Psoriasis:

A Study of the Skin Transcriptome and Microbiome

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This thesis is dedicated to my parents Yalcin and Sabahat Tanes and my sister Zeynep Tanes.

Their encouragements and unconditional support has kept me going.

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Abstract

Psoriasis: A Study of the Skin Transcriptome and Microbiome

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Psoriasis is a complex autoimmune skin disorder characterized by dry, scaly plaques and painful flares. Even though genetic contribution and environmental factors are suspected, the exact trigger of psoriasis is not well understood. The chronic condition of the disease and the lack of effective and definitive treatments are burdens on the patients. Recent emergence of transcriptome and genomic datasets for the host, as well as the taxonomic datasets for the microbiome has enabled the use of bioinformatics approaches to investigate altered gene circuits in psoriasis.

As a first step, open source microarray datasets of psoriasis were analyzed in context of other skin conditions. The analysis showed that upregulated genes in the psoriasis transcriptome included those involved in epidermal differentiation complex and antimicrobial processes, while the top downregulated genes were involved in lipid metabolism. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways that were enriched with significantly altered genes point to the upregulation of both innate and adaptive immune responses. The psoriasis gene signature was distinctive from other inflammatory skin conditions and it resembled the wound healing process in terms of keratinization and immune response signals.

On the microbiome side, over-abundance of opportunistic bacteria on the psoriasis microbiome was observed compared to controls. Virulence genes were consistently in high abundance across different body sites. Bacterial invasion of epithelial cells gene pathway was crowded with both significantly altered genes on the host side and high-abundance orthologs on the microbiome side. The findings suggested bacterial involvement in the initiation or maintenance of psoriasis flares.

Genetic components also play a role in susceptibility to psoriasis. Human Leukocyte Antigen (HLA) is one of the regions that has previously been associated with psoriasis through Genome Wide Association studies. The Single Nucleotide Polymorphisms (SNPs) typed in the HapMap dataset (11 ethnic populations) within the HLA region have been analyzed using extended haplotype homozygosity based tests to identify positive selection on polymorphisms that have not yet reached fixation. Results showed regional specificity of positive selection signals on the sub-classes of HLA. The positive selection signals in Class I sub-region showed European ancestry specificity with intronic SNPs on a psoriasis related gene PSORS1C1 as well as on TCF19, MUC22, TRIM10, and TRIM15. The region specific selection signals were also seen in the Class III region for the East Asian populations and in the Class II region for African ancestry populations. Similar to single population tests, the cross population tests showed that the significant SNPs were concentrated in the Class II region for African ancestry populations, whereas for European ancestry populations, they were concentrated in the Class I region. The results show how positive selection of a SNP can encourage genetic hitchhiking of the susceptibility SNPs for a disease along with a SNP that is under positive selection.

This research thesis bridges large scale transcriptome datasets of the host and operational taxonomy unit abundance datasets of the microbiome, opening up new avenues for drug repositioning studies by pointing out specific host-microbiome genes as drug targets.

Introduction

Motivation

This research is dedicated to investigating psoriasis as a complex disease through gene expression, microbiome, and genetic susceptibility analysis. Although the literature contains psoriasis research using these approaches individually, the bridge between the three perspectives is still lacking. There is hence a need to integrate between multiple microarray datasets to investigate psoriasis in the broad spectrum of skin conditions and bridging them with microbiome functional perturbations. The combination of the results from the two analyses can provide a comprehensive perspective of the cross-talk between host and the microbiome. Furthermore the genomic aspect of psoriasis can be identified by positive selection regions in the highly disease and psoriasis associated HLA region.

Psoriasis has an extensive number of open source datasets available for meta-analysis, however the datasets that have been curated for this study for both microarray and microbiome will continue to expand over time. Therefore, the methodology and approach described in this thesis will have the ability to incorporate new datasets and study other autoimmune conditions as they become available in the future.

Background Information

Skin Disorders and Psoriasis

Autoimmune disorders currently affect 5% of the Western population and are on the rise, with more than seventy subtypes [1]. Current drugs on the market prescribed to manage autoimmune diseases are of the anti-inflammatory type used primarily to subdue the symptoms and does not provide complete treatment. These disorders are hypothesized to arise when the immune system reacts to self, in addition to non-self antigens through the relaxation of negative selection for T-cells and B-cells. This results in the accumulation of clones that recognize major histocompatibility complex (MHC) molecules. The resulting inflammation damages and can eventually destroy the affected tissues [2]. In our lab, inflammatory bowel disease has been extensively studied using transcriptome data to provide a deeper understanding of autoimmune disease mechanisms [3].

The skin is an organ commonly affected by autoimmune conditions, either as the primary target such as psoriasis, atopic dermatitis, and allergic contact dermatitis, or through secondary manifestations such as dermatomyositis or sarcoidosis. Due to their chronic nature, they have negative effects on the quality of life of the patient in terms of economic burden, pain management, social discrimination, and mental status [4, 5].

Among the skin related autoimmune conditions, psoriasis has a 1.4 – 3% prevalence across Europe and the United States [6]. It is a chronic inflammatory condition in which the keratinocytes proliferate and differentiate resulting in skin thickening [7]. Even though the exact trigger for psoriasis is not fully understood, genetic disposition [8] and environmental conditions

[9] are known risk factors. Psoriatic patients (approximately 70%) have a peak onset between the ages of 16-22 [10]. The symptoms for psoriasis can be alleviated with anti-inflammatory drugs, topical treatments, and dietary regimen [11, 12]. Psoriasis treatment adherence is challenging due to concerns regarding long-term safety and drug efficacy [6]. The chronic nature of the disease calls for life-long maintenance and high healthcare cost [13]. Approximately 20% of patients with psoriasis are also diagnosed with psoriatic arthritis within a decade of initial diagnosis [6]. Comorbidities including cardiovascular conditions [14, 15], psoriatic arthritis [16], type II diabetes [17], and inflammatory bowel disease [18] cause additional long term physical and economical strain for the patient [19].

In order to study psoriasis, high throughput technologies are required to understand the complex and multi-level nature of the disease. Microarray technologies that quantify the amount of mRNA in the samples are used to observe gene expression changes in the hyperproliferating keratinocytes in the context of other skin conditions. SNPchip microarrays are used to detect the polymorphisms in different populations. The genotypes can be utilized to better understand the susceptibility differences observed across populations. Finally, high throughput sequencing technologies are useful in confirming suspected microbiome contributions to the psoriasis phenotype. Understanding the pathology of psoriasis through transcriptomic and genomic datasets will aid in creating better, more effective treatments to increase patient quality of life and decrease associated life-long economic burdens.

DNA Microarrays

DNA microarrays are high throughput technologies to detect the relative concentration of nucleic acid sequences that are extracted from a sample of interest. In DNA microarrays, short

nucleic acid sequences, called probes, are bound to a platform. They rely on DNA base pairing between the probes and the tested sequences. Relative concentrations are calculated from fluorescent labeling techniques. This technology has been used for manufacturing high throughput gene expression, transcription binding, and genotyping platforms [20].

Gene expression levels in a sample are detected using mRNA microarrays. One of the major companies that produce microarrays is Affymetrix. Their core technology identifies and synthesizes species specific 25 nucleotide length oligonucleotide sequences onto the microarray platform surface. The RNA segments extracted from the samples of interest are processed and labeled with fluorescent markers. The microarray platform is washed with the extracted sequences to promote hybridization and fluorescence intensities are subsequently quantified by confocal microscopy. The intensities correlate directly to corresponding gene expression levels [20].

The single nucleotide polymorphism genotyping platforms manufactured by Affymetrix rely on a similar method where pairs of oligonucleotides are used to represent the alleles on the genome. Each pair of oligonucleotides differ at only one position in the middle of the sequence that represent the two alleles of a SNP. The DNA is extracted from the samples, labeled with fluorescent markers, and hybridized onto the platform synthesized with the oligonucleotides. The image that is captured from the fluorescent labeling can then determine if the sample of interest is homozygous in one of the two alleles or heterozygous.

Both the expression and genotyping arrays have been utilized in this research to understand the psoriasis phenotype. The expression arrays have been utilized to detect mRNA perturbations in

psoriasis skin. Genotype data from the HapMap project [21] have been used to discover the genetic contribution to the susceptibility differences observed in psoriasis.

Sequencing Technologies

Since the draft of the human genome in 2001, sequencing technologies have improved to provide more sequence reads per run at a significantly lower cost. This has paved the way for large scale efforts such as the 1000 Genomes Project [22] and Human Microbiome Project [23]. Through these efforts, genomes of bacteria and model organisms have been decoded. Besides genomic characterization of organisms in their healthy state, sequencing technologies have also made it possible to depict disease states. Various projects have been funded such as decoding the genome of cancer patients [24] and sequencing the microbiome communities of patients with autoimmune disorders [25]. Whereas microarrays can only test for what they are designed to detect, sequencing allows for the detection of less annotated regions of the genome and can therefore be used to detect novel biomarkers [26].

Current sequencing technologies follow the same essential steps with slight variations depending on the manufacturer. First, adapters are ligated at the ends of the DNA fragments to be sequenced. The fragments are then amplified on a solid surface such as a glass slide or a microbead by a polymerase mediated process. The nucleotides that are added are detected automatically using fluorescence. The detection procedure varies for different technologies and ranges from measuring bioluminescent signals to four-color imaging of single molecular events [26, 27].

One application of sequencing technologies is for detecting the bacterial composition of an environment by sequencing 16S ribosomal RNA. 16S rRNA is a component of the ribosome that is present in every organism. It is made up of conserved regions that can be selectively targeted during sequencing, but it is also composed of variable regions ideal for differentiating between organisms. Depending on the capabilities of the technology used, sequencing one or more of the variable regions makes it possible to record the bacterial composition of an environment when mapped against a 16S library. To date 16S sequencing has been utilized to categorize bacteria into their appropriate taxonomy using sequence similarity instead of phenotypic traits. It has also been used to identify novel bacterial species and diagnose culture negative infections [28].

Specific Aim I: Analyzing Psoriasis Transcriptome

The primary aim of this chapter is to uncover statistically significant gene expression alterations in psoriasis lesions compared to uninvolved skin and healthy controls. My hypothesis is that the meta-analysis of multiple psoriasis microarray datasets will aid in generating a robust gene expression signature for psoriasis. Psoriasis will likely share gene expression markers with other inflammatory skin conditions, while having a set of distinct genes that can be used as biomarkers. Higher level functional annotations of skin conditions will provide similarities between the conditions pointing to common inflammatory responses in the skin conditions of interest.

Introduction

Psoriasis is a complex inflammatory condition characterized by keratinocyte hyperproliferation, epidermal differentiation, and immune cell infiltration [7]. It has also been defined as aberrant wound healing due to uncontrolled thickening of the epidermis [29]. To understand the disease as a whole, a systemic approach using microarray expression analysis of psoriasis lesions in context with other skin conditions is necessary.

Various microarray studies have been conducted to investigate the lesional and non-lesional psoriasis expression profiles compared to healthy controls. Inflammatory signatures altered in psoriasis lesions have been identified such as TNF-alpha, IFN-gamma signaling [30], and Th17 immune response [31]. Among the genes that have psoriasis associated SNPs positioned on

them, the highest expression was seen in neutrophils, signifying a genetic component for the inflammatory response [32]. Meta-analysis of the psoriasis microarray datasets revealed that the gene signature is most consistent within the same microarray platform [33, 34]. The studies selected in this chapter were hybridized onto the widely used and comprehensive Affymetrix HGU133+2 microarray platform. The results were later compared to a study hybridized onto PIQOR platform for validation.

Since psoriasis only effects humans and primates [35], animal models have not been insightful on the full spectrum of the disease. Comparing microarray gene signatures of human psoriasis biopsies and mouse disease models showed that even though there are expression similarities between the progression of the disease in terms of keratinization and epidermal differentiation, each mouse model had slight variations on psoriasis inflammatory signatures [36]. In general, there have been conflicting studies on the correlation of mouse and human inflammatory signatures [37, 38]. In order to evaluate the most genuine representation of psoriasis flares, this chapter will only focus on the expression profiles of human skin biopsies.

Psoriasis and atopic dermatitis are the two most common autoimmune skin conditions. Small inflammatory expression differences could be observed between psoriasis and atopic dermatitis [39]. Meta-analysis of psoriasis, atopic dermatitis, nickel allergy, and acne transcriptome signatures showed a unifying gene signature that was T cell mediated but with different sets of chemokines upregulated for each condition [40, 41]. For example, while psoriasis was Th17 mediated, atopic dermatitis was Th2 mediated. However they both had neutrophil chemoattractant expressions in common [39]. The results were further confirmed with a microarray study including five inflammatory skin conditions where IL-17 and TNF-alpha signals were exclusively predominant in psoriasis lesions [42]. Among the comparative studies in

literature, a microarray meta-analysis of multiple skin conditions is needed to reach a unifying conclusion about the commonalities and differences between psoriasis and other skin conditions.

Wound healing is significantly accelerated in patients with psoriasis compared to healthy controls [43]. The two conditions also show similarities with respect to increased keratinocyte proliferation and differentiation. During wound healing, chemoattractants are released to regulate neutrophils and IL-8 is essential in re-epithelialization signaling [44]. Due to their similarities, when gene targets and biomarkers are selected for psoriasis, it is crucial to analyze wound healing transcriptome alongside of inflammatory skin conditions to differentiate between which altered genes are beneficial for the organism to heal wounds and the genes that result in an aberrant wound healing response such as psoriasis.

Merkel cell carcinoma is an aggressive cutaneous cancer with mortality rate greater than 30%. It has been observed that 75% of Merkel Cell carcinomas contain Merkel cell polyomavirus. Clonal integration of the virus to the host cells implicates viral origins of the cancer [45]. Psoriasis and atopic dermatitis are autoimmune conditions that have suspected bacterial involvements [46]. Therefore the Merkel Cell carcinoma dataset was included in the analysis even though the pathology of the disease varies from autoimmune definition.

Since the development of microarray technologies, sequencing has become cheaper and available, making way for high-throughput complementary DNA sequencing (RNA-seq) as an alternative to microarray studies. RNA-seq study by Bingshan et al. has expanded the psoriasis transcriptome, especially in the immune system process [47]. Even though RNA-seq data would provide a more sensitive analysis of the expression profiles of skin conditions, not enough

studies have been performed to carry out a meta-analysis. Therefore, in order to put psoriasis in context of other skin conditions, microarray datasets have been utilized as they provide a rich source of data.

Overall, this chapter focuses on the meta-analysis of psoriasis microarray datases in context of other inflammatory conditions (atopic dermatitis and allergic contact dermatitis), an aggressive skin cancer with suspected viral origins and natural wound healing process making it the most comprehensive analysis of psoriasis transcriptome in literature.

Methods

Microarray Datasets

NCBI Gene Expression Omnibus (GEO) [48] was queried with the search term "skin" and "GPL570" for skin biopsy microarray datasets hybridized onto Affymetrix Human Genome U133 Plus 2.0 GeneChip Array. This platform is commonly used and is a comprehensive gene chip representing almost 19,000 genes. The datasets (before January 1st 2014) obtained were further narrowed down by selecting skin condition studies which include at least five skin biopsy samples, each from at least two of the flare-up (A), uninvolved skin (U), and healthy control (C) categories. The search criteria yielded 14 datasets as summarized in Table 1. From these datasets, only the untreated samples have been included in the study. The datasets were processed using robust multiarray averaging (RMA) background adjustment and quantile normalization [49]. The resulting probe expression data were mapped onto Entrez Gene IDs using the custom chip description file (cdf) [50] using median polishing [49].

The microarray dataset GSE63741 hybridized onto PIQOR Skin 2.0 platform was also included in the analysis due to its extensive coverage of inflammatory skin conditions [42]. The dataset represented 30 samples each from healthy controls, as well as psoriasis, atopic dermatitis, and allergic contact dermatitis patients. In this chapter the dataset has been used to compare the robustness of expression profiles across platforms as well as validate the gene signatures obtained for each skin condition. The normalized expression values for the dataset were downloaded from NCBI GEO [48] for further processing.

Gene Signatures of the Host

For the Affymetrix datasets, gene signatures were obtained for the following comparisons: active involved versus active uninvolved samples (A/U), active involved versus healthy controls (A/C), and active uninvolved versus healthy controls (U/C). Lists of significantly upregulated and downregulated genes were obtained for each comparison using (a) Significance Analysis of Microarray (SAM) [51] and (b) Rank Product (RP) method [52]. Significant gene lists determined from the analyses had a q-value cutoff of 0.01 and fold change cutoff above 1.5. The intersection of the SAM and RP tests were considered as the gene signature for a given comparison in a single dataset. A consensus gene list was obtained with r^{th} ordered p-value meta-analysis method [53] over multiple datasets by finding the genes that are significant in more than half of the datasets.

GSE #	Condition	Publication	C	U	A	Age	Treatment Criteria for Eligibility	Severity	Biopsy diameter
14905	Psoriasis	Yao *et al.,* 2008	21	28	33	-	-	moderate to severe	-
13355	Plaque Psoriasis	Swidell *et al.*, 2009	64	58	58	21-69 (PS) 18-45 (C)	No systemic treatments 2 weeks prior, no topical treatments 1 week prior to study	≥1% of body effected	6 mm
34248	Plaque Psoriasis	Bigler *et al.*, 2013	0	14	14	19-55	No treatment	moderate to severe ≥10% of body effected PASI ≥10	5 mm
41663	Plaque Psoriasis	Bigler *et al.*, 2013	0	15	15	19-55	No treatment	moderate to severe ≥10% of body effected PASI ≥10	-
41662	Plaque Psoriasis	Bigler *et al.*, 2013	0	24	24	19-55	No treatment	moderate to severe ≥10% of body effected PASI ≥10	6 mm
30999	Plaque Psoriasis	Suarez-Farinaz *et al*, 2012.	0	85	85	-	No systemic treatments 4 weeks prior, no topical treatments 2 weeks, no biological agent 3 months prior to study	moderate to severe ≥10% of body effected	4 mm
63741	Plaque Psoriasis	D'Erme et al., 2015	30	0	30	-	No treatment	-	-
32924	Atopic dermatitis	Suarez-Farinaz *et al.*, 2011	8	12	13	16-81	No treatment 4 weeks prior to study	SCORAD 28-97.5 11-63% of body effected	-
36842	Atopic dermatitis	Gittler *et al.*, 2012	15	8	16	20-67	No treatment 4 weeks prior to study	SCORAD 40-63	-
27887	Atopic dermatitis	Tintle *et al.*, 2011	0	8	9	24-51	Patients allowed to use emollients	SCORAD 28-97.5	4-6 mm

16161	Atopic dermatitis	Guttman-Yassky *et al*, 2009.	9	0	9	28-54 (AD) 24-69 (C)	No treatment 4 weeks prior to study	SCORAD 20-70	-
63741	Atopic dermatitis	D'Erme et al., 2015	30	0	30	-	No treatment	-	-
6281	Allergic contact dermatitis	Pedersen *et al.*, 2007	16	9	9	33-49 (ACD) 31-55 (C)	No immunosuppressants during study	At least 2+ reaction to 5% nickel sulfate	4 mm
63741	Allergic contact dermatitis	D'Erme et al., 2015	30	0	30	-	No treatment	-	-
39612[a]	Merkel cell carcinoma	Harms *et al.*, 2013	64	16	19	59-88	-	Stage I-III	-
28914[b]	Wound healing	Nuutila *et al.*, 2012	6	6	5	20-75	NA	NA	3 mm

Table 2 Summary of skin condition transcriptome datasets obtained from NCBI GEO database obtained before January 1st 2014. The numbers of control (C), active uninvolved (U) and active lesion (A) skin biopsy samples are presented for each dataset. Only the skin biopsies are included in this study. Inclusion criteria such as age, severity of the condition and treatment status are also included. The samples are hybridized onto Affymetrix HGU133+2 platform with the exception of GSE63741 dataset which is hybridized onto PIQOR Skin 2.0 Microarray. [a] For the Merkel cell carcinoma dataset, U stands for tumor biopsies that tested negative for MCPyV and A stands for tumor biopsies that tested positive for MCPyV. [b] For the wound healing dataset, C stands for acute wound; U stands for biopsy at day 3 of healing process; and A stands for biopsy at day 7 of healing process.

