An Association Study Exploring the Genetic Relationship of Psoriatic Arthritis and Obesity

by © Cassidy Welsh A thesis submitted

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Abstract

Objective: To determine if there is a genetic component causing psoriatic arthritis (PsA) patients to have higher BMIs when compared to the general population.

Method: 696 obese samples were identified from a previous genetic study on obesity which were genotyped using a PsA SNP panel. 650 PsA patients who were examined for PsA related anthropometric measures were genotyped using an obesity SNP panel. An obesity panel was created using a gene prioritization method to create a 46 SNP obesity-weighted panel. Two separate quantitative trait analyses were performed to obtain the association between BMI and genotype of the subsequent panels using a linear regression model. Bonferroni correction was used to adjust for multiple comparisons.

Results: Genotypes of two PsA-weighted SNPs, rs10782001 (*FBXL19*) and rs3131382 (HLA-B*39), showed a significant difference with BMI. Patients with the *FBXL19* variant had an average BMI in the presence of GG genotype of 37.2 kg/m^2 vs 34.3 kg/m^2 for the AA genotype (p=0.0007). Patients with the *HLA-B*39:05* variant had an average BMI with the TT genotype of 47.1 kg/m^2 vs 35.4 kg/m^2 for the CC genotype (p=0.00005). One obesity-weighted SNP, rs11915371 (*SAMMSON/FOXP1)*, showed a significant difference of BMI between genotypes in PsA patients. The average BMI of those with the CC genotype was 32.42 kg/m^2 compared to an average BMI of 29.77 kg/m^2 with the TT genotype (p=0.0009).

Conclusion: Homozygotes for the minor allele of SNPs within *HLA-B*39*, *FBXL19*, and *SAMMSON/FOXP1* have shown to have an increased BMI, suggesting a potential genetic link between these genes and PsA and obesity.

General Summary

Psoriatic arthritis is an inflammatory form of arthritis which causes psoriasis and joint pain. PsA patients tend to have higher BMIs when being compared to rheumatoid arthritis, psoriasis, and the general population. There have been multiple hypothesis linking obesity and PsA, one being a shared genetic background. For this project, a cohort of patients who were considered obese were genotyped for PsA related variants. A novel obesity SNP panel was then designed, consisting of 46 SNPs which have been associated with obesity. A PsA cohort was then genotyped using this obesity SNP panel. An analysis was completed to determine if there were differences between BMIs and genotypes of obesity and PsA variants. Three variants had a significant difference in BMI across genotypes- *HLA-B*39, FBXL19,* and *SAMMSON/FOXP1*. An increase in BMI was seen within the homozygous genotypes. These SNPs should be further explored as a potential link between PsA and obesity.

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List of Abbreviations

A	Adenine
ACR20/50/70	American College of Rheumatology 20/50/70
ACTH	Adrenocorticotropic Hormone
ADCY3	Adenylate Cyclase 3
ADCY5	Adenylate Cyclase 5
ADCY8	Adenylate Cyclase 8
ADCY9	Adenylate Cyclase 9
AGR3	Anterior Gradient 3
AgRP	Agouti-related Peptide
AHR	Aryl Hydrocarbon Receptor
AKT3	Protein Kinase B Serine/Threonine Kinase 3
AS2	Antisense RNA 2
ATP	Adenosine Triphosphate
BDNF	Brain-derived Neurotrophic Factor
BDX	BigDye Xterminator
BLAST	Basic Local Alignment Search Tool
BLAT	BLAST-like Alignment Tool
BMI	Body Mass Index
BMP	Bone Morphogenetic Protein
C	Cytosine
C8orf86	Chromosome 8 Open Reading Frame 86
CAMP	Cyclic Adenosine Monophosphate
CART	Cocaine-and Amphetamine-Regulated Transcript
CASPAR	Classification Criteria for Psoriatic Arthritis
CD14+	Cluster of Differentiation 14
CD4+	Cluster of Differentiation 4
CD8+	Cluster of Differentiation 8
CDH20	Cadherin 20
CEP164	Centrosomal Protein 164
CREB	cAMP Response Element Binding Protein
CREB1	cAMP Responsive Element Binding Protein 1
CSF3	Colony Stimulating Factor 3
DEXA	Dual-energy X-ray Absorptiometry
DGKG	Diacylglycerol Kinase Gamma
DIP	Distal Interphalangeal Joint
DMARD	Disease Modifying Anti-Rheumatic Drugs
DNA	Deoxyribonucleic Acid

