

# Masters Thesis

## SKIN

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# APPROVAL OF THESIS

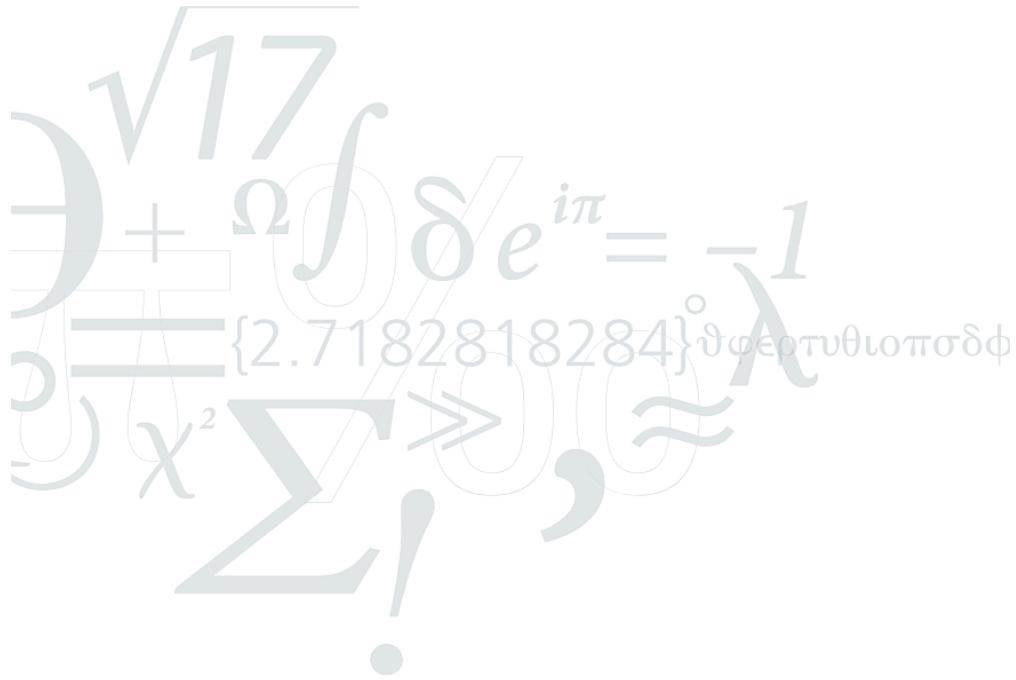
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# **STATEMENT OF THESIS ORIGINALITY**

## **Declaration of Authorship**

I, Miss Oriade Latifah Simpson, hereby declare that the present master's thesis is my own original work and has been written independently. This thesis has not been submitted, either in whole or in part, for the award of any academic degree or qualification at any other institution.

All sources of information and ideas that are not my own have been appropriately acknowledged and referenced. I affirm that this work complies with the ethical and academic standards required for submission at the Technical University of Denmark.

This thesis is submitted in partial fulfilment of the requirements for the Master's Programme at the Department of Health Technology, Technical University of Denmark.

## **Abstract**

This section provides a concise summary of the research, including the central research question, the methodology employed, key findings and the main conclusions drawn from the analysis. The abstract does not exceed 500 words. It consists of 3 paragraphs each of which contains research problems and objectives research method and research results. The abstract is typed italicised.

The keywords related to the thesis are listed. ( Write this at the end. )

## Acknowledgements

First and foremost, I would like to express my deepest gratitude to my mother, Veronika Quintyne, for her unwavering support and encouragement throughout my academic journey. Her belief in me has been a constant source of strength.

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# **Chapter I**

## **INTRODUCTION**

### **1.1 Background of the Problem**

The rationale facts and observations that are important. The research problem and why the research problem is important and needs to be researched. Apply new knowledge from the program and present a piece of work that involves thinking. The subject should relate to the program and specific specialisation.

Set the scene and motivate the problem being studied. It describes a domain and indicates a problem in general terms.

What is the general area being addressed? What is the motivation for studying a particular problem?

What makes it worth the effort?

Is it a real problem in everyday life?

Is it a theoretical problem that is worth solving?

Would anyone care if I solved this?

### **1.2 Formulation of the problem**

The situation or phenomenon that needs to be solved and requires an answer through thorough research and in depth thinking using scientific tools.

The research has a sense of clarity and authenticity and it is in line with the research objectives it is an important matter and worthy of research and it provides implications for empirical studies. It is supported by primary or secondary data. Based on the research problem research questions can be formulated.

### **1.3 Objective and Benefits of the Research**

The research objectives reveals the results to be achieve through the research process. The research objective answers the research problem and reflects the scope of the research, the methods used and the expected results.

### **1.4 Systematics of Writing**

A brief description of the things in each chapter.

This thesis is submitted in fulfilment of the requirements for a master's degree in bioinformatics and serves as a demonstration of advanced research competences. It aims to exhibit the ability to define a clear research question, conduct a comprehensive review of the literature and apply appropriate research methodologies.

The objective of this study is to critically evaluate existing academic work in the field and to contribute new insights or perspectives that may advance scholarly understanding or have practical relevance.

The thesis presents an opportunity to dive deeply into a specific topic and enhance my expertise and understanding of that area. This process has also provided an opportunity for the development of academic communication skills, both written and oral, as a part of preparing, presenting and defending the thesis findings.

Through the formulation of a coherent research narrative and the integration of evidence based conclusions, this thesis seeks to generate original contributions with the chosen area of inquiry.

The master's thesis contributes original knowledge or insights to a specific discipline which can be beneficial for academic and practical applications.

The references are made using Zotero<sup>1</sup>.

## **Chapter II**

### **LITERATURE REVIEW**

Reviews existing research related to the topic, highlighting gaps that your study aims to address.

The literature review contains the theoretical basis and discussion of the results of previous similar studies. A framework of thought and hypothesis can also be put forward.

### **2.1 Theoretical Foundations and Previous Research**

The theories supporting the hypothesis are said. The research problem has not been answered or solved satisfactorily.

What is the research context and discipline the thesis fits within?

Who has looked at this area before?

What is the state of the art of methods and solution to the problem?

What other work complements this research ?

### **2.2 Framework**

The problems to be studied are explained. There is a research hypothesis. This explanation is included in the form of a schematic to clarify the purpose of the study. This is a series of thought arrangements about what should happen so the intended hypothesis arises.

### **2.4 Hypotheses | Problem Statement | Research Question**

The hypothesis is a short statement that is concluded from the literature review and it is a temporary answer to the problem under study. The hypothesis is supported by theories or references from previous studies.

This is a statement of the hypothesis and problems. The hypothesis is the highest level problem or goal you are going to address.

The problems should be unambiguous. The importance of the problem should be mentioned if it was not already done so. You can develop a new approach for solving a well known problem or replicate a method in the literature.

**Hypothesis I:** Analysing the genomic sequence of *Streptococcus pyogenes* (GAS) can reveal key virulence factors and their regulation mechanisms, providing insights into potential targets for vaccine development and therapeutic interventions.

**Hypothesis II:** Determine the structure of the M Protein.

**Hypothesis:** High-quality genomic sequences of GAS strains will reveal conserved and strain-specific genetic features associated with virulence.

**Hypothesis:** Virulence-associated genes in GAS are enriched in GO terms related to immune evasion, adhesion, and toxin activity, suggesting coordinated biological roles in host-pathogen interaction.

**Network Analysis:** Construct gene/protein interaction networks and analyze relationships **Hypotheses:** Network analysis will reveal key hub proteins that coordinate multiple virulence pathways in GAS. Virulence factors form tightly connected modules in protein-protein interaction networks, indicating co-regulation or functional synergy.

**Hypothesis:** Phylogenetic clustering of GAS strains will correlate with emm types and virulence gene profiles, reflecting evolutionary adaptations to host immune pressures.

Hypotheses:emm gene diversity across GAS strains correlates with distinct virulence gene repertoires. Certain emm types are associated with the presence of unique virulence genes, which may contribute to tissue tropism or infection severity.

Hypotheses:Structural variants of the M protein display differential binding affinities to human skin cell receptors. Key amino acid residues in the M protein are conserved across strains and are essential for receptor binding, representing potential therapeutic targets.

## RESEARCH AIMS

- To collect and curate high-quality genomic sequences of *Streptococcus pyogenes* (GAS) strains from diverse clinical and geographical sources. To characterize the functional roles of identified virulence genes via GO enrichment analysis using tools such as DAVID and GSEA.
- To construct gene and protein interaction networks to explore the regulatory relationships between virulence factors using Cytoscape.
- To compare virulence gene content and emm-type distribution across multiple GAS strains to uncover patterns of genomic diversity.
- To identify and characterize virulence genes across multiple GAS strains. Hypothesis: GAS strains share a conserved core of virulence factors, with additional strain-specific genes that correlate with clinical severity.

# The Skin

## The Function of the Skin

The skin is the largest organ of the human body and is comprised of a diverse array of specialised cell types. It serves as a critical barrier that protects the internal organs from bacteria invasion, environmental pathogens, ultraviolet (UV) radiation and various biochemical agents. In addition to its protective role, the skin plays a fundamental part in thermoregulation by modulating body temperature and enabling adaptation to fluctuating environmental conditions.<sup>2</sup>

Furthermore, the skin facilitates the excretion of sweat, sebum, and metabolic waste products through its glandular structures<sup>2</sup>. It possesses wound-healing capabilities, allowing for the repair of abrasions, lacerations and other forms of tissue injury<sup>2</sup>. The subcutaneous fat layer functions as a mechanical cushion, providing shock absorption and an additional line of defence against infection<sup>2</sup>.

The skin also contributes to endocrine function through its role in the synthesis of vitamin D upon exposure to UV radiation<sup>2</sup>. Additionally, it plays a vital sensory role, continuously transmitting information to the central nervous system regarding the external environment<sup>3</sup>. The skin is integrated with the nervous system to enable the perception of thermal stimuli, tactile sensations, and other sensory inputs essential for survival and interaction with the environment<sup>2</sup>.

## The Structure of the Skin

The skin is composed of three primary layers: the **epidermis**, the **dermis**, and the **hypodermis** (also known as the subcutaneous fat layer). Each layer performs specific functions essential to maintaining homeostasis, immunity and overall health.

### The Epidermis

The **epidermis** is the outermost layer of the skin and is primarily composed of keratinocytes, which are specialised cells responsible for the synthesis of keratin, cytokines, growth factors and interleukins. This layer provides the first line of defence against environmental pathogens and is organised into four distinct strata, arranged from superficial to deep.

- *The Stratum corneum*
- *The Stratum granulosum*
- *The Stratum spinosum*
- *The Stratum basale* (also referred to as the *stratum germinativum* or the basal cell layer).

An illustrative representation of the epidermis is provided below<sup>4</sup>.

The **stratum corneum** consists of terminally differentiated keratinocytes. Terminally differentiated cells exit the cell cycle as they can no longer divide. The keratinocytes become corneocytes in the stratum corneum. Corneocytes are non-viable, enucleated cells<sup>5</sup>.

The corneocytes function to minimise transepidermal water loss and provide protection against mechanical and microbial damage. Keratin produced in the underlying layers accumulates in the corneocytes, which are eventually shed through a natural process known as desquamation.

The skin surface is interspersed with pores, which serve as conduits for the excretion of sweat and sebum via eccrine and sebaceous glands, respectively<sup>3</sup>.

The **stratum spinosum**, or *prickle cell layer*, lies above the stratum basale and consists of keratinocytes connected by desmosomes, which provide structural support. In this layer, keratinocytes begin producing cytokeratins that form tonofibrils. Langerhans cells, involved in immune defence, are also present in this layer.

