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Parental treatment and CTRA gene and expression

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Abstract

Here we examined how a person's exposure to negative parenting (NP) and their cognitive bias - their tendency to interpret ambiguous social events as threatening - is associated with their peripheral blood gene expression, with and without adjustment for sex, race and body-mass index (BMI). Each participant's exposure to negative parenting was measured by in-house observation and their "cognitive bias" was captured from their responses to the Chen videos. Our results suggest that CTRA may indeed relate to individual cognitive responses to social ambiguity: lower expression of CTRA interferons in particular was associated with a tendency to interpret the Chen Videos as more threatening, controlling sex and race. This relationship becomes insignificant when BMI is introduced. A similar pattern was observed in the relation between negative parenting and CTRA expression. From these preliminary results we speculate on the possibility that cognitive phenotype mediates the impact of negative parenting on immune status.

Context

We first consider how cognitive bias (Chen score) and negative parenting (NP) are associated with CTRA gene expression in isolation, adjusting for sex, race and bmi. Then we tentatively address whether cognitive biases might partially mediate the effect of adverse parenting on CTRA gene expression.

Preprocessing

31 PaxGene blood RNA tubes were processed in the Fry lab, UNC, using the PaxGene Blood miRNA extraction kit per manufacturer's instructions. All 31 RNA samples were submitted to the UNC Genomics core to be run on the Affymetrix platform. Samples showed decreased overall RNA yield, with several samples not meeting minimum concentration requirements so samples were processed using the Affymetrix Pico Kit prior to hybridization to the HuGene 2.0 ST arrays. Signal intensities of the .cel files were then normalized using RMA processing (B. M. Bolstad et al. 2003, R. A. Irizarry et al. (2003)).

These normalized samples were assembled - together with coarse demographic/phenotype data - into the Bioconductor ExpressionSet format in the Shanahan lab, UZH. The marginal, empirical distributions of our CTRA genes are depicted in the violin plot below.

Results

Chen analyses

Sample phenotype

Of 31 participants with gene expression data, 25 also had a Chen score.

Of these, one participant (1092) had somewhat anomolous gene expression measurements according to various metrics, including the hierarchical clustering distance (Euclidian, agglomerative) and regression residuals, see plots in the Appendix of URL, JC. Regarding the latter for example, of those residuals which exceeded three standard deviations, 14 out of the 16 were due to subject 1092. Despite these anomolies, removing this subject does not qualitatively change our conclusions. Our analysis therefore does not omit this subject. To examine the consequence of omission, readers can find the corresponding analysis, including this one subject, at URL, JC.

Table 1a: Sex and race

	Black	Mixed	White
F	3	1	7
M	6	0	8

Table 1b: Sex and BMI

	1st	2nd	3rd	4th
F	4	2	3	2
M	3	4	3	4

Table 1c: Race and BMI

	1st	2nd	3rd	4th
Black	2	1	2	4
Mixed	0	0	1	0
White	5	5	3	2

Table 1 describes the demographics of our sample of 25 subjects in terms of self-reported race (black, white, mixed), gender (male, female) and bmi (real-valued in the range [12.6, 19.7]). For the sake of presentation, this table discretizes bmi into 4 quartile bins (1st-4th). We found no evidence that these three demographic variables covary in the general population of our sample: A Fishers exact test on the race x sex contingency table ($p = 0.535$), and two linear-Gaussian regressions of bmi on sex ($p = 0.449$) and race ($p = 0.313$) revealed no detectable relations.

We then asked whether race, sex or bmi related to Chen score. Three seperate linear-Gaussian regressions indicated that bmi (p = 0.00329) - but not race (p = 0.227) or sex (p = 0.31)- related to Chen scores. Similarly, bmi - but not sex or race - predicted Chen conditionally (adjusting for the remaining covariates) at the $\alpha = 0.05$ level.

Overview: Chen and the CTRA

We asked various questions about the relation of Chen to CTRA: (Q1) does any gene in the CTRA set relate to Chen (Q2) which specific CTRA gene(s) relate to Chen (Q3) under what assumptions can we interpret these relations causally. Each question will require a different method, as discussed next.

To formalize Q1, we performed a global score test (see Information Box) of the null hypothesis that no CTRA gene predicts Chen. To formalize Q2, we tested the gene-by-gene components of the global score statistic used to test Q1. For comparison, we also tested the null hypotheses of zero linear relation between Chen and each gene in turn. This latter is not based on a multiple regression of Chen on the CTRA set, but on moderated univariate regressions of each gene on Chen, corrected for multiple comparisons via Benjamini and Hochberg's method to control the false discovery rate (Smyth, Thorne, and Wettenhall 2005). (Moderated regression reduces variance in the estimation of standard errors by drawing them all towards a common value within a simple Bayesian model.) To formalize Q3, we relate the above regression equations to putative causal equations of the data generating process, see Pearl (2009). To constrain the set of possible causal interpretations, this discussion is based on parallel analyses which adjust for the covariates sex, race and bmi.

To briefly anticipate our results here, we can conclude that at least one CTRA interferon gene predicts Chen (Q1). We cannot say much more regarding internal and external specificity or causality (Q2-3).

Q1: Does CTRA predict Chen?

Our analysis necessarily involves many parameters, or equivalently one high-dimensional parameter, which relate Chen to each CTRA molecule. Sadly, there is no uniformly most powerful test of a high-dimensional parameter (Jelle J. Goeman, Geer, and Houwelingen 2006). Different tests are sensitive to detect different alternative hypotheses (different possible settings of the unknown p-dimensional parameter of interest). When testing a multidimensional alternative, it is therefore important to choose a test which has power for those alternative hypotheses which are most plausible. We consider two cases. Our primary test has optimal power to detect many small effects - weak relations between Chen and many members of the CTRA set - and our secondary test is designed to detect a few large effects (amoung mostly zeros).

The first is the global score test (Jelle J. Goeman, Geer, and Houwelingen 2006), based on a linear model of Chen on CTRA. Note that there are more target parameters than participants, 25 participants < 50 CTRA molecules, so we need some constraints to estimate the model. Namely, our model assumes the regression coefficients weighing each CTRA element follow a zero-mean Gaussian distribution (a.k.a. random coefficients). Our

null hypothesis is $H_0: \beta = 0$, where $\beta = (\beta_1, \ldots, \beta_p)$ is the weight of each CTRA molecule. Note that this global score test is two-tailed: the alternative hypothesis is that one or more CTRA interferons have either negative or positive (partial) relation to Chen score.

Column p1 of Table 2 reports the p-value from the global score test of this hypothesis that no gene in the CTRA is associated with Chen (similarly for all 3 CTRA subsets: interferon, inflammatory and antibody). We can reject the hypothesis that CTRA interferons has no association with Chen. Column p2 of Table 2 shows that this association remains after adjusting for self-reported sex and race. Column p3 of Table 2 shows that this association weakens when additionally adjusting for a linear effect of bmi. Clearly, bmi is associated with gene expression as well as with Chen score. Interestingly, a relation between CTRA antibodies and Chen is unmasked after controlling for sex, race and bmi. For a tentative interpretation of these results please refer to Q4 below.

Table 2: Our analysis of the relation between Chen, the CTRA gene set and its component subsets, with and without covariate adjustment (see Q1). This table presents p-values from a global score test on a hyperparameter in an empirical Bayesian model, and is an alternative to classical tests of a point null hypothesis against a high dimensional alternative, even when the number of genes exceeds the number of samples. This test has optimal expected power in the neighbourhood of the null hypothesis. We used a permutation null distribution which requires the assumption that there is no relationship between gene expressions on the one hand, the covariates (bmi, sex and race) and the censoring mechanism on the other hand: permuting destroys these associations. The main advantage of the permutation-based P-value is that it gives an 'exact' P-value, which is guaranteed to keep the alpha level provided enough permutations are used. This is especially useful for smaller sample sizes like ours, where we may not trust the normality of the distribution of our score statistic. Note that a significant global test does not mean that every interferon gene is associated with Chen. It means that the subjects with similar Chen have relatively similar CTRA interferon expression profile. It also means that there is potential to predict Chen from interferon gene expression.

	p1	p2	p3
Inflammatory	0.787	0.556	0.801
Interferon	0.032	0.073	0.265
Antibody	0.690	0.254	0.046
All	0.033	0.084	0.285

To better understand the relation between Chen and CTRA interferons, we plot the relation between Chen and each CTRA molecule in Appendix "Chen and CTRA". This plot shows a negative relation between Chen and interferon on average. There are various ways to quantify this negative relationship. For illustration purposes, we chose a mixed model complementary to that in the preceding paragraph, namely CTRA on Chen rather than Chen on CTRA. In particular, we infered the fixed effect of Chen on CTRA gene expression in a multilevel linear mixed model with independent random intercepts for both participant and CTRA gene (95% confidence interval = [-0.353, -0.00235]). This gives some confidence that the set of CTRA coefficients is negative on average.

Our secondary analysis also aimed to answer Q1, but with more sensitivity to detect sparse alternative hypotheses (a few large effects amoung mostly zeros). We did a permutation test of the claim that Chen scores carry no information about the CTRA interferons. This test was based on the maximum absolute t-statistic (over the set of pairwise regressions of each gene in turn on Chen). This procedure was designed for its power to detect sparse alternative hypotheses, i.e. a few large parameters relating CTRA to Chen, among mostly zero parameters (Jelle J. Goeman, Geer, and Houwelingen 2006). Note that if the alternative is not sparse, then this procedure is provably less powerful than the global score test above (which is optimal to detect many small effects). In practice, we seperately regressed each gene in the 50 CTRA on Chen, and calculated the maximum absolute t-statistic over this set. We assessed the significance of this relation with reference to the empirically-derived permutation null distribution arising from 1000 permutations of the Chen scores. This procedure yielded a p-value of p = 0.2. This p-value is larger than that under the global test above, which likely reflects the test's lower power to detect the many true small associations between CTRA genes and Chen.

Q2: Which specific genes predict Chen?

To gain more internal specificity, we decomposed CTRA interferon global score test result into it's gene-specific components. Figure 2 depicts which of these genes contribute positively to a high value of the test statistic and which do not contribute. It shows that the relation between Chen and CTRA interferons may be narrowed down to a subset of the 29 CTRA interferons (the subtree beneath the bold root branch). We would not want to overstate this specificity, because it was not observed in our parallel analysis (described above, which ommitted subject 1092 could not reduce the significant effect of CTRA interferons to any CTRA interferon subset). JC: no bold line

To assess which specific CTRA genes - as opposed to CTRA (sub)sets - could be related to Chen, we regressed each gene on Chen in turn in the linear-Gaussian framework. A visualization of the linear relation between Chen and interferon is given in the Appendix, where we also report the multiplicity-corrected p-values. In brief, no single CTRA gene is marginally significant at the corrected $\alpha = 0.05$ level. In light of the significant relation described in the previous section, between Chen and CTRA subsets *en masse*, this null result can be viewed as a false negative, which reflects insufficient power to detect individual gene-wise relations over noise. In contrast, our gene-set global score test achieves a power boost by pooling across multiple weak signals.

Q3: What about causality?

In principle, (predictive) regression equations can be used as a tool to identify/test *causal* parameters of the underlying data generating process, see Pearl (2009). This is generally true in the setting of randomized experiments. In the non-experimental setting however, this identification is only possible conditional on a well-specified structural model, i.e. when the underlying causal structure of the data generating system can be safely assumed, up to it's unknown paramaters. These structural assumptions are often untested or untestable. Nonetheless, this framework offers an elegant way to precisely express the conditions required for a predictive (regression) parameter to be interpreted as a causal

(structural) parameter. Conversely, it offers an elegant way to enumerate different causal models which are consistent with the data. From these general considerations it should be clear that our specific data will not perfectly distinguish between different underlying causal models, as we discuss informally below.

Our observation that Chen and CTRA interferons are related only if we do not adjust for bmi is consistent with - at least - three underlying causal models, see Figure 3. In one, bmi confounds the relation between Chen and CTRA interferons (top plot, below). In the other two, bmi somehow mediates this relation (middle, bottom plot). Formally these three cases are difficult to distingish because the imply the same conditional independence structure on observables (namely, independence of Chen and CTRA interferons conditional on bmi, but marginal dependence between Chen and CTRA otherwise). Note that the preceding interpretations rest on the - very strong - assumption that our observed covariates - bmi, sex, race - are the only confounding causes in the data generative process (i.e. common linear causes of both gene expression and Chen). Below we will revisit mediation more formally, both full mediation (conditional independence between treatment and outcome given the mediator) and partial mediation (both a response to mediator and a response to treatment).

We also noted above that a relation between CTRA antibodies is unmasked when we adjust for all three covariates: bmi, sex and race. One interpretation here is that adjusting for these covariates increases statistical power by explaining/eliminating some independent residual variation in Chen. One puzzling feature of this relation is that Chen relates negatively to one of the two CTRA antibodies, but positively to the other. It will be interesting to assess whether this result generalizes to future datasets.

Whole-chip Chen analysis

We addressed 3 questions about the relation of CTRA subsets to Chen scores above. Here we move beyond this special focus, using a global test to ask whether the entire 53617-gene expression profile - not just CTRA - could predict Chen scores. This agnostic approach yeilded a p-value of 0.226. We then asked whether, adjusting for race, sex and bmi, the whole-chip could predict Chen scores ($p = 0.36$).