The average rank of a significant gene for a given comparison was calculated based on the SAM fold changes in order to find the set of consistently upregulated and downregulated genes across multiple datasets of a condition. The top 25 genes from the significantly upregulated and downregulated psoriasis ranked sets were used as biomarker genes and their functional memberships were further characterized. The fold changes were clustered using hierarchical clustering to determine if the genes can be used to distinguish psoriasis from other skin conditions.

The expression values of the disease samples hybridized onto PIQOR platform were compared against the control samples using SAM and RP methods in order to get significantly altered genes with a Benjamini cutoff of 0.01 and fold change cutoff of 1.5. The overlap of the two methods composed the disease profiles for the PIQOR platform, consistent with the steps taken to analyze Affymetrix datasets.

Functional Analysis of Gene Signatures

The pair wise gene signature intersections were quantified with Soerensen coefficient ($SC=2\|A\cap B\|/(\|A\|+\|B\|)$, where A and B are two separate sets) [54] to test the similarity between different conditions as well as the consistency between the datasets of the same condition. The Bioconductor GOstats package [55] was used for statistical enrichment of Gene Ontology Biological Process (GO-BP) [56] and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [57] in consensus signatures. The p-values were corrected with the Benjamini-Hocheberg method [58] to account for false discovery. The representative condition enrichments were obtained by assigning the smallest identified p-value from the three comparisons (A/C, A/U, U/C). KEGG pathways were mapped into their BRITE categories [59],

which provided a hierarchical classification of pathways. The same enrichment steps were taken to perform functional annotation of the PIQOR significant gene lists. Significantly enriched KEGG pathways were annotated with virulence factors using the Virulence Factors Database (VfDB) [60, 61], while the viral and bacterial proteins that are known to bind with the human proteins were identified using Pathogen-Host Interaction Search Tool (PHISTO) [62].

Signature Comparison between Psoriasis and Wound Healing

Due to the observed expression similarities between psoriasis lesions and wound healing process, the gene signatures of both conditions were further examined. The consensus A/C and A/U gene signature of psoriasis were overlapped with reconstructive (A/C) and inflammatory (U/C) gene signatures of wound healing process. The comparison of gene signatures were grouped as follows: reconstructive stage of wound healing genes overlapped with any psoriasis genes, inflammatory stage of wound healing genes overlapped with any psoriasis genes, common inflammatory and reconstructive stages of wound healing genes overlapped with any psoriasis genes, wound healing specific genes and psoriasis specific genes. The top five GO-BP Level 5 processes enriched were found. The gene signatures have also been projected onto the cytokine-cytokine receptor interaction KEGG pathway in order to visualize the differences in immune function in both processes.

Signature Comparison between Affymetrix and PIQOR Platforms

The gene signatures obtained for psoriasis, atopic dermatitis and allergic contact dermatitis were compared between Affymetrix and PIQOR platforms using hypergeometric test to evaluate the robustness of gene signatures across microarray platforms. For the test, only the genes that

are represented on both platforms have been considered. The KEGG, GO-BP and GO-MF enrichments (Benjamini < 0.01) of the gene signatures obtained from both platforms were also compared.

Results

Fourteen publicly available microarray datasets were analyzed for the host consisting of 977 samples (Table 1). Each skin condition included samples from healthy controls (C), active uninvolved (U), and active involved (A) stages. Transcriptome datasets for five different skin conditions were utilized: psoriasis (PS) [19, 30, 34, 36], atopic dermatitis (AD) [63-66], allergic contact dermatitis (ACD) [67], Merkel cell carcinoma (MCC) [68], and wound healing (WND) [69].

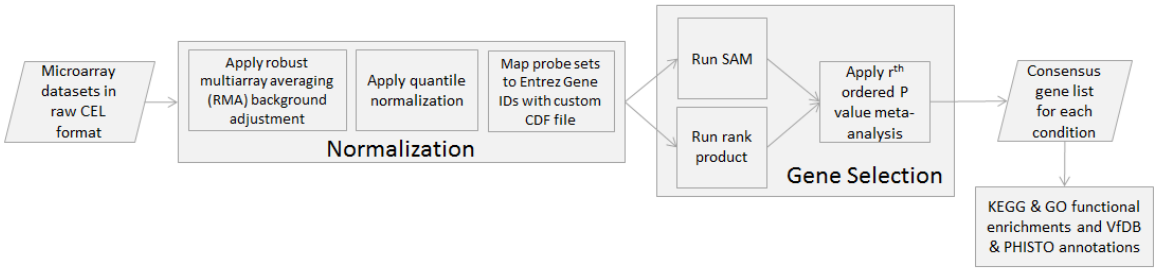

Figure 1 Flowchart of microarray analysis steps. The raw microarray samples are downloaded and normalized. Significant genes are obtained through statistical analysis and processed though functional annotation datasets.

Host Gene Signatures for Skin Diseases

The microarray datasets shown in Table 1 were normalized and analyzed as described in Methods and summarized in Figure 1. The numbers of significant genes obtained for each microarray dataset, as well as the consensus signatures over all datasets for a given disease are shown in Table 2. Psoriasis signatures were highly similar across the six datasets yielding 801 consensus upregulated and 578 downregulated genes in active/control (A/C) comparison among the 18,959 genes on the microarray chip. MCC had the largest significant gene list, a common feature of signatures associated with cancer [70].

		A/C		A/U		U/C	
Condition	Dataset	UP	DWN	UP	DWN	UP	DWN
PS	GSE14905	1376	1317	1101	936	182	244
	GSE13355	1008	880	929	666	11	7
	GSE34248			956	1081		
	GSE41663			1074	1266		
	GSE41662			1359	1584		
	GSE30999			1265	1187		
	consensus	**801**	**578**	**907**	**836**	**4**	**1**
	GSE63741	197	169				
AD	GSE32924	705	514	0	0	217	272
	GSE36842	2280	2175	0	0	1217	1513
	GSE27887			0	0		
	GSE16161	759	720				
	consensus	**644**	**551**	**0**	**0**	**66**	**165**
	GSE63741	104	72				
ACD	GSE6281	790	1103	311	106	219	379
	GSE63741	168	139				
MCC	GSE39612	2592	2873	538	566	2594	3108
WND	GSE28914	505	394	4	1	659	405

Table 2 Gene signatures obtained from host microarray dataset comparisons. A: Active, U: Uninvolved, C: Control; UP: upregulated; DWN: downregulated. The numbers in the table indicate the number of genes with significantly altered expression in a comparison. The numbers of consensus genes for multiple Affymetrix datasets in the same comparison are shown in bold. All the datasets except for GSE63741 have been hybridized onto Affymetrix HGU 133 Plus 2 chip. Grey boxes indicate comparisons for which transcriptome data did not exist.

Similarity of gene signatures across datasets was considered by calculating the Soerensen coefficient (SC) [54] for the top 200 most significant genes from sets of pairwise gene signatures. Heatmap diagrams in Figure 2 point to high levels of similarity between the six psoriasis datasets in A/U and A/C comparisons. Averaging over the six datasets, SC was equal to 0.65 for upregulated and 0.45 for downregulated genes. Approximately half of the top 200 genes for psoriasis and for process of wound healing were the same. In contrast, atopic dermatitis gene signatures for A/C and U/C comparisons were more heterogeneous. Moreover there was little overlap between gene signatures of allergic contact dermatitis and Merkel cell carcinoma.

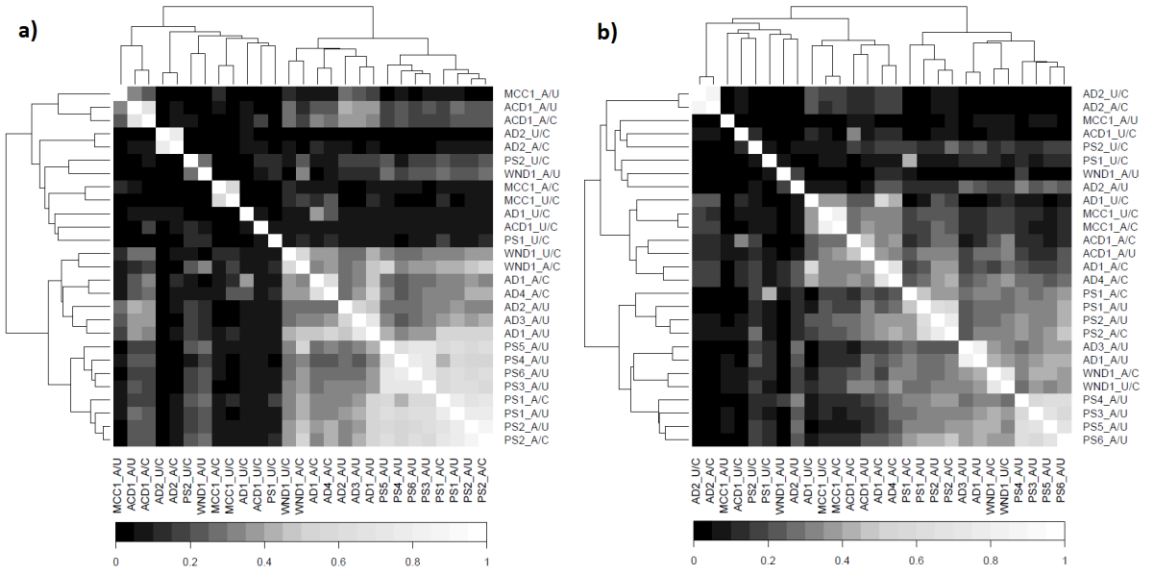

Figure 2 Soerensen Coefficient for Pairs of Comparisons. Soerensen similarity coefficient calculated for the a) 200 upregulated and b) 200 downregulated genes with the highest fold change for each pair of gene signatures. Coefficient ranges in value from 0 (no overlap, shown in black) to 1 (perfect overlap, shown in white). The conditions under study are as follows: psoriasis (PS), atopic dermatitis (AD), allergic contact dermatitis (ACD), Merkel cell carcinoma (MCC) and wound healing (WND). A: Active involved, U: active uninvolved, C: healthy control samples

Biomarker Genes with Drastic Expression Changes in Psoriasis

The top 25 most upregulated genes in the consensus list for psoriasis were altered up to a fold-change of 300. As such, they comprised a candidate biomarker set for psoriasis. The fold change patterns of these genes were investigated across the six skin disorders being studied. Figure 3 shows that biomarker candidate genes for psoriasis undergo much less dramatic expression changes in other skin disorders. Hierarchical clustering of the identified gene fold changes showed close clustering of A/U and A/C psoriasis comparisons confirming the distinctive role of those genes as psoriasis biomarkers. Wound healing U/C and A/C comparisons which represent the inflammatory and reconstructive stages of wound healing also cluster closely with psoriasis datasets.

The upregulated biomarker genes in Figure 3 were previously associated with psoriasis through expression studies [33, 71-75]. However the downregulated genes identified (TSPAN8, SCGB1D2, C5orf46, IL37, RBP4, PPARGC1A, CA6, NR3C2, ZSCAN18, and GSTA3) have not been linked to psoriasis since the emphasis of previous studies were predominantly on upregulated genes as possible biomarkers. The top upregulated genes include those part of the epidermal differentiation complex (EDC) (S100A7A, S100A9, S100A12, SPRR2A, SPRR2C); antimicrobial peptides (LCN2, TMPRSS11D, PI3, S100A7A, S100A9, OASL), structural proteins (KRT6C, KRT16, GDA), metalloproteinases (ADAMDEC1, MMP12), and the serpine peptidase inhibitors (SERPINB3 and SERPINB4). Top downregulated genes including THRSP, H19, LPL, PPARGC1A, RBP4, and HSD11B1 play important roles in lipid processes. PPARGC1A, known to interact with PPARgamma has previously been associated with psoriasis [76]. PPARs are a family of genes that communicate between lipid metabolic disorders, Th17 response, and innate immunity [77]. HSD11B1 codes a microsomal enzyme that catalyzes the conversion of the stress hormone

cortisol to the inactive metabolite cortisone. The downregulated biomarkers showed similarities to Merkel cell carcinoma and wound healing.

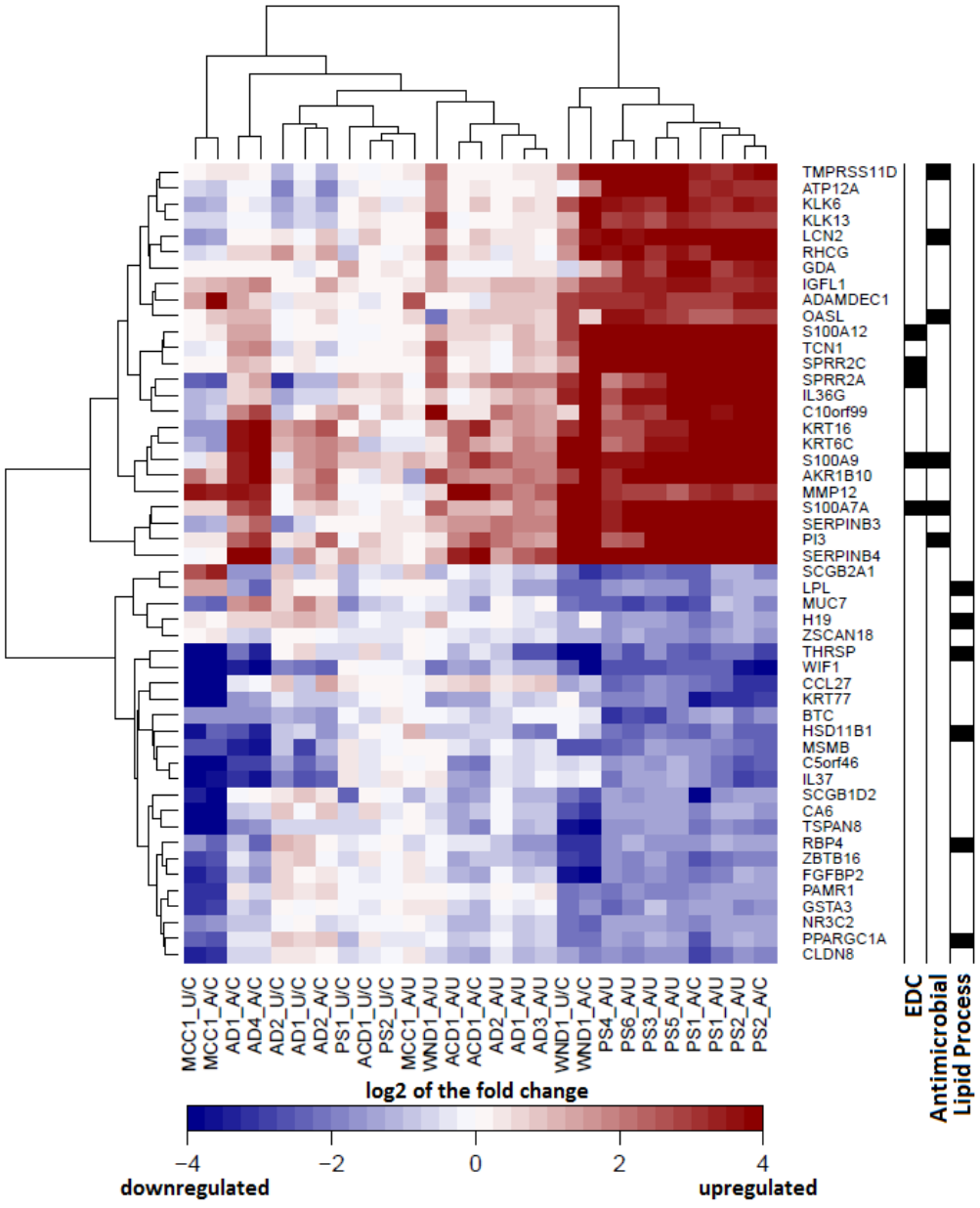

Figure 3 Top genes in psoriasis signature and the corresponding log 2 transformed fold changes in all comparisons. The upregulated genes are indicated with shades of red and the downregulated genes are indicated with shades of blue. The genes with epidermal differentiation complex (EDC), antimicrobial and lipid processing functions are marked in black. PS: psoriasis, AD: atopic dermatitis, ACD: allergic contact dermatitis, SA: sarcoidosis, MCC: Merkel cell carcinoma, WND: wound healing. A: Active involved, U: active uninvolved, C: healthy control samples

Functional Annotation of Gene Signatures

The consensus gene lists obtained for each skin condition were annotated using statistical enrichment of KEGG pathways [57] and GO-BP [56]. Figure 4 shows KEGG pathways enriched with significantly upregulated (pink) and significantly downregulated (blue) genes in psoriasis, with a Benjamini cutoff of 0.05. The outer edge of the figure shows the pathways enriched for the other five skin conditions. The three most upregulated KEGG pathways for psoriasis are cytokine-cytokine receptor interaction, cell cycle, and NOD-like receptor signaling pathways. These three pathways illustrate the involvement of the innate and adaptive immune system mechanisms in psoriasis, as well as increased cell overturn in the progression of the flares. Functional enrichment of other skin conditions revealed adaptive and innate inflammatory pathways that are also commonly upregulated with psoriasis. The downregulated pathways in psoriasis include organismal pathways such as the PPAR signaling pathway, which is a hormone-signaling pathway activated by fatty acids. The significantly downregulated pathways are typically psoriasis specific and show less commonality with other conditions.

Overall, the pathway enrichment results confirm previous findings [78, 79] and reveal new psoriasis enrichment results, including cytosolic DNA sensing and RIG-I like receptor signaling. In addition, as shown in Figure 4, a number of disease pathways were statistically enriched with psoriasis significant genes. These disease pathways include rheumatoid arthritis, malaria, and cardiovascular disease. The KEGG disease pathways annotated with psoriasis gene signatures can be seen in Appendix A and Appendix B. The latter of which were previously linked to psoriasis in the literature [19, 33]. Furthermore, malaria treatment has a risk of triggering

psoriasis [80]. Genes marked in these disease pathways shed light on the molecular mechanisms linking psoriasis to its comorbidities.

