NIMBus: a Negative Binomial Regression based Integrative Method for Mutation Burden Analysis

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ABSTRACT

Background

Identifying highly mutated regions is a key way that scientists can use sequencing on a population scale to discover key genomic regions associated with complex diseases such as cancer. Nevertheless, it is challenging to identify such regions because severe mutation rate heterogeneity across different genome regions gives rise to highly over-dispersed mutation counts. Moreover, it is known that part of this heterogeneity relates to confounding genomic features, such as replication timing and chromatin organization.

Results

Here, we address these issues with a Negative binomial regression based Integrative Method for mutation Burden analysis (NIMBus). This approach uses a Gamma-Poisson mixture model to capture the mutation rate heterogeneity across different individuals, and consequently models the over-dispersed mutation counts as a negative binomial distribution. Furthermore, the model regresses the mutation counts against 381 genomic features extracted from REMC and ENCODE to accurately estimate the local background mutation rate. This framework can be readily extended to accommodate additional genomic features in the future. NIMBus was used to analyze 649 whole-genome cancer sequences. It successfully controlled P value inflation and identified well-known coding and noncoding drivers, such as TP53 and the TERT promoter. In addition, NIMBus was used for mutation burden tests of KEGG pathways. It successfully found known cancer

related pathways, such as TP53 signaling pathway and apoptosis pathway, to be significantly mutated.

Conclusion

Integrative frameworks are becoming rapidly necessary to understand the relationship between whole genome sequencing data and the functional characteristics of such data. Identifying mutational hotspots as driver candidates in complex diseases will better allow biologists and clinicians to understand the underlying biological mechanisms of these diseaeses. We make NIMBus available and release our results as an online resource (nimbus.gersteinlab.org).

KEYWORDS

Somatic mutation burden

Cancer driver

Mutation rate heterogeneity

Mutation rate estimation

Mutation count overdispersion

1 BACKGROUND

Population level analysis, which looks for regions mutated more frequently than expected, is one of the most powerful ways to identify deleterious mutations for diseases [1-3]. Recent developments of whole genome sequencing (WGS) and personal genomics have provided unprecedented statistical power to perform such analyses.

Therefore, an accurate quantification of mutation burden is important to uncover the genetic cause of various diseases, which in turn would allow for targeted therapies in clinical studies. However, mutation burden tests for somatic variants remain challenging for several reasons.

First, some of the pioneer work analyzing WGS assumed a constant mutation rate across different regions or cancer genomes and ignored that somatic genomes are highly heterogeneous [4]. Under such assumption, the positional level mutation counts often demonstrate larger than expected variance, known as overdispersion. This assumption results in poor data fitting and generates numerous false positives [5], so it is necessary to introduce more sophisticated models to handle mutation rate heterogeneity.

Second, numerous genomic features have been reported to largely affect the mutation process [6], necessitating careful correction in burden analysis. Unfortunately, none of the few current methods that considered such effects systematically explored these genomic features in a tissue-specific way, and their models demonstrated very limited extensibility to accommodate new features in the future. For instance, MutSigCV tried to correct the effects of several features, such as gene expression and replication timing, by only using a small neighborhood of genes with similar covariate values. However, as the covariate number increases, it is usually difficult to find a meaningful neighborhood in a high dimension space.

Lastly, many *state-of-the-art* methods are only optimally designed for analysis of coding regions [6], which represent less than 2 percent of the human genome. Nowadays, a myriad of studies have shown that noncoding mutations can serve as driver events for diseases. For example the mutations in the TERT promoter were found to be associated

with cancer progression [7]. Hence, unified analysis of coding and noncoding regions is needed to give a thorough annotation of discovered hotspots.

We propose a Negative binomial regression based Integrative Method for mutation Burden analysis (NIMBus) that solves the three problems mentioned above. It first intuitively treats mutation rates from different individuals as random variables with a gamma distribution, and resultantly models the pooled mutation counts from a heterogeneous population as a negative binomial distribution to handle overdispersion. Furthermore, to capture the effect of covariates, NIMBus integrates extensive features in all available tissues from Roadmap Epigenomics Mapping Consortium (REMC) and the Encyclopedia of DNA Elements (ENCODE) project to create a covariate matrix to predict the local mutation rate with high precision through regression. In addition, it also customizes the most comprehensive noncoding annotations from ENCODE to facilitate interpretation of results. This integrative approach employed in NIMBus enables us to effectively pinpoint mutation hotspots associated with disease progression and to better understand the biological mechanisms therein.

