Uncovering association networks through an eQTL analysis involving human miRNAs and lincRNAs

Paulo R. Branco¹,², Gilderlanio S. de Araújo¹, Júnior Barrera³, Guilherme Suarez-Kurtz⁴ & Sandro José de Souza¹,⁵

Non-coding RNAs (ncRNA) have an essential role in the complex landscape of human genetic regulatory networks. One area that is poorly explored is the effect of genetic variations on the interaction between ncRNA and their targets. By integrating a significant amount of public data, the present study cataloged the vast landscape of the regulatory effect of microRNAs (miRNA) and long intergenic noncoding RNAs (lincRNA) in the human genome. An expression quantitative trait loci (eQTL) analysis was used to identify genetic variants associated with miRNA and lincRNA and whose genotypes affect gene expression. Association networks were built for eQTL associated to traits of clinical and/or pharmacological relevance.

Materials and Methods
An overview of the whole analysis workflow and data source is depicted in Fig. 1. Our strategy comprises three broad steps: (1) construction of a catalog of SNPs mapped to miRNA seeds, miRNA-binding sites, and lincRNA genes; (2) eQTL analysis of genetic variants and (3) identification of variants possibly associated to either clinical or pharmacological features.

¹Bioinformatics Multidisciplinary Environment (BioME), Instituto Metrópole Digital, UFRN, Natal, Brazil. ²Ph.D Program in Bioinformatics, Instituto Metrópole Digital, UFRN, Natal, Brazil. ³Instituto de Matemática e Estatística, Universidade de São Paulo, São Paulo, Brazil. ⁴Instituto Nacional do Câncer, Rio de Janeiro, Brazil. ⁵Instituto do Cérebro, UFRN, Natal, Brazil. Correspondence and requests for materials should be addressed to S.J.d.S. (email: sandro@neuro.ufrn.br)
For the first step, the genomic position of each SNP was compared to the genome coordinates of genes coding for miRNAs and lncRNAs as well as with the coordinates of miRNA-binding sites in mRNAs. Data for approximately 153 million SNPs were retrieved from the Single Nucleotide Polymorphism Database (dbSNP), a public-domain archive for a large collection of single genetic polymorphisms (https://www.ncbi.nlm.nih.gov/snp/), release 150. SNPs were then mapped to almost 6,000 miRNA families that were obtained from the release 21 of the miRBase dataset (http://www.mirbase.org) while TargetScan, release 7.1, was used to retrieve the position of miRNA-binding sites in mRNAs, which resulted in over 123,000 sites predicted. Genome positions for 4,519 lincRNAs were retrieved from release 89 of Ensembl (http://grch37.ensembl.org/index.html). Based on the size distribution of annotated lincRNAs (Ensembl), a threshold was defined to exclude extremely long
linCRNs. Only SNPs mapped to linCRNs longer than 200 bp and shorter than 100Kbp were used in the subsequent analysis. This filter generated a set of 4,059 linCRNs and ~1,9 million mapped SNPs.

In the second step of the workflow shown in Fig. 1, an eQTL analysis was performed to measure the contribution of cataloged SNP in the expression level of genes. RNA-seq data for 373 Europeans - CEPH (CEU), Finns (FIN), British (GBR), Toscani (TSI) - were extracted from gEUVADIS (Genetic European Variation in Health and Disease) (http://www.geuvadis.org/web/geuvadis)21. The individuals in gEUVADIS are a subset of the individuals available from the 1000 Genomes Project22. A Spearman correlation coefficient test was used to evaluate any putative association between genotype and gene expression. An adjusted p-value was generated after correction for multiple testing using the Benjamini-Hochberg method (using a threshold of 0.05 for the adjusted p-value). Additionally, we integrated the Genotype-Tissue Expression (GTEx) Project (version 6) that provides correlations between genotypes and tissue-specific gene expression (https://www.gtexportal.org/home/). Over 2 million GTEx eQTL grouped by 44 tissues were extracted from the project repository23 and compared to the cataloged SNPs.