The **stratum granulosum** contains flattened keratinocytes that undergo terminal differentiation. Keratinocytes accumulate keratohyalin granules, involved in keratin aggregation, and lamellar bodies, which secrete lipids

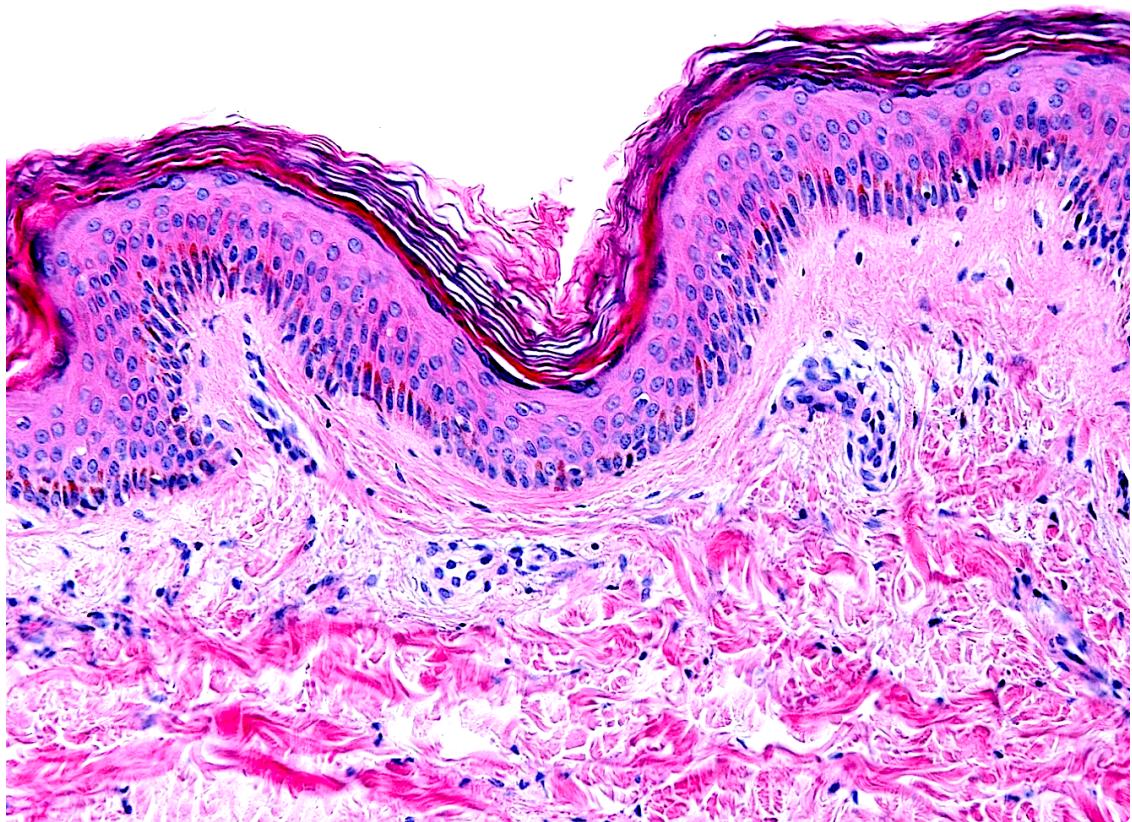


Figure 1: Structure of the epidermis with the different strata, resting on the dermis (Source: Shutterstock.com, Jose Luis Calvo, News Medical 2025)

that form a barrier to water loss. Keratinocytes in this layer begin to lose their nuclei and organelles as they prepare for transformation into dead corneocytes of the uppermost layer ;the stratum corneum.

The **stratum basale** (or stratum germinativum) is the deepest layer of the epidermis and plays a central role in skin regeneration. This layer has mitotically active keratinocytes, which divide to replenish the upper layers. In addition to keratinocytes, several other specialised cells are found within this layer :

- **Melanocytes**, which produce melanin, the pigment responsible for skin colour and protection against ultraviolet (UV) radiation<sup>6</sup>.
- **Langerhans Cells**, (LCs) a type of dendritic cell (DC) that originate from hematopoietic stem cells in the bone marrow. They have a role in immune surveillance by recognising antigens and initiating T-cell responses<sup>5</sup>.
- **Merkel cells**, which are mechanoreceptors involved in the sensation of touch.
- **Dendritic cells**, which also play a defence role in the immune response as they differentiate into macrophages<sup>5</sup>.

Within the **stratum basale** UV radiation stimulates the conversion of provitamin  $D_3$  into pre-vitamin  $D_3$  that initiates the cutaneous synthesis of vitamin D. Subsequent hydroxylation in the liver and kidneys leads to the production of the active form of vitamin D<sup>5</sup>.

The epidermis is not only a structural barrier but also a site of pathological relevance. Several dermatological and systemic conditions occur in this layer including **seborrhoeic dermatitis** (dandruff), **psoriasis**, **atopic dermatitis** (eczema), melanoma, **acne vulgaris** , **actinic keratoses** and pressure ulcers (decubitus ulcers)<sup>3</sup>.

## The Dermis

The dermis is the middle layer of the skin, situated beneath the epidermis, and serves as the primary site of structural and functional support. It contains many essential components including blood vessels such as capillaries<sup>5</sup>, lymphatic vessels, sweat glands, sebaceous glands, hair follicles, nerve endings, and specialised sensory receptors. The dermis is primarily composed of **collagen** and **elastin**, two fibrous proteins that confer tensile strength and elasticity, respectively.

The dermis is subdivided into two distinct layers:

- **The papillary dermis**, the superficial layer, which is composed of loose connective tissue and contains capillaries and sensory neurons.
- **The reticular dermis**, the deeper layer, composed of dense irregular connective tissue rich in collagen and elastin fibres, glands, hair follicles, and larger blood vessels.

The dermis contains sweat glands, sebaceous glands, blood vessels, lymphatic vessels, and other structures critical to skin function. These glands play essential roles in thermoregulation, lubrication, and excretion. The eccrine glands are responsible for sweat production, while the sebaceous glands secrete sebum to maintain skin hydration and barrier function. Both are embedded within the dermal layer and are regulated by hormonal and neural signals.

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Fibroblasts, the predominant cell type in the dermis, are responsible for synthesising collagen proteins, and other components of the extracellular matrix, which maintains the structural framework of connective tissues. In addition to their structural role, fibroblasts are actively involved in **wound healing** through production of signalling molecules and matrix proteins<sup>7</sup>.

Collagen is the most abundant protein in the human body and is found not only in the skin but also in muscles, bones, tendons, ligaments, blood vessels, internal organs and the gastrointestinal lining<sup>8</sup>. The primary amino acids in collagen include glycine, proline and hydroxyproline, which assemble into a characteristic triple-helix structure to form collagen fibrils. The biosynthesis of this structure requires several cofactors, including **vitamin C, zinc, copper and manganese**<sup>8</sup>.

Among the specialised mechanoreceptors in the dermis are Meissner's corpuscles and Pacinian corpuscles, which detect mechanical stimuli such as touch, pressure, and vibration. These corpuscles are multicellular structures (of multiple cell types) consisting of a sensory nerve ending surrounded by specialised Schwann cells.

The vascular network within the dermis plays a crucial role in thermoregulation by adjusting blood flow in response to temperature changes<sup>5</sup>. The nerve endings transmit sensory information such as touch, pain, and temperature<sup>5</sup>. Dermal immune cells contribute to the inflammatory response following injury or infection.

The dermis contains a diverse population of cells, including fibroblasts, immune dendritic cells, macrophages, T lymphocytes, mast cells, innate lymphoid cells, neutrophils, eosinophils, and natural killer cells, neuronal cells and endothelial cells<sup>9</sup>.

Among the immune cells, T lymphocytes are predominantly located in close proximity to blood vessels, vessels, hair follicles and sweat glands within the dermis. Subsets of T cells perform distinct immunological functions: **Th1 cells** secrete cytokines that enhance the capacity of other immune cells to target and eliminate pathogens; however, dysregulation of Th1 activity may contribute to the development of autoimmune disorders<sup>9</sup>. **Th2 cells** are primarily involved in the mediation of allergic responses. **Th17 cells** play a crucial role in defending against bacterial and fungal infections and are implicated in the pathogenesis of inflammatory skin diseases such as eczema and psoriasis.

In contrast, **regulatory T cells (Tregs)** modulate immune responses by suppressing excessive inflammation through the release of inhibitory signals and by eliminating over-active immune cells, thereby maintaining immune homeostasis with the dermis<sup>9</sup>.

Several conditions originate within the dermis, including wrinkles (due to collagen degradation), cellulitis (a bacterial skin infection), dermoid cysts (which may contain hair or teeth), sebaceous cysts, and dermatofibromas.

An illustrative representation of the dermis containing sweat glands, sebaceous glands, blood vessels, lymphatic vessels is shown below<sup>10</sup>.

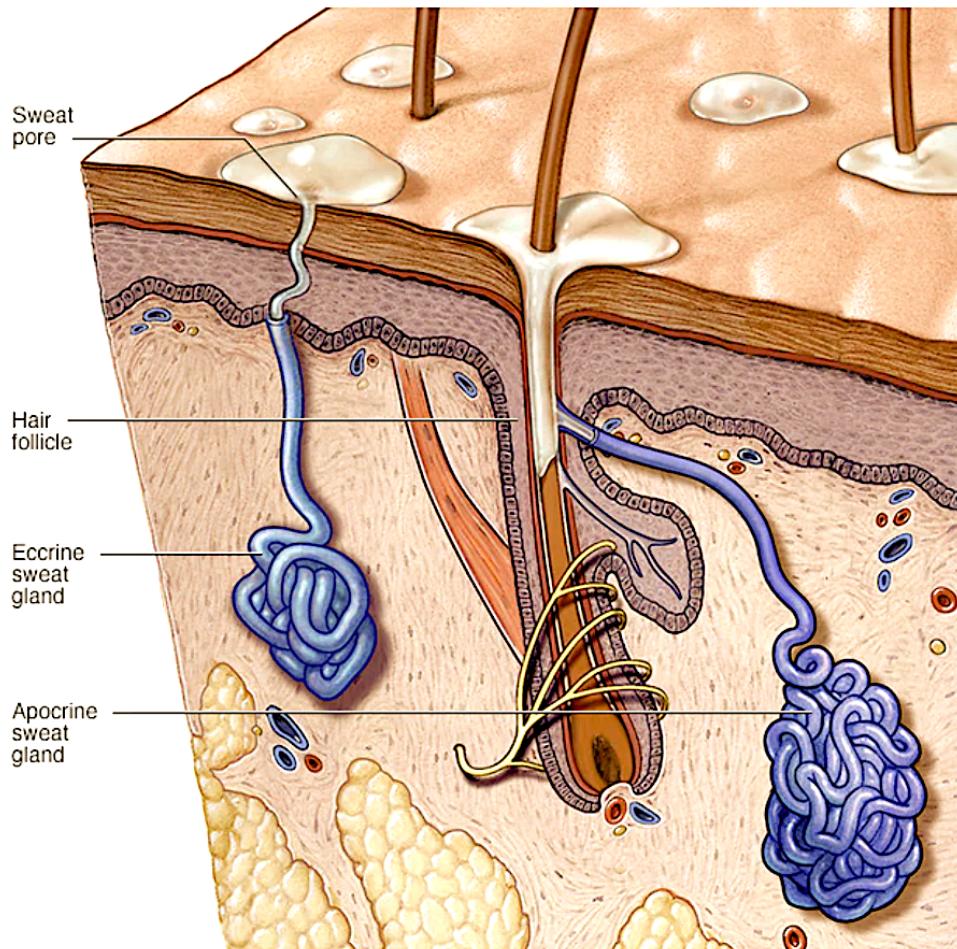


Figure 2: Eccrine & sebaceous glands in the dermis (Source: Mayo Foundation, 2025)

## The Hypodermis

The **hypodermis**, also known as the subcutaneous layer of fat, lies beneath the dermis and primarily consists of adipose tissue. This layer is composed of lipocytes that function to insulate the body, maintain thermoregulation, and serve as an energy reserve. The hypodermis also plays a crucial role in absorbing mechanical shock and protecting underlying muscles and organs.

Structurally, the hypodermis includes the following key components:

- Fibroblasts: Cells responsible for the production of collagen<sup>11</sup>. They also regulate the immune response to producing cytokines and chemokines<sup>9</sup>.

- Adipose tissue: Specialised are fatty tissues composed of lipocytes<sup>11</sup>
- Connective tissue: A network of collagen and elastin fibres that support and anchors, and gives structure to other tissues<sup>11</sup>.
- Blood vessels: Including arteries, veins and capillaries that supply the skin with oxygen rich blood and nutrients, while facilitating thermoregulation<sup>11</sup>.
- Lymphatic vessels: Structures involved in maintaining fluid homeostasis and transporting lymph, a fluid containing immune cells and waste products<sup>11</sup>.
- Hair follicles: Structures that anchor individual hair shafts and are associated with sebaceous glands and nerve endings.
- Nerve fibres: Sensory neurons the body's sense of position and movement in space.

The hypodermis functions as a supportive and protective layer and has important vascular, immune and sensory roles.

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# **Streptococcus pyogenes**

## **Streptococcus pyogenes: Taxonomy, Morphology & Clinical Relevance**

*Streptococcus pyogenes* is a Gram-positive, anaerobic bacterium commonly referred to as Group A Streptococcus (GAS)<sup>13</sup>.

The Gram-positive nature is due to a thick peptidoglycan layer in its cell wall, which retains the crystal violet stain.

Structurally, *S.pyogenes* is characterised by its beta-hemolytic activity, meaning it causes complete lysis of red blood cells on blood agar plates. Morphologically, the cells are small, spherical, and typically arranged in chains, a feature that distinguishes them from other bacterial species<sup>13</sup>.

Taxonómically, *S.pyogenes* is classified as follows:

- **Domain:** *Bacteria*
- **Kingdom:** *Bacillati*
- **Phylum:** *Bacillota*
- **Class:** *Bacilli*
- **Order:** *Lactobacillales*
- **Family:** *Streptococcaceae*
- **Genus:** *Streptococcus*
- **Species:** *S.pyogenes*<sup>13</sup>

As a highly adaptable pathogen, *S.pyogenes* is capable of causing a wide range of clinical diseases, from mild superficial infections to severe invasive conditions. Its chain-like cellular arrangement and distinct beta-hemolytic properties are key identifiers in both clinical and microbiological contexts.

