Investigating differences in the GWAS-based protein-protein interaction network of blood pressure regulation due to ancestry or transcript consequence severity

Evridiki-Pandora G. Tsare *, Maria I. Klapa *, Nicholas K. Moschonas *, **

**Metabolic Engineering and Systems Biology Laboratory, Institute of Chemical Engineering Sciences, Foundation for Research and Technology-Hellas (FORTH/ICE-HT), Patras, Greece, (Tel: +30-2610-965230; e-mail: tsare@upatras.gr; Tel: +30-2610-965249; e-mail: mklapa@iceht.forth.gr)*

***Department of General Biology, School of Medicine, University of Patras, Patras, Greece (e-mail: n_moschonas@med.upatras.gr)*

Abstract: Genome-wide association studies (GWAS) have been valuable for the identification of genetic factors associated with complex diseases or traits. GWAS findings can be enhanced if analysed in the context of protein-protein interaction (PPI) networks, as the related pathophysiology results from dysfunction of interacting polyprotein pathways. In a recent study, we reconstructed the PPI network of blood pressure regulation (BP) based on a systematically-curated GWAS meta-database and network extension principles. Our meta-database enables the selection of GWAS-data of different ancestries and/or different Ensembl-defined transcript consequence severities. Thus, this study aimed at investigating any differences in the BP PPI network, due to (a) ancestry, by comparing the most-abundant European and Asian ancestry GWAS datasets, and (b) variant consequence severity, by excluding single nucleotide polymorphisms (SNPs) involved only in "modifier" categories, of difficult to predict impact. We identified that 82% of the collected BP-SNPs are from European-specific studies, with only 11% from Asian-specific, validating the need to augment the GWAS-data from other than European ancestries. Thus, only 7% vs 83% of the 1170 BP GWAS-proteins originate from Asian- and European-specific studies, respectively, with 2% (24) identified as Asian-specific vs ~45% (524) as European-specific GWAS-proteins. In the second part of the study, we found that the vast majority (85%) of the BP-SNPs with protein-coding transcript consequences are only intron variants ('modifier' category). Hence, the PPI network based on the SNPs in other than "modifier" categories included 142 proteins, with 12 in the largest connected component. However, while the relevant interactome is much smaller than the full, when extended based on our shortest-path approach, it revealed the same BP-significant pathways. This result supports the need to upgrade the information content of GWAS-data through network analysis.

Keywords: Blood pressure regulation, GWAS, Human protein–protein interactions, PPI network analysis, Network Medicine

1. INTRODUCTION

Since the late 2000s, genome-wide association studies (GWAS) have served as a crucial tool in genomic analysis and have contributed to furthering our understanding of the genetic basis of complex traits and phenotypes by uncovering a plethora of associated genetic variants (Hettiarachchi and Komar, 2022). Network biology and medicine studies have demonstrated in recent years that the information content of GWAS data can be significantly upgraded if analysed in the context of biomolecular networks, e.g. (Yan *et al.*, 2017; Ratnakumar *et al.*, 2020; Tsare *et al.*, 2024), as the investigated physiology is the result of multiple interacting pathways. In a recent study of our group (Tsare *et al.*, 2024), we reconstructed the protein-protein interaction (PPI) network of blood pressure regulation (BP), using a systematically literature-curated GWAS meta-database that we developed, while extending it by a proposed shortest-path approach. Network analysis revealed BP-significant pathways, while a prioritized protein-set was identified and ranked by an integrated set of GWAS- and network-based criteria.

Despite the remarkable progress of GWAS, a major limitation remains the limited representation of individuals of non-European ancestry. The majority of the collected samples and the SNP microarrays constructed to-date represent genome-wide genetic diversity corresponding mainly to populations of European ancestry (Loos, 2020). The only other ancestry that corresponds to an increasingly large GWAS dataset are Asians (Fitipaldi and Franks, 2023), while there exist substantially limited data for the African ancestry. Our BP GWAS meta-database enables the selection of ancestry-specific studies and corresponding single nucleotide polymorphisms (SNPs). Another characteristic of our BP GWAS-data curation is that we have associated the collected SNPs with all their transcript consequences independently of severity. Ensembl (Martin *et al.*, 2023) has defined a list of variant consequences, of decreasing level of severity (high, medium, low) for coding regions, along with