In the third step of our workflow (Fig. 1), eQTL from both gEUVADIS and GTEx analyses were compared to data from the NGHRI/EBI GW AS Catalog24 (release 1.0.1) and from PharmGKB25 (data download in September 2017) to select eQTL associated to genes of clinical and/or pharmacological interest.

The clinically-relevant eQTL were used to model two association networks (illustrated in Fig. 2, as defined below:

(a) The miRNA association network is a multi-graph $G = (V, E)$ (Fig. 2a), in which the node set $V$ comprises four disjoint subsets of SNPs, genes, miRNA, and phenotypes, and the edge set $E$ can be decomposed in five node relationships, such as “located in/sig. eQTL” that links a SNP located in a miRNA binding site to a gene; “located in” links a SNP that is located in a gene; “sig. associated with” links SNPs with phenotypes, if there is an genetic association reported on GWAS Catalog; “associated with” links a gene and a phenotype also based in a given association reported by GWAS Catalog; “regulated by” links a gene to a miRNA based on the TargetScan binding sites prediction; and “LD” links two SNPs if the index of linkage disequilibrium ($R^2$) is greater than or equal to 0.8.

(b) The linCRNA association network also is a multi-graph $G = (V, E)$ (Fig. 2b), in which the node set $V$ comprises four disjoint subsets of SNPs, genes, linCRNAs, and phenotypes. The edge set $E$ can be decomposed in five node relationships, such as “located in” that links a SNP located in a linCRNA; “sig. associated with” that links a SNP with a given phenotype if there is an association reported in GWAS catalog; “associated with” links a gene and a phenotype also based in a given association reported by GWAS Catalog; “sig.
eQTL” links a SNP to a gene if there is an eQTL reported in GTEx; and “LD” links two SNPs if the index of linkage disequilibrium (R²) is greater than or equal to 0.8. Since there is no predicted gene targets for lincRNAs, a direct association between lincRNA and a given gene could not be established (as we have done for miRNAs).

The adjacency lists were loaded in Cytoscape v3 for graphical representations and layout editing. All Python scripts developed here are available at https://github.com/paulorobertobranco/Uncovering-association-networks-through-an-eQTL-analysis-involving-human-miRNAs-and-lincRNAs.

Results and Discussion
Catalog of SNPs mapped to lincRNA, miRNA seeds and miRNA-binding sites. SNPs were mapped to miRNA seed regions, to their putative binding sites in all human mRNAs and to lincRNA-coding genomic regions to identify genetic variants that could affect gene expression. By integrating dbSNP, miRBase, TargetScan and Ensembl (Fig. 1), our method identified 40,009 SNPs located in miRNA binding sites (Supplementary Table S1 for a complete list of all SNPs), 129 SNPs in miRNA seed regions (Supplementary Table S2) and 1,964,426 SNPs in lincRNAs-coding genomic regions (Supplementary Table S3).

Enrichment analysis using data from the Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed to identify, in the set of genes where the mapped SNPs were located, any possible enrichment for biological pathways. Among the most significant (p-adjusted < 0.05) enriched pathways, associations with some disease-related pathways, such as cancer, diabetes, and depression were found (see Supplementary Fig. S1).