## **Infection of Human Skin**

*Streptococcus pyogenes* is a significant pathogen responsible for a wide spectrum of clinical diseases. Prompt diagnosis and treatment of *S.pyogenes* infections are critical due to the organism's capacity to cause both superficial and systemic illnesses.

Skin infections caused by *S.pyogenes* range from localised conditions such as impetigo to more severe and invasive diseases, including necrotising fasciitis, a life-threatening infection of the deep dermal and subcutaneous tissues<sup>13</sup>.

In addition to skin infections, *S.pyogenes* is known to cause pharyngitis, pneumonia, scarlet fever, acute post-streptococcal glomerulonephritis, and the autoimmune condition rheumatic fever. In chronic cases, *S.pyogenes* may also contribute to the development of rheumatic heart disease.

## **Virulence Factors of Streptococcus pyogenes**

Virulence factors are molecules produced by pathogens that facilitate infection, survival and damage within the host. *S.pyogenes* expresses a diverse array of virulence factors that enable its pathogenicity, immune system invasion, and tissue invasion<sup>13</sup>.

1. **Capsules** The bacterium produces a capsule that protects it from being engulfed by the host immune cells.
2. **Adherence Factors** Adherence factors (Adhesins), including lipoteichoic acid (LTA) and fibronectin-binding proteins help the bacterium to attach to host epithelial cells and tissues.

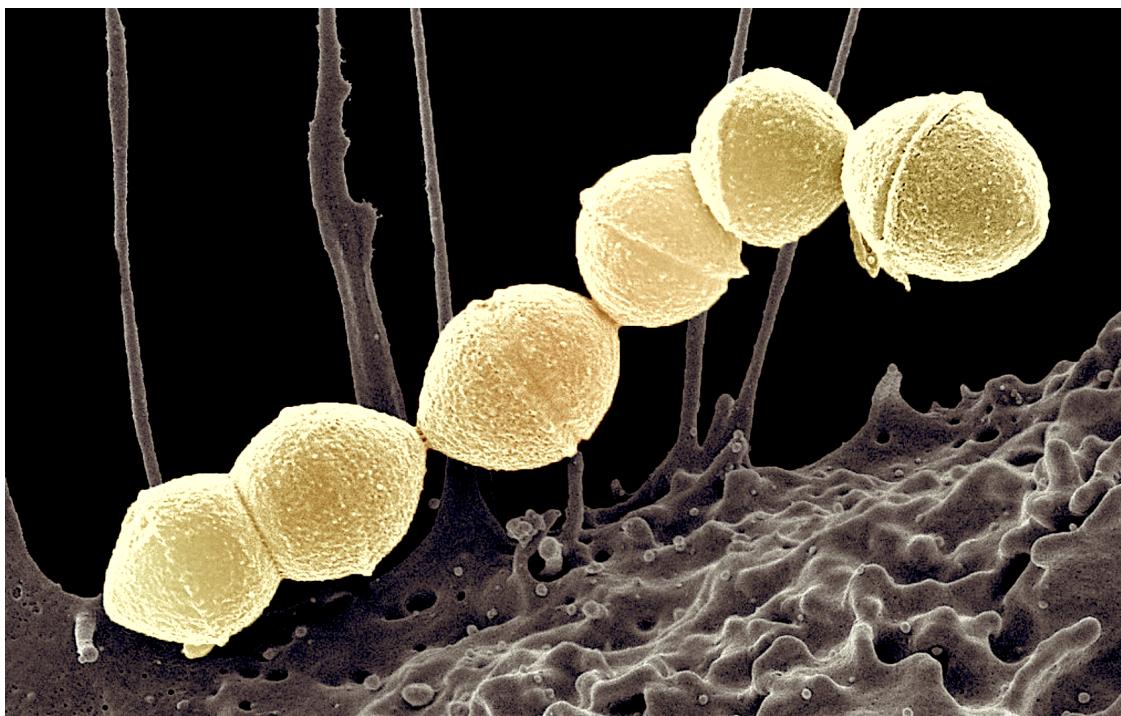


Figure 3: *Streptococcus pyogenes* (Source:National Institute of Allergy and Infectious Diseases (NIAID), Flickr, December 29, 2022)

**3. Surface Proteins** Surface proteins such as M protein and related members (e.g. Mrp and Enn) play crucial roles in immune evasion. These proteins have variable antigens which allow the pathogen to avoid recognition by the host immune system.

**4. Enzymes** *S.pyogenes* secretes several enzymes that degrade host tissues and promote bacterial invasion. These include:

- **Streptokinase:** Converts plasminogen to plasmin, aiding in the breakdown of fibrin blood clots.
- **Hyaluronidase:** Degrades hyaluronic acid in connective tissue, facilitating bacterial spread.
- **DNases:** Break down extracellular DNA.

**5. Toxins** *S.pyogenes* produces streptolysins (SLO and SLS), exotoxins that lyse red blood cells and other host cells. Additionally, streptococcal pyrogenic exotoxins (SPEs) are super-antigens that activate T cells and induce a massive immune response. At least three distinct SPEs have been identified<sup>13</sup>.

### The M Protein

The M protein is a major virulence factor encoded by the emm gene family, which is present in all *S.pyogenes* strains<sup>12</sup>. These surface-anchored proteins are involved in adherence, immune evasion, and resistance to phagocytosis.

The M protein is considered one of the most important virulence factors.

- **Structure:** The M protein is a coiled-coil molecule anchored in the bacterial membrane, with a highly variable N-terminal region responsible for antigenic diversity.
- **Function:** It interferes with opsonisation and complement activation, making it a key player in immune system evasion. The M-protein changes surface antigens to make it harder for the host to recognise the pathogen.

- **Variants:** M-related proteins such as Mrp and Enn, along with fibronectin-binding proteins, are also expressed and contribute to pathogenicity.

## F proteins

F proteins are another group of surface adhesins produced by *S.pyogenes*. These include fibrinogen-binding and fibronectin-binding proteins, which facilitate tight adherence to host tissues and are critical in the early stages of infection.

### Streptolysins and Exotoxins

- Streptolysin O (SLO) and Streptolysin S (SLS) are cytolytic toxins that cause hemolysis and contribute to tissue damage during infection.
- Streptococcal pyrogenic exotoxins (SPEs) are potent super-antigens activate T cells and stimulate a massive immune response, often leading to severe systemic symptoms.

### Lipoteichoic Acid and Vaccine Targets

Lipoteichoic acid is a key surface molecule involved in adherence and immune activation, and it is under investigation as a potential target for vaccine development<sup>13</sup>.

### Antimicrobial Resistance

*S.pyogenes* also harbours genes associated with antimicrobial resistance. Notable among these are:

- **lmrP:** Encodes a multidrug efflux pump
- **tetM and tetL:** Confer resistance to tetracyclines.
- **tgfT:** involved in resistance to specific antimicrobial agents<sup>15</sup>.

These resistance genes highlight the need for continuous surveillance and prudent use of antibiotics in treating *S.pyogenes* infections.

### Biofilm Formation and Quorum Sensing in *Streptococcus pyogenes*

Biofilms are structured microbial communities encased within a self-produced extracellular matrix. In *Streptococcus pyogenes*, biofilm formation facilitates communication between cells and contributes to bacterial survival, particularly under host immune response and exposure to antibiotics. This communication is mediated by a mechanism known as quorum sensing, which regulates gene expression in response to cell density.

In *S.pyogenes*, one of the key quorum sensing pathways involved in biofilm development is the Rgg2/3 pathway. This pathway controls the expression of genes involved in biofilm formation through the modulation of short hydrophobic peptides, which act as quorum sensing pheromones, also referred to as autoinducers.

Short hydrophobic peptides are initially synthesised in an immature form within the bacterial cell. To become functionally active, these peptides undergo a two-step processing mechanism. First, an intracellular metalloprotease enzyme processes the SHPS. Subsequently, they undergo further processing in the extracellular environment to reach their mature, biologically active form.

The specific transport mechanism responsible for the SHP export and the identity of the extracellular processing factor(s) remain to be elucidated.

The Rgg2/3 pathway is essential for biofilm maturation and plays a central role in *S.pyogenes* pathogenesis, particularly in facilitating persistent infections by enhancing resistance to host immune defences and antimicrobial agents.

# Melanoma

## Introduction to Melanoma

Melanoma is a highly prevalent form of skin cancer originating from the melanocytes<sup>19</sup>.

As mentioned in the earlier section, Melanocytes are cells that contribute to skin colouration due to the creation of melanin<sup>20</sup>. A tumour occurs when the DNA mutates inside of the Melanocyte cells<sup>21</sup>. Melanoma is notable for its high metastatic potential<sup>22</sup>.

Melanoma has several distinct subtypes, including Acral Melanoma<sup>23</sup>, Mucosal Melanoma and Uveal Melanoma, each arising in different anatomical sites and with unique molecular profiles<sup>21</sup>. In contrast to keratinocyte carcinomas which include basal cell carcinoma and squamous cell carcinoma, these melanomas originate from melanocytes<sup>23</sup>.

## Related Skin Cancers

Other rare cutaneous malignancies include Sebaceous Carcinoma and Apocrine Adenocarcinoma, as well as Merkel Cell Carcinoma, a neuroendocrine tumour strongly linked with exposure to ultraviolet light. Although these cancers are less common than melanoma, they use similar methods for diagnosis and molecular testing.

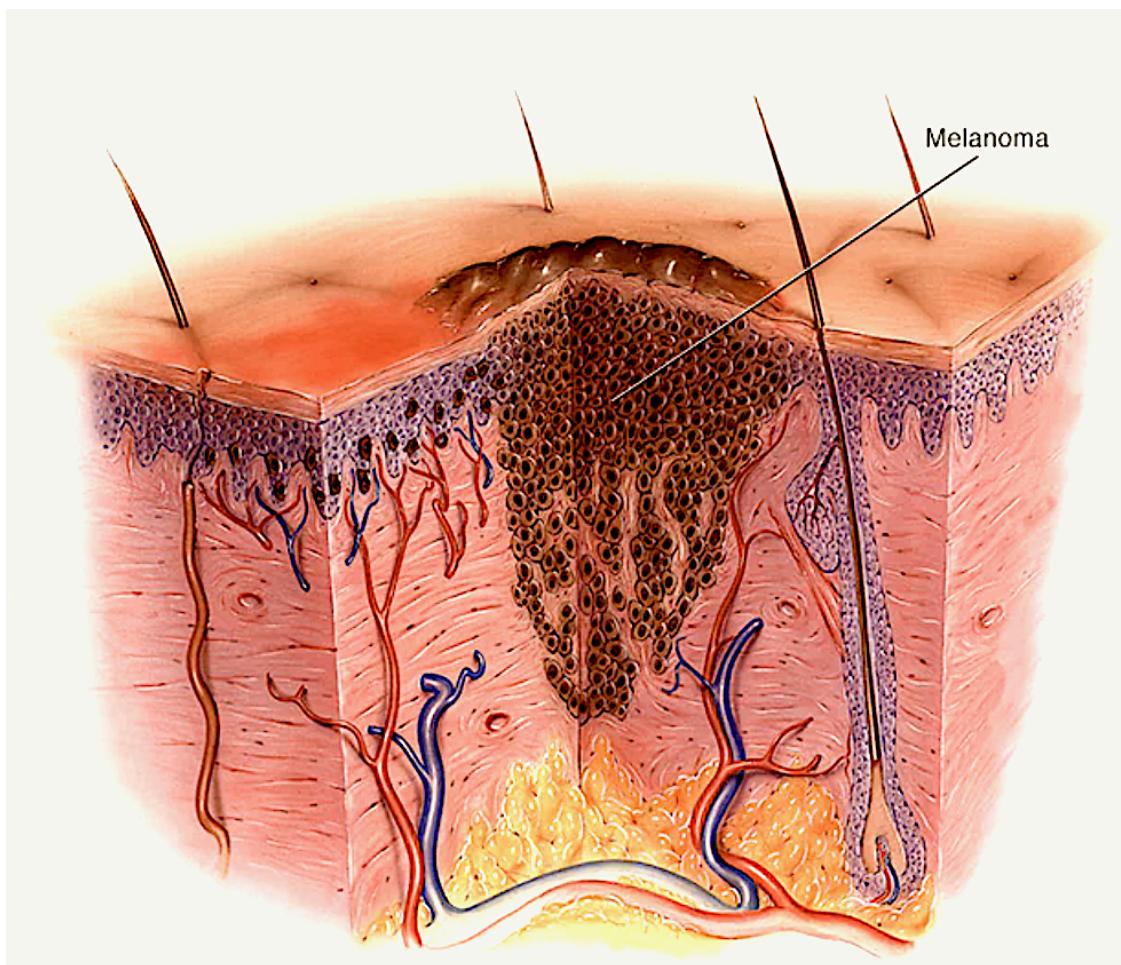


Figure 4: Melanoma , Diseases and Conditons (Source Mayo Clinic, June 2025)

## Melanocyte Development and Key Pathways

The development of Melanocytes from neural crest cells (NCCs) is regulated by a network of growth factors and intracellular signalling pathways<sup>6</sup>.