Summary of Chen analyses

Our analysis attempted to infer the relation between Chen and CTRA subsets. Accordingly, the analysis corrected for some systematic and random sources of error, by appropriate covariate and multiplicity adjustment. In general, covariate adjustment offers one way to limit systematic error, i.e. causal false positives attributable to omitted confounding variables, while multiplicity adjustments and regularization limit sampling error, i.e. false positives due to sampling variance, aggregated across many parameters or tests.

We rejected the null hypothesis that Chen is unrelated to CTRA interferons by means of a global score test. This holds when correcting for sex and race, but not bmi.

Applied considerations

From the perspective of its utility to physicians and physician researchers, in the fields of allergy, immunology etc. He thought to frame the results as follows:

The change to interferons may indicate that the host defense capability, which would protect the host, is diminished or perturbed. Modified host defenses could account for a. increased asthma (not sure if he meant exacerbations or susceptibility to asthma onset) b. modified/exacerbated response to air pollution or other environmental insults c. exacerbation of illness, and d.perhaps malignancies in predisposed populations. That BMI was observed to abolish the relationship between the CTRA sets and the Chen score raises some interesting questions, both at the molecular and sociological level.

Overview of negative parenting NP and CTRA analyses

Putting Chen aside for the moment, this section examines the relation between negative parenting and CTRA. This section follows the same structure as the preceeding discussion of Chen and CTRA.

Here we ask whether a person's experience of negative parenting (NP) relates to their peripheral blood CTRA gene expression. Each participant's experience of NP was captured by a single real-valued score. We examined the relation of this NP score to CTRA gene expression profiles within peripheral whole blood, with and without adjustment for sex, race and body-mass index (BMI).

Sample phenotype

Of 31 participants with gene expression data, 28 also had an NP score.

We asked whether race, sex or bmi related to NP score. Three seperate linear-Gaussian regressions indicated that neither bmi ($p = 0.902$) nor sex ($p = 0.251$) predicted negative parenting, but self-reported race did ($p = 0.00151$), see supplementary Figure "NP and race".

Q1: Does CTRA predict NP?

Column p1 of Table 3 reports the p-value from the global score test of the hypothesis that no gene in the CTRA is associated with NP (similarly for all 3 CTRA subsets: interferon, inflammatory and antibody). The table shows that CTRA *en masse* has some association with NP, but that this cannot be reduced to any CTRA subset (although CTRA interferons are marginally significant). Column p2 of Table 3 shows that this association is diminished after adjusting for self-reported sex and race. Column p3 of Table 3 shows that this association is also weaker when additionally adjusting for a linear effect of bmi.

Table 3:The relation between NP, the CTRA gene set and its component subsets, with and without covariate adjustment (see Q1).

	p1	p2	p3

Inflammatory	0.168	0.950	0.872
Interferon	0.061	0.228	0.107
Antibody	0.604	0.915	0.915
All	0.030	0.200	0.091

To better understand the relation between NP and CTRA interferons, we plot the relation between NP and each CTRA molecule in the Appendix "Negative parenting and CTRA". Despite no statistically significant relation between NP and interferons, this plot does show some indication of a negative relation between NP and interferons (only 3 of 29 interferons had a positive relationship with NP). For comparison with the preceding analysis of Chen on CTRA interferons, we chose to quantify this with a mixed model complementary to that in the preceding paragraph, namely CTRA on NP rather than NP on CTRA. In particular, we infered the fixed effect of NP on CTRA gene expression in a multilevel linear mixed model with independent random intercepts for both participant and CTRA gene (95% confidence interval = [-0.28, 0.00792]). Finally, for comparison with the preceding analysis of Chen on CTRA, we seperately regressed each gene in the 50 CTRA on NP, and calculated the maximum absolute t-statistic over this set. We assessed the significance of this relation with reference to the empirically-derived permutation null distribution arising from 1000 permutations of the NP scores. This procedure yielded a p-value of p = 0.111. This p-value is larger than that under the global test above, which likely reflects the test's lower power to detect the many true small associations between CTRA genes and NP.

Q2: Which specific genes predict NP?

For comparability with our analysis of Chen, we decomposed CTRA interferon global score test result into it's gene-specific components. Figure 4 depicts which of these genes contribute positively to a high value of the test statistic and which do not contribute.

Q3: What about causality?

Our observation that NP and CTRA interferons are related only before adjustment for covariates (sex, race, bmi) may reflect that one covariate is a confounding variable, see Figure 3 for an analogy.

Whole-chip NP analysis

In the preceding analysis, we addressed 3 questions about the relation of CTRA subsets to NP scores. Here we move beyond this special focus, using a global test to ask whether the entire 53617-gene expression profile - not just CTRA - could predict NP scores. This agnostic approach yeilded a p-value of 0.271 . We then asked whether, adjusting for race, sex and bmi, the whole-chip could predict NP scores (p = 0.832).

Summary of NP analyses

Our analysis attempted to infer the relation between NP and CTRA subsets. We rejected the null hypothesis that NP is unrelated to CTRA interferons by means of a global score test.

Relating Chen, negative parenting and the CTRA subsets

Motivation

Adverse parenting has an enduring impact on how children come to cognitively interpret social events - their cognitive "bias" or "schemata". It also leaves an enduring biological trace, notably immune gene expression in the 53-gene 'Conserved Transcription Response to Adversity' set (CTRA), comprised of interferon, inflammatory and antibody genes. It is unclear whether these cognitive and immune outcomes are independent consequences of NP, or for example, whether NP-induced cognitive bias mediates immune changes through some brain-immune interactions. In this latter case, Chen and CTRA might be proximal and distal effects of NP, and aim to quantify the "natural indirect effect" of NP on CTRA, that is mediated by Chen. (The natural mediated effect, is roughly the expected change in the CTRA when we let the Chen change *as if* the NP status had changed, when in fact we have (counterfactually) fixed NP. Similarly for the natural direct effect of NP on CTRA.

In practice, it is impossible for us to identify causal mediation without invoking strong and untestable assumptions about the underlying data generating process, see Information Box: What is identification?. These include no unobserved heterogeniety - e.g. no differences among subjects in CTRA reactions to NP and Chen, or Chen to NP, no confounding - e.g. common causes of Chen and CTRA, and no selection effects - e.g. not simple random sampling of subjects. It is important that these assumptions are conceptually meaningful and unambiguous, and that we can evaluate sensitivity to these assumptions when possible.

We therefore first define the target quantity or causal estimand we wish to infer, then we assess identifiability of this estimand, then discuss statistical inference.

Practicalities

Table 4: Naive mediation analysis of NP effect on CTRA gene expression. Column 1 recapitulates the main result of the preceding section: CTRA is significantly associated with NP. Column 2 tests this same hypothesis on a smaller sample of subjects, those for whom we have both Chen and NP scores. Column 3, reexamines this relation having partialled out a linear effect of Chen. It more fair to compare this relation with that in Column 2 than Column 1 (which has a different, larger sample.)

28 subjects	23 subjects (a)	23 subjects (b)
0.168	0.660	0.528
0.061	0.269	0.602
0.604	0.419	0.280
0.030	0.195	0.469

Two additional issues complicate the analysis of this mediation hypotheses. First, there is no clear statistical framework for mediation analysis with for gene-sets (or high-dimensional outcome variables in general). Traditional mediation analysis is not designed to contend with this issue. We believe this issue is ripe for theoretical contributions. Our

provisional work-around is simply to apply mediation methods designed for univariate outcomes repeatedly - one for each CTRA molecule - then simply correct for multiple comparisons.

Our second complication is an artifact of our specific, small sample. In particular, the pattern of missing measurements for negative parenting and Chen. Recall that there are 31 subjects with CTRA measurements. 28 have NP score. 25 have Chen score. *With these respective sample sizes*, we can summarize the basic results detailed above as:

(1) CTRA is significantly related to negative parenting (without any adjustments) at the 0.05 level. (2) CTRA interferon gene set is significantly related to Chen (without any adjustments) at the 0.05 level.

Unfortunately, neither of these conclusions hold at the conventional 0.05 level if we restrict the sample only to those subjects who have both NP and Chen scores. This complicates the most naive mediation analysis, whereby we compare the effect of NP on CTRA with and without adjustment for Chen (see (Baron and Kenny 1986)). Without adjustment the effect of NP is insignificant (Baron and Kenny 1986).

A slightly less naive approach than (Baron and Kenny 1986), is due to Sobel (Sobel 1982). This Sobel test nonetheless is known for its low power in small samples. The null distribution depends on an asymptotic Gaussian distribution, which is known to fail for small samples such as ours, see (MacKinnon et al. 2002). This motivates non-parametric methods, such as the bootstrap (Preacher and Hayes 2004). However we postpone such an analyses, partly because all these preceding methods have been challenged by statisticians and epidemiologists, based on concerns about identification, rather than inference (Bullock, Green, and Ha 2010; Greenland and Robins 1986; J. S. Kaufman, Maclehose, and Kaufman 2004; Pearl 2009). Thus, while some of these methods produce better standard errors, they do not address systematic bias with respect to underlying causal parameters, due to counfounding, selection and heterogeneity (Bullock, Green, and Ha 2010).

Another approach to mediation

Mediation effects can be defined formally in terms of potential outcome or causal graphs (see Information box "Modern mediation"). Under certain conditions, mediation effects defined in this way can be identified from data. Here we focus on the average causal mediation effects (ACME) of mediator Chen in the relation between NP and CTRA. This ACME represents the population averages of subject-specific causal mediation. In particular, this ACME (aka 'natural indirect effect', the term "total indirect effect" is used by Robins (2003)) quantifies how much NP acts through mediator Chen to alter CTRA expression (Greenland and Robins 1986; Pearl 2009). We address the high-dimensionality of CTRA, by assessing the average causal mediation effect ACME for each gene seperately, then report simultaneous (multiplicity corrected) confidence intervals for the 50 counterfactual parameters of interest. Unfortunately for the mediation hypothesis, there are no non-zero ACMEs, even before correction for multiplicity (see Appendix "ACME"). There are no non-zero ACMEs after adjusting for pretreatment covariates sex, race and bmi.

Identification conditions required by (Baron and Kenny 1986)

Traditional "operational definitions" of mediation (Baron and Kenny 1986) are based on linear models (see (Kraemer et al. 2001) for a critique). These definitions are not general, but are tied to specific inference strategies, e.g. testing parameters derived from a set of linear equations (by Sobel test, Bootstrapping, Monte Carlo, etc) (Preacher and Hayes 2004). In fact there do exist conditions which gaurentee that the popular product of coefficients estimator (Baron and Kenny 1986), which is based only on observed outcomes, has a causal interpretation (in terms of unobserved potential outcome parameters). These assumptions can be articulated in different vocabulary: conditional sequential ignorability, exogeneity, independent errors, etc, and pose a formidable obstactle to a causally interpretable mediation analysis (Bullock, Green, and Ha 2010). The following bullet points detail these conditions informally, and discuss their (im)plausibility in our application.

- No omitted confounds on any of the three paths: i.e. all common causes of Chen and CTRA, NP and Chen, and NP and CTRA measured and controlled (including common "shared" method effects).

This assumption can be represented as $Cov(U_{Chen}, U_{CTRA}) \neq 0$ where U_{Chen}, U_{CTRA} are ommitted/unobserved causes of Chen and CTRA molecules respectively. We must explicitly justify the assumption that ommitted causes of Chen are unrelated to ommitted causes of CTRA, when Chen is fixed. (Interestingly, even though experimentally randomized NP treatment would ensure independence of NP with spurious causes of CTRA, denoted U_{CTRA}, it says nothing about whether Chen is independent of these causes, which is crucial for unbiasedness. This illustrates that mediation analysis is demanding **even in randomized experiments**. Thus a method is biased due to unobservables that covary with the treatment, in particular those which also influence outcome.)

- No feedback loops (simultanious reverse causation) - Chen does not effect NP - Chen and CTRA do not cause NP - CTRA does not cause Chen

In fact, it is likely that NP is an artifact of Chen, because of the dyadic interactions between parent and child. CTRA of the child might affect NP, if for example sick children make for tired and impatient parents. CTRA may influence Chen through the well-known effect of immune factors on the brain, and on subjective affect. It is possible that suitable instrumental variables may be found to address this problem, though we do not pursue this here.

- No unobserved heterogeneity (no non-additive interaction between NP treatment and subject index): the average treatment effect therefore equals the unit treatment effect.

