

Table 9: Observations with largest leverage and Cook's distance for Model 2.

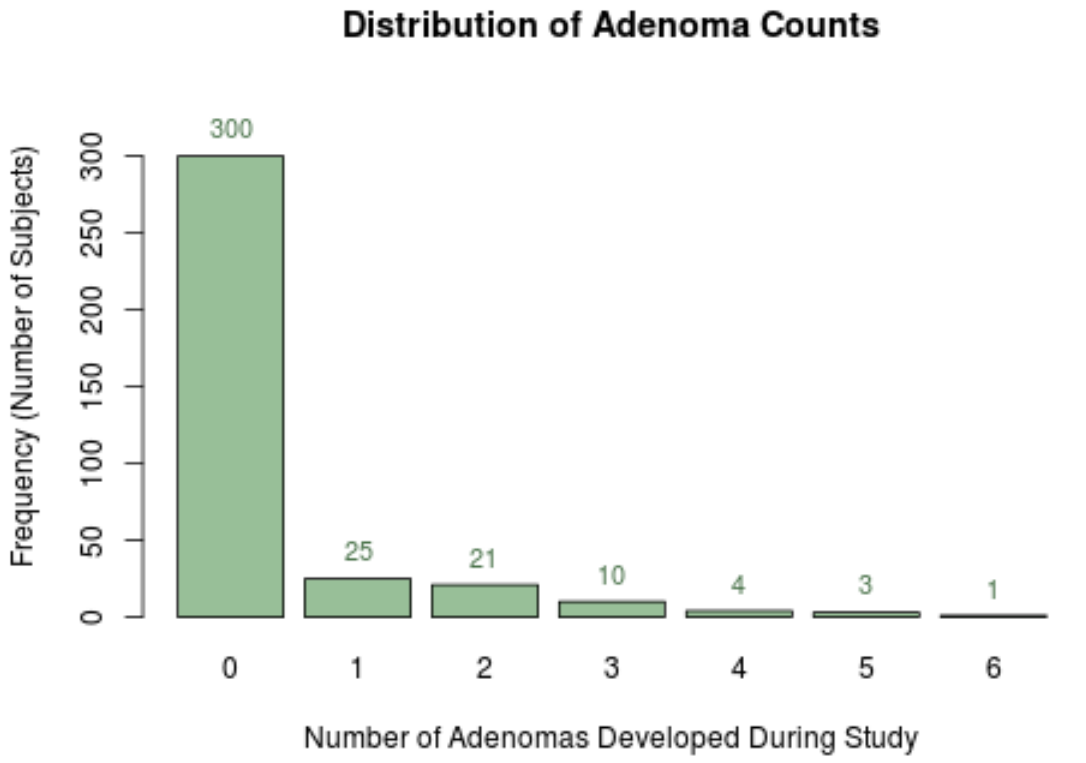
Treatment	Aspirin Use	
	None	Daily
	IRR Est. (95% CI)	IRR Est. (95% CI)
Placebo	1.0 (—)	1.528 (0.914, 2.555)
D-CARB	0.142 (0.047, 0.428)	0.027 (0.002, 0.472)

Table 10: Incidence rate ratio estimates from Model 3, unadjusted Poisson regression for treatment and aspirin interaction effect. Confidence intervals calculated using robust variance estimates. Estimates given are with respect to the referent group Placebo/No Aspirin.