The most important regulatory molecules are endothelins and stem cell factors. Stem cell factors are ligands for the c-Kit receptor. Other critical growth factors include members of the Wnt protein family and Neuregulin-1 (NRG1).

### Neuregulin-1

Neuregulin-1 (NRG1) is a growth factor that is known for its pleiotropic effects<sup>16</sup>. In addition to its role in melanocyte biology, NRG1 is a key growth factor within the nervous system. It promotes development of Schwann cells, glial cells that form the myelin sheath, while also supporting neuron growth and enhancing synaptic plasticity.

Furthermore, Neuregulin-1 contributes to the repair of the cardiac and vascular tissues and acts via ErbB receptors.

### The Mitogen-Activated Protein Kinase (MAPK) signalling pathway

The Mitogen-Activated Protein Kinase (MAPK) signalling pathway is central to the development of melanocytes<sup>19</sup>. This pathway plays a fundamental role in cell survival, proliferation and differentiation. Signalling pathways are cascades of protein interactions that respond to growth factors to direct the cells.

The MAPK pathway is activated when stem cell factors bind to the c-Kit receptors on the melanoblast cell surface. The binding event initiates a signalling cascade that leads to the activation of extracellular signal-regulated kinases (ERKs). These kinases move into the nucleus and activate gene expression processes required for melanocyte development and melanin biosynthesis.

Understanding how melanocytes normally develop is important for figuring out how melanoma begins and spreads, since problems in these signalling pathways are often involved in the disease process.

## Diagnosis and Imaging Techniques

Magnetic Resonance Imaging (MRI) is the primary diagnostic tool for Melanoma. MRI can be conducted with or without the use of contrast agents to enhance lesion visualisation. In cases where imaging results are inconclusive or suspicious, a biopsy is performed to obtain a tissue sample for histological and molecular examination<sup>17</sup>.

Histopathological analysis typically involves immunohistochemical staining protocols, where tissue samples are incubated in biotin and stained using haematoxylin blue/black nuclear stain and eosin pink cytoplasmic stain to highlight cellular architecture and pathology<sup>17</sup>. To preserve biological integrity for subsequent analyses, the specimen are often cryopreserved.

## Molecular and Genetic Characterisation

The identification of genetic mutations and molecular pathways involved in melanoma is pivotal for both diagnosis and the development of targeted therapies. Several genes are recognised as therapeutic biomarkers and are investigated for the presence of mutations<sup>17</sup>.

Minimal Residual Disease (MRD) relates to the small number of cancer cells that may remain in the body during or after treatment for Melanoma and potentially lead to recurrence. Minimal Residual Disease is typically assessed via liquid biopsy, a minimally invasive technique that analyses circulating tumour DNA (ctDNA) in cerebrospinal fluid, blood or urine.

This ctDNA analysis is referred to as fragmentomics and it uses advanced techniques such as Digital PCR (dPCR) and Droplet Digital PCR (ddPCR) to detect mutations.

Micro-RNAs (microRNA) also play a significant role in melanoma pathogenesis. For example, miR-21 is often up-regulated in patients with Melanoma and breast cancer. These non-coding RNAs are regulators of gene expression and may serve as diagnostic and prognostic biomarkers<sup>17</sup>.

## **Omics Approaches in Melanoma Research**

Comprehensive molecular profiling using omics based technologies has changed the classification and understanding of melanoma<sup>16</sup>. Transcriptomics, which investigates the full range of RNA transcripts expressed in tumour cells, provides insight into the functional state of cancerous tissues<sup>16</sup>. Methylomics, focusing on DNA methylation patterns reveals epigenetic modifications that contribute to the oncogenesis and may serve as early diagnostic indicators. Microarray based transcription profiling is employed for high throughput analysis of gene expression, while cell surface proteomic analysis enables the identification of differentially expressed membrane proteins.

## **Molecular Subtyping and Future Directions**

Molecular subtyping of melanoma has become a cornerstone of personalised oncology. By integrating data from genomics, transcriptomics and proteomics, clinicians and researchers can classify tumours into subtypes that inform prognosis and therapeutic response. This systems biology approach not only enhances diagnostic precision but also opens up avenues for novel targeted treatments<sup>24</sup>. Bioinformatics studies may provide a clearer understanding of the molecular mechanisms behind melanoma metastasis<sup>24</sup>.

Further insights into the overlap between cutaneous and Uveal Melanoma have been gained through bioinformatics approaches which identify shared gene expression signature and signalling pathways<sup>21</sup>. Such comparative analyses deepen the understanding of melanoma heterogeneity and path the way for cross-subtype therapeutic strategies<sup>24</sup>.

# Chapter III : RESEARCH METHODS

## Overview of Research Design

This thesis investigates two biologically distinct but methodologically complementary topics:

- The analysis of gene expression and immune-related pathways in melanoma
- The genomic and structural analysis of virulence genes in *Streptococcus pyogenes*.

Despite being different biological systems, both studies rely on bioinformatics and integrative genomic approaches.

## Topic I: Melanoma --- Gene Expression and Pathway Analysis

### Topic 1: Melanoma Data and Methods

#### Data Sources

- National Centre for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) dataset

The data used in this study were obtained from publicly available databases and include the *Cutaneous Malignant Melanoma* dataset. Specifically, I utilized gene expression data and associated clinical information from tumour samples available through the National Centre for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO).

- Clinical information, GPL96 Affymetrix Microarray Data, gene expression

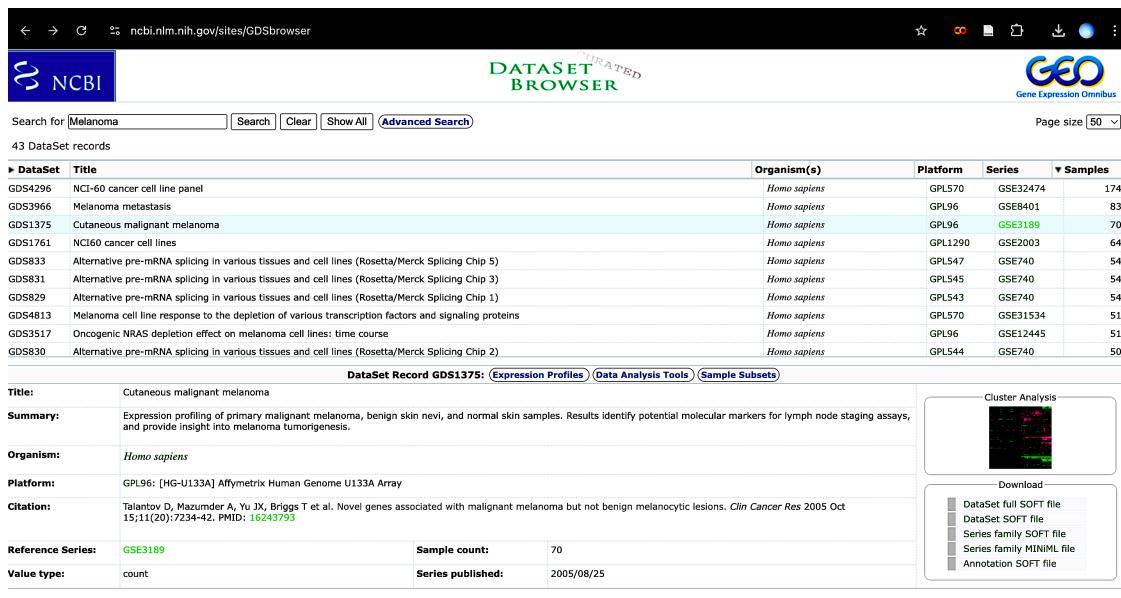


Figure 5: Cutaneous Malignant Melanoma dataset (Source:GDS Browser, 2025)

#### Data Preprocessing

Normalization, filtering, preprocessing tools

## **Analytical Methods**

- Linear Models for Microarray and Omics Data (LIMMA) : Differential Gene Expression
  - Principal Component Analysis
  - KEGG/Pathway analysis
  - GSVA / ssGSEA
  - Tumour Micro-Environment Analysis
- 

# **Topic II: Streptococcus pyogenes --- Virulence Gene & Protein Analysis**

## **Data Sources**

- Public databases (NCBI, etc.)
- Genomic Data Acquisition
- Download of genomic and protein sequence data of multiple *S. pyogenes* strains from NCBI
- Standardized file naming and merging into emm.fasta.
- Include Table of emm genes and strains

## **Data Preparation**

- Renaming FASTA files
- Merging into emm.fasta

## **Analytical Methods**

- MUSCLE alignment of emm genes
- WebLogo motif analysis
- Phylogenetic Tree Construction or comparative genomics
- Use aligned sequences to infer phylogeny and strain relationships.
- Evaluate whether emm-type clusters correlate with virulence gene presence.
- Functional Enrichment (GO/KEGG)
- Use DAVID or GSEA to perform enrichment analysis for virulence-related genes.
- Gene/Protein Interaction Network
  - Construct virulence gene networks using STRING or Cytoscape.
  - Identify hub genes/modules.
- Protein Structure and Docking
  - Use Swiss-Model and PyMOL to model M protein structures. -Perform docking simulations to assess receptor interactions.
- Comparative Genomics and Analysis of Virulence Factors
  - Create virulence factor heatmaps, assess emm-type distribution across strains

## Chapter IV: RESULTS AND ANALYSIS

### RESULTS - Topic I: Melanoma

#### Overview of the Dataset

The dataset selected for this study is the *Cutaneous Malignant Melanoma* dataset, identified by GEO Data Set (GDS) accession number **GDS1375** and series number **GSE3189**, comprised of a total of 70 samples. This dataset was made publicly available on the 25th day of August in 2005. It consists of microarray gene expression profiles from three distinct sample groups: 45 malignant melanoma samples, 18 benign skin nevus samples and 7 normal skin samples.

For the purposes of this analysis, the SOFT file was downloaded and both the phenotype data and gene expression counts were extracted and processed. From the full dataset, a subset was selected for differential analysis, comprising of 21 malignant melanoma samples and 14 skin nevus samples. The 7 normal skin samples were excluded from this comparative analysis.

The screenshot shows the NCBI GEO Accession Display page for Series GSE3189. At the top, there are search and navigation links. Below the header, the series information is displayed:

Series	GSE3189
Status	Public on Aug 25, 2005
Title	Novel genes associated with malignant melanoma but not benign melanocytic lesions
Organism	Homo sapiens
Experiment type	Expression profiling by array
Summary	A number of melanoma specific genes were identified to differentiate clinical relevant tissue samples containing benign from malignant melanocytes. Keywords: disease state analysis
Overall design	7 normal skin, 18 nevi and 45 melanoma samples
Citation(s)	Talantov D, Mazumder A, Yu JX, Briggs T et al. Novel genes associated with malignant melanoma but not benign melanocytic lesions. <i>Clin Cancer Res</i> 2005 Oct 15;11(20):7234-42. PMID: 16243793

Below this, there is a section titled "Analyze with GEO2R" which lists contact information and organization details. At the bottom, it shows the platforms used and the number of samples.

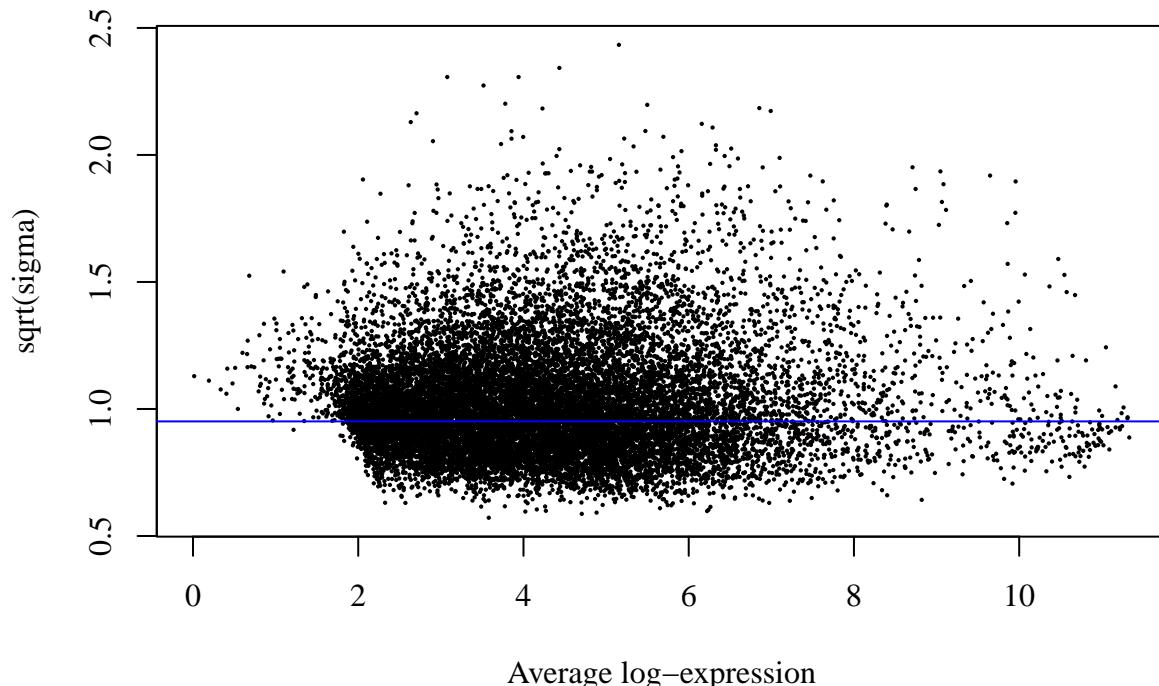
Platforms (1)	GPL96 [HG-U133A] Affymetrix Human Genome U133A Array
Samples (70)	GSM71671 430MM GSM71672 425MM ... More...

