



**HAL**  
open science

**Synonymous mutation rs1129293 is associated with PIK3CG expression and PI3K $\gamma$  activation in patients with chronic Chagas cardiomyopathy**

Maria Cláudia Silva, Carlos Alessandro Fuzo, Isadora Marques Paiva, Naira Lopes Bibó, Maykon Tavares de Oliveira, Hellen Anastácia da Silva Soares, Christophe Chevillard, Jorge Kalil, Edecio Cunha-Neto, Thiago Mattar Cunha, et al.

► **To cite this version:**

Maria Cláudia Silva, Carlos Alessandro Fuzo, Isadora Marques Paiva, Naira Lopes Bibó, Maykon Tavares de Oliveira, et al.. Synonymous mutation rs1129293 is associated with PIK3CG expression and PI3K $\gamma$  activation in patients with chronic Chagas cardiomyopathy. *Immunobiology*, 2022, 227 (5), pp.152242. 10.1016/j.imbio.2022.152242 . hal-03934958

**HAL Id: hal-03934958**

**<https://hal.science/hal-03934958>**

Submitted on 17 Jan 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## Short communication

## Synonymous mutation rs1129293 is associated with *PIK3CG* expression and PI3K $\gamma$ activation in patients with chronic Chagas cardiomyopathy

Maria Cláudia Silva<sup>a</sup>, Carlos Alessandro Fuzo<sup>b</sup>, Isadora Marques Paiva<sup>c</sup>, Naira Lopes Bibó<sup>c</sup>, Maykon Tavares de Oliveira<sup>d</sup>, Hellen Anastácia da Silva Soares<sup>a</sup>, Christophe Chevillard<sup>e</sup>, Jorge Kalil<sup>f,g</sup>, Edecio Cunha-Neto<sup>f,g</sup>, Thiago Mattar Cunha<sup>c,\*</sup>, João Santana Silva<sup>a,h,\*</sup>

<sup>a</sup> Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

<sup>b</sup> Department of Clinical Analyses, Toxicology and Food Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

<sup>c</sup> Department of Pharmacology of Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

<sup>d</sup> Department of Internal Medicine, Cardiology Division, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

<sup>e</sup> INSERM, UMR\_1090, Aix Marseille Université, TAGC Theories and Approaches of Genomic Complexity, Institut MarMaRa, Marseille, France

<sup>f</sup> Laboratory of Immunology, Heart Institute, School of Medicine, University of São Paulo, São Paulo, Brazil

<sup>g</sup> Institute for Investigation in Immunology (iii), INCT, 05403-001, São Paulo, Brazil

<sup>h</sup> Fiocruz-Bi-Institutional Translational Medicine Platform, Ribeirão Preto, SP, Brazil

## ARTICLE INFO

## Keywords:

Synonymous mutation

Chagas disease

PI3K $\gamma$

Single nucleotide polymorphism

rs1129293

## ABSTRACT

Single nucleotide polymorphisms (SNPs) that do not change the composition of amino acids and cause synonymous mutations (sSNPs) were previously considered to lack any functional roles. However, sSNPs have recently been shown to interfere with protein expression owing to a myriad of factors related to the regulation of transcription, mRNA stability, and protein translation processes. In patients with Chagas disease, the presence of the synonymous mutation rs1129293 in phosphatidylinositol-4,5-bisphosphate 3-kinase gamma (*PIK3CG*) gene contributes to the development of the chronic Chagas cardiomyopathy (CCC), instead of the digestive or asymptomatic forms. In this study, we aimed to investigate whether rs1129293 is associated with the transcription of *PIK3CG* mRNA and its activity by quantifying AKT phosphorylation in the heart samples of 26 chagasic patients with CCC. Our results showed an association between rs1129293 and decreased *PIK3CG* mRNA expression levels in the cardiac tissues of patients with CCC. The phosphorylation levels of AKT, the protein target of PI3K, were also reduced in patients with this mutation, but were not correlated with *PIK3CG* mRNA expression levels. Moreover, bioinformatics analysis showed that rs1129293 and other SNPs in linkage disequilibrium (LD) were associated with the transcriptional regulatory elements, post-transcriptional modifications, and cell-specific splicing expression of *PIK3CG* mRNA. Therefore, our data demonstrates that the synonymous SNP rs1129293 is capable of affecting the *PIK3CG* mRNA expression and PI3K $\gamma$  activation.

### 1. Introduction

Single nucleotide polymorphisms (SNPs) are considered to be the simplest type of DNA mutations. Substitution of one single nucleotide with another can change or not the encoded amino acid. SNPs that change a codon for another synonymous codon and do not alter the

amino acid sequence of the translated protein are called synonymous mutations. These type of mutations were previously considered to be “silent,” without any functional consequences to the organisms; however, recent studies have shown that synonymous mutations can interfere with several points of protein synthesis (Plotkin and Kudla, 2011).

Synonymous SNPs (sSNPs) can alter the protein expression,

**Abbreviations:** SNP, single nucleotide polymorphism; sSNP, synonymous single nucleotide polymorphism; GWAS, genome-wide association studies; CCC, chronic Chagas cardiomyopathy; PI3K, phosphatidylinositol 3-kinase; eQTL, expression quantitative trait loci; sQTL, splicing quantitative trait loci; HWE, Hardy-Weinberg equilibrium.

\* Corresponding authors. at: Ribeirão Preto Medical School - University of São Paulo, Bandeirantes Avenue, 3900, 14049-900, Ribeirão Preto, SP, Brazil. Fiocruz-Bi-Institutional Translational Medicine Platform, Bandeirantes Avenue, 3900, 14049-900 Ribeirão Preto, SP, Brazil.

E-mail addresses: [thicunha@usp.br](mailto:thicunha@usp.br) (T. Mattar Cunha), [jdsilva@fmrp.usp.br](mailto:jdsilva@fmrp.usp.br) (J. Santana Silva).

<https://doi.org/10.1016/j.imbio.2022.152242>

Received 30 March 2022; Received in revised form 23 June 2022; Accepted 6 July 2022

Available online 9 July 2022

0171-2985/© 2022 Elsevier GmbH. All rights reserved.

conformation, and/or function via several mechanisms. sSNPs can disrupt the spliceosome machinery, and affect intron removal during the RNA processing (Cartegni et al., 2002), as well as the availability of anticodons in a specific tissue or cell type (Chamary and Hurst, 2005). Micro-RNA (miRNA) binding sites can also be altered by synonymous mutations, which interfere with the post-translational modifications of proteins (Brest et al., 2011). In addition, synonymous changes can interfere with the mRNA expression and also reduce mRNA stability (Eyre-Walker and Bulmer, 1993; Gutman et al., 2021; Gu et al., 2010). In addition, although some sSNPs do not impact on splicing, miRNA regulation, or mRNA expression and stability, they impact protein translation (Kim et al., 2020).

Our group has already showed that when people carrying the sSNP rs1129293 are infected with *Trypanosoma cruzi*, they exhibit an increased risk of developing the severe cardiac form of Chagas disease, instead of the asymptomatic or digestive forms (Silva et al., 2021). In general, studies involving SNPs have primarily focused on non-synonymous mutations; however, sSNPs are involved in disease mechanisms similar to non-synonymous SNPs (Chen et al., 2010). In fact, sSNPs are associated with >50 human diseases. This discovery was possible due to the development of computational methods to analyze these data using genome-wide association studies (GWAS) or approaches that use arrays with a very high number of genes (Sauna and Kimchi-Sarfaty, 2013). These findings reveal the importance of performing association studies along with functional studies. Here, using functional and computational studies, we investigated the effects of the synonymous mutation rs1129293 on *PIK3CG* gene expression and protein function in the heart samples of chagasic patients with chronic Chagas cardiomyopathy (CCC).