2 METHODS

2.1 WGS variants data used

We collected 649 whole genome variant calls from public resources and collaborators. This data contains a broad spectrum of 7 different cancer types (details in Text S1 section 1).

2.2 Local background mutation rate estimation

(A) Human genome gridding and covariate matrix calculation

First we divide the whole genome into bins with fixed length l. In this stage, l is usually large, such as 1 Mb. Any bins overlapping either of the two blacklist regions are removed. Then, 381 features are extracted from both REMC and ENCODE, and the average signal in the bins is calculated (details in Text S1 Section S2). We let $x_{i,j}$ denote the average signal strength for the i^{th} bin and j^{th} covariate, where $i = 1, \cdots, n$ and $j = 1, \cdots, m$.

(B) Use negative binomial distribution to handle mutation count overdispersion

Suppose there are $d = 1, \cdots, D$ different diseases (or disease types) in the collected WGS data, and $s = 1, \cdots, s_d$ unique samples for each disease (or disease type) d. Let $y_i^{d,s}$ and $\lambda_i^{d,s}$ denote the mutation count and rate for the i^{th} bin defined in section 2.2 (A) for sample s in disease d. In previous efforts, scientists assume that mutation rate $\lambda_i^{d,s}$ is constant across different regions of the human genome, samples, and diseases, so they have that $\lambda_i^{d,s} \triangleq \lambda$ for $\forall\, i, d, s$. Hence $y_i^{d,s}$ follows a Poisson distribution with the probability mass function (PMF) given in equation (1).

$$p\left\{Y_i^{d,s} = y_i^{d,s}\right\} = \frac{e^{-\lambda_i^{d,s}}\left(\lambda_i^{d,s}\right)^{y_i^{d,s}}}{y_i^{d,s}!} \triangleq \frac{e^{-\lambda}\lambda^{y_i^{d,s}}}{y_i^{d,s}!} \tag{1}$$

However, somatic genomes are highly heterogeneous because mutation rates vary considerably among various diseases, samples, and regions of the same genome, severely violating the assumption in equation (1). As a result, fitting of $y_i^{d,s}$ is usually very poor because overdispersion is often observed [5]. Simply assuming a constant mutation rate

will generate numerous false positives. Instead, in our model we assume that different $\lambda_i^{d,s}$ are i.i.d random variables that follow a Gamma distribution with probability density function (PDF)

$$P\left\{\lambda_i^{d,s}=x\right\}=\frac{1}{\Gamma\left(c_i^d\right)\left(\upsilon_i^d\right)^{c_i^d}}x^{\left(c_i^d-1\right)}e^{-\frac{x}{\upsilon_i^d}} \quad (2),$$

where $c_i^d>0$ and $\upsilon_i^d>0$. In equation (2), c_i^d and υ_i^d are the shape and scale parameters respectively. Assume that $\lambda_i^d=\sum_{s=1}^{s_d}\lambda_i^{d,s}$ is the overall mutation rate from all samples in bin i of disease d. Its distribution can be readily obtained through convolution as

$$P\left\{\lambda_i^d=x\right\}=\frac{1}{\Gamma\left(s_d c_i^d\right)\left(\upsilon_i^d\right)^{s_d c_i^d}}x^{\left(s_d c_i^d-1\right)}\exp\left(-\frac{x}{\upsilon_i^d}\right) \quad (3).$$

If we let $y_i^d=\sum_{s=1}^{s_d}y_i^{d,s}$ represent the total mutation counts in region i from all disease samples, d, then the conditional distribution of y_i^d given λ_i^d can be written as

$$P\left(y_i^d\middle|\lambda_i^d\right)=\frac{\left(\lambda_i^d\right)^{y_i^d}\exp\left(-\lambda_i^d\right)}{\left(y_i^d\right)!} \quad (4).$$

By integrating (3) into (4), the marginal distribution of y_i^d can be denoted as a negative binomial distribution ([8], page 50 in [9]).

$$P\left(y_i^d\middle|c_i^d,\upsilon_i^d\right)=\left(\frac{1}{1+\upsilon_i^d}\right)^{s_d c_i^d}\frac{\Gamma\left(s_d c_i^d+y_i^d\right)}{\Gamma\left(s_d c_i^d\right)\left(y_i^d\right)!}\left(\frac{\upsilon_i^d}{1+\upsilon_i^d}\right)^{y_i^d} \quad (5a).$$