Figure 6: Selection of GSE3189 Series Data (Source:GDS Browser, 2025)

## Differential Gene Expression

Mean-variance trend

**Mean variance trend, GSE3189**



This is a diagnostic plot where each point represents a gene. The x-axis shows the average log2-counts per million (logCPM) for each gene. The y-axis shows the estimated variance (or square root of standard deviation) of the log2 expression values for each gene. The trend line is plotted over the points to estimate the mean-variance relationship in the dataset. The mean-variance trend shows a relatively stable variance across expression levels, indicating the normalization and statistical modelling are appropriate for differential expression analysis.

Table 1: (Table of Top 20 Significantly Up-regulated Genes)

Gene Symbol	Log FC	Ave Expr	P-Value	Adj P-Value
NTRK3	5.574	5.693	0.0000129	0.0001071
WFDC1	5.222	4.888	0.0000264	0.0001952
HEY1	5.047	4.436	0.0000000	0.0000000
GDF15	4.964	5.666	0.0000000	0.0000000
NTRK3	4.946	5.498	0.0001644	0.0009026
PHACTR1	4.654	4.232	0.0000001	0.0000024
NTRK3	4.639	5.913	0.0000029	0.0000314
SPP1	4.363	5.332	0.0000000	0.0000004
ABHD2	4.228	4.999	0.0000002	0.0000035
NTRK3	3.960	6.619	0.0000000	0.0000011
KIF23	3.849	2.064	0.0001005	0.0005954
MIR6872 /// SEMA3B	3.819	3.665	0.0000005	0.0000077
RNFT2	3.800	1.402	0.0000763	0.0004709
NTRK3	3.792	6.718	0.0000001	0.0000016
RASGRF1	3.698	1.962	0.0044561	0.0144427
NTRK3	3.668	6.624	0.0000001	0.0000014
UBE2S	3.557	5.534	0.0000000	0.0000003
ATP6V0E2	3.540	6.915	0.0000000	0.0000000
BCL2A1	3.531	4.655	0.0000000	0.0000000
ARHGAP8 /// PRR5-ARHGAP8	3.522	2.658	0.0147619	0.0385832

Table 2: (Table of Top 20 Significantly Down-regulated Genes)

Gene Symbol	Log FC	Ave Expr	P-Value	Adj P-Value
KRT15	-8.013	3.912	0.0000000	0.0000000
KRT14	-7.298	6.514	0.0000000	0.0000000
LOR	-6.931	5.048	0.0000000	0.0000000
LGALS7 /// LGALS7B	-6.740	5.159	0.0000000	0.0000000
KRT1	-6.686	6.289	0.0000000	0.0000000
SERPINB5	-6.510	3.593	0.0000000	0.0000000
PKP1	-6.493	3.489	0.0000002	0.0000028
KRT5	-6.399	6.329	0.0000000	0.0000000
LY6D	-6.246	3.688	0.0000000	0.0000000
S100A7	-6.224	4.062	0.0000008	0.0000109
FGFR3	-6.213	3.892	0.0000002	0.0000035
HOPX	-6.075	4.480	0.0000000	0.0000000
AQP3	-5.998	5.094	0.0000000	0.0000000
SFN	-5.965	3.973	0.0000000	0.0000010
TACSTD2	-5.873	5.161	0.0000000	0.0000000
PPL	-5.805	3.711	0.0000000	0.0000000
SPRR1A	-5.713	2.635	0.0031335	0.0107551
CHL1	-5.709	3.169	0.0000000	0.0000000
DSP	-5.691	5.536	0.0000000	0.0000000
FLG	-5.622	4.183	0.0000002	0.0000032