## 2. Materials and methods

### 2.1. Subjects and heart samples

We studied 26 heart samples collected *postmortem* from chagasic patients exhibiting at least two positive serological tests (hemagglutination, immunofluorescence, or ELISA) for Chagas disease, as recommended by Dias et al. (Dias et al., 2016). All the patients were diagnosed with CCC. All samples were collected at the Heart Institute, São Paulo, Brazil and stored in TRIzol™ Reagent (ThermoFisher Scientific) (RNA extraction) or RIPA with a cocktail of protease and phosphatase inhibitors (protein extraction) at  $-80^{\circ}\text{C}$ . It is important to emphasize that the samples were processed as soon as possible to avoid degradation of the biological material.

### 2.2. Ethics statement

The research protocol used in this study was approved by the Institutional Ethics Committee of the CAPesq-Heart Institute, University Hospital of the Faculty of Medicine of São Paulo, Brazil (Protocol #0265/10).

### 2.3. DNA extraction and genotyping

Genomic DNA was extracted from the heart samples of patients with CCC using the specific kit Wizard® Genomic DNA Purification from Promega®, according to the manufacturer's instructions. The concentration and purity of isolated genomic DNA were measured using the NanoDrop™ One/One spectrophotometer (Thermo Scientific). The genotyping experiment of rs1129293 (C > T) were carried out using the TaqMan genotyping assays on the StepOnePlus real-time PCR system (Applied Biosystems®, California, USA). This SNP is a C/T substitution located on chromosome 7, at a location that encodes the catalytic subunit of the *PI3K $\gamma$*  gene (*PIK3CG*). SNP genotyping probes for rs1129293 [VIC/FAM] TGAAATTTGAACCATACCATGATAG [C/T] GCCCTTGCCAGATTCTGCTGAAGC, respectively (Catalog number: 4351379) were

purchased from ThermoFisher Scientific. Real-time PCR reactions were performed in a total final volume of 10  $\mu\text{L}$  contained the following: 6.25  $\mu\text{L}$  Taqman genotyping master mix (ThermoFisher Scientific), 5 ng DNA sample, 0.7  $\mu\text{M}$  TaqMan probe (Thermo Fisher Scientific) and add distilled water to 11.5  $\mu\text{L}$ . Real time fluorescence quantitative PCR was performed in triplicate per sample with the following conditions: An initial denaturation at  $95^{\circ}\text{C}$  for 10 min, followed by 40 cycles of denaturation at  $95^{\circ}\text{C}$  for 15 s and annealing at  $60^{\circ}\text{C}$  for 1 min. Genotypes were analyzed using the StepOnePlus software.

### 2.4. RNA extraction and real-time quantitative PCR (qPCR) assay

Total RNA was extracted from the heart samples of patients with CCC using the SV Total RNA Isolation System kit from Promega®, and the cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems®, California, USA), according to the manufacturer's instructions. PCR to detect the mRNA expression of *PI3K $\gamma$*  gene was carried out using the StepOnePlus real-time PCR system (Applied Biosystems®, California, USA) along with the SYBR Green GoTaq qPCR MasterMix (Promega®). qPCR data were analyzed by the Ct delta-delta method, using the *HPRT* as an endogenous control for transcript expression normalization. The sequences of *PI3K $\gamma$*  and *HPRT* primers are listed in Table 1.

### 2.5. Western blotting analysis

Protein lysates from the heart samples of patients with CCC were prepared in the RIPA buffer (Sigma-Aldrich®) supplemented with protease and phosphatase inhibitors (Cell Signaling®). The same amount of total protein for each sample was loaded and separated in 4–20% Mini-PROTEAN® TGX™ Precast Protein Gel (BIORAD). After electrophoresis, the proteins were electrotransferred onto nitrocellulose membranes (0.2  $\mu\text{m}$ ; GE Healthcare) and incubated for 1 h with 5% non-fat dry milk (Cell Signaling®), followed by overnight incubation at  $4^{\circ}\text{C}$  with the primary antibodies. After washing, the membranes were incubated for 1 h with horseradish peroxidase (HRP)-conjugated secondary antibodies. Immunoblots were visualized after membrane incubation with the Immobilon® Forte HRP Substrate (Millipore) using a ChemiDoc™ XRS System (BIORAD). The primary antibodies used in this study were phospho-AKT (Thr308) (1:2000; Cell Signaling; #13038); and AKT (pan) (1:2000; Cell Signaling; #4691). The images of the western blot bands were analyzed using the program Image Lab (BIORAD).

### 2.6. Statistical and bioinformatics analyses

The normal distributions for *PIK3CG* transcript quantification by qPCR pAKT levels by western blotting were determined by using the Kolmogorov–Smirnov test. Hardy-Weinberg equilibrium (HWE) was determined using the Haldane exact test with SELOME p-value calculation (Haldane, 1954) using the R package, *HardyWeinberg* (<https://www.jstatsoft.org/v64/i03/>) and  $p < 0.05$  as a significant threshold for HWE deviation. We tested the genotype association of rs1129293C > T with *PIK3CG* levels and pAKT using multiple linear regression considering additive (CC, CT, and TT) and recessive (CC and CT + TT) genetic models, assuming C as the major allele, T as the minor allele, and sex and age as covariates. SNPs in linkage disequilibrium with

**Table 1**  
Sequence of primers used in the study.

Gene	Primer sequence
<i>PIK3CG</i>	Fwd: 5'- CATATTGACTTCGGGCACATTCTTG – 3' Rev: 5'- GTCTCTGCAAACTCGATCTGATC – 3'
<i>HPRT</i>	Fwd: 5'- GCGATGTCAATAGGACTCCAG – 3' Rev: 5'- TTGTTGTAGGATATGCCCTTGA – 3'

Fwd = forward primer; Rev = reverse primer.

rs1129293 within  $R^2 > 0.8$  were obtained using the LDProxy tool from Ldlink webservice (Machiela and Chanock, 2015) using genotype data from phase 3 of the 1000 Genomes Project (Auton et al., 2015). The functional effects of genomic variants on PI3KCG transcripts were predicted using the Ensembl Variant Effect Predictor (VEP) (McLaren et al., 2016). Expression quantitative trait loci (eQTLs) and splicing quantitative trait loci (sQTLs) for rs1129293, and SNPs in LD annotated to immune cells and cardiac tissues of the EBI eQTL Catalogue (Kerimov et al., 2021) were obtained from Fivex (Kwong et al., 2021) with  $p < 0.01$  as the threshold for the size significance of the effect. Illustrations of annotation heatmaps, genomic regions, and associations were made using the packages, pheatmap, Gviz (Hahne and Ivanek, 2016), and ggplot2.