"modifier" variant categories, identified mainly as non-coding variants or associated with non-coding genes and of difficult to predict or unknown impact. A characteristic of GWAS that is still valid today is that the majority of the identified susceptibility variants are indeed mapped in non-protein coding regions, such as intergenic or intronic regions (Maurano *et al.*, 2012). The main GWAS data repository, GWAS Catalog (Buniello *et al.*, 2019), opts to store for each SNP only the most severe transcript consequence. We support that including all transcript consequences for a SNP increases the information acquired by the GWAS data, especially in an era where we are still investigating the impact of most identified variants. By its structure, our BP-GWAS meta-database enables us to search for differences in the resulted PPI network if certain variant consequences are considered compared to the full dataset.

In this context, in this study, we aimed at investigating further the BP PPI network, resulting from the GWAS data with respect to (a) ancestry-specific differences, focusing on the two most abundant GWAS sub-sets, i.e. of the European and the Asian ancestries, and (b) the variant consequence severity, excluding SNPs that are involved only in "modifier" categories.

2. METHODS

2.1 GWAS data

The BP GWAS meta-database developed by our group (Tsare *et al.*, 2024) was used to retrieve the GWAS data based on the ancestry of the involved individuals and the variant consequence severity. To obtain clear results, we selected only ancestry-specific studies, combining also ancestry sub-groups (e.g. East Asians, South Asians, etc.). If an ancestry is mentioned only in the context of mixed ancestry studies, it was not considered as a separate group (i.e. Hispanic or Latin American). SNPs associated only with mixed (complex)-ancestry studies are included in the 'Other' ancestry group. It is noted that SNPs associated with individuals of European or Asian or African ancestries are present in the 'Other' category, but since they cannot be clearly assigned to specific ancestries, they are not considered in the ancestry-specific analysis. For the transcript consequence severity analysis, we excluded all BP-associated SNPs that have been characterized as 'modifier' variants based on Ensembl (version 97). The ancestry-specific BP GWAS-protein sets were compared with the full set of 1170 proteins (Tsare *et al.*, 2024).

2.2 PPI networks

Ancestry-specific GWAS-deduced PPI networks were extracted from the full PPI network (Tsare *et al.*, 2024) and compared. Similarly, the GWAS-deduced PPI network of the proteins associated with the BP SNPs in the new severity threshold group was reconstructed. To investigate how different the full BP-associated PPI network is in this case, we proceeded with extending the GWAS-deduced interactome with the shortest PPI paths that connect all proteins in one component. More details about the used algorithm are provided in Tsare *et al.*, 2024. All PPI networks are based on the human protein interactome of the PPI meta-database PICKLE v.2.4 (www.pickle.gr) (Klapa *et al.*, 2013; Gioutlakis *et al.*, 2017; Dimitrakopoulos *et al.*, 2021, 2022). in which the experimental PPIs in human are integrated in the context of the genetic information ontology network of the UniProt/SwissProt-defined reviewed human complete proteome (RHCP) (https://www.uniprot.org/).

2.3 Network and Pathway Enrichment Analysis

PPI network visualization was carried out using Cytoscape version 3.8.2 (https://cytoscape.org/) (Shannon *et al.*, 2003). Network analysis was carried out with the relevant Cytoscape plugin. Pathway enrichment analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) Knowledgebase v2023q4 (https://david.ncifcrf.gov/) (Sherman *et al.*, 2022).

3. RESULTS AND DISCUSSION

3.1 BP-GWAS data analysis based on ancestry

Our BP GWAS meta-database (Tsare *et al.*, 2024) records 6687 SNPs (SNP-trait association p-value < $5x10^{-8}$). From these SNPs, 82% (5495), ~11% (727) and only 1% (86) have been, respectively, identified in European, Asian and African ancestry-specific studies (Table 1). This result was expected as the largest studies conducted so far involved mainly European-ancestry individuals, while the next relatively well-represent descent in the GWAS is the Asian (including all sub-groups), while the African ancestry cohorts have so far been very limited.