## Volcano Plot

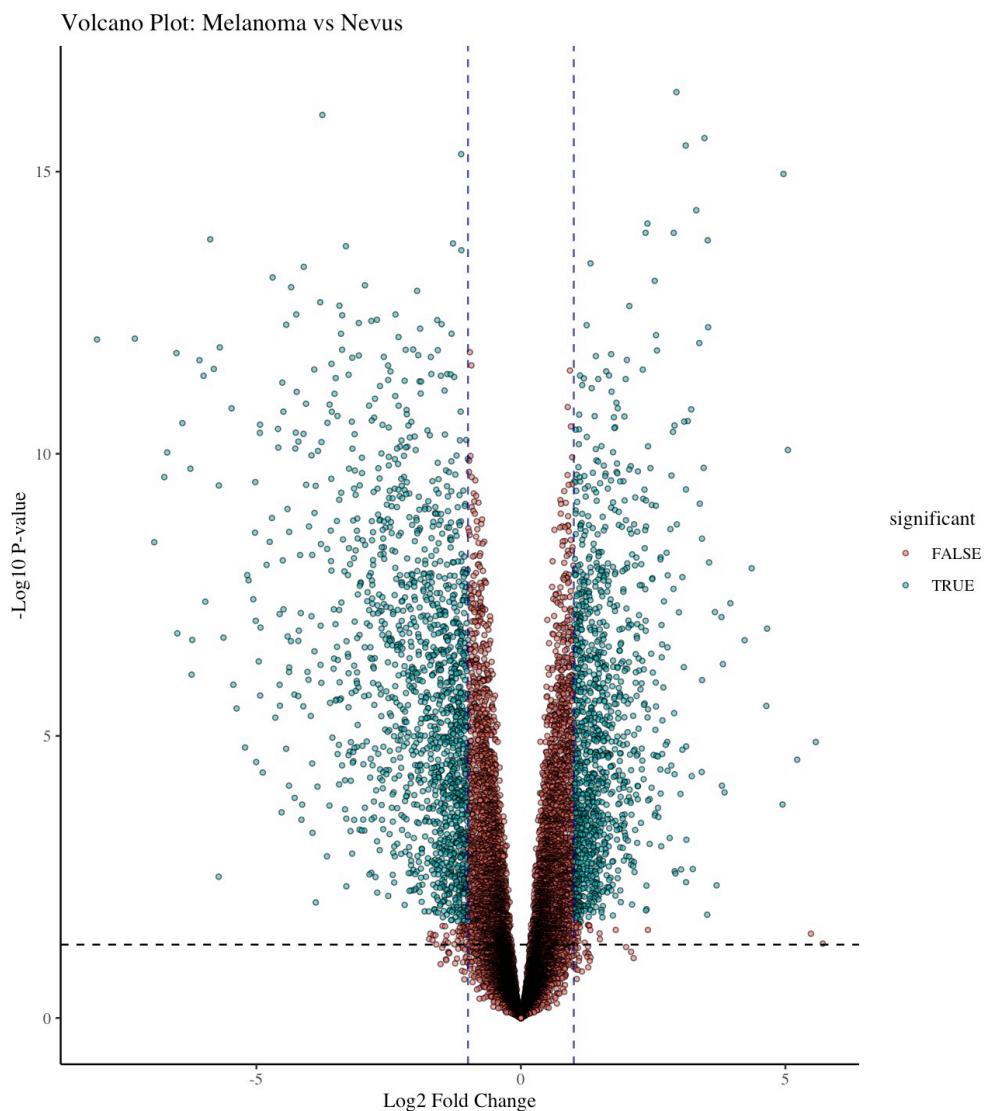


Figure 7: Volcano Plot I

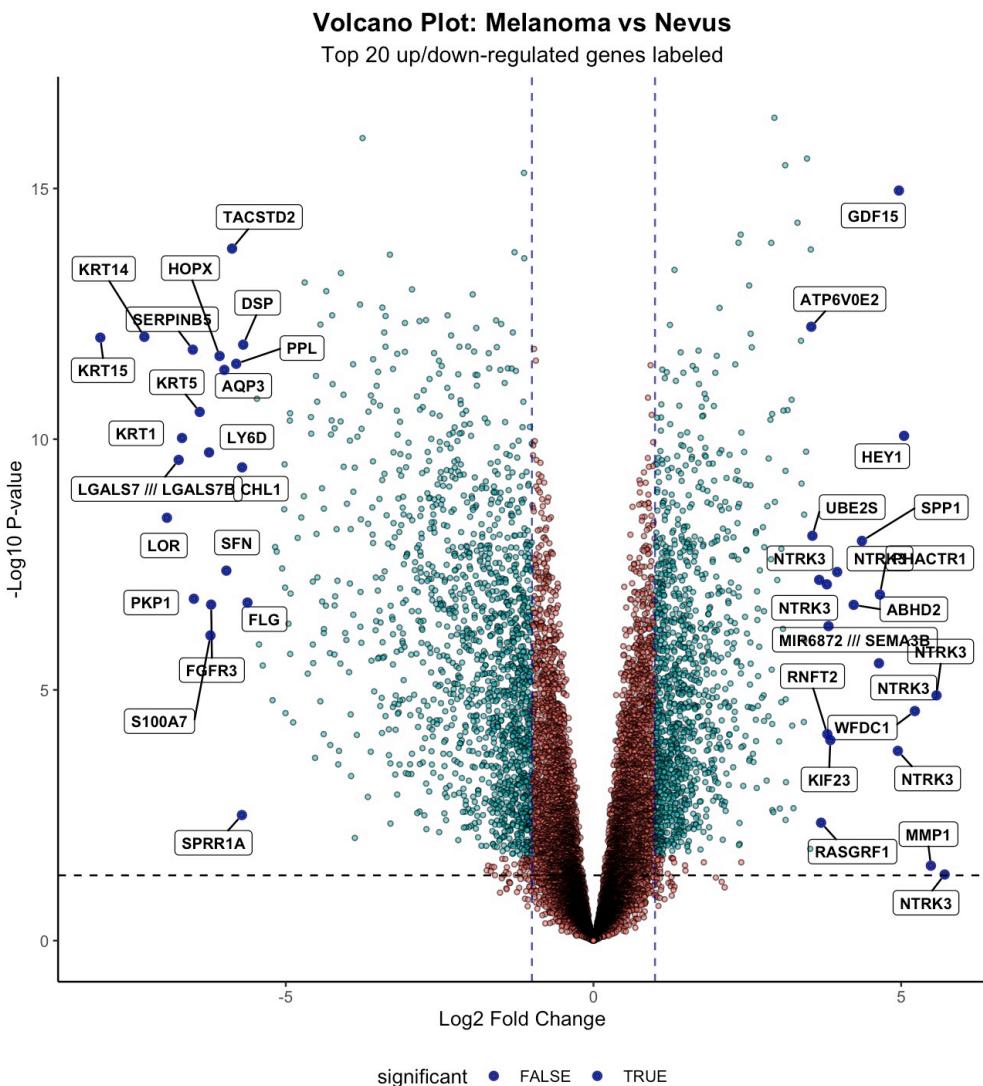


Figure 8: Volcano Plot II

## MA Plot

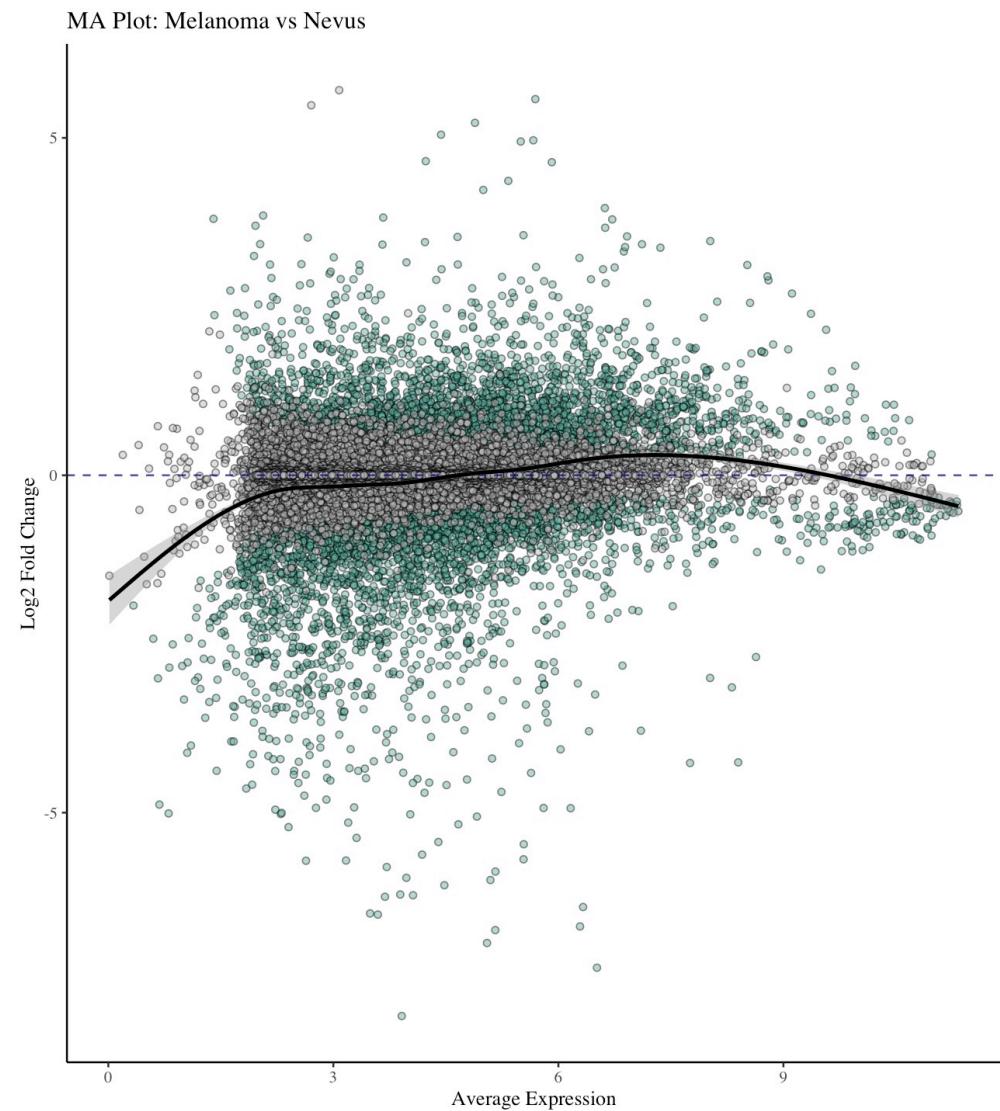


Figure 9: MA Plot

## Principal Component Analysis (PCA) & Clustering

### Sample Correlation Heatmap

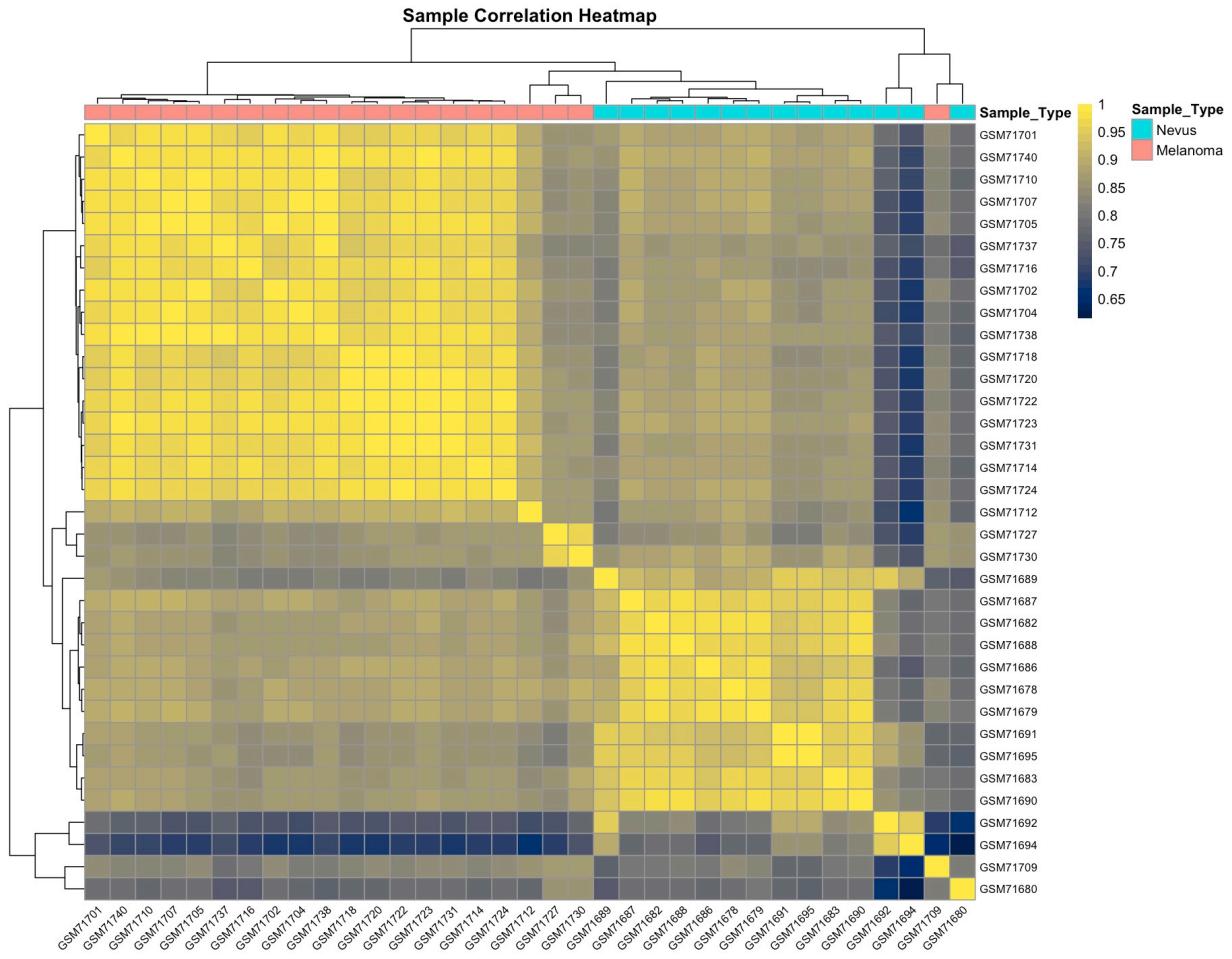


Figure 10: Sample Correlation Heatmap

This heatmap shows the pairwise correlations between the samples in the dataset. Yellow signifies a strong correlation and blue signifies no correlation. The rows and columns represent the same set of samples. Samples are hierarchically clustered based on their correlation. Similar samples are grouped together. However, there is 1 melanoma sample that is clustering with the nevi skin samples.

### Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a dimensionality reduction technique used to simplify complex datasets by transforming correlated variables into a smaller set of uncorrelated variables called principal components.

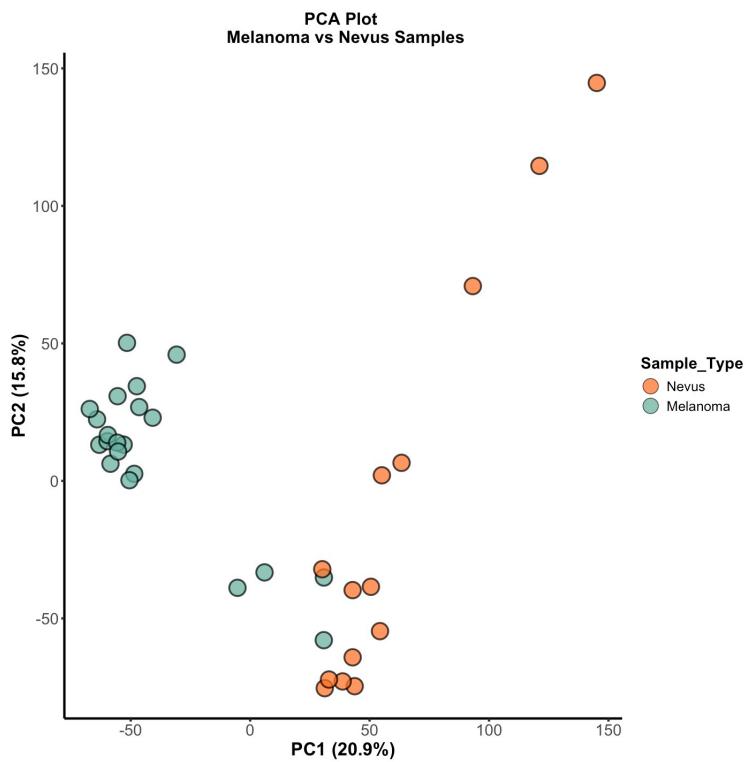


Figure 11: Principal Component Analysis (PCA)

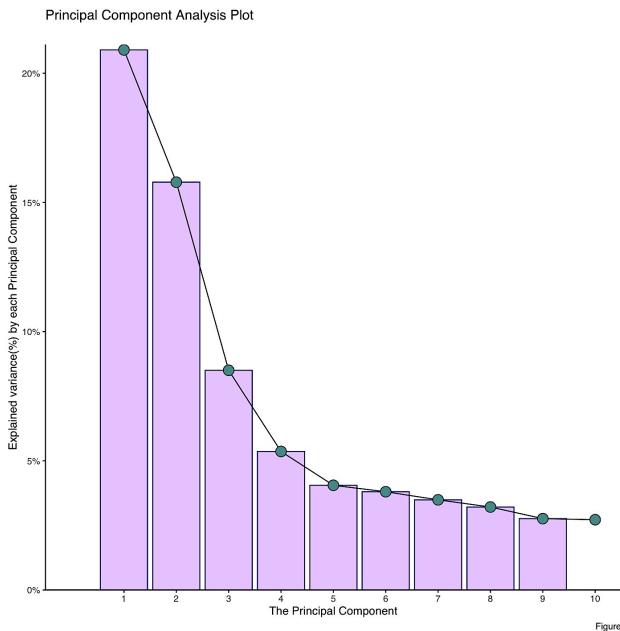


Figure 12: Scree Plot

## Uniform Manifold Approximation and Projection (UMAP)

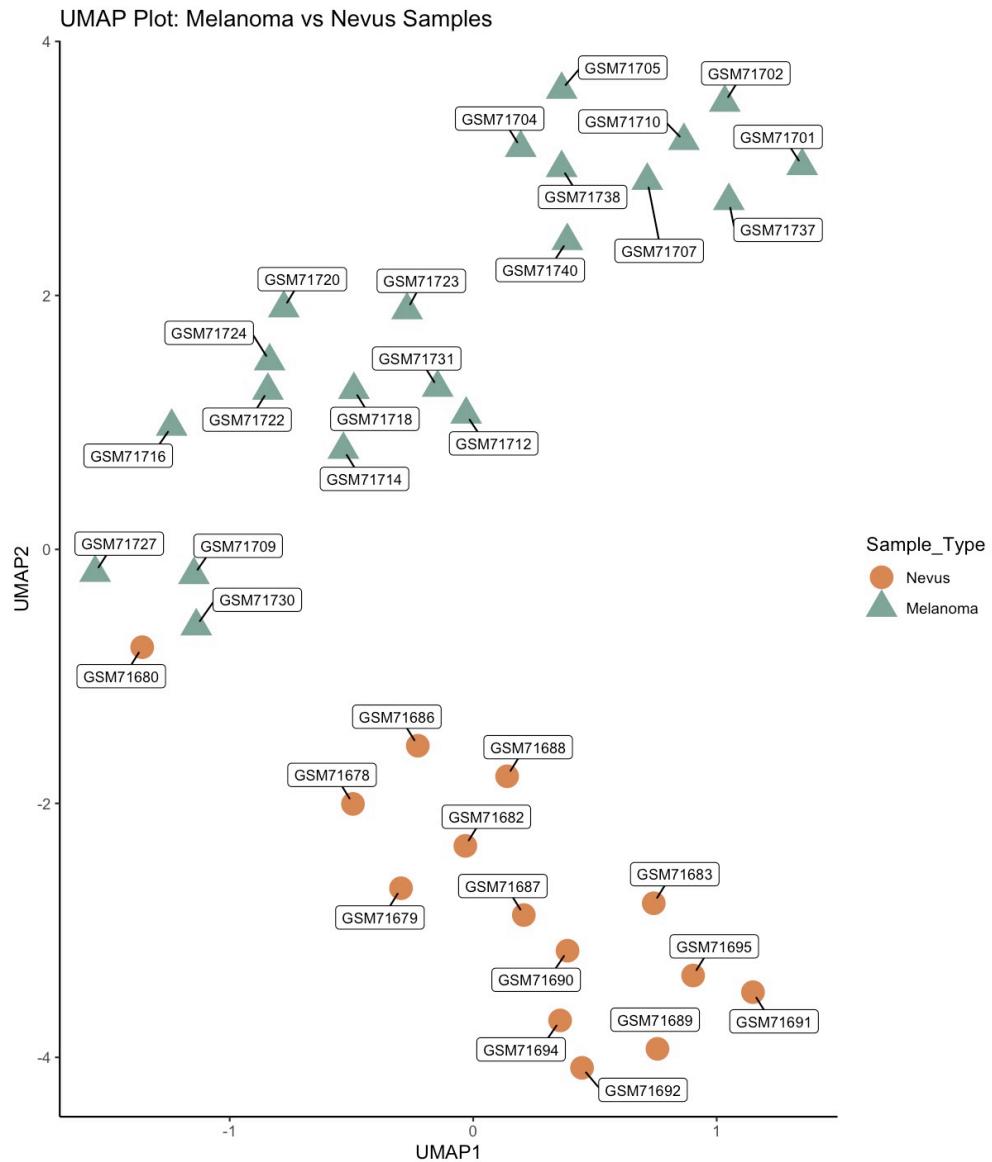


Figure 13: Uniform Manifold Approximation and Projection

## Pathway Analysis

KEGG / GO enrichment KEGG pathways identified to look at genes in melanoma samples Biological Process and Pathway Analysis

The clusterProfiler package was used for pathway analysis.