### 3. Results

#### 3.1. Decrease in PIK3CG mRNA transcription is associated with SNP rs1129293

The 26 chagasic patients from whom we collected the heart samples did not differ in sex or age (Table 2). All the patients were diagnosed with CCC (Table 2). First, the subjects were classified according to the occurrence of SNP rs1129293. DNA samples were collected from the cardiac tissues, and the genotype distribution did not deviate from HWE ( $p = 0.631$ ), with 50.0% of patients exhibiting the ancestral genotype CC, 46.15% of patients exhibiting the mutant genotype CT, and 3.85% of patients exhibiting the genotype TT (Table 3). Samples from subjects with and without the mutation rs1129293 were evaluated for the mRNA expression of PIK3CG. The associations between ancestral and mutant genotypes and mRNA transcription levels, tested in the additive genetic model, indicated that the occurrence of the mutation rs1129293 was associated with decreased PIK3CG mRNA transcript levels ( $p = 0.044$ ) (Fig. 1A).

#### 3.2. Synonymous mutation rs1129293 is associated with low levels of phosphorylated AKT in the cardiac tissue

The main protein phosphorylated after the activation of PI3K is the AKT (Burgering and Coffey, 1995). We investigated PI3K $\gamma$  activation in patients with or without the mutation in PIK3CG gene by determining the levels of pAKT at the threonine 308 (T308) site. Interestingly, heart samples from patients carrying the mutation showed decreased phosphorylation of AKT when tested in the recessive genetic model ( $p = 0.040$ ) (Fig. 1B and C). Although PI3K $\gamma$  expression and pAKT level were decreased in patients carrying this SNP, the two variants were not directly proportional to each other ( $r = 0.216$ ;  $p = 0.289$ ) (Fig. 1D), indicating that even when the PIK3CG mRNA is highly expressed, some additional post-transcriptional modifications can affect the AKT phosphorylation.

**Table 2**  
Gender and clinical form of the studied population.

Gender (n, %)	
F	13, (50.0%)
M	13, (50.0%)
Age (Years, $\pm$ SD)	
F	51, ( $\pm$ 9.7)
M	47, ( $\pm$ 13.7)
Clinical form (n, %)	
CARD	26, (100.0%)

F = female; M = male; CARD = cardiac; SD = standard deviation.

**Table 3**  
Genotype of patients used in the study.

Genotype (n, %)	
CC	13, (50.00%)
CT	12, (46.15%)
TT	1, (3.85%)

#### 3.3. rs1129293 and other SNPs in LD are predicted to interfere with the expression and post-transcriptional modification of PIK3CG

After bioinformatics analysis using the 1000 Genome data in LDProxy service, five SNPs were found to be in LD with rs1129293, one of them was localized upstream of PIK3CG and the other four in the intronic regions of the protein-coding transcripts of this gene (Fig. 2A). Among the SNPs in LD, rs4730204 was located in the upstream region and predicted to affect the regulatory promoter region of the PIK3CG, while intronic rs6963859 was predicted to be located in the promoter flanking region (Fig. 2A). Thereafter, we searched for significant eQTLs and sQTLs for PIK3CG associated with these SNPs in the immune cells and cardiac tissues found in the FIVEX database (Fig. 2B). Due to the high LD between these SNPs, the association values were very close. For eQTLs we observed only a small mean positive effect of 0.063 annotated for the expression of PIK3CG in Treg naïve cells from the Schmiedel\_2018 study (Schmiedel et al., 2018). Interestingly, we observed bigger effects for sQTLs datasets showing distinct tissue and cell expression splicing variants, which were compiled by considering the mean effects of all SNPs in Fig. 2B. The biggest effect associated with allelic variations was a decrease in transcript ENST00000496166 expression with mean effect size  $-0.68$  for CD4\_T\_cell\_anti-CD3-CD28 from the Schmiedel\_2018 study. Decreased levels of transcript ENST00000440650 were observed in T-cell (BLUEPRINT study) and naïve macrophage (Alasoo\_2018 study) (Alasoo et al., 2018). Interestingly, decreased and increased levels of the transcripts, ENST00000440650 and ENST00000496166, respectively, were associated with SNPs in both monocytes and neutrophils in the BLUEPRINT study. The same behavior was observed for the pair of transcripts, ENST00000473541 and ENST00000496166, in the artery aorta from GTEx study (Fig. 2B).

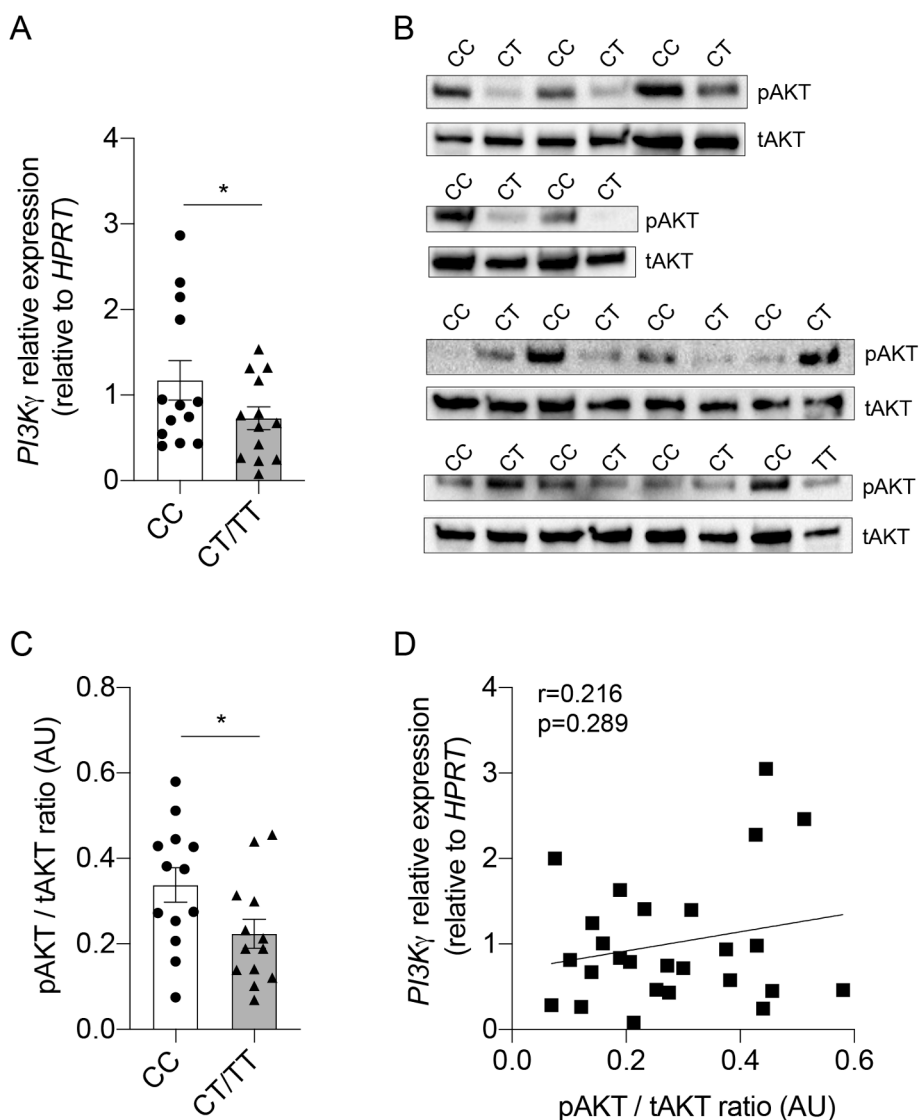
### 4. Discussion

The activation of PI3K isoforms has controversial roles in the protection in different models of infection in humans and mice (Olayinka-Adefemi et al., 2021; Hsu et al., 2015; Silva et al., 2018; Xiang and Wang, 2018). Following PI3K activation, AKT is phosphorylated and activated, executing its kinase function, which is essential for the PI3K signaling pathway (Burgering and Coffey, 1995). Modifications at any step of the protein expression, including the occurrence of sSNPs, can interfere with the protein function. Here, we used experimental and bioinformatics analyses to evaluate the functional effects of the synonymous SNP rs1129293 in PIK3CG, gene. Since our group have showed that this sSNP is highly prevalent in chagasic patients with CCC (Silva et al., 2021), we used in this study only samples provided from patients with the cardiac form of Chagas disease, in order to obtain a considerable number of samples with the mutant genotype CT or TT.

