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Software Engineering Department

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Capstone Project Phase A

Gender-Based Analysis of Biological Pathways in Gene Expression

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Git repository link:

https://github.com/avtn96/Final-Project.git

Abstract

Bioinformatics is an interdisciplinary field that combines biology, computer science, and statistics to analyze large-scale biological data, such as genomics. Genes, as sequences of DNA, carry instructions for cellular functions, and differential gene expression refers to how these genes are expressed at varying levels under different conditions or across groups, including between males and females. Sex-based differences at the gene level, influenced by sex chromosomes and hormones, are thought to shape various biological pathways—complex sequences of molecular interactions that lead to specific cellular outcomes.

This research aims to explore how gender differences influence gene expression across multiple tissues, using data from the Genotype-Tissue Expression (GTEx) project. By applying advanced bioinformatics techniques, we expect to identify genes and biological pathways that may exhibit sex-biased expression patterns. By analyzing gene regulatory networks (GRNs) and applying machine learning models, such as XGBoost and SVM, we aim to investigate the roles of transcription factors and their potential contribution to these differences.

While the specific regulatory mechanisms remain to be uncovered, we anticipate revealing insights into how gender-specific factors might influence key biological processes. This study ultimately seeks to understand how gender impacts biological pathways, providing a foundation for the development of more precise, individualized medical interventions that take gender-related genetic differences into account.

Introduction

Gender differences significantly influence biological processes underlying development and disease. For instance, autoimmune diseases are more prevalent in females, whereas non-reproductive cancers are more common in males(Morrow, 2015)[18]. These differences are rooted in molecular and genetic variances between male and female cells, driven by sex steroids and chromosomes. Previous studies

have shown that the human transcriptome exhibits sex-biased expression across various tissues(Gershoni & Pietrokovski, 2017)[5].

Bioinformatics, an interdisciplinary field merging biology, computer science, and statistics, is crucial for analyzing large-scale genomic data. This research utilizes data from the Genotype-Tissue Expression (GTEx) project, which provides extensive RNA sequencing data across numerous human tissues. The GTEx project is instrumental in exploring how genetic variation influences gene expression and regulation, facilitating the study of sex-differential gene expression(Oliva et al., 2020)[15].

Biological pathways are a series of actions among molecules in a cell that lead to a specific product or change, such as metabolic or signal transduction pathways. Understanding these pathways in the context of gender differences involves identifying genes with sex differential expression (SDE), meaning they exhibit varying expression levels between males and females. Systems and network biology approaches are increasingly used to identify disease targets based on the complexity of biological systems, revealing that sex shapes biological networks (Hartman et al., 2021)[6]. Machine learning techniques, such as XGBoost and SVM, have also been applied to classify receptor functions in tissue-specific pathways, expanding our understanding of how metabolic and inflammatory functions differ between sexes (Yehuda & Somekh, 2022)[14].

Gene regulatory networks (GRNs) consist of genes, transcription factors (TFs), and other molecular regulators that interact to control gene expression. Transcription factors are proteins that bind to specific DNA sequences, regulating the transcription of target genes. Analyzing GRNs helps identify regulatory network structures that differ between genders, providing insights into the molecular mechanisms underlying sex-specific gene expression(Lopes-Ramos et al., 2020)[12].

Sex differences in gene expression have profound implications for health and disease. For example, sex-biased target genes and gender-biased regulatory targeting patterns can influence susceptibility to diseases and responses to treatments. Understanding these differences can lead to the development of gender-specific

therapeutic strategies, enhancing the effectiveness of treatments and reducing adverse effects(Lopes-Ramos et al., 2020)[12]. This research aims to uncover these fundamental biological differences, contributing to personalized medicine and improved health outcomes for both genders.

The primary goal of this research is to identify and analyze specific genes and pathways that show differential expression between males and females. By leveraging genomic data from the GTEx project and employing advanced bioinformatics tools, this study seeks to uncover biological differences that could inform gender-specific healthcare practices. This exploration is expected to provide valuable insights into gender-specific traits and conditions, enhancing our understanding of human biology and contributing to the development of more targeted medical interventions.