Equation (5a) is the PDF of a negative binomial distribution with $E\left(y_i^d\right)=s_d c_i^d\upsilon_i^d$ and $Var\left(y_i^d\right)=s_d c_i^d\upsilon_i^d\left(1+\upsilon_i^d\right)$. To better interpret (5a), we define $\upsilon_i^d=\mu_i^d\sigma_i^d$ and $s_d c_i^d=1/\sigma_i^d$. Then equation (5a) can be rewritten as (5b).

$$p_{Y_i^d}\left(y_i^d \middle| \mu_i^d, \sigma_i^d\right) = \left(\frac{1}{1+\sigma_i^d \mu_i^d}\right)^{1/\sigma_i^d} \frac{\Gamma\left(y_i^d + 1/\sigma_i^d\right)}{\Gamma\left(1/\sigma_i^d\right)\Gamma\left(y_i^d + 1\right)} \left(\frac{\sigma_i^d \mu_i^d}{1+\sigma_i^d \mu_i^d}\right)^{y_i^d} \quad (5b)$$

The mean and variance of y_i^d from (5b) can be described as μ_i^d and $\mu_i^d\left(1+\mu_i^d \sigma_i^d\right)$ respectively. Our model in equation (5b) is convenient due to its explicit interpretability. First, it assumes that the individual mutation rates are heterogeneous by modeling $\lambda_i^{d,s}$ as i.i.d. Gamma distributed random variables. Unlike the constant mutation rate assumption where $E\left(y_i^d\right) = Var\left(y_i^d\right)$, our model captures the extra variance of y_i^d due to population heterogeneity. Our model in (5b) also clearly separates the two main parameters μ_i^d and σ_i^d with physically interpretable meanings: the mean and overdispersion, respectively. Here a larger σ_i^d indicates a more severe degree of overdispersion, which is usually due to larger differences in mutation rates.

(C) Accurate local background mutation rate estimation by regression

After modeling y_i^d with a negative binomial distribution in 2.2 (B), we then estimate the local mutation rate by correcting the covariate matrix $\boldsymbol{X}$ described in 2.2 (A). Again $x_{i,j}$ denotes the average signal strength in the i^{th} bin and j^{th} covariate, where $i = 1, \cdots, n$ and $j = 1, \cdots, m$. Because the genomic features in the covariate matrix are highly correlated and may introduce multicollinearity if directly used in regression, we first apply principal component analysis (PCA) to matrix $\boldsymbol{X}$. We define $\boldsymbol{X}'$ to be the covariate matrix after PCA and $x'_{i,j}$ as each element in $\boldsymbol{X}'$.

A generalized regression scheme is used here. Suppose g_1 and g_2 are two link functions. We then use linear combinations of covariate matrix $\boldsymbol{X}'$ to predict the transformed mean parameter, μ_i^d, and overdispersion parameter, σ_i^d, as

$$\begin{aligned} g_1\left(\mu_i^d\right) &= \log\left(\mu_i^d\right) = \beta_0^d + \beta_1^d x'_{i,1} + \cdots + \beta_j^d x'_{i,j} + \cdots + \beta_j^d x'_{i,m} \\ g_2\left(\sigma_i^d\right) &= \log\left(\sigma_i^d\right) = \alpha_0^d + \alpha_1^d x'_{i,1} + \cdots + \alpha_j^d x'_{i,j} + \cdots + \alpha_m^d x'_{i,m} \end{aligned} \quad (6).$$

Here we use a log link function for both g_1 and g_2, so the regression model in (6) is a negative binomial regression. Note that $\boldsymbol{X}$ contains 381 genomic features in all available tissues. In the following analysis, we use all features to run the regression in (6) to achieve better performance. The GAMLSS package in R is used to estimate the parameters in (6) as $\hat{\alpha}_0^d, \cdots, \hat{\alpha}_m^d, \hat{\beta}_0^d, \cdots, \hat{\beta}_m^d$. Generally, there are biological reasons to explain how μ_i^d changes with covariates. For example, single-stranded DNA in the later replicated regions usually suffers from accumulative damage resulting in larger μ_i^d. It is more difficult to interpret such a relationship with σ_i^d. Hence, we simplify equation (6) by assuming σ_i^d is constant in our real data analysis.