	Est. for Time Effect				Est. for Time-DCARB Interaction			
Ear, Freq. (Hz)	Est. Coeff.	95% CI Low	95% CI High	P-value	Est. Coeff.	95% CI Low	95% CI High	P-value
Left 250	0.624	0.240	1.009	0.002	-0.635	-1.189	-0.082	0.025
Left 500 2	0.824	0.480	1.168	< 0.001	-0.407	-0.902	0.088	0.107
Left 1000	0.773	0.484	1.062	< 0.001	-0.356	-0.774	0.061	0.095
Left 2000	1.509	1.161	1.856	< 0.001	-0.381	-0.880	0.119	0.136
Left 3000	1.813	1.482	2.143	< 0.001	-0.649	-1.120	-0.177	0.007
Left 4000	1.894	1.521	2.268	< 0.001	-0.606	-1.145	-0.067	0.028
Left 6000	1.153	0.746	1.560	< 0.001	-0.388	-0.963	0.186	0.185
Left 8000	1.059	0.617	1.502	< 0.001	-0.568	-1.208	0.072	0.082
Right 250	0.240	-0.107	0.587	0.175	0.106	-0.393	0.605	0.678
Right 500	0.361	0.037	0.685	0.029	-0.031	-0.497	0.435	0.896
Right 1000	0.697	0.413	0.982	< 0.001	-0.155	-0.564	0.255	0.459
Right 2000	1.272	0.949	1.595	< 0.001	0.025	-0.440	0.489	0.917
Right 3000	1.095	0.745	1.445	< 0.001	0.273	-0.225	0.771	0.283
Right 4000	1.060	0.685	1.435	< 0.001	-0.017	-0.556	0.522	0.951
Right 6000	0.921	0.503	1.340	< 0.001	0.448	-0.144	1.040	0.139
Right 8000	0.882	0.425	1.340	< 0.001	0.479	-0.178	1.136	0.154

Table 11: Estimated coefficients from Model 6 for all ears and frequencies. Effect estimates are with respect to time measured in years.

Appendix B: Figures



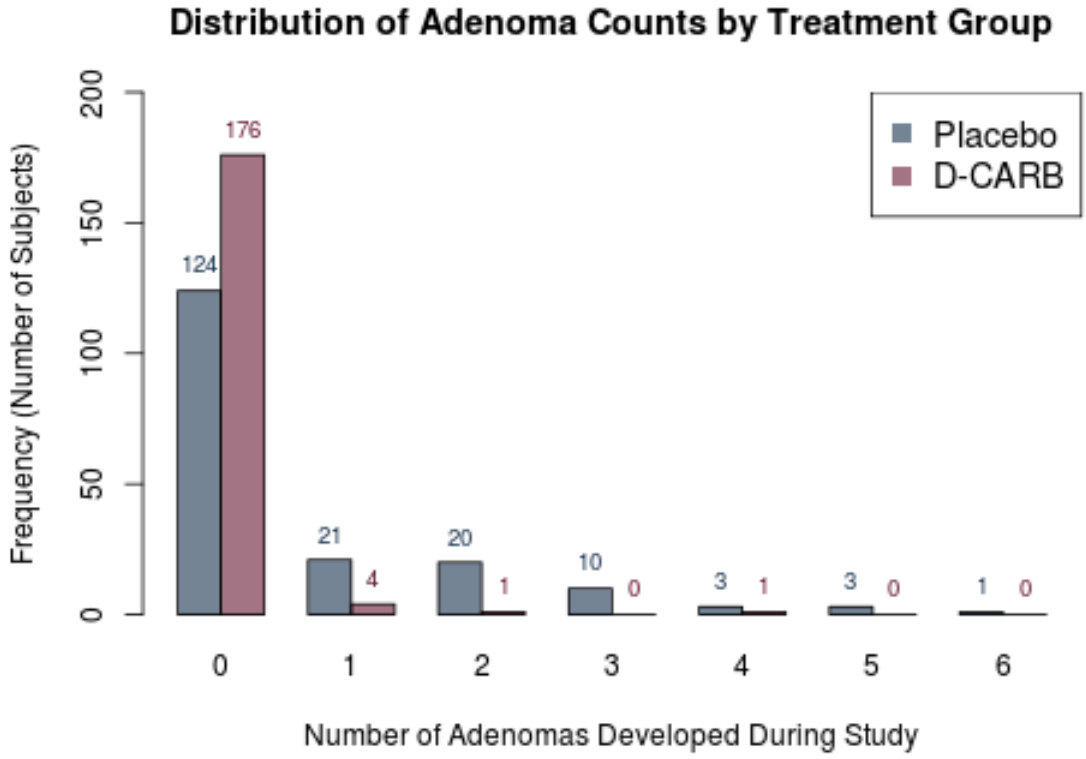


Figure 1: Empirical distribution of observed adenoma counts for patients on study.