In conclusion, this research project aims to advance our understanding of gender-based biological pathways differences in gene expression. By analyzing genomic data and identifying differentially expressed genes, we aim to uncover key regulatory mechanisms and pathways that differ between males and females. This knowledge has the potential to inform personalized medicine and improve health outcomes for both genders, highlighting the importance of considering sex as a biological variable in biomedical research.

Background & Related Work

General Studies on Sex Differences in Gene Expression

Sex-based differences in gene expression and biological pathways have profound implications for understanding gender-specific health outcomes. Numerous studies have highlighted the molecular mechanisms behind these differences using diverse approaches, from whole-genome sequencing to gene regulatory network analysis. Mayne et al. (2016)[13] explored genetic differences across human populations, focusing on how these variations contribute to phenotypic diversity and disease susceptibility. The study uncovered sex-biased pathways related to immune system regulation, energy metabolism, and growth, with immune-related pathways more highly expressed in females and energy production pathways in males. This research lays the groundwork for understanding evolutionary pressures that shape sex-differential gene expression.

Hartman et al. (2021)[6] analyzed sex-dependent gene co-expression patterns using RNA-seq data from the GTEx project. They found that 29.5% of genes displayed sex-dependent co-expression, particularly in immune-related and inflammatory pathways more frequently expressed in females, potentially explaining female susceptibility to autoimmune diseases like Hashimoto's thyroiditis.

Yehuda and Somekh (2022)[14] developed a machine learning-based framework to classify metabolic and inflammatory receptor functions in adipose tissues. By applying XGBoost, SVM, and k-NN models to gene co-expression patterns, they accurately predicted receptor functions, with XGBoost performing the best. This study underscores the potential of bioinformatics and machine learning for analyzing tissue-specific gene networks and offers applications in receptor function prediction and drug development

Tissue-Specific Studies

Several studies have examined how sex differences manifest in gene expression across various tissues, offering insights into tissue-specific regulatory mechanisms. Kassam et al. (2019)[8] identified over 30,000 transcripts exhibiting tissue-specific sex differences (TSSDs) using GTEx data. Their study emphasized the importance of

androgen and estrogen regulatory elements and cis-expression quantitative trait loci (eQTLs) in shaping tissue-specific gene expression in males and females.
Oliva et al. (2020)[15] provided a comprehensive analysis of sex-biased gene expression across 44 tissues. They demonstrated that 37% of all genes exhibit sex-biased expression in at least one tissue. Notably, this study linked sex-biased gene expression to important biological functions such as drug metabolism and immune response, highlighting its relevance to precision medicine.
Warren et al. (2020)[1] focused on sex differences in gene expression in subcutaneous adipose tissue. They found robust sex-biased expression in 162 genes, impacting pathways like oxidative phosphorylation, contributing to a better understanding of sex-specific cardiometabolic diseases.
Gautam et al. (2021)[4] investigated sex differences in gene expression among asthmatic patients. The study identified differentially expressed genes (DEGs) in sex-biased pathways, such as hypoxia-inducible factor 1 (HIF-1) signaling in males and IL-17/chemokine signaling in females. These findings provide valuable insights into the molecular mechanisms behind sex differences in asthma susceptibility and offer potential targets for personalized asthma therapies.
InanlooRahatloo et al. (2021)[7] studied gene expression in the left ventricles of deceased male and female heart donors. They found significant sex differences in inflammatory gene expression, with females showing higher immune-related gene expression. This research highlights how these differences may influence susceptibility to cardiovascular diseases, underscoring the need for sex-specific approaches in cardiac research.
Galiuto and Patrono (2020)[11] explored sex differences across 44 tissues, finding that 37% of genes displayed sex-biased expression. Their study identified that transcription factor binding and epigenetic modifications are key drivers of sex-biased gene expression. This research emphasizes the importance of considering sex as a variable in precision medicine, especially in the context of drug metabolism and immune response.

Regulatory Networks and Mechanisms Influencing Sex Differences

Regulatory networks play a crucial role in mediating sex differences, with transcription factors and gene regulatory networks contributing to tissue-specific and systemic biological differences.