2.3 Somatic burden tests using local background mutation rate

(A) Background mutation rate calculation for target regions

Suppose there are K regions to be tested. We use the local mutation rate to evaluate the mutation burden. For the k^{th} target region ($k = 1, \cdots, K$), optimally we should extend it into length l (illustrative figure given in Fig. S2). Then we calculate the average signal for feature j as $x_{k,j}, j = 1, \cdots m$ for this extended bin, and after PCA projection let $x'_{k,j}$ represent the value for the j^{th} PC. The local mutation parameters $\hat{\mu}_k^d$ and $\hat{\sigma}_k^d$ in the extended bin for the k^{th} target region can be calculated as

$$\hat{\mu}_k^d = \exp\left(\hat{\beta}_0^d + \hat{\beta}_1^d x'_{k,1} + \cdots + \hat{\beta}_j^d x'_{k,j} + \cdots + \hat{\beta}_m^d x'_{k,m}\right)$$
$$\hat{\sigma}_k^d = \exp\left(\hat{\alpha}_0^d + \hat{\alpha}_1^d x'_{k,1} + \cdots + \hat{\alpha}_j^d x'_{k,j} + \cdots + \hat{\alpha}_m^d x'_{k,m}\right) \quad (7).$$

In reality, the length of the k^{th} test region l_k is much shorter than the length of the training bins (up to 1Mb). Hence $\hat{\mu}_k^d$ needs to be adjusted by a factor of l_k/l. Then $\hat{\sigma}_k^d$ and the adjusted $\hat{\mu}_k^d$ can be used to calculate the disease specific P value, p_k^d. This optimal scheme is usually computationally expensive because there are usually millions of target regions to be tested. Therefore, we also propose an approximation method to replace the optimal $\hat{\mu}_k^d$ and $\hat{\sigma}_k^d$ in our analysis (details see section S4 in Text S1).

(B) Combining P values for multiple disease types

Sometimes it is necessary to analyze several related diseases (or disease types) to provide a combined P value. One typical example is in pan-cancer analysis. In section 2.3 (A), we calculated the P value for disease/disease type d as p_k^d for test region k. Fisher's method can be used to combine these P values. Specifically, the test statistic is

$$T_k = -2\sum_{d=1}^{D} \ln\left(p_k^d\right) \sim \chi^2(2D) \quad (8).$$

Here T_k follows a centered chi-square distribution with $2D$ degrees of freedom, where D is the total number of diseases/disease types. The final P value, p_k, can be calculated from T_k.

To better illustrate how NIMBus works, Figure 1 gives its workflow.

Figure 1. Flowchart of NIMBus.

2.4 Noncoding annotations customized for NIMBus

We customized the full list of noncoding annotations from both ENCODE annotations and our previous efforts in the 1000 Genomes Project to make it suitable for burden analysis. More details are given in Text S1.

2.5 Mutation burden test for networks in the genome

In addition to testing single target regions, it is useful to extend our analysis to testing of networks of target regions of the genome. Given a network of regions consisting of K ($k = 1, \cdots, K$) individual regions, each with y_k mutations, we can determine the p-value (p_k) associated with each individual region based on μ_k and γ_k, and then combine these p-values to produce a single p-value (p_{comb}) associated with the network. To do this, we use Fisher's method for combining p-values.

$$p_k = \Pr\{Y_k \geq y_k | \mu_k, \gamma_k\}$$
$$T = -2\sum_{k=1}^{K} \ln(p_k), \quad T \sim \chi^2_{2K} \tag{9}$$
$$p_{network} = \Pr_{\chi^2_{2K}}\{T \geq t\}$$

We took the KEGG pathway as a natural biological application of our network analysis. Each coding gene represents a target region in the genome, and the gene set that makes up a pathway represents a network of genes. Since a KEGG pathway may consist of genes that are located on different chromosomes or regions of the genome, the mutation burden for a pathway will be heterogeneous. Therefore, for each pathway, we first determine the p-value of each coding gene in the pathway list using the local mutation burden calculations from NIMBus, and then combine them using Fisher's method for a pathway associated p-value. This example can be seen in Figure 2.

Figure 2. Schematic plot of network analysis: a gene set is first selected in a network of genes. The associated values of μ_k, γ_k are extracted from NIMBus for each of the k genes, potentially located on different chromosomes. A single p-value, p_k, is obtained for each gene. Fisher's method is used to combine all of the p-values into a final p-value for the network, $p_{network}$.

3 RESULTS

3.1 Heterogeneity from various sources leads to large overdispersion in mutation counts data

Pioneer genome wide somatic burden analysis usually assumes a homogeneous mutation rate per nucleotide, which consequently uses binomial tests to calculate P values [4]. However, we found that mutation count data usually violates this assumption because there is severe mutation rate heterogeneity from various sources. To demonstrate this, we collected WGS variants from 649 cancer patients and 7 cancer types (Fig. S1).