eQTL analysis of gEUVADIS data and tissue-specific eQTL from GTEx. Next, we investigated whether genetic variants in miRNA seeds, miRNA-binding sites and lincRNAs-coding genomic regions could affect gene expression. Transcriptome and genome data available from gEUVADIS were used to perform an eQTL analysis, as described in Material and Methods. As a result, a set of 27 SNPs in miRNA binding sites were found to be significantly associated with the expression of the corresponding genes (genes where the respective miRNA-binding site was present). A Manhattan plot resulting from the eQTL analysis can be seen in Fig. 3a and details of all 27 significant SNPs are shown in Supplementary Table S4. The three most significant eQTL were: rs33664 (p-adjusted = 9.1e–16, correlation coefficient = 0.44) that may affect the binding between miR-30-5p (a tumor suppressor miRNA) and transcripts from TCFL5 (Fig. 3b); rs11680458 (p-adjusted = 1.8e–10, correlation coefficient = 0.37) possibly affecting the binding between miR-141-3p and WDR43 (Fig. 3c); and rs3828609 (p-adjusted = 9.5e–09, correlation coefficient = 0.34) that may affect the affinity between miR-155-5p and CSF1R (Fig. 3d). TCFL is a transcription factor whose expression seems to be a prognostic factor for childhood acute lymphoblastic leukemia26; miR-141-3p is also a cancer-related miRNA acting either as a tumor suppressor27,28 or an oncogene29, and WDR43 has been recently associated with the etiology of estrogen receptor (ER)-negative breast cancer30. CSF1R has been associated with several hematological-related traits31, while a relationship between miR-155-5p and papillary thyroid carcinoma diagnosis was described by Jahanbani et al.32.

No significant eQTL was found in the analysis involving SNPs mapped to miRNA seeds. On the other hand, the large number of SNPs mapped to lincRNA regions made the eQTL analysis computationally intractable, since a whole-genome eQTL analysis should be calculated by each one of the ~1,9 M SNPs. This limitation of the present work is being considered for future studies.

Using the available eQTL data from GTEx, our strategy identified 180 significant SNPs mapped to mRNA binding sites and 26,052 significant SNPs mapped to lincRNA-coding genomic regions (Supplementary Tables S5 and S6, respectively). No SNP mapped to miRNA seed matched any significant eQTL in the GTEx dataset. Thyroid and testis were the tissues that presented the highest number of e-QTL in both miRNA-binding sites and lincRNAs (Supplementary Fig. S2). By analyzing both tissues, it is possible to notice that some eQTL mapped to miRNA-binding sites diverge from the mean distribution of effect size (Fig. 4). The effect size of a given eQTL is defined as the slope of the linear regression and is computed as the effect of the alternative allele relative to the reference allele (allele reported in the human genome reference sequence). This suggests that these eQTL associated with miRNA-binding sites may have a higher influence on gene expression when compared to eQTL in general. A KEGG enrichment analysis was also performed on such eQTL and returned some common disease-related pathways such as cancer, diabetes, asthma and tuberculosis (Supplementary Fig. S3 for miRNA-binding sites and Supplementary Fig. S4 for lincRNAs).

Association networks for clinically-relevant eQTL. To assess whether the eQTL identified here could be associated with clinically relevant genes and/or clinically relevant traits, a comparison was made with data from the NHGRI/EBI GWAS Catalog and PharmGKB. Besides a direct comparison, querying the clinical databases with our eQTL, we have also checked whether any of our eQTL were in linkage disequilibrium with any variation present in the clinical databases.

To simplify the interpretation of the resulting data, eQTL identified using the gEUVADIS and GTEx data were pooled together. Clinically relevant eQTL were used to build association networks involving genes and traits linked to a given eQTL. Direct comparison of eQTL associated with miRNA binding sites and the clinical databases identified three variations associated with GWAS studies (see Fig. 5 and Supplementary Table S7): (i) rs1051424 that affects the expression of RP56KB1 (p-adjusted = 2.2e–05) in skeletal muscle tissue and has been associated to obesity-related traits33; (ii) rs11191548 that affects the expression of NT5C2 in two different tissues (esophagus and blood with p-adjusted equals to 3.6e–05 and 5.6e–05, respectively), and has been associated to blood pressure in four different studies34–36; and finally, (iii) rs7132908 that affects the expression of FAIM2 (p-adjusted = 3.0e–05) in testis, and has been associated to childhood body mass37. To provide a better overview
Figure 3. gEUVADIS eQTL analysis results. (a) Manhattan plot derived from gEUVADIS eQTL mapped to miRNA-binding sites. Significant SNPs are identified by green dots and threshold (p-adj $< 0.05$) is represented by the blue line. (b) Boxplot of TCFL5 expression level (as measured by RPKM) in gEUVADIS samples grouped by rs3664 genotype. (c) Boxplot of WDR43 expression level in gEUVADIS samples grouped by rs11680458 genotype. (d) Boxplot of CSF1R expression level on gEUVADIS samples grouped by rs3828609 genotype.