Table 1. Ancestry-based BP-GWAS data statistics

	Total $p<5x10^{-8}$	**RHCP-protein coding $p<5x10^{-8}$**	
Ancestry	#SNPs (specific)	#SNPs (specific)	#Proteins (specific)
African	86 (37)	25 (12)	23 (8)
Asian	727 (145)	373 (70)	86 (24)
European	5495 (1943)	3110 (1054)	976 (524)
Other	4544 (951)	2592 (526)	617(167)

Note: The data correspond to studies that were specific to each of the depicted ancestries. Other involves mixed-ancestry studies. Specific depicts #SNPs or #proteins that have been identified as BP-related in the particular ancestry only.

As anticipated, the relative numbers between ancestries are of similar differences, when the associated proteins are considered. Specifically, of the 1170 RHCP-proteins associated with the BP-SNPs (Tsare *et al.*, 2024), the vast majority (83%, 976) originate from European ancestry-specific studies, while 7% (86) and 2% (23), respectively, from Asian-specific and African-specific studies (Table 1). Furthermore, by comparing the GWAS-protein sets of the two most represented descents, with the rest considered in a third (Other & African) group, we observed that ~45% of the BP GWAS-proteins (524) have been identified as BP-related only in individuals of European ancestry, while the respective numbers for the Asian and African ancestry are 24 and 8, respectively (Table 1, Fig. 1). Finally, 59 GWAS-proteins

have been identified as BP-related in both European and Asian descent individuals, with 5 have not been identified in any of the other mixed or African ancestry studies (Fig. 1). More GWAS on other than the European ancestries are needed to validate any ancestry-specific variants or proteins. Still the results are important in pointing out some genetic differentiation between ancestries with respect to BP.

Figure 2 shows the BP-related interactomes of the GWAS-protein sets that were identified only in European-specific or Asian-specific studies (Figs. 2B, 2C, respectively), compared to the full BP GWAS-deduced PPI network (Fig. 2A). In the latter network, the protein-nodes that were connected in a large component were named "blue nodes" (BNs), while the rest were named "green nodes" (GNs) (Tsare *et al.*, 2024). As anticipated, the vast majority of BNs and GNs are part of the European-related PPI network and consequently, same is true for the PPIs. Actually, the European-related GWAS-deduced PPI network is similar to the full interactome, and its four most connected proteins, i.e. P53, ESR1, UBC9 and SMAD3, are also hubs of the full network. This is not of course the case for the Asian-related network, in which only 44, 32 and 7, respectively, BNs, GNs and PPIs of the full network are included.

The European-specific BP GWAS-protein set (Fig. 2D) includes almost half of the nodes of the full network, i.e. 301 out of 672 BNs and 173 out of 393 GNs, but the 186 BNs that remain connected interact only through 293 (17% of 1707) edges. Interesting, the protein with the highest number of interactions in the European-specific BP GWAS-deduced PPI network is Ataxin 1 (ATX1), mainly associated with neurodegenerative diseases (Ma and Didonna, 2021) and recently linked with BP (Wang *et al.*, 2023).

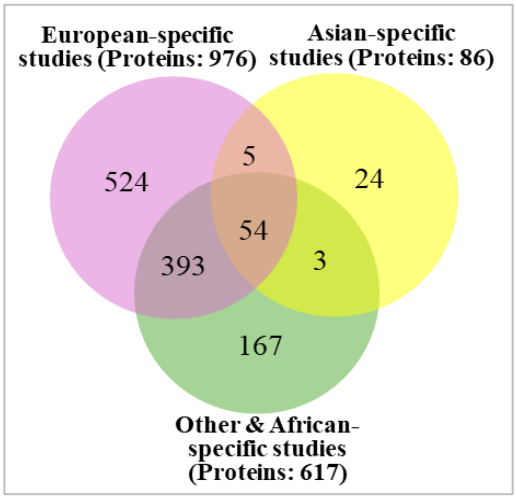

Figure 1. Venn diagram of the BP-GWAS proteins based on ancestry.