Differences in quality and time spent with parents (i.e. amount of NP treatment), or other intrinsic resilience factors, may buffer the impact of NP for some children but not others. Differences in the impact of NP on the child's viral or bacterial environment also pose a problem. It may be misleading to estimate the average direct or indirect effects for the entire sample. Sadly problems of unobserved heterogeneity - of the effects of NP on Chen and Chen on CTRA - cannot be resolved by *repeated interventions of (NP, Chen)* and repeated measures of CTRA, because of the nature of these variables. While homogeneity

assumptions do little harm when infering total causal effects, it ruins inference on indirect effects: regression of CTRA on NP and Chen will not recover the average direct effect of NP, even if both NP and Chen had been experimentally manipulated! (see Glynn, 2009; Pearl, 2001; Robins, 2003). One consequence of heterogeneity is that our approach produces estimates of indirect effects that apply not to the entire sample but only to those subjects whose Chen was affected by NP (e.g., Angrist, Imbens, & Rubin, 1996). To see why, consider a clinical trial that we conduct to learn the effect of a pill. All treatment-group subjects are asked to take the pill. Some refuse. We have no way of learning how these "noncompliers" would have been affected by the pill if they had taken it. We are therefore unable to estimate the average effect of taking the pill for all subjects in our sample. We cannot know which subjects' Chen does not change with parental NP, which is on par with refusing to take a treatment in a randomized trial. Thus, in addition to being unable to estimate an average indirect effect for all of our subjects, we often cannot even know which subjects our estimates apply to when we conduct a mediation analysis.

- No observable heterogeniety due to treatment x mediator interaction (i.e. linear mediation or "no moderation", which is a strong restriction).

Mediator–treatment interactions are easily accomodated in modern approachs, such as (Imai, Keele, and Yamamoto 2010). We must assume that NP does not change the impact of Chen on CTRA. Otherwise, we should estimate average indirect effects for homogeneous subgroups rather than a single average for the entire sample. Discussions of "moderated mediation" (Muller et al., 2005) consider causal heterogeneity but seldom discuss the problem that it poses to the calculation of average mediation effects. If NP and Chen have been experimentally manipulated, and if their effects can be modeled as functions of observed variables and *independent random factors*, and if one has a sufficient number of subjects for each level of sensitivity to changes in NP and Chen, then methods of estimating moderated mediation can solve the problem posed by causal heterogeneity.

- Linear parametric mediation analysis also makes all of the standard assumptions of the general linear model (i.e., linearity, normality, homogeneity of error variance, and independence of errors).

Misspecification may bias the indirect and direct effects even if identification holds. So, using a linear effects model despite underlying nonlinearity will bias an identified indirect effect.

- No measurement error for Chen and CTRA (e.g. there is no measurement error in Chen).

This will downwardly bias the mediation effect of Chen. The underlying data-generating process involves latent variables such as cognitive bias, which we estimate - imperfectly - by Chen. Note that we can neither observe, nor manipulate cognitive bias. In theory, one solution is to adjust estimates - using Structural Equation Modeling - or conduct sensitivity analyses assuming different values of reliability. We do not pursue this here.

- Sample selection is not influenced by NP, CTRA or CTRA.

Identification conditions for our mediation analysis

We now discuss these conditions again with a slightly different vocabulary, which may be more familiar to some readers.

The literature on mediation based on a potential/counterfactual framework has primarily focused on identification rather than estimation, see Information box: What is identification? Indirect and direct effects can be nonparametrically identified under two assumptions called "sequential ignorability" (Imai, Keele, and Yamamoto 2010).

The first assumption is that NP assignment must be conditionally ignorable, i.e. statistically independent of potential CTRA outcomes and potential Chen mediators, given some set of pretreatment confounds. This is akin to saying that factors influencing treatment NP assignment are independent of factors influencing CTRA and Chen, when NP is fixed by intervention (Pearl 2014). This independence would hold unconditionally, if NP were randomized or as-if randomized in a natural experiment, or conditionally, given subjects matched on any "backdoor set" of observed pretreatment confounder variables (or the propensity score).

The second assumption is that Chen is as-if randomized (independent of the potential outcomes of CTRA), given observed pretreatment confounds. This is akin to saying that the causes of Chen are independent of the causes of CTRA, having fixed NP and Chen within each subpopulation defined by levels of the pretreatment covariates/confounds. This can be achieved if and when we can condition (match) on another backdoor set of pretreatment covariates that affect both the Chen and CTRA.

Under sequential ignorability, we must assume that all the joint pretreatment causes of Chen and CTRA in the data generating process are known and observable, otherwise indirect and direct effects will not be identified. This assumption is strong (cannot be tested by observed data), and we cast doubts on this in the previous section.

Inference (assuming the identifiability above)

In the traditional mediation literature (Baron and Kenny 1986), our mediation effects would be estimated using a model for the mediator (Chen, given NP) and a model for the outcome (CTRA, given NP and Chen), as given in the last two equations from this triplet:

$$\begin{aligned} T_i &= U_{T_i} \\ M_i &= a_M + b_{M,T}T_i + c_{M,Z}Z + U_{M_i} \\ Y_i &= a_Y + b_{Y,T}T_i + c_{Y,Z}Z + d_{Y,M}M + U_{Y_i} \end{aligned}$$

for subjects i. Where T_i, M_i, Y_i are the value of the treatment NP, mediator Chen and CTRA outcome for subject i. Z_i is a vector of pretreatment covariates/confounds, ideally a backdoor set. We ommit an index for different CTRA molecules for simplicity (recall that CTRA is in fact a vector valued outcome). These equations satisfies sequential ignorability if

$Cov(U_{M_i}, U_{Y_i}|Z_i) = Cov(U_{T,i}, U_{M,i}|Z_i) = Cov(U_{T_i}, U_{Y_i}|Z_i) = 0$ (Imai, Keele, and Yamamoto 2010; Pearl 2014).

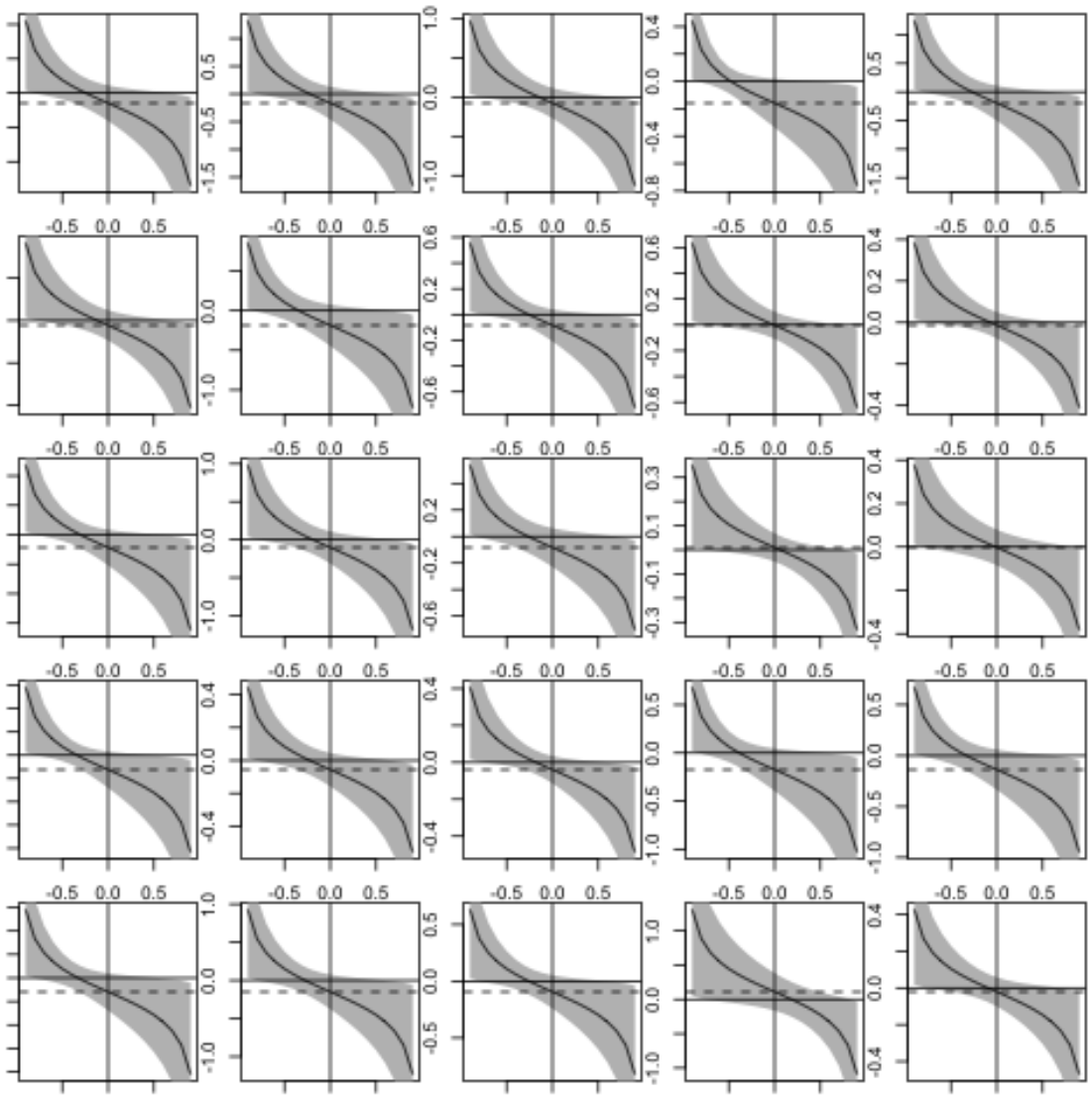
The indirect effect is estimated as the product of $\hat{d}_{Y,M}\ \hat{b}_{M,T}$.

Our naive analysis above assumes that NP does not moderate the effect of Chen, that both models are linear.

Sensitivity of inference to identification assumptions

Our sensitivity analysis is based on the fact that - if NP is as good as randomized - then the second sequential ignorability assumption can be encoded in the correlation coefficient between the two error terms from the mediator and outcome models. Let ρ represent this correlation. When $\rho = 0$, the two error terms are uncorrelated, implying that sequential ignorability holds. By varying ρ and observing how the indirect and direct effects change under different possible violations of sequential ignorability, we can assess sensitivity to this assumption.

The general strategy is to infer the indirect effect assuming different values of the unidentifiable sensitivity parameter, i.e. the strength of Chen-CTRA confounding. We check the robustness of our conclusion, obtained under the sequential ignorability assumptions, via changing the assumed covariance or correlation or between U_M, U_Y. A nonzero correlation parameter can be interpreted as the existence of omitted variables that influence both mediator $Chen_i$ and the potential outcomes $CTRA_i$.



ACME versus latent endogeneity: correlated innovations U_{CHEN}, U_{CTRA}

In particular, ρ is a sensitivity parameter representing the non-identified correlation between the residuals. A sensitivity analysis is obtained by varying the fixed ρ. The sensitivity analysis varies the ρ from -0.9 to $+0.9$. A graph of the indirect effect is shown in Figure, including a 95% confidence interval. This shows that there is no evidence for a non-zero ACME under any condition.

Information box: Defining and representing causation

Modern definitions of total, and path-specific effects - direct and indirect - are general, and not tied to any specific statistical model.

A causal variable is defined as any variable which changes the potential outcome of another variable. This idea can be interpreted as follows. First suppose we know the equations

which dictate the natural directions of causation between variables in some system. Next override the equation governing one focal variable, and instead switch this variable between two different values. By definition, this focal variable is a cause of any variable which responds to this intervention (through the remaining equations). The difference between these definitions is purely notational; potential outcome definitions can easily be converted to structural definitions. Potential outcomes can be viewed as a short hand notation for general structural equations (not necessarily linear or parametric). For example, take the following trivial, linear parametric structural equation model: we can abbreviate the structural causal equations $CTRA_i(X_i = 1) = d + c + e_i$, and $CTRA_i(X_i = 0) = d + e_i$ as $CTRA_i(1)$ and $CTRA_i(0)$ respectively. Note that only one potential outcome can be observed, the other is counterfactual. Causal inference, i.e. on $CTRA_i(1) - CTRA_i(0)$, thus requires identifying conditions which justify imputing the missing counterfactual. See (Pearl 2014) to explicitly compare the structural formulation of mediation side by side with the potential outcome formulation.

Causation is defined *ceteris parabis*, i.e. at the level of each individual "unit" subjected to intervention. Various statistical methods aim to infer population parameters of these unit-level causal effects, such as propensity score matching and nearest-neighbor matching (which often uses the Mahalanobis metric, also called Mahalanobis matching), attempt to correct for the assignment mechanism by finding control units similar to treatment units on variables which confound causal effects (implied by ceteris parabis).

Information box: what is identification?

A parameter is said to be identified if different parameter settings of the underlying data generating process imply different distributions over observed variables. This identifiability - or lack thereof - is not a statistical problem related to the challenges of statistical inference with small samples. Pearl (2009) provides one way to think about identification. Dependence between observed variables reflects some unknown mix of causal and noncausal ("backdoor") effects. A causal effect is identified when the observed association can been adjusted somehow to remove these noncausal components. For nonparametric identification, the analyst would describe the set of assumptions that will allow us to identify a causal effect without any distributional or functional form assumptions.

To take a famous example, randomized treatment and the SUTVA identification (Rubin 1974) together nonparametrically identify the average total effect. To identify the indirect and direct effects, additional assumptions are necessary, e.g. "sequential ignorability".

Causal identification assumes the investigator has domain knowledge to judge the plausibility of no confounding type of assumptions which underly all mediation methods, whether under the rubric of sequential ignorability (e.g., Imai et al., 2010b), uncorrelated error terms, or graphical criterea. The assumptions identifying mediation can be stated most succinctly in the latter.