Figure 4. Distribution of effect size for significant eQTL (derived from GTEx). Y-axis represents the putative SNP effect size (slope) over gene expression. X-axis represents the SNPs grouped by the gene where they are located. SNPs mapped to miRNA binding sites are represented by red or green dots for thyroid (a) and testis (b) tissues. Green dots represent those SNPs that are outliers for the respective distribution ($z$-score $> 2$ or $z$-score $<-2$).
of the associations, networks for all three individual eQTL, as well as their distribution of expression levels according to the respective genotypes, are seen in Fig. 5.

Our workflow identified one miRNA-binding site eQTL in the PharmGKB database. SNP rs712 is located in the target site of miR-877-5p and may affect the expression of an important pharmacogene, KRAS. Besides its involvement in several normal physiological processes, KRAS is related to response to cetuximab and panitumumab, two epidermal growth factor receptor (EGFR) inhibitor drugs used in the treatment of some types of...
cancer. Additionally, LD analysis reported that rs712 is in high linkage disequilibrium \( (R^2 > 0.8) \) with a set of 35 SNPs (Supplementary Table S8), also located in KRAS.

LD analysis identified 16 pairs of miRNA-binding sites eQTL showing an LD relationship with a variant present in the GW AS Catalog repository. Two of the most interesting ones are: (a) rs6664467 (mapped to gene MRPL9-TDRKH) is associated with carotid plaque burden in GWAS Catalog and is in high LD \( (R^2 = 0.85) \) with rs6683364, an eQTL \( (p\text{-adjusted} = 0.004) \) identified here; (b) the second pair is rs11680458 and rs67073037 with high linkage disequilibrium \( (R^2 = 0.97) \). The eQTL rs11680458 possibly affects the expression of WDR43 \( (p\text{-adjusted} = 1.8e^{-10}) \) and rs67073037 was recently associated with breast cancer in GWAS Catalog\(^{30}\). For more details on the additional eQTL, please see Supplementary Table S9.

A comparison of eQTL mapped to lincRNAs and present in NGHRI/EBI GWAS Catalog identified 101 SNPs directly associated with GWAS studies (Supplementary Table S10). Interestingly, 11 of them were related to 7 different types of cancer: breast cancer (rs2016394), colorectal cancer (rs1372474, rs2293582), endometrial cancer (rs13328298, rs1777220, rs2797160), Ewing sarcoma (rs4924410), Hodgkin's lymphoma (rs1432295), prostate cancer (rs11672691, rs8014671), and testicular germ cell cancer (rs4561483). Besides cancer, some other disease-related phenotypes were also reported, such as allergy, asthma, blood pressure, hepatitis C and HIV, and neuropsychiatric traits, such as schizophrenia, bipolar disorder, migraine and response to antipsychotic treatments.

