

# A way to analyse GWAS data using exons

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## Abstract

Genome Wide Association Studies aim to analyse the link between frequency of single nucleotide polymorphism and phenotype by comparing to a reference population. The final goal is to find relevant biomarkers involved in predisposition to diseases.

The first step of this analyse is evaluating how the p-values of SNPs resulting from comparison are transferred to genes. The commonly used methods consider genes in totality and base their computing on  $\chi^2$  statistics. However, in the genome, genes are divided into exons and are built by a mechanism where splicing is a key step. The SNPs close to splice sites, which are near exons, disturb the structure of the concerned genes. The distances from SNPs to exons are a factor influencing the effect of SNPs.

Using summary statistics of GWAS, here method is based on the statistical relation between positions of SNPs and positions of exons. The obtained weights are introduced in the Stouffer's Z-score formula. So, the p-values transferred from SNPs to genes become a function of distance from SNPs to exons.

Moreover, a simple geometric analysis of the distance and Z-score couple shows an area of extreme values. The analysis can be directed to a subset of SNPs which probably plays a role in difference of genotype.

**Keywords:** GWAS, SNP, exon, intron, gene, splice

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# Aim and principle

Genome Wide Association Studies allow to analyse the link between frequency of single nucleotide polymorphism and phenotype (for instance predisposition to specific disease) by comparing to a reference population. Their results are synthesized in the summary statistics used here. They provide a p-value measuring the gap between the frequency of every SNP in the studied population and the reference population whose features are similar but without the phenotype. Thier goal is to find biomarkers which are presumed to be involved in the phenotype.

The aim of study is to find a function giving p-values of genes from p-values of SNPs based on the consistency of genes and how they are along the genome. Genes are divided into exons which are transformed in messenger RNA (mRNA) by the mechanism of splicing. So, this method is very different from methods usually used softwares such as VEGAS2 [11] or MAGMA [3] based on statistical comparisons using  $\chi^2$  statistic (see Comparison to MAGMA).

The elements of human genome on which are focused are the coding sequences (a tiny part less than 2% of genome) and, between them, the introns, interfering in gene expression by splicing (about 30% of genome). The other parts of the genome: regulatory DNA sequences, repetitive DNA sequences and mobile genetic elements are neglected. Notably, other effects are possible by the regulation of expression by regulatory DNA sequences which is not yet well known. Taking them into consideration is difficult.

Base position of exons are extracted by chromosomes from *ncbi.nlm.nih.gov* (acces by [19]). The computed number and length are globally:

- 20,108 genes divided in 1 to 190 exons (repartition fig 1)
- 193,532 exons covering 1,1 % of genome
- exons and introns covering 34 % of genome
- only about 12% of exons include one gene

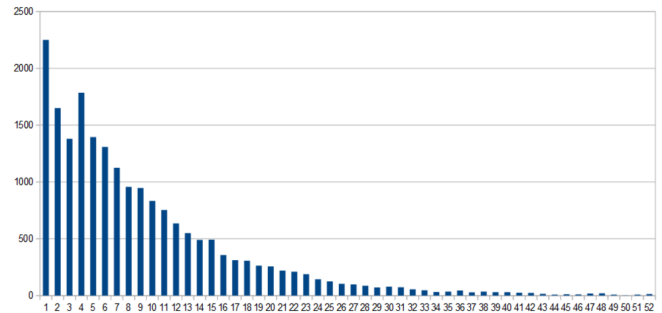


Figure 1: Number of exons by genes, until 52 exons

Cellular machinery builds proteins from messenger RNA. A primary transcript of DNA is transformed in mRNA by removing introns between exons and binding exons. This process is splicing. An exon can be used to make several genes. Here the analysis is not about the detailed mechanism but is based on statistics about relative position of mutations and genes.

SNPs are punctual and some studies are about repartition of SNPs along the genome [5] and [7], but they do not take into consideration the position of other elements involved in building genes.

The relative position of SNPs in respect of exons implies different ways of influencing gene expression. With the reasonable hypothesis: the effect of SNPs is not able to jump over neighboring exons, six cases are distinguished:

1. inside an exon: effect on thin structure of the gene;
2. between 2 exons of the same gene: effect on global structure of the gene by disturbance of splicing;
3. between 2 exons of 2 different genes and included in the 2 genes: effect on structure of 2 genes by splicing;
4. between 2 exons of 2 genes and included only in 1 gene: effect on structure of the gene by splicing, no effect on the other;
5. between 2 exons and outside any gene: no effect;
6. before the first exon and after the last exon: no effect.