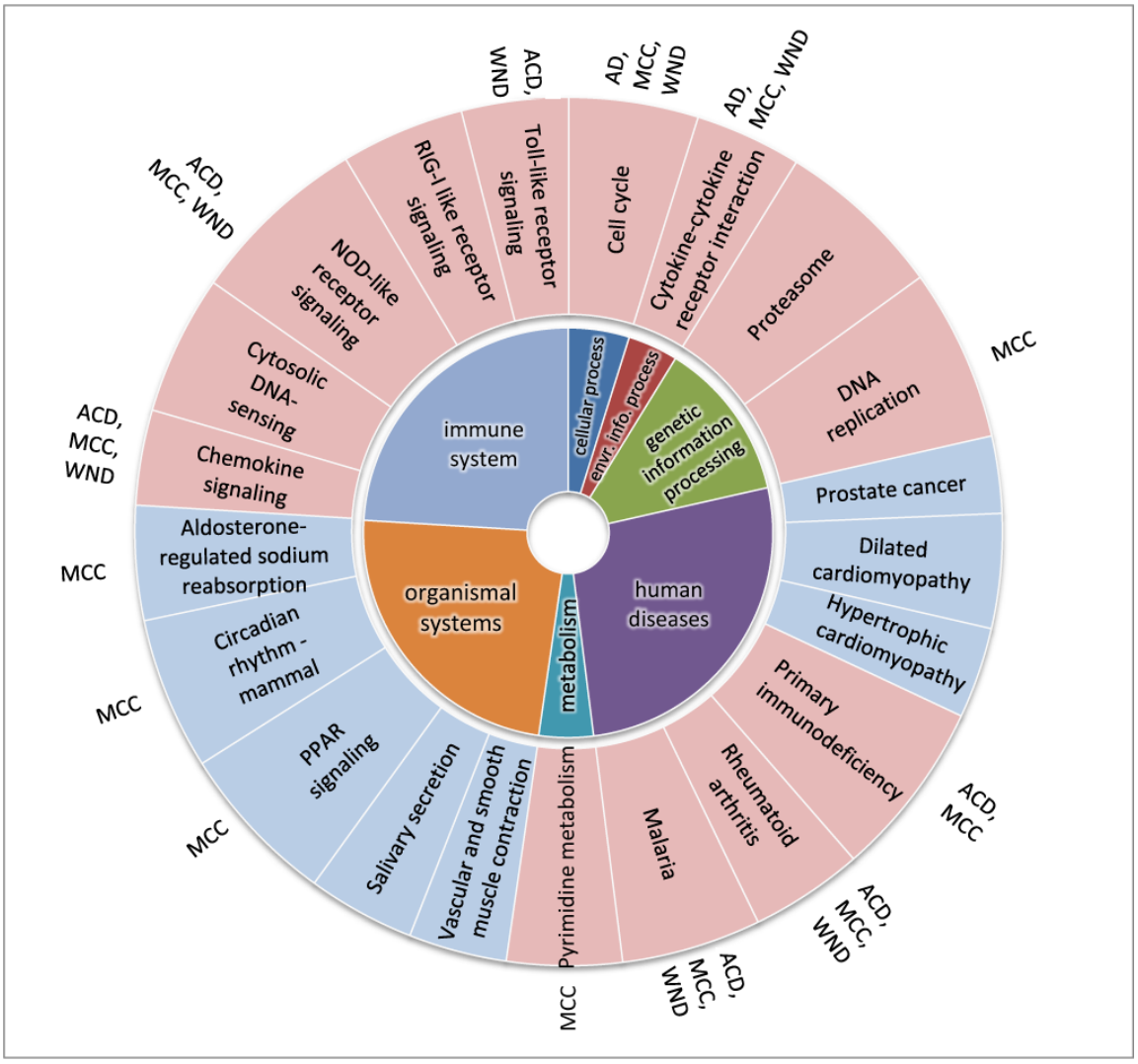

Figure 4 Significantly upregulated (pink) and downregulated (blue) KEGG pathways (Benjamini<0.05) of the psoriasis microarray datasets of the host. The width of each slice is proportional to the ratio of significant genes in the pathway. The pathways are categorized according to KEGG-BRITE hierarchy (inner circle). Pathways significantly altered in other skin conditions are marked outside of the corresponding pathway slices. PS: psoriasis, AD: atopic dermatitis, ACD: allergic contact dermatitis, MCC: Merkel cell carcinoma, WND: wound healing

Functional enrichment analysis of the disease signatures for GO-BP [56] terms show upregulation of type I interferon signaling in all skin conditions except Merkel cell carcinoma. The GO annotations for downregulated genes show clear functional segregation, in which terms related to muscle development and cell adhesion are downregulated in psoriasis. Keratinization shows heterogeneity with respect to skin disease subtype, where it is upregulated in psoriasis and wound healing and downregulated in atopic dermatitis and Merkel cell carcinoma. GO-BP term for defense response to virus is enriched in significant gene lists for psoriasis, atopic dermatitis, allergic contact dermatitis, and sarcoidosis, pointing to potential pathogenic involvement in autoimmune conditions.

Expression Similarities between Psoriasis and Wound Healing

As observed in Figure 3, the fold change profiles of the reconstructive (A/C) and inflammatory (U/C) stages of the wound healing process clustered with psoriasis biomarker profiles. This was further investigated by comparing significantly expressed genes involved in wound healing and psoriasis gene signatures. The overlapping number of genes between the profiles and their top five significant GO-BP Level 5 enriched terms can be seen in Figure 5. There are 448 genes associated with wound healing that overlap between either A/C or A/U psoriasis gene signatures. The GO-BP annotations of these genes show functional differences depending on which wound gene signature they overlap with. The psoriasis gene signature that overlaps exclusively with A/C wound signature is mainly related to keratinization and epidermis development. This is consistent with epidermal regeneration that is observed in wound healing and high keratinization of psoriasis lesions. The genes that overlap with the U/C wound signature exclusively are related to T cell regulatory functions such as CD80, CD83, IL2RG and IL4R. The genes that are exclusive to wound healing have further regulation of inflammatory

response such as IL10, IL6, TNFRSF1B and TLR8. Finally the genes that are common between psoriasis gene signatures and both stages of wound healing have genes related to mitosis. This correlates to the high cell overturn that is observed in both conditions. The genes that are common for downregulated genes did not show functional coherence except for psoriasis exclusive genes, which were related to muscle development.

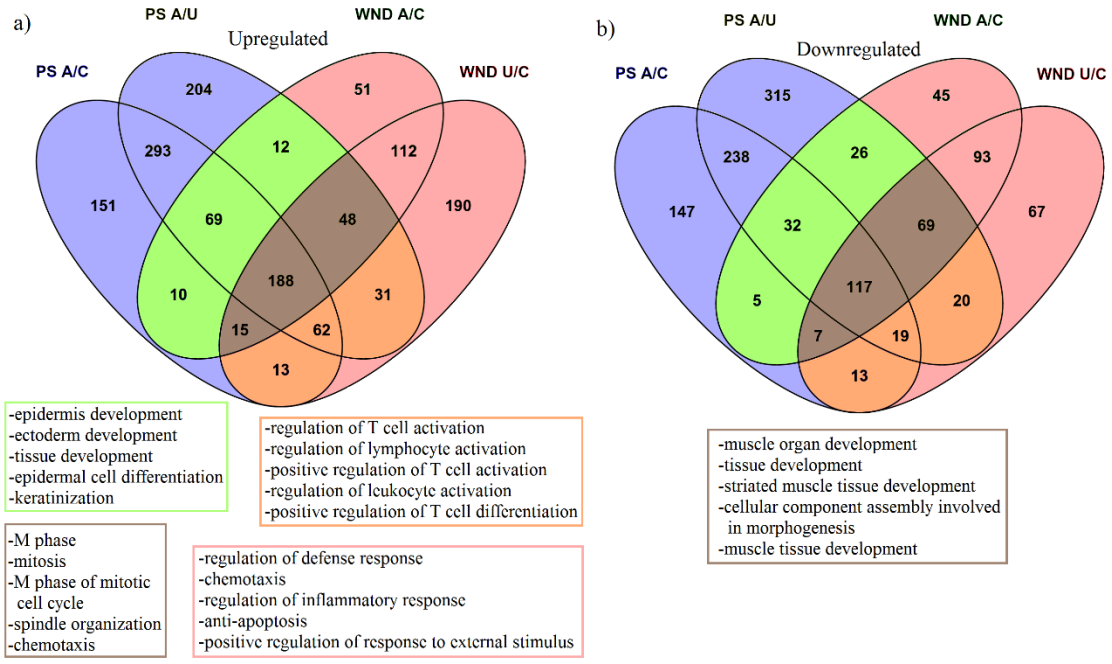

Figure 5 Number of genes that are common between the consensus psoriasis gene signatures (A/C/ and A/U) and wound healing gene signatures (A/C and U/C) for a) upregulated and b) downregulated genes. The genes in the color coded regions have been annotated with GP-BP Level 5 terms. The top five enrichments are reported in their corresponding colored boxes. The colored regions that don't have associated annotations provided did not have any significant enrichments (Benjamini < 0.01).

The psoriasis and wound healing gene signatures have been projected onto the cytokine-cytokine receptor interaction pathway to visualize the expression of cytokines in both conditions as illustrated in Figure 6. Cytokines CXCL1, CXCL2, IL7R, and IL1B were expressed commonly between psoriasis and wound healing, whereas CXCL5, CXCL6, CCR1, IL6, IL10, IL24, and IL1A were expressed exclusively in wound healing, and CXCL9, CXCL11, IL12, IL19, IL20, IL26, and TNFSF10 were expressed exclusively in psoriasis lesions.

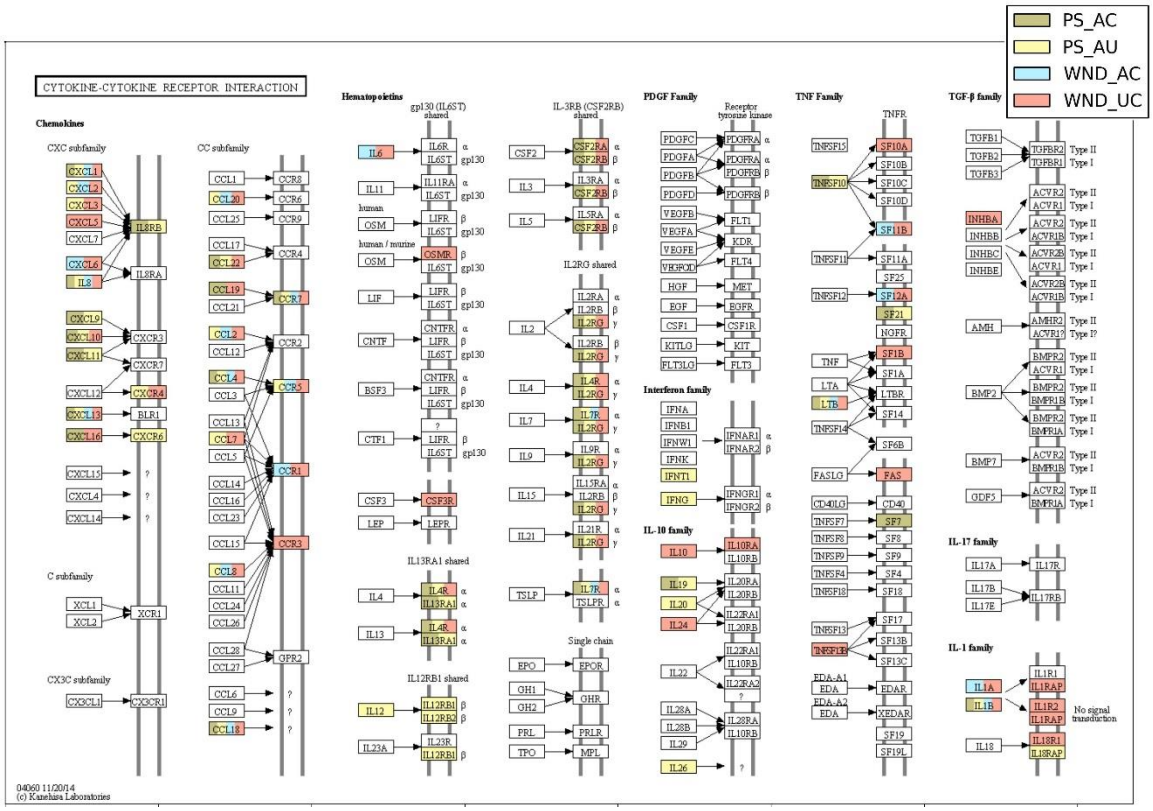

Figure 6 Psoriasis (A/C, A/U) and wound (A/C, U/C) upregulated genes (Benjamini < 0.01 and fold change > 1.5) overlapped with the KEGG pathway cytokine-cytokine receptor interaction pathway. The cytokines that are commonly upregulated as well as the ones that are exclusive to one conditions can be seen. PS_AC: psoriasis A/C consensus gene signature, PS_AU: psoriasis A/U consensus gene signature, WND_AC: wound healing reconstructive stage gene signature, WND_UC: wound healing inflammatory stage gene signature.

Comparison of Gene Signatures between Affymetrix and PIQOR Datasets

Even though Affymetrix Human Genome U133 Plus 2 is a comprehensive microarray chip that is widely used, other microarray technologies such as PIQOR have been used for skin studies. In order to assess the robustness of gene signatures across platforms, a study hybridized onto PIQOR array was analyzed. The microarray chip used for the GEO dataset GSE63741 [42] is PIQOR Skin 2.0 Microarray which represents 1542 skin specific genes. When the consensus gene signatures obtained from Affymetrix datasets were compared against PIQOR gene signatures for each condition, the hypergeometric tests were all significant with p-values smaller than 3e-4. This signifies that, among the genes that were common between the two platforms, the mRNA perturbations observed were equivalent.

Between the two microarray platforms, there were discrepancies with the functional annotations done through KEGG and GO databases, most likely due to the reduced number of genes that the PIQOR chip covers. The details of the terms enriched can be seen in Table 3. For example if the upregulated gene signatures of psoriasis are considered, even though there were 18 pathways that were enriched with Affymetrix obtained genes and four pathways that were enriched with PIQOR obtained genes, the only consensus between the two platforms were NOD-like receptor signaling pathway. There was a higher number of overlap for the GO-BP functional enrichments. For the psoriasis upregulated genes, the commonly enriched terms included keratinization, type I interferon-mediated signaling pathway, negative regulation of viral genome replication, and regulation of response to stress.

Database	Direction	Term	PIQOR	Affymetrix
KEGG	up	NOD-like receptor signaling pathway	0.12	0.27
GO-MF	up	interleukin-1 receptor binding	0.25	0.38
GO-MF	up	serine-type endopeptidase inhibitor activity	0.08	0.15
GO-MF	up	peptidase inhibitor activity	0.06	0.13
GO-MF	up	endopeptidase regulator activity	0.06	0.13
GO-MF	down	glutathione transferase activity	0.30	0.35
GO-MF	down	heparin binding	0.06	0.13
GO-BP	up	keratinization	0.36	0.50
GO-BP	up	type I interferon-mediated signaling pathway	0.32	0.49
GO-BP	up	peptide cross-linking	0.29	0.25
GO-BP	up	response to type I interferon	0.17	0.30
GO-BP	up	negative regulation of viral genome replication	0.16	0.37
GO-BP	up	interferon-gamma-mediated signaling pathway	0.14	0.27
GO-BP	up	negative regulation of endopeptidase activity	0.09	0.22
GO-BP	up	regulation of response to stress	0.04	0.11
GO-BP	down	extracellular matrix organization	0.07	0.13

Table 3 Common significant enrichments (Benjamini < 0.01) between datasets hybridized onto PIQOR and Affymetrix platforms. The values represent the percentage of significant genes within the corresponding term. Up: upregulated genes, down: downregulated genes, KEGG: Kyoto Encyclopedia of Genes and Genomes, GO: Gene Ontology, BP: Biological Process, MF: Molecular Function

Discussion

Psoriasis is a complex autoimmune condition with a high economic and psychological burden on the patients. Microarray studies conducted on various skin conditions allow for a systems approach to mRNA perturbations. In this chapter, multiple psoriasis microarray studies have been analyzed in context of other skin conditions to compare and contrast their expression profiles.

We find that psoriasis has robust and consistent upregulation of gene circuit patterns across a multitude of microarray datasets. Psoriasis signature was specific enough that the top upregulated genes could differentiate psoriasis accurately from other skin diseases based on

unsupervised clustering of fold changes. Psoriasis lesions, when compared to healthy controls, shared inflammatory and reconstructive processes with the process of wound healing, as evidenced by the shared upregulated biomarkers.

Host pathways significantly enriched with upregulated genes in psoriasis corresponded to the immune system, cellular processes, environmental information processing, genetic information processing, and metabolic categories. Upregulated psoriasis genes were also significantly enriched in disease pathways of psoriasis comorbidities such as rheumatoid arthritis. For example, proteins related to the Th17 response [81] in rheumatoid arthritis pathway such as CXCL1, IL8, CCL20, CCL2, and CD80/86 are also upregulated in psoriasis. Shared inflammatory features in psoriasis and arthritis [82] suggest the presence of a cascade eventually causing joint degeneration [83]. Genes contributing to joint cartilage destruction such as MMP1/3 and CTSL are upregulated across psoriasis datasets. The genes that are commonly upregulated should be further investigated to better understand the link between psoriasis and its comorbidities.

Host pathways enriched with downregulated psoriasis genes include a number of disease pathways such as dilated or hypertrophic cardiomyopathy as presented in Appendix A. In these pathways, genes coding the transmembrane protein sarcoglycan and contractility proteins actin, troponin, tropomyosin, and desmin are downregulated in psoriasis. The consequences of the downregulation of sets of transmembrane proteins in psoriasis are yet to be fully explored.

This chapter shows genes involved in antimicrobial function are consistently upregulated in psoriasis. These findings are consistent with the skin's role as the major physical barrier between self and non-self, with these antimicrobial genes providing the first line of defense against bacterial and viral pathogens [84]. Antimicrobial genes provide defense against a multitude of

pathogens: gram positive and gram negative bacteria as well as viral agents [46, 85]. The upregulation in the antimicrobial peptides on the skin correlates with recent studies on perturbed skin microbial flora in psoriasis and other skin diseases [86-88]. Biopsy studies show bacterial presence in the lower levels of the skin [87] and in the blood of patients with psoriasis [89], suggesting pathogenic invasion. The upregulated Th17 response is crucial in host response to viral infection [90].

Psoriasis has been defined as aberrant wound healing due to abnormal thickening of the epidermis [29]. As with psoriasis, wound healing has an inflammatory and a reconstructive component. The results of this chapter show that there are differences in the immune responses mounted between psoriasis and wound healing processes. Understanding the differences between the two can be beneficial in developing psoriasis drugs with more mild side effects. For example an immunosuppressive agent, Sirolimus, has been investigated to treat psoriasis with positive results [91]. However one of the side effects is impaired wound healing and wound dehiscence [92]. Treatments with wound healing specific genes and agonists for psoriasis specific genes can mitigate the side effects of therapies and provide a healthier inflammatory response. IL-10 which is exclusive to wound healing has been associated with decreased inflammatory response in wound healing and reduced scar formation [93]. IL10 treatment for psoriasis has already been underway to test its effectiveness [94]. Even though IL6 expression was exclusive to wound healing in our analysis and can possibly be a drug target for psoriasis treatment, there is evidence of IL6 expression in both psoriasis and wound healing in literature [95, 96]. It is claimed that the high expression of the gene prevents T cells from responding to regulation in psoriasis. As for the psoriasis specific genes, there are already prescriptions for IL-12 agonist [97]. IL-20 has been explored as psoriasis treatment with no efficacy [Clinical Trials:

NCT01261767]. On the other hand IL-19, CXCL9 and CXCL11 have not yet been explored as drug targets.

Specific Aim II: Functional Perturbations in Psoriasis Microbiome

The primary aim of this chapter is to identify the microbiome perturbations of the psoriatic skin compared to healthy control to identify the bacterial Operational Taxonomic Units (OTUs) and the functional perturbations that are significantly altered in psoriatic lesions. The microbiome of each individual is likely to contribute to the psoriasis phenotype. The second hypothesis is that the functional changes in the microbiome will be mirrored by the processes occurring in the host skin. Despite the varying OTUs across the sampled body sites, core orthologs in the psoriasis microbiome is expected to become altered.

Introduction

Psoriasis has been evaluated through genetic, epigenetic, transcriptome, and microbiome assays. However, the functional perturbations of the host and the microbiome has not yet been bridged. In this chapter the transcriptome gene signature of the host along with the functional perturbations on the microbiome side are studied to further the understanding of the host-microbiome interactions on psoriatic skin.