First, we found that the mutation count per genome varies across diseases and samples. For instance, the median number of variants can be as low as 70 in Pilocytic Astrocytoma (PA) and as high as 21287 in Lung adenocarcinoma (LUAD). Even within the same cancer type, mutation counts vary dramatically from sample to sample (lowest at 1743 and highest at 145500 in LUAD, Fig. 2A). In addition, there are also large regional mutation rate differences within the same sample (Fig. S4). Therefore, distributions based on constant mutation rate assumption usually fit poorly to the real mutation counts data (Fig. 2B, dashed lines with +, Fig. S3 in Text S1). In light of these issues, we utilized a two-parameter negative binomial distribution to further capture the

over-dispersed nature of mutation counts data, which improves fitting to real data significantly (dashed lines with star in Fig. 3B).

Figure 3. (A) Disease and sample mutation rate heterogeneity; (B) improved fitting by negative binomial distribution of mutation counts in 1mb bins in breast cancer (BRCA) and Medulloblastoma (MB).

3.2 Local mutation rate is confounded by many genomic features

Somatic mutation rate has been reported to be confounded by several genomic features [6, 10]. For example, single-stranded DNA during replication usually suffers from endogenous DNA damage, such as oxidation and deamination. Therefore, the accumulative damage effect in the later replicated regions will result in increased mutation rate. We have observed a similar trend in our data. For example, the Pearson correlation between normalized mutation counts and replication timing values in BRCA is as high as 0.67 in the first 70 1mb bins (Fig. S4A). Another example is that the chromatin organization, which arranges the genome into heterochromatin- and euchromatin-like domains, has a dominant influence on regional mutation rate variation in human somatic cells [10]. Consistently, we also find that mutation counts are significantly associated with the DNase-seq signal (Pearson correlation= -0.61, P=1.52×10^{-8}, Fig S4B in Text S1). Therefore, it is important to estimate local background mutation rate for accurate mutation burden analysis.

3.3 Negative binomial regression precisely estimates local mutation rates by correcting many genomic features

(A) Features in matched tissues usually provide best prediction accuracy but features in unmatched tissue still help

It has been reported that the most accurate local mutation rate prediction can be achieved by using features from matched tissue [11]. Hence, we specifically selected variants in two distinct cancer types, BRCA and MB, and predicted their local mutation rates with a few features from matched (or loosely matched) and unmatched tissues (Table S2 in Text S1). Relative error, defined as the normalized difference of observed and predicted value (equation s3 in Text S1), was used to assess model performance. Consistent with previous conclusions, we find that features in matched tissues usually outperform those from unmatched tissues. For example, the relative error is only 0.128 by using breast tissue related features to predict BRCA mutation rates, which is noticeably smaller than an error of 0.195 when using brain related features (Table S3 in Text S1). Similarly, brain related features have more predictive power compared to breast related ones for MB mutation rates (error of 0.135 VS. 0.183).

However, biologically meaningful tissue matching remains challenging and usually is not an optimal process for researchers without enough domain knowledge. Specifically, if samples of distinct hidden subtypes were pooled together for a certain disease, tissue matching would be more difficult. Furthermore, even after the best-matched tissue has been identified, we frequently need to handle missing features in that tissue. We noticed

that many genomic features are highly correlated both within and across tissues (correlation plot in Fig. S6A), which leads to suboptimal but still decent regression performance (scatter plots given in Fig. S6B). This is extremely helpful when processing WGS from diseases without matched features. For example, there are no prostate related tissue features in REMC, but features in other tissues still help to estimate the local mutation rates.

(B) Pooling features from multiple tissues significantly improves local background mutation rate prediction

In light of the correlated nature of covariates, especially those epigenetic features [12], we first performed principal component analysis (PCA) on the covariate matrix to overcome the multicollinearly problem during regression. The correlation of each PC with the mutation counts data varies significantly across different cancer types (boxplots in Fig. S7B in Text S1). For example, the first PC demonstrates a Pearson correlation of 0.653 in LICA, which is much higher than 0.352 in PRAD. Therefore, it is necessary to run a separate regression model for each cancer type.