Although non-synonymous mutations commonly affect protein function, several studies have shown that sSNPs can alter the mRNA expression (Chen et al., 2020; Kerna et al., 2013), post-transcriptional modifications (Duan and Antezana, 2003; Meijer et al., 2013; Mueller et al., 2015), and protein functions (Karakostis et al., 2019; Simhadri et al., 2017).

mRNA expression is not always related to protein expression or function. This point is extremely relevant because contextual changes, such as temporal changes, can affect the mRNA–protein correlation, (Buccitelli and Selbach, 2020). Our results showed that, during the





**Fig. 1.** PI3K $\gamma$  expression and function in subjects with and without the mutation rs1129293. (A) Total RNA was isolated from heart samples of patients with CCC carrying the ancestral CC (n = 13) and mutant CT/TT (n = 13) genotypes and the RT-PCR analysis of the mRNA expression of *PIK3CG* was performed. The mRNA expression of *HPRT* was used as housekeeping control. (B) Proteins were extracted from heart samples of patients with CCC carrying the ancestral CC (n = 13) and mutant CT/TT (n = 13) genotypes and the phosphorylation levels of AKT (pAKT) were evaluated via western blot assay. The expression of total AKT (tAKT) was used as housekeeping control. (C) Quantitative western blot analysis using arbitrary units (AU). (D) Correlation between *PIK3CG* mRNA expression and pAKT/tAKT ratio in the heart samples of patients with CCC. \* $p < 0.05$ . The data were normally distributed according to the Kolmogorov-Smirnov test.

chronic phase of Chagas disease, patients with the polymorphism rs1129293 presented reduced *PIK3CG* mRNA expression levels, and also reduced PI3K $\gamma$  activity, that is, reduced AKT phosphorylation compared to those without the mutation. However, it was not surprising to observe that there is no positive correlation between the enzyme mRNA expression, and enzyme function. Recent papers have showed that, independently of the amount of mRNA, SNPs can affect the translation process (Robert and Pelletier, 2018) and the phosphorylation of proteins (Feng et al., 2020). Furthermore, using computational analysis, Wang et al. showed that sSNPs can change miRNA-mediated gene regulation affecting protein function (Wang et al., 2015).

Polymorphisms can affect the AKT signaling pathway in different ways. Since this signaling pathway is activated downstream of several receptors (Liem et al., 2017; Schabbauer et al., 2008; Zhang et al., 2007) in response to different stimuli, polymorphisms in several proteins can affect AKT activation (Brognard et al., 2009; Ma et al., 2017). Moreover, mutations in PI3K gene (Wang et al., 2016) and the occurrence of LD can interfere with this signaling pathway. However, only a few studies have shown the effects of synonymous mutations on PI3K signaling.

LD is a non-random association of alleles in the same or different genes (Lewontin and Kojima, 1960). The occurrence of SNPs in LD with rs1129293 in *PIK3CG* makes it difficult to identify the specific phenotype-associated SNPs. Moreover, bioinformatics analysis of sQTL

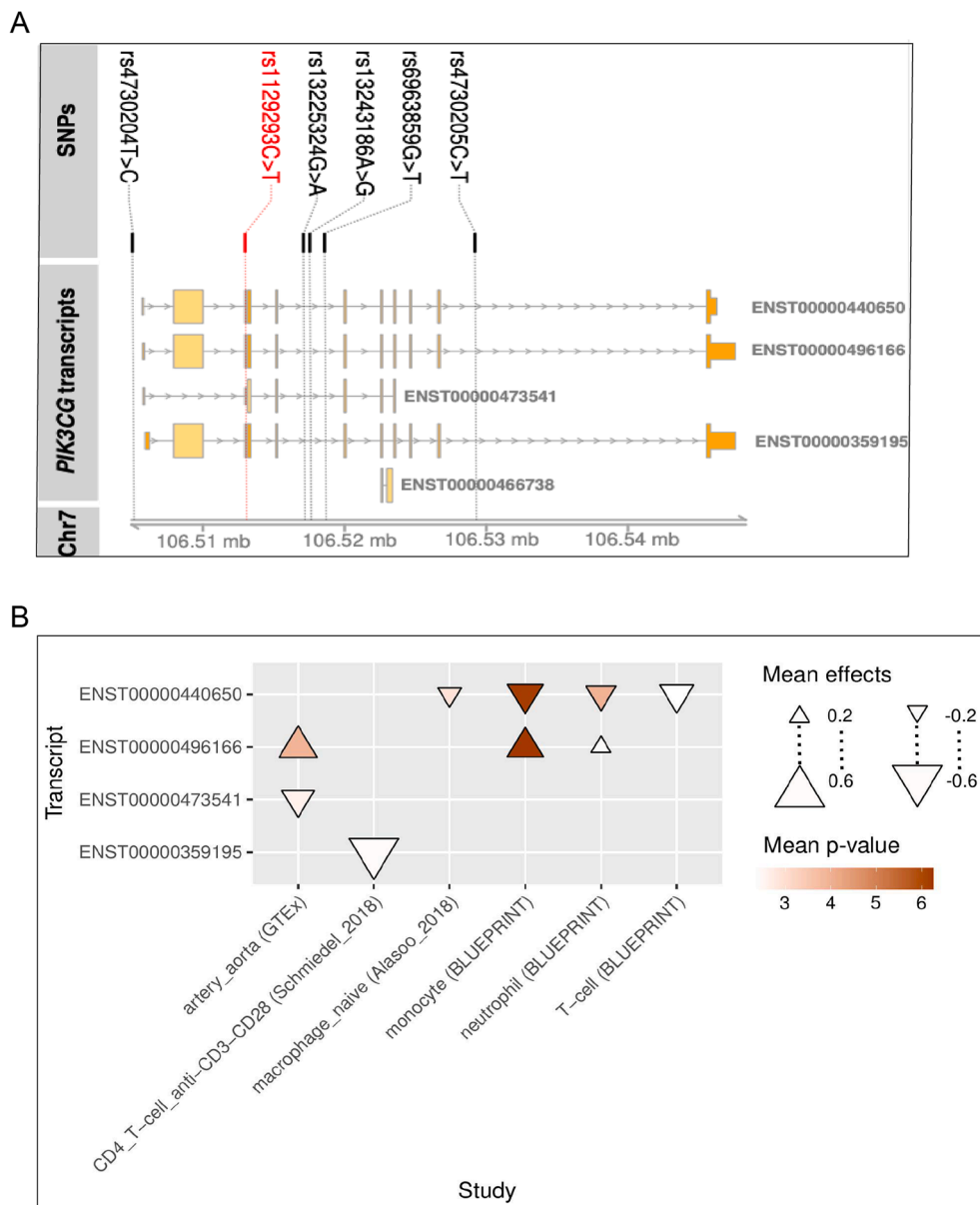
indicated that mutations in LD with the rs1129293 can interfere with the splicing of *PIK3CG*. Recent studies have shown that several SNPs located in sQTL are related to disease development (Guo et al., 2020; Liu et al., 2020).