The effect of SNPs to genes is different according to the relative position, confirmed by some publication of effect by SNPs on splicing ([9] [12] and [10]). For the case 'no direct effect', other effects are possible by the regulation of expression by regulatory DNA sequences which are neglected here.

## Observations about Relative Positions of SNPs and Exons

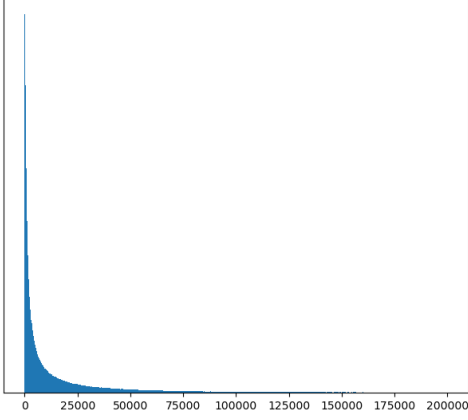


Figure 2: Histogram showing the numbers of SNPs by bins of minimal distances from SNPs to exons (GCST006719 from ebi [18])

The repartition of SNPs appears not uniform along the genome. It can be observed visually and reported in [8]. Further the proximity of exons seems to increase the density of SNPs. The proximity of exons plays a role in its variations: more SNPs are near exons, more they are numerous. So, the chosen variable for evaluating these variations is the minimal distance from SNPs to exons. Exons are considered as anchors in the genome. The histogram 2 confirms this observation.

The profile of this relation is specified by drawing the normalised cumulative number of SNPs in function of the minimal distance from SNPs to exons (fig 3). Obviously, these inside genes are nearer to exons than these outside genes. In the following, only the SNPs inside the genes will be taken into account.

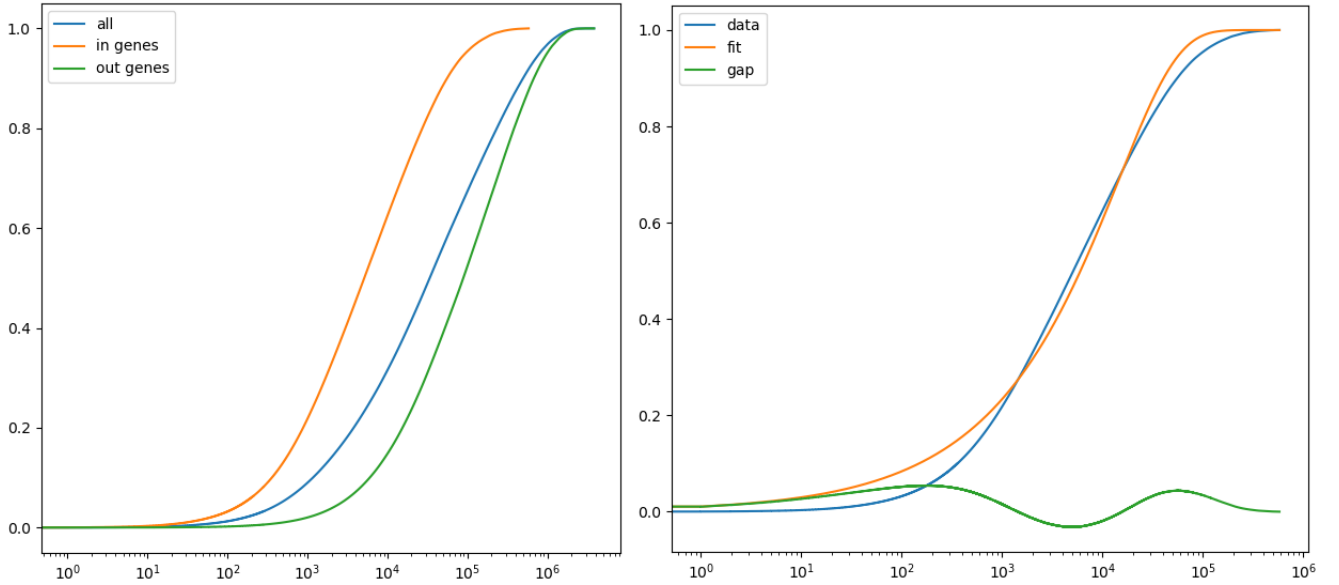


Figure 3: On the left, position of SNPs relative to exons (orange in genes, green out genes, blue all), normalized cumulative number in function of minimal distance of SNPs to exons, logarithmic scale on the abscissa.

On the right, fitting a normalized cumulative number in function of minimal distance of SNPs to exons, logarithmic scale on the abscissa.