Lopes-Ramos et al. (2020)[12] analyzed gene regulatory networks across 29 tissues and identified significant sex-biased targeting patterns of transcription factors. These differences impact tissue-specific functions and disease mechanisms, emphasizing the need for sex-based approaches in precision medicine.

Saha et al. (2021)[16] investigated sex differences in gene regulatory networks in lung adenocarcinoma (LUAD). Their findings revealed significant sex-biased regulation of key biological pathways, exacerbated by tobacco smoking. Additionally, they identified small-molecule drugs with sex-biased efficacy, stressing the importance of incorporating sex-specific considerations in lung cancer treatment and drug development.

Blencowe et al. (2022)[2] explored the roles of sex hormones, chromosomes, and gonads in regulating gene expression in liver and adipose tissues. They found that activational hormone levels had the strongest influence on gene expression, followed by the organizational effects of gonads and sex chromosomes, which are crucial for understanding diseases like coronary artery disease.

Gershoni and Pietrokovski (2017)[5] explored sex-differential gene expression across 53 tissues and found that genes with sex-biased expression are subject to different evolutionary selection pressures, particularly in reproductive tissues. This work highlights the evolutionary consequences of sex-biased gene expression.

Health Implications and Personalized Medicine

Understanding sex-biased gene expression is key to addressing gender-specific health issues and developing personalized therapeutic strategies.

Kurt et al. (2018)[9] studied sexual dimorphisms in NAFLD, identifying key metabolic and inflammatory pathways that differ between sexes. Male-specific pathways included phospholipid metabolism, while females had pathways related to vitamin and cofactor metabolism. These findings are essential for developing sex-specific treatments for NAFLD.

Li et al. (2021)[10] identified sex-biased genes implicated in synaptic transmission and G-protein coupled receptor signaling pathways in depression, offering potential therapeutic targets. This study underscores the importance of considering sex differences in treating neurological disorders like depression.

Sousa et al. (2020)[17] explored sex-specific gene deregulation in gastric and thyroid cancers, which are more common in males and females, respectively. Their study

identified sex-biased genes in both normal and tumor tissues, providing insights into gender-specific cancer treatment strategies.
Fisher et al. (2023)[3] examined sex-specific differences in drug metabolism and constructed gene regulatory networks related to sex-biased adverse events (SBAEs). Their findings emphasize the need for personalized pharmacological approaches to account for sex differences in drug efficacy and safety.

Summary
Sex differences in gene expression have critical implications for gender-specific health outcomes. Research highlights sex-biased pathways such as immune regulation and energy metabolism, as well as co-expression patterns linked to autoimmune diseases. Tissue-specific studies reveal how sex influences gene expression across various tissues, impacting biological functions like drug metabolism. These findings emphasize the importance of considering sex differences in personalized medicine and treatment strategies.

Research Process

Our research process involves several key steps: data preprocessing, Weighted Gene Co-expression Network Analysis (WGCNA), enrichment analysis and module correlations, and then machine learning (ML) algorithms. These steps are designed to extract meaningful biological insights, but the process is inherently dynamic and may evolve as the research progresses. This flexibility is necessary to adapt to emerging challenges, such as data quality issues, discovering unexpected patterns, or refining analysis methods.

Data Preprocessing

Data preprocessing is a fundamental stage where raw data from the Genotype-Tissue Expression (GTEx) project is prepared for further analysis. This step involves handling missing values, correcting batch effects, and removing outliers to ensure the data is suitable for network analysis and machine learning.