Bacterial culture based studies showed correlations of certain bacterial infections and increased severity of psoriasis. A study done by Gudjonsson et al showed that patients with psoriasis were more likely to contract Streptococcus infections, which in turn exacerbated plaque psoriasis [98]. A similar study demonstrated that even though the prevalence of H. pylori was not higher in psoriasis than in the control group, those infected by it had a greater severity of plaque

psoriasis [99]. The strain of the bacteria colonization is also significant in the severity of psoriasis plaques. Patients with psoriasis that were colonized with enterotoxin positive Staphylococcus aureus bacteria had higher severity scores than toxin negative psoriasis patients or patients without S. aureus colonization [100]. These observations were further supported clinically, with patients showing better recovery when they were prescribed antibiotics as part of their treatment regimen [99]. Even though culture based studies capture the perturbations of well-known pathogens, they can miss the effects of non-culturable bacteria on psoriasis severity or pathogenesis. It is also unclear whether skin microbiome perturbations have an effect on the abundance of virulence factors known to cause human disease.

In stark contrast to culture-based studies, recent advancements in high throughput sequencing technologies and enhanced RNA extraction methods have enabled large-scale 16S based microbiome studies, evaluating alterations in bacterial abundance on psoriatic skin. The current 16S microbiome studies have concluded that the diversity and composition of bacterial flora is altered on psoriasis flares. Phylum level abundance differences can be observed for Firmicutes, Actinobacteria, and Proteobacteria [86, 87, 101]. The studies that focus on analyzing Operational Taxonomic Units (OTUs) alone do not allude the undergoing functional differences of the microbiome. The microbiome differences between individuals [102] also make it difficult to evaluate the contributions of low-abundance bacteria.

Even though whole genome shotgun metagenomics sequencing of the skin microbiome would provide a more complete picture of bacterial abundance and diversity, such a study has not yet been reported and the skin microbiome studies have been limited to 16S data. This is due to the low number of reference genomes for the skin isolates, difficulty in culturing certain skin specific bacteria, and the challenges in obtaining sterile host DNA samples [102]. Therefore this chapter

focuses on extrapolating 16S OTU level abundance data using the database of decoded bacterial genomes to predict the ortholog composition of the samples. This provides a preview of the functional perturbations that are observed on the skin and is applicable to study cross talk interactions in other skin diseases with suspected microbiome involvement such as atopic dermatitis [101]. A follow up study can be conducted once robust methods have been developed for skin whole genome shotgun metagenomics sequencing.

Methods

Obtaining the Microbiome Datasets

NCBI Short Read Archive (SRA) [103] was queried with the search terms “skin metagenome psoriasis” or “skin microbiome psoriasis”. This resulted in two datasets (before June 2014) belonging to the Human Microbiome Project [23, 25] and the American Gut Project [104]. The number of samples present in each of these datasets is recorded in Table 4.

Analogous to the samples obtained for the microarrays in Chapter 1, the Human Microbiome Project samples represented swabs from active psoriasis lesions (A), uninvolved contralateral skin (U), and from subjects without any skin conditions (C). The dataset represented samples from 14 body sites. Among the body sites of origin only the elbow, knee, back, forearm, and leg had five or more samples each from at least two categories (A, U or C). Skin microbiome composition is highly dependent on the body site [102]. Therefore the analyses were conducted separately for each body site containing enough samples. The V1-3 regions of the 16S rRNA bacterial sequences were extracted and sequenced with Roche 454 GS FLX System. Eligible

patients had moderate to severe psoriasis. The control samples were age matched to patients with psoriasis (18-75 years). Eligibility criteria excluded subjects with systemic antibiotics, corticosteroids, cytokine treatments, large consumption of probiotics one month prior to the study, and topical antibiotic or steroid applications one week prior.

American Gut Project samples consisted of swabs from subjects with no skin condition (C) and with psoriasis (U). The samples were collected from the hands and forehead. However only the forehead samples had more than five samples in both C and U categories. 16S rRNA reads from bacterial rDNA V4 regions sequenced on Illumina MiSeq platforms were downloaded from the NCBI Short Read Archive (SRA) database [103].

Body Site	**Number of Samples (Usable Samples / Total)**				**Dataset**	**16S Region**
	Control	Uninvolved Psoriasis	Active Psoriasis	Total		
Elbow	35/37	21/22	23/23	79/82	HMP	V1-3
Knee	28/28	11/11	11/11	50/50	HMP	V1-3
Back	0	12/13	12/13	24/26	HMP	V1-3
Leg	0	8/8	8/8	16/16	HMP	V1-3
Forearm	1/1	7/7	7/7	15/15	HMP	V1-3
Other	12/15	15/15	15/15	42/45	HMP	V1-3
Forehead	46/69	5/5	0	51/74	AGP	V4
Total	**122/150**	**79/81**	**76/77**	**277/308**		

Table 4: The number of distinct tissue samples with 16S rRNA sequence datasets presented by the Human Microbiome Project (HMP) and American Gut Project (AGP). The 16S rRNA sequence reads obtained from skin swabs of active psoriasis lesions, uninvolved contralateral skin from psoriatic patients, and subjects without any skin conditions (Control) were included in the analysis. The remaining samples used for further analysis after quality control steps (Usable Samples) as well as the total number of samples in the dataset before the quality control step (Total) are reported.

Processing the Microbiome Reads

From the samples that were included in this study, low quality sequences were filtered with the Quantitative Insights into Microbial Ecology (QIIME) [105] tool. Since the two datasets were sequenced with different technologies, optimal quality control procedures were followed as described in QIIME pipelines for each dataset. The sequences from HMP samples, sequenced with the Roche 454 platform, were selected for further processing if they were above an average Phred score of 25, and if the sequence lengths were between 200 and 1000 amino acids. For the AGP dataset, sequenced with the Illumina MiSeq platform, the quality threshold was set at a Phred score of 20. The sequences were truncated after every three consecutive low quality reads. After the truncation step, if 75% of the consecutive reads passed the quality threshold, the sequence was kept for further processing.

The sequences retained after the quality control step were assigned Operational Taxonomic Units (OTUs) with 97% identity using the 16S reference database: Green Genes version 13_5 [106]. The samples in the AGP dataset were then rarefied with an OTU threshold of 15,000. Samples in the HMP were rarefied to an OTU threshold of 1500 to have uniform sequencing depth. These threshold values were chosen to retain sufficient samples while preserving diversity of the skin flora.

The abundance of the OTUs in active psoriasis samples were compared to healthy control samples with Mann-Whitney U test for all the body sites combined. The significance test was then repeated for each body site separately to see the contribution of individual body sites to the OTU significance.

Metagenome Prediction

The OTU tables for each body site were fed into the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) [107] pipeline for further analysis. The abundances were normalized with this pipeline to account for 16S copy number variations for each OTU. The metagenomes were then inferred by multiplying the normalized OTU abundances with the precalculated gene content predictions. The process resulted in estimations of the ortholog gene contents of the microbiome and subsequent categorization by KEGG pathways. The accuracy of the metagenome predictions were calculated with Nearest Sequenced Taxon Index (NSTI) scores. The ortholog genes that were significantly altered in A/C, A/U, and U/C comparisons were identified using Mann-Whitney U test with a p-value threshold of 0.01. Mann-Whitney U test [108] was also used for A/C, A/U, and U/C comparisons for each body site to identify the KEGG Pathways with significantly different abundance levels.

The fold change of the metagenome predictions of the mentioned comparisons were calculated to evaluate the set of orthologs that are commonly altered across body sites. The resulting fold changes of each comparison were ranked. The top ranking 25 high and 25 low abundance orthologs were visualized.

Commonly Altered Genes between the Host and the Microbiome

Expression profiles of the host have been obtained from the meta-analysis of psoriasis studies hybridized onto the Affymetrix platform, as described in the previous chapter. Gene signatures from active involved versus active uninvolved (A/U), active involved versus healthy controls (A/C), and active uninvolved versus healthy controls (U/C) comparisons have been used in this

chapter. The gene expression of psoriatic skin does not correlate with body site or age onset [109], therefore the psoriasis samples from microarray have been annotated as either uninvolved (U) or active (A) regardless of body site.

To find the genes that were commonly altered between the host and the microbiome, the corresponding Entrez IDs of the KEGG orthologs were extracted from the KEGG database. The genes that have been significantly altered ($p < 0.01$) both in the host and the microbiome were recorded.

Results

Psoriasis is an autoimmune condition with suspected microbial involvement. In an effort to explore the perturbations of bacterial composition, the 16S sequences extracted from the psoriasis lesions, uninvolved and healthy skin, have been sequenced in previous studies as part of the Human Microbiome Project [25] and the American Gut Project [104]. This chapter utilizes the mentioned studies and the annotated bacterial genomes to extrapolate from OTU abundances to microbial gene contents and functional perturbations in psoriasis lesions. Alterations of the microbiome's orthologous gene content in psoriasis lesions were analyzed using QIIME [105] and PICRUST [107] packages. The overview of the methods is illustrated on the flowchart in Figure 7.

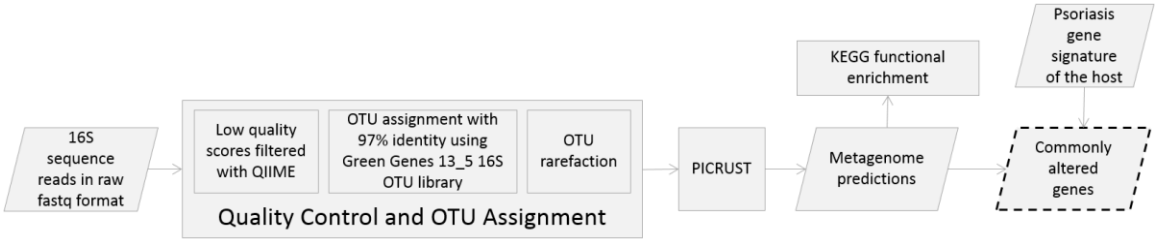

Figure 7 Flowchart of microbiome analysis steps. The 16S rRNA sequence reads were downloaded, passed through quality control steps and assigned to OTUs. The metagenome was predicted through PICRUST package. The host gene expression signatures obtained for the first specific aim have been used to find the commonly altered genes in both the host and the microbiome.

Significantly Altered OTUs

After the quality control steps, OTUs were assigned through the QIIME software package. The average relative frequencies of the phyla that are found in the microbiome are shown in Figure 8. The majority of the skin microbiome phyla in both control subjects and microbiome samples consisted of Proteobacteria, Firmicutes, and Actinobacteria. Even though the abundance levels of the phyla showed minor perturbations, the differences were not statistically significant between psoriasis and healthy control samples for individual body sites. Figure 9 shows the class level breakdown of the samples collected from the elbow. There are ten main classes of bacteria that are commonly present in all samples with varying abundance levels. The abundance perturbations of these major phyla or classes in any of the body sites were not significant according to the Mann-Whitney U test with a p-value of 0.01.

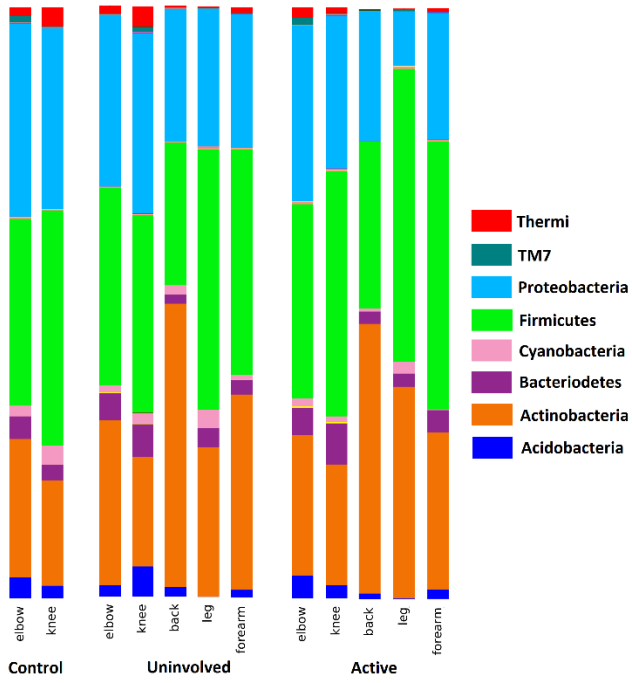

Figure 8: Average bacterial phylum level abundance of microbiome in patients with active psoriasis, contralateral uninvolved skin and healthy controls from different body sites. The phyla Actinobacteria, Firmicutes and Proteobacteria dominate each body site.

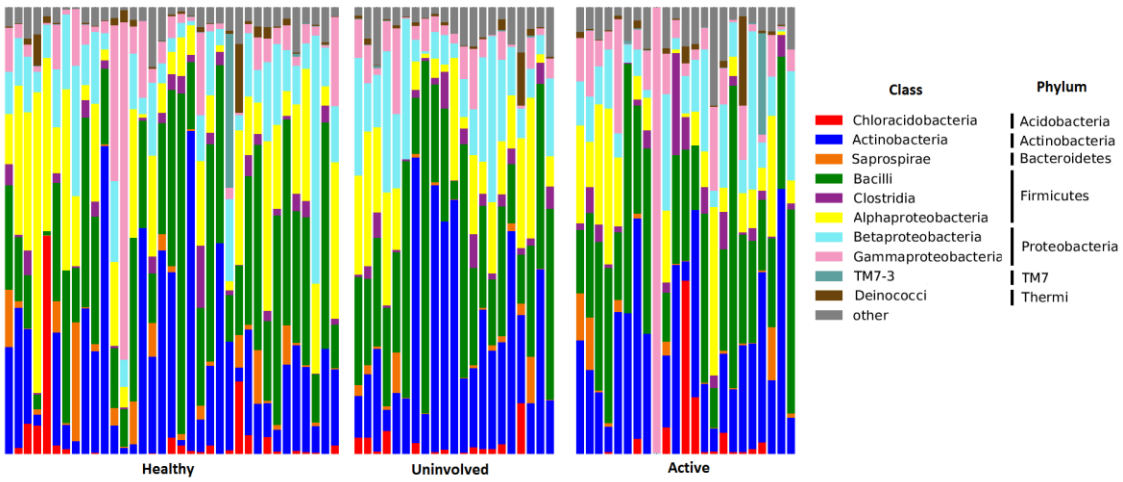

Figure 9 Bacterial class level abundance of microbiome in patients with psoriasis and healthy controls for only the elbow samples based on the Human Microbiome Project data. Bacterial classes that have more than 10% abundance in more than one sample were labeled in the bar graph. The rest of the classes are categorized as "other". The bacterial classes that are shown were annotated with their respective phylum memberships.

Significant differences in bacterial OTU abundance can be observed between psoriasis lesions and healthy skin as quantified by the non-parametric Mann-Whitney U test and corrected for false discovery rate with the Benjamini false discovery rate correction method. When the samples from each studied body site were pooled and tested for significance, all but two OTUs belonged to the phylum Proteobacteria. Within the Proteobacteria phylum, multiple OTUs belonging to the Methylobacteriaceae family had significantly lower abundance in psoriasis microbiome. The combined phylogenetic tree of the significantly altered OTUs can be seen in Figure 10. Most of the body sites in the HMP dataset had less than five samples for each category. Therefore the same significance test was repeated excluding the body sites that didn't have enough samples. When the test was repeated with only the pooled samples from elbow, knee, back, leg, and forearm body sites, the OTUs belonging to the family Methylobacteriaceae continued to be in low abundance in psoriasis active flares compared to healthy controls.

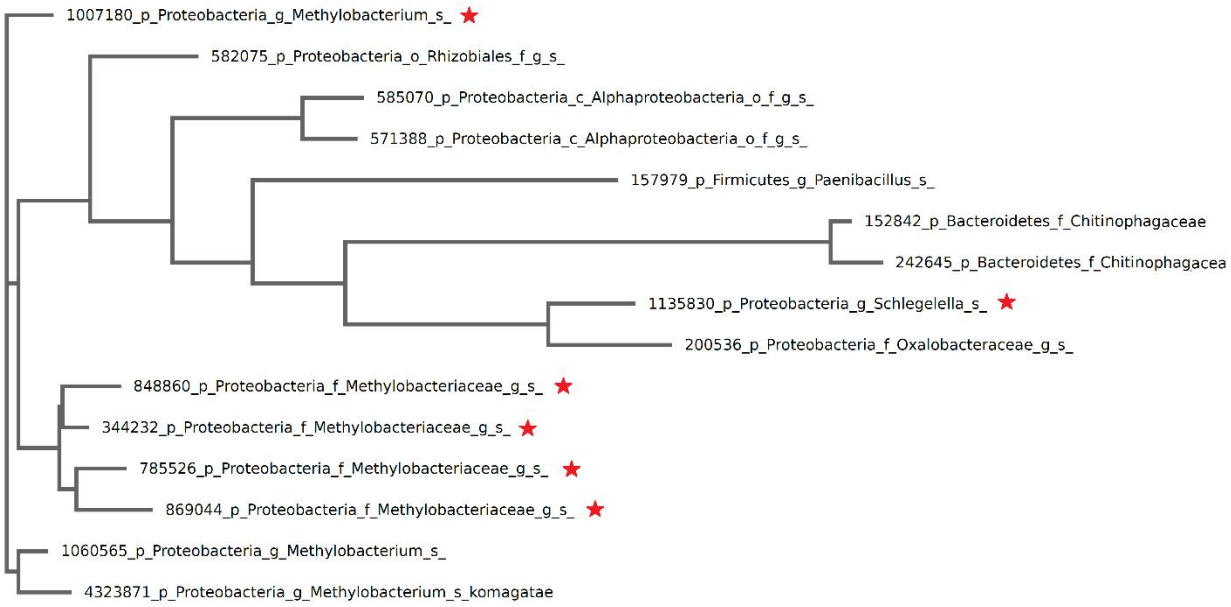

Figure 10: The OTUs significantly altered (Benjamini < 0.05) in psoriasis lesions compared to healthy controls in the human skin microbiome for the pooled samples from all body sites. All the OTUs were lower in abundance in psoriasis lesions. The OTUs that are statistically significant (Benjamini < 0.05) for the non-singleton body sites (elbow, knee, back, leg, forearm) are labeled with red stars.

Due to the bacterial composition differences across body sites [102], the significance of the OTUs were tested separately for each body site. Most differences involved higher abundance of OTUs on the knee and lower abundance in the elbow for psoriasis lesions compared to healthy controls. The OTUs, such as the ones belonging to the genus Corynebacterium and Staphylococcus were known opportunistic bacteria with respect to infection. Although there was no OTU commonly altered in more than one skin site, their toxonomy converged at a higher phylum level. They belonged to the phyla Firmicutes, Proteobacteria, and Actinobacteria. The OTUs that are altered in specific body sites are visualized in Figure 11.

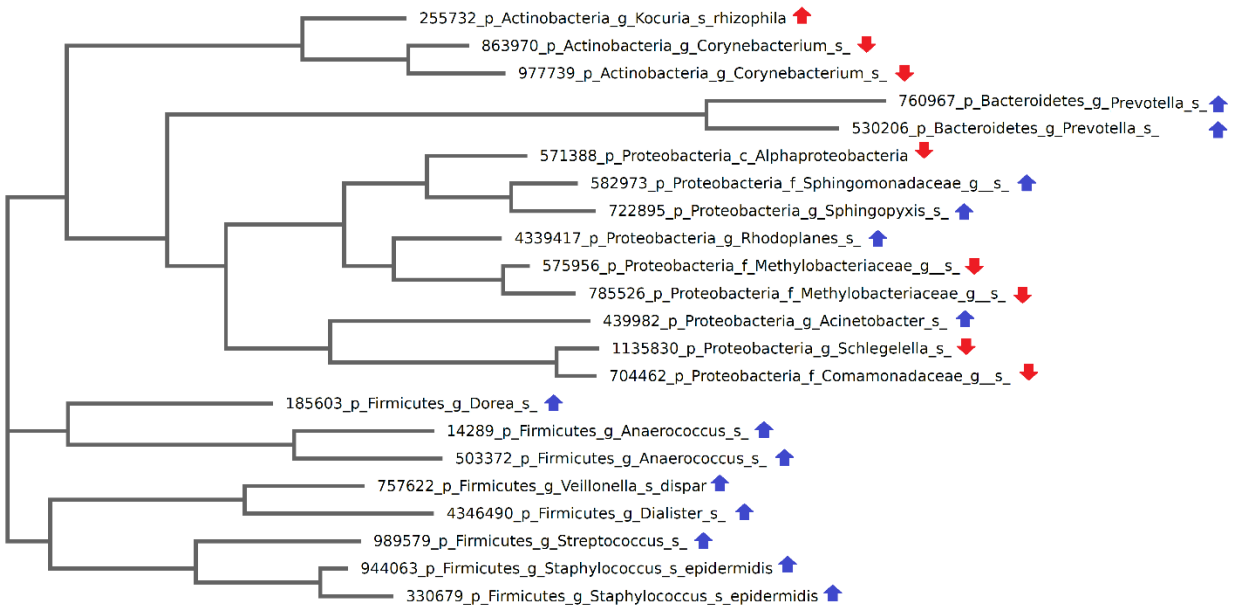

Figure 11: The OTUs significantly altered (p<0.01) in psoriasis lesions compared to healthy controls in the human skin microbiome. The nodes are labeled with Green Genes OTU numbers, the phyla, and the most specific level of categorization that is available with the OTUs such as species (s), genus (g), and family (f). The abundance perturbations are noted with an up arrow for higher abundance in psoriasis and a down arrow for lower abundance OTUs. The colors represent the body site where the OTU is significant (red: elbow, blue: knee).