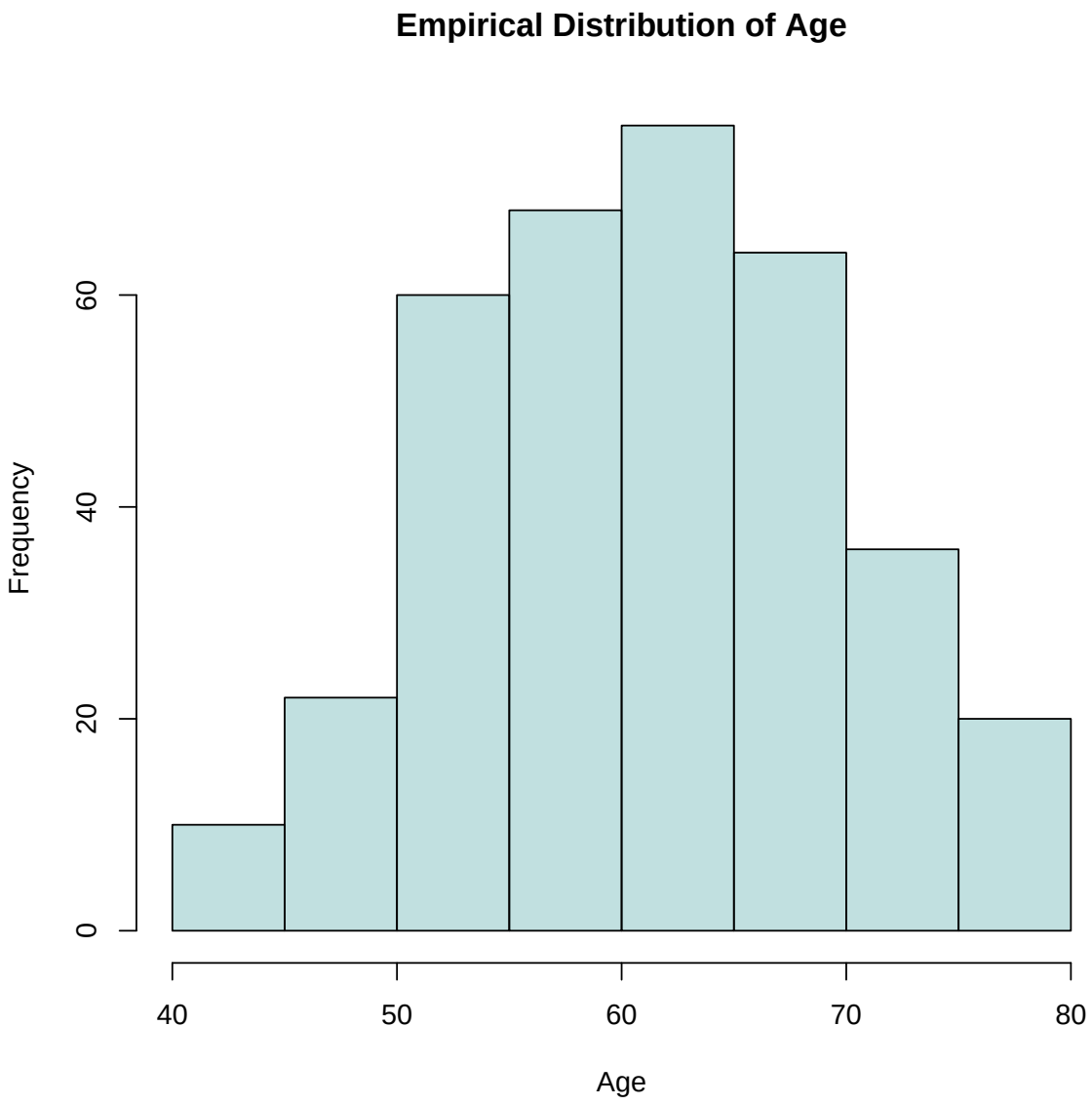


Figure 2: Empirical distribution of age for patients on study.