In Tsare *et al.*, 2024 we identified 335 proteins as BP-significant based on GWAS-based and network-based criteria, 124 of which are GWAS-proteins. Of these 124, 117 are identified in European-specific studies, including the top-10 prioritized: ESR1 INSR, PTN11, CDK6, CSK, NOS3, SH2B3, ATP2B1, FES, FINC and thirty-four are identified in Asian-specific studies, from which FES and FINC belong to the common protein-set between the two ancestries. Furthermore, 12 of the prioritized proteins (VAC14, CCN3, PDILT, MK01, HSP74, RAF1, FBW1A, TAU, LATS2, NCOR2, PTEN, XRCC6) are identified as European-specific and one prioritized protein (HDAC4) as Asian-specific. Investigating any known BP-association of the European-specific BP-prioritized GWAS-proteins, we identified five involved in BP-related pathways: FBW1A (Hippo pathway), LATS2 (Hippo and Wnt pathway), PTEN (insulin resistance, diabetic cardiomyopathy), RAF1 (cGMP-PKG, cAMP, PI3K-Akt and insulin signaling pathways) and HSP74 (lipid and atherosclerosis). The role of HDAC4 in the hypertension pathology has already been discussed (Tsare *et al.*, 2024).

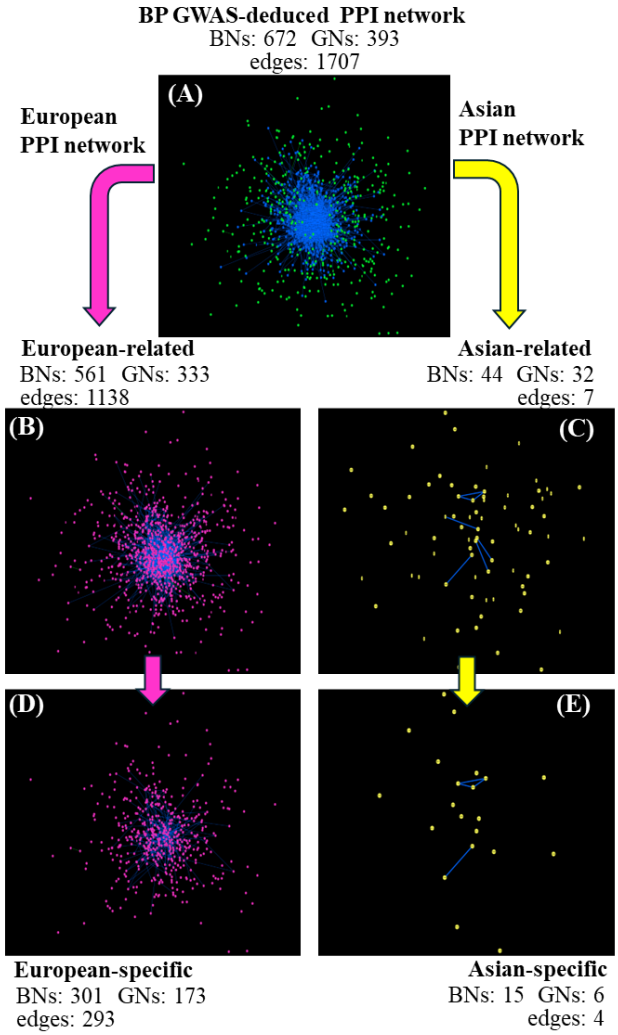

Figure 2. The full (A), European-related (B), Asian-related (C), European-specific (D), and Asian-specific (E), BP GWAS-deduced BP PPI Networks. Blue nodes (BNs) and green nodes (GNs) are named and respectively colored in network (A) the proteins that are connected in one component (BNs) and the rest (GNs). All protein nodes in (B) and (D) are shown in pink, whereas protein nodes in (C) and (E) are shown in yellow.

Pathway enrichment analysis was performed on the European-specific, Asian-specific and common datasets, investigating any ancestry-specific BP mechanisms based on the available GWAS data so far. Thirty KEGG-defined

pathways were significantly enriched ($q < 0.05$) in European-specific BP GWAS-proteins. These indeed include pathways that have been strongly associated with BP regulation, as the renin and insulin secretion, the aldosterone and the cortisol synthesis and secretion and the vascular smooth muscle contraction pathways, as well as the cGMP-PKG, cAMP, PI3k-Akt and estrogen signaling pathways and cell–cell junctions, including the Gap junction (Tsare *et al.*, 2024). This is not surprising, as many of the BP GWAS-proteins in the full dataset have been identified as European-specific. The Asian-specific and the common between the two ancestry protein sets are small, thus no pathway was identified as significantly enriched in any of the two groups based on the false discovery rate ($q < 0.05$) threshold. However, using the p-value < 0.05 as significance threshold, we identified eleven pathways as enriched in the Asian-specific BP GWAS-proteins, including the cGMP-PKG and PI3K-Akt signaling pathways, the vascular smooth muscle contraction and the Gap Junction and five pathways enriched in the common proteins of the two ancestries, including the calcium signaling, the aldosterone and the cortisol synthesis and secretion pathways. These results further support the need to analyse the GWAS data in the context of pathways and networks unravelling connections and related mechanisms even in relatively sparse datasets.

