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## **Identification of Influential Variants in Significant Aggregate Rare Variant Tests**

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**Running title:** Identification of Influential Rare Variants

**Keywords:** rare variants, localization, genetic association tests, idiopathic pulmonary fibrosis

1 **Abstract**

2

3 Introduction: Studies that examine the role of rare variants in both simple and complex  
4 disease are increasingly common. Though the usual approach of testing rare variants in  
5 aggregate sets is more powerful than testing individual variants, it is of interest to  
6 identify the variants that are plausible drivers of the association. We present a novel  
7 method for prioritization of rare variants after a significant aggregate test by quantifying  
8 the influence of the variant on the aggregate test of association.

9

10 Methods: In addition to providing a measure used to rank variants, we use outlier  
11 detection methods to present the computationally efficient Rare Variant Influential  
12 Filtering Tool (RIFT) to identify a subset of variants that influence the disease  
13 association. We evaluated several outlier detection methods that vary based on the  
14 underlying variance measure: interquartile range (Tukey fences), median absolute  
15 deviation and standard deviation. We performed 1000 simulations for 50 regions of size  
16 3kb and compared the true and false positive rates. We compared RIFT using the Inner  
17 Tukey to two existing methods: adaptive combination of p-values (ADA) and a Bayesian  
18 hierarchical model (BeviMed). Finally, we applied this method to data from our targeted  
19 resequencing study in idiopathic pulmonary fibrosis (IPF).

20

21 Results: All outlier detection methods observed higher sensitivity to detect uncommon  
22 variants ( $0.001 < \text{MAF} < 0.03$ ) compared to very rare variants ( $\text{MAF} < 0.001$ ). For  
23 uncommon variants, RIFT had a lower median false positive rate compared to the ADA.

24 ADA and RIFT had significantly higher true positive rates than that observed for  
25 BeviMed. When applied to two regions found previously associated with IPF including  
26 100 rare variants, we identified six polymorphisms with the greatest evidence for  
27 influencing the association with IPF.

28

29 Discussion: In summary, RIFT has a high true positive rate while maintaining a low false  
30 positive rate for identifying polymorphisms influencing rare variant association tests.

31 This work provides an approach to obtain greater resolution of the rare variant signals  
32 within significant aggregate sets; this information can provide an objective measure to  
33 prioritize variants for follow-up experimental studies and insight into the biological  
34 pathways involved.

## 1 **Introduction**

2

3 The number of studies that investigate the role of rare genetic variants in complex  
4 diseases has been steadily increasing. This is in part due to the hypothesis that rare  
5 variant effects might account for the discrepancy between estimated heritability for  
6 complex traits and that accounted for by common variant associations [1]. In addition,  
7 sequencing technologies have become less expensive such that more studies have the  
8 ability to study rare variation in large numbers of individuals or families. A study of  
9 circulating adiponectin levels found that among Hispanics, low frequency variants  
10 explained more variation in the trait than common variants [2]. Rare variants have been  
11 shown to have modest [3] or large effect sizes [1,4]. Rare variants can be associated  
12 with increased [5] or decreased risk of disease [6]. Often rare variants influencing a trait  
13 are only observed in a few families [7]. Thus, rare variants exhibit a range of association  
14 patterns and can exhibit an important contribution to human trait variation.

15

16 Studies of rare variation often require either whole genome sequencing or targeted  
17 sequencing on large study populations. Even with large sample sizes, there often  
18 remains insufficient power for testing rare and uncommon variants individually [8,9].  
19 This is due to the low frequency of observations as well as the higher testing burden, as  
20 there are far more rare variants compared to common variants in the genome. To  
21 overcome this issue, methods for testing rare/uncommon variants often involve  
22 aggregating information across a genomic region [10]. Variants may be grouped into  
23 sets based on genomic information (e.g. gene bodies, exons), linkage disequilibrium

1 blocks, association windows, or into overlapping sliding windows of a fixed size in terms  
2 of physical distance or number of rare variants included. Methods for testing rare  
3 variants in aggregate fall into three main types: burden tests, variance component tests,  
4 and combined burden and variance component tests. The underlying assumption of  
5 burden tests is that the effects of the rare variants within a given set are in the same  
6 direction. Variance component tests ease this constraint and have higher power to  
7 detect disease association with a mixture of protective and deleterious rare variants.  
8 Given the underlying model by which rare variants are associated with disease in a  
9 given region is unknown, tests which combine the burden and variance component  
10 approaches weight the given contributions of the burden and variance components to  
11 improve power.

12  
13 Once a set of variants is found to be associated with a phenotype of interest, a logical  
14 next step is the identification of the plausible drivers of the association that might be the  
15 best statistical candidates for functional studies. Experimental validation of all rare  
16 variants in a significant set is generally unreasonable based on time and expense. The  
17 ability to narrow down the list of rare variants to those most likely to be contributing to  
18 the association signal could help target experimental validation. Additionally, reporting  
19 the rare variants most likely to be causal within a significant set of variants focuses  
20 functional efforts and can aid in comparison of results across studies.

21  
22 Several methods have been proposed for statistically identifying the most likely causal  
23 variants [11–15]. One method uses a classic backward elimination procedure; a variant

1 is removed from the set if its removal decreases the aggregate test p-value, and this  
2 process is repeated until no improvement in the p-value from removing a variant is  
3 observed [11]. Related stepwise procedures could be envisioned that use either the p-  
4 value or various information criteria (e.g., Bayes information criteria, Akaike information  
5 criterion). Another method considers the problem of prioritizing variants in aggregate  
6 tests as a variable selection problem using kernel machine methods [16]. Although this  
7 method was developed for prioritization of common variants rather than rare variants,  
8 conceptually it is reasonable for rare variants. There are also previously published  
9 methods which test each variant individually, such as using a Fisher's Exact Test and  
10 applying the adaptive combination of p-values procedure (referred to as ADA) to the  
11 resulting p-values [12]. This method has been shown to be more powerful than the  
12 backward elimination method proposed by Ionita-Laza, et. al., 2014. Most recently, the  
13 BeviMed method applies a Bayesian hierarchical model and makes inference on  
14 whether a rare variant is causal based on power posteriors [15]. Methods like the  
15 backward elimination method and adaptive combination of p-values are iterative in  
16 nature and therefore computationally intensive. In addition, the Bayesian approach is  
17 developed for genome-wide testing of rare variant associations in rare Mendelian  
18 disorders under specific (unknown) modes of inheritance rather than being more  
19 broadly applicable.

20

21 We present a novel method for prioritization of rare variants within a given set of  
22 variants after the set of variants is found to be significant using aggregate testing  
23 methods. Building on the rich outlier-detection statistical literature, we present a

1 computationally efficient approach to be applied following identification of a set of  
2 variants that is agnostic to putative function. Our approach, which we refer to as RIFT  
3 for Rare Variant Influential Filtering Tool, leverages the influence of the variant on the  
4 aggregate test of association by quantifying the change in the aggregate test when that  
5 variant is removed. It is particularly well suited for rare and uncommon variants, the  
6 most common applications of aggregate tests, but is applicable to aggregate testing of  
7 variants of all frequencies. When applied to a significant set of rare/uncommon variants,  
8 RIFT provides a scheme for quantifying the contribution of an individual variant to the  
9 overall association signal, while adjusting for covariates. This method also provides a  
10 quantitative measure by which to rank variants for further investigation and several  
11 visualizations to aid in evaluation of a region of interest.