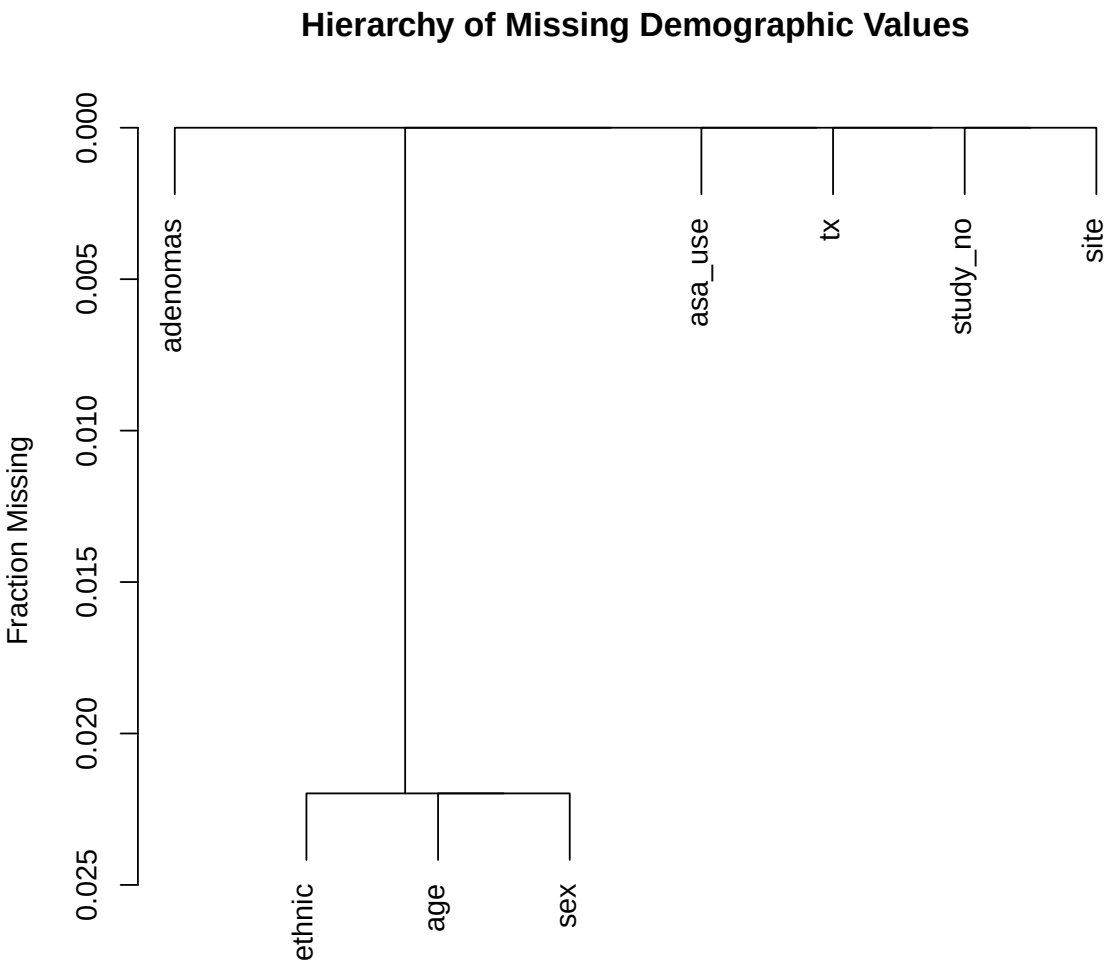


Figure 3: Distribution of missing demographic values.