(GCST006719 from ebi [18])

This distribution can be fitted by a  $\gamma$  distribution whose probability density function (pdf) is:

$$\gamma.pdf(x, shape, scale) = \frac{1}{\Gamma(shape) * scale^{shape}} * x^{shape-1} * e^{-\frac{x}{scale}}$$

The fitted curve (fig 3) is the cumulative density function (cdf) of  $\gamma$  law whose parameters are explained below .

This distribution can be interpreted as a heterogeneous spatial Poisson process. The intensity  $\lambda$ , average number of points by length, depends on the location:

$$\lambda(d) = \frac{\gamma \cdot pdf(d)}{\gamma \cdot cdf(d)}.$$

The inhomogeneous Poisson process is a counting process  $n(t)$  in a time interval where the intensity  $\lambda$  gives the number of events in an interval by:

$$E(n(t+h) - n(t)) = \int_t^{t+h} \lambda(\tau) d\tau$$

This expression with  $h \rightarrow 0$  is brought closer to the law from observation (N number of SNPs):

$$\frac{dn(t)}{dt} = \frac{1}{N} * \gamma \cdot pdf(t)$$

Considering  $t$  as the minimal distances from SNPs to exons, the expression of  $\lambda$  is:

$$\lambda(t) = \frac{1}{N} * \gamma \cdot pdf(t)$$

This function is used only as weight, the term  $\frac{1}{N}$  can be removed without changing the result.

Therefore the intensity  $\lambda$  is the parameter of Poisson law which is not constant contrary to the well known law. This can be pictured by cars driving along a straight line where there are villages which slow the traffic (traffic light, speed limit). Exons are villages, SNPs are cars at a moment and intensity in the instantaneous car flow.

The fitting normalized cumulative number in function of minimal distance of SNPs to exons (fig 3) gives these values of parameters for special data: shape = 0.44924 scale = 32738. This observation does no concern only this example as the above array show for different sources [18] [16] (only SNPs inside genes) ; the parameters obtained are close to each other:

data source	shape	scale	mse*	phenotype
CIDR_AFRO	0.44718	34050	$8.6 \cdot 10^{-4}$	breast cancer
GCST004988	0.44924	32738	$8.9 \cdot 10^{-4}$	breast cancer
GCST006719	0.45382	34094	$8.2 \cdot 10^{-4}$	breast cancer
GCST007236	0.44931	33066	$8.8 \cdot 10^{-4}$	breast cancer
GCST90011804	0.44956	32842	$8.9 \cdot 10^{-4}$	breast cancer
GCST90011808	0.44960	32863	$8.9 \cdot 10^{-4}$	prostate cancer
GCST90011811	0.44956	32839	$8.9 \cdot 10^{-4}$	colon cancer

\* mse: mean square error

Shape is the critical parameter for the link between intensity and distance: if shape = 1,  $\gamma$  is exponential, if shape < 1, its form is hyperbolic, if shape > 1,  $\gamma$  increase after that decrease (confer  $\gamma$  distribution for different values of shape [17]). Here the shape is far less than 1, so SNPs tend to agglomerate preferably close to exons. It leads to notice accumulation of SNPs near exons as the histogram 2 shows. Finding a  $\gamma$  distribution is not surprising, it is common in nature [4].  $\gamma$  distribution is the maximum entropy distribution for a positive variable, given its mean value and the mean value of its logarithm.

These observations raise two questions. Could this distribution be the consequence of a statistical effect ? Can the fit be improved?

The statistical effect can be analysed by simulation. The SNPs are almost evenly distributed. Their repartition respects a gamma law with a significant dispersion of values (shape = 0.75 - 0.82 and scale = 230 - 650). The uniform distribution corresponds to shape equals to one. The law of intervals between values uniformly distributed is exponential. The distribution of SNPs seems to be the result of linkage disequilibrium. The correlation between loci decreases with the distance between loci as measures of LD show [15] [1]. The consequence is that the variants contributing to an analog effect tend to agglomerate as

shown by a simulation (Toy Simulation of Linkage Disequilibrium in supplementary information).

From this basis, SNPs are drawn along a female genome with shape = 0.80 and scale = 640. To simplify, exons are taken as punctual at their middle. The minimal distances from SNPs to exons is computed in both cases: this random distribution and the found law  $\gamma$  with shape = 0.45 and scale = 33400. A ratio between the both is computed:

minimal distance	$10^1$	$10^2$	$10^3$	$10^4$	$10^5$	$10^6$
ratio	22.6	3.8	2.1	1.7	1.2	1.1

These values confirm the accumulation of SNPs near close to exons.

