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Abstract: Social biases may concentrate attention of researchers on a small number of well-known molecules/mechanisms leaving others underexplored. In accordance with this view, central to mechanistic toxicology is a narrow range of molecular pathways that are assumed to be involved in significant part of responses to toxicity. It is unclear, however, if there are other molecular mechanisms which play important role in toxicity events but are overlooked by toxicology. To identify overlooked genes sensitive to chemical exposures we used publicly available databases. First, we used data on published chemical-gene interactions for 17,338 genes to estimate their sensitivity to chemical exposures. Next, we extracted data on publication numbers per gene for 19,243 human genes from *Find My Understudied Genes* database. Threshold were applied to both datasets using our algorithm to identify chemically sensitive and chemically insensitive genes and well-studied and underexplored genes. 1,110 underexplored genes highly sensitive to chemical exposures were used in GSEA and Shiny GO analyses to identify enriched biological categories. Metabolism of fatty acids, amino acids, and glucose were identified as underexplored molecular mechanisms sensitive to chemical exposures. These findings suggest that future effort is needed to uncover the role of xenobiotics in the current epidemics of metabolic diseases.

Keywords: publication bias; underexplored pathways; toxicity mechanisms

1. Introduction

Existing research demonstrates that the distribution of research efforts focusing on specific genes, proteins, and molecular mechanisms is biased by social factors that are not always related to the importance of corresponding molecules for living organisms. For example, analysis of temporal patterns of publications on specific genes suggests that some genes received exceptional attention due to societal needs [cure for an important disease] [1]. Only a fraction of protein kinases, ion channels, G protein-coupled receptors, and nuclear receptors received significant research attention, not explained by their more important roles in cells [2–4].

Surprisingly the list of proteins [3] and genes [5] which attracted most of the research attention did not change over the period of 20 years. A high level of research attention to a selected list of molecules may be partly explained by trends in the scientific community resulting from dynamics of social networks [1]. These trends are successfully modeled based on vast publication data demonstrating that researchers predominantly publish on genes that are already popular in research literature [6,7]. At least partly these trends may originate from the social phenomena known as "famous for being famous" in popular culture. Additional social bias results from a catch-22 situation when research hypotheses are being developed using molecular pathways and functional biological categories based on well-annotated molecules. The resulting hypotheses focus further research efforts on the same set of molecules and pathways [8]. A recent analysis of gene features in association with the number of published studies showed that features that hinder our ability to study specific genes using traditional methodologies are associated with a smaller number of papers [5].

Taken together these studies suggest that medico-biological research is prone to bias due to a broad range of factors, which all together concentrate the attention of researchers on a small number of well-known molecules/mechanisms leaving others underexplored. In accordance with this view, central to mechanistic toxicology is a narrow range of molecular pathways that are assumed to be involved in a significant part of toxicities. It is unclear however if there are other molecular

mechanisms overlooked by previous research which also play a significant role in toxicity events. A recent attempt to identify in an unbiased manner molecular mechanisms most sensitive to a broad range of chemical exposures demonstrated that indeed a range of molecular mechanisms poorly covered by toxicological research may be as sensitive to toxins as those that received significant attention [9,10].

Toxicology started to use molecular biology tools in the late 1970s [11], however, the rapid transformation into a molecular discipline was triggered by the publication of *Toxicity Testing in the 21st Century: a Vision and a Strategy* in 2007 by the US National Research Council [12]. One important component of this transformation consists in the understanding of molecular mechanisms that causally link exposures with adverse outcomes - toxicity pathways or adverse outcome pathways (AOP) [12–16]. Characterization of AOP is critically important for the transition to pathway-based toxicity testing [17]. Thus, a systematic effort is needed to identify and characterize AOP and ensure that no important mechanisms linking exposures and toxicities have been overlooked.

In this report, we attempt to use minimally biased approaches to identify underexplored genes and molecular mechanisms sensitive to chemical exposures to inform toxicological community on the important directions of the future research.