### Top GO Biological Processes

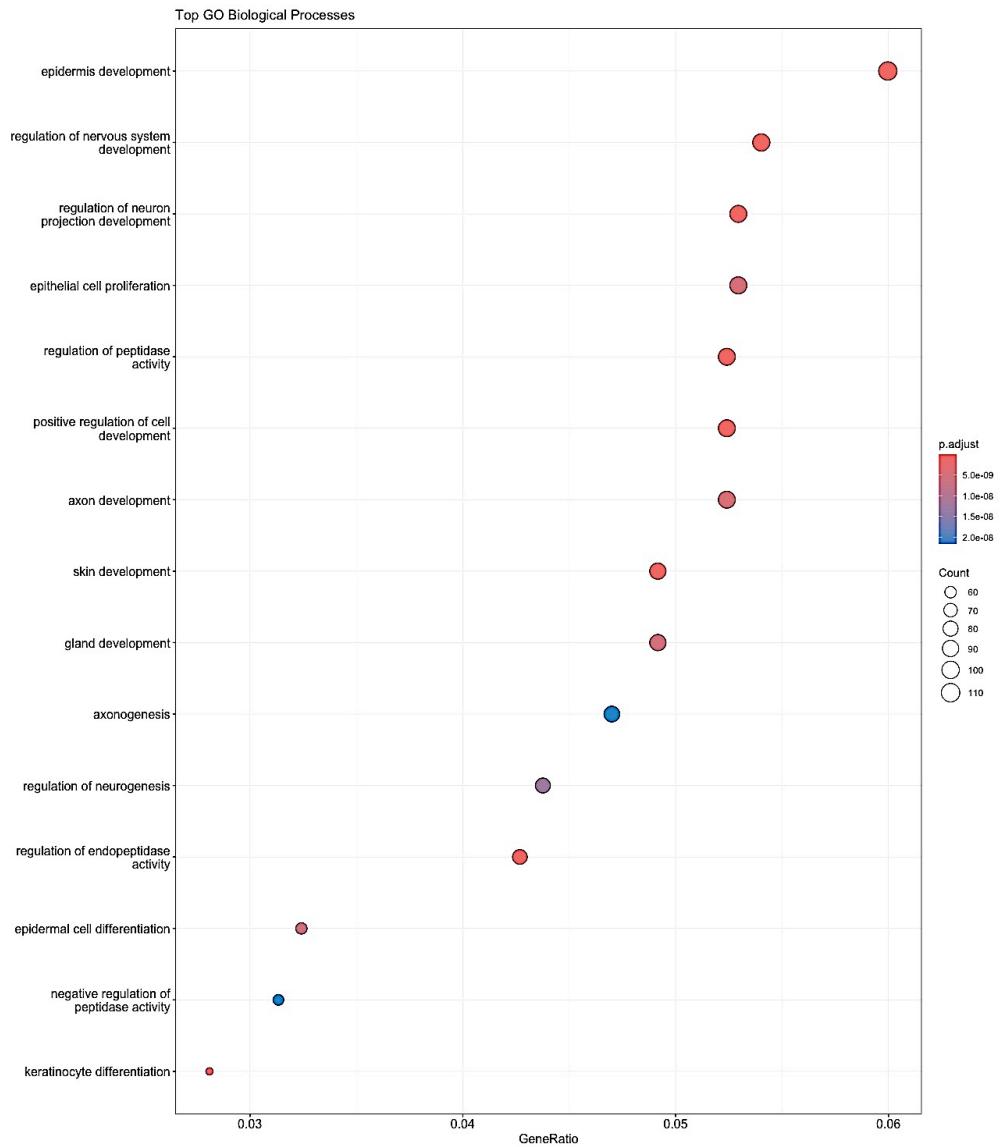


Figure 14: Gene Ontology Biological Processes

## Top KEGG Pathways

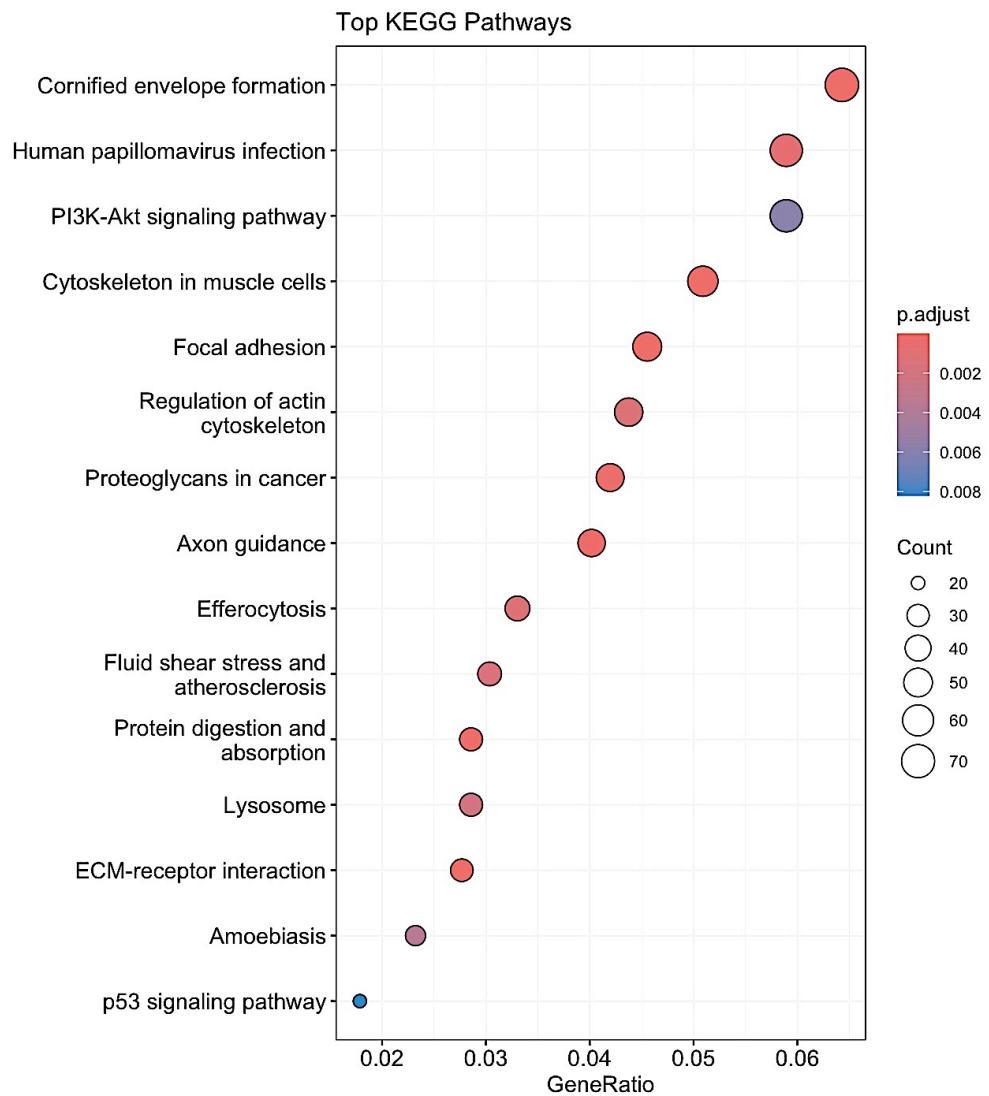


Figure 15: KEGG Pathways

## Gene Signature Enrichment

- GSVA / ssGSEA results
- GSVA to perform ssGSEA analysis on signature genes with GSVA Package.

Table 3: (ssGSEA Enrichment Summary)

Gene Set	Mean Difference (Mel-Nevus)	P-value	Adjusted P-value	Significance
Melanoma Upreg Top20	0.524	1.51e-14	4.53e-14	Higher enrichment in Melanoma
Melanoma Downreg Top20	-0.7301	1.12e-10	1.68e-10	Lower enrichment in Melanoma
All Top DE Genes	-0.0661	1.08e-02	1.08e-02	Lower enrichment in Melanoma (small diff)