DNAJC27	DNAJ Heat Shock Protein Family Member C27
dNTP	Deoxynucleoside Triphosphate
DPYD	Dihydropyrimidine Dehydrogenase
EFR3A	EFR3 Homolog A
EHMT1	Euchromatic Histone Lysine Methyltransferase 1
ERAP1	Endoplasmic Reticulum Aminopeptidase 1
ETV5	ETS Variant Transcription Factor 5
FBXL19	F-Box and Leucine Rich Repeat Protein 19
FGFR1	Fibroblast Growth Factor Receptor 1
FKBP5	FK506 Binding Protein Prolyl Isomerase 5
FOXP1	Forkhead Box P1
FTO	Fat Mass and Obesity Associated Protein
g	Gravitational force
G	guanine
GATA4	GATA Binding Protein 4
GIPR	Gastric Inhibitory Polypeptide Receptor
GNAT2	G Protein Subunit Alpha Transducing 2
GPCR	G Protein-Coupled Receptor
GRIK2	Glutamate Ionotropic Receptor Kainite Type Subunit 2
GTF2I	General Transcription Factor II
GWAS	Genome-Wide Association Study
H2O	Nuclease-free Water
HACE1	HECT Domain and Ankyrin Repeat Containing E3 Ubiquitin Protein Ligase 1
HAQ-DI	Health Assessment Questionnaire-Disability Index
HLA	Human Leukocyte Antigen
HLA-A	Human Leukocyte Antigen A
HLA-A*02	Human Leukocyte Antigen A 02
HLA-B	Human Leukocyte Antigen B
HLA-B*08	Human Leukocyte Antigen B 08
HLA-B*09	Human Leukocyte Antigen B 09
HLA-B*27	Human Leukocyte Antigen B 27
HLA-B*38	Human Leukocyte Antigen B 38
HLA-B*39	Human Leukocyte Antigen B 39
HLA-C	Human Leukocyte Antigen C
HLA-C*06	Human Leukocyte Antigen C 06
HLA-DQB1	Human Leukocyte Antigen DQ beta 1
HLA-DRB1	Human Leukocyte Antigen DR beta 1

HPLC	High Performance Liquid Chromatography
HPRD	Human Protein Reference Database
HWE	Hardy-Weinberg Equilibrium
IBD	Irritable Bowel Disease
IDT	Integrated DNA Technologies
IFN-gamma	Interferon-gamma
IL-10	Interluekin-12
IL-12	Interleukin-12
IL-17	Interleukin-12
IL-17A	Interleukin-17a
IL-22	Interleukin-22
IL-23	Interleukin-23
IL-6	Interleukin-6
IL-8	Interleukin-8
IL19	Interleukin-19
IL23R	Interleukin-23R
JNK	c-Jun N-terminal Kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
kg	Kilogram
KIR	Killer Cell Immunoglobulin Like Receptor
KIR2DS2	Killer Cell Immunoglobulin Like Receptor, Two Ig Domains and Short Cytoplasmic Tail 2
KMT2A	Lysine Methyltransferase 2A
LADA	Latent Autoimmune Diabetes in Adults
LCE3A	Late Comified Envelope 3A
LEPR	Leptin Receptor
lncRNA	Long Non-Coding RNA
m	Metres
MAF	Minor Allele Frequency
MAP2K1	Mitogen-Activated Protein Kinase Kinase 1
MAP2K5	Mitogen-Activated Protein Kinase Kinase 5
MC3R	Melanocortin-3 Receptor
MC4R	Melanoncortin-4 Receptor
MCR	Melanocortin Receptors
MDA	Minimal Disease Activity
MEF2C	Myocyte Enhancer Factor 2C
MgCl2	Magnesium Chloride
MHC	Major Histocompatibility Complex
MICA	MHC Class I Polypeptide-Related Sequence A