2. Materials and Methods

2.1. Sensitivity of Genes to Chemical Exposures

Previous research developed an approach to identify genes sensitive to chemical exposures in an unbiased way [9,18]. In short, transcriptomic data were extracted from toxicological experiments in which gene expression changes in responses to chemical exposures were analyzed using high-throughput methods. Transcriptomic information from 2,169 individual in-vivo and in-vitro studies using human, rat, or mouse cells or tissue covering experiments with 1,239 chemical compounds was extracted from the Comparative Toxicogenomic Database (CTD) [19]. Genes that are not present in the genomes of all three species were excluded from further analysis. The number of published chemical-gene interactions (CGI) was calculated for 17,338 genes to represent their sensitivity to chemical exposures. It is important to note that ranked sensitivities of genes to chemical exposures do not depend on the composition of chemicals used for the identification of CGI numbers [9]. The full list of genes with their corresponding CGI numbers is available through Mendeley Data [20]. The threshold between genes highly sensitive to chemical exposures and genes with low sensitivity was determined using a method for the identification of cutoff points in descriptive high-throughput omics studies [21]. This approach identifies an inflection point in a ranked distribution of variables if this distribution follows a biphasic pattern. In the current study, the method identified a cutoff between the big number of genes with low CGI numbers (< 73) and the smaller group of genes with high CGI numbers ($\geq$ 73).

2.2. Number of Publications Per Gene

The level of research attention for every human gene was evaluated by the number of PubMed publications that mentioned the gene in the title and/or abstract. This analysis was done by T. Stoeger's group using PubTator [22]. As a result of this research, the authors created a database and a tool *Find My Understudied Genes* (FMUG) (https://fmug.amaral.northwestern.edu/) which contains the number of publications per every human gene. This information for 19,243 genes was downloaded for our analysis. We used the same method for cutoff point identification as described in the previous paragraph [21] to determine the threshold between underexplored (< 200 publications/gene) and well-explored ($\geq$ 200 publications/gene) genes.

2.1. Underexplored Pathways Sensitive to Chemical Exposures

To analyze biological categories enriched by underexplored genes sensitive to chemical exposures the list of all genes sensitive to chemical exposures (CGI number $\geq$ 73] with their respective publication numbers was used for gene set enrichment analysis (GSEA) [23,24]. GSEA was developed

to characterize the cumulative shift of genes in a particular pathway towards an increase or decrease of expression. As such, it was designed for an input in which values of gene expression changes have positive and negative values. To prepare datasets suitable for GSEA, in accordance with our threshold of 200 publications/gene we subtracted 200 from the values of publications/gene, to achieve negative publication values for underexplored genes and positive for well-explored genes. The resulting gene list with publication values was uploaded to GSEA and analyzed against three independent databases: Reactome [25,26], KEGG [27], and Gene Ontology [28,29]. Additionally, the shortlist of top genes with the highest number of CGIs (CGI number ≥ 76) and the 10th percentile lowest number of publications (≤ 20 publications/gene) was uploaded to ShinyGO 8.0 [30], and enriched functional terms were explored with default settings.

3. Results

The overlap between the CGI/gene dataset and publication numbers/gene dataset consisted of 16,095 genes. Out of this list, 1,333 (8.3%) and 14,768 (91.7%) genes had high (≥ 73 CGIs) and low (< 73 CGIs] sensitivity to chemical exposures respectively; and 555 (3.5%] and 15,540(96.5%] genes were well explored (≥ 200 publications/gene) and underexplored (< 200 publications/gene) respectively. Among 1,333 chemically sensitive genes, 223 (16.7%) were well explored and 1,110 (83.3%) genes were underexplored. The distribution of chemical sensitivities vs the number of publications per gene is shown in Figure 1A.

GSEA analysis conducted against three databases of biological pathways/categories retrieved coherent results demonstrating that chemically sensitive underexplored genes are enriched significantly with metabolic categories. Categories that were enriched with FDR q < 0.1 are shown in Table 1. Specifically, a range of biological categories related to lipid metabolism was significantly enriched. Enriched categories also included the metabolism of amino acids, glucose, and nucleosides (see Figure 1B-D for representative enrichment plots).