Identification conditions can be expressed in diverse ways, e.g. judging conditional independencies among counterfactual variables, often called strong ignorability,

conditional ignorability , or sequential ignorability, presents a formidable task without structural models. Efforts to replace ignorability vocabulary - with notions such as no unmeasured confounders, no unmeasured confounding, as if randomized, effectively randomly assigned, or essentially random - create ambiguity. First, the notion of a confounder varies significantly from author to author. Some define a confounder (say of the NP-CTRA relationship) as a variable that affects both NP and CTRA. Some define confounder as a variable that is associated with both NP and CTRA. Others allow for a confounder to affect NP and be associated with CTRA. Worse yet, the expression no unmeasured confounders is sometimes used to exclude the very existence of such confounders and sometimes to affirm our ability to neutralize them by controlling other variables, not necessarily confounders. Second, the interpretations have taken sequential ignorability as a starting point and consequently are overly stringent – sequential ignorability is a sufficient but not necessary condition for identifying natural effects. Weaker conditions can be articulated in a transparent and unambiguous language which provide a greater identification power and a greater conceptual clarity.

Information box: Alternatives to sequential ignorability conditions for

identification

Instrumental variables offer a very different answer from a causal mediation analysis (Keele 2015). Mechanisms based on IV have the advantage that one can allow for the possibility of unobserved confounding between the mediator and the outcome. However, to identify the indirect effect, one must assume that the direct effect is zero. The assumption that the direct effect is zero is widely referred to as the exclusion restriction (Angrist, Imbens, & Rubin, 1996). Thus, one must assume that there is only an indirect effect, which implies that the effect of the treatment is entirely mediated. Under this form of mechanism, we must assume that the effect of a NP only works through Chen: There cannot be any other mechanisms for the intervention.

Statistically "controlling" for M in the analysis (by including M in the regression equation) does not physically disable the paths going through M ; it merely matches samples with equal M values, and thus induces spurious correlations among other factors in the analysis, see (Pearl 2014). This can be readily shown using classical path-tracing rules. Such dependence cannot be detected by statistical means, so theoretical knowledge must be invoked to identify the sources of these correlations and control for common causes (so called "confounders") of M and CTRA whenever they are observable. This approach to mediation has two major drawbacks. One (mentioned above) is its reliance on the untested assumption of uncorrelated errors, and the second is its reliance on linearity and, in particular, on a property of linear systems called effect constancy (or no interaction): The effect of one variable on another is independent of the level at which we hold a third. This property does not extend to nonlinear systems; in such systems, the level at which we control M would in general modify the effect of T on CTRA. For example, if the output CTRA requires both T and M to be present, then holding M at zero would disable the effect of T on CTRA , while holding M at a high value would enable the latter.

Information Box: Modern mediation

Although one could define mediation statistically, we follow the causal definition.

The conventional mediation analysis entails fitting a set of linear regression models: ``mediation effects'' are defined in terms of these estimated model parameters. One problem with *defining* mediation in terms of statistical changes induced by adding a third mediator variable into a regression equation, is that mediation is inherently a causal notion hence should not be defined in statistical terms. Modern approaches therefore define mediation in terms of potential outcomes, or equivalently causal graphs. In the language of the latter, a mediator is then an intermediate variable that lies on the causal path from the treatment to the outcome. This definition is grounded in the notion of a causal path and emphasizes the difference between "fixing a variable" and "statistically adjusting for" (conditioning on) a variable as in regression.

To illustrate our measure of ACME more formally, consider a binary measure of negative parenting, a variable we call t which takes 0 or 1. We will now define indirect effect of NP - via mediator Chen M - within the modern framework. $M_i(t)$ is the effect of NP on Chen for subject i under treatment (NP) status t. Let $CTRA_i(t, m)$ denote the potential outcome if NP and Chen took values t, m respectively. We only observe one of these potential outcomes $CTRA_i(t_i, M_i(t_i))$, where $M_i(t_i)$ is the observed value of Chen at the observed NP level t_i. $CTRA_i(t, M_i(t))$ is the effect of t on CTRA, which in general and be transmitted both indirectly, through $M_i(t)$, and "directly" (i.e. not through M but possibly through some independent mediators). Let the total causal effect for unit (subject) i be

$$\tau_i = CTRA_i(1, M_i(1)) - CTRA_i(0, M_i(0))$$

and the unit-level indirect effect be

$$\delta_i = CTRA_i(t, M_i(1)) - CTRA_i(t, M_i(0)).$$

This latter relates to the following counterfactual question: how would CTRA change in this indidividual if we were to physically (counterfactually) change Chen's value under $t = 0$ (no negative parenting) to that under $t = 1$ (negative parenting), while keeping NP at its observed value t? Because these two values of Chen would naturally occur as responses to changes in NP, this quantity formalizes the notion of a causal mechanism that the causal effect of the treatment is transmitted through changes in the mediator of interest. Similarly, we define the unit direct effect, corresponding to all other possible causal mechanisms (sometimes refered to en masse as the "direct effect"), as:

$$\gamma_i = CTRA_i(1, M_i(t)) - CTRA_i(0, M_i(t)).$$

The counterfactual question here is: how would CTRA respond to NP change $T_i = 0$ to $T_i = 1$, if (counterfactually) Chen was held constant?

Mediation analysis creates an identification problem. The quantity $CTRAi(1, M_i(0))$, for example, is unobservable, but to estimate the mediation effect we need assumptions which link this unobserved counterfactual to observed quantities. We examine these assumptions.

Such definitions can easily be extended to continuous treatments (NP not binary) (Imai, Keele, and Yamamoto 2010).

Information Box: CTRA gene set analysis

Three broad approaches have been historically used to test whether some observable phenotype, outcome or treatment relates *en masse* to the gene expression across a gene set: (i) tests of independence in a cross-classification table, which counts genes belonging both to the prespecified gene-set and to the differentially-expressed gene set, e.g. Fishers exact test (ii) tests which make no hard classification of genes into differentially expressed versus not differentially expressed, but rather use the p-value of every gene-phenotype correlation, e.g. gene set enrichment analysis (Subramanian et al. 2005) (iii) high-dimensional regression models which regress phenotype directly on the raw gene expression levels of all genes in the gene-set, e.g. global score test. A serious case can be made for the latter (Jelle J Goeman and Bühlmann 2007). P-values derived from these models have a clear interpretation with respect to sampling variation over participants - not over genes. Unlike many popular alternatives, these p-values do not depend on the (obviously false) assumption that gene expression measurements are independent. This class of models bears a close relation to hierachical and empirical Bayesian models, penalized likelihood, whose behavior has been well studied.

The 53-gene "CTRA" set - discussed in prior literature - is of primary interest to us here. It is listed below and includes (a) 19 proinflammatory genes which are upregulated in CTRA "on average" (b) 31 genes involved in type I IFN responses down-regulated in the CTRA (c) 3 genes involved in antibody synthesis down-regulated in the CTRA. These molecules have been historically designated by their HGNC names (HUGO gene nomenclature committee). IL1A, IL1B, IL6, IL8, TNF, PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA, RELB, GBP1, IFI16, IFI27, IFI27L1, IFI27L2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1, IFIT2, IFIT3, IFIT5, IFIT1L, IFITM1, IFITM2, IFITM3, IFITM4P, IFITM5, IFNB1, IRF2, IRF7, IRF8, MX1, MX2, OAS1, OAS2, OAS3, OASL, IGJ, IGLL1, IGLL3. Our chip covers a subset of these molecules (our chip is different from that used in the Cole lab historically, i.e. the Illumina Human HT-12 v4 BeadArray). In particular, 50 of the 53 CTRA were on this chip. Inflamatory: IL1A, IL1B, IL6, CXCL8, TNF, PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA, RELB; Interferon type-I: IFI16, IFI27, IFI27L1, IFI27L2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1, IFIT2, IFIT3, IFIT5, IFIT1B, IFITM1, IFITM2, IFITM3, IFITM4P, IFITM5, IFNB1, IRF2, IRF7, IRF8, MX1, OAS1, OAS2, OAS3, OASL. Antibody: JCHAIN, IGLL1. Note that 4 of the original 53 CTRA have been renamed: IL8, IFIT1L, IGJ, IGLL3 are now CXCL8, IFIT1B, JCHAIN, IGLL3P.

Figures

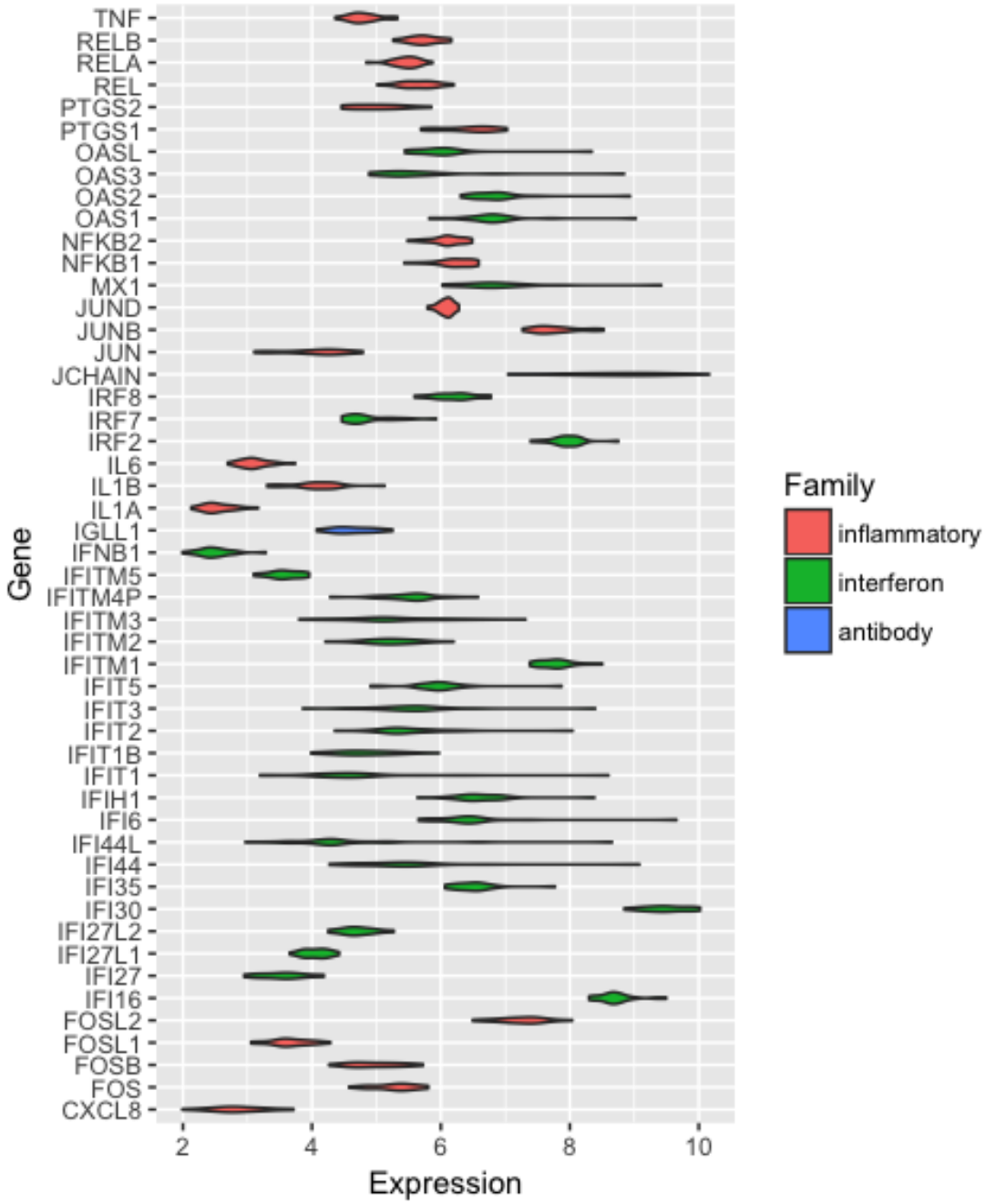


Figure 1: The observed marginal distribution of gene expression for each CTRA molecule.