3.2 BP GWAS data analysis based on stricter variant consequence severity threshold

We analysed the 3738 BP SNPs associated with the 1170 RHCP-proteins with respect to their consequences (Table 2). Just 183 SNPs and 157 RHCP-proteins are associated with transcript consequences in other than modifier categories only. The 'stop gained' is the variant consequence of the highest severity for the BP SNPs and only five BP SNPs have such high impact on five proteins.

Table 2. RHCP-associated BP-GWAS data statistics based on severity level

Variant consequence (impact)	total #SNPs up to the particular level	total #Proteins up to the particular level
stop gained (high)	5	5
inframe deletion (moderate)	6	6
missense (moderate)	137	118
splice region (low)	151	130
synonymous (low)	183	157
5 prime UTR (modifier)	224	184
3 prime UTR (modifier)	352	258
intron (modifier)	3738	1170
NMD transcript (modifier)	3738	1170

Note: SNPs may be associated with multiple transcript consequences, thus at each level we present the number of unique SNPs or proteins associated with consequences up to that level. If all proteins associated with a consequence level are also associated with other previous levels, there is no change in the total number of SNPs or proteins of this level compared to its previous.

These are: DPB1, GEM, AMPE, KCNJ11 and ZC21C, of which only AMPE is associated with only this type of variant consequence. SESQ1 is the only protein in the next variant consequence category ('inframe deletion') of moderate impact. AMPE (glutamyl aminopeptidase) is involved in the strongly BP-related renin-angiotensin system (Nehme *et al.*, 2019). KCNJ11 (ATP-sensitive inward rectifier potassium channel 11) has been reported as associated with hypertension-induced heart failure (Kane *et al.*, 2006). GEM (GTP binding protein) has been shown to regulate the activity of the serine/threonine Rho kinases (ROCKs) that play an important role in cardiovascular system and hypertension pathology (Wirth, 2010). On the other hand, we identified that the vast majority of SNPs with RHCP-coding transcript consequences (~85%, 3168) are only intron variants ('modifier' category). As a result, 912 of the 1170 BP GWAS-proteins are associated with intron variants and 856 are considered as BP-related based on this type of SNPs only.

We proceeded to reconstruct the BP PPI network based on the 157 proteins associated with variant consequences in other than modifier categories to investigate whether essential information about BP regulation is retained even by considering only this small fraction of the full BP GWAS protein set. The reconstruction process is described in detail in (Tsare *et al.*, 2024). We note that 33 of the 157 proteins are among the 103 GWAS-prioritized proteins of the full protein set (Tsare *et al.*, 2024). The GWAS-deduced PPI network includes 142 proteins (with at least one PPI of high-confidence of being direct in the human protein interactome). This comprises of one 12-protein component with 11 PPIs (excluding self-interactions), one trimer, few heterodimers and many monomers (Fig. 3A). According to our network reconstruction process, we consider as "blue nodes" (BNs) the 12 proteins in the largest connected component and we proceed in extending the PPI network by the shortest paths connecting all proteins in one component (Fig. 3B). The shortest-path intermediates are named "yellow nodes" (YNs) and considered as BP-related. The final "GWAS-reconstructed by the shortest-path approach" (GWAS-RbSP) PPI network comprised 797 protein-nodes (655 YNs) with 9826 PPIs. As expected by the full BP PPI network, we observed that almost all GNs are at most second neighbors of a BN.

Network analysis showed that the extended BP PPI network reconstructed from the 157 proteins associated with SNPs of most impactful transcript consequences follows a scale-free structure with a moderate fit ($R^2 = 74\%$) (Fig. 4). The P53 protein (YN) is the node with the highest number of interactions (161), while six more proteins (all YNs) have ≥ 120 interactors: A4, EGFR, AKT1, UBC, EP300 and ESR1. These proteins are also hubs in the full BP PPI network (Tsare *et al*., 2024). Interestingly, proteins P53 and ESR1, which are BNs in the full BP PPI network, do not belong to the 157 proteins and are YNs in this case. This shows that through the 'guilt by association' PPI network extension principle that we used, they are still identified as BP-related and included in the final BP-related protein interactome.