We further used the shortlist of chemically sensitive genes selected based on a stringent criterion for the level of knowledge availability (≤ 20 publications/gene) to identify enriched categories using ShinyGO 8.0. The results of this analysis (Figure 1E) confirm that underexplored chemically sensitive genes enrich metabolic pathways, including the metabolism of lipids, amino acids, and glucose, along with categories representing the existing focus of toxicology (e.g., glutathione metabolism, cytochrome P450).
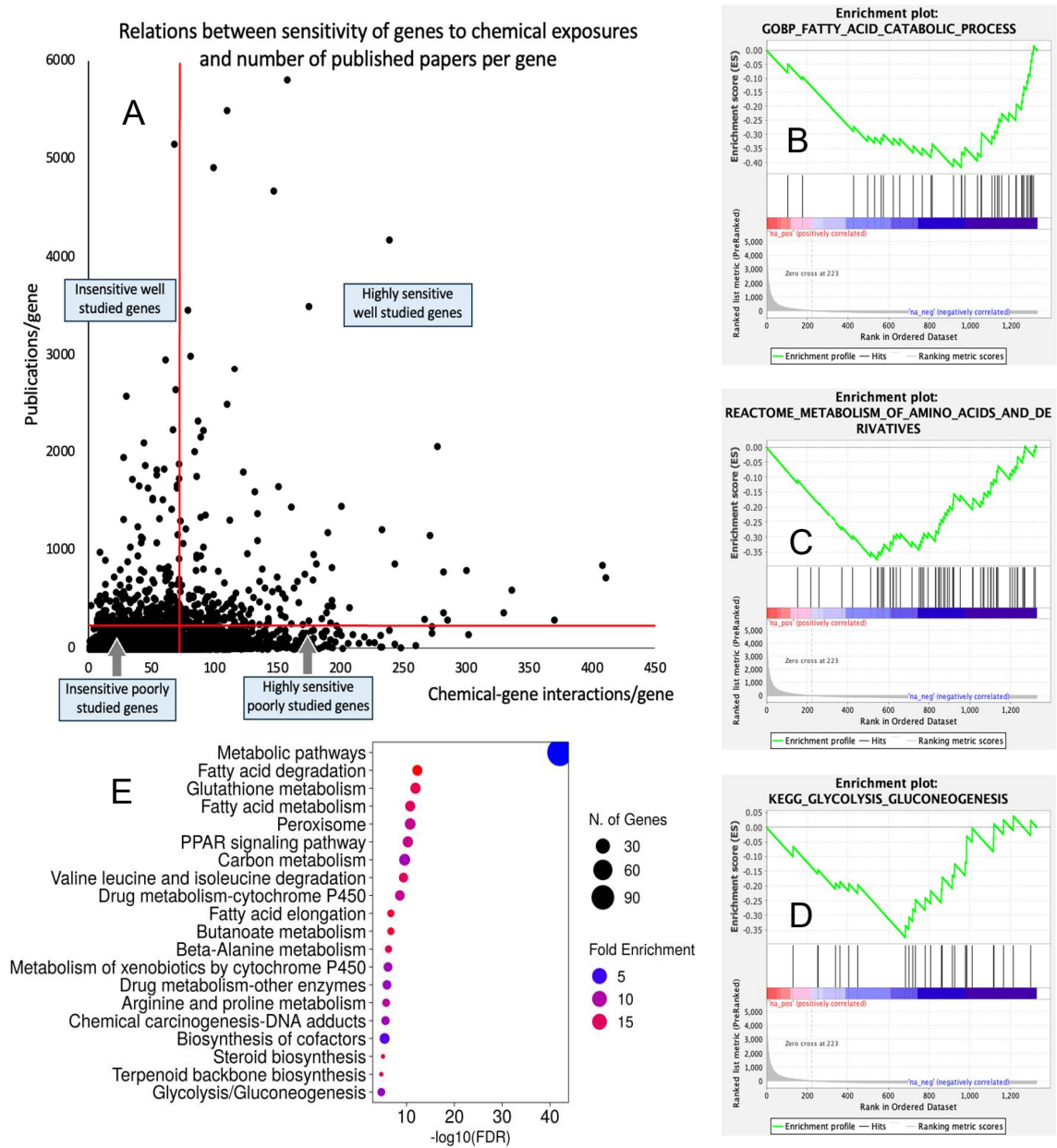
Figure 1. Underexplored molecular mechanisms of toxicity. A - Distribution of 16,095 genes in accordance with their sensitivity to chemical exposures [number of chemical-gene interactions] and level of research attention (publications/gene). B-D - Representative GSEA enrichment plots illustrating enrichment of lipid metabolism (B], amino-acid metabolism (C), and glucose metabolism (D] with chemically sensitive underexplored genes. E - Biological categories enriched with chemically sensitive underexplored genes identified by ShinyGO 8.0.