The SNPs predicted via GWAS were mostly located in the non-coding regions of the gene (Nishizaki and Boyle, 2017). Our results showed that most SNPs in LD with rs1129293 were located in introns, that is, the non-coding DNA regions. Interesting, mutations in these regions can also be associated with protein changes and promote various diseases (Linhares et al., 2020; Taheri et al., 2020).

Overall, our results showed that the sSNP rs1129293 in *PIK3CG* gene, which is highly prevalent in patients with CCC, is related to decreased *PIK3CG* expression and PI3K $\gamma$  activation. Moreover, our target SNP was in LD with other SNPs also able to interfere in the gene expression and splicing. Therefore, further studies are necessary to identify the specific mutations responsible for the observed phenotype in the encoded protein.

## 5. Conclusion

The findings of our study revealed that the occurrence of the synonymous SNP rs1129293 in patients with CCC is related to decreased *PIK3CG* expression and PI3K $\gamma$  activation. We further showed that this



**Fig. 2.** Effect prediction and association data for rs1129293 by considering linkage disequilibrium. (A) SNPs in LD with rs1129293 ( $R^2 > 0.8$ ) along *PIK3CG* gene transcripts along intron (dashed lines) and exon regions (close boxes). The coordinates for *PIK3CG* at chromosome 7 are shown at the bottom. (B) sQTL associations with rs1129293 and SNPs in LD over immune cells and tissue retrieved with FIVEx webtool over EBI eQTL Catalogue studies. The up and down triangles represent the direction of the association, and its size represent the mean effect for associations over rs1129293 and the others in LD. The color bar gradient is related to the p-value of associations transformed to  $-\log_{10}$  scale.

mutation is in LD with several other SNPs that are located in sQTLs and are able to interfere with the post-transcriptional modifications, such as splicing.

### Funding

The research leading to these results received funding from the São Paulo Research Foundation (FAPESP) under grants agreement numbers 2013/08216-2 (Center for Research in Inflammatory Diseases – CRID) and 2018/20130-0; and the Coordination for the Improvement of Higher Educational Personnel (CAPES) with finance code 001.

### CRedit authorship contribution statement

**Maria Cláudia Silva:** Conceptualization, Methodology, Formal analysis, Writing – original draft. **Carlos Alessandro Fuzo:** Conceptualization, Methodology, Writing – review & editing. **Isadora Marques Paiva:** Methodology, Writing – review & editing. **Naira Lopes Bibó:** Methodology, Writing – review & editing. **Maykon Tavares de Oliveira:** Methodology, Writing – review & editing. **Hellen Anastácia da**

**Silva Soares:** Methodology, Writing – review & editing. **Christophe Chevillard:** Writing – review & editing. **Jorge Kalil:** Writing – review & editing. **Edecio Cunha-Neto:** Conceptualization, Writing – review & editing. **Thiago Mattar Cunha:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision. **João Santana Silva:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

We would like to thank Andréia Kuramoto for the technician support providing the patients samples and data.