Improving fit is possible by using the generalized gamma distribution:

$$gen\gamma.pdf(x, a, c) = \frac{c}{\Gamma(a)} * x^{ca-1} * e^{-x^c}$$

In addition to the parameters a and c, loc (localization) and scale are used to shim and scale the function. The fit result is better (fig 4), the mean square error is divided by 30, above the detail for the breast cancer GWAS:

data src	a	c	loc	scale	mse
GCST006719	4.53286	0.236350	59.2710	12.65112	2.8E-05
CIDR_AFRO	5.13095	0.219329	51.4059	4.16039	2.8E-05
GCST004988	5.91680	0.204237	49.2646	1.13130	2.5E-05
GCST007236	5.75427	0.207206	50.3181	1.48760	2.6E-05
GCST90011804	5.80228	0.206401	50.4562	1.37293	2.6E-05

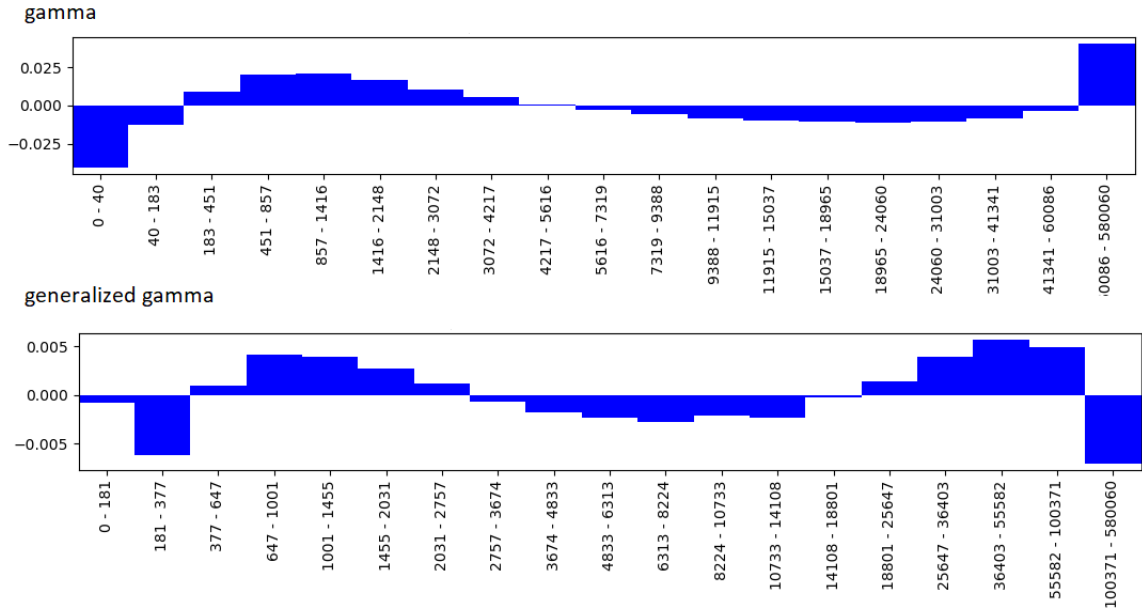


Figure 4: Quantile quantile plot as slice of 5% of theoretical number of gamma and generalized gamma law, the usual qqplot gives a distorted vision because the density of points decreases dramatically along the value axis (GCST006719 from ebi [18])

The dispersion of parameters is greater than fit by  $\gamma$  law. The relative standard deviations of the parameters are 9.7%, 5.6%, 7.0%, 105% for generalized gamma law and 0.5%, 1.8% for  $\gamma$  law. This is the result of an overfitting which complicates the method without providing real better precision. This is why the simple  $\gamma$  law is kept.

Also intervals between exons follow a generalized gamma distribution whose parameters are a = 4.223377, c = -0.313196, loc = -89.94722, scale = 144093.2 and mean square error 8.2E-05. This observation is not

used here. It shows the generalized gamma distribution is a very versatile law.

The accumulation of SNPs close to exons is a real phenomenon. The SNPs located preferentially inside genes and near exons according to a  $\gamma$  law with a scale much lower than 1, dramatically decreasing with the distance, which suggest that effect of SNPs on genes decreases also when the distances from SNPs to exons increases. The relative positions of SNPs and exons play a leading role in the effect by SNPs on genes. So, the method to evaluate the quantitative link between SNPs and genes is based on the following rules:

- Only the direct effect is kept for SNPs inside genes, the indirect regulation is neglected
- The scope of the effect by SNPs is limited to the neighboring exons and does not jump over exons
- The resulting p-values of genes are computed by the Stouffer's Z-score method
- The weights used in Z-score formula are the probability density function of gamma law

## Method

It follows that using the probability density function as weights to transfer the p-value to genes seems natural. The resulting p-values of genes are computed by the Stouffer's Z-score method ([21]) (Z-score is the number of standard deviations between value and the mean value):

$$Z = \frac{\sum_i w_i * z_i}{\sqrt{\sum_i w_i^2 + 2 * \sum_{i < j} r_{ij} * w_i * w_j}}$$

The Z-score of every gene is computed from the Z-score of  $SNP_i$  in the scope. The weights  $w_i$  are the probability density function at the distance between  $SNP_i$  and exons of this gene. They are one or two according to the case because the effect of SNPs does not jump over genes.