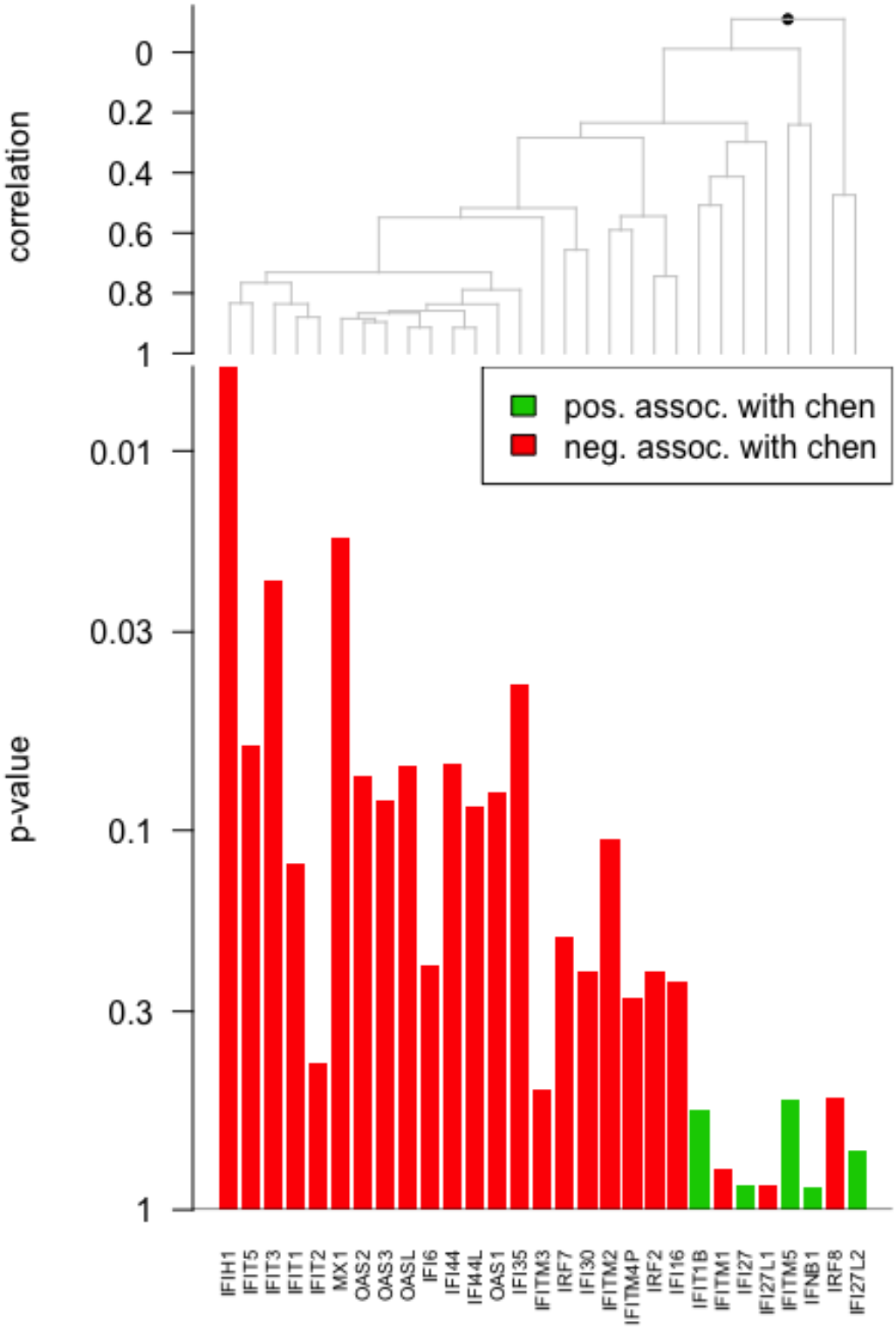

correlation
0
0.2
0.4
0.6
0.8
1
p-value
0.01
0.03
0.1
0.3
1
pos. assoc. with chen
neg. assoc. with chen
IFIH1
IFIT5
IFIT3
IFIT1
IFIT2
MX1
OAS2
OAS3
OASL
IFI6
IFI44
IFI44L
OAS1
IFI35
IFITM3
IRF7
IFI30
IFITM2
IFITM4P
IRF2
IFI16
IFIT1B
IFITM1
IFI27
IFI27L1
IFITM5
IFNB1
IRF8
IFI27L2

Figure 2: A decomposition of the CTRA interferon global score test. The global score test statistic can be interpreted as a weighted average (partial) correlation between each gene and the residuals of Chen (with respect to covariates). These contributions themselves constitute tests. In addition, we can test all nested subsets of the CTRA interferons, as induced by agglomerative hierarchical clustering (based on correlation distance). This analysis showed that there is little specificity to our results: some CTRA interferons were related to Chen, but we cannot confidently narrow this conclusion further to some smaller subset of CTRA interferons, or to any individual interferons.

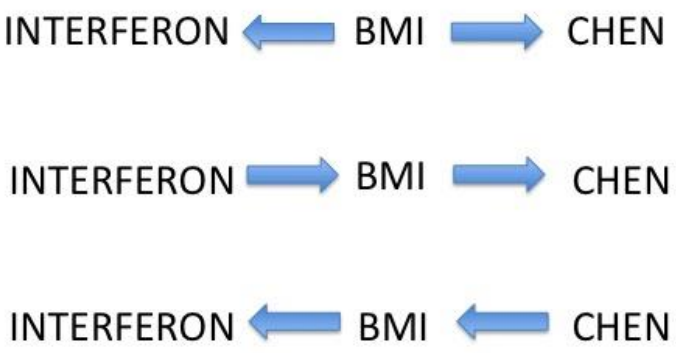


Figure 3: Possible causal interpretations of the fact that Chen and CTRA interferons are related, but only before accounting for bmi.

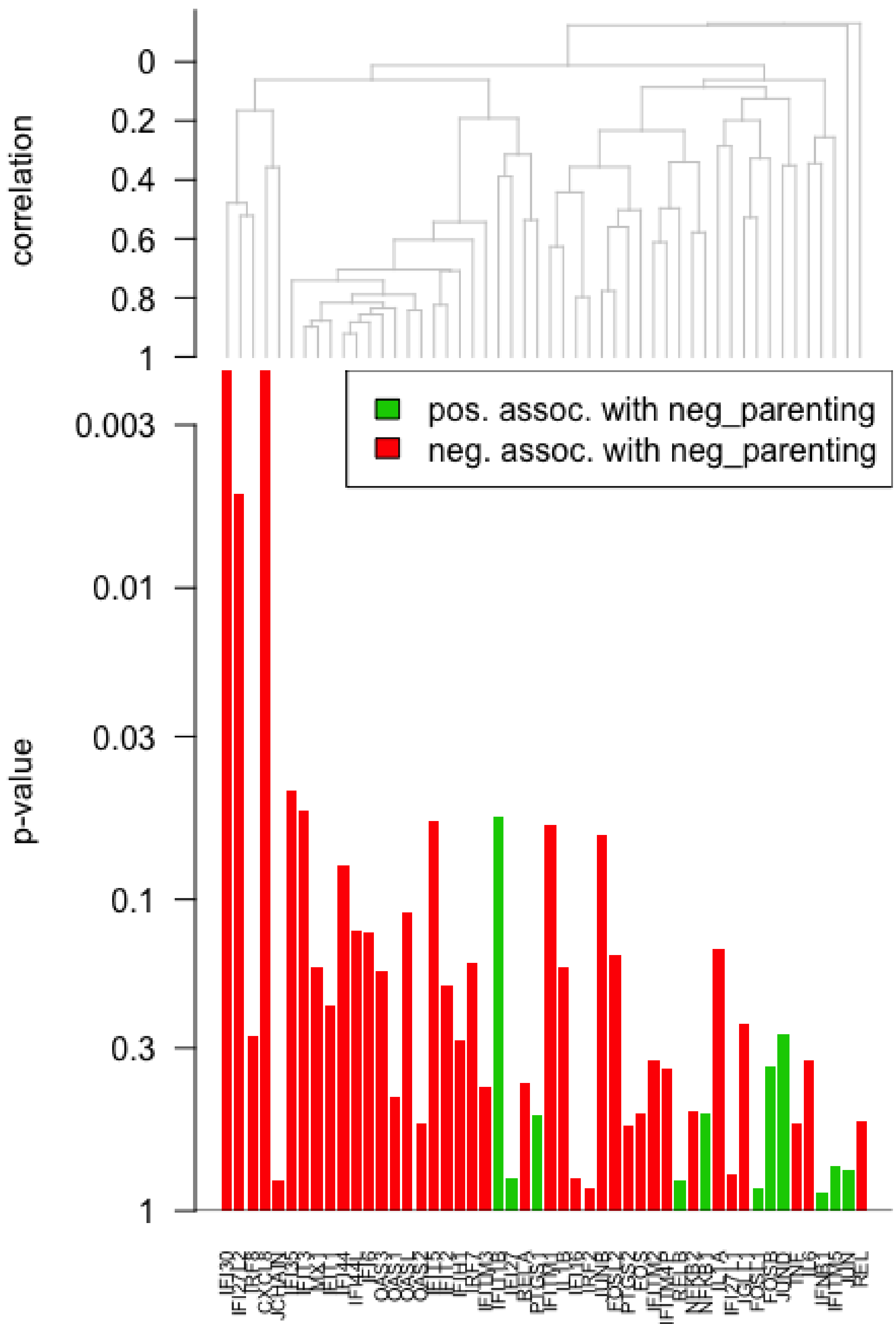
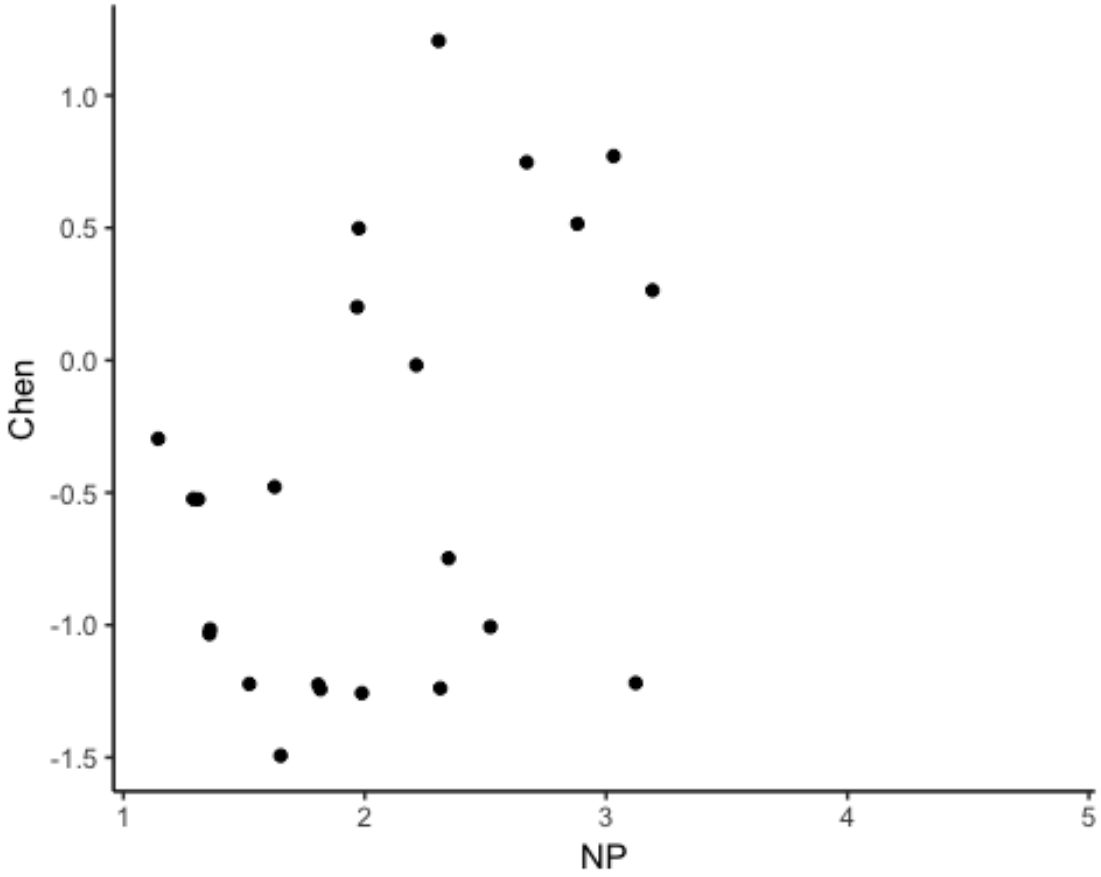

correlation
0
0.2
0.4
0.6
0.8
1
p-value
0.003
0.01
0.03
0.1
0.3
1
pos. assoc. with neg_parenting
neg. assoc. with neg_parenting

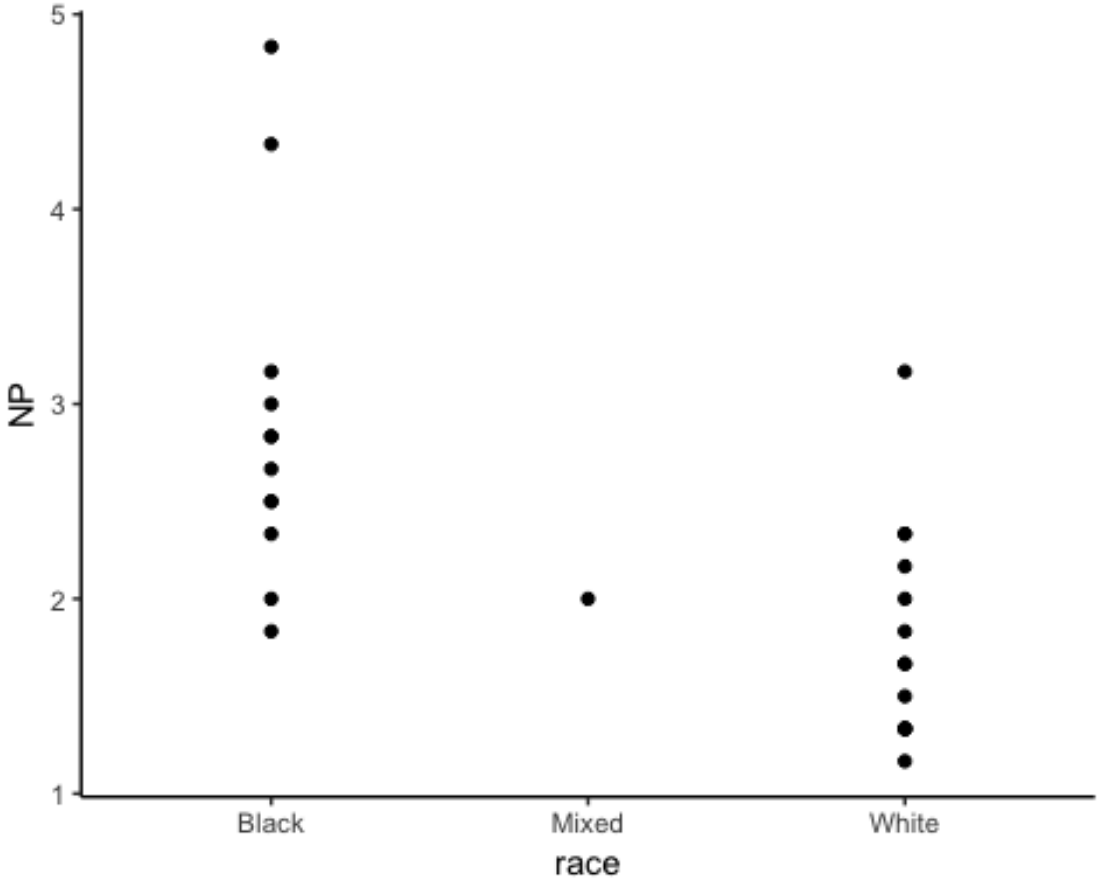
Figure 4: Our observed association between CTRA and NP cannot be parsimoniously reduced to an association between any CTRA subset - defined by agglomerative clustering as in Figure 2 - and NP.