A comparison of eQTL mapped to lincRNAs and present in PharmGKB database analysis resulted in 92 SNPs (Supplementary Table S11) that may affect the expression of some critical pharmacogenes, including \( BRCA1 \), \( CYP2D6 \), \( CYP4F2 \), \( DPDY \), \( DRD2 \), \( HLA-B \). Two eQTL (rs7223460, rs1396510) were negatively associated with the expression of \( BRCA1 \). \( CYP2D6 \) gene is associated with response to medications used to treat a number of mental illnesses (depression, anxiety disorder, attention deficit hyperactivity disorder and bipolar disorder), heart-related diseases (congestive heart failure, left ventricular dysfunction and high blood pressure), allergic conditions (rhinitis and urticaria) and others. Fifteen significant SNPs that regulate \( CYP2D6 \) expression levels

Figure 6. Association network built using all GWAS-linked eQTL mapping to miRNA-binding sites. (a) Sub-network distribution size for the association network. (b) Distribution of the number of regulated genes and the number of SNPs based on the sub-network size. The largest sub-network is shown in red. (c) Graphical representation of the largest sub-network identified by the red bin in (b).
Figure 7. Association network built using all GWAS-linked eQTL mapping to lincRNA. (a) Sub-network distribution size for the network. The X-axis represents the component size and Y-axis represents the frequency of the component. (b) Distribution of regulated genes based on the sub-network size and the number of SNPs. The X-axis represents the sub-network size, Z-axis represents the number of SNPs and Y-axis represents the number of regulated genes. Largest sub-network is shown in red. (c) Graphical representation of the largest sub-network identified by the red bin on (b).

Figure 8. Impact of the removal of individual eQTL in the topology of association network. Impact on miRNA and lincRNA networks is shown in (a and b), respectively. Iteration zero means the initial state of the network topology. Each subsequent iteration represents the removal of an individual and independent eQTL (X-axis). The number of sub-networks resulted from the respective eQTL removal is shown on the Y-axis.
were reported. Three eQTL influence the expression of CYP4F2, a gene related to blood pressure diseases and associated with anticoagulant drugs, such as warfarin. rs5776391 and rs2000920 may significantly influence the expression of DPYD and DRD2 genes, respectively. DPYD is associated with response to capcitabine, fluorouracil and tegafur, all of them used to treat different types of cancer. DRD2 is associated with antipsychotic medications used to manage schizophrenia, bipolar disorder and related diseases. At least, 70 SNPs related to HLA-B gene were reported. They were associated with 12 drugs, such as abacavir, used to prevent and treat HIV/AIDS.

Indirectly, 79 lincRNA eQTL are in high linkage disequilibrium ($R^2 > 0.8$) with 80 GWAS associated SNPs (Supplementary Table S12). From this analysis, disease-related phenotypes such as asthma, bipolar disorder, blood pressure, colorectal cancer, depression or major depressive disorder and endometrial cancer were observed. Also, indirectly, 73 SNPs mapped to lincRNA are in high LD ($R^2 > 0.8$) with 72 eQTL (Supplementary Table S13) that may regulate some important pharmacogenes, including BRCA1, CYP2D6, CYP4F2 and HLA-B.

To uncover any other possible relationship between clinically relevant eQTL, we decided to model two association networks including all respective GWAS-linked eQTL, one for miRNA and the other for lincRNA. The association network for miRNA presented 146 nodes and 2010 edges distributed in four sub-networks (Fig. 6a,b). The largest sub-network is shown in Fig. 6c, while the remaining sub-networks are shown in Supplementary Fig. S5. The association network for lincRNA was more fragmented with 42 sub-networks comprising 629 nodes and 887 edges (Fig. 7a,b). The largest sub-network is shown in Fig. 7c while all the remaining sub-networks are shown in Supplementary Fig. S6.

To evaluate the impact of individual eQTL in the topology of each network, we exhaustively removed all eQTL from the network in an iterative way. For the association network for eQTL mapped to miRNA-binding sites, removal of rs11191548-NT5C2 had the most significant impact in the network increasing the number of sub-network from 4 to 11 (Fig. 8a). For the association network for eQTL mapped to lincRNA, removal of six eQTL had a similar impact on the network increasing the number of sub-network from 41 to 61 (Fig. 8b). All eQTL involved the SNP rs35181953.