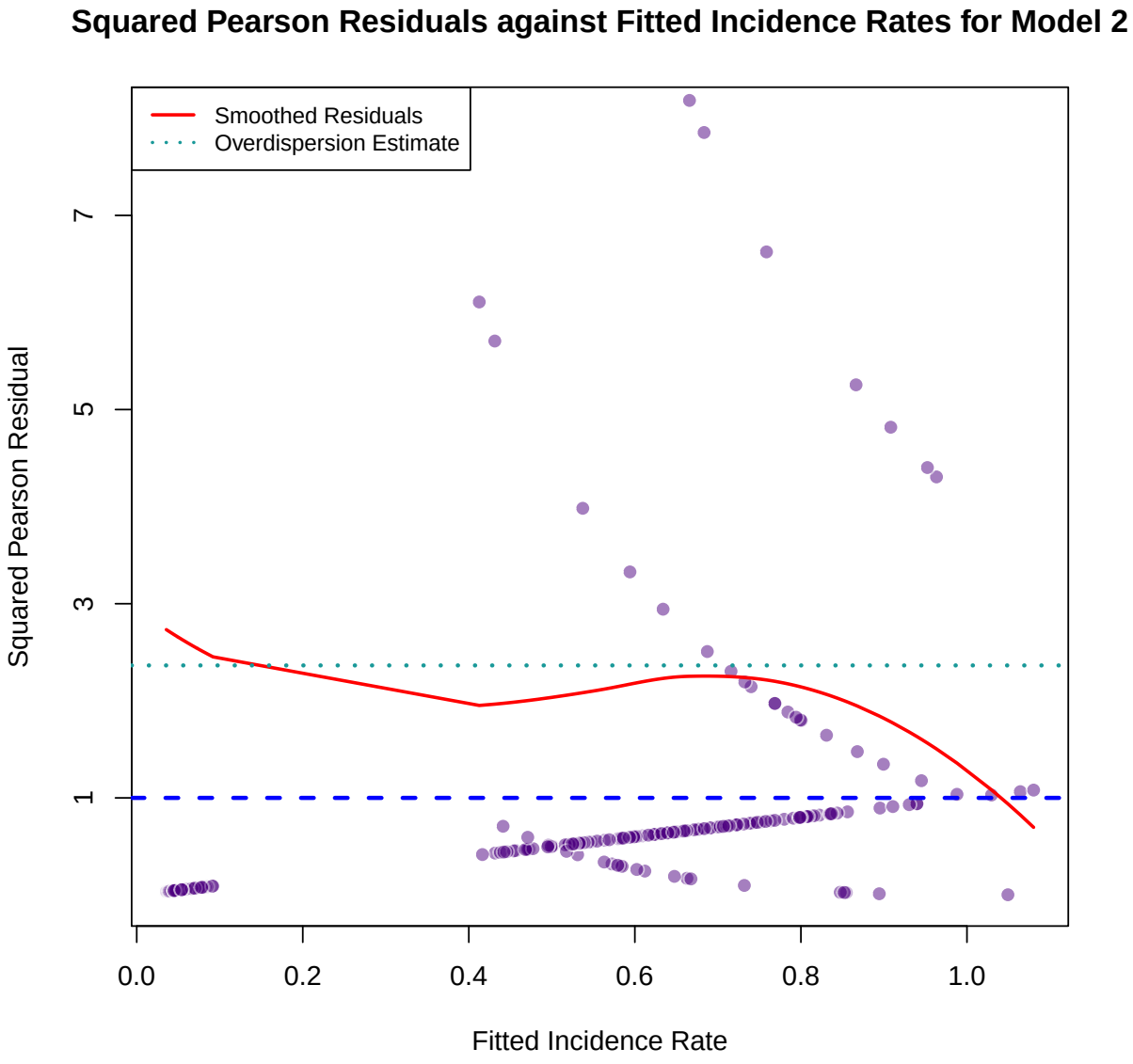


Figure 4: Squared Pearson residuals vs fitted values for Model 2.