Obstacles in Data Preprocessing:

1. **Incomplete Data**: Large-scale datasets often suffer from missing or incomplete data, which can hinder accurate analysis. If this issue is not addressed properly, it can lead to biased or misleading results, particularly when analyzing co-expression networks or applying machine learning algorithms. To mitigate this, statistical imputation techniques will be employed to estimate missing values. In cases where large portions of data are missing, careful evaluation will be conducted to determine whether the incomplete data can be meaningfully used or should be excluded from the analysis. This will ensure that the dataset remains robust and does not compromise the reliability of network analysis or machine learning tasks.
2. **Batch Effects**: Variability introduced during the data generation process can obscure real biological signals. Without correction, batch effects can result in artificial differences between samples, which may mislead the analysis of sex-based gene expression patterns. To account for this, normalization methods such as Combat or RUV will be applied to minimize the impact of batch effects while preserving true biological variability. This approach ensures that comparisons between male and female gene expression profiles reflect genuine differences, free from technical noise that could distort the analysis.
3. **Outliers**: Outliers—whether due to experimental errors or biological anomalies—can significantly distort results, particularly in network-based approaches like WGCNA. Outliers can lead to false connections between genes and obscure genuine biological relationships. Statistical techniques such as z-score normalization or PCA will be utilized to detect and remove outliers, preventing them from skewing the co-expression networks. This will help maintain the integrity of the gene modules identified, ensuring they represent valid biological relationships.

Why Adjustments May Be Required:

- **New Biological Insights**: During exploratory analysis, new patterns or outliers might emerge, requiring additional preprocessing or re-analysis to

ensure these findings are valid. This might involve refining gene selection or adjusting thresholds in co-expression analysis.

- **Changing Data Requirements**: As the research advances, different stages may call for varying levels of data granularity. For instance, early stages may focus on broad patterns across tissues, but later stages might require more detailed, tissue-specific data or adjusted normalization techniques to accommodate refined research goals.
- **Algorithmic Refinement**: As new findings arise, machine learning models like XGBoost or SVM might need to be fine-tuned or replaced by more suitable methods to better capture subtle sex-biased gene expression differences.

Importance of Data Quality

High-quality data is vital to ensure the success of subsequent steps such as WGCNA, enrichment analysis, and machine learning. Preprocessing helps to resolve data quality issues by addressing missing values, correcting batch effects, and mitigating the influence of outliers. Ensuring that the data is appropriately prepared allows for:

- **Accurate Biological Interpretation**: Properly processed data helps ensure that any patterns or gene co-expression modules identified reflect genuine biological phenomena rather than technical noise.
- **Reliable Model Performance**: Machine learning models rely heavily on the quality of input data. If preprocessing is inadequate, model predictions can be inaccurate or misleading.
- **Robustness of Network Analysis**: In WGCNA, the formation of gene modules is sensitive to outliers and noise. Ensuring clean and reliable data allows for the identification of biologically meaningful modules that can be further analyzed for gender-based differences.

WGCNA and Enrichment Analysis

After data preprocessing, WGCNA is used to identify clusters of co-expressed genes that may display gender-specific patterns. Enrichment analysis or module correlations follow to link these gene modules to specific biological pathways. The