Conclusions

The approach and data reported here provide a catalog of eQTL mapped to miRNA seeds, miRNA-binding sites and lincRNAs that supposedly affect gene expression of clinical and important pharmacogenes. By taking advantage of heterogeneous biological data sources, as NGHRI/EBI GWAS Catalog and PharmGKB, our workflow and data allow a series of promising new investigations, such as the replication of eQTL analysis in other populations and the study of the selection forces acting on regulatory networks, among others.

References

1. Ha, M. & Kim, V. N. Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell Biol. 15, 509–524 (2014).
2. Winter, J., Jung, S., Keller, S., Gregory, R. I. & Diederichs, S. Many roads to maturity: MicroRNA biogenesis pathways and their regulation. Nat. Cell Biol. 11, 228–234 (2009).
3. Meng, Y., Quan, L. & Liu, A. Identification of key microRNAs associated with diffuse large B-cell lymphoma by analyzing serum microRNA expressions. Gene 462, 205–211 (2018).
4. Qu, R. et al. MicroRNA-374b reduces the proliferation and invasion of colon cancer cells by regulation of LRH-1/Wnt signaling. Gene 462, 354–361 (2018).
5. Hayes, J., Peruzzi, P. P. & Lawler, S. MicroRNAs in cancer: Biomarkers, functions and therapy. Trends Mol. Med. 20, 460–469 (2014).
6. Chen, K. & Rajewsky, N. Natural selection on human microRNA binding sites inferred from SNP data. Nat. Genet. 38, 1452–1456 (2006).
7. Peng, Z. et al. Genome-wide analyses of long noncoding RNA expression profiles in lung adenocarcinoma. Sci. Rep. 7, 1–11 (2017).
8. Prensner, J. R. & Chinnaiyan, A. M. The emergence of lncRNAs in cancer biology. Cancer Discov. 1, 391–407 (2011).
9. Shi, X., Sun, M., Liu, H., Yao, Y. & Song, Y. Long non-coding RNAs: A new frontier in the study of human diseases. Cancer Lett. 339, 159–166 (2013).
10. Bhattacharya, A., Ziebarth, J. D. & Cui, Y. PolymiRTS Database 3.0: Linking polymorphisms in microRNAs and their target sites with disease associations. Nucleic Acids Res. 42, 86–91 (2014).
11. Liu, C. et al. MirSNP, a database of polymorphisms altering miRNA target sites, identifies miRNA-related SNPs in GWAS SNPs and eQTLs. BMC Genomics 13 (2012).
12. Deveci, M., Çatalyürek, Ü. V. & Toland, A. E. MirSNP: Software to detect SNP effects on microRNA binding sites. BMC Bioinformatics 15 (2014).
13. Vísa, U., Esko, T., Kasela, S. & Annul, T. Altered gene expression associated with microRNA binding site polymorphisms. Plos One 10, 1–24 (2015).
14. Bruno, A. E. et al. miRdSNP: A database of disease-associated SNPs and microRNA target sites on 3′UTRs of human genes. BMC Genomics 13 (2012).
15. Griffiths-Jones, S. The microRNA Registry. Nucleic Acids Res. 32, 109D–111 (2004).
16. Griffiths-Jones, S., Grocock, R. J., van Dongen, S., Bateman, A. & Enright, A. J. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 34, D140–D144 (2006).
17. Griffiths-Jones, S., Saini, H. K., Van Dongen, S. & Enright, A. J. miRBase: Tools for microRNA genomics. Nucleic Acids Res. 36, 154–158 (2008).
18. Kozomara, A. & Griffiths-Jones, S. MiRBase: Integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res. 39, 152–157 (2011).
19. Griffiths-Jones, S., Kozomara, A. & Patro, R. miRBase: Annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 42, 68–73 (2014).
20. Agarwal, V., Bell, G. W., Nam, J. W. & Bartel, D. P. Predicting effective microRNA target sites in mammalian mRNAs. Elife 4, 1–38 (2015).
21. Lappalainen, T. et al. Transcriptome and genome sequencing uncovers functional variation in humans. Nature 501, 506–511 (2013).