12

## 13 **Methods**

14

### 15 *Overview*

16 We present the Rare Variant Influential Filtering Tool (RIFT) to quantify the effect of  
17 each variant on an aggregate test of association (Figure 1). In our simulations, we  
18 consider variants with a minor allele frequency (MAF) of <3% (i.e., rare and  
19 uncommon), but note that the method is directly applicable to any set of variants,  
20 including common variants or mixtures of rare and common variants. First, we describe  
21 our jackknife (leave-one-out) approach to obtain a score for each variant when applied  
22 to a set of rare variants from an aggregate test. We then describe several outlier  
23 detection methods that aim to identify influential variants (IVs) when applied to the



1 variant scores within a set. We perform simulations to evaluate the jackknife scores and  
2 the ability of the outlier detection methods to identify variants simulated to be associated  
3 with the outcome. Finally, we apply RIFT to recently published rare variant regions  
4 found to be significantly associated with idiopathic pulmonary fibrosis [17].

5

### 6 *Localization Approach*

7 For a given set of rare variants, we define the p-value resulting from the aggregate test  
8 as  $p_{(0)}$ . For rare variant  $j$ , we calculate the p-value from the aggregate test of the  
9 variants within the set excluding variant  $j$  and refer to this p-value as  $p_{(-j)}$ . P-values are  
10 transformed into chi-square statistics (df = 1) using the inverse cumulative distribution  
11 function (CDF) of the chi-square distribution (Equation 1), where  $\chi^2_{(-j)}$  corresponds to  
12  $p_{(-j)}$  and  $\chi^2_{(0)}$  corresponds to  $p_{(0)}$ . For each rare variant  $j$ , we calculate a delta chi-  
13 square score, denoted  $\Delta\chi^2_{(-j)}$ , which represents the change of the chi-square statistic  
14 when the rare variant  $j$  is removed (Equation 2). For variant  $j$ ,  $\Delta\chi^2_{(-j)}$  provides a relative  
15 measure of how the results of the aggregate test compare with and without variant  $j$ .  
16 Larger negative values indicate larger contributions to the overall test statistic.

17

18 Equation 1: Inverse CDF function of the chi-square distribution (df = 1) can be written in  
19 terms of the inverse CDF of the normal distribution,  $N(0,1)$  denoted as  $\Phi^{-1}$ .

$$20 \quad \chi^2_{(-j)} = \left[ \Phi^{-1} \left( \frac{p_{(-j)} + 1}{2} \right) \right]^2 \quad (1)$$

21 Equation 2: Delta chi-square score

$$22 \quad \Delta\chi^2_{(-j)} = \chi^2_{(-j)} - \chi^2_{(0)} \quad (2)$$

1

## 2 *Outlier Detection for Identifying IVs*

3 The delta chi-square score provides a quantitative measure to rank the rare variants  
4 within a significant set according to the impact of that variant on the aggregate test of  
5 association. In addition to ranking, it is also desirable to identify the subset of  
6 polymorphisms influencing the set's statistical association. We considered this as an  
7 outlier detection objective, whereby unusually large delta chi-square scores across a  
8 significant set of variants correspond to the set of association influencing  
9 polymorphisms. Sample values more extreme than a pre-specified cutoff are  
10 determined to be outliers. Outlier detection methods are rooted in using an estimate of  
11 the sample variance that is robust to outliers, and robust measures of spread are often  
12 applied for that purpose. We considered two non-parametric approaches to identifying  
13 outliers and compared these to a parametric approach.

14

## 15 *Non-parametric variance estimation approaches*

16 In 1977, Tukey defined the commonly-known descriptive univariate boxplot that displays  
17 the interquartile range (IQR) as a measure of spread (IQR = distance between the first  
18 [Q1] and third [Q3] quartiles; [18]). Boundaries based on the IQR are referred to as  
19 "fences", and observations lying outside the fences are considered outliers. The inner  
20 fence is defined by  $Q1 - 1.5 \cdot IQR$  and  $Q3 + 1.5 \cdot IQR$ ; the outer fence is defined by  $Q1 -$   
21  $3 \cdot IQR$  and  $Q3 + 3 \cdot IQR$ . Note that the outer fence boundary is further away from the  
22 median than that of the inner fence boundary, and is therefore more conservative in  
23 classification of outliers. The IQR is robust to extreme values in the data and therefore

1 identification of outliers using the IQR are superior to methods that rely on parametric  
2 variance estimators in the presence of outliers [19]. Additionally, the IQR and  
3 corresponding Tukey fences do not make any distributional assumptions and have been  
4 shown to be effective as long as the data are not highly skewed [19].

5  
6 Another measure of spread that does not assume a parametric distribution for the data  
7 is the median absolute deviation (MAD). Identification of outliers based on the MAD is  
8 provided in Equation 3 (further details can be found in Leys, Ley, Klein, Bernard, &  
9 Licata, 2013). Similar to the IQR, the MAD as a measure of spread is robust to extreme  
10 values compared to the standard deviation, which is greatly influenced by extreme  
11 values. The MAD requires specification of a constant and a cutoff; as others have used  
12 for criteria in outlier detection, we set the constant corresponding to the normal  
13 approximation ( $b = 1.4826$ ) and a cutoff value of three; observations more than three  
14 MAD away from the median are considered outliers [21,22].

15 Equation 3:

$$16 \quad MAD = b \cdot \text{median}(|X_j - \tilde{X}|); \tilde{X} = \text{median}(X) \quad (3)$$

17  
18 For completeness, we compare the cutoffs based on both the Inner and Outer Tukey  
19 fences and MAD to that of the standard deviation (SD), in which observations more  
20 extreme than three standard deviations from the mean are considered outliers. This  
21 method is often referred to as the three sigma rule [23]. Given that we expect causal  
22 variants to reduce the chi-square statistic when removed, we further limited  
23 classification of IVs to variants having a negative delta chi-square score.

1

## 2 *Simulated Data*

3 We follow a previously developed simulation approach to generate rare variant data  
4 with a binary outcome [24,25]. Specifically, to generate case-control rare variant data  
5 under both null and alternative hypotheses, we simulated 10,000 haplotypes for a 1MB  
6 genomic region under a coalescent model with parameters consistent with a European  
7 population using the software package COSI [26]. We considered 50 different genomic  
8 regions of size 3kb and included only rare variants with  $MAF < 0.03$ . To generate  
9 samples of cases and controls from the population, we repeatedly selected two  
10 haplotypes at random and converted these haplotype data to genotypes at each variant  
11 location. We determined the probability of subject  $k$  being a case, defined as  $p_k$ , based  
12 on a logistic regression equation where  $\beta_k$  represents the coefficient for variant  $k$   
13 (Equation 4).  $G_{jk}$  corresponds to number of minor alleles carried by subject  $k$  for variant  $j$   
14 and  $\beta_0$  the disease prevalence. For all simulations, the disease prevalence was fixed at  
15 0.05. Case status,  $Y_k$ , is specified using the Bernoulli ( $p_k$ ) distribution.

16 Equation 4:

$$17 \quad \text{logit}(p_k) = \beta_0 + \sum_{j=1}^J \beta_j G_{jk} \quad (4)$$

$$18 \quad Y_k \sim \text{Bernoulli}(p_k)$$

19 Consistent with previously published simulation of rare variant data, we defined the  
20 coefficient for variants under the alternative (Equation 5) to be a function of the  
21 population minor allele frequency ( $MAF_j$ ) for variant  $j$  [24,25]. This relationship between  
22 the coefficient and the population MAF results in larger odds ratios for more rare

1 variants. The constant,  $c$ , defines the strength of association between the causal  
2 variants and the outcome.

3 Equation 5:

$$4 \quad \beta_j = c |\log_{10} MAF_j| \quad (5)$$

5 With  $c$  set to 0.4, this corresponds to an odds ratio of 3.32 for a variant with a MAF of  
6 0.001 and 7.39 for a variant with a MAF of 0.00001. After determining case status for a  
7 large sample of individuals, we sub-sampled to obtain a specified number of cases and  
8 controls. As expected, the sub-sampling of the population results in some variants (both  
9 under the null and alternative) to be no longer observed in a given sample.