The simple formula of Z-score does not contain the term  $2 * \sum_{i < j} r_{ij} * w_i * w_j$ . This formula is commonly used in meta analysis. But in this case this term must be taken into account. Indeed  $r_{ij}$  is the correlation coefficient between the data used for computing the p-values of  $SNP_i$  and  $SNP_j$ . The SNPs contribute to the same phenotype, so the correlation coefficient is not null and must be evaluated.

Computing every  $r_{ij}$  is out of reach. Only one value is assigned to the correlation coefficient  $r_{ij}$ . It is computed by linear adjustment of  $\log(\text{p-values})$  and  $\log(\text{cumulative number})$  (fig 5), which is justified by the beta uniform mixture model. The distribution of p-values can be described by a  $\beta(a, 1)$  law to which the noise is added (beta uniform mixture model [13]). The expression of  $\beta$  is:

$$\beta.pdf(x) = a * x^{a-1} \text{ and } \beta.cdf(x) = x^a$$

The formula with noise  $\lambda$  is:

$$bum.pdf(x) = \lambda + (1 - \lambda) * a * x^{a-1}$$

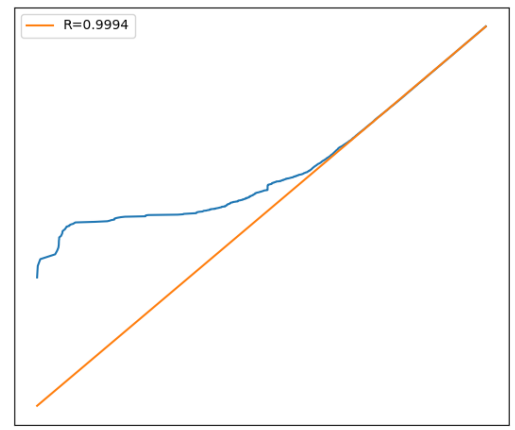
The noise ruins the correlation between p-values. So, the linear fitting log-log on cumulative normalized values gives the correlation coefficient between p-values.

To evaluate the effect of SNPs inside exons, the weights are taken infinite, which cancels the effect of any SNPs outside exons. The formula of Z-score method for n Z-score to be weighted with R as the common correlation coefficient. When the weights are infinity, it becomes, :

$$Z = \frac{\sum_i w_i * z_i}{\sqrt{n + R * n * (n - 1)}}$$

On a practical level, Python cannot compute the Z-score below  $5.55 * 10^{-17}$ . So the p-value between  $6.7 * 10^{-133}$  and  $3.7 * 10^{-17}$  can be taken into account. They are considered as outlier values. Other outlier values are also eliminated in tails to enter in calculable p-values by Python. This is the case of GCST006719 and GCST007236 [18].

The effects of SNPs outside exons and inside genes are computed separately. This computing allows getting two lists of genes sorted by decreasing computed Z in two cases: SNPs inside exons and SNPs inside genes but not inside exons. Their effects may be added for genes influenced in two ways by the Z-score method (sum divided by  $\sqrt{2}$ ). But the kind of effect, necessary for analysing mechanisms, is lost.



In addition, the contributions of every SNP can be detailed by every term of the Z-score formula, obviously on a restricted sublist of genes (size of output). This detailed results show how a set of SNPs can reinforce its effects.

Figure 5: Linear log log fitting of normalized cumulative number of SNPs and their p-values (GCST006719 from ebi [18])

## Extreme values of Z-score and minimal distances

The Z-score of SNPs in function of minimal distance of SNPs to exons (fig 6) shows a feature of extreme values: the higher the Z of SNP, the closer SNP is to the exon. This interesting observation of extreme values provides confirmation that the distance of SNPs to exons plays a role in the effect of SNPs.