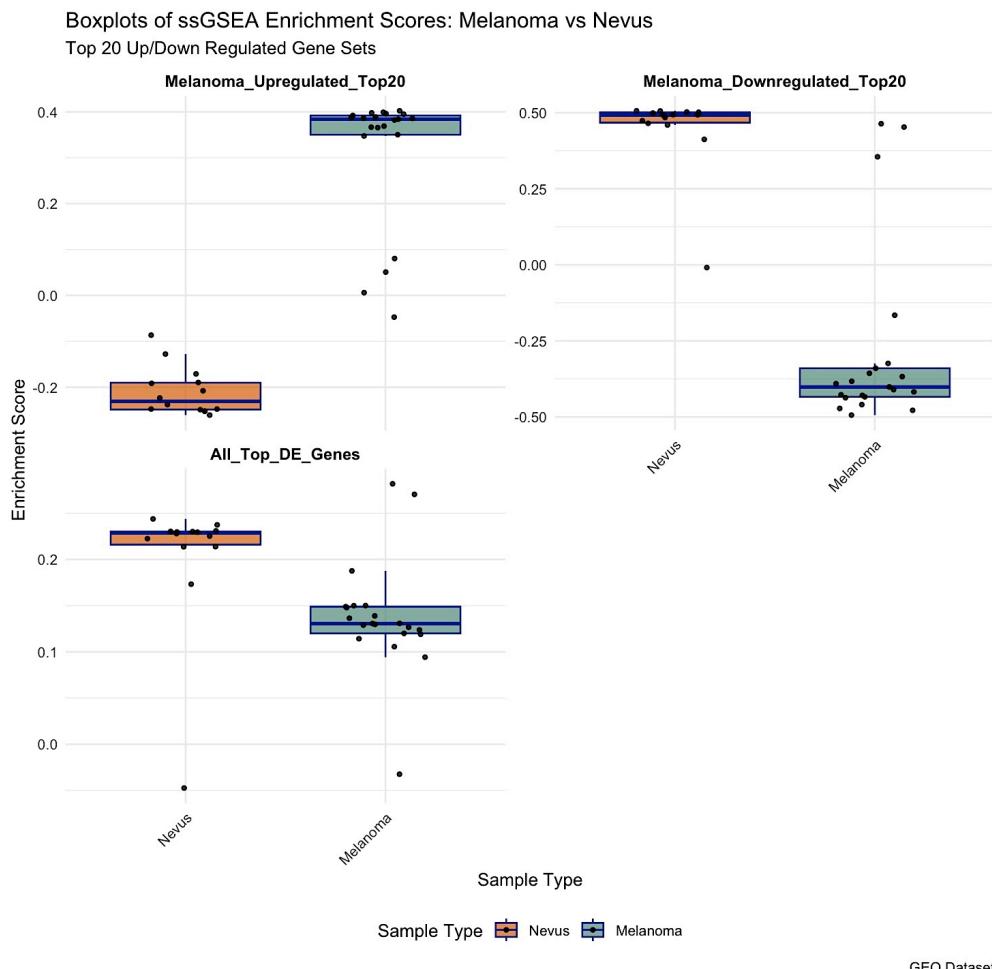


Figure 16: ssGSEA Enrichment Scores

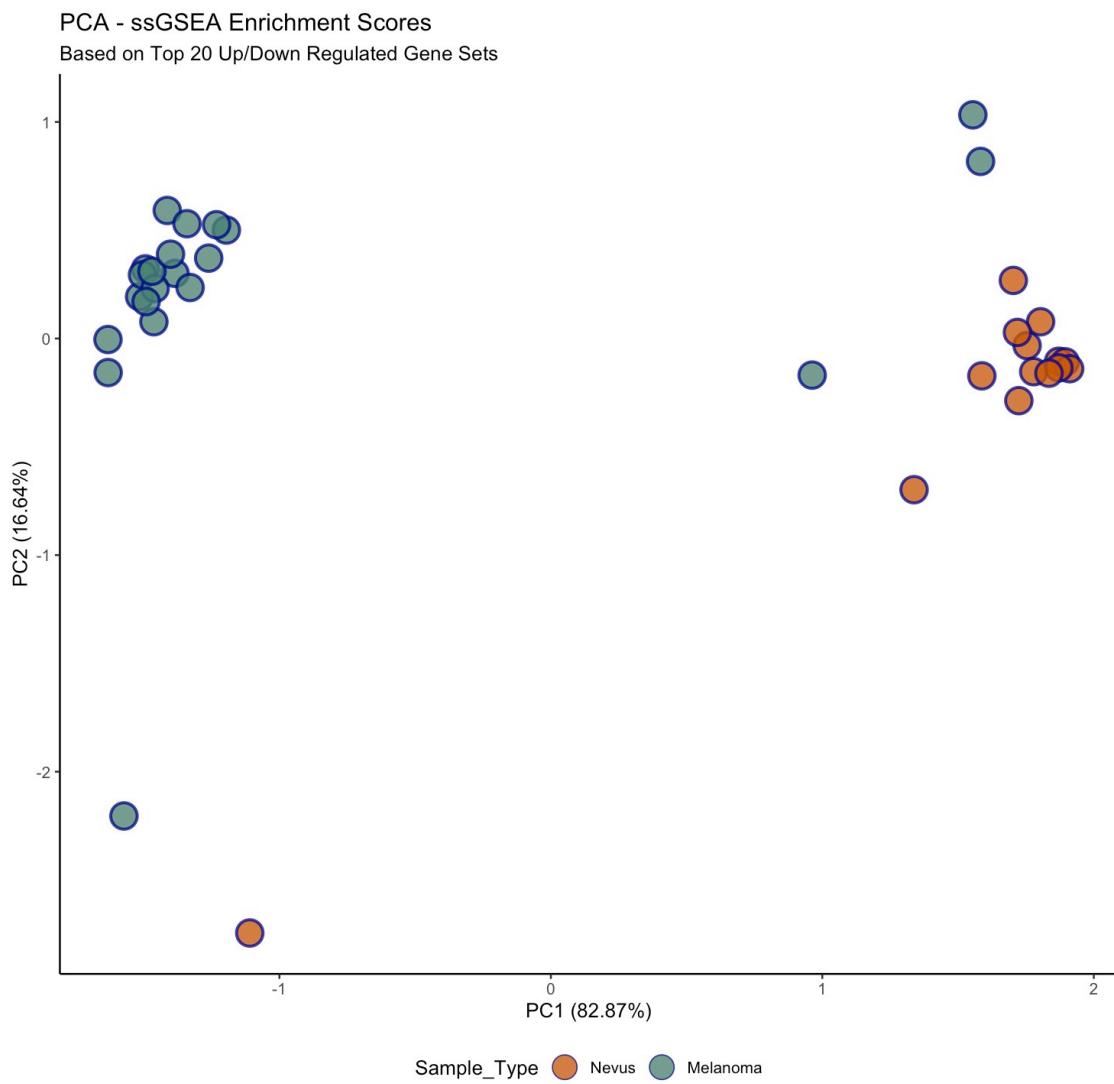


Figure 17: PCA from GSVA

## Tumor Purity Estimation for Melanoma vs Nevus Samples

Table 4: (ESTIMATE Score Comparison: Melanoma vs Nevus)

Score	Melanoma_Mean	Nevus_Mean	P_Value
StromalScore	-602.188	-486.441	0.6347
ImmuneScore	-302.864	-505.272	0.5063
ESTIMATEScore	-905.052	-991.713	0.8710
TumorPurity	0.869	0.879	0.8334

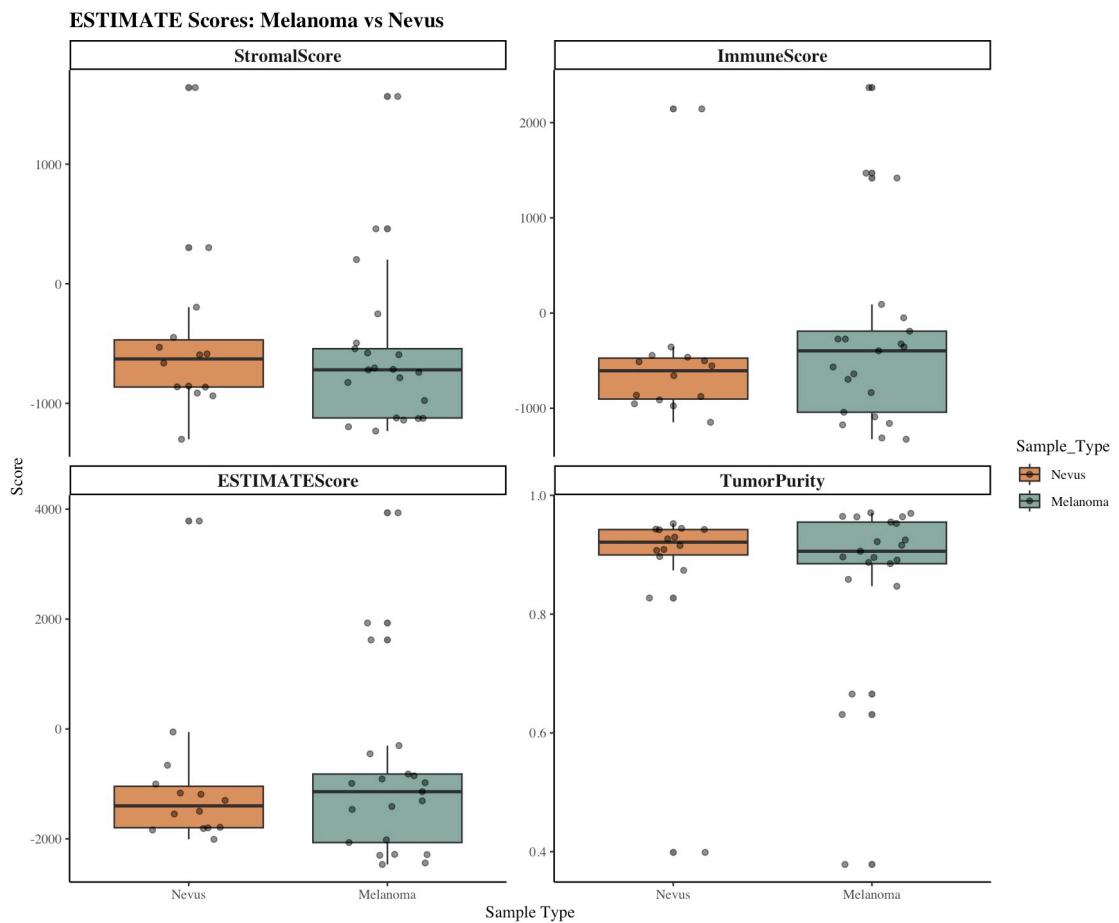


Figure 18: ESTIMATE Scores

(\*\*\*not this part yet 11.15 )

## RESULTS -- Topic II: *Streptococcus pyogenes*

### Sequence Dataset Overview

Summary table of GAS strains and emm genes.

Total number of strains, collection dates, accession numbers.

Table 5: (Table of *Streptococcus pyogenes* genomes)

Name.	Accession Number	Strain	Collection Date	Link
S. Pyogenes	AE014074.1	MGAS315	31-JAN-2014	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AE014074.1">https://www.ncbi.nlm.nih.gov/nuccore/AE014074.1</a>
S. Pyogenes	CP000017.2	MGAS5005	01-APR-2014	<a href="https://www.ncbi.nlm.nih.gov/nuccore/CP000017.2">https://www.ncbi.nlm.nih.gov/nuccore/CP000017.2</a>
S. Pyogenes	CP155740.1	1851/03	06-AUG-2024	<a href="https://www.ncbi.nlm.nih.gov/nuccore/CP155740.1">https://www.ncbi.nlm.nih.gov/nuccore/CP155740.1</a>

Table 6: (Table of *Streptococcus pyogenes* emm genes) *Streptococcus pyogenes* emm gene for M protein, complete cds of various strains – Collection Date: 15-JAN-2014

Accession Number	Gene Name	Strain	Link
AB548437.1	emm1 gene for M protein	RE014	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548437.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548437.1</a>
AB548438.1	emm28 gene for M protein	RE015	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548438.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548438.1</a>
AB548441.1	emm1 gene for M protein	RE020	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548441.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548441.1</a>
AB548442.1	emm1 gene for M protein	RE025	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548442.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548442.1</a>
AB548444.1	emm28 gene for M protein	RE031	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548444.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548444.1</a>
AB548445.1	emm1 gene for M protein	RE032	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548445.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548445.1</a>
AB548446.1	emm49 gene for M protein	RE037	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548446.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548446.1</a>
AB548447.1	emm49 gene for M protein	RE039	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548447.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548447.1</a>
AB548448.1	emm28 gene for M protein	RE041	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548448.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548448.1</a>
AB548449.1	emm89 gene for M protein	RE050	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548449.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548449.1</a>
AB548450.1	emm1 gene for M protein	RE059	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548450.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548450.1</a>
AB548451.1	emm12 gene for M protein	RE066	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548451.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548451.1</a>
AB548452.1	emm49 gene for M protein	RE076	<a href="https://www.ncbi.nlm.nih.gov/nuccore/AB548452.1">https://www.ncbi.nlm.nih.gov/nuccore/AB548452.1</a>

Accession Number	Gene Name	Strain	Link
AB548453.1	emm49 gene for M protein	RE080	https://www.ncbi.nlm.nih.gov/nuccore/AB548453.1
AB548454.1	emm49 gene for M protein	RE104	https://www.ncbi.nlm.nih.gov/nuccore/AB548454.1
AB548456.1	emm49 gene for M protein	RE121	https://www.ncbi.nlm.nih.gov/nuccore/AB548456.1
AB548503.1	emm4 gene for M protein	RE342	https://www.ncbi.nlm.nih.gov/nuccore/AB548503.1
AB548508.1	emm12 gene for M protein	RE366	https://www.ncbi.nlm.nih.gov/nuccore/AB548508.1
AB548516.1	emm75 gene for M protein	RE436	https://www.ncbi.nlm.nih.gov/nuccore/AB548516.1
AB549960.1	emm58 gene for M protein	RE614	https://www.ncbi.nlm.nih.gov/nuccore/AB549960.1

Table 7: (Outgroup *Streptococcus pyogenes* emm50 type - emm gene for M protein, partial cds. of various strains – Collection Date: 26-JUN-2013

Accession Number	Gene Name	Strain	Link
JX028641.1	emm gene, emm50 type	GLS244	https://www.ncbi.nlm.nih.gov/nuccore/JX028641

### Multiple Sequence Alignment and Motif Identification

A multiple alignment of emm genes was constructed using MUSCLE software.

```
muscle -in emm.fasta -out emm_aligned.fasta
```

Alignment log (MUSCLE summary table).

Table: (Summary of MUSCLE Alignment Log for emm Genes Isolated from *Streptococcus pyogenes*)

MUSCLE v3.8.1551 by Robert C. Edgar | <http://www.drive5.com/muscle> This software is donated to the public domain. Please cite: Edgar, R.C. Nucleic Acids Res 32(5), 1792-97.

Time	Memory	Iteration	Progress	Step
00:00:00	1 MB(0%)	1	100.00%	K-mer dist pass 1
00:00:00	1 MB(0%)	1	100.00%	K-mer dist pass 2
00:00:00	22 MB(0%)	1	100.00%	Align node
00:00:00	22 MB(0%)	1	100.00%	Root alignment
00:00:00	24 MB(0%)	2	100.00%	Refine tree
00:00:00	24 MB(0%)	2	100.00%	Root alignment
00:00:00	24 MB(0%)	2	100.00%	Root alignment
00:00:01	24 MB(0%)	3	100.00%	Refine biparts
00:00:03	24 MB(0%)	4	100.00%	Refine biparts
00:00:03	24 MB(0%)	5	100.00%	Refine biparts
00:00:03	24 MB(0%)	5	100.00%	Refine biparts

## **WebLogo output --- identify conserved and variable regions.**

Interpretation: conserved motifs likely under selective pressure; variable ones may contribute to immune evasion.

### **4.2.3 Phylogenetic Analysis**

Phylogenetic tree showing clustering by emm type.

Interpretation: Strain evolution reflects functional divergence.

### **4.2.4 Virulence Gene Profiles and Comparative Genomics**

Heatmaps of virulence gene presence/absence.

Association between emm type and gene content.

### **4.2.5 Functional Enrichment (GO/KEGG)**

Tables and graphs of enriched pathways for virulence genes.

Immune response and adhesion-related terms highlighted.

### **4.2.6 Protein-Protein Interaction Networks**

Network plots from Cytoscape.

Interpretation: central hubs as potential drug targets.

### **4.2.7 M Protein Structure and Docking**

PyMOL screenshots and docking affinity results.

Interpretation: key conserved residues in binding interfaces.

## **4.3 Integrated Interpretations**

Comparative reflection on results:

Immune evasion is a key mechanism in both melanoma and GAS.

Multi-omic data can guide precision medicine or vaccine development.

## **4.4 Implications and Future Directions**

Melanoma: prognostic gene sets, TME insights.

GAS: M protein as vaccine candidate; deeper exploration of strain diversity. Summarise what you think is an important find or contribution. What you do about the problem you have identified.

### **Future work:**

experimental validation, longitudinal data, more strains/tumours.

---

# **CHAPTER V**

## **CONCLUSION**

### **4.1 Conclusion**

The conclusion is a brief presentation of what has been obtained from the discussion. Summarises the key findings and their importance offering final thoughts on the research.

### **4.2 Limitations**

The limitations of the study describe the weaknesses and shortcomings found after analysis and interpretations of the results.

### **4.3 Suggestion**

Suggestions for future research.

## Appendices

Supplementary material, such as raw data, questionnaires or additional charts that are relevant to the thesis but not critical in the main sections.

## Bibliography

Lists all the sources cited in the thesis in a consistent format.

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