10

11 Note that our approach can be applied to any such aggregate test (for a review of  
12 methods for rare variant aggregate tests, please see Seunggeung Lee, Abecasis,  
13 Boehnke, & Lin, 2014). For exposition of the method, we use a combined burden and  
14 variance component test, the Sequence Kernel Association Optimal Unified Test  
15 (SKAT-O), due to its popularity in rare variant analyses [25]. We used the  
16 recommended settings – including a linear weighted kernel, estimation of p-values using  
17 the “davies” method and the variant weights as a function of the MAF using the  
18 Beta(1,25) distribution. If the SKAT-O p-value met the significance level (here, alpha  
19 level of 0.05), we then applied our localization method to obtain a chi-square statistic  
20 and delta chi-square score for each variant. We considered the proportion of the total  
21 1000 samples with a SKAT-O p-value that met the significance as an estimate of the  
22 power of SKAT-O for that region. SKAT-O performs a grid search to determine the  
23 optimal value of the parameter  $\rho$  that weights the relative influence of the burden and

1 variance statistic. The parameter  $\rho$  determined to be optimal for the full data was then  
2 fixed when calculating the leave-one-out p-values. This insured that within a significant  
3 set, the jackknife p-values and corresponding chi-square statistics are comparable. To  
4 identify IVs, we applied the previously described outlier detection methods to the  
5 resulting delta chi-square scores. For every variant, we calculated the proportion of  
6 times it was labelled as an IV across the samples it was observed; for variants under  
7 the alternative, this corresponds to a true positive rate and for variants under the null,  
8 this corresponds to a false positive rate.

9

#### 10 *Comparison with existing methods*

11 We compared the performance of RIFT to that of ADA and BeviMed by applying both  
12 methods to simulated datasets. Due to the superior performance and computational  
13 efficiency of ADA compared to the backward selection procedure, we chose to limit our  
14 comparisons to these two methods [12]. We followed our simulation approach above,  
15 where we simulated 50 genomic regions of size 3kb with 10% of rare variants under the  
16 alternative and effect size parameter of 0.4. Given RIFT is developed for regions which  
17 are previously found to be associated via an aggregate test of association, we restricted  
18 our comparisons of methods to regions where the SKAT-O was significant at 0.05. We  
19 performed ADA using the default parameters: 1) a MAF threshold of 0.05, 2) calculation  
20 of p-values using Fisher's exact test, 3) assuming an additive model and 4) 1000  
21 permutations for calculation of the final region ADA test p-value. For each replicate, the  
22 final set of variants (per-site p-value smaller than the optimal value) were classified as  
23 IVs. We applied BeviMed using the default parameters: 1) for each variant, a prior

1 probability of association of 0.01 and b) prior probability of a dominant model of  
2 inheritance of 0.5. BeviMed returns the posterior probability of association for both the  
3 dominant and recessive models of inheritance. For each of these models, we classified  
4 any variant with a posterior probability greater than 0.9 as a IV. We compared the above  
5 methods to our localization method (RIFT) using both the Inner Tukey and MAD criteria  
6 applied to the delta chi-square scores (referred to as RIFT:Inner Tukey and RIFT:MAD).  
7 To compare the performance of the above methods, we calculated the true positive rate  
8 and false positive rate across samples in which a variant was observed.

9

#### 10 *Impact of Varying Sample Characteristics on RIFT Performance*

11 To determine the robustness of RIFT performance to varying characteristics of a given  
12 sample, we performed additional simulations that varied the following parameters: #  
13 haplotypes simulated (1,000 and 100,000), sample size (5,000 cases and 5,000  
14 controls), region size (0.75kb), disease prevalence (10%), coefficient of disease  
15 association ( $c = 0.8$ ) and proportion of alternative variants (20%). For each, we  
16 simulated 500 samples for each of 10 regions while fixing the other simulation  
17 parameters to those used in the comparisons with the ADA and BeviMed methods. We  
18 summarized these different conditions by the structure of the resulting genotype data  
19 (number of variants, etc.), number of samples meeting SKAT-O significance level  
20 (power of SKAT-O) and finally in the performance of RIFT such as the relationship  
21 between delta chi-square score, MAF and true positive rate (Supplemental Table 1).

22

#### 23 *Application to Rare Variant Regions Associated with IPF*

1 We applied our leave-one-out localization method to data from a recently published  
2 targeted resequencing study in idiopathic pulmonary fibrosis (IPF) [27] as part of the  
3 Global IPF Collaborative Network  
4 (<http://www.ucdenver.edu/academics/colleges/medicalschoo/department/medicine/GloballIPF/Pages/GloballIPF.aspx>). We applied RIFT with the goal of identifying rare  
5 variants within regions associated with IPF to focus follow-up experimental validation  
6 studies. We report results for two regions, each containing 50 rare variants. To provide  
7 insight into putative function, we include functional annotation information obtained from  
8 SNPDOC (<https://wakegen.phs.wakehealth.edu/public/snpdoc3/index.cfm>) and  
9 HaploReg v4.1 [28].  
10

11

## 12 **Results**

13

### 14 *Characteristics of simulated regions*

15 Among the 50 simulated 3kb regions, each region included between 43 and 73 rare  
16 variants (MAF < 3%) with a median of 58.5 variants. Though we explored a range of the  
17 proportion of variants assumed to be under the alternative, we report the results for  
18 simulations where 10% of the variants in a given region were simulated to be under the  
19 alternative. Results are qualitatively very similar for higher proportions of variants under  
20 the alternative (Supplemental Table 2). After drawing random samples and selecting  
21 1000 cases and 1000 controls to reflect the sampling process, the average proportion of  
22 variants under the alternative across the 50 regions ranged from 9.7% to 15.2% with a  
23 median of 12.3%. Samples for a given region often contained higher than 10%



1 alternative variants due to the over-sampling of cases to obtain an equal number of  
2 cases and controls (Supplemental Table 2). We applied our localization method to  
3 samples that had a SKAT-O p-value less than 0.05. With the low proportion of variants  
4 (10%) simulated under the alternative and effect size parameter,  $c$ , of 0.4, the observed  
5 median power of SKAT-O across the 1000 simulated samples was 22.0%. As  
6 expected, increasing the effect size parameter to 0.8 for the same 50 regions  
7 dramatically increased the median power of SKAT-O to 99.1% (Supplemental Table 2).