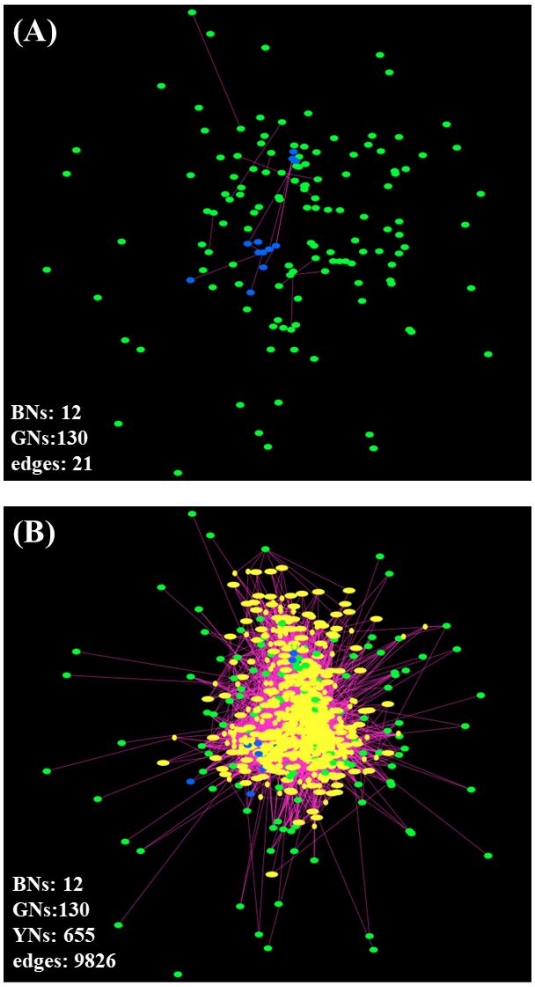

Figure 3. The GWAS-deduced (A), and the extended by shortest-path approach (B) BP PPI network of the 157 proteins associated with SNPs in non-modifier consequence categories. Blue nodes (BNs) are the proteins in the largest connected component of the (A) network, while the rest are green nodes (GNs). Yellow nodes (YNs) are the shortest path intermediates in the (B) network.

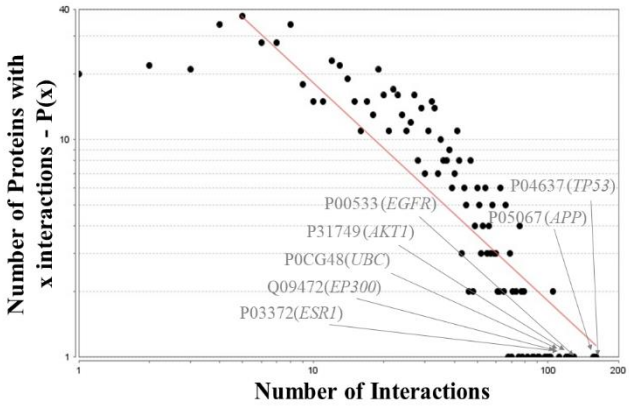

Figure 4. Degree distribution of the extended PPI network reconstructed based on the 157 proteins associated with the most impactful SNPs. The most connected proteins of the network are also shown.

Pathway enrichment analysis of the BP PPI network based on the 157 proteins associated with the most impactful SNPs indicated BP-protein enrichment in the most of the same pathways as the full BP PPI network, described in Tsare *et al.*, 2024, presenting the same perspective despite their differences in starting proteins and size. The enriched pathways include the cardiomyopathies' pathways, signaling pathways, among which the adrenergic signaling in cardiomyocytes and the PI3K-Akt, the cGMP-PKG, the cAMP, the HIF-1 and the calcium signaling pathways and focal adhesion/axon guidance-related pathways. Additionally, the pathways strongly associated with BP including the aldosterone synthesis and secretion, the renin-angiotensin system, the insulin resistance and secretion and the thyroid hormone synthesis are also among the BP-enriched. Finally, almost half (176) of the 335 prioritized proteins of the full BP-protein set are included in the extended BP PPI network derived based on a stricter variant consequence severity score.