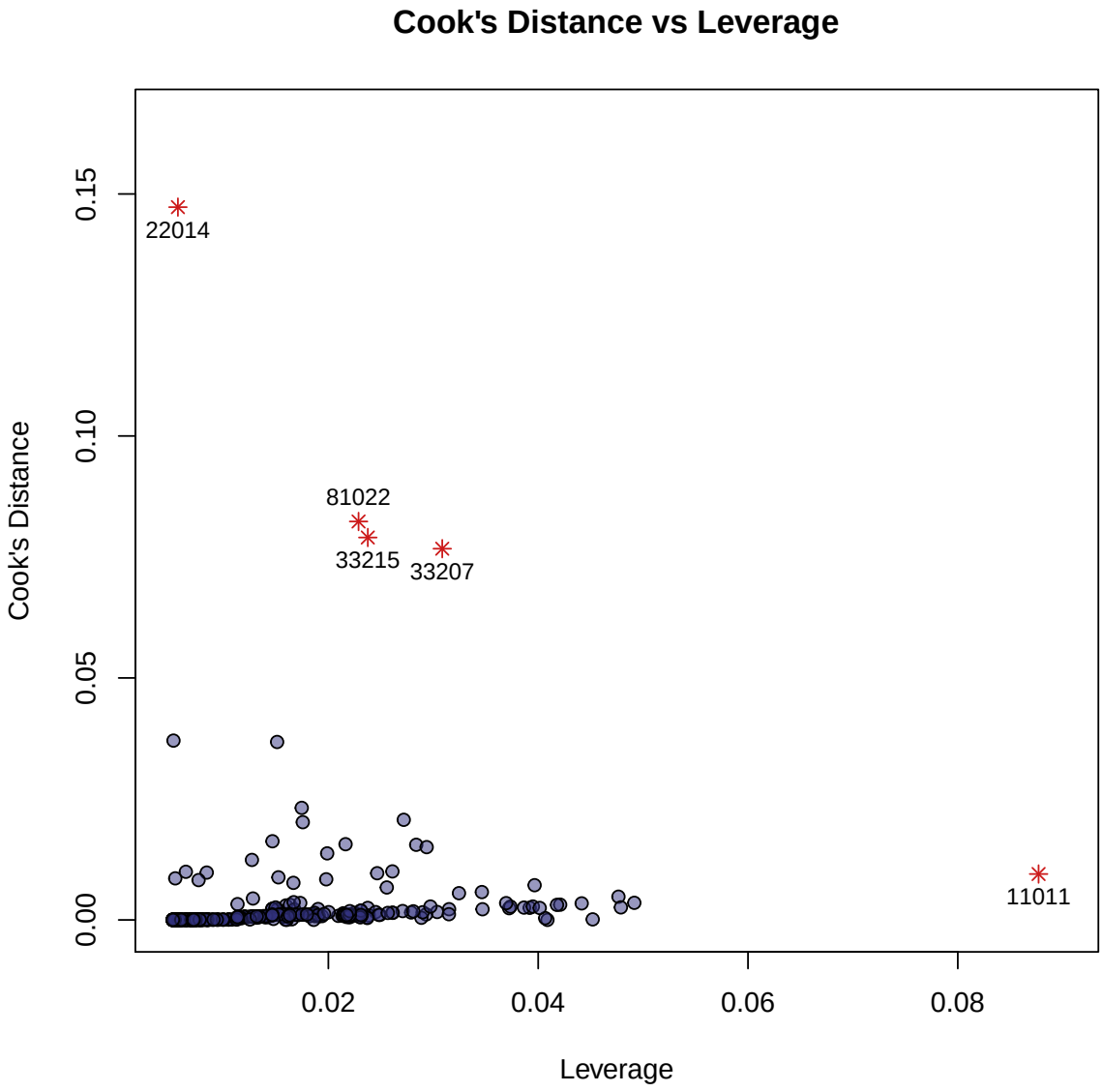


Figure 5: Plot of Cook's Distance vs Leverage with potential outliers and high leverage points observations identified.