MIR1302-4	Micro RNA 1302-4
µL	Micro Litre
MLXIPL	MLX Interacting Protein Like
µM	Micro Molar
mM	Millimolar
MPP6	MAGUK p55 subfamily member 6
MS	Multiple Sclerosis
MSH	Melanocyte Stimulating Hormone
NCBI	National Centre of Biotechnology Information
NCOR1	Nuclear Receptor Corepressor 1
NEDD1	Neural Precursor Cell Expressed, Developmentally Down-Regulated 1
NFkB	Nuclear Factor Kappa Light Chain Enhancer of Activated B Cells
NK	Natural Killer
NL	Newfoundland and Labrador
NPY	Neuropeptide Y
NTC	No Template Control
OMIM	Online Mendelian Inheritance in Man
OPRM1	Opioid-Receptor Mu 1
OR	Odds Ratio
PAMPs	Pathogen-Associated Molecular Pattern
PASDAS	Psoriatic Arthritis Disease Activity Score
PASI	Psoriasis Area and Severity Index
PC1	Proprotein Convertase 1
PCR	Polymerase Chain Reaction
PCSK1	Proprotein Convertase Subtilisin/Lexin-Type 1
PDE4D	Phosphodiesterase 4D
PMAIP1	Phorbol-12 Myristate 13 Acetate Induced Protein 1
POMC	pro-opiomelanocortin
PPARG	Peroxisome Proliferator-Activated Receptor Gamma
PRO	Patient Reported Outcomes
PRPH2	Peripherin 2
Ps	Psoriasis
PsA	Psoriatic Arthritis
PSORS1	Psoriasis Susceptibility Locus 1
PTBP2	Polypyrimidine Tract Binding Protein 2
QTL	Quantitative Trait Locus
RAC1	Ras-Related C3 Botulinum Toxin Substrate 1

RANK	Receptor Activator of NF-kB
RANKL	Receptor Activator of NF-kB Ligand
RARB	Retinoic Acid Receptor Beta
REL	V-Rel Avian Reticuloendotheliosis Viral Oncogene Homolog
RGS17	Regulator of G Protein Signalling 17
RGS6	Regulator of G Protein Signalling 6
RMST	Rhabdomyosarcoma 2 Associated Transcript
RNA	Ribonucleic Acid
SAMMSON	Survival Associated Mitochondrial Melanoma Specific Oncogenic Non-Coding RNA
SAP	shrimp alkaline phosphatase
SCAPER	S-Phase Cyclin A Associated Protein In The ER
SDCCAG8	Serologically Defined Colon Cancer Antigen 8
SES	Socioeconomic Status
SIPAL1L1	Signal Induced Proliferation Associated 1 Like 1
SKOR1	SKI Family Transcriptional Corepressor 1
SNP	Single Nucleotide Polymorphism
SNR	Signal to Noise Ratio
SpA	Spondylarthritis
STAT2	Signal Transducer and Activator of Transcription 2
T	Tyrosine
TBE	Tris-Borate-Ethylenediaminetetraacetic Acid
TH1	T-helper 1
TH17	T-helper 17
TLR	Toll-like Receptor
TLR9	Toll-like Receptor 9
TNF alpha	Tumour Necrosis Factor Alpha
TNFAIP3	Tumour Necrosis Factor Alpha Induced Protein 3
TNFRSF9	Tumour Necrosis Factor Receptor Superfamily Member 9
TNIP1	Tumour Necrosis Factor Alpha Induced Protein 3 Interacting Protein 1
TNKS	Tankyrase
TRAF3IP2	Tumour Necrosis Factor Receptor Associated Factor 3 Interacting Protein 2
TULP1	Tubby-Related Protein 1
TYK2	Tyrosine Kinase 2
UCSC	University of California Santa Cruz
UEP	Unextended Extend Primer