8

### 9 *Performance of Our Localization Methods*

10 Across all regions, we found an interesting relationship between the delta chi-square  
11 score and the MAF (Figure 2). Delta chi-square scores were more extreme (and more  
12 variable) for variants with smaller odds ratios and correspondingly higher MAFs. The  
13 delta chi-square score is most sensitive to uncommon variants ( $0.03 > \text{MAF} \geq 0.001$ )  
14 with modest effect sizes and least sensitive to rare variants ( $\text{MAF} < 0.001$ ) with stronger  
15 effect sizes. Positive delta chi-square scores were observed for some variants under the  
16 alternative; however, the average delta chi-square score was negative for all but three  
17 variants out of a total of 288 variants simulated under the alternative across the 50  
18 regions. The general directionality of the delta chi-square score is consistent with what  
19 we expect for causal variants, where the removal of a causal variant results in a larger  
20 p-value, smaller chi-square statistic and therefore a negative delta chi-square score.  
21 The relationship between the delta chi-square score and MAF was consistent when  
22 varying the number of haplotypes simulated; however, we observed an even stronger

1 relationship when increasing the sample size from 1,000 cases and controls to 5,000  
2 cases and controls (Supplemental Figure 2).  
3  
4 Consistent with what was observed for the delta chi-square score, all outlier detection  
5 methods observed higher sensitivity to detect uncommon variants ( $0.03 > \text{MAF} \geq 0.001$ )  
6 compared to very rare variants ( $\text{MAF} < 0.001$ ). The Inner Tukey fence had the highest  
7 true positive rate (correctly labeling a variant under the alternative as an IV) compared  
8 to other methods (Figure 3). For variants with a  $\text{MAF} \geq 0.001$ , corresponding to an odds  
9 ratio less than 3.32, the Inner Tukey observed a median true positive rate of 0.60 (IQR:  
10 0.51, 0.87) compared to the SD method having a median of 0.30 (IQR: 0.10, 0.59;  
11 Supplemental Table 4). Among the 288 variants under the alternative, the Inner Tukey  
12 fence obtained the highest true positive rate 94.1% of the time and obtained a higher  
13 true positive rate than the other three methods 74.7% of the time. The false positive rate  
14 (inaccurately labeling a null variant as an IV) was remarkably low across all four  
15 methods, with the SD method having the lowest rate. For variants with  $\text{MAF} \geq 0.001$ , the  
16 SD method had a median false positive rate of 0 (IQR: 0, 0) and the Inner Tukey a  
17 median of 0.09 (IQR: 0.06, 0.18; Supplemental Figure 1, Supplemental Table 4). Taken  
18 together, the Inner Tukey has the best characteristics for correctly labeling a variant as  
19 an IV, as evidenced by the high true positive rate and low false positive rate. The SD  
20 method was substantially more conservative in labeling variants as IVs under the  
21 alternative, especially for those variants having a  $\text{MAF} \geq 0.0001$ .

22

23

## 1 *Comparison with existing methods*

2 Similar to RIFT, we observed a trend among existing localization methods in terms of  
3 having increased ability to identify IVs at uncommon allele frequencies compared to  
4 rare. (Figure 4, Table 1). While comparing the ADA and the BeviMed to the Inner Tukey  
5 and MAD, the ADA had the highest true positive rate across the entire MAF spectrum  
6 we considered, whereas BeviMed (dominant and recessive) observed the lowest true  
7 positive rate. For uncommon variants ( $MAF \geq 0.001$ ), the ADA had a median true  
8 positive rate of 0.92 (IQR: 0.78, 0.98) compared to BeviMed under the dominant model  
9 having a median of 0.12 (IQR: 0.00, 0.35). The higher sensitivity of the ADA to correctly  
10 label IVs for rare MAFs is at the cost of having a high false positive rate (Figure 5;  
11 median false positive rate of 0.22 [IQR: 0.17, 0.34]). As described above, the Inner  
12 Tukey observed a lower median false positive rate of 0.09 (IQR: 0.06, 0.18). In  
13 summary, both of our leave-one-out methods (Inner Tukey and MAD) produced a high  
14 true positive rate for rare variants with  $MAF > 0.001$  (comparable to ADA) while  
15 maintaining low false positive rates across the entire spectrum of MAF.

16

## 17 *Influential Variants in IPF Associated Loci*

18 In a rare variant analysis of targeted sequencing in 3,017 idiopathic pulmonary fibrosis  
19 (IPF) cases and 4,093 controls, we found several sets of rare variants associated with  
20 IPF. Rare variants were grouped into gene-sets or sliding windows and tested for  
21 association using SKAT-O. We applied RIFT to two significant rare variant windows,  
22 each of which contained 50 rare variants. The most significant window is located on  
23 chromosome 5 and spans the 5' UTR, exon 1 and intronic regions of the *TERT* gene.

1 This window had a Bonferroni-adjusted SKAT-O p-value of  $9.21 \times 10^{-16}$  after adjusting  
2 for sex and the most strongly association common variant in the region, rs4449583.  
3 After applying RIFT, there were three variants called outliers by both the Inner Tukey  
4 fence and MAD cutoffs (Figure 6). Annotation with SNPDOC found the variant with the  
5 largest delta chi-square (-28.7) to have unknown function and the second ranked IV  
6 (delta chi-square = -17.5) to be in a non-coding RNA transcript in the 5' untranslated  
7 region of *TERT* (Table 2). We additionally applied the localization method to a window  
8 with a less significant association (SKAT-O Bonferroni-adjusted p-value = 0.0215;  
9 adjusted for sex only as there is not a top common variant in the region) as an example  
10 of a region with a more moderate aggregate rare variant association signal. This  
11 window is located in the *RTEL* gene on chromosome 20 spanning both exons and  
12 introns. Our localization method identified 3 IVs by both the Inner Tukey fence and MAD  
13 cutoffs with the most outlying variant having a negative delta chi-square score = -6.77  
14 (Figure 7; Table 2). This variant was annotated to be located in an intron of the *RTEL*  
15 gene and based on annotation with HaploReg, has enhancer histone marks identified in  
16 8 tissues including lung and a lung carcinoma cell line. The other two IVs were each  
17 annotated to be in the coding region of *RTEL* and are nonsense mutations.

18

## 19 **Discussion**

20

21 The delta chi-square score we outline here provides an estimate of the contribution of a  
22 given variant to the aggregate test statistic for a set of variants. This measure can be  
23 used to rank variants in order to prioritize follow-up studies. We also compared several

1 outlier detection methods to identify variants as having a disproportionate impact on the  
2 aggregate test of association, likely increasing the probability of capturing a causal  
3 variant. We found the inner Tukey fence to have the greatest sensitivity, and we  
4 recommend this method to obtain a set of variants most likely to be driving the  
5 aggregate signal. As expected, our method has higher sensitivity to detect uncommon  
6 variants ( $0.001 < \text{MAF} < 0.03$ ) compared to extremely rare variants ( $\text{MAF} < 0.001$ ). This  
7 underscores the difficulty in detecting extremely rare variants individually and thoughtful  
8 alternative weighting schemes might provide leverage to better capture very rare  
9 variants. The ADA method obtained the highest sensitivity for uncommon variants;  
10 however, this was at a cost of low specificity. We found BeviMed lacked sufficient  
11 sensitivity in the classification of IVs under our simulation framework. We recognize this  
12 may be due to using their recommended parameter of 0.90 posterior probability for  
13 classification of IVs. However, it is unclear how to determine an optimal value for the  
14 posterior probability, which likely depends on the features of each region (e.g., number  
15 of true causal variants, effect size of the variants, total number of variants tested).  
16 Without requiring user optimization or selection of parameters, RIFT with the Inner  
17 Tukey classification approach achieves higher sensitivity and specificity to ADA and  
18 BeviMed, respectively.

19

20 The rare variants identified by our method to have the greatest evidence for influencing  
21 the association with IPF have been previously found to be associated with other  
22 diseases (Table 2). Common, non-coding variants in the promoter region of the *TERT*  
23 gene have been found in several human cancers [29,30]. Specifically, the two IVs with

1 the largest delta chi-square score (rs398123017 and rs373740199) have been identified  
2 in studies of familial and sporadic melanoma and thyroid cancer [31–34]. These  
3 mutations have been found to increase transcription of *TERT* and maintain telomerase  
4 activity resulting in long telomeres, thereby promoting tumorigenesis [35].

5  
6 On chromosome 20, two of the three rare variants identified by our method are  
7 missense mutations in the *RTEL* gene and have been previously identified in  
8 dyskeratosis congenita and the related Hoyeraal-Hreidarsson syndrome, both of which  
9 are diseases that result due to failures in telomere maintenance [36–38]. Though  
10 extensive research was not performed for all variants within our significant sets (as  
11 we've previously noted is often prohibitive), identification of these variants in other  
12 diseases supports the ability of our approach to detect plausible impactful rare variants  
13 that drive our observed association with IPF.