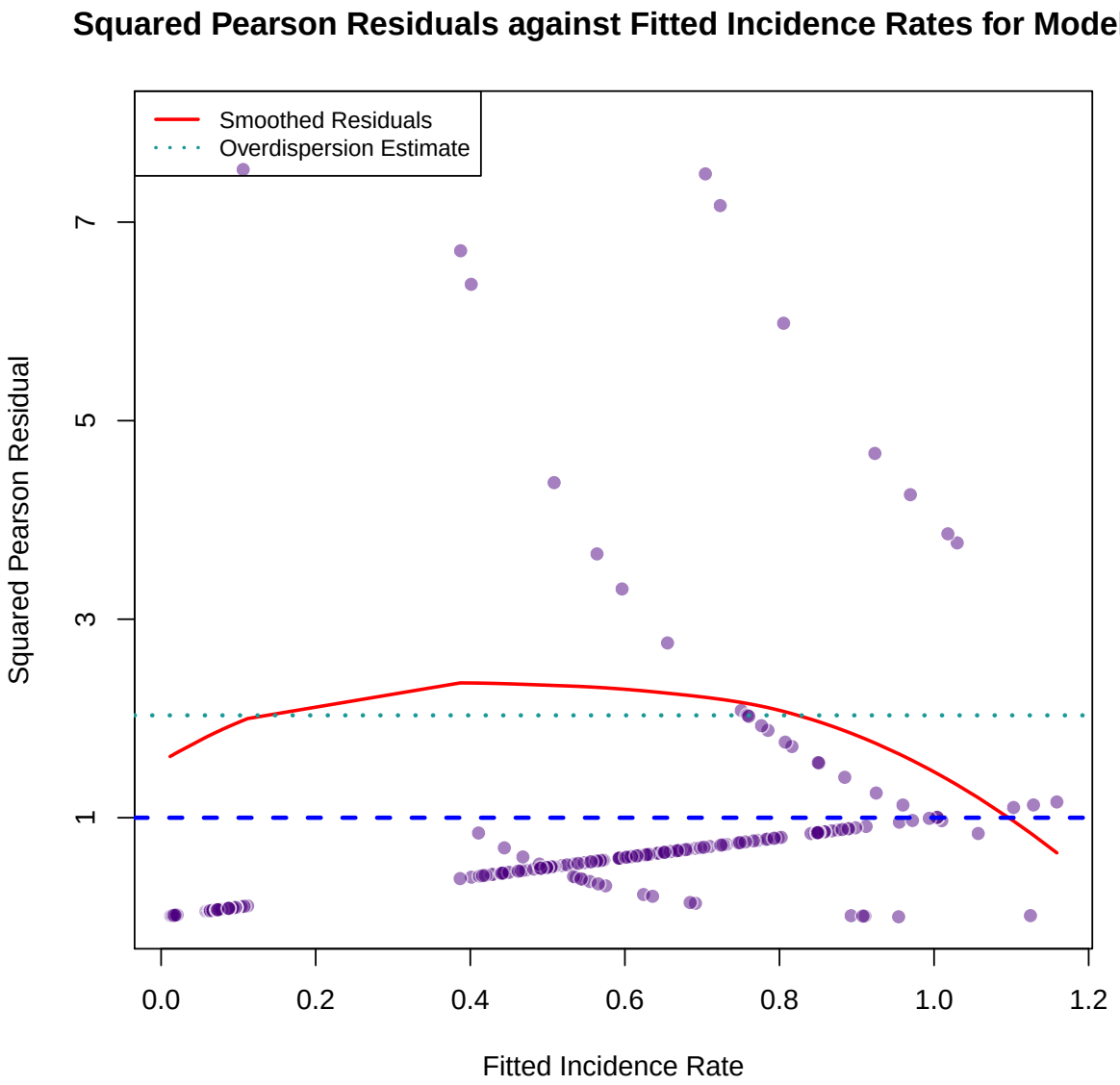


Figure 6: Squared Pearson residuals vs fitted values for Model 4.