Metagenome Predictions

OTU level abundance data alone does not indicate functional alterations that are present in the microbiome. Ideally, whole genome shotgun metagenomics of the microbiome need to be carried out in order to map the composition of the genes. However, such data is not yet available due to technical limitations of sequencing technologies and DNA extraction methods [102]. Nonetheless, the genomes that have already been decoded can provide an estimation of the gene content of the microbiome [107]. This idea has been implemented in the PICRUST software package and utilized in this study.

The assigned OTUs have been processed with the PICRUST metagenome prediction package. The availability of nearby genome representatives for each microbiome samples were quantified with the Nearest Sequenced Taxon Index (NSTI), which is based on the phylogenetic distance of each organism in the OTU table to its nearest relative sequenced reference genome [107]. The NSTI values quantify the error that is introduced with the prediction algorithm. The average NSTI values for each category is shown in Table 5. All NSTI values were within acceptable ranges for accurate predictions (NSTI<0.17) according to PICRUST publication [107].

Body Site	**NSTI values (average ± standard deviation)**		
	Control	Uninvolved Psoriasis	Active Psoriasis
Elbow	0.082±0.052	0.065±0.026	0.078±0.052
Knee	0.078±0.034	0.092±0.033	0.072±0.023
Back	NA	0.048±0.037	0.040±0.020
Leg	NA	0.068±0.024	0.050±0.021
Forearm	NA	0.045±0.015	0.055±0.019
Forehead	0.088±0.044	0.151±0.070	NA

Table 5 Average Nearest Sequenced Taxon Index (NSTI) for the microbiome datasets based on the phylogenetic distance for each organism in the OTU table to its nearest relative sequenced reference genome. All values were within an acceptable range for accurate prediction.

Even though the OTU abundance profiles varied with body site, the gene content of the microbiome as a whole demonstrated consistent alterations across the sampled sites. These genes were identified by calculating the rank product of the abundance fold changes. The top 25 orthologs with high abundance and top 25 orthologs with low abundance obtained are illustrated in Figure 12. The genes that were commonly altered between the body sites include pathogenic genes that were higher in abundance such as yeeJ (adhesion / invasion), yscF, sctF, yscW, sctW (type III secretion proteins), pagC (putative virulence related protein), pla, ompT

(plasminogen related genes), gspS (type II secretion proteins), and acfC (accessory colonization factor). On the other hand, iga (Type V secretion system) and rtxB (Type I secretion system) were in lower abundance in psoriasis microbiome.

The rest of the genes were involved in various metabolic processes. For example, the high abundance orthologs RRM1 (ribonucleoside-diphosphate reductase subunit 1) and yjjG (5'-nucleotidase) and lower abundance orthologs NUDT2 (bis(5' nuxleosidyl)-tetraphosphatase) are part of the pyrimidine and purine metabolism pathways. ACLY is involved in citrare cycle. Orthologs that belong to the fructooligosaccharide transport system (msmE, msmF, msmG) were in lower abundance.

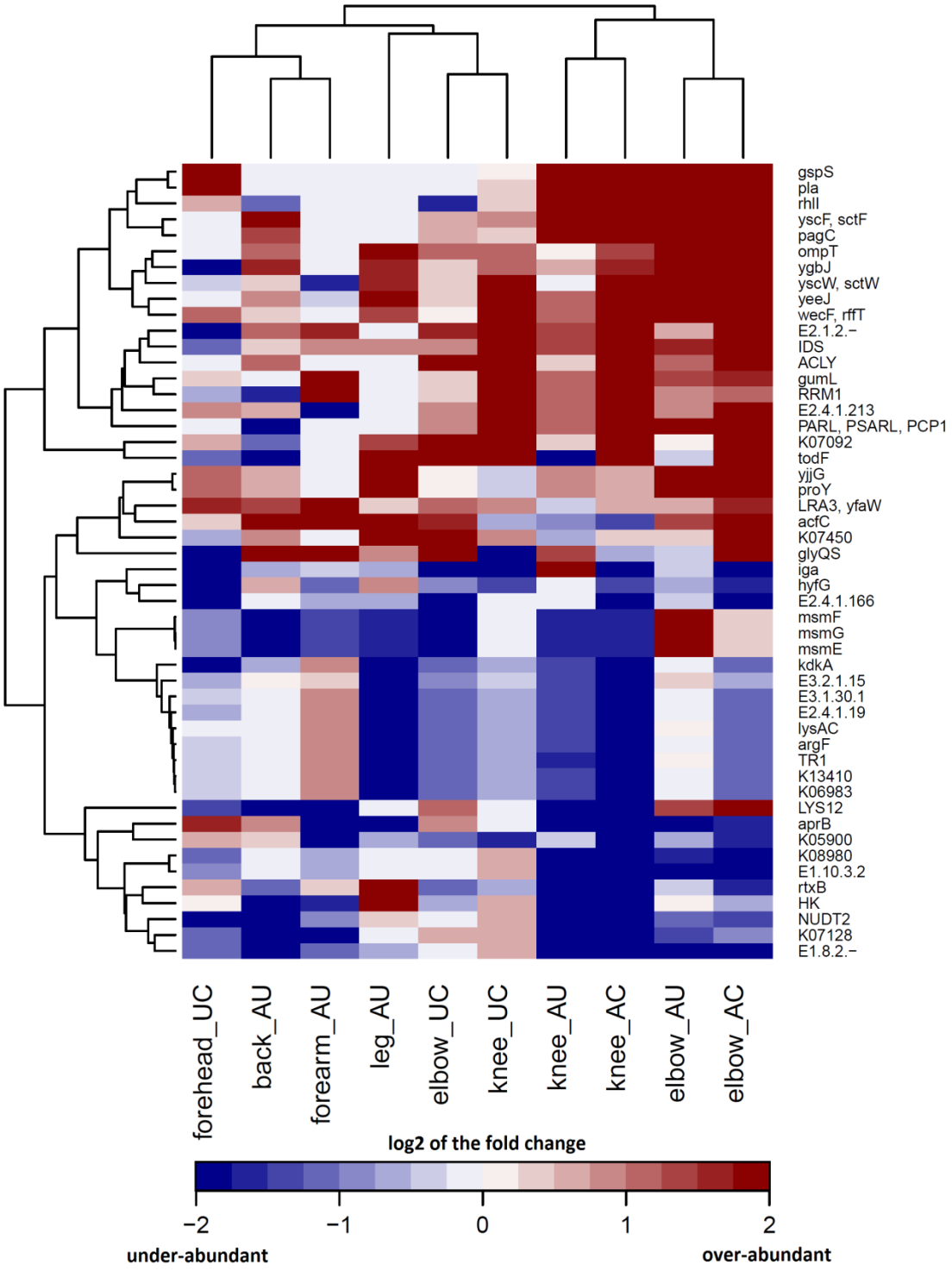

Figure 12 the orthologous genes that are consistently altered in the microbiome comparisons. The values represent the log2 transformed fold changes of the metagenome predicted through PICRUST software package. The high abundance genes are indicated with shades of red and the low abundance genes are indicated with shades of blue.

Significantly Altered Pathways in Psoriasis Microbiome

The pathways with top altered abundance in psoriasis microbiome were identified using the Mann-Whitney U test [108]. Statistical significance of vibrio cholera infection, amoebiasis and beta-lactam resistance pathways suggest an increase in disease causing orthologs in the psoriasis microbiome. (Table 6). Other pathways with increased gene abundance were xylene degradation and sporulation.

Body Site	Comparison	KEGG Pathway	P value	Fold Change
Elbow	U/C	Vibrio cholera infection	0.0047	1.10
Knee	U/C	Xylene degradation	0.0087	1.30
Forehead	U/C	Sporulation	0.0064	1.63
Forehead	U/C	Amoebiasis	0.0086	2.32
Forehead	U/C	Beta-Lactam resistance	0.0094	1.83

Table 6 KEGG pathways that have significantly different ($p < 0.01$) abundance levels in psoriasis microbiome compared to controls calculated with PICRUST [107]. The significance was only observed in the microbiome comparisons between uninvolved skin (U) and healthy controls (C). The table shows for each listed KEGG pathway, the p-value calculated using the Mann-Whitney U test and the abundance fold changes.

Orthologous Genes that are Commonly Altered between Host and the Microbiome

The orthologs that were commonly altered both on the host and the microbiome pointed to common processes in psoriasis. Orthologous genes with significant abundance changes in psoriasis microbiome and the host compared to healthy controls are shown in Table 7. ATOX1 is a copper chaperone that also functions as antioxidant. AMD1 is involved in polyamine synthesis,

GMPPB catalyzes the conversion of mannose-1-phosphate to GDP-mannose, and AKR1B10 is a member of the aldo/keto reductase superfamily.

Comparison	Symbol	Body Site	FC Microbiome	FC Host	KEGG Pathways
AC	ATOX1	elbow	0.71	1.94	Mineral absorption
AU	AMD1	knee	0.61	1.89	Cysteine and methionine metabolism, Arginine and proline metabolism
	GMPPB	knee	0.66	1.67	Fructose and mannose metabolism, Amino sugar and nucleotide sugar metabolism, Biosynthesis of secondary metabolites
	AKR1B10	forearm	0.17	20.82	Pentose and glucuronate interconversions, Fructose and mannose metabolism, Galactose metabolism, Glycerolipid metabolism

Table 7: Significantly over- and under-represented genes in psoriasis in the host and the microbiome for the A/C and A/U comparisons (p<0.01 for host and p<0.05 for microbiome). No commonalities were observed for the U/C comparison. The table reflects the fold changes of the commonly altered genes in the microbiome and the host, and p-values resulting from Mann-Whitney U test. A: active involved skin, U: active uninvolved skin, C: control skin, FC: fold change.

Altered KEGG Pathway Expression for Bacterial Invasion of Epithelial Cells in Relation to Skin Disease Host Genes

Bacterial invasion of epithelial cells KEGG pathway [57] depicts mechanisms that bacteria use to hijack the actin structure of the cell. Using the Virulence Factor Database (VfDB) [60], bacterial genes with pathogenic potential were identified within the pathway, enabling the annotation of crosstalk linkages between the host and the psoriasis microbiome. Figure 13 shows the pathway, annotated with color markers for upregulated and downregulated genes. Host genes

Cbl, SHC1, and ARP 2/3 were upregulated in psoriasis whereas Dynamin, SHC4, PI3K, and Septin were downregulated. The pathogenic orthologs that bind to fibronectin (FnBPA, FnBPB, Pfb, Sfb1, invasin) used by Staphylococcus, Streptococcus, and Yersinia species were in high abundance in psoriasis microbiome, potentially indicating an increase in virulence factors in the psoriasis microbiome.

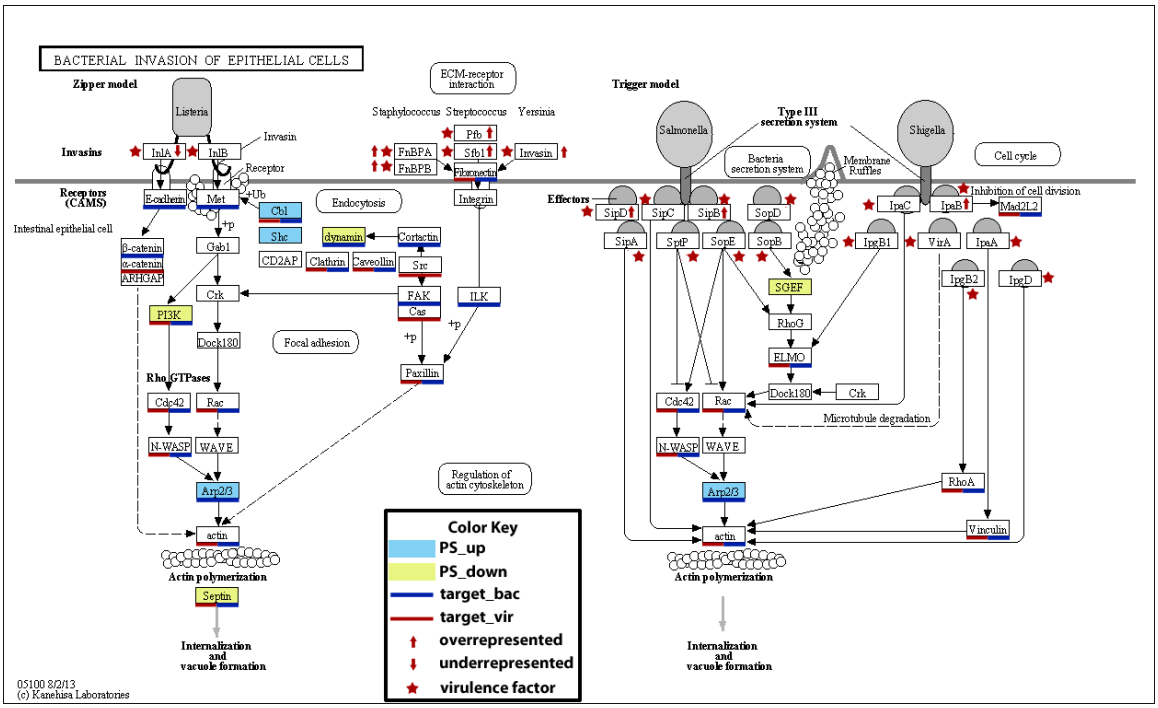

Figure 13 Bacterial invasion of epithelial cells KEGG pathway annotated with the up and downregulated gene signatures for psoriasis. The genes targeted by bacteria (blue) and viruses (red) obtained from PHISTO database are indicated with lines under the gene boxes. The virulence factors obtained from VfDB database are indicated with red stars. The over and underrepresented microbial genes obtained from metagenome analysis of the psoriasis microbiome are marked with red arrows. PS: psoriasis, up: upregulated genes, down: downregulated genes, target_bac: bacterial target, target_vir: viral target.

Discussion

Psoriasis is an autoimmune disease with suspected microbial involvement. The gene contents of skin microbiome were predicted from 16S sequencing data in order to understand the functional perturbations in psoriasis flares across different body sites. The altered metagenome was compared with the microarray gene signatures obtained in Specific Aim 1 to draw parallels between the two sides of the coin.

The OTUs that were significantly altered were members of the phyla, Proteobacteria, Firmicutes, Actinobacteria, and Bacteriodetes. The higher abundances of opportunistic pathogens belonging to these phyla in psoriasis microbiome suggest pathogenic contributions to the maintenance of the psoriatic lesions. For example, Staphylococcus epidermidis had significantly higher abundance in knee psoriasis microbiome compared to healthy controls. Even though it is usually labeled as a skin commensal, recent evidence shows that due to intravenous catheter insertions S. epidermidis is responsible for 22% of the bloodstream infections of the intensive care unit patients and is capable of forming biofilms on medical device surfaces [110]. Streptococcus genus, which had high abundance in the knee psoriasis microbiome, is notorious for having pathogenic strains. Another opportunistic pathogen with high abundance was Anaerococcus, which has been observed in chronic diabetic wounds, suggesting the involvement of bacteria in chronic skin conditions [111]. The pathogenic membership of the bacteria can only be determined accurately with strain level information which can't be obtained with 16S data. However, the genus level information was suggestive of pathogenic contributions to psoriasis.

The OTUs that were significantly altered in the psoriasis microbiome such as Kochuria, Methylobacterium, and Schlegelella are commonly found in soil [112, 113]. Soil associated

bacteria have previously been found on the hand microbiome of Tanzanian women and not on women residing in the United States. This implicates the effect of environment in shaping the microbial skin communities [114]. The abundance differences of soil associated bacteria on psoriasis lesions suggests involvement of environmental factors in psoriasis.

Pathogenic contributions can also be observed in the commonly altered bacterial orthologs across different body sites. While selected members of Type II and Type III Secretion Systems were among the higher abundance orthologs, Type I and Type V Secretion System orthologs were in lower abundance. Type III secretion system utilizes needle like proteins to secrete bacterial proteins to its host. The highly abundant orthologs in psoriasis YscF is a protein that influences the host immune response [115] and is recognized by Toll-like receptors [116]. As mentioned in the first chapter, Toll-like receptor pathway is upregulated in the host psoriasis gene signature. Another high abundance protein YscW is responsible for inducing immunodeficiency during infection as shown in mice [117]. It is also required for the biogenesis of bacterial protein secretion [118]. Type II secretion system is composed of gated channels in the outer membrane and require pilotin proteins to assemble around the channels [119]. A pilotin protein GspS was consistently altered in the psoriasis microbiome. The virulence membrane protein pagC was also highly abundant in psoriasis microbiome. It is regulated by phoP-phoQ and is an essential protein in the pathogenesis process [120]. It is also indirectly involved in the serum resistance of bacteria [121]. Finally, the genes related to plasminogen were in high abundance such as pla and ompT. During infection, pla cleaves the host protein plasminogen activator inhibitor (PAI-1). The gene PAI-1 regulates plasminogen which dissolves fibrin in clots and degrades laminin. However coagulation activates immune response and may help restrain bacteria. Hence, cleaving PAI-1 helps the bacteria hinder the immune system of the

host [122]. The pathogenic genes that were consistently in high abundance in the psoriasis microbiome suggest mechanisms as to how the immune system may be triggered due to dysbiosis of the microbiome.

On the host side, bacterial invasion of epithelial cells pathway was crowded with significantly altered psoriasis genes, consistent with the microbiome signatures observed. For example, the ARP2/3 complex was elevated in psoriasis. The complex, involved in actin polymerization, has been associated with cell crawling motility with the help of N-WASP and WAVE [123], especially for migration of immune cells [124]. ARP2/3 is also significant in organizing tight junctions and maintaining epidermal integrity [125]. ARP2/3 upregulation potentially points to hijacking the host mechanism of bacterial protein transport using actin structures [126, 127]. The high abundance of microbiome genes in this pathway also suggest microbiome involvement. A pathogenic component to psoriasis is also suggested by the shared immune response with Staphylococcus [128, 129] and Streptococcus [130] skin invasions. Together, these results point to a pivotal role the microbial agents play in the initiation and maintenance of psoriatic phenotypes.

The genes that are commonly altered between the host and the microbiome involved metabolic genes. GMPPB and AKR1B10 are both involved in the fructose and mannose metabolism pathway. They were upregulated in the host and were in low abundance in psoriasis microbiome. This finding suggests metabolic competition of the host and the microbiome on the skin. Another metabolic gene commonly altered was AMD1, which is an intermediary enzyme to produce spermidine. This polyamine spermidine, responsible for keratinocyte proliferation, was shown to be significantly higher in psoriasis patients [131]. The analysis also showed that microbiome mirrors and complements the oxidative stress of the host. The gene ATOX1 plays a

role in copper homeostasis. The alteration of the gene's expression can be resulting in elevated copper levels in the serum of psoriasis patients [132].