14  
15 Though we have only evaluated the delta chi-square statistic and corresponding outlier  
16 detection methods for data containing unrelated individuals, RIFT is not restricted to  
17 aggregate tests for this specific study design. Although we illustrated our approach  
18 using SKAT-O in a case-control framework, the methods are widely applicable to other  
19 aggregate tests and study designs. For example, the method could be applied using  
20 tests developed for samples of related individuals such as famSKAT [39].

21  
22 Unlike many other localization methods, RIFT does not rely on outside functional  
23 information and is able to distinguish among variants with no known function that may

1 be contributing to disease risk. We recognize that incorporating functional information  
2 can increase the power to identify causal rare variants. However, methods which rely  
3 on functional information are limited by the depth and accuracy of the annotation. Our  
4 method complements methods that utilize functional information to provide increased  
5 coverage in capturing plausible causal rare variation. Outlier detection methods to  
6 identify influential variants will eventually break down if a large proportion of the variants  
7 are under the alternative. It is plausible that in certain situations, the proportion of  
8 variants that are under the alternative is higher for a set of variants that all have putative  
9 function compared to a set that is selected agnostic of function. Since it is unlikely that  
10 the majority of putatively functional variants in a gene are associated with a given trait, it  
11 is also unlikely that filtering to functional variants prior to testing with an aggregate test  
12 would be problematic for the outlier detection methods implemented in RIFT. While  
13 RIFT maintains and perhaps even improves sensitivity for smaller numbers of variants  
14 included in the aggregate test (Supplemental Table 5), pre-filtering variants after the  
15 aggregate test is likely to be counter-productive. We recommend not filtering to variants  
16 with putative function either before or after aggregate testing, but instead applying RIFT  
17 agnostic of putative function and, if desired, further prioritizing those identified IVs based  
18 on putative function.

19

20 RIFT is available as an R package from <https://github.com/rachelzoeb/RIFT>. The time to  
21 complete an analysis with RIFT is dependent on the computation time of the aggregate  
22 test used. SKAT-O is fast for sets with a relatively small number of variants (e.g., < 100  
23 rare variants) and requires parallelization for large significant sets. Future work will

1 require optimization of RIFT for larger groups of variants such as sets based on gene  
2 boundaries.  
3  
4 Greater resolution of rare variant signals within significant sets of variants can provide  
5 valuable insight into the mechanism of disease and narrow the number of variants to  
6 prioritize for follow-up experimental studies. Often, aggregate tests contain such a large  
7 number of rare variants that follow up of each rare variant within the set would be  
8 prohibitive, even after filtering down to variants with functional evidence for causality. In  
9 addition, filtering to variants with known function precludes the ability to identify new  
10 variants with as-yet unknown functional roles. In addition to being agnostic to functional  
11 information, RIFT can be applied after application of any aggregate test, including those  
12 that include common variants. The ease and flexibility of this approach will aid  
13 investigators in post-aggregate association testing in a wide range of genetic studies.



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## **STATEMENT OF ETHICS**

For the IPF resequencing data, all of the subjects provided written informed consent as part of institutional review board (IRB)- approved protocols for recruitment at their respective institution, and the resequencing study was approved by the National Jewish Health IRB and the University of Colorado Combined IRB.

## **DISCLOSURE STATEMENT**

Dr. Schwartz reports personal fees from Eleven P15, Inc., outside the submitted work; In addition, Dr. Schwartz has a patent Compositions and Methods of Treating or Preventing Fibrotic Diseases pending, a patent Biomarkers for the diagnosis and treatment of fibrotic lung disease pending, and a patent Methods and Compositions for Risk Prediction, Diagnosis, Prognosis, and Treatment of Pulmonary Disorders issued. Dr. Fingerlin reports consulting fees from Eleven P15, Inc., outside the submitted work and a patent Methods and Compositions for Risk Prediction, Diagnosis, Prognosis, and Treatment of Pulmonary Disorders issued. All remaining authors besides Dr. Schwartz and Dr. Fingerlin declare no competing interest.

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## **AUTHOR'S CONTRIBUTIONS**

CDL, TEF, and RZB designed the study and developed the conceptual approaches to data analysis; TEF and DAS provided resequencing data. RZB performed the simulations and data analysis; RZB wrote the manuscript; TEF, DAS, and CDL reviewed the manuscript.

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## TABLES

**Table 1. Summary of true positive and false positive rates, stratified by MAF for each localization method.** The outlier detection methods for RIFT (MAD and Inner Tukey) were applied to the delta chi-square scores. Simulation included 1000 samples per region, across 50 regions. Data were simulated to have 10% variants under the alternative with effect size parameter  $c = 0.4$  (see Equation 5).

Method	True Positive Rate - Median (IQR)		False Positive Rate – Median (IQR)	
	MAF < 0.001	MAF $\geq$ 0.001	MAF < 0.001	MAF $\geq$ 0.001
<b>RIFT:MAD</b>	0.03 (0.01, 0.11)	0.54 (0.43, 0.85)	0.00 (0.00, 0.01)	0.08 (0.04, 0.15)
<b>RIFT:Inner Tukey</b>	0.04 (0.01, 0.15)	0.60 (0.51, 0.87)	0.00 (0.00, 0.01)	0.09 (0.06, 0.18)
<b>BeviMed:DOM</b>	0.00 (0.00, 0.00)	0.12 (0.00, 0.35)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
<b>BeviMed:REC</b>	0.00 (0.00, 0.00)	0.02 (0.00, 0.27)	0.00 (0.00, 0.00)	0.00 (0.00, 0.01)
<b>ADA</b>	0.17 (0.08, 0.35)	0.92 (0.78, 0.98)	0.01 (0.00, 0.05)	0.22 (0.17, 0.34)

**Table 2. Influential Variants identified in two rare variant loci previously found associated with IPF**

Chr:Pos <sup>a</sup>	MA <sup>b</sup>	MAF <sup>c</sup>	Delta Chi-Square	rs#	SNP-DOC annotation	Nearest Gene	Publications/Clinical Notes
chr5:1294704	A	0.00056	-3.65		coding-synon,ncRNA	TERT	
chr5:1295161	G	0.00353	-17.50	rs878855297	ncRNA,untranslated-5	TERT	<ul style="list-style-type: none"> <li>- Familial and sporadic melanoma (Horn et al., 2013)</li> <li>- High penetrance, early onset melanoma (Harland et al., 2016)</li> <li>- Cancer cell lines, found to abrogate telomerase silencing and promote tumorigenesis (Chiba et al., 2015)</li> </ul>
chr5:1295228	A	0.00705	-28.68		unknown	TERT	<ul style="list-style-type: none"> <li>- Familial and sporadic melanoma (Horn et al., 2013; Huang et al., 2013)</li> <li>- Cancer cell lines, found to abrogate telomerase silencing and promote tumorigenesis (Chiba et al., 2015)</li> <li>- Thyroid cancer (Liu and Xing, 2016)</li> </ul>
chr20:62324391	A	0.05008	-6.77	rs41308092 <sup>d</sup>	intron	RTEL	
chr20:62324564	T	0.00197	-3.25	rs398123017	ncRNA,nonsense	RTEL	<ul style="list-style-type: none"> <li>- Dyskeratosis congenital (Ballew et al., 2013; Walne et al., 2013)</li> <li>- Hoyeraal-Hreidarsson syndrome (Deng et al., 2013)</li> <li>- Familial interstitial pneumonia (Cogan et al., 2015)</li> <li>- Idiopathic pulmonary fibrosis (Todd et al., 2017)</li> </ul>
chr20:62324600	T	0.00098	-2.60	rs373740199	coding-synon,ncRNA,nonsense	RTEL	<ul style="list-style-type: none"> <li>- Dyskeratosis congenital (Ballew et al., 2013)</li> <li>- Hoyeraal-Hreidarsson syndrome (Moriya et al., 2016)</li> <li>- Idiopathic pulmonary fibrosis (Todd et al., 2017)</li> <li>- Early-onset Inflammatory Bowel Disease (Petersen et al., 2017)</li> </ul>