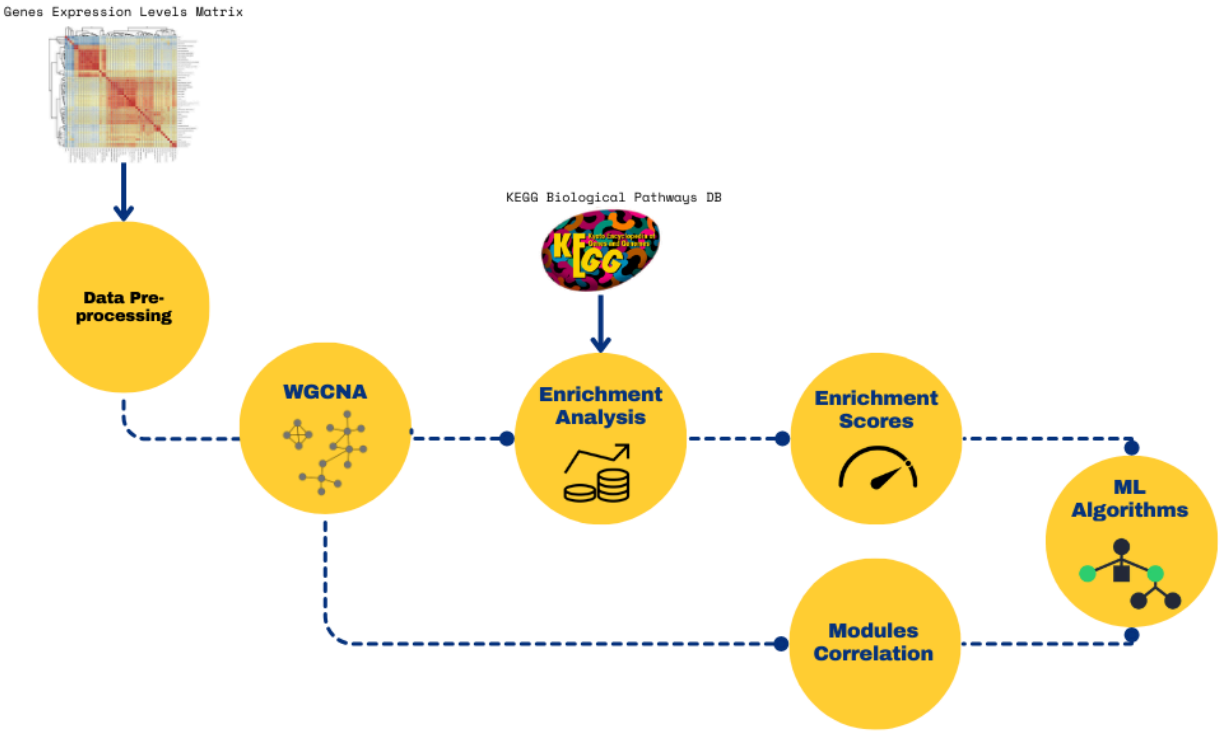
Kyoto Encyclopedia of Genes and Genomes (KEGG) database will be employed to annotate these gene clusters with relevant pathways, helping to contextualize their biological significance. This stage is focused on identifying whether the gene modules are involved in known biological processes, particularly those that may show gender-specific characteristics.

Machine Learning Algorithms

Machine learning algorithms such as XGBoost and SVM are applied to further examine the identified modules and predict sex-biased regulatory mechanisms. These algorithms can be adjusted or refined as needed to improve their performance or interpretability, ensuring that the research remains aligned with its objectives.

Conclusion

The research process for this project is both structured and adaptable. While it follows a defined series of steps—data preprocessing, WGCNA, enrichment analysis, and machine learning—it remains flexible to accommodate changes based on the research's evolving needs. Ensuring high data quality is fundamental to deriving meaningful biological insights, and the ability to adjust the process ensures the research can respond effectively to new challenges, discoveries, or emerging trends.



Evaluation Plan

To ensure the validity and reliability of the results obtained from our analysis of biological pathways, we will employ a multi-step evaluation process. This evaluation plan is designed to critically assess whether the identified pathways and their biological relevance align with established scientific understanding and provide meaningful insights.

1. **Literature-Based Validation**
   For each biological pathway identified in our analysis, we will conduct an extensive literature review. By comparing our results with existing studies, we aim to confirm whether the biological functions of the pathway align with known mechanisms in the relevant tissues or conditions. This step will help verify that our findings are consistent with prior scientific discoveries and not spurious results.
2. **Biological Plausibility**
   Beyond literature comparison, we will assess the biological plausibility of the identified pathways. This involves critically analyzing whether the role of the pathway, as suggested by our results, is feasible within the context of the tissue or biological process being studied. We will consider factors such as:
   - The role of genes in the pathway.
   - Known interactions between genes, transcription factors, and signaling molecules.
   - The broader biological context, such as developmental stages, disease mechanisms, or metabolic processes.
3. **Cross-Referencing with Pathway Databases**
   To further evaluate the significance of the pathways, we will cross-reference our results with established pathway databases, such as KEGG. This will allow us to determine if the pathways identified in our study have been previously implicated in similar biological processes, adding a layer of credibility to our findings.

4. **Internal Consistency with Experimental Data**
   Finally, we will examine whether our results show internal consistency with the experimental data. This includes ensuring that the gene expression patterns in the pathways align with what is observed in biological experiments or datasets such as GTEx. Pathways that demonstrate strong correlation with known experimental results will be considered more reliable.