The OTU and metagenome alterations in the psoriasis microbiome allude to a higher abundance of virulence factors, which can be responsible for triggering or the maintaining the immune response observed in psoriasis flares. The metabolic contributions of the microbiome and the crosstalk should be further evaluated with metabolic analyses.

Specific Aim III: Positive Selection Signals in Psoriasis

The goal of this chapter is to map the selection patterns on the HLA region for populations with different ethnic backgrounds. Due to the geographic differences, populations are subject to different evolutionary pressures. The HLA region is highly involved with immune response and is linked to psoriasis phenotype. I hypothesize that there are population subtype specific regions that are under positive selection. Those regions can be the key to explaining the susceptibility differences that are observed in psoriasis prevalence.

Introduction

Susceptibility to disease is dependent on population history in many cases. GWA studies provide insight into genetic loci responsible for disease pathogenesis. National databases curate GWAS results in order to provide a global view of disease associated polymorphisms [133, 134]. Human Leukocyte Antigen (HLA) region, a 4 Mb long region located on the chromosomal position 6p21, is crowded with immune genes [135] and multiple polymorphisms [136], which creates and extensive repertoire for pathogen identification during immune defense. The region is associated with a multitude of conditions through GWA studies. These associations mainly consist of autoimmune disorders such as psoriasis [137], rheumatoid arthritis [138] and ulcerative colitis [139] as well as infectious diseases such as Hepatitis B [140]and HIV [141, 142]. However, GWA studies are typically limited to a small number of patient ethnic population as representative subjects, which do not capture susceptibility differences between ethnic populations. In this study we investigated the interplay between the SNPs, diseases and

population subtypes within the HLA region using open access genomics datasets and computational tools.

Psoriasis has a strong genetic component. Twin studies show that genetic factors explain 68% of the variation in psoriasis susceptibility and concordance is higher in monozygotic twins than dizygotic twins [143]. Online Mendelian Inheritance in Man (OMIM) [144] has curated 15 regions associated with psoriasis including the HLA region. Within the different HLA haplotypes, HLA-Cw6 and HLA-Bw7 have specifically been associated with psoriasis susceptibility [145].

Prevalence differences in psoriasis can be observed in people with different ethnic and genetic background. A study done on the United States psoriasis patient population shows that Caucasians have the highest prevalence of 3.6%, followed by African Americans with 1.9% and Hispanics with 1.4% prevalence [146]. The susceptibility differences are also reflected in global studies with lowest incidence in Taiwan (0%) and highest in Denmark (8.5%) [4]. These observed susceptibility differences between ethnic populations lead to questions about the selection pressures behind the genetic component of diseases. This can be achieved by understanding the population history of the highly disease associated HLA region in different ethnic populations.

Identifying disease causing SNPs in the HLA region in GWAS studies can be misleading due to high linkage disequilibrium (LD) in the region [147, 148]. Recombination hotspots have been established using linkage disequilibrium based tests in North European [149-151], East Asian [152] and African [153] populations. An integrative study has shown that recombination hotspots vary for different populations, which explains the high diversity of haplotypes [148] as well as the susceptibility differences between populations. This is a comparative study that

follows up on those findings and focuses on identifying positive selection in the HLA region by making use of the haplotype backgrounds of SNPs typed in different ethnic populations.

Recent advances have enabled whole genome sequencing, and SNP detection to be cheaper and faster, which led to large scale projects to catalog common human genetic variants. The HapMap Project provides genotype information on 11 ethnic populations with African, European and Asian ancestry [21, 154]. The main objective of the project was to identify the haplotypes in different ethnic populations to aid in more accurate association results in GWA studies. The diversity of population backgrounds in HapMap make it ideal to study susceptibility differences based on haplotype based statistical tests.

It has been observed by Sabeti et al. that under neutral evolution, low frequency alleles are on longer haplotypes and high frequency alleles are on short haplotypes since haplotypes are broken down by recombination over time as the allele frequencies rise. The long range haplotype test is a method which identifies alleles that have risen to moderate frequency and are on unusual length haplotype blocks [155]. Voight et al. has standardized the detection of positive selection by comparing the heterozygosity of a SNP to the other SNPs in the genome that fall within the same frequency bin [156]. Tang et al. has utilized these ideas to detect positions on the genome that have different selection histories in pairwise population comparisons [157]. In this study, these tests have been utilized to identify alleles of interest in the HLA region.

In this study, SNPs undergoing recent positive selection in a population and SNPs that have different recombination backgrounds in pairwise population comparisons were identified. In order to explore this, polymorphisms typed in Phase III HapMap populations [21, 154] were

analyzed. Pairwise population comparisons were conducted to find SNPs that were statistically significant between populations in order to illuminate prevalence differences. The resulting regions of interest were annotated with disease associations through literature searches. The results are novel as it produces new insights into the effect of positive selection on disease susceptibility in different populations as well as annotation of the HLA region with different evolutionary histories through pairwise population comparisons.

Methods

Identifying Gene Lists

Genes in the HLA region and their sub-region annotations of class I, class II, extended class II and class III were obtained from Shiina et al. [158]. The genomic coordinates of these sub-regions obtained from the hg19 build of the human genome [159] can be seen in Table 8. The Bioconductor GOstats package [55] was used for statistical enrichment of Gene Ontology Biological Process (GO-BP), Gene Ontology Molecular Function (GO-MF) [56] and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [57] for the genes in the whole HLA region and genes in each sub-region.

HLA class	Genic Region (hg19) on Chr 6	# of SNPs in Phased Data	# of SNPs with Ancestral Allele Information
Class I	29680000-31480000	2723	1611
Class III	31480000-32400000	1216	1161
Class II	32400000-33100000	1141	766
Class II extended	33100000-33378000	204	201
Total	29680000-33378000	5284	3739

Table 8: The HLA region coordinates on chromosome 6 partitioned according to their functional class annotations as described in Shiina et al. The coordinates are based on the hg19 build of the genome. The number of SNPs included in the phased genotypes of the HapMap populations as well as the number of phased SNPs that have ancestral annotations according to the EPO pipeline.

Obtaining Population Level Data

The phased genotypes and frequency data were downloaded for each ethnic population typed in Phase III of the HapMap [21, 154] project. The HapMap database includes populations with East Asian ancestry (Japanese in Tokyo-JPT (91 samples), Han Chinese in Beijing-CHB (90 samples), Chinese in Denver-CHD (100 samples)), European ancestry (Europeans of Northern and Western Ancestry-CEU (180 samples) and Toscana- TSI(100 samples)), African ancestry (Yorubans-YRI (180 samples), Masaai-MKK (180 samples), Luhya-LWK (100 samples), and African Americans-ASW(90 samples)) and other ethnic groups (Gujaratis in Houston-GIH (100 samples), and Mexicans in Los Angeles-MEX(90 samples)), making it ideal for identifying susceptibility SNPs. The SNP positions were lifted over from hg18 to hg19 build using UCSC tools [160]. The hg19 build of the human ancestral genome was acquired from the 6-way primate genome alignment using the Enredo-Pecan-Ortheus (EPO) pipeline [22, 161, 162].

Population Differences

The SNPs that are common between all populations were extracted from the frequency data. For each pair of populations, pairwise allele frequency distribution for each subclass as well as for the whole HLA region were calculated with Kolmogorov-Smirnov (KS) test [163]. The p values were corrected for false discovery rate (FDR) using Benjamini-Hocheberg correction [58]. The same test was carried out for 20 concatenated autosomal loci that are under neutral selection as described by Wall et al. [164] in order to observe neutral estimate of allelic variation and to establish that the frequency differences seen at the HLA regions are not a result of demographic forces but signify selection.

For a population, the Integrated Extended Haplotype Homozygosity (IHH) [155] and the Integrated Site-specific EHH (iES) [157] of the ancestral and derived alleles were calculated for each typed SNP in order to find the transmission of extended haplotypes without recombination. The IHH of a SNP was standardized with respect to the results from the whole genome that fall within the same 0.025 frequency bin using the Standardized IHH (iHS) test [156]. SNPs with a minor allele frequency of 0.05 were discarded from the analysis since the iHS method is not applicable for alleles that are fixed or almost fixed in a population. Finally standardized ratio of iES from pairwise populations (Rsb) were calculated for each SNP [157]. The resulting p values of the iHS and Rsb tests were adjusted for multiple testing using Benjamini-Hochberg FDR correction [58]. The HLA region of interest was then extracted from the whole genome analyses.

Polymorphism Annotations

The SNPs were annotated according to their location as intergenic, intron, coding, UTR and promoter [165]. The intergenic SNPs were annotated with genes present within a 20 Kb upstream and downstream window. The SNPs that are under positive selection and the genes they are on are also cross-referenced with diseases they are associated with through MalaCards [166] and National Human Genome Research Institute GWAS Catalog [133].

Results

This study presents a systematic approach to detect positive selection in the extended HLA region in order to explain demographic susceptibility differences of HLA linked conditions. The SNPs that are associated with a condition can be hitchhiking on the haplotype blocks that are under selection. By detecting the driving SNPs on the haplotype blocks, more information on the hitchhiking SNPs can be obtained.

The analysis utilizes genotypes of 11 HapMap populations [21, 154] consisting of 2022 haplotypes with 12622 typed SNPs in the HLA region. Among these typed SNPs, 5284 of them have phased data and 3739 have ancestral alleles deduced from the EPO pipeline [22, 161, 162]. The definitions of the sub-classes of the HLA region as well as the breakdown of the number of SNPs typed in each sub-region can be seen in Table 8. The phased data of the SNPs are then used to find positive selection in each HapMap population, as well as to find SNPs that have significantly different population histories. The resulting SNPs of interest are then annotated

with diseases they are associated with through GWA studies. The overall workflow is summarized in Figure 14.

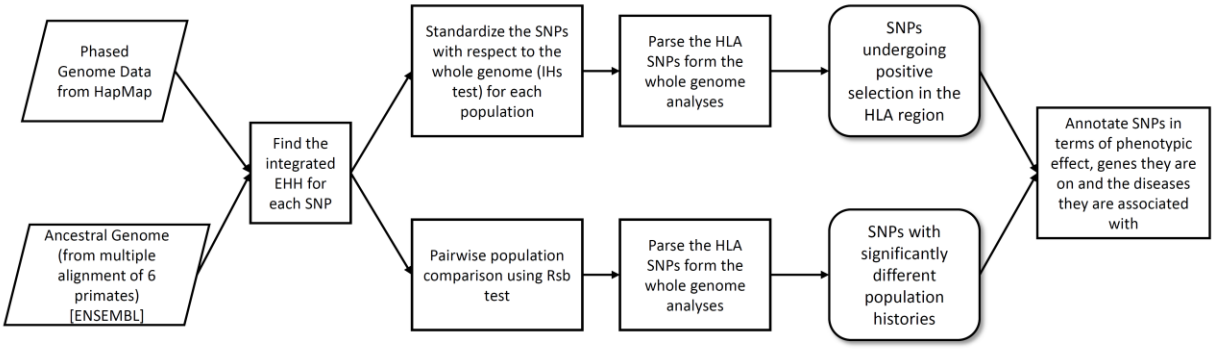

Figure 14 Flowchart of the analysis shows the steps taken to obtain the SNPs that are under positive selection in the HLA region for each population as well as identifying the SNPs that have significantly different population histories.

Functional Annotation of the HLA Region

The HLA is a dense genic region with exclusive immune function. This is represented by the functional annotations of genes in this region through Gene Ontology [56] and KEGG pathways [57]. The KEGG annotations are represented in the Table 9. KEGG pathways that are enriched with genes in the HLA region are involved in autoimmune conditions such as type-I diabetes, asthma and rheumatoid arthritis as well as infections such as Leshmanaiasis, Staphylococcus aureus and Toxoplasmosis. The biological processes they are involved in include interferon-gamma-mediated signaling pathway, antigen processing and presentation and positive regulation of immune response. Top molecular function terms that are enriched with HLA genes include peptide antigen binding, MHC class II receptor activity and amide binding. The

annotations are primarily for the Class I and Class II, while Class III and extended Class II region do not have any significant terms associated with them.

Pathways	Class I	Class II	all HLA
Antigen processing and presentation	0.08	0.18	0.33
Allograft rejection	0.16	0.32	0.51
Autoimmune thyroid disease	0.12	0.23	0.35
Cell adhesion molecules (CAMs)	0.05	0.09	0.14
Graft-versus-host disease	0.15	0.29	0.46
Phagosome	0.05	0.09	0.14
Type I diabetes mellitus	0.14	0.28	0.47
Viral myocarditis	0.09	0.17	0.26
Asthma		0.40	0.43
Endocytosis	0.03		0.04
Intestinal immune network for IgA production		0.25	0.25
Leishmaniasis		0.17	0.18
Natural killer cell mediated cytotoxicity	0.04		0.06
Rheumatoid arthritis		0.13	0.16
Staphylococcus aureus infection		0.22	0.27
Systemic lupus erythematosus		0.09	0.11
Toxoplasmosis		0.09	0.12
ABC transporters		0.05	
Hematopoietic cell lineage		0.03	
Primary immunodeficiency		0.06	
Proteasome		0.05	
Spliceosome			

Table 9: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways that are enriched with all HLA genes and sub-classes of genes. The terms are significant (Benjamini < 0.01) in at least 1 gene list. The terms are listed as most commonly enriched to region specific terms. The values represent the ratio of genes in the term that overlap with the gene list. The cells that are left blank signifies that the corresponding term is not enriched for that gene list. There were no terms enriched for extended class II region, hence its corresponding column is not shown.

Allele Frequency Distributions of HLA sub-Region

The allele frequency distributions of the HLA region were compared between populations with Kolmogorov-Smirnov (KS) test in order to find the populations that have distinguishing patterns of allele histories. The –log10 of the Benjamini corrected p values of pairwise tests are represented as heatmaps in Figure 15. The results for the whole HLA region show significantly different allele frequency distributions for CEU, TSI and JPT populations compared to all other ethnic populations. When the HLS region is broken down into sub-regions, it is seen that each sub-region has a different population history, Class I region being the greatest contributor to frequency distribution diversity between populations. European ancestry (CEU and TSI) difference is apparent in Class I region whereas East Asian (JPT+CHB+CHD) and CEU difference dominates in the Class II region. In the extended Class II region, regional coherence patterns can be observed where the allele frequency distributions are similar within African, East Asian and European descent populations. When the test is applied to neutrally evolving autosomal regions in the genome [164], there were no significant differences between the populations. This signifies that the allele frequency distribution variations are not a result of population demography but can explain different selection signals in populations.

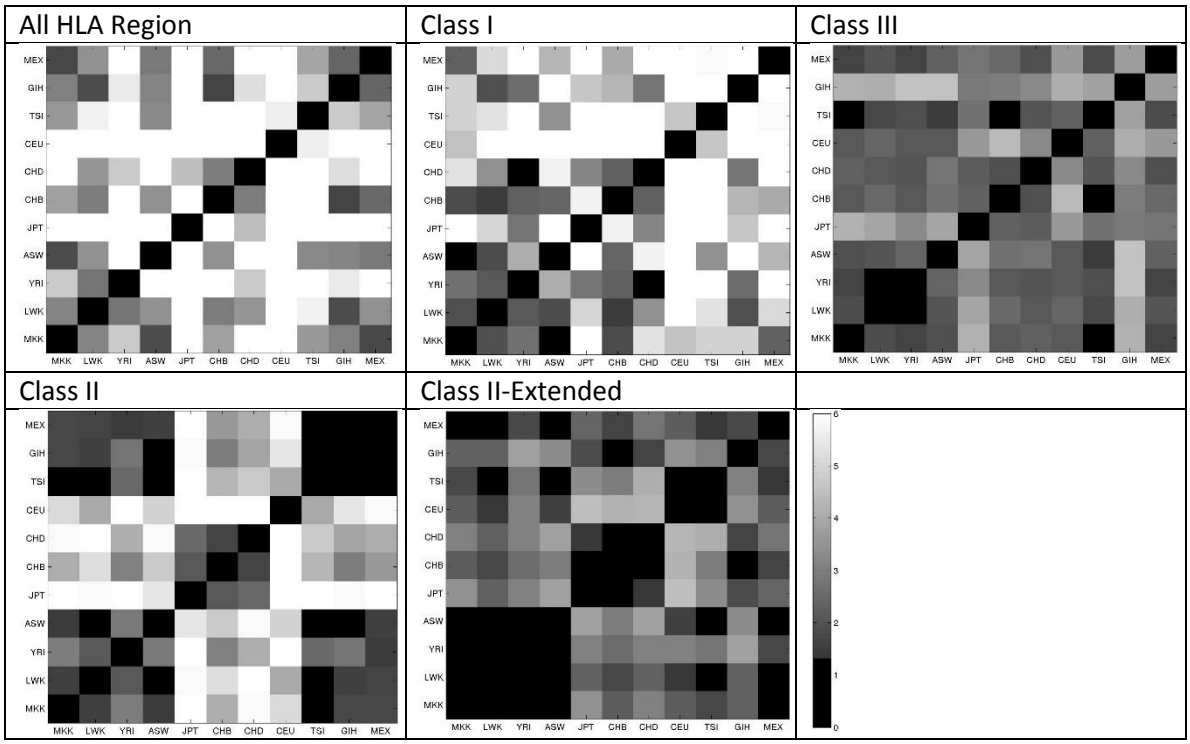

Figure 15: Heatmaps of pairwise SNP allele frequency distribution comparisons using the Kolmogorov-Smirnov (KS) test for each cluster. The values represent the -log10 of the Benjamini FDR corrected KS-test p values. The comparisons that are not significant (Benjamini >0.05) are in black.

Polymorphisms on the HLA that are Under Positive Selection

To further investigate the selection signals, recent positive selection on the HLA variants that have not yet reached fixation have been calculated with the iHS test. The –log10 of the corrected p values for different ethnic populations have been overlapped on the scatter plot in Figure 15. A complete and more detailed annotations of the significant SNPs, as well as the genes they fall within are provided in Appendix C. The tests are also able to detect if the selection is on the ancestral or the derived allele. With the exception of five SNPs, selection signals are on derived alleles. Similar to the results obtained from the KS heatmaps, there are

European ancestry specific selection peaks in Class I region. Coding and intronic SNPs that are on PSORS1C1, TCF19, MUC22, TRIM10 and TRIM15 are under positive selection with a Benjamini cutoff of 0.01. The polymorphisms with the highest significance value, rs3130557, is driving the selection in both CEU and TSI populations. The SNP is located on the intronic region on the PSORS1C1 gene. In the Class III region, there is positive selection signals for the East Asian populations, especially on the genes NOTCH4, C6orf10 and BTNL2. The driving polymorphism across populations are rs3132946, rs3830041, rs8192565 and rs12055568 which are intronic or promoter SNPs on the NOTCH4 gene. The East Asian specificity continues on to the Class II genes with positive selection on TAP2. There is African ancestry specific positive selection signals on Class II genes, specifically HLA-DOA and HLA-DPA, both HLA class II alpha chain paralogues expressed in antigen presenting cells. The driving SNPs in this region are rs3129304, rs3129303 and rs10947368 which are found on 3' UTR and coding region of the HLA-DOA gene. The SNPs of interest from the iHS test and the genes that they fall under have been annotated through the MalaCards database and summarized in Table 10. The associations range from autoimmune conditions such as psoriasis, rheumatoid arthritis and celiac disease to infections such as HIV-I.