<sup>a</sup>NCBI Build 37 coordinates

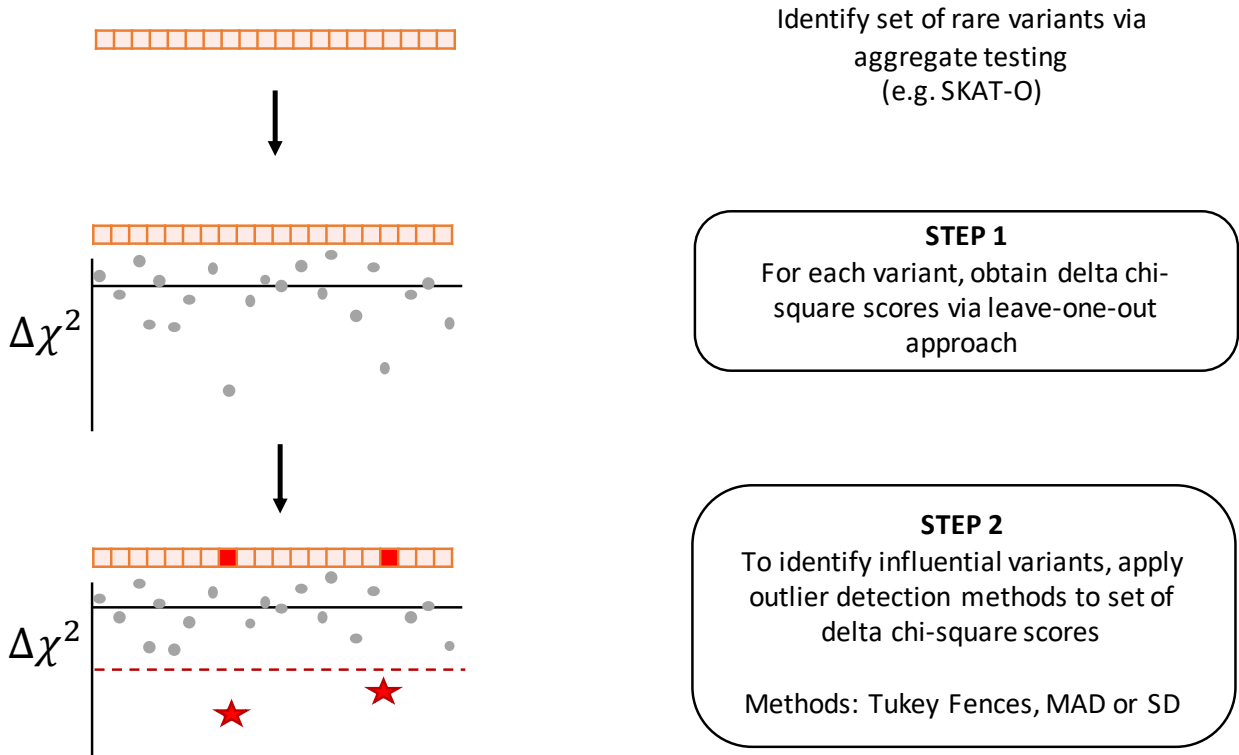
<sup>b</sup>Minor allele observed in our data

<sup>c</sup>Minor allele frequency observed in our data

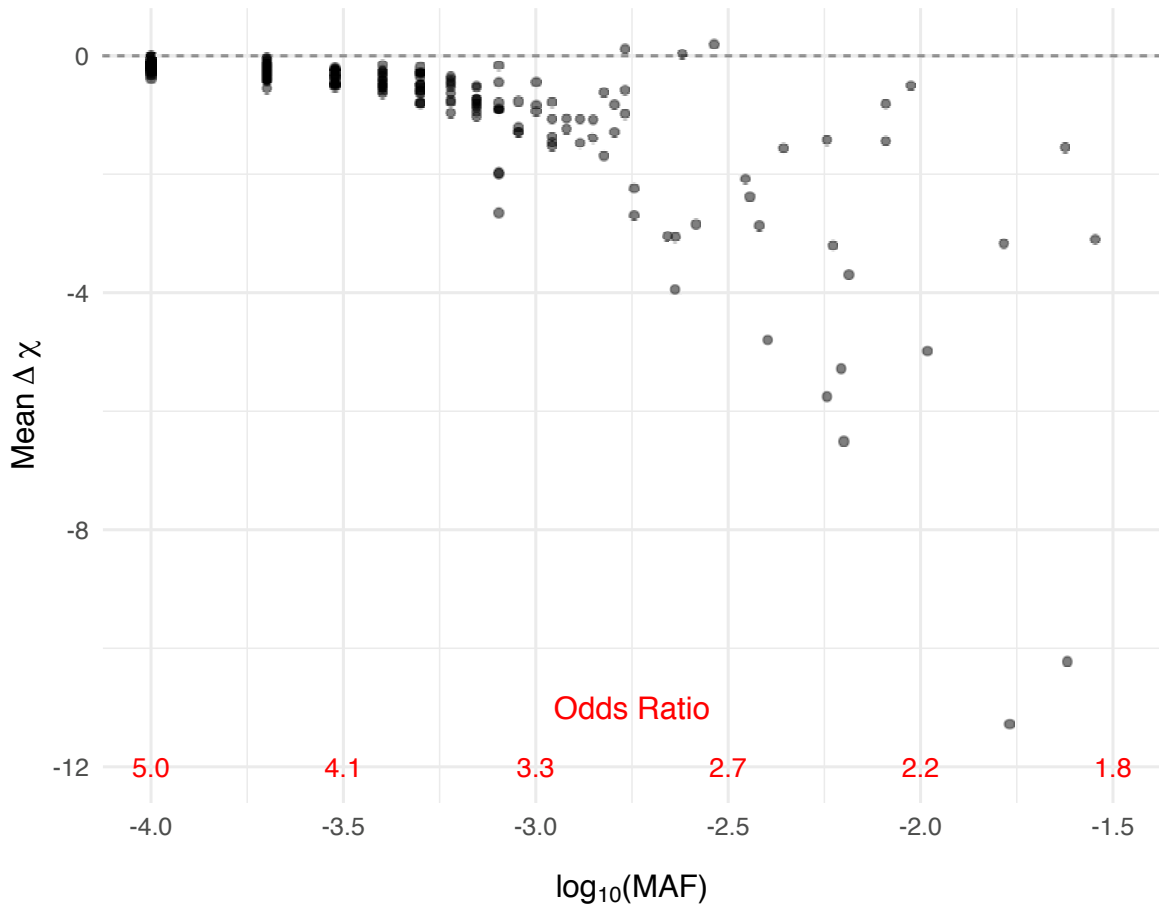
<sup>d</sup>HaploReg results: promoter histone marks found in skin, GI and enhancer histone marks found in 8 tissues including lung and a lung carcinoma cell line

## FIGURES

Figure 1. Flow chart of RIFT.