Figure 4. (A) Regression performance by correcting different number of PCs; (B) Regression performance vs. total number of variants used in all cancer types

Since numerous PCs have been shown to be associated with mutation rates, we tried to investigate the joint effect of multiple PCs to predict the local mutation rates. Particularly, for each cancer type, we first ranked the individual PCs by their correlations

with mutation rates, and then selected the top 1, 30, and 381 PCs to estimate the local mutation rate. Fig. 4A shows that using more PCs can noticeably boost prediction accuracy in all cancer types. For example, in BRCA the Pearson correlation is only 0.472 if 1 PC is used in regression, but rises to 0.655 and 0.709 if 15 and 30 PCs are used respectively. The correlation increases to 0.818 after using all 381 PCs. As a result, in all of the following analyses, we used all 381 PCs for accurate local mutation rate estimation.

As shown in Fig. 4B, we achieved good prediction accuracy through regression against all PCs of the covariate matrix in all cancer types. The Pearson correlations of the observed mutation counts and the predicted $\hat{\mu}_i^d$ vary from 0.668 in PA to 0.958 in LICA. Scatter plots are given in Fig. S8 in Text S1. It is worth mentioning that although there are no features matching prostate tissue in REMC, we can still achieve a very high correlation of 0.81 with the help of 381 unmatched but correlated features. This indicates that our model could still provide acceptable performance even when somatic WGS of a disease is given without optimally matched covariates.

In addition, the number of available variants obviously affects prediction performance, though it is not the only factor. As shown in Fig. 4B, limited number of variants, such as those in quiet somatic genomes of PA, can usually restrict our prediction precision (lowest correlation at 0.668 among 7 cancer types). However, other factors, such as the number of effective covariates, quality of mutation calls, and molecular similarity of pooled samples of the same disease can also influence the prediction performance considerably. For instance, although there are fewer variants in MB than

those in BRCA, our regression for MB still outperforms that of BRCA (0.865 vs 0.818, Fig. 4B).

3.4 Coding region calibration for NIMBus

(A) Single gene target region analysis

Since coding regions have been investigated in more detail than the noncoding regions, we first applied NIMBus on coding regions. First, we extracted coding regions from the GENCODE annotation v19 and ran NIMBus on both real and simulated datasets (details in section S11 in Text S1). We found that in all cancer types analyzed, NIMBus effectively controlled P value inflation compared to the method mentioned in [4]. For example, in LUAD the P values for real data fall on the diagonal with the uniform P values, apart from a few outliers that represent the true significant signals (black dots on the right sides in Fig. 5). After P value correction using the Benjamini–Hochberg method, only 11 genes are reported as highly mutation in LUAD, while none were discovered on the simulated data (orange dots in Fig. 5). On the other hand, the method using a constant mutation rate assumption (as in [4]) reported 6023 genes to be significantly mutated, indicating severe P value inflation.

Figure 5. Q-Q plots of P values of real and simulated WGS data.

Table 1. Top genes after *p*-value combination

Rank	Gene	Adjust P	PubMed ID
1	TP53	4.33E-139	17401424
2	DDX3X	3.65E-18	22820256
3	KRAS	2.56E-06	19847166
4	MUC4	4.47E-06	19935676
5	CDH1	3.07E-05	10973239
6	ARID1A	2.36 E-04	22037554
7	SMARCA4	3.78 E-04	18386774
8	FGFR1	7.43 E-04	23817572

We also used Fisher's method to combine P values from all cancer types. In total, 15 genes were discovered to be significantly mutated. Twelve of them are well documented as related with cancer progression. The top genes are shown in Table 1 and their PubMed ID is given in the last column for reference. These results showed that NIMBus is able to find sensible mutational hotspots as cancer drivers.

(B) Mutation burden of KEGG pathways

Using the KEGG pathway dataset, consisting of 288 unique pathways, we performed a network mutation burden test on each pathway for each cancer type to discover significantly mutated pathways. We found that of the seven cancer types analyzed, four cancer types exhibited significantly mutated KEGG pathways ($p_{adj} < 0.05$). In particular, we found 5 significant pathways in BRCA, 5 in LICA, 10 in GACA, and 3 in LUAD. No significant pathways were found in MB, PA, or PRAD. The significant pathways and their associated cancer types are seen in Table 2, as well as the Benjamini-Hochberg adjusted *p*-value. The significant pathway list includes pathways associated with the p53 signaling pathway, apoptosis, and cell growth – all of which are

well known KEGG pathways associated with cancer. In addition to these well-studied pathways, we were able to discover many novel pathways, including other signaling and disease-associated pathways. These results demonstrate a novel way to use NIMBus as a way to conduct mutation burden tests in biologically meaningful networks in the genome.