The tests were followed up with pairwise population comparisons of haplotype background using the Rsb test. The SNPs that are statistically significant between populations from different geographical backgrounds are visualized in Figure 17. The results from the iHS test have been confirmed with the Rsb test. The SNPs that distinguished between ethnic regions were on PSORS1C1, NOTCH4, C6orf10 and HLA-DOA. The SNP rs3130557 on PSORS1C1 gene that is under positive selection for the European populations show differences in ASW and TSI populations. The SNPs that are on HLA-DOA gene under positive selection for the LWK population also shows significant difference with the TSI population. These SNPs can be responsible for the

susceptibility differences that is observed between populations for the diseases associated with the genes.

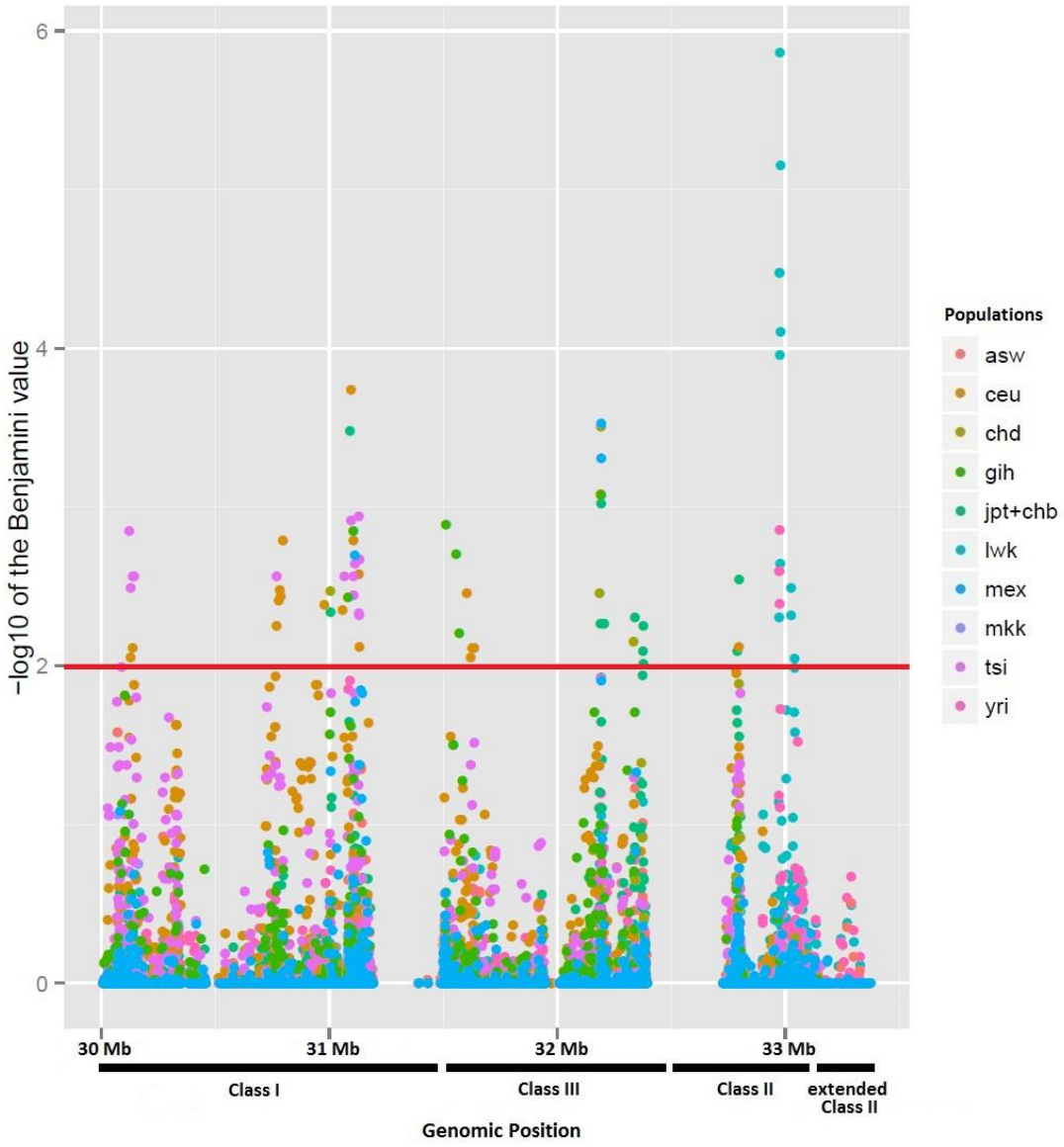

Figure 16: Superposition of –log10 of the Benjamini corrected iHS p-values of all HapMap populations along the HLA region. The horizontal red line represents cutoff for significance (Benjamini < 0.01). Class I region shows evidence of positive selection with European (CEU, TSI) specificity, non-specific ethnic background positive selection in Class III, African (LWK, YRI) specificity in the Class II region and no positive selection in extended Class II region.

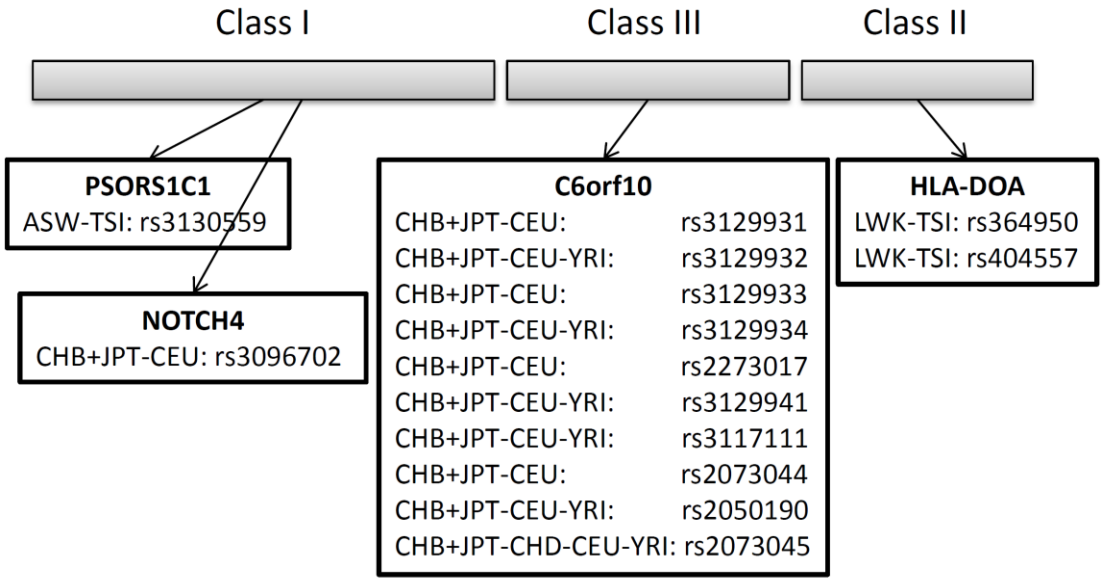

Figure 17: The SNPs with significantly different evolutionary backgrounds between at least two populations belonging to different geographical regions (Benjamini < 0.01). The standardized ratio of integrated site-specific EHH from pairwise populations (Rsb) on the HLA region were calculated. Each box represents a gene annotated with the SNPs that have significantly different evolutionary backgrounds. The genes are labeled with the HLA sub-regions they belong to.

Overall, the study shows that there are discrete patterns of selection for populations that belong to different geographical regions. Selections signals are concentrated in the Class I region for the European descent populations and Class II for the African descent populations.

	Gene	Associated Disease				
		autoimmune	infection	inherited	cancer	other
Class I	TRIM31		lymphocytic choriomeningitis			
	TRIM26				endodermal sinus tumor	seizure disorder
	HCG22				follicular lymphoma	
	CDSN	guttate psoriasis		hypotrichosis simplex, peeling skin syndrome		
	C6orf15				follicular lymphoma	
	PSORS1C1	psoriasis, epidermal necrolysis, stevens-johnson syndrome, rheumatoid arthritis				
	CCHCR1	psoriasis				
	TCF19			tetraploidy		
	PSORS1C3	psoriasis				
	POU5F1				Pineoblastoma, germinoma, teratoma	seborrheic keratosis
Class III	DDX39B	rheumatoid arthritis				
	LST1					hyperbilirubinemia
	PRRC2A	rheumatoid arthritis, diabetes mellitus				coronary artery aneurysm
	APOM	type 2 diabetes mellitus				limb ischemia
	CSNK2B	egg allergy				
	NOTCH4				salivary gland tumor	schizophrenia, pseudobulbar palsy
	BTNL2	sarcoidosis, wegener's granulomatosis				
Class II	TAP2	wegener-like granulomatosis, ankylosing spondylitis, cardiac sarcoidosis, celiac	echinococcosis	type I bare lymphocyte syndrome	melanoma	
	HLA-DOA		HIV-I			
	HLA-DPA1	chronic berylliosis, wegener's granulomatosis, myelitis, neonatal lupus erythematosus, rheumatoid arthritis	rubella			pulmonary hypertension
	HLA-DPB1			dysplasia		

Table 10: Diseases that are associated with the genes under positive selection (iHS Benjamini < 0.01) in at least one HapMap population. The diseases associated with the gene under positive selection are separated as autoimmune, infection, inherited, cancer and other. Genes are sorted according to their genomic positions. The associated diseases are obtained through the MalaCards database.

Discussion

Psoriasis is a skin condition with a strong genetic component. HLA region has shown associations with the condition through GWAS studies. In this chapter, the SNPs that are undergoing positive selection in the HLA region have been identified through haplotype based statistical methods.

The overall results showed partitioning of the positive selection signals according to ethnic background of populations. Positive selection on the Class I region was observed exclusively in populations with European background. Genes that are on the MHC Class I region are mainly to differentiate self from non-self. Hence they are highly associated with autoimmune conditions. Positive selection on the Class II region could be observed in populations with African ancestry. Genes on this region are necessary for establishing an immune response. The positive selection patterns are consistent with the infection based evolutionary pressures that are present in the African ancestry populations [167].

The SNPs that are on the intronic region of PSORS1C1 are undergoing positive selection in the European ancestry populations. The gene has been associated with psoriasis [168] and its comorbidity rheumatoid arthritis [169]. These results are consistent with the observation that psoriasis has a higher prevalence in the Caucasian ethnic population than African ancestry populations [4]. The driving SNP with the highest p value for positive selection signal is also on the PSORS1C1 gene. The SNP rs3130557 is associated with multiple autoimmune conditions such as ulcerative colitis [170], Crohn's [171] and coeliac diseases [134] as well as association with glycemic traits [172]. It also shows significant difference between the TSI and ASW populations according to the cross population test performed. The positive selection in the

region may be the driving force behind the high psoriasis rates in the Caucasian population through genetic hitchhiking.

The gene undergoing positive selection in the East Asian ancestry populations is NOTCH4. It is a gene that is responsible for cell fate regulation. NOTCH4 has been associated with schizophrenia in the Chinese Zhuang and Chinese Han population for the SNP rs3131296 [173]. Even though that SNP is not significant in our analysis, it is within 20kb of the positively selected region. This may again indicate that the SNP that is associated with a condition may be a result of positive selection in the neighboring regions for a population.

Genes that are under positive selection in the African population comprise of HLA-DOA. It is a gene that is expressed in B cells. Even though the nucleotide sequences are well conserved among these species in order to conserve function [174], positive selection has been observed in the African ancestry population. It has also been associated with HIV [166] which poses as a recent environmental pressure in the geographical region.

A limitation of this method is the dependence of the calculations on the ancestral genome build. The ancestral genome obtained from the EPO pipeline is not mapped for genes such as HLA-C and HLA-B genes potentially due to the high variance in those genes. Therefore the selection signatures on the coding regions of those genes could not be obtained. However, positive selection detected on the non-coding regions can contribute to the regulation of the genes and provide equally valuable information.

Overall, the positive selection signatures on the HLA region can help understand the driving pressures behind susceptibility to conditions in different ethnic populations, including a complex disease with genetic origins such as psoriasis.

Conclusions

Psoriasis is a complex disease with genetic and environmental dimensions. Psoriasis is linked to a combination of multiple risk factors such as the genetic susceptibility polymorphisms in the HLA region and the suspected microbial involvement. In order to understand the interplay between the different factors, an integrated survey of the high throughput datasets need to be conducted. This thesis presents the first study to integrate the microarray and microbiome results for psoriasis in order to evaluate the cross talk between the host and the microbiome. It is also the first study to provide a metagenome analysis of the skin microbiome even if the gene content was predicted from 16S abundance data.

In this thesis, psoriasis transcriptome was studied using open source mRNA microarray datasets which provided a large cohort of samples to analyze psoriasis in the context of other inflammatory skin conditions, skin cancer, and wound healing. Psoriasis transcriptome was highly consistent across different studies which suggests that the manifestation of the disease is uniform across different patient cohorts and the technologies that have been used were reliable. The genes that are consistently altered across different datasets provided a distinctive signature for psoriasis from other inflammatory skin conditions. On the other hand, transcriptome similarities could be observed between psoriasis and wound healing. This was consistent with clinical observations that psoriasis is a disease of hyper-proliferating keratinocytes with an inflammatory component. The pathways that were enriched with significantly altered psoriasis genes showed inflammatory signatures. The genes that are exclusive to either psoriasis or wound healing such as IL-19, CXCL9, and CXCL11 can be explored as drug targets. This would reduce the side effects and balance between the healthy

inflammatory responses needed for healing and the inflammatory response that is responsible for the autoimmune aspect. Psoriasis gene signature also suggested a pathogenic component to the disease. Its gene signature overlapped with pathogenic KEGG pathways such as bacterial invasion of epithelial cells. The upregulation of immune response and antimicrobial genes as well as their overlap with pathogenic pathways required a microbiome analysis to illuminate the role of crosstalk between the host and the microbiome in the maintenance of psoriatic flares.

The microbiome of psoriasis has been evaluated through multiple 16S studies to compare the psoriasis flares as well as healthy control cohorts. Even though 16S data is very useful in understanding the bacterial composition of the skin, 16S alone does not answer questions about the functional perturbations. Until the DNA extraction and technologies are developed to reliably conduct a metagenomics analysis of the skin microbiome, 16S and previously annotated bacterial genomes can be used to predict the genomic content of a microbial community. This thesis provides a novel approach to understand the functional perturbations caused by the microbiome in psoriasis flares. The results from the analysis of open source 16S psoriasis datasets show overabundance of opportunistic bacteria such as the OTUs belonging to the genera Corynebacterium and Staphylococcus in the psoriasis microbiome. The functional perturbations that are common across different body sites show an overabundance of virulence factors in the psoriasis microbiome such as yeeJ (adhesion / invasion), yscF, sctF, yscW, sctW (type III secretion proteins), pagC (putative virulence related protein), pla, ompT (plasminogen related genes), gspS (type II secretion proteins), and acfC. The overabundance of the virulence factors and their intersection with the bacterial invasion of epithelial cells pathway suggests virulence factors affecting the pathogenesis or maintenance of the psoriasis flares.

Aside from the environmental factors affecting psoriasis, genetic factors also play great part in the susceptibility to psoriasis. Extended HLA is a 4 Mb long region located on the chromosomal position 6p21. It is a gene dense region with critical immune functions for initiating the adaptive immune response and distinguishing self from non-self. Single nucleotide polymorphisms (SNPs) and genes in this region have been associated in literature with many conditions, including psoriasis. In order to identify susceptibility SNPs in the HLA region, the SNPs typed in the HapMap data (11 ethnic populations) within the HLA region have been analyzed using extended haplotype homozygosity based tests to identify positive selection on polymorphisms that have not yet reached fixation. Results show regional specificity of positive selection signals on the sub-classes of HLA. The positive selection signals in Class I sub-region show European ancestry specificity with intronic SNPs on genes PSORS1C1, TCF19, MUC22, TRIM10 and TRIM15. In the Class III region, there is positive selection signals for the East Asian populations, especially on the genes NOTCH4, C6orf10 and BTNL2. Finally, there is African ancestry specific positive selection signals on Class II genes, specifically HLA-DOA and HLA-DPA; both HLA class II alpha chain paralogues expressed in antigen presenting cells. The cross population tests show there are more haplotype differences within the same geographical populations than between. Similar to single population tests, cross population test results show that the significant SNPs are concentrated in the Class II region for African ancestry populations, whereas for European ancestry populations, they are concentrated in the Class I region.

Three techniques were developed and applied in this thesis to explore a complex skin condition. The methods used in the microarray analysis of psoriasis revealed that psoriasis had consistent gene signatures that showed upregulation of immune function, keratinization, and antimicrobial activity. The microbiome analysis showed that there were composition differences in psoriasis

patients compared to healthy cohorts. The analysis also demonstrated that the functional perturbations presented an overabundance of virulence factors. Scanning the HLA region for positive selection across populations with different ethnic backgrounds indicated that there are region specific signals for each class of HLA genes. Together, these approaches comprise a systems biology approach to tackle complex diseases. The methods that are explored in this thesis can be applied to other conditions to better understand their mechanisms, explore the environmental effects, and identify new drug targets.

Future Work

This research describes a pipeline to evaluate the gene expression and microbiome perturbations linked to psoriasis as well as the genetic implications of a complex disease. The explained pipeline can be extended to evaluate other autoimmune disease and complex conditions as more open source data accumulates.

The biomarkers for psoriasis expression in the host that have been identified through computational analysis need to be validated for accuracy and efficacy in patient population. The microbiome genes identified through metagenome prediction in the second chapter also need to be validated through deep sequencing, preferably for the same patient population. These require a large patient cohort, IRB approval and assay development, which is beyond the scope of the project.