Appendix

Chen and NP

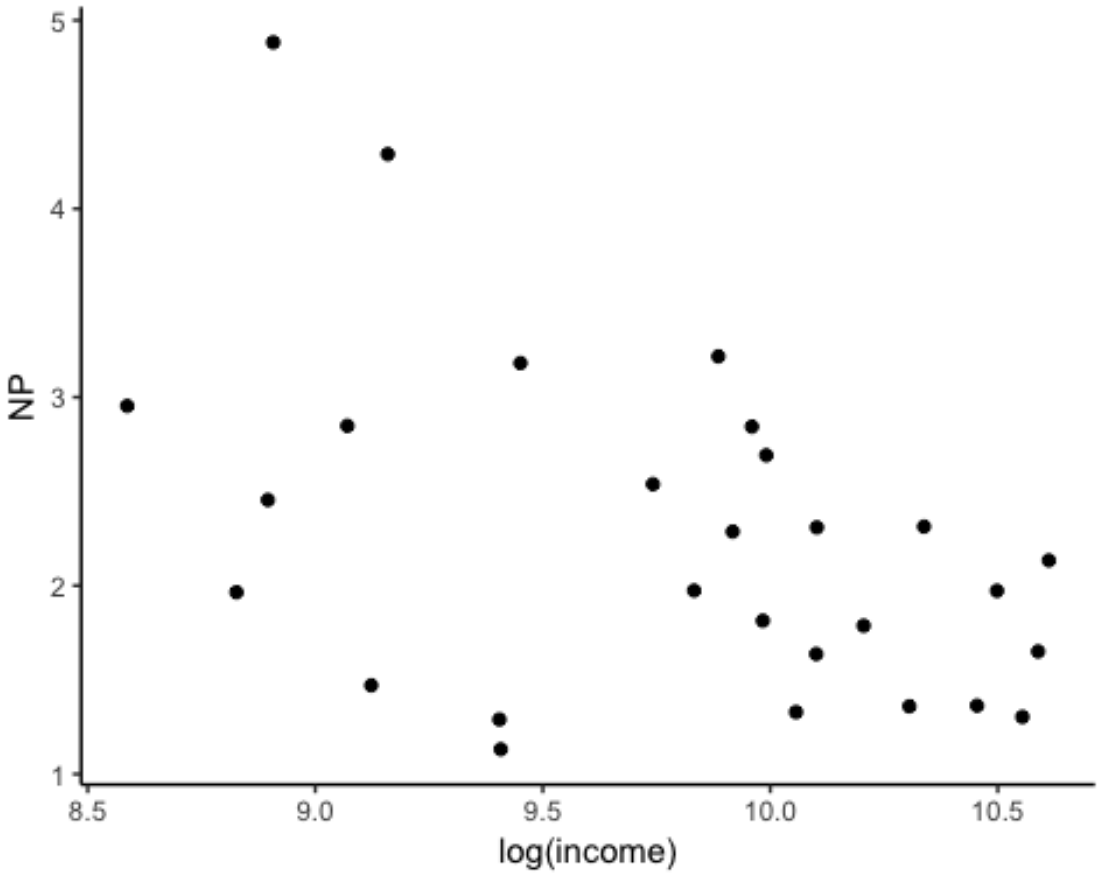


NP and race



NP and (log) income

28 subjects had both income and NP scores. There was a significant negative relation between income and NP in our data ($r(28) = -0.4632759$, $p = 0.0130358$), see Figure 0b.



Chen and CTRA

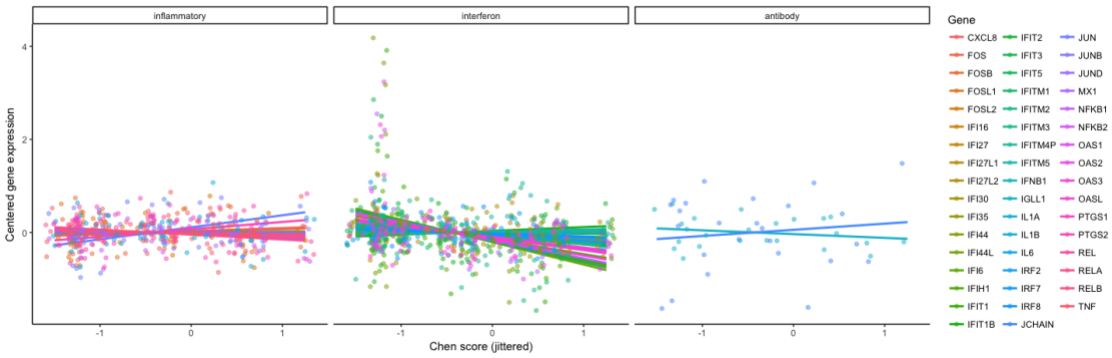


Figure : Linear effect of Chen on CTRA.

Table: Mass univariate regression of CTRA genes on chen (Limma in R).

	logFC	AveExpr	t	P.Value	adj.P.Val	B
IFIH1	-0.325	6.625	-3.001	0.006	0.290	-2.245
MX1	-0.389	6.870	-2.545	0.017	0.306	-3.037
IFIT3	-0.447	5.539	-2.442	0.022	0.306	-3.207

JUN	0.258	4.075	2.321	0.028	0.306	-3.402
IFI35	-0.176	6.507	-2.095	0.046	0.306	-3.751
IFI44	-0.420	5.437	-1.948	0.062	0.306	-3.963
IFIT5	-0.244	5.977	-1.946	0.062	0.306	-3.966
OAS1	-0.266	6.823	-1.909	0.067	0.306	-4.018
OASL	-0.251	6.025	-1.903	0.068	0.306	-4.027
OAS2	-0.226	6.871	-1.851	0.075	0.306	-4.098
IFI44L	-0.481	4.473	-1.842	0.077	0.306	-4.111
RELA	-0.105	5.462	-1.831	0.078	0.306	-4.126
OAS3	-0.346	5.605	-1.824	0.080	0.306	-4.135
IFITM2	-0.178	5.238	-1.752	0.091	0.326	-4.231
IFIT1	-0.408	4.687	-1.669	0.107	0.356	-4.337
PTGS2	0.158	5.019	1.587	0.124	0.388	-4.439
IRF7	-0.128	4.848	-1.388	0.177	0.520	-4.669
IFI6	-0.244	6.482	-1.315	0.200	0.555	-4.746
IFI30	-0.105	9.498	-1.210	0.237	0.585	-4.852
IRF2	-0.084	7.956	-1.189	0.245	0.585	-4.872
IFITM4P	-0.134	5.522	-1.171	0.252	0.585	-4.889
NFKB1	-0.086	6.222	-1.139	0.265	0.585	-4.918
IFI16	-0.083	8.707	-1.129	0.269	0.585	-4.928
IGLL1	-0.081	4.634	-0.965	0.343	0.716	-5.067
NFKB2	-0.064	6.105	-0.932	0.360	0.718	-5.093
IFIT2	-0.154	5.545	-0.906	0.373	0.718	-5.112
TNF	-0.057	4.802	-0.859	0.398	0.737	-5.146
IFITM3	-0.156	5.268	-0.797	0.433	0.754	-5.189
FOS	0.066	5.288	0.754	0.457	0.754	-5.216
IRF8	-0.061	6.214	-0.699	0.490	0.754	-5.250
RELB	-0.045	5.734	-0.684	0.500	0.754	-5.259
IL1B	-0.069	4.053	-0.674	0.506	0.754	-5.264
IFIT1B	0.081	4.825	0.671	0.508	0.754	-5.266
IFITM5	0.043	3.585	0.643	0.526	0.754	-5.281
JCHAIN	0.132	8.678	0.640	0.528	0.754	-5.283
CXCL8	0.053	2.849	0.499	0.622	0.864	-5.350
FOSL2	-0.042	7.254	-0.441	0.663	0.885	-5.373
PTGS1	-0.045	6.436	-0.427	0.673	0.885	-5.378
IFI27L2	0.028	4.709	0.380	0.707	0.906	-5.394

REL	-0.027	5.651	-0.354	0.726	0.908	-5.403
JUND	-0.014	6.079	-0.316	0.755	0.920	-5.413
IFITM1	-0.020	7.778	-0.274	0.786	0.928	-5.424
JUNB	-0.017	7.777	-0.191	0.850	0.928	-5.440
FOSL1	0.015	3.672	0.185	0.855	0.928	-5.441
IFI27	0.017	3.547	0.183	0.856	0.928	-5.442
IFI27L1	-0.010	4.061	-0.178	0.860	0.928	-5.442
IFNB1	0.012	2.488	0.163	0.872	0.928	-5.445
FOSB	-0.006	4.980	-0.060	0.953	0.987	-5.454
IL6	-0.002	3.105	-0.033	0.974	0.987	-5.455
IL1A	-0.001	2.542	-0.016	0.987	0.987	-5.456

Table: Regression of CTRA genes on chen, adjusting for sex and race.

	logFC	AveExpr	t	P.Value	adj.P.Val	B
JCHAIN	0.476	8.678	2.050	0.051	0.880	-4.009
IFIH1	-0.243	6.625	-1.809	0.083	0.880	-4.189
RELA	-0.127	5.462	-1.761	0.091	0.880	-4.223
IGLL1	-0.177	4.634	-1.740	0.094	0.880	-4.238
FOS	0.163	5.288	1.548	0.134	0.880	-4.368
IFITM2	-0.190	5.238	-1.482	0.151	0.880	-4.410
NFKB1	-0.127	6.222	-1.332	0.195	0.880	-4.501
IFI35	-0.138	6.507	-1.298	0.206	0.880	-4.521
IFI27L1	-0.087	4.061	-1.256	0.221	0.880	-4.544
IL1A	-0.097	2.542	-1.236	0.228	0.880	-4.555
IRF8	-0.118	6.214	-1.222	0.233	0.880	-4.562
OASL	-0.195	6.025	-1.175	0.251	0.880	-4.588
OAS1	-0.206	6.823	-1.172	0.252	0.880	-4.589
PTGS2	0.147	5.019	1.161	0.257	0.880	-4.595
MX1	-0.211	6.870	-1.144	0.264	0.880	-4.604
NFKB2	-0.087	6.105	-0.991	0.331	0.919	-4.677
IFIT3	-0.202	5.539	-0.930	0.362	0.919	-4.704
FOSB	-0.106	4.980	-0.852	0.403	0.919	-4.736
IFI44L	-0.276	4.473	-0.852	0.403	0.919	-4.737
OAS2	-0.122	6.871	-0.811	0.425	0.919	-4.752
IFI44	-0.209	5.437	-0.802	0.430	0.919	-4.756
JUN	0.099	4.075	0.764	0.452	0.919	-4.769
CXCL8	0.102	2.849	0.760	0.454	0.919	-4.771