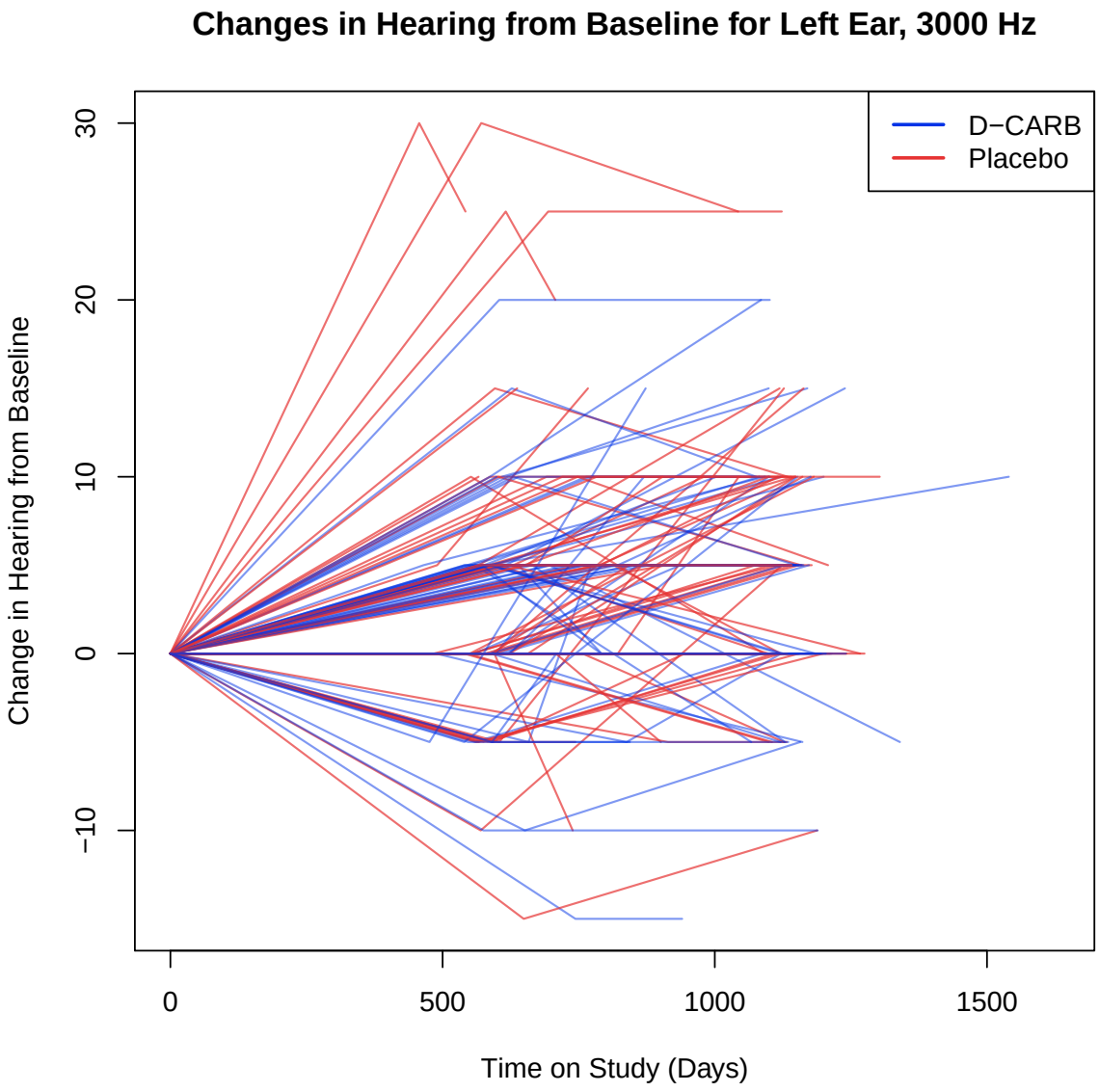


Figure 7: Plot of hearing changes with respect to baseline for left ear, 3000 Hz.

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