Table 1. Biological categories enriched with chemically sensitive underexplored genes as identified with GSEA analysis against Reactome, KEGG, and Gene Ontology databases [NES – normalized enrichment score, FDR – false discovery rate].

Biological Category	NES	FDR q
Reactome		
Glycerophospholipid biosynthesis	-2.16	0.014
Metabolism of amino acids and derivatives	-2.15	0.007
Peroxisomal protein import	-2.06	0.009

Cholesterol biosynthesis	-2.06	0.007
Metabolism of steroids	-1.94	0.018
Fatty acid metabolism	-1.82	0.046
Activation of gene expression by SREBF SREBP	-1.79	0.048

KEGG

Butanoate metabolism	-2.03	0.007
Valine leucine and isoleucine degradation	-2.02	0.004
Fatty acid metabolism	-1.85	0.017
Peroxisome	-1.65	0.062
Glycolysis gluconeogenesis	-1.59	0.073

Gene Ontology [Biological Process]

Organic acid catabolic process	-2.33	0.004
Monocarboxylic acid catabolic process	-2.12	0.016
Fatty acid catabolic process	-2.03	0.028
Fatty acid beta oxidation	-1.98	0.037
Fatty acid derivative metabolic process	-1.93	0.05
Nucleoside bisphosphate metabolic process	-1.93	0.044
Amino acid metabolic process	-1.85	0.069
Cellular modified amino acid metabolic process	-1.83	0.069
Lipid oxidation	-1.83	0.065
Thioester metabolic process	-1.81	0.072
Alpha amino acid metabolic process	-1.78	0.08

4. Discussion

Our results demonstrate that metabolic pathways, especially the metabolism of fatty acids and amino acids, and to a lesser degree metabolism of glucose are underexplored molecular mechanisms sensitive to chemical exposures. These results are concordant with the traditional structure of toxicology. The field of "metabolic disruption" started to take shape only recently. Indeed the term "metabolic disruption" was first proposed by Casals-Casas and Desvergne in 2011 [31] and it was further promoted by the group of multidisciplinary experts in 2015 [32]. PubMed search conducted on 05/16/2024 with the key word "metabolic toxicity" retrieved only 247 studies. For comparison, searchers with the key words "endocrine disruption", "reproductive toxicity", "neurotoxicity", and "carcinogenicity" resulted in 4273, 4944, 91356, and 228470 studies. The Society of Toxicology does not have a specialty section in its structure focusing on metabolic toxicity and most toxicological textbooks do not have chapters focusing on metabolic toxicity as well. The importance of the focus of future efforts on the identification of metabolic molecular mechanisms affected by chemical exposures is dictated by the current epidemic of metabolic disease, one of the biggest public health issues in the modern-day [33–37].

The major limitation of this study consists of the use of existing gene annotations to identify molecular mechanisms associated with underexplored genes. This approach does not allow identification of toxicological mechanisms that are not present in current annotations of underexplored genes. It is reasonable to assume that due to the underexplored nature of these genes, their annotations are far from being complete, and future research may identify other functions of currently underexplored genes. Another limitation is that our crude approach does no allow identification of the role of each gene in the development of toxicity outcomes. Some chemically sensitive genes may be causally involved in toxicities, others may be involved in compensatory

responses, and some other genes may be involved in neither but represent a side effect of AOP or compensatory mechanisms activation. Despite these limitations, we suggest that the current analysis identifies underexplored area of toxicology. Additional research effort in this area may provide significant progress and important discoveries.

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Data Availability Statement: All data on chemical-gene interactions per gene used in this study are available through Mendeley Data [20]. All data on PubMed publications numbers per gene used in this study are available through *Find My Understudied Genes* [FMUG] database [https://fmug.amaral.northwestern.edu/].

Conflicts of Interest: Alexander Suvorov reports a relationship with ReGENE LLC that includes board membership, equity or stocks, and funding grants. Olatunbosun Arowolo declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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