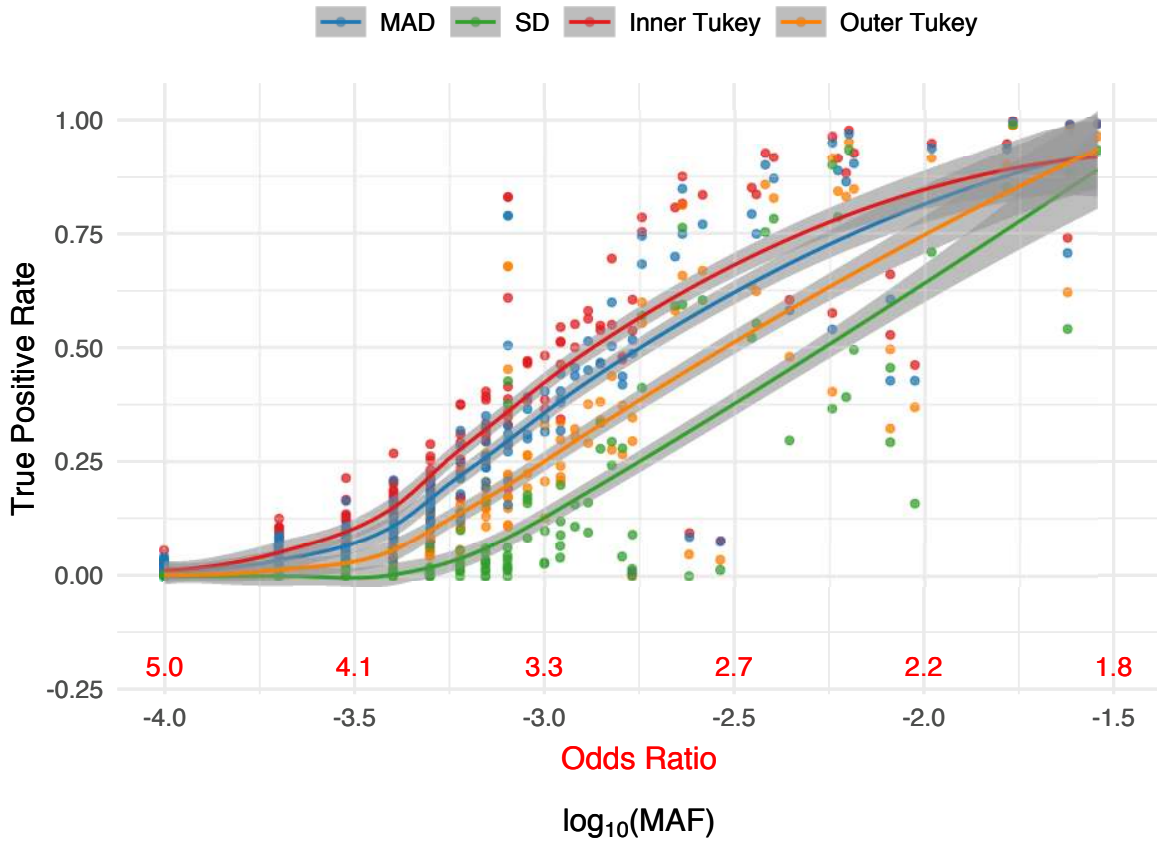


**Figure 2. Delta chi-square score more sensitive to the more frequently observed rare variants with correspondingly smaller effect sizes.** Mean delta chi-square score plotted by odds ratio for variants simulated under the alternative across all 50 regions. Data were simulated to have 10% variants under the alternative with effect size parameter  $c = 0.4$  (see Equation 5).