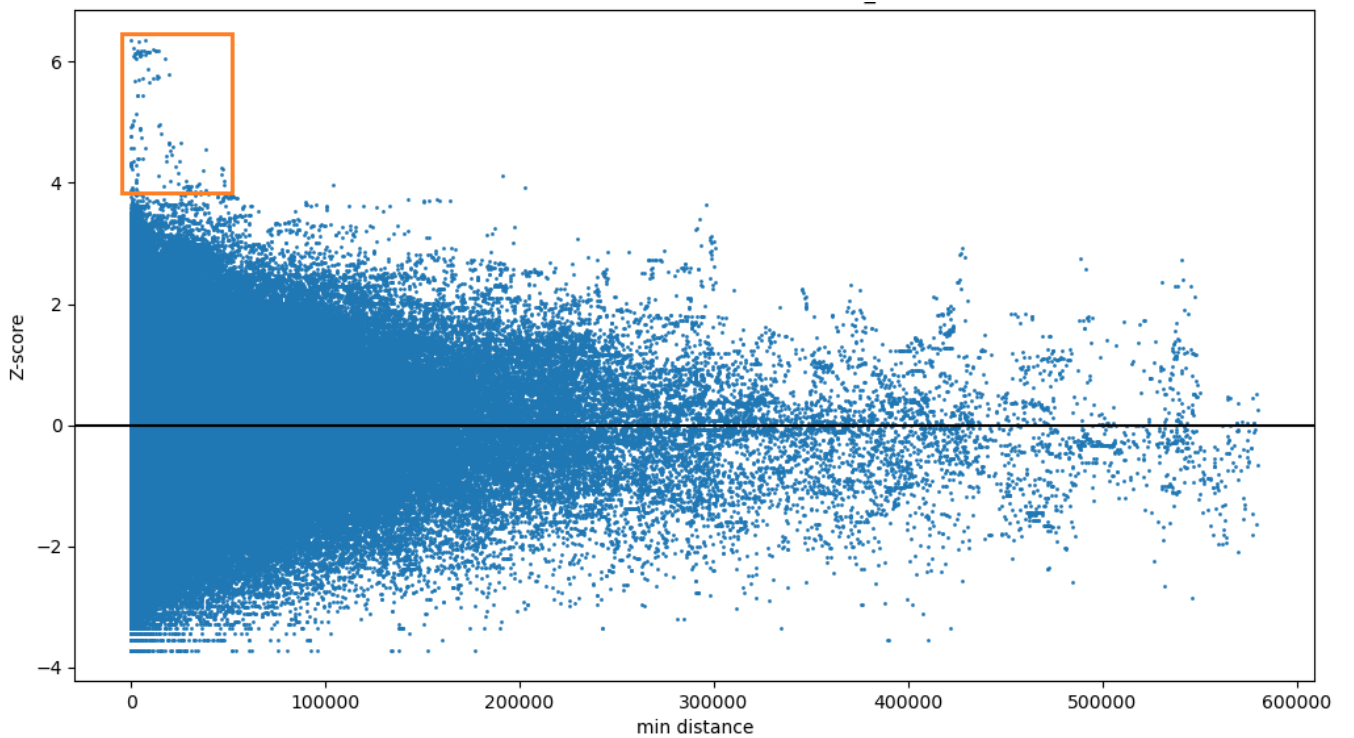


Figure 6: Z-score of SNPs in function of their minimal distances to exons (GCST006719 from ebi [18])

This analysis of the effect of SNPs on genes can be focused on this subset of SNPs. The scatter plot by gene as figure 6 graphically shows the effect of SNPs to genes by the p-value under the form of Z-score. It can be used for starting an accurate biological study. An incidental remark: the partial use of the weighting formula may give surprising results about genes because the evaluation of effect by not extreme SNPs can be negative (contribution by a set of SNPs > 100%).

# Comparison to MAGMA

MAGMA [3] is based on  $\chi^2$  statistics, a commonly used method. The method of MAGMA is of the same type as those implemented in PLINK [14] and VEGAS [11]. There are some small differences between them. MAGMA has the advantage of being computationally performant and easy to be used.

The results by MAGMA are far from these by SNP exons method. The difference of results is confirmed by the two graphs: gap for every gene sorted by Z-score and the histogram of gaps fig 7. The histogram shows a non completely gaussian difference. But, these differences upset the ranking of genes. To number these differences, the Euclidean distance between the resulting Z-score is equal to 66 and Kendall's  $\tau$  coefficient is equal to 0.70.