## References

- Alasoo, K., Rodrigues, J., Mukhopadhyay, S., Knights, A.J., Mann, A.L., Kundu, K., Hale, C., Dougan, G., Gaffney, D.J., 2018. Shared genetic effects on chromatin and gene expression indicate a role for enhancer priming in immune response. *Nat. Genet.* 50, 424–431. <https://doi.org/10.1038/S41588-018-0046-7>.
- Auton, A., Abecasis, G.R., Altshuler, D.M., Durbin, R.M., Bentley, D.R., Chakravarti, A., Clark, A.G., Donnelly, P., Eichler, E.E., Flück, P., Gabriel, S.B., Gibbs, R.A., Green, E. D., Hurler, M.E., Knoppers, B.M., Korbel, J.O., Lander, E.S., Lee, C., Lehrach, H., Mardis, E.R., Marth, G.T., McVean, G.A., Nickerson, D.A., Schmidt, J.P., Sherry, S.T., Wang, J., Wilson, R.K., Boerwinkle, E., Doddapaneni, H., Han, Y., Korchina, V., Kovar, C., Lee, S., Muzny, D., Reid, J.G., Zhu, Y., Chang, Y., Feng, Q., Fang, X., Guo, X., Jian, M., Jiang, H., Jin, X., Lan, T., Li, G., Li, J., Li, Y., Liu, S., Liu, X., Lu, Y., Ma, X., Tang, M., Wang, B., Wang, G., Wu, H., Wu, R., Xu, X., Yin, Y., Zhang, D., Zhang, W., Zhao, J., Zhao, M., Zheng, X., Gupta, N., Gharani, N., Toji, L.H., Gerry, N. P., Resch, A.M., Barker, J., Clarke, L., Gil, L., Hunt, S.E., Kelman, G., Kulesha, E., Leinonen, R., McLaren, W.M., Radhakrishnan, R., Roa, A., Smirnov, D., Smith, R.E., Streeter, L., Thormann, A., Toneva, I., Vaughan, B., Zheng-Bradley, X., Grocock, R., Humphray, S., James, T., Kingsbury, Z., Sudbrak, R., Albrecht, M.W., Amstislavskiy, V.S., Borodina, T.A., Lienhard, M., Mertes, F., Sultan, M., Timmermann, B., Yaspo, M.L., Fulton, L., Ananiev, V., Belaia, Z., Beloslyudtsev, D., Bouk, N., Chen, C., Church, D., Cohen, R., Cook, C., Garner, J., Hefferon, T., Kimelman, M., Liu, C., Lopez, J., Meric, P., O'Sullivan, C., Ostapchuk, Y., Phan, L., Ponomarev, S., Schneider, V., Shekhtman, E., Sirotkin, K., Slotta, D., Zhang, H., Balasubramanian, S., Burton, J., Danecek, P., Keane, T.M., Kolb-Kococinski, A., McCarthy, S., Stalker, J., Quail, M., Davies, C.J., Gollub, J., Webster, T., Wong, B., Zhan, Y., Campbell, C.L., Kong, Y., Marcketta, A., Yu, F., Antunes, L., Bainbridge, M., Sabo, A., Huang, Z., Coin, L.J.M., Fang, L., Li, Q., Li, Z., Lin, H., Liu, B., Luo, R., Shao, H., Xie, Y., Ye, C., Yu, C., Zhang, F., Zheng, H., Zhu, H., Alkan, C., Dal, E., Kahveci, F., Garrison, E.P., Kural, D., Lee, W.P., Leong, W.F., Stromberg, M., Ward, A.N., Wu, J., Zhang, M., Daly, M.J., DePristo, M.A., Handsaker, R.E., Banks, E., Bhatia, G., Del Angel, G., Genovesse, G., Li, H., Kashin, S., McCarroll, S.A., Nemes, J.C., Poplin, R.E., Yoon, S.C., Li, H., Makarov, V., Gottipati, S., Keinan, A., Rodriguez-Flores, J.L., Rausch, T., Fritz, M.H., Stütz, A.M., Beal, K., Datta, A., Herrero, J., Ritchie, G.R.S., Zerbino, D., Sabeti, P.C., Shlyakhter, I., Schaffner, S.F., Vitti, J., Cooper, D.N., Ball, E.V., Stenson, P.D., Barnes, B., Bauer, M., Cheatham, R.K., Cox, A., Eberle, M., Kahn, S., Murray, L., Peden, J., Shaw, R., Kenny, E.E., Batzer, M.A., Konkel, M.K., Walker, J.A., MacArthur, D.G., Lek, M., Herwig, R., Ding, L., Koboldt, D.C., Larson, D., Ye, K., Gravel, S., Swaroop, A., Chew, E., Lappalainen, T., Erlich, Y., Gymrek, M., Willems, T.F., Simpson, J.T., Shriver, M.D., Rosenfeld, J.A., Bustamante, C.D., Montgomery, S.B., De La Vega, F. M., Byrnes, J.K., Carroll, A.W., DeGortor, M.K., Lacroite, P., Maples, B.K., Martin, A. R., Moreno-Estrada, A., Shringarpure, S.S., Zakharia, F., Halperin, E., Baran, Y., Cerveira, E., Hwang, J., Malhotra, A., Plewczynski, D., Radew, K., Romanovitch, M., Zhang, C., Hyland, F.C.L., Craig, D.W., Christoforides, A., Homer, N., Izatt, T., Kurdoglu, A.A., Sinari, S.A., Squire, K., Xiao, C., Sebat, J., Antaki, D., Gujral, M., Noor, A., Ye, K., Burchard, E.G., Hernandez, R.D., Gignoux, C.R., Haussler, D., Katzman, S.J., Kent, W.J., Howie, B., Ruiz-Linares, A., Dermitzakis, E.T., Devine, S. E., Kang, H.M., Kidd, J.M., Blackwell, T., Caron, S., Chen, W., Emery, S., Fritsche, L., Fuchsberger, C., Jun, G., Li, B., Lyons, R., Scheller, C., Sidore, C., Song, S., Sliwerska, E., Taliun, D., Tan, A., Welch, R., Wing, M.K., Zhan, X., Awadalla, P., Hodgkinson, A., Li, Y., Shi, X., Quaidamo, A., Lunter, G., Marchini, J.L., Myers, S., Churchhouse, C., Delaneau, O., Gupta-Hinch, A., Kretschmar, W., Iqbal, Z., Mathieson, I., Menelaou, A., Rimmer, A., Xifara, D.K., Oleksyk, T.K., Fu, Y., Liu, X., Xiong, M., Jorde, L., Witherspoon, D., Xing, J., Browning, B.L., Browning, S.R., Hormozdiari, F., Sudmant, P.H., Khurana, E., Tyler-Smith, C., Albers, C.A., Ayub, Q., Chen, Y., Colonna, V., Jostins, L., Walter, K., Xue, Y., Gerstein, M.B., Abzyov, A., Balasubramanian, S., Chen, J., Clarke, D., Fu, Y., Harman, A.O., Jin, M., Lee, D., Liu, J., Mu, X.J., Zhang, J., Zhang, Y., Hartl, C., Shakir, K., Degenhardt, J., Meiers, S., Raeder, B., Casale, F.P., Stegle, O., Lameijer, E.W., Hall, I., Bafna, V., Michaelson, J., Gardner, E.J., Mills, R.E., Dayama, G., Chen, K., Fan, X., Chong, Z., Chen, T., Chaisson, M.J., Huddleston, J., Malig, M., Nelson, B.J., Parrish, N.F., Blackburne, B., Lindsay, S.J., Ning, Z., Zhang, Y., Lam, H., Sisu, C., Challis, D., Evani, U.S., Lu, J., Nagaswamy, U., Yu, J., Li, W., Habegger, L., Yu, H., Cunningham, F., Dunham, I., Lage, K., Jaspersen, J.B., Horn, H., Kim, D., Desalle, R., Narechania, A., Sayres, M.A. W., Mendez, F.L., Poznik, G.D., Underhill, P.A., Mittelman, D., Banerjee, R., Cerezo, M., Fitzgerald, T.W., Louzada, S., Massaia, A., Yang, F., Kalra, D., Hale, W., Dan, X., Barnes, K.C., Beiswanger, C., Cai, H., Cao, H., Henn, B., Jones, D., Kaye, J.S., Kent, A., Kerasidou, A., Mathias, R., Ossorio, P.N., Parker, M., Rotimi, C.N., Royal, C. D., Sandoval, K., Su, Y., Tian, Z., Tishkoff, S., Via, M., Wang, Y., Yang, H., Yang, L., Zhu, J., Bodmer, W., Bedoya, G., Cai, Z., Gao, Y., Chu, J., Peltonen, L., Garcia-Montero, A., Orfao, A., Dutil, J., Martinez-Cruzado, J.C., Mathias, R.A., Hennis, A., Watson, H., McKenzie, C., Qadri, F., LaRoque, R., Deng, X., Asogun, D., Folarin, O., Hapci, C., Omoniwa, O., Stremmler, M., Tariyal, R., Jallow, M., Joof, F.S., Corrah, T., Rockett, K., Kwiatkowski, D., Kooner, J., Hien, T.T., Dunstan, S.J., ThuangHan, N., Focant, R., Garry, R., Kanneh, L., Moses, L., Schieffelin, J., Grant, D.S., Gallo, C., Poletti, G., Saleheen, D., Rasheed, A., Brooks, L.D., Felsenfeld, A.L., McEwen, J.E., Vaydylevich, Y., Duncanson, A., Dunn, M., Schloss, J.A., 2015. A global reference for human genetic variation. *Nature*. 526, 68–74. <https://doi.org/10.1038/nature15393>.