Table 2: Significant Pathways and *p*-values

KEGG ID	Description	LICA	GACA	LUAD	BRCA
04115	p53 signaling pathway	****	****	-	****
04210	Apoptosis	****	****	-	****
04110	Cell cycle; growth and death	*	-	-	-
04919	Thyroid hormone signaling	***	****	*	**
05014	Amyotrophic lateral sclerosis	****	****	**	****
04310	Wnt signaling pathway	-	****	-	-
04722	Neurotrophin signaling	-	****	-	****
04010	MAPK signaling pathway	-	***	-	-
05216	Thyroid cancer pathway	-	*	-	-
05219	Bladder cancer, urothelial tumor	-	*	-	-
04742	Taste transduction	-	-	*	-

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$

3.5 NIMBus discovered a list of highly mutated noncoding regions from cancer WGS data

We applied NIMBus on WGS variant calls for all seven cancer types to predict the individual somatic burden P values, and compared these results to those from global and local Binomial models (details in Text S1).

Similar to the results in the coding region analysis, both global and local Binomial models generated too many burdened regions in all noncoding annotation categories, as evidenced by the poor fitting in Fig. 3B. For example, in liver cancer after P value correction, NIMBus identified 21 promoters as highly mutated, while local and global

binomial models identified 79 and 641, respectively. Hence, our negative binomial assumption in NIMBus effectively captured the overdispersion and controlled the number of false positives. To further demonstrate this, we provided the Q-Q plots of P values in promoter regions for all seven cancer types in Fig. 6B as a quality check. In theory, if no significantly burdened regions are detected, the P values should follow uniform distribution. As shown in Fig. 6B, the majority of our P values follow the uniform assumption, with the exception of a few outliers representing the true signals, indicating reasonable P value distributions for all cancer types. Similar results were also observed in other noncoding annotations (data not shown). We release our burden test results on nimbus.gersteinlab.org as an online resource for the whole community.

Figure 6 (A) number of overly mutated promoter regions in all cancer types; (B) Q-Q plots of P values for promoter regions; (C) total number of burdened regions in all noncoding annotations after merging P values from 7 cancer types. B_local: local Binomial Model, B_global: global Binomial Model

To summarize the mutation burdens from all cancer types, we used Fisher's method to calculate the final P values for all three models. Similar to P values from a single cancer type, the combined P values are severely inflated in both global and local Binomial models, but are rigorously controlled by NIMBus (table C in Fig. 6). For example, NIMBus reported only 39 transcription start sites (TSS) as burdened, compared to 164 and 263 for the other two methods. Additionally, out of the 39 TSS elements, several of them have been experimentally validated or computationally predicted in other

work to be associated with cancer. For instance, TP53 is a well-studied tumor suppressor gene that is involved in many cancer types, and the combined P value for the TP53 TSS is ranked second in our analysis ($P=4.26\times10^{-14}$). Also, LMO3 interacts with the tumor suppressor TP53 and regulates its function, and it is ranked fourth in our analysis ($P=3.25\times10^{-13}$). Similar to previous reports, we also identified the AGAP5 TSS site as a mutation hotspot, ranking third ($P=7.07\times10^{-14}$) in our analysis. Another important example is the TSS site in TERT, which is ranked fifth in our results ($P=1.55\times10^{-10}$) and has been experimentally validated to be associated with multiple types of cancer progression [7]. The discovery of such results shows that NIMBus can serve as a powerful tool for mutation driver event discovery in genetic diseases.

4 CONCLUSION

Thousands of somatic genomes are now available due to the fast development of whole genome sequencing technologies, providing us with increasing statistical power to scrutinize the somatic mutation landscape. At the same time, thanks to collaborative efforts of big consortia, such as REMC and ENCODE, tens of thousands of functional characteristic experimental results on human genomes have been released for immediate use to the whole community. Hence, integrative frameworks are of urgent need in order to explore the interplay between WGS data and these functional characteristic data. It will not only be important to accurately search for mutational hotspots as driver candidates for complex diseases but also to better interpret the underlying biological mechanisms of diseases for clinicians and biologists.