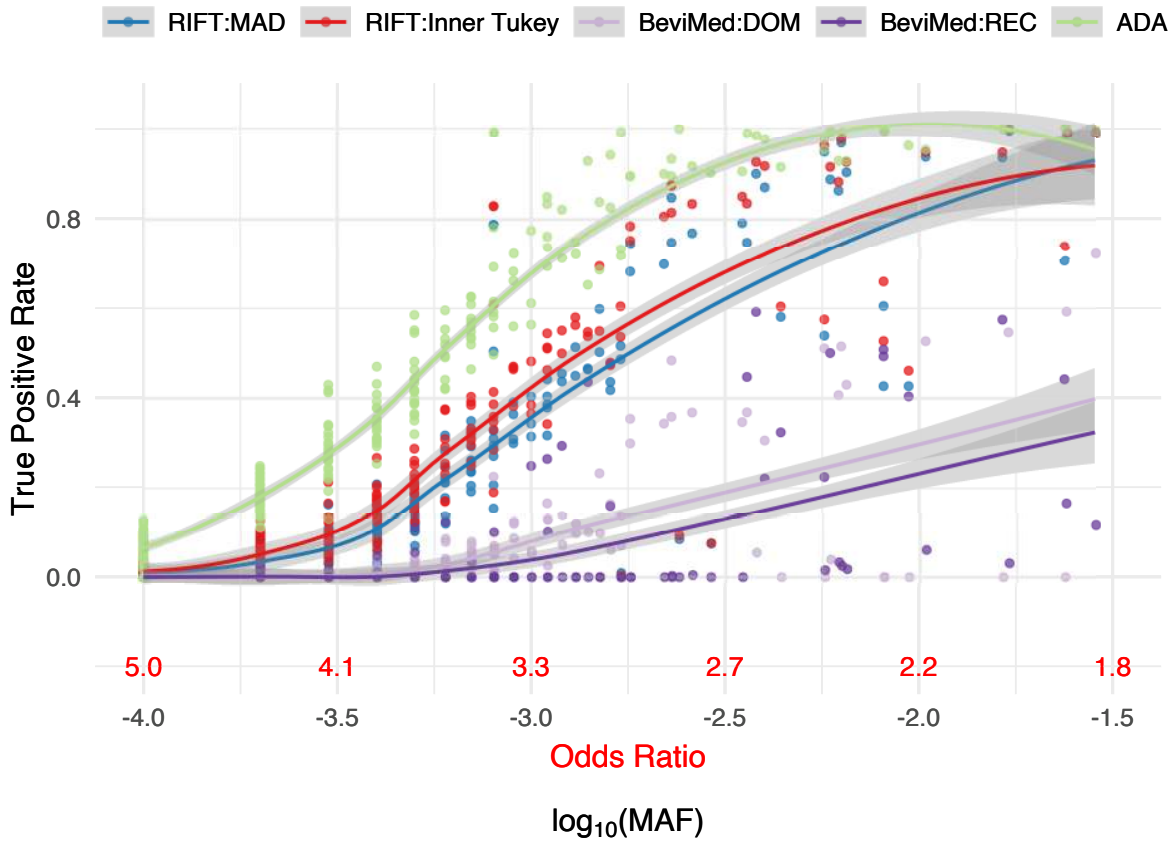




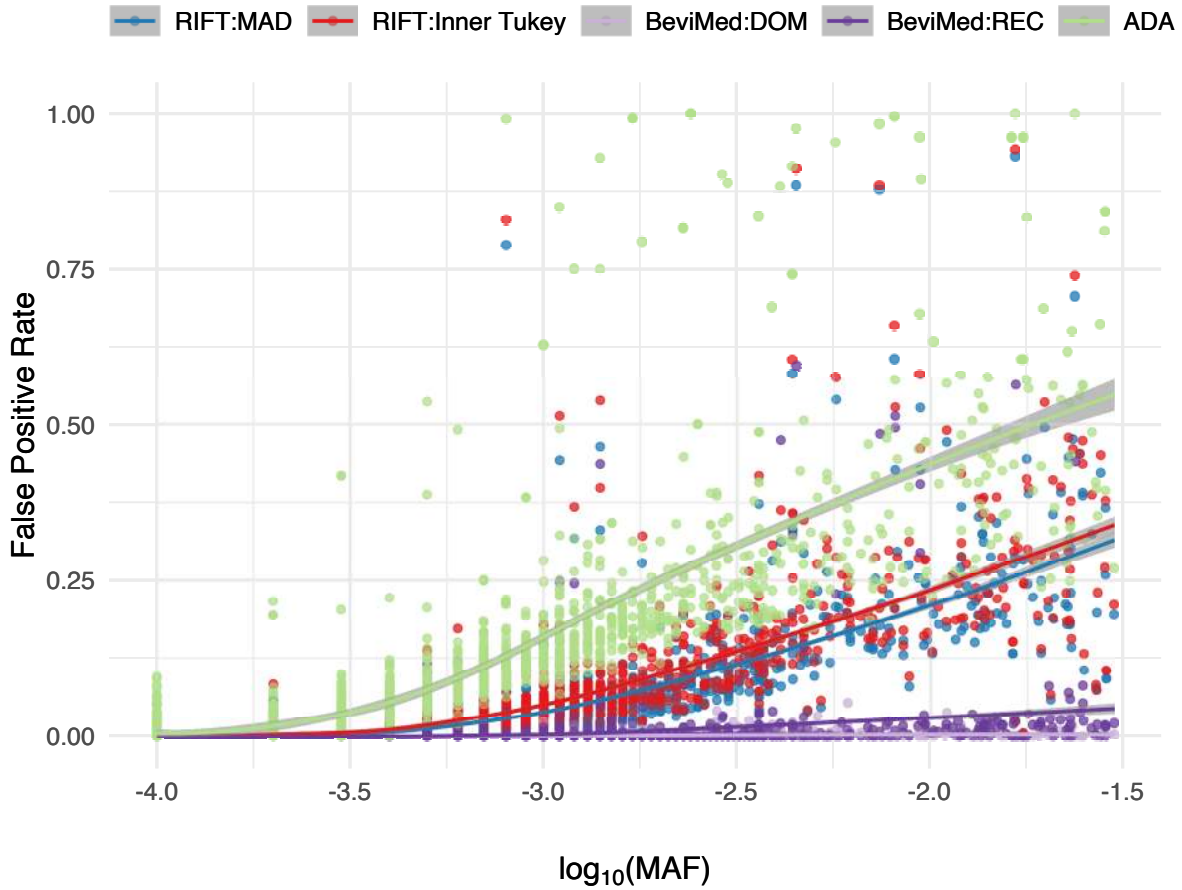
**Figure 3. True positive rate (proportion correctly labeled IV) for variants under the alternative for each outlier detection method of RIFT as a function of MAF.** Corresponding odds ratio is also provided for reference. Smoothed line and confidence band provided by the loess method. Data were simulated to have 10% variants under the alternative with effect size parameter  $c = 0.4$  (see Equation 5).



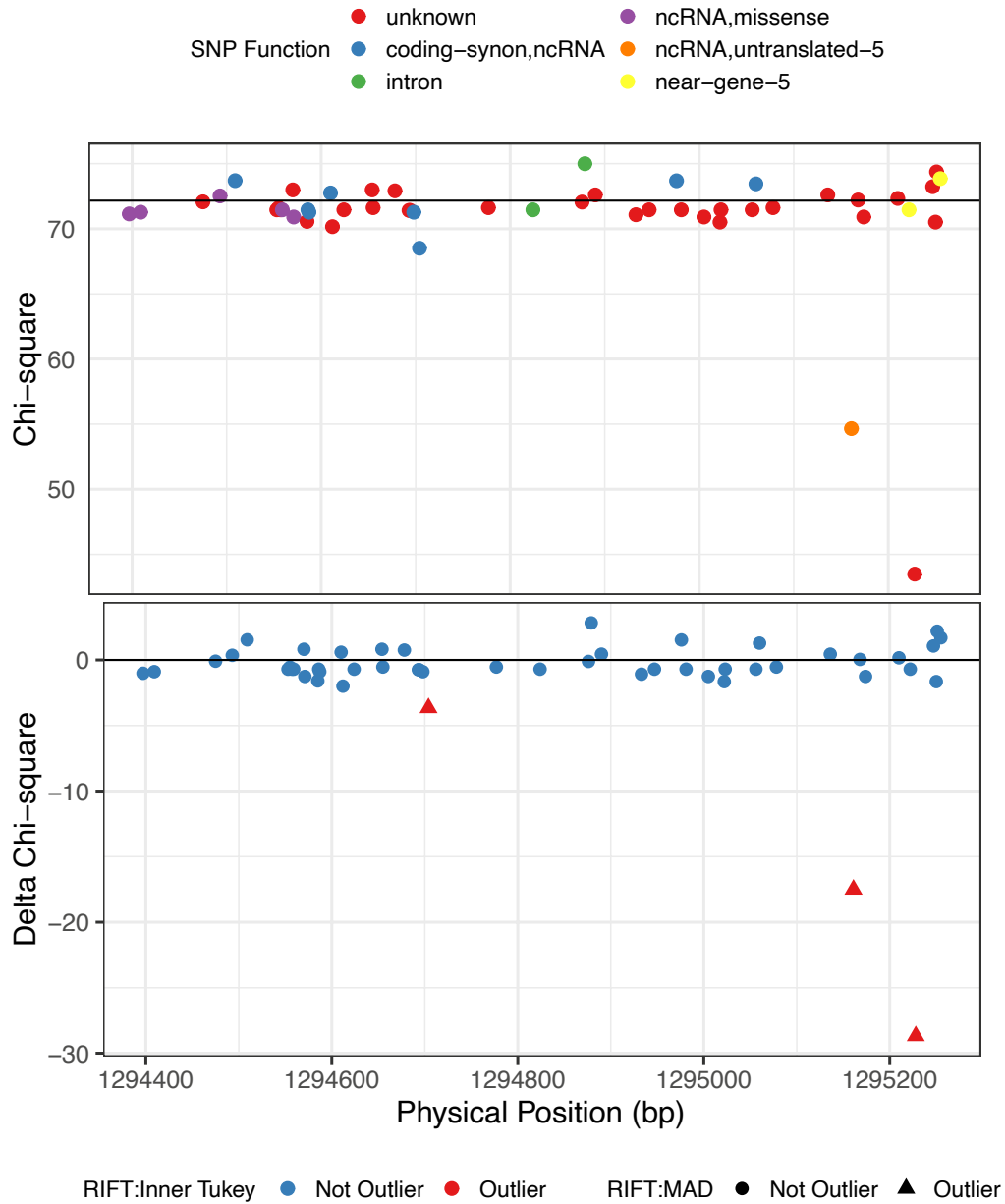
**Figure 4. True positive rate (proportion correctly labeled IV) for variants under the alternative for each localization method as a function of MAF. Corresponding odds ratio is also provided for reference. Smoothed line and confidence band provided by the loess method. Data were simulated to have 10% variants under the alternative with effect size parameter  $c = 0.4$  (see Equation 5).**



**Figure 5. False positive rate (proportion incorrectly labeled IV) for variants under the null for each localization method as a function of MAF. Smoothed line and confidence band provided by the loess method. Data were simulated to have 10% variants under the alternative with effect size parameter  $c = 0.4$  (see Equation 5).**



**Figure 6. Chi-square (top and delta chi-square scores (bottom) by genomic position for the IPF-associated rare variant loci on chr5 (bp: 1294397-1295255).** Color for the top plot corresponds to SNP-DOC functional annotation and for the bottom plot, color corresponds to outlier by the Inner Tukey method and shape corresponds to outlier by the MAD method.



**Figure 7. Chi-square (top and delta chi-square scores (bottom) by genomic position for the IPF-associated rare variant loci on chr20 (bp: 62324166-62324601).** Color for the top plot corresponds to SNP-DOC functional annotation and for the bottom plot, color corresponds to outlier by the Inner Tukey method and shape corresponds to outlier by the MAD method.