22. Ahlbuler, D. M. & et al. An integrated map of genetic variation from 1,092 human genomes. Nature 491, 56–65 (2012).
23. The GTEx Consortium. et al. The Genotype-Tissue Expression (GTEx) pilot analysis: multivariate gene regulation in humans. Science (80-) 348, 648–660 (2015).
24. Welter, D. et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res. 42, 1001–1006 (2014).
25. Whirl-Carrillo, M. et al. Pharmacogenomics Knowledge for Personalized Medicine. Clin. Pharmacol. Therapeutics 92, 414–417 (2012).
26. Silveira, V. S. et al. Gene expression pattern contributing to prognostic factors in childhood acute lymphoblastic leukemia. *Leuk. Lymphoma* **54**, 310–314 (2013).
27. Wang, N. et al. MiR-141-3p suppresses proliferation and promotes apoptosis by targeting GLI2 in osteosarcoma cells. *Oncol. Rep.* **39**, 747–754 (2018).
28. Huang, S. et al. Downregulation of miR-141-3p promotes bone metastasis via activating NF-κB signaling in prostate cancer. *J. Exp. Clin. Cancer Res.* **36**, 173 (2017).
29. Zhou, X. et al. MicroRNA-141-3p promotes glioma cell growth and temozolomide resistance by directly targeting p53. *Oncotarget* **8**, 71080–71094 (2017).
30. Couch, F. J. et al. Identification of four novel susceptibility loci for oestrogen receptor negative breast cancer. *Nat. Commun.* **7** (2016).
31. Astle, W. J. et al. The Allelic Landscape of Human Blood Cell Trait Variation and Links to Common Complex Disease. *Cell* **167**, 1415–1429.e19 (2016).
32. Jahanbani, L., Al-Abdallah, A., Ali, R., Al-Brahim, N. & Mojiminiyi, O. Discriminatory miRNAs for the management of papillary thyroid carcinoma and noninvasive follicular thyroid neoplasms with papillary-like features. *Thyroid* thy.2017.0127, https://doi.org/10.1089/thy.2017.0127 (2018).
33. Comuzzie, A. G. et al. Novel Genetic Loci Identified for the Pathophysiology of Childhood Obesity in the Hispanic Population. *Plos One* **7** (2012).
34. Studies, T. I. C. for B. P. G.-W. A. Genetic Variants in Novel Pathways Influence Blood Pressure and Cardiovascular Disease Risk, *Nature* **478**, 103–109 (2012).
35. Newton-Cheh, C. et al. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* **41**, 666–676 (2009).
36. Wain, L. V. et al. Novel Blood Pressure Locus and Gene Discovery Using Genome-Wide Association Study and Expression Data Sets from Blood and the Kidney. *Hypertension* **70**, e4–e19 (2017).
37. Kato, N. et al. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in East Asians. *Nat. Genet.* **43**, 531–538 (2011).
38. Felix, J. F. et al. Genome-wide association analysis identifies three new susceptibility loci for childhood body mass index. *Hum. Mol. Genet.* **25**, 389–403 (2016).
39. Pott, J. et al. Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. *Atherosclerosis* **259**, 32–40 (2017).

**Acknowledgements**

This work was supported by a CAPES grant (23038.004629/2014-19) to SJS. PRBL was supported by a CAPES fellowship and GSA was supported by a CNPq fellowship.

**Author Contributions**
P.R.B.L. and G.S.A. processed the data; P.R.B.L., G.S.A., G.S., J.B., S.J.S. analyzed and interpreted the data; S.J.S. supervised the project; P.R.B.L., G.S.A. and S.J.S. wrote the paper. All authors have read and approved the manuscript.

**Additional Information**

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-33420-z.

**Competing Interests:** The authors declare no competing interests.

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018