In this paper, we proposed a new integrative framework called NIMBus to analyze somatic genomes. Due to the heterogeneous nature of various somatic genomes, our method treated the individual mutation rate as a gamma distributed random variable to mimic the varying mutational baseline for different patients. Resultantly, it modeled the mutation counts data using a two parameter negative binomial distribution, which improved data fitting dramatically as compared to previous work (Fig. 3B). It then uses a negative binomial regression to capture the effect of a widespread list of genomic features on mutation processes for accurate somatic burden analysis.

Unlike previous efforts, which use very limited covariates to estimate local mutation rate in very qualitative way, we explored the whole REMC and ENCODE data and extracted 381 features that best describe chromatin organization, expression profiling, replication status, and context effect in all possible tissues to jointly predict the local mutation rate at high precision. In terms of covariate correction, NIMBus demonstrated three obvious advantages: 1) It incorporates the most comprehensive list of covariates in multiple tissues to achieve accurate background mutation rate estimation; 2) It provides an integrative framework that can be extended to any number of covariates and successfully avoids the high dimensionality problem of other methods [6], which is extremely important due to the rapidly growing amount of available functional characteristic data available and the drop in cost of sequencing technologies; 3) It automatically utilizes the genomic regions with the highest credibility for training purposes, so users do not have to be concerned about performing carefully calibrated training data selection and complex covariate matching processes.

The length of training bins l is an important parameter for NIMBus. On one side, a shorter bin size will be advantageous in the P value evaluation as it can remove the mutational heterogeneity across regions more effectively at a higher resolution. On the other side, a smaller l sometimes will result in worse mutation rate prediction performance for two reasons. First, sensible mutation rate quantification is necessary in each single bin for the regression purpose. However, somatic mutations are usually sparsely scattered across the genome due to limited number of disease genomes available at the moment. In the extreme case, when l is so small that most bins have zero mutations, it is difficult for the regression model to capture the relationship between mutations and covariates. Second, some of the covariates are only reported to be functional on a large scale [10], so reducing l will not necessarily boost prediction precision. Optimal bin size selection is still a challenging problem that needs further case-by-case investigation. In our analysis, we used a 1 Mb bin size for all cancer types.

Noncoding regions represent more than 98% of the whole human genome, and are investigated less mainly due to limited knowledge of their biological functions. NIMBus is also designed to explore the most comprehensive collection of noncoding annotations. Therefore, it collects the up-to-date, full catalog of noncoding annotations of all possible tissues from ENCODE and our previous efforts from the 1000 Genomes Project. Furthermore, it further customizes these annotations specifically for somatic burden analysis. All these integrated internal annotations of NIMBus can be either tested for somatic burden or used to annotate the user specific input regions.

We applied NIMBus to 649 cancer genomes of seven different types collected from public data and collaborators. The burden test P values for each cancer type were

deduced and Fisher's method was used to calculate the combined P values. We first evaluated the performance of NIMBus on coding regions, which have been investigated with much detail by researchers. Many well-documented cancer associated genes were discovered by NIMBus (Table 1 and Table S3). We also repeated the same analysis on a simulated dataset and found no significant genes. These results demonstrate that NIMBus is able to find overly mutated genes effectively while rigorously controlling false positives.

In addition to single gene burden analysis tests, we were able to detect significantly mutated KEGG pathways, including the TP53 signaling pathway and apoptosis pathway, both of which are implicated in cancer progression. The adaptability of NIMBus to analysis of gene networks may prove useful in determining significantly mutated regions of the genome that are not physically adjacent.

Furthermore, numerous noncoding elements were also reported as significantly mutated (Table C in Fig. 6). Included were some well-known regions, such as the TP53, LMO, and TERT TSS, proving the effectiveness of NIMBus in indentifying disease-associated regions.

It is worth mentioning that although we demonstrate the effectiveness of NIMBus mostly on somatic mutation analysis, NIMBus can be immediately extended to germline variant analysis as well. In summary, NIMBus is the first method that integrates comprehensive genomic features to analyze the mutation burdens of disease genomes. Such external data does not only help to better estimate the background mutation rate for successful false positive and negative control, but also provides the most extensive noncoding annotations for users to interpret their results. It serves as a powerful

computation tool to accurately predict driver events in human genetic diseases and potentially identify biological targets for drug discovery.

Data availability

Additional files

Additional file 1: Supplemental document including details of all statistical methods used. Appropriate supplemental sections and figures are referenced in the paper.

Abbreviations

breast cancer (BRCA), gastric cancer (GACA), liver cancer (LICA), Lung adenocarcinoma (LUAD), prostate cancer (PRAD), Medulloblastoma (MB), and Pilocytic Astrocytoma (PA)

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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