IFITM5	0.059	3.585	0.750	0.460	0.919	-4.774
OAS3	-0.165	5.605	-0.710	0.485	0.919	-4.788
JUND	-0.035	6.079	-0.683	0.501	0.919	-4.797
IL1B	-0.071	4.053	-0.582	0.566	0.919	-4.827
IFI30	-0.061	9.498	-0.559	0.581	0.919	-4.833
IFITM3	-0.135	5.268	-0.545	0.591	0.919	-4.837
PTGS1	-0.070	6.436	-0.525	0.604	0.919	-4.842
TNF	-0.039	4.802	-0.474	0.640	0.919	-4.854
IFIT2	0.089	5.545	0.469	0.643	0.919	-4.855
IFIT5	-0.064	5.977	-0.444	0.661	0.919	-4.861
IFI27	-0.044	3.547	-0.403	0.690	0.919	-4.869
IFI6	-0.090	6.482	-0.397	0.694	0.919	-4.870
IFITM4P	-0.055	5.522	-0.394	0.697	0.919	-4.871
IL6	0.031	3.105	0.374	0.711	0.919	-4.874
IFI27L2	-0.029	4.709	-0.341	0.736	0.919	-4.880
IFNB1	-0.029	2.488	-0.315	0.756	0.919	-4.884
FOSL2	0.036	7.254	0.309	0.760	0.919	-4.885
IFITM1	-0.025	7.778	-0.288	0.776	0.919	-4.888
JUNB	0.031	7.777	0.269	0.790	0.919	-4.891
IFIT1B	0.037	4.825	0.269	0.790	0.919	-4.891
RELB	-0.018	5.734	-0.223	0.826	0.938	-4.896
IRF2	-0.016	7.956	-0.195	0.847	0.941	-4.899
IFIT1	-0.045	4.687	-0.163	0.872	0.948	-4.902
REL	0.013	5.651	0.134	0.894	0.951	-4.904
IFI16	-0.008	8.707	-0.090	0.929	0.952	-4.906
FOSL1	0.009	3.672	0.085	0.933	0.952	-4.907
IRF7	0.000	4.848	-0.002	0.998	0.998	-4.908

Table: Regression of CTRA genes on chen, adjusting for sex, race and BMI.

	logFC	AveExpr	t	P.Value	adj.P.Val	B
JCHAIN	0.560	8.678	2.321	0.030	0.815	-3.825
IFIH1	-0.273	6.625	-1.907	0.069	0.815	-4.134
IGLL1	-0.190	4.634	-1.781	0.088	0.815	-4.222
RELA	-0.131	5.462	-1.708	0.102	0.815	-4.271
IFITM2	-0.209	5.238	-1.655	0.112	0.815	-4.306
IFI35	-0.161	6.507	-1.513	0.144	0.815	-4.395
MX1	-0.268	6.870	-1.388	0.179	0.815	-4.469

IFIT3	-0.282	5.539	-1.365	0.186	0.815	-4.483
PTGS2	0.164	5.019	1.337	0.195	0.815	-4.498
IFI27L1	-0.098	4.061	-1.334	0.196	0.815	-4.500
NFKB1	-0.134	6.222	-1.325	0.199	0.815	-4.505
OAS1	-0.235	6.823	-1.276	0.215	0.815	-4.532
OASL	-0.211	6.025	-1.180	0.251	0.815	-4.582
FOS	0.119	5.288	1.169	0.255	0.815	-4.587
IRF8	-0.120	6.214	-1.157	0.259	0.815	-4.593
IL1A	-0.095	2.542	-1.154	0.261	0.815	-4.595
JUN	0.141	4.075	1.105	0.281	0.826	-4.619
IL6	0.080	3.105	1.066	0.298	0.827	-4.637
IFI44L	-0.328	4.473	-0.943	0.356	0.863	-4.691
IFI44	-0.251	5.437	-0.897	0.379	0.863	-4.710
OAS2	-0.140	6.871	-0.870	0.394	0.863	-4.721
IFITM5	0.071	3.585	0.848	0.405	0.863	-4.729
NFKB2	-0.078	6.105	-0.842	0.409	0.863	-4.732
IFI27	-0.088	3.547	-0.832	0.414	0.863	-4.736
OAS3	-0.186	5.605	-0.744	0.465	0.866	-4.767
FOSB	-0.092	4.980	-0.729	0.473	0.866	-4.772
CXCL8	0.092	2.849	0.715	0.482	0.866	-4.776
IFIT5	-0.100	5.977	-0.677	0.505	0.866	-4.788
IFITM1	-0.057	7.778	-0.641	0.528	0.866	-4.799
IFITM3	-0.149	5.268	-0.591	0.561	0.866	-4.813
IRF2	-0.046	7.956	-0.531	0.601	0.866	-4.829
IFITM4P	-0.076	5.522	-0.521	0.607	0.866	-4.831
IL1B	-0.065	4.053	-0.509	0.616	0.866	-4.834
IFI30	-0.058	9.498	-0.507	0.617	0.866	-4.835
REL	0.045	5.651	0.477	0.638	0.866	-4.842
JUND	-0.026	6.079	-0.472	0.642	0.866	-4.843
IFI6	-0.112	6.482	-0.459	0.651	0.866	-4.845
IFIT1	-0.130	4.687	-0.448	0.658	0.866	-4.848
IFI16	-0.035	8.707	-0.390	0.700	0.881	-4.859
TNF	-0.033	4.802	-0.380	0.708	0.881	-4.861
FOSL1	-0.038	3.672	-0.359	0.723	0.881	-4.864
IFIT2	0.063	5.545	0.315	0.755	0.896	-4.871
IFI27L2	-0.026	4.709	-0.286	0.777	0.896	-4.875

PTGS1	-0.034	6.436	-0.244	0.810	0.896	-4.881
RELB	-0.020	5.734	-0.230	0.820	0.896	-4.882
JUNB	0.025	7.777	0.224	0.825	0.896	-4.883
IFNB1	-0.018	2.488	-0.184	0.856	0.911	-4.887
FOSL2	0.014	7.254	0.130	0.898	0.935	-4.891
IFIT1B	0.012	4.825	0.088	0.930	0.944	-4.893
IRF7	-0.008	4.848	-0.071	0.944	0.944	-4.893

Negative parenting and CTRA

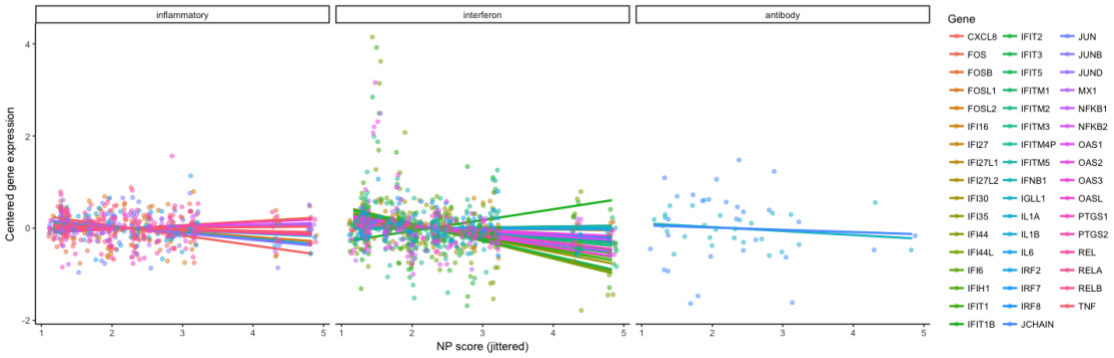


Figure : Linear effect of NP on CTRA.

Table: Mass univariate regression of CTRA genes on NP.

	logFC	AveExpr	t	P.Value	adj.P.Val	B
IFI30	-0.298	9.428	-3.374	0.002	0.099	-1.364
IFI27L2	-0.188	4.666	-3.127	0.004	0.099	-1.923
CXCL8	-0.215	2.744	-2.951	0.006	0.103	-2.308
IFIT3	-0.348	5.548	-2.292	0.029	0.284	-3.639
IFI35	-0.149	6.498	-2.232	0.033	0.284	-3.750
IFIT5	-0.212	5.998	-2.202	0.036	0.284	-3.804
IFIT1B	0.236	4.875	2.139	0.041	0.284	-3.917
IFI44	-0.363	5.458	-2.040	0.050	0.284	-4.090
IFITM1	-0.126	7.729	-2.034	0.051	0.284	-4.101
JUNB	-0.147	7.764	-1.960	0.060	0.298	-4.226
IFI44L	-0.380	4.507	-1.747	0.091	0.414	-4.565
OASL	-0.176	6.031	-1.667	0.106	0.420	-4.684
IFI6	-0.245	6.512	-1.651	0.109	0.420	-4.706
IL1B	-0.131	3.992	-1.572	0.127	0.432	-4.819
MX1	-0.200	6.925	-1.540	0.134	0.432	-4.863

OAS3	-0.237	5.683	-1.523	0.139	0.432	-4.887
FOSL2	-0.109	7.245	-1.456	0.156	0.432	-4.976
IRF7	-0.105	4.864	-1.431	0.163	0.432	-5.008
IL1A	-0.067	2.498	-1.427	0.164	0.432	-5.013
IFIT2	-0.184	5.551	-1.385	0.177	0.441	-5.067
IFIT1	-0.271	4.681	-1.322	0.196	0.467	-5.144
IRF8	-0.082	6.188	-1.156	0.257	0.553	-5.333
IFIH1	-0.116	6.689	-1.141	0.263	0.553	-5.348
IGLL1	-0.086	4.642	-1.135	0.266	0.553	-5.355
IFITM2	-0.102	5.168	-1.009	0.321	0.622	-5.481
FOSB	0.087	5.034	0.994	0.328	0.622	-5.494
IFITM4P	-0.095	5.520	-0.956	0.347	0.622	-5.528
IL6	-0.050	3.078	-0.953	0.349	0.622	-5.532
IFITM3	-0.147	5.322	-0.915	0.368	0.634	-5.565
JUND	0.029	6.079	0.837	0.410	0.653	-5.630
RELA	-0.044	5.478	-0.830	0.413	0.653	-5.635
OAS1	-0.098	6.824	-0.821	0.418	0.653	-5.642
PTGS1	0.073	6.540	0.714	0.481	0.677	-5.719
NFKB2	-0.040	6.107	-0.707	0.485	0.677	-5.724
FOS	-0.050	5.257	-0.686	0.498	0.677	-5.738
NFKB1	0.043	6.217	0.671	0.507	0.677	-5.748
REL	-0.041	5.643	-0.655	0.518	0.677	-5.758
PTGS2	-0.057	5.030	-0.651	0.520	0.677	-5.760
OAS2	-0.065	6.868	-0.619	0.541	0.677	-5.780
TNF	-0.033	4.822	-0.618	0.541	0.677	-5.780
IFITM5	0.020	3.563	0.371	0.713	0.851	-5.897
JUN	0.033	4.069	0.339	0.737	0.851	-5.908
IFI27L1	-0.014	4.055	-0.298	0.768	0.851	-5.921
IFI16	-0.017	8.720	-0.282	0.780	0.851	-5.925
RELB	0.017	5.773	0.279	0.782	0.851	-5.926
JCHAIN	-0.049	8.682	-0.278	0.783	0.851	-5.926
IFI27	0.020	3.600	0.251	0.804	0.855	-5.933
IRF2	-0.010	7.952	-0.178	0.860	0.882	-5.948
FOSL1	0.013	3.696	0.169	0.867	0.882	-5.950
IFNB1	0.009	2.461	0.149	0.882	0.882	-5.953

Table: Regression of CTRA genes on NP, adjusting for sex and race.

	logFC	AveExpr	t	P.Value	adj.P.Val	B
IFI27L2	-0.210	4.666	-3.413	0.002	0.054	-1.320
IFI30	-0.315	9.428	-3.384	0.002	0.054	-1.387
CXCL8	-0.236	2.744	-3.112	0.004	0.072	-1.992
IFIT1B	0.283	4.875	2.655	0.013	0.163	-2.956
IFIT3	-0.351	5.548	-2.428	0.022	0.220	-3.404
IFI35	-0.155	6.498	-2.321	0.028	0.233	-3.607
IFIT5	-0.186	5.998	-2.079	0.047	0.336	-4.042
JUNB	-0.152	7.764	-1.912	0.066	0.415	-4.323
IFI44	-0.324	5.458	-1.844	0.076	0.416	-4.431
IRF8	-0.122	6.188	-1.797	0.083	0.416	-4.504
IFITM1	-0.103	7.729	-1.635	0.113	0.445	-4.748
IFI44L	-0.352	4.507	-1.626	0.115	0.445	-4.760
MX1	-0.192	6.925	-1.625	0.116	0.445	-4.762
OASL	-0.168	6.031	-1.532	0.137	0.472	-4.892
OAS3	-0.231	5.683	-1.492	0.147	0.472	-4.946
FOSL2	-0.117	7.245	-1.478	0.151	0.472	-4.965
IL1A	-0.071	2.498	-1.432	0.163	0.480	-5.024
IFI6	-0.200	6.512	-1.353	0.187	0.509	-5.124
IRF7	-0.097	4.864	-1.333	0.194	0.509	-5.149
IL1B	-0.097	3.992	-1.154	0.258	0.616	-5.353
IFITM2	-0.119	5.168	-1.117	0.274	0.616	-5.391
IFIT1	-0.207	4.681	-1.113	0.275	0.616	-5.395
IFIT2	-0.144	5.551	-1.094	0.283	0.616	-5.415
IGLL1	-0.079	4.642	-0.989	0.331	0.672	-5.517
IFIH1	-0.094	6.689	-0.980	0.336	0.672	-5.526
FOSB	0.087	5.034	0.938	0.356	0.676	-5.563
RELA	-0.051	5.478	-0.908	0.372	0.676	-5.589
IL6	-0.050	3.078	-0.895	0.378	0.676	-5.600
TNF	-0.047	4.822	-0.832	0.413	0.692	-5.652
IFITM4P	-0.083	5.520	-0.794	0.434	0.692	-5.681
IFI27	0.058	3.600	0.762	0.452	0.692	-5.704
PTGS1	0.079	6.540	0.750	0.459	0.692	-5.713
PTGS2	-0.066	5.030	-0.718	0.479	0.692	-5.735
NFKB1	0.048	6.217	0.710	0.484	0.692	-5.741