4. CONCLUSIONS

The systematic curation of all available GWAS data for particular complex traits or pathophysiologies through the development of specialized meta-databases and the analysis of GWAS data in the context of PPI networks can significantly upgrade the information content of the GWAS data, enabling also their investigation based on various criteria and parameters. In this study, we identified the European- and Asian- specific BP PPI networks, supporting the fact that most of the currently available BP-GWAS data are based on individuals of European ancestry. Still, although scarce, the Asian-specific BP GWAS dataset pointed to eleven BP-related pathways, showing that the resolution of the BP (de)regulation at the pathway-level is less dependent of the GWAS dataset size. As more BP GWAS data from other ancestral backgrounds become available, our meta-database is appropriately structured to enable the selection of ancestry-specific GWAS information and contribute to more specific studies that may lead to valuable ancestry-specific insights about BP. Furthermore, our meta-database enables the selection of proteins associated with SNPs of varying consequence severity. In this study, we reconstructed the BP PPI network starting from the 157 proteins of the most impactful SNPs (non-modifier categories). In this case too, it was observed that despite smaller than the full PPI network, the new network revealed the same BP-significant pathways, "recruiting" important BP-proteins that are not in the initially considered 157 through the "guilt by association" extension principle that we used. Extending upon the work of Tsare et. al. (2024), this study also supports the significance of integrating genetic with functional knowledge in the context of biomolecular networks, as this combined approach diminishes the impact of false positives in any of the involved datasets. Furthermore, the identified as prioritized genes and/or pathways provide targets for experimental investigation to prove their functional association with BP.

ACKNOWLEDGMENTS

This work was supported by a Hellenic Foundation for Research and Innovation (HFRI) PhD scholarship grant to EPGT (#2082), by the BrainPrecision (TAEDR 0535850) project that is funded by the European Union-Next Generation EU, Greece 2.0 National Recovery and Resilience

plan, National Flagship Initiative "Health and Pharmaceuticals", by Greek national projects ELIXIR-GR (MIS 5002780), “EATRIS-GR (MIS 5028091), BBMRI-GR (MIS 5028275), “INSPIRED (MIS 5002550) implemented under the Action “Reinforcement of the Research and Innovation Infrastructure”, funded by the Operational Program “Competitiveness, Entrepreneurship and Innovation” (NSRF 2014–2020) and co-financed by Greece and the European Union (European Regional Development Fund), by the EU-funded H2020 projects “DIAGONAL (ID 95152), “JOINTPROMISE: (ID 874837) and “AIDPATH: (ID 101016909).

REFERENCES

Buniello, A., MacArthur, J.A.L., Cerezo, M., Harris, L.W., Hayhurst, J., Malangone, C., *et al.* (2019) ‘The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019’, *Nucleic Acids Research*, 47(D1), pp. D1005–D1012. doi:10.1093/nar/gky1120.

Dimitrakopoulos, G.N., Klapa, M.I. and Moschonas, N.K. (2021) ‘PICKLE 3.0: enriching the human meta-database with the mouse protein interactome extended via mouse–human orthology’, *Bioinformatics*. Edited by P.L. Martelli, 37(1), pp. 145–146. doi:10.1093/bioinformatics/btaa1070.

Dimitrakopoulos, G.N., Klapa, M.I. and Moschonas, N.K. (2022) ‘How Far Are We from the Completion of the Human Protein Interactome Reconstruction?’, *Biomolecules*, 12(1), p. 140. doi:10.3390/biom12010140.

Fitipaldi, H. and Franks, P.W. (2023) ‘Ethnic, gender and other sociodemographic biases in genome-wide association studies for the most burdensome non-communicable diseases: 2005–2022’, *Human Molecular Genetics*, 32(3), pp. 520–532. doi:10.1093/hmg/ddac245.

Gioutlakis, A., Klapa, M.I. and Moschonas, N.K. (2017) ‘PICKLE 2.0: A human protein-protein interaction meta-database employing data integration via genetic information ontology’, *PLOS ONE*. Edited by B. Oliva, 12(10), p. e0186039. doi:10.1371/journal.pone.0186039.