- Brest, P., Lapaquette, P., Souidi, M., Lebrigand, K., Cesaro, A., Vouret-Craviari, V., Mari, B., Barbry, P., Mosnier, J.F., Hébuterne, X., Harel-Bellan, A., Mograbi, B., Darfeuille-Michaud, A., Hofman, P., 2011. A synonymous variant in IRGM alters a binding site for miR-196 and causes deregulation of IRGM-dependent xenophagy in Crohn's disease. *Nat. Genet.* 43, 242–245. <https://doi.org/10.1038/NG.762>.
- Brogard, J., Niederst, M., Reyes, G., Warfel, N., Newton, A.C., 2009. Common polymorphism in the phosphatase PHLPP2 results in reduced regulation of Akt and protein kinase C. *J. Biol. Chem.* 284, 15215–15223. <https://doi.org/10.1074/JBC.M901468200>.
- Buccitelli, C., Selbach, M., 2020. mRNAs, proteins and the emerging principles of gene expression control. *Nat. Rev. Genet.* 21, 630–644. <https://doi.org/10.1038/S41576-020-0258-4>.
- Burgering, B.M., Coffey, P.J., 1995. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* 376, 599–602. <https://doi.org/10.1038/376599A0>.
- Cartegni, L., Chew, S.L., Krainer, A.R., 2002. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat. Rev. Genet.* 3, 285–298. <https://doi.org/10.1038/nrg775>.
- Chamary, J.V., Hurst, L.D., 2005. Evidence for selection on synonymous mutations affecting stability of mRNA secondary structure in mammals. *Genome Biol.* 6 <https://doi.org/10.1186/GB-2005-6-9-R75>.
- Chen, R., Davydov, E.V., Sirota, M., Butte, A.J., 2010. Non-synonymous and synonymous coding SNPs show similar likelihood and effect size of human disease association. *PLoS One* 5, e13574.
- Chen, K., Guo, R., Wei, C., 2020. Synonymous mutation rs2515641 affects CYP2E1 mRNA and protein expression and susceptibility to drug-induced liver injury. *Pharmacogenomics* 21, 459–470. <https://doi.org/10.2217/PGS-2019-0151>.
- Dias, J.C.P., Ramos, A.N., Gontijo, E.D., Luquetti, A., Shikanai-Yasuda, M.A., Coura, J.R., Torres, R.M., Melo, J.R. da C., Almeida, E.A. de, Oliveira, W. de, Silveira, A.C., Rezende, J.M. de, Pinto, F.S., Ferreira, A.W., Rassi, A., Fragata, A.A., Sousa, A.S. de, Correia, D., Jansen, A.M., Andrade, G.M.Q., Britto, C.F.D.P. de C., Pinto, A.Y. das N., Rassi, A., Campos, D.E., Abad-Franch, F., Santos, S.E., Chiari, E., Hasslocher-Moreno, A.M., Moreira, E.F., Marques, D.S. de O., Silva, E.L., Marin-Neto, J.A., Galvão, L.M. da C., Xavier, S.S., Valente, S.A. da S., Carvalho, N.B., Cardoso, A.V., Silva, R.A.E., Costa, V.M. da, Vivaldini, S.M., Oliveira, S.M., Valente, V. da C., Lima, M.M., Alves, R.V., 2016. [Brazilian Consensus on Chagas Disease, 2015]. *Epidemiol. e Serv. Saude Rev. do Sist. Unico Saude do Bras.* 25, 10.5123/S1679-49742016000500002.
- Duan, J., Antezana, M.A., 2003. Mammalian mutation pressure, synonymous codon choice, and mRNA degradation. *J. Mol. Evol.* 57, 694–701. <https://doi.org/10.1007/S00239-003-2519-1>.
- Eyre-Walker, A., Bulmer, M., 1993. Reduced synonymous substitution rate at the start of interbacterial genes. *Nucleic Acids Res.* 21, 4599–4603. <https://doi.org/10.1093/NAR/21.19.4599>.
- Feng, T., Feng, N., Zhu, T., Li, Q., Zhang, Q., Wang, Y., Gao, M., Zhou, B., Yu, H., Zheng, M., Qian, B., 2020. A SNP-mediated lncRNA (LOC146880) and microRNA (miR-539-5p) interaction and its potential impact on the NSCLC risk. *J. Exp. Clin. Cancer Res.* 39 <https://doi.org/10.1186/S13046-020-01652-5>.
- Gu, W., Zhou, T., Wilke, C.O., 2010. A universal trend of reduced mRNA stability near the translation-initiation site in prokaryotes and eukaryotes. *PLoS Comput. Biol.* 6, e1000664.
- Guo, Z., Zhu, H., Xu, W., Wang, X., Liu, H., Wu, Y., Wang, M., Chu, H., Zhang, Z., 2020. Alternative splicing related genetic variants contribute to bladder cancer risk. *Mol. Carcinog.* 59, 923–929. <https://doi.org/10.1002/MC.23207>.
- Gutman, T., Goren, G., Efroni, O., Tuller, T., 2021. Estimating the predictive power of silent mutations on cancer classification and prognosis. *NPJ genomic Med.* 6 <https://doi.org/10.1038/S41525-021-00229-1>.
- Hahne, F., Ivanek, R., 2016. Visualizing genomic data using Gviz and bioconductor. In: *Methods in Molecular Biology*. Humana Press Inc., pp. 335–351. [https://doi.org/10.1007/978-1-4939-3578-9\\_16](https://doi.org/10.1007/978-1-4939-3578-9_16).
- Haldane, J.B.S., 1954. An exact test for randomness of mating. *J. Genet.* 52, 631–635. <https://doi.org/10.1007/BF02985085>.
- Hsu, A.C.Y., Starkey, M.R., Hanish, I., Parsons, K., Haw, T.J., Howland, L.J., Barr, I., Mahony, J.B., Foster, P.S., Knight, D.A., Wark, P.A., Hansbro, P.M., 2015. Targeting PI3K-p110 $\alpha$  suppresses influenza virus infection in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 191, 1012–1023. <https://doi.org/10.1164/RCCM.201501-01880C>.
- Karakostis, K., Gnanasundaram, S.V., López, I., Theriou, A., Wang, L., Nylander, K., Olivares-Illana, V., Fähræus, R., 2019. A single synonymous mutation determines the phosphorylation and stability of the nascent protein. *J. Mol. Cell Biol.* 11, 187–199. <https://doi.org/10.1093/JMCE/MJY049>.
- Kerimov, N., Hayhurst, J.D., Peikova, K., Manning, J.R., Walter, P., Kolberg, L., Samoviča, M., Sakthivel, M.P., Kuzmin, I., Trevanion, S.J., Burdett, T., Jupp, S., Parkinson, H., Papatheodorou, I., Yates, A.D., Zerbino, D.R., Alasoo, K., 2021. A compendium of uniformly processed human gene expression and splicing quantitative trait loci. *Nat. Genet.* 53, 1290–1299. <https://doi.org/10.1038/S41588-021-00924-W>.
- Kerna, I., Kisand, K., Tamm, A., Tamm, A., 2013. Synonymous SNP influences Adam12 mRNA expression level in synovial tissue. *J. Mol. Genet. Med.* 7, 321–322. <https://doi.org/10.4172/1747-0862.1000057>.
- Kim, A., Le Douce, J., Diab, F., Ferozova, M., Dubourg, C., Odent, S., Dupé, V., David, V., Diambra, L., Watrin, E., de Tayrac, M., 2020. Synonymous variants in holoprosencephaly alter codon usage and impact the Sonic Hedgehog protein. *Brain* 143, 2027–2038. <https://doi.org/10.1093/BRAIN/AWAA152>.
- Kwong, A., Boughton, A.P., Wang, M., VandeHaar, P., Boehnke, M., Abecasis, G., Kang, H.M., 2021. FIVEx: an interactive eQTL browser across public datasets. *Bioinformatics* 38, 559–561. <https://doi.org/10.1093/BIOINFORMATICSBT/AB614>.
- Lewontin, R., Kojima, K., 1960. The evolutionary dynamics of complex polymorphisms. *Evolution* 14, 458–472. <https://doi.org/10.1111/j.1558-5646.1960.tb03113.x>.
- Liem, M., Ang, C.S., Mathivanan, S., 2017. Insulin mediated activation of PI3K/Akt signalling pathway modifies the proteomic cargo of extracellular vesicles. *Proteomics* 17. <https://doi.org/10.1002/PMIC.201600371>.