Future Work

Our research provides valuable insights into the influence of gender on gene expression and the associated biological pathways. However, there are several avenues for future exploration that can build upon the results of this research, benefiting the broader bioinformatics and genomics communities.

1. **Extension of Data Sources and Tissues Studied**: While we have utilized the GTEx dataset to explore sex-biased gene expression, future studies could expand the data sources by incorporating additional genomic databases. Integrating datasets from more diverse populations and underrepresented tissue types would allow for a broader understanding of gender-based gene expression.
2. **Exploring Additional Biological Pathways and Disease Models**: Our study identifies pathways influenced by gender, but future research could focus on disease-specific models, particularly where sex differences play a critical role. For example, cardiovascular diseases, autoimmune conditions, and metabolic disorders display sex-based variations in prevalence and progression, which may be tied to differential gene expression. Expanding our models to include disease-specific pathways could provide targeted insights for more effective medical interventions, similar to related research in cardiovascular genetics .
3. **Refinement of Machine Learning Models**: While we have employed advanced models like XGBoost and SVM, future work could refine these approaches by incorporating newer techniques such as deep learning. Additionally, comparing different machine learning models alongside

unsupervised clustering techniques could help identify more subtle patterns in gender-specific gene expression. Further optimization of these computational models may lead to more precise predictions and the identification of previously undiscovered sex-biased regulatory mechanisms.

4. **Functional Validation of Regulatory Networks**: Our study identifies potential transcription factors involved in gender-based gene expression differences. However, experimental validation of these findings is needed. Techniques like CRISPR/Cas9 gene editing or RNA interference (RNAi) could confirm the biological relevance of these transcription factors in both males and females. Such experiments would solidify our computational predictions, providing concrete evidence of how gender-specific factors influence key biological processes.
5. **Clinical Implications and Precision Medicine**: A significant goal of this research is to contribute to the development of gender-specific medical interventions. Future studies could translate these findings into clinical applications, particularly in diseases that exhibit gender-specific differences, such as breast cancer or osteoporosis. Identifying pathways that differ between males and females could enable the development of more personalized treatment strategies that take gender-related biological processes into account.

By pursuing these future directions, our research could deepen the understanding of how gender influences gene expression and biological pathways, advancing the field of bioinformatics and contributing to the development of more individualized medical treatments.

References

1. Anderson, W. D., Soh, J. Y., Innis, S. E., Dimanche, A., Ma, L., Langefeld, C. D., Comeau, M. E., Das, S. K., Schadt, E. E., Björkegren, J. L. M., & Civelek, M. (2020). Sex differences in human adipose tissue gene expression and genetic regulation involve adipogenesis. Genome Research, 30(10), 1379–1392. https://doi.org/10.1101/gr.264614.120
2. Blencowe, M., Chen, X., Zhao, Y., Itoh, Y., McQuillen, C. N., Han, Y., Shou, B. L., McClusky, R., Reue, K., Arnold, A. P., & Yang, X. (2022). Relative contributions of sex hormones, sex chromosomes, and gonads to sex differences in tissue gene regulation. Genome Research. https://doi.org/10.1101/gr.275965.121
3. Fisher, J. L., Clark, A. D., Jones, E. F., & Lasseigne, B. N. (2023). Sex-biased gene expression and gene-regulatory networks of sex-biased adverse event drug targets and drug metabolism genes. Cold Spring Harbor Laboratory. http://dx.doi.org/10.1101/2023.05.23.541950
4. Gautam, Y., Afanador, Y., Abebe, T., López, J. E., & Mersha, T. B. (2019). Genome-wide analysis revealed sex-specific gene expression in asthmatics. Human Molecular Genetics, 28(15), 2600–2614. https://doi.org/10.1093/hmg/ddz074
5. Gershoni, M., & Pietrokovski, S. (2017). The landscape of sex-differential transcriptome and its consequent selection in human adults. BMC Biology, 15(1). https://doi.org/10.1186/s12915-017-0352-z
6. Hartman, R. J. G., Mokry, M., Pasterkamp, G., & den Ruijter, H. M. (2021). Sex-dependent gene co-expression in the human body. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-98059-9
7. InanlooRahatloo, K., Liang, G., Vo, D., Ebert, A., Nguyen, I., & Nguyen, P. K. (2017). Sex-based differences in myocardial gene expression in recently deceased organ donors with no prior cardiovascular disease. PLOS ONE, 12(8), e0183874. https://doi.org/10.1371/journal.pone.0183874
8. Kassam, I., Wu, Y., Yang, J., Visscher, P. M., & McRae, A. F. (2019). Tissue-specific sex differences in human gene expression. Human Molecular Genetics, 28(17), 2976–2986. https://doi.org/10.1093/hmg/ddz090
9. Kurt, Z., Barrere-Cain, R., LaGuardia, J., Mehrabian, M., Pan, C., Hui, S. T.,