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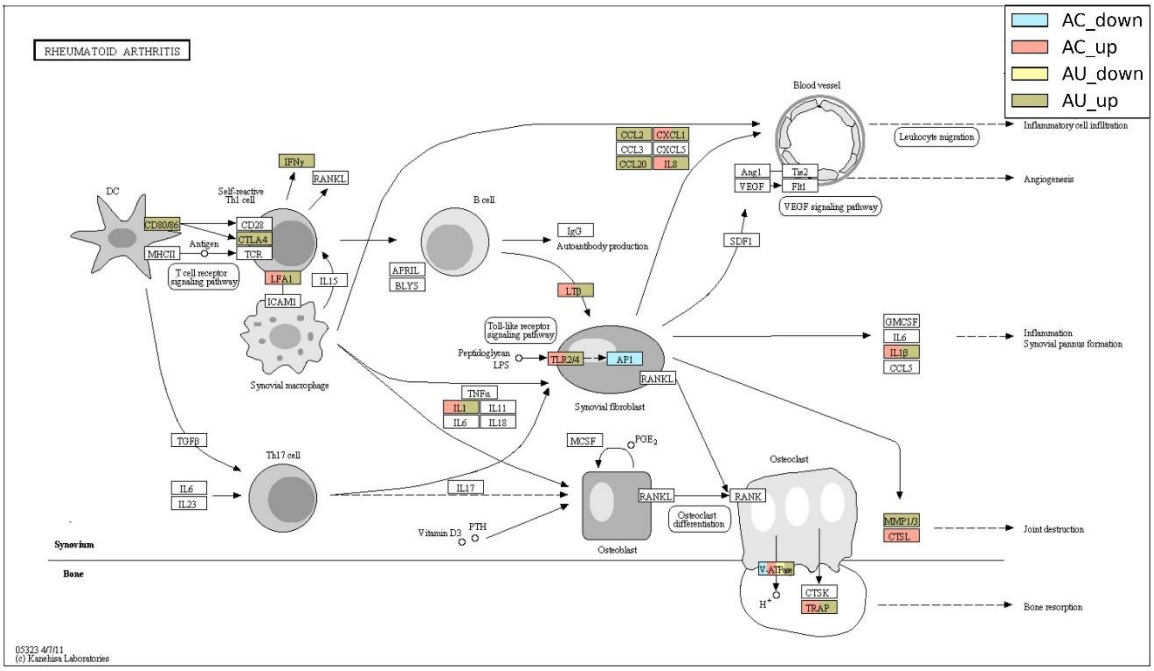
Appendices

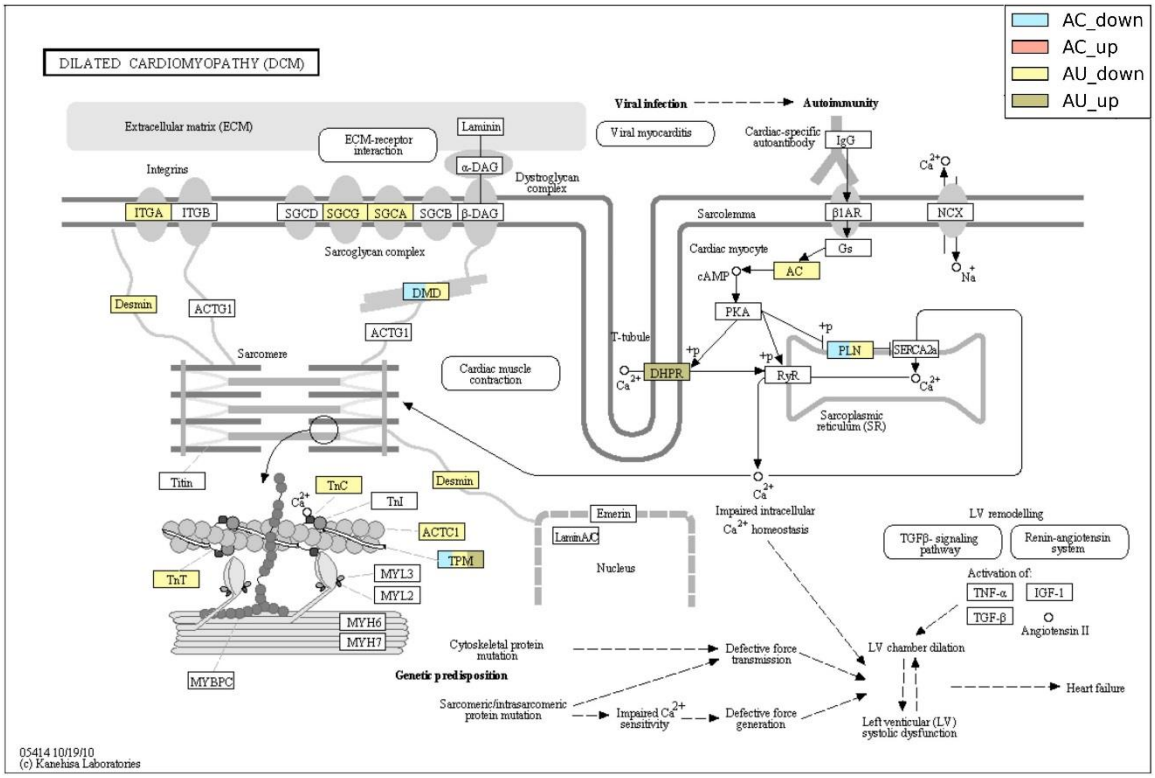
Appendix A

Psoriasis upregulated genes (Benjamini < 0.01 and fold change > 1.5) overlapped with Rheumatoid Arthritis KEGG pathway. Genes belonging to IL-17 immune response are commonly altered between psoriasis and rheumatoid arthritis. AC_down: psoriasis A/C consensus downregulated gene signature, AC_up: psoriasis A/C consensus upregulated gene signature, AU_down: psoriasis A/U downregulated consensus gene signature, AU_up: psoriasis A/U upregulated consensus gene signature.



Appendix B

Psoriasis upregulated genes (Benjamini < 0.01 and fold change > 1.5) overlapped with Dilated Cardiomyopathy KEGG pathway. The pathway is crowded with downregulated genes in psoriasis transcriptome. AC_down: psoriasis A/C consensus downregulated gene signature, AC_up: psoriasis A/C consensus upregulated gene signature, AU_down: psoriasis A/U downregulated consensus gene signature, AU_up: psoriasis A/U upregulated consensus gene signature.



Appendix C

The average frequencies of the HLA alleles that are under positive selection (iHS Benjamini < 0.01) in at least one population. The SNPs under positive selection are in bold. The SNPs are labeled according to the sub-regions they are located on (Class I, Class III, Class II). The allele column represents if the selection is on the ancestral allele (A) or derived allele (D). The SNPs are sorted according to their genomic positions. They are also annotated with the gene they are located on (intron, coding, UTR, promoter) or around (intergenic within 20K basepairs). The iHS is not calculated for frequencies above 0.95 and below 0.05. The SNPs that don't have any frequency data for a population are left blank.

			Average Frequencies of the Alleles under Selection														For intergenic SNPs	
	SNPs	**Allele**	**mkk**	**lwk**	**yri**	**asw**	**chb**	**jpt**	**chd**	**ceu**	**tsi**	**gih**	**mex**	**position**	**location**	**Gene Symbol**	**Preceding Gene**	**Following Gene**
Class I	rs9261434	D	0.042	0.023	0.044	0.026	0.004	0.033	0.009	0.106	**0.123**	0.079	0.052	30087776	intergenic		TRIM40	TRIM31
	rs3094134	D		0.005	0	0.009	0	0.015	0.009	0.093	**0.108**	0.03	0.043	30122154	coding	TRIM10		
	rs1573296	D	0.042	0.005	0		0	0		**0.111**	**0.049**		0.026	30127805	intron	TRIM10		
	rs2523729	D	0.022	0.064	0.058	0.035	0	0		**0.111**	**0.049**		0.017	30137387	intron	TRIM15		
	rs9261538	D		0.005		0.009		0.015	0.009	0.088	**0.103**	0.035	0.043	30143491	intergenic		TRIM26	TRIM10, TRIM15
	rs3129988	D			0	0.009	0	0		**0.084**	**0.059**	0.005	0.026	30769478				
	rs1264361	D	0.01	0.023	0.02	0.053		0.007	0.005	**0.128**	0.049	0.015	0.043	30777498				
	rs886424	D	0.01	0.023	0.021	0.053	0	0.007	0.005	**0.128**	0.05	0.015	0.043	30782002				
	rs1264353	D			0.024		0	0		**0.172**				30787762				
	rs1264350	D	0.013	0.027	0.02	0.061	0.004	0.044	0.037	**0.125**	0.083	0.005	0.043	30796545				
	rs1634721	D	0.006	0.018	0.003		0	0		**0.116**	0.044		0.026	30977680	intron	MUC22		
	rs9357105	D			0.014		**0.066**	**0.117**	**0.101**	0	0.005	0.005	0.009	31003923	intergenic		HCG22	MUC22
	rs3130544	D		0.018	0.003		0	0		**0.115**	0.054		0.026	31058340				

	rs2256962	D				0.018				0.102	**0.054**	0.005	0.035	31065620	intergenic		CDSN, PSORS1C1, C6orf15	
	rs2233956	D	0.029	0.023	0.01	0.053	0.009	0.026	0.028	0.204	0.137	**0.208**	0.086	31081205	promoter	PSORS1C1		
	rs4410768	D	0.026	0.041	0.003	0.018	**0.106**	**0.022**	0.014	0.009	0.005	0.094	0.009	31089708	intron	PSORS1C1		
	rs3130557	D		0.032	0.024	0.035	0	0		**0.115**	**0.069**		0.026	31094703	intron	PSORS1C1		
	rs28732100	D	0.022			0.018	0.009	0.029	0.028	0.084	0.049	**0.198**	0.017	31104593	intron	PSORS1C1		
	rs2233952	D			0	0.035	0.031	0.069	0.046	**0.133**	**0.098**	0.059	0.086	31105891	intron	PSORS1C1		
	rs1265097	D	0.101		0.222	0.102	0.022	0.102	0.043	0.099	**0.167**	0.126	0.245	31106459	coding	PSORS1C1		
	rs1265083	D	0.022	0.009	0.054		0	0		0.004	0.015		**0.069**	31111347	intron	CCHCR1		
	rs130072	D	0.019	0.05	0.082	0.026	0.022	0.077	0.041	0.071	**0.137**	0.1	0.147	31112484	coding	CCHCR1		
	rs7750641	D	0.013	0.023	0.003		0.004	0.004	0.005	**0.112**	**0.059**	0.005	0.034	31129310	coding	TCF19		
	rs2073724	D	0.019	0.05	0.092	0.035	0.022	0.077	0.041	0.071	**0.137**	0.099	0.147	31129707	coding	TCF19		
	rs17190776	D	0.019	0.05	0.092	0.035	0.022	0.077	0.041	0.071	**0.137**	0.099	0.147	31130865	threeUTR	TCF19		
	rs3130933	D	0.048		0	0.026	0.062	0.029	0.009	**0.159**	**0.093**	0.03	0.043	31132085	intergenic	NA	PSORS1C3, POU5F1	CCHCR1, TCF19
Class III	rs9267487	D	0.157	0.068	0.017	0.061	0.018	0.033	0.018	0.066	0.064	**0.163**	0.121	31511350	promoter	DDX39B		
	rs28732144	D	0.105	0.055	0.003	0.018		0.018	0.014	0.058	0.029	**0.158**	0.034	31556205	intron	LST1		
	rs17207190	D	0.099	0.055	0.003	0.018		0.018	0.009	0.053	0.029	**0.15**	0.034	31569520	intergenic		AIF1, PRRC2A	NCR3, LTB, LST1
	rs35502919	D				0.018				**0.058**	0.01			31604355	coding	PRRC2A		
	rs3117582	D	0.048	0.014	0.041	0.026	0	0		**0.08**	0.064		0.026	31620520	intron	APOM		
	rs3132449	D	0.049	0.023	0.044	0.026				**0.08**	0.064		0.035	31626013	intergenic		CSNK2B, C6orf47, LY6G5B, GPANK1, LY6G5C	APOM, BAG6
	rs9267531	D	0.045	0.023	0.048	0.026	0	0		**0.08**	0.064		0.034	31636742	threeUTR	CSNK2B		
	rs2854050	D	0.042	0.037	0.027	0.035	0.221	0.199	**0.174**	0.062	0.034	0.069	0.123	32185605	intron	NOTCH4		
	rs2071282	D		0.005	0.003		**0.102**	**0.062**	0.032	0.004		0.01	0.018	32188943	coding	NOTCH4		
	rs3132946	D			0.007	0.018	0	0.018	0.009	**0.168**	0.074	0.03	0.052	32190028	intron	NOTCH4		
	rs3830041	D	0.256	0.2	0.218	0.123	0.195	0.12	0.092	0.084	0.108	**0.183**	**0.121**	32191339	intron	NOTCH4		
	rs8192565	D	0.035	0.005	0.031	0.079	**0.133**	**0.088**	0.096	0.075	0.113	0.153	0.052	32191607	intron	NOTCH4		
	rs12055568	D	0.074	0.046	0.007		0.128	0.193	**0.197**	0.013	0.039	0.005	0.121	32192083	promoter	NOTCH4		

	rs549182	D	0.105	0.138	0.106	0.096	**0.15**	**0.106**	0.069	0.009	0.044	0.11	0.086	32205045	intergenic			NOTCH4
	rs549270	D	0.125	0.164	0.126	0.158	**0.15**	**0.109**	0.087	0.013	0.07	0.149	0.138	32205072	intergenic			NOTCH4
	rs2073047	A			0		0.084	0.15	**0.156**	0			0.026	32335899	intron	C6orf10		
	rs2076535	D	0.003	0.005			**0.181**	**0.077**	0.018	0.004	0.005	0.054	0.017	32339511	fiveUTR	C6orf10		
	rs3763309	D	0.077	0.041	0.071	0.088	**0.084**	**0.102**	0.11	0.265	0.162	0.198	0.267	32375973	promoter	BTNL2		
	rs3763310	D			0		**0.066**	**0.047**	0.046	0				32376103	promoter	BTNL2		
	rs3763312	D	0.077	0.036	0.071	0.088	**0.085**	**0.103**	0.11	0.265	0.158	0.198	0.267	32376348	promoter	BTNL2		
Class II	rs9784876	A	0.154	0.195	0.272	0.246	**0.106**	**0.018**	0.009	0.058	0.094	0.104	0.086	32788878	intron	TAP2		
	rs4148876	D	0.064		0	0.018	0.167	0.085	0.042	**0.093**	0.049	0.035	0.103	32796793	intron	TAP2		
	rs2228391	D	0.006	0.005			**0.049**	**0.089**	0.087			0.045		32797773	coding	TAP2		
	rs3128935	D	0.157	**0.359**	0.293	0.228	0.164	0.172	0.165	0.04	0.054	0.104	0.095	32972404	threeUTR	HLA-DOA		
	rs3129304	D	0.053	**0.252**	**0.219**	0.205	0.04	0.084	0.041	0.159	0.114	0.03	0.371	32973743	threeUTR	HLA-DOA		
	rs3129303	D	0.074	**0.264**	**0.221**	0.211	0.04	0.088	0.046	0.159	0.114	0.03	0.371	32973878	threeUTR	HLA-DOA		
	rs10947368	D	0.051	**0.243**	**0.221**	0.105	0	0		0				32975341	coding	HLA-DOA		
	rs364950	A	0.087	**0.405**	**0.299**	0.193	0	0		0.04	0.044		0.034	32975896	coding	HLA-DOA		
	rs404557	D	0.087	**0.405**	0.306	0.193	0	0		0.04	0.044		0.034	32976927	intron	HLA-DOA		
	rs3763342	A	0.201	**0.366**	0.4	0.36	0.354	0.233	0.192	0.133	0.213	0.273	0.172	32978997	promoter	HLA-DOA		
	rs3763341	A	0.125	**0.268**	0.226	0.184	0.353	0.19	0.154	0.106	0.153	0.267	0.112	32979020	promoter	HLA-DOA		
	rs2116263	D	0.056	**0.255**	0.243	0.158	0.465	0.471	0.573	0.031	0.02	0.079	0.017	33025493	intergenic		HLA-DPA1, HLA-DPB1	
	rs4551215	D	0.099	**0.257**	0.252	0.184	0.465	0.533	0.61	0.04	0.025	0.149	0.026	33025700	intergenic		HLA-DPA1, HLA-DPB1	
	rs4582419	D	0.055	**0.257**	0.245	0.184	0.478	0.537	0.602	0.045	0.025	0.149	0.034	33040138	intron	HLA-DPA1		

Curriculum Vitae

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- Advisor: Aydin Tozeren, Ph.D.
- Thesis: Psoriasis and Skin Disorders: A Study of Transcriptome and Microbiome
- Deployed open-source microarray analysis workflows in R Bioconductor package to evaluate mRNA expression similarities between skin autoimmune conditions.
- Performed 16S NGS analysis to explore skin microbiome composition using QIIME package.
- Investigated microbiome-host interactions in psoriasis pathogenesis by metagenome functional prediction of microbiome using PICRUST software package.
- Developed, maintained and optimized population genetics workflow to identify positive selection in HLA genic region in 11 ethnic populations.
- Inspected expression profiles of biomarker pairs in different types of lung cancer using microarray datasets.

Undergraduate Research Assistant 09/2008 – 12/2009
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- Explored the regulation of extravasation genes in cancer leukocytes after treating them with engineered metabolic oligosaccharides (sialic acid precursors specifically) and three dimensional growth substrate.

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Software Engineer Intern 06/2010-08/2010
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- Developed file sharing web applications to improve physician collaboration and decision making.
- Modified web applications to ensure compatibility with mobile phones and different browsers.

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- Calibrated biomedical devices and t for the hospital including defibrillators, patient monitors, infusion pumps, ECG, patient beds and pulse oximeters.
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Publications

- **C. Tanes**, and A. Tozeren. Crosstalk between host and microbiome in psoriasis: a system analysis based on genomics datasets. (in prep)
- **C. Tanes**, and A. Tozeren. HLA region SNPs and their modulation of population subtypes in disease. (in prep)
- L. Jackson, **C. Tanes,** and A. Tozeren. Addiction Drugs Cluster Functionally in the Genome with Concomitant Variation in Human Populations (submitted)
- K. Chen, K. Wu, S. Cai, W. Zhang, J. Zhou, J. Wang, A. Ertel, Z. Li, H. Rui, A. Quong, M.P. Lisanti, A. Tozeren, **C. Tanes**, S. Addya, M. Gormley, C. Wang, S.B. McMahon, and R.G. Pestell. Dachshund binds p53 to block the growth of lung adenocarcinoma cells. Cancer Res. 2013 Jun 1;73(11):3262-74.

Conference Abstracts and Presentations

- **C. Tanes**, L. Jackson and A. Tozeren. Epidermal Differentiation Complex Exhibits Population Subtype Differences in SNP Frequencies. Poster session presented at: 64th Annual Meeting of the American Society of Human Genetics; 2014 October 18-22; San Diego, CA.
- L. Jackson, **C. Tanes** and F. Jackson. Genomic Analysis of Blood-mediated Disorders in African Americans. Poster session presented at: 64th Annual Meeting of the American Society of Human Genetics; 2014 October 18-22; San Diego, CA.
- T. Purwin, Y. Lan, **C. Tanes**, and A. Tozeren. Integrative Analysis of Autoimmune Diseases and Possible Drug Targets. Poster session presented at: Drexel University's 16th Annual Research Day; 2012 April 19; Philadelphia, PA.

Leadership Experience

- Bioinformatics Mentor, Mastery Charter Schools 01/2014-06/2014
- Judge, 9th Annual DNA Day Essay Contest, American Society of Human Genetics 04/2014
- Vice President, Turkish Student Association, Drexel University 2011 - 2012
- President, Turkish Student Association, Johns Hopkins University 2009 - 2011

Teaching Experience

Graduate Teaching Assistant / Lab Instructor 01/2012 - present

Drexel University, Philadelphia, PA

1. Quantitative Systems Biology
2. Genome Information Engineering
3. Computational Bioengineering

- Coordinated and weekly lab sections to help students utilize MATLAB to tackle bioinformatics problems.
- Created weekly assignments and solutions.
- Mentored students in efficient code writing in MATLAB and Python.
- Lectured on microarray analysis, ordinary differential equations and sequence alignment

Graduate Teaching Assistant 01/2012 - present

Drexel University, Philadelphia, PA

1. Biosimulations
2. Biocomputational Languages
3. Programming and Modeling for Biomedical Engineering II
4. Programming and Modeling for Biomedical Engineering I

Undergraduate Course Assistant / Lab Leader 01/2010-05/2011

Johns Hopkins University, Baltimore, MD

- Introduction to Programming with JAVA

Professional Societies

- Society for Mathematical Biology 2013 - present
- Society for Molecular Biology and Evolution (SMBE) 2014 - present
- American Society for Human Genetics 2014 - present

Honors and Awards

- Graduate Travel Subsidy, Drexel University: $250 awarded to attend ASHG Meeting 2014
- Nominated for Outstanding Teaching Assistant Award, Drexel University 2012 & 2014
- Calhoun Fellowship awarded for academic excellence, Drexel University 2011 - present
- International Scholarship awarded for academic excellence, Johns Hopkins University 2007 - 2011
- Dean's List, Johns Hopkins University Spring 2008 & 2010

Other Skills

Computer programs: R, MATLAB, Python, Java , C, C++, asp.net MVC, Linux environment

Bioinformatics: Bioconductor package, microarray analysis, gene expression analysis, gene set enrichment analysis, next-generation sequencing, QIIME, BLAST, sequence alignment, clustering, agent based modeling, big data mining, principal component analysis, SVM, naïve Bayes, neural networks

Lab: cell culture, RNA extraction, cDNA synthesis, qPCR, LD50 assay, glycolipid detection with flow cytometry and resorcinol assays

Languages: Turkish (native speaker), German (intermediate), Japanese (beginner)