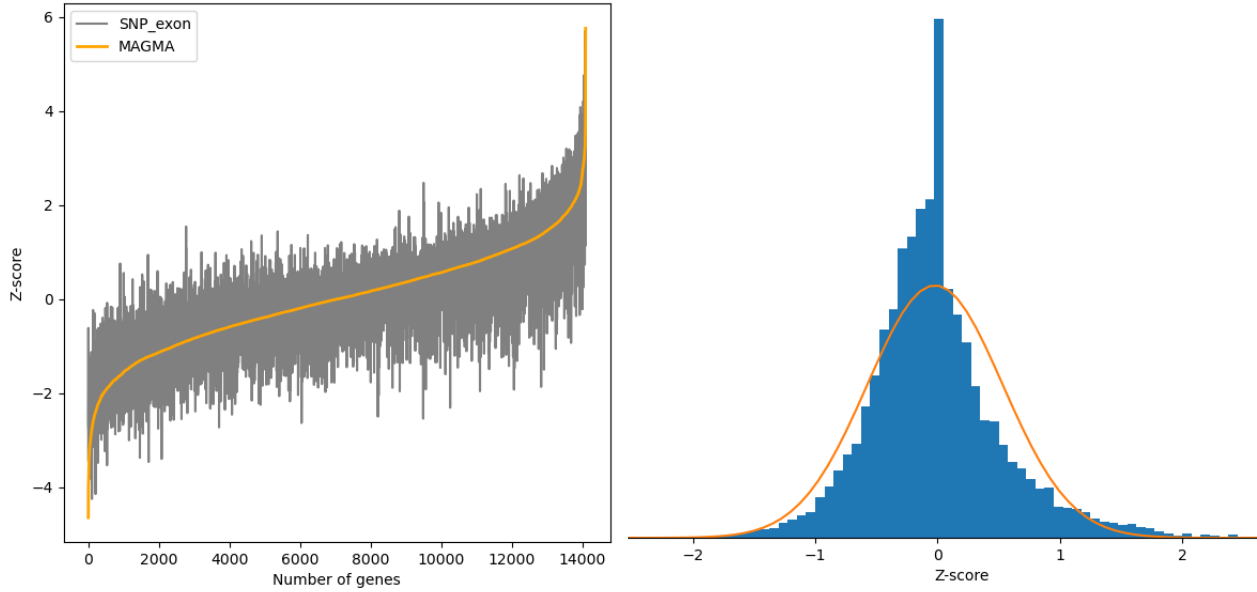


Figure 7: Cumulative Z-score of genes and histogram show the difference of outcome between MAGMA and SNP exon method, MAGMA is reference (GCST006719 from ebi [18])

A surprising ascertainment: the moving average of a hundred gene Z-score almost coincides with the result, which can be explained by a similar process of distributing the Z-score of SNPs to genes. But this ascertainment has a weak interest to explain these differences.

To identify the origin of differences, a way is checking the sensitivity of results to data by playing with both variables: p-value and base position. So, artificial data is created to evaluate the sensitivity to data. Firstly all p-values are made equal to the geometric mean of p-values. Secondly, SNPs are distributed uniformly along the segments of chromosomes common to genes, so SNPs stay inside genes where they were and variations of distances are canceled. Genes taken from first exon to last exon may cover each other, certainly it is infrequent. The Z-scores of genes obtained with these artificial data are compared to the Z-score obtained with real data.

At the sight of these histograms 8, the sensitivities to p-values of MAGMA and SNP exon method are very closed, but MAGMA is insensitive to positions of SNPs except to these inside the margin of proximity. Regardless of the position of SNPs inside a gene, MAGMA gives the same Z-score to the gene. On the contrary, the SNP exon method modulates the effect of SNPs by the position measured by the distance from SNPs to exons. The question is: is the effect of SNP on genes independent of the position of SNPs inside genes ?