Norheim, F., Zhou, Z., Hasin, Y., Lusis, A. J., & Yang, X. (2018). Tissue-specific pathways and networks underlying sexual dimorphism in non-alcoholic fatty liver disease. Biology of Sex Differences, 9(1). https://doi.org/10.1186/s13293-018-0205-7

10. Li, X., Su, X., Liu, J., Li, H., Li, M., Li, W., & Luo, X.-J. (2021). Transcriptome-wide association study identifies new susceptibility genes and pathways for depression. Translational Psychiatry, 11(1). https://doi.org/10.1038/s41398-021-01411-w
11. Liuzzo, G., & Galiuto, L. (2020). Ubiquitous sex differences in tissue gene expression: The dawn of a new era for gender medicine. European Heart Journal, 41(42), 4090–4091. https://doi.org/10.1093/eurheartj/ehaa877
12. Lopes-Ramos, C. M., Chen, C.-Y., Kuijjer, M. L., Paulson, J. N., Sonawane, A. R., Fagny, M., Platig, J., Glass, K., Quackenbush, J., & DeMeo, D. L. (2020). Sex differences in gene expression and regulatory networks across 29 human tissues. Cell Reports, 31(12), 107795. https://www.cell.com/cell-reports/fulltext/S2211-1247(20)30776-2?dgcid=raven_jbs_etoc_email
13. Mayne, B. T., Bianco-Miotto, T., Buckberry, S., Breen, J., Clifton, V., Shoubridge, C., & Roberts, C. T. (2016a). Large scale gene expression meta-analysis reveals tissue-specific, sex-biased gene expression in humans. Frontiers in Genetics, 7. https://doi.org/10.3389/fgene.2016.00183
14. Arye Yehuda, G., Somekh, J. (2022) A methodology for classifying tissue-specific metabolic and inflammatory receptor functions applied to subcutaneous and visceral adipose. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0276699
15. Oliva, M. (2020). The impact of sex on gene expression across human tissues. Science. https://www.science.org/doi/full/10.1126/science.aba3066
16. Saha, E., Guebila, M. B., Fanfani, V., Fischer, J., Shutta, K. H., Mandros, P., DeMeo, D. L., Quackenbush, J., & Lopes-Ramos, C. M. (2023). Gene regulatory Networks Reveal Sex Difference in Lung Adenocarcinoma. Cold Spring Harbor Laboratory. http://dx.doi.org/10.1101/2023.09.22.559001
17. Sousa, A., Ferreira, M., Oliveira, C., & Ferreira, P. G. (2020). Gender differential transcriptome in gastric and thyroid cancers. Frontiers in Genetics, 11. https://doi.org/10.3389/fgene.2020.00808

18. Morrow, E. H. (2015). The evolution of sex differences in disease. Biology of Sex Differences, 6(1). https://doi.org/10.1186/s13293-015-0023-0

AI Tools:

https://openai.com/chatgpt/
Prompts:
Explain what is biological pathways
Explain how the WGCNA technique works
What is the KEGG database?

https://copilot.microsoft.com/
Prompts:
Generate document summary
Generate key insights from this document
Are there any limitations to this study?
Tell me more about the methods of this study