- Linhares, N.D., Pereira, D.A., Conceição, I.M.C.A., Franco, G.R., Eckalbar, W.L., Ahituv, N., Luizon, M.R., 2020. Noncoding SNPs associated with increased GDF15 levels located in a metformin-activated enhancer region upstream of GDF15. *Pharmacogenomics* 21, 509–520. <https://doi.org/10.2217/PGS-2020-0010>.
- Liu, Y., Shen, H., Greenbaum, J., Liu, A., Su, K.J., Zhang, L.S., Zhang, L., Tian, Q., Hu, H.G., He, J.S., Deng, H.W., 2020. Gene expression and RNA splicing imputation identifies novel candidate genes associated with osteoporosis. *J. Clin. Endocrinol. Metab.* 105, e4742.
- Ma, X., Ma, Z., Jiao, X., Hejtmančík, J.F., 2017. Functional non-coding polymorphism in an EPHA2 promoter PAX2 binding site modifies expression and alters the MAPK and AKT pathways. *Sci. Rep.* 7 <https://doi.org/10.1038/S41598-017-10117-3>.
- Machiela, M.J., Chanock, S.J., 2015. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics* 31, 3555–3557. <https://doi.org/10.1093/BIOINFORMATICS/BTV402>.
- McLaren, W., Gil, L., Hunt, S.E., Riat, H.S., Ritchie, G.R.S., Thormann, A., Flicek, P., Cunningham, F., 2016. The ensembl variant effect predictor. *Genome Biol.* 17, 122. <https://doi.org/10.1186/s13059-016-0974-4>.
- Meijer, J., Nakajima, Y., Zhang, C., Meinsma, R., Ito, T., Van Kuilenburg, A.B.P., 2013. Identification of a novel synonymous mutation in the human  $\beta$ -Ureidopropionase Gene UPB1 affecting pre-mRNA splicing. *Nucleosides. Nucleotides Nucleic Acids* 32, 639–645. <https://doi.org/10.1080/15257770.2013.847189>.
- Mueller, W.F., Larsen, L.S.Z., Garibaldi, A., Hatfield, G.W., Hertel, K.J., 2015. The silent sway of splicing by synonymous substitutions. *J. Biol. Chem.* 290, 27700–27711. <https://doi.org/10.1074/JBC.M115.684035>.
- Nishizaki, S.S., Boyle, A.P., 2017. Mining the Unknown: Assigning Function to Noncoding Single Nucleotide Polymorphisms. *Trends Genet.* 33, 34–45. <https://doi.org/10.1016/J.TIG.2016.10.008>.
- Olayinka-Adefemi, A., Onyilagha, C., Jayachandran, N., Jia, S.H.P., Uzonna, J., Marshall, A.J., 2021. Critical roles of phosphoinositide 3-kinase  $\delta$  in the humoral immune response to *Trypanosoma congolense* infection. *J. Immunol.* 207, 1401–1410. <https://doi.org/10.4049/JIMMUNOL.2100311>.
- Plotkin, J.B., Kudla, G., 2011. Synonymous but not the same: the causes and consequences of codon bias. *Nat. Rev. Genet.* 12, 32–42. <https://doi.org/10.1038/NRG2899>.
- Robert, F., Pelletier, J., 2018. Exploring the Impact of Single-Nucleotide Polymorphisms on Translation. *Front. Genet.* 9, 507. <https://doi.org/10.3389/FGENE.2018.00507>.
- Sauna, Z.E., Kimchi-Sarfaty, C., 2013. Synonymous Mutations as a cause of human genetic disease. *eLS*. <https://doi.org/10.1002/9780470015902.A0025173>.
- Schabbauer, G., Luyendyk, J., Crozat, K., Jiang, Z., Mackman, N., Bahram, S., Georgel, P., 2008. TLR4/CD14-mediated PI3K activation is an essential component of interferon-dependent VSV resistance in macrophages. *Mol. Immunol.* 45, 2790–2796. <https://doi.org/10.1016/J.MOLIMM.2008.02.001>.
- Schmiedel, B.J., Singh, D., Madrigal, A., Valdovino-Gonzalez, A.G., White, B.M., Zapardiel-Gonzalo, J., Ha, B., Altay, G., Greenbaum, J.A., McVicker, G., Seumois, G., Rao, A., Kronenberg, M., Peters, B., Vijayanand, P., 2018. Impact of genetic polymorphisms on human immune cell gene expression. *Cell* 175, 1701–1715. <https://doi.org/10.1016/J.CELL.2018.10.022>.
- Silva, M.C., Davoli-Ferreira, M., Medina, T.S., Sesti-Costa, R., Silva, G.K., Lopes, C.D., Cardozo, L.E., Gava, F.N., Lyroni, K., Dias, F.C., Frade, A.F., Baron, M., Nakaya, H.I., Figueiredo, F., Alves-Filho, J.C., Cunha, F.Q., Tsatsanis, C., Chevillard, C., Cunha-Neto, E., Hirsch, E., Silva, J.S., Cunha, T.M., 2018. Canonical PI3K signaling in myeloid cells restricts *Trypanosoma cruzi* infection and dampens chagasic myocarditis. *Nat. Commun.* 9, 1513. <https://doi.org/10.1038/s41467-018-03986-3>.
- Silva, M.C., Medina, T.S., Fuzo, C.A., Dias, F.C., Freitas-Castro, F., Fukutani, K.F., Donadi, E.A., Cunha-Neto, E., Cunha, T.M., Silva, J.S., 2021. Polymorphism in the catalytic subunit of the PI3K gene is associated with *Trypanosoma cruzi*-induced chronic chagasic cardiomyopathy. *Infect. Genet. Evol.* 88 <https://doi.org/10.1016/J.MEEGID.2020.104671>.
- Simhadri, V.L., Hamasaki-Katagiri, N., Lin, B.C., Hunt, R., Jha, S., Tseng, S.C., Wu, A., Bentley, A.A., Zichel, R., Lu, Q., Zhu, L., Freedberg, D.I., Monroe, D.M., Sauna, Z.E., Peters, R., Komar, A.A., Kimchi-Sarfaty, C., 2017. Single synonymous mutation in factor IX alters protein properties and underlies haemophilia B. *J. Med. Genet.* 54, 338–345. <https://doi.org/10.1136/JMEDGENET-2016-104072>.
- Taheri, M., Noroozi, R., Sadeghpour, S., Omrani, M.D., Ghafouri-Fard, S., 2020. The rs4759314 SNP within Hotair lncRNA is associated with risk of multiple sclerosis. *Mult. Scler. Relat. Disord.* 40 <https://doi.org/10.1016/J.MSARD.2020.101986>.
- Wang, Y., Qiu, C., Cui, Q., 2015. A large-scale analysis of the relationship of synonymous SNPs changing MicroRNA regulation with functionality and disease. *Int. J. Mol. Sci.* 16, 23545–23555. <https://doi.org/10.3390/IJMS161023545>.
- Wang, M.Y., He, J., Zhu, M.L., Teng, X.Y., Li, Q.X., Sun, M.H., Wang, X.F., Yang, Y.J., Wang, J.C., Jin, L., Wang, Y.N., Wei, Q.Y., 2016. A Functional Polymorphism (rs2494752) in the AKT1 Promoter Region and Gastric Adenocarcinoma Risk in an Eastern Chinese Population. *Sci. Rep.* 6 <https://doi.org/10.1038/SREP20008>.
- Xiang, K., Wang, B., 2018. Role of the PI3K-AKT-mTOR pathway in hepatitis B virus infection and replication. *Mol. Med. Rep.* 17, 4713–4719. <https://doi.org/10.3892/MMR.2018.8395/HTML>.
- Zhang, H., Bajraszewski, N., Wu, E., Wang, H., Moseman, A.P., Dabora, S.L., Griffin, J.D., Kwiatkowski, D.J., 2007. PDGFRs are critical for PI3K/Akt activation and negatively regulated by mTOR. *J. Clin. Invest.* 117, 730–738. <https://doi.org/10.1172/JCI28984>.