OAS1	-0.088	6.824	-0.709	0.484	0.692	-5.742
NFKB2	-0.040	6.107	-0.663	0.513	0.697	-5.772
REL	-0.043	5.643	-0.641	0.527	0.697	-5.785
IFITM3	-0.107	5.322	-0.636	0.530	0.697	-5.788
OAS2	-0.058	6.868	-0.574	0.571	0.717	-5.825
FOS	-0.043	5.257	-0.569	0.574	0.717	-5.827
JUND	0.020	6.079	0.537	0.595	0.726	-5.844
FOSL1	0.023	3.696	0.301	0.765	0.911	-5.939
IFI16	0.013	8.720	0.231	0.819	0.930	-5.958
IFI27L1	-0.009	4.055	-0.184	0.856	0.930	-5.967
JUN	0.015	4.069	0.168	0.868	0.930	-5.970
IRF2	0.009	7.952	0.161	0.873	0.930	-5.971
JCHAIN	-0.030	8.682	-0.160	0.874	0.930	-5.971
IFITM5	-0.007	3.563	-0.120	0.906	0.943	-5.976
RELB	0.003	5.773	0.042	0.967	0.986	-5.983
IFNB1	0.001	2.461	0.014	0.989	0.989	-5.983

Table: Regression of CTRA genes on NP, adjusting for sex, race and BMI.

	logFC	AveExpr	t	P.Value	adj.P.Val	B
IFI27L2	-0.200	4.666	-2.310	0.029	0.487	-3.759
IFI44	-0.521	5.458	-2.170	0.040	0.487	-3.880
IFI30	-0.272	9.428	-2.087	0.047	0.487	-3.950
MX1	-0.315	6.925	-1.970	0.060	0.487	-4.045
IRF8	-0.183	6.188	-1.954	0.062	0.487	-4.059
IFI44L	-0.574	4.507	-1.936	0.064	0.487	-4.073
CXCL8	-0.193	2.744	-1.894	0.070	0.487	-4.107
IFIT3	-0.349	5.548	-1.834	0.078	0.487	-4.153
OAS1	-0.291	6.824	-1.776	0.088	0.487	-4.197
JUN	0.184	4.069	1.533	0.138	0.581	-4.373
OAS3	-0.311	5.683	-1.436	0.163	0.581	-4.437
IFIT5	-0.171	5.998	-1.412	0.170	0.581	-4.452
REL	-0.119	5.643	-1.406	0.172	0.581	-4.456
IFI6	-0.287	6.512	-1.387	0.178	0.581	-4.469
OASL	-0.209	6.031	-1.353	0.188	0.581	-4.490
IFITM1	-0.117	7.729	-1.337	0.193	0.581	-4.500
IFIT1B	0.195	4.875	1.323	0.198	0.581	-4.508
IFIT1	-0.316	4.681	-1.239	0.227	0.583	-4.558

IFI35	-0.110	6.498	-1.213	0.236	0.583	-4.572
IFIH1	-0.160	6.689	-1.200	0.241	0.583	-4.580
IFITM3	-0.274	5.322	-1.191	0.245	0.583	-4.585
IL6	-0.076	3.078	-1.130	0.269	0.612	-4.619
RELA	-0.067	5.478	-0.847	0.405	0.852	-4.753
IFITM5	0.057	3.563	0.761	0.453	0.852	-4.786
OAS2	-0.106	6.868	-0.749	0.461	0.852	-4.791
IRF7	-0.074	4.864	-0.728	0.474	0.852	-4.799
PTGS2	0.086	5.030	0.714	0.482	0.852	-4.804
NFKB1	0.064	6.217	0.678	0.504	0.852	-4.816
TNF	-0.053	4.822	-0.676	0.505	0.852	-4.816
PTGS1	0.097	6.540	0.655	0.519	0.852	-4.824
IGLL1	-0.069	4.642	-0.618	0.542	0.852	-4.835
IFI27	-0.062	3.600	-0.613	0.546	0.852	-4.837
JUNB	-0.059	7.764	-0.559	0.581	0.873	-4.853
JUND	0.027	6.079	0.523	0.606	0.873	-4.862
NFKB2	-0.043	6.107	-0.515	0.611	0.873	-4.864
IL1B	0.053	3.992	0.480	0.635	0.882	-4.873
IFITM2	0.059	5.168	0.417	0.680	0.915	-4.887
IFI16	0.030	8.720	0.396	0.695	0.915	-4.892
IFIT2	-0.058	5.551	-0.322	0.750	0.951	-4.905
IL1A	-0.021	2.498	-0.308	0.760	0.951	-4.907
FOSL2	0.027	7.245	0.274	0.786	0.959	-4.912
IRF2	0.017	7.952	0.225	0.824	0.961	-4.918
IFITM4P	0.031	5.520	0.221	0.827	0.961	-4.919
RELB	0.013	5.773	0.145	0.886	0.978	-4.926
IFNB1	0.009	2.461	0.108	0.915	0.978	-4.928
JCHAIN	0.027	8.682	0.102	0.919	0.978	-4.929
FOSL1	-0.008	3.696	-0.078	0.938	0.978	-4.930
FOSB	0.010	5.034	0.077	0.939	0.978	-4.930
FOS	-0.003	5.257	-0.035	0.972	0.992	-4.931
IFI27L1	0.000	4.055	-0.005	0.996	0.996	-4.931

ACME

Table: ACME of NP on CTRA, via Chen.

	2.5%	97.5%

IL1A	-0.0492691	0.1226263
IL1B	-0.2292277	0.0999527
IL6	-0.1210280	0.1166459
CXCL8	-0.0736483	0.2732447
TNF	-0.1445491	0.0562754
PTGS1	-0.1932632	0.1559906
PTGS2	-0.0350711	0.3398224
FOS	-0.0639153	0.1694459
FOSB	-0.2043235	0.1479612
FOSL1	-0.1652599	0.1806089
FOSL2	-0.1481825	0.1198619
JUN	-0.0164303	0.4133509
JUNB	-0.0862870	0.1294175
JUND	-0.0622679	0.0180468
NFKB1	-0.2157946	0.0147637
NFKB2	-0.1948588	0.0563514
REL	-0.1191951	0.1028080
RELA	-0.1949663	0.0330904
RELB	-0.1669905	0.0575605
IFI16	-0.1682092	0.0188644
IFI27	-0.1854050	0.1873231
IFI27L1	-0.0929109	0.0981262
IFI27L2	-0.0371968	0.1049537
IFI30	-0.1594560	0.0765764
IFI35	-0.2018739	0.0202945
IFI44	-0.5666978	0.1349518
IFI44L	-0.6984903	0.1324819
IFI6	-0.3766706	0.1287165
IFIH1	-0.4163483	0.0245304
IFIT1	-0.6507310	0.1106404
IFIT2	-0.3665873	0.1825211
IFIT3	-0.6060644	0.0681605
IFIT5	-0.2659560	0.0300885
IFIT1B	-0.1897296	0.1455182
IFITM1	-0.1056862	0.0778914
IFITM2	-0.2654835	0.0253557

IFITM3	-0.4447370	0.1407896
IFITM4P	-0.3049322	0.0727289
IFITM5	-0.0608937	0.0968012
IFNB1	-0.1230881	0.1269578
IRF2	-0.1768799	0.0097599
IRF7	-0.2096993	0.0484995
IRF8	-0.1525945	0.0351236
MX1	-0.5053552	0.0275972
OAS1	-0.4666046	0.0599672
OAS2	-0.3319225	0.0193999
OAS3	-0.5234441	0.0546053
OASL	-0.3529898	0.0445261
JCHAIN	-0.2314463	0.5212531
IGLL1	-0.1449014	0.0793230

References

Baron, Reuben M, and David A Kenny. 1986. "The Moderator-Mediator Variable Distinction in Social Psychological Research: Conceptual, Strategic, and Statistical Considerations." *Journal of Personality and Social Psychology* 51 (6): 1173–82. http://webcom.upmf-grenoble.fr/LIP/Perso/DMuller/GSERM/Articles/Journal of Personality and Social Psychology 1986 Baron.pdf.

Bolstad, B M, R A Irizarry, M Astrand, and T P Speed. 2003. "A comparison of normalization methods for high density oligonucleotide array data based on variance and bias." *Bioinformatics* 19 (2): 185–93. http://www.stat.berkeley.edu/bolstad/normalize/.

Bullock, John G., Donald P. Green, and Shang E. Ha. 2010. "Yes, but what's the mechanism? (don't expect an easy answer)." *Journal of Personality and Social Psychology* 98 (4). American Psychological Association: 550–58. doi:10.1037/a0018933.

Goeman, Jelle J, and Peter Bühlmann. 2007. "Analyzing gene expression data in terms of gene sets: methodological issues." *Bioinformatics (Oxford, England)* 23 (8). Oxford University Press: 980–7. doi:10.1093/bioinformatics/btm051.

Goeman, Jelle J., Sara A. van de Geer, and Hans C. van Houwelingen. 2006. "Testing against a high dimensional alternative." *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* 68 (3). Blackwell Publishing Ltd: 477–93. doi:10.1111/j.1467-9868.2006.00551.x.

Greenland, Sander, and James M Robins. 1986. "Identifiability, Exchangeability, and Epidemiological Confounding." *International Journal of Epidemiology* 15 (3): 413–19. doi:10.1093/ije/15.3.413.

Imai, Kosuke, Luke Keele, and Teppei Yamamoto. 2010. “Identification, Inference and Sensitivity Analysis for Causal Mediation Effects.” *Statistical Science* 25 (1): 51–71. doi:10.1214/10-STS321.

Irizarry, Rafael A, Benjamin M Bolstad, Francois Collin, Leslie M Cope, Bridget Hobbs, and Terence P Speed. 2003. “Summaries of Affymetrix GeneChip probe level data.” *Nucleic Acids Research* 31 (4). Oxford University Press: e15. http://www.ncbi.nlm.nih.gov/pubmed/12582260.

Kaufman, Jay S, Richard F Maclehose, and Sol Kaufman. 2004. “A further critique of the analytic strategy of adjusting for covariates to identify biologic mediation.” *Epidemiologic Perspectives & Innovations* 1 (4). doi:10.1186/1742-5573-1-4.

Keele, Luke. 2015. “Causal Mediation Analysis.” *American Journal of Evaluation* 36 (4). SAGE PublicationsSage CA: Los Angeles, CA: 500–513. doi:10.1177/1098214015594689.

Kraemer, Helena, Eric Stice, Alan Kazdin, David Offord, and David Kupfer. 2001. “How Do Risk Factors Work Together? Mediators, Moderators, and Independent, Overlapping, and Proxy Risk Factors.” *Am J Psychiatry* 1586 (158): 848–56. http://ajp.psychiatryonline.org/doi/pdf/10.1176/appi.ajp.158.6.848.

MacKinnon, David P., Chondra M. Lockwood, Jeanne M. Hoffman, Stephen G. West, and Virgil Sheets. 2002. “A comparison of methods to test mediation and other intervening variable effects.” *Psychological Methods* 7 (1). American Psychological Association: 83–104. doi:10.1037/1082-989X.7.1.83.

Pearl, Judea. 2009. “Causal inference in statistics: An overview.” *Statistics Surveys* 3: 96–146. doi:10.1214/09-SS057.

———. 2014. “Interpretation and identification of causal mediation.” *Psychological Methods* 19 (4): 459–81. doi:10.1037/a0036434.

Preacher, Kristopher J., and Andrew F. Hayes. 2004. “SPSS and SAS procedures for estimating indirect effects in simple mediation models.” *Behavior Research Methods, Instruments, & Computers* 36 (4). Springer-Verlag: 717–31. doi:10.3758/BF03206553.

Rubin, Donald B. 1974. “Estimating causal effects of treatments in randomized and nonrandomized studies.” *Journal of Educational Psychology* 66 (5): 688–701. doi:10.1037/h0037350.

Smyth, Gordon, Natalie Thorne, and James Wettenhall. 2005. “Limma: linear models for microarray data.” In *Bioinformatics and Computational Biology Solutions Using R and Bioconductor.*, 397–420. Springer New York.

Sobel, Michael E. 1982. “Asymptotic Confidence Intervals for Indirect Effects in Structural Equation Models.” *Sociological Methodology* 13: 290. doi:10.2307/270723.

Subramanian, Aravind, Pablo Tamayo, Vamsi K Mootha, Sayan Mukherjee, Benjamin L Ebert, Michael A Gillette, Amanda Paulovich, et al. 2005. “Gene set enrichment analysis: a

knowledge-based approach for interpreting genome-wide expression profiles." *Proceedings of the National Academy of Sciences of the United States of America* 102 (43). National Academy of Sciences: 15545–50. doi:10.1073/pnas.0506580102.