Hettiarachchi, G. and Komar, A.A. (2022) ‘GWAS to Identify SNPs Associated with Common Diseases and Individual Risk: Genome Wide Association Studies (GWAS) to Identify SNPs Associated with Common Diseases and Individual Risk’, in *Single Nucleotide Polymorphisms*. Cham: Springer International Publishing, pp. 51–76. doi:10.1007/978-3-031-05616-1_4.

Kane, G.C., Behfar, A., Dyer, R.B., O’Cochlain, D.F., Liu, X.-K., Hodgson, D.M., *et al.* (2006) ‘KCNJ11 gene knockout of the Kir6.2 K ATP channel causes maladaptive remodeling and heart failure in hypertension’, *Human Molecular Genetics*, 15(15), pp. 2285–2297. doi:10.1093/hmg/ddl154.

Klapa, M.I., Tsafou, K., Theodoridis, E., Tsakalidis, A. and Moschonas, N.K. (2013) ‘Reconstruction of the experimentally supported human protein interactome: what can we learn?’, *BMC systems biology*, 7, p. 96. doi:10.1186/1752-0509-7-96.

Loos, R.J.F. (2020) ‘15 years of genome-wide association studies and no signs of slowing down’, *Nature Communications*, 11(1), p. 5900. doi:10.1038/s41467-020-19653-5.

Ma, Q. and Didonna, A. (2021) ‘The novel multiple sclerosis susceptibility gene ATXN1 regulates B cell receptor signaling in B-1a cells’, *Molecular Brain*, 14(1), p. 19. doi:10.1186/s13041-020-00715-0.

Martin, F.J., Amode, M.R., Aneja, A., Austine-Orimoloye, O., Azov, A.G., Barnes, I., *et al.* (2023) ‘Ensembl 2023’, *Nucleic Acids Research*, 51(D1), pp. D933–D941. doi:10.1093/nar/gkac958.

Maurano, M.T., Humbert, R., Rynes, E., Thurman, R.E., Haugen, E., Wang, H., *et al.* (2012) ‘Systematic Localization of Common Disease-Associated Variation in Regulatory DNA’, *Science*, 337(6099), pp. 1190–1195. doi:10.1126/science.1222794.

Nehme, A., Zouein, F.A., Zayeri, Z.D. and Zibara, K. (2019) ‘An Update on the Tissue Renin Angiotensin System and Its Role in Physiology and Pathology’, *Journal of Cardiovascular Development and Disease*, 6(2), p. 14. doi:10.3390/jcdd6020014.

Ratnakumar, A., Weinhold, N., Mar, J.C. and Riaz, N. (2020) ‘Protein-Protein interactions uncover candidate “core genes” within omnigenic disease networks’, *PLOS Genetics*. Edited by X. Liu, 16(7), p. e1008903. doi:10.1371/journal.pgen.1008903.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., *et al.* (2003) ‘Cytoscape: A software Environment for integrated models of biomolecular interaction networks’, *Genome Research*, 13, pp. 2498–2504. doi:10.1101/gr.1239303.

Sherman, B.T., Hao, M., Qiu, J., Jiao, X., Baseler, M.W., Lane, H.C., *et al.* (2022) ‘DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update)’, *Nucleic Acids Research*, 50(W1), pp. W216–W221. doi:10.1093/nar/gkac194.

Tsare, E.-P.G., Klapa, M.I. and Moschonas, N.K. (2024) ‘Protein–protein interaction network-based integration of GWAS and functional data for blood pressure regulation analysis’, *Human Genomics*, 18(1), p. 15. doi:10.1186/s40246-023-00565-6.

Wang, W., Yao, J., Li, W., Wu, Y., Duan, H., Xu, C., *et al.* (2023) ‘Epigenome-wide association study in Chinese monozygotic twins identifies DNA methylation loci associated with blood pressure’, *Clinical Epigenetics*, 15(1), p. 38. doi:10.1186/s13148-023-01457-1.

Wirth, A. (2010) ‘Rho kinase and hypertension’, *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1802(12), pp. 1276–1284. doi:10.1016/j.bbadis.2010.05.002.

Yan, J., Risacher, S.L., Shen, L. and Saykin, A.J. (2017) ‘Network approaches to systems biology analysis of complex disease: integrative methods for multi-omics data’, *Briefings in Bioinformatics* [Preprint]. doi:10.1093/bib/bbx066.