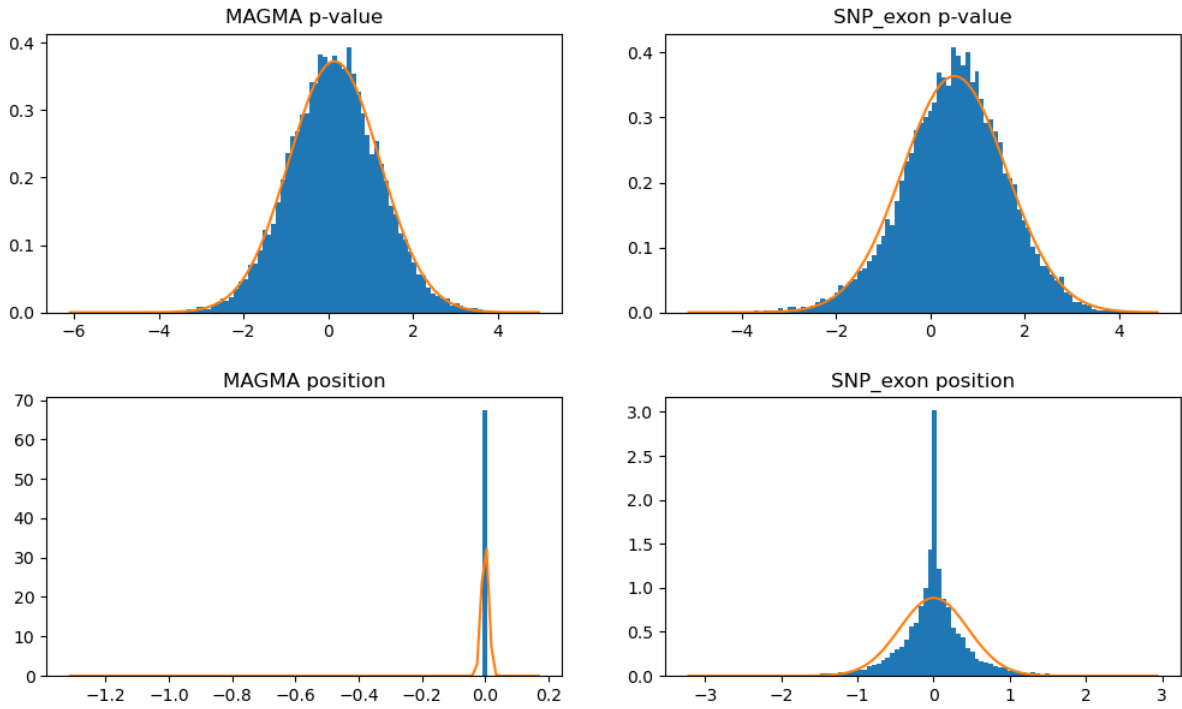


Figure 8: On the left Z-scores of genes got by MAGMA with same p-value and homogeneous position compared to result from real data as histogram, on the right same comparison for SNP exon method(GCST006719 from ebi [18])

## Biological knowledge

This observation must be confronted with knowledge in biology. The presence of a mutation within an exon can modify the shape of the active site or the protein folding. This obviously leads to modifications or disappearances of functions as [9].

Likewise several publications show the effect of SNPs on the mechanism of splicing as [6] and [20]. That last publication identifies an area of exonic splicing enhancers located at less than 69 base position. In this area, the density is multiplied by 7 according to the above simulation. These considerations underline the proximity between effective SNPs and exons.

A gene consists of multiple small exons (fig 1) separated by exons by a distance much bigger than the sizes of exons. The splice machinery has the task of finding small right exons among much longer exons. This is the role of splice sites. Splice site sequences that drive exon recognition are located at the begin and the end of introns [2]. So SNPs in or the near splice site can disturb splicing and affect building of genes.

Positions of SNPs in exons or in introns are not neutral to the phenotype.

## Discussion

The main drawback is that the results of the SNP exon method are far from those obtained by the commonly used method (based on  $\chi^2$  statistics). The differences are difficult to explain, except for the anecdotal coincidence of smoothed curves. Because their logics are far from each other. The  $\chi^2$  statistics method ignores the effect of positions of SNPs inside genes.

The SNP exon method has some advantages. The Z-scores or p-values of genes are computed from Z-scores or p-values of SNPs by a simple continuous function. All parameters are computed from available data being rid of outlier values. No hyper-parameter is necessary. Here the method is a way to create a bridge between

a global approach in order to be a nearer biological mechanism.

The main critique of the SNP exon method is about the use of Stouffer Z-score method where used weights are the probability density function  $\gamma$ . It is a too simple mode to use these observations. It needs to be deepened in a more rigorous way.

But, trying to get closer to biological reality, this method ignores some important phenomenons. The indirect mechanisms of regulation by regulatory DNA are neglected. SNPs in these areas probably disturb the expression of genes. Here method is based on the linear distances in the genome as 23 segments and so it obscures the spatial storage of folded DNA. It goes part of the way to bring closer the statistical approach and the biological mechanisms.

The  $\chi^2$  method and SNP exon method are rather complementary. Facing their both results must enlarge searching the cause of phenotype. In addition, the focus on extreme values in Z-score of SNPs in function of minimal distances from SNPs to exons allows to identify a supposedly set of SNPs. These analyses completed by positioning in pathways must enlighten the biological mechanism involved in the studied phenotype.

## Tools

The Python scripts for data analysis and gene p-values with the manual are available at [https://github.com/drovera/SNP\\_exon](https://github.com/drovera/SNP_exon) (except scripts about comparison and simulation which look like trials, if interested, contact author)

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