UofT	University of Toronto
VPS37D	Vacuolar protein sorting-associated protein 37D
Wnt	Wingless/Integrated
YWHAZ	Tyrosine 3 Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta
ZNF816	Zinc Finger Protein 816

Chapter 1 Introduction

1.1 Psoriatic Arthritis

1.1.1 Epidemiology

Psoriatic arthritis (PsA) is an immune-mediated disease characterized by inflammatory arthritis among psoriasis patients. PsA is associated with peripheral joint pain and axial pain, stiffness, and cutaneous involvement. Psoriasis, the major cutaneous feature of PsA, is defined as the build-up of skin cells on the surface, forming red, scaly, hardened patches. About 21% of patients who have psoriasis will go on to develop PsA (Alinaghi *et al.* 2019). Psoriasis is often the first symptom of disease, followed by additional features including joint involvement. Globally, the prevalence of psoriasis is 2-3%. The prevalence of PsA is 133 per 100,000 and the incidence is 83 per 100,000 (Scotti *et al.* 2018). Both diseases are typically more common in Caucasian populations and are equally present in men and women (Gladman, Chandran, Rosen, 2014*).*

1.1.2 Clinical Features

PsA is an inflammatory arthritis associated with psoriasis. Patients often present with joint stiffness and pain, particularly in the morning, which improves with exercise. Joint symptoms often starts at the entheseal insertion of joints and synovial membrane, leading to joint pain and swelling (Gladman 2015). The joints that are most commonly involved are the small joints of the feet and hands, followed by the knees, wrists, ankles, and shoulders. Joints are typically warm to touch, tender to palpate, swollen and sometimes discoloured when examined (Gladman 2015). Some individuals with PsA tend to have axial involvement, associated with prolonged stiffness. As there are no clear visible clinical indications, such as swelling, axial involvement is sometimes missed during diagnostic assessment (Gladman 2015).

PsA is a heterogeneous disease with varying clinical presentations. Moll and Wright described five clinical patterns, all of which can evolve and be overlapping. The first pattern are patients with classical PsA where the distal interphalangeal (DIP) joints are predominantly involved, which is the first knuckle from the top of the finger. This clinical group is relatively uncommon (5% of patients) (Moll & Wright, 1973). Next, is a severe, erosive, and deforming pattern, arthritis mutilans, characterized by marked osteolysis. This is also a relatively uncommon clinical group (5% of patients) (Moll & Wright, 1973). The two most common forms of PsA are symmetrical polyarthritis (five or more joints) and asymmetrical oligoarthritis (2 to 4 joints). The symmetrical pattern of polyarthritis, affecting the same joints on each side of the body, is indistinguishable from rheumatoid arthritis (Moll & Wright, 1973). Patients with single or few fingers or toe joints involved are considered asymmetrical oligoarticular arthritis and the most common clinical group at disease presentation. This form affects different joints on each side of the body. Lastly, patients with the predominant feature of axial inflammation, like ankylosing spondylitis, with their peripheral joints sometimes being involved (5% of patients) (Moll & Wright, 1973).

Typically, patients will first present with psoriasis. Plaque psoriasis is the most common form of psoriasis, affecting about 90% of those with psoriasis (National Clinical Guideline Centre (UK), 2012). Plaque psoriasis is characterized by well-delineated red, scaly plaques which can be found at multiple locations, most commonly extensor sites of the elbows and knees. Flexural psoriasis refers to psoriasis at flexion points, which is generally less scaly than plaque psoriasis. Guttate psoriasis typically is preceded by a streptococcal infection and is characterized by small bumps which last for about a month. Pustular psoriasis appears as raised bumps, which are filled with fluid or pus. An

aggressive, systemic form of psoriasis that affects the entire body is called erythrodermic psoriasis (National Clinical Guideline Centre (UK), 2012). Nail involvements are seen in 70% of patients with PsA. Nail lesions are the most consistently documented risk factor for PsA. Lesions often include pits, onycholysis, hyperkeratosis, and nail bed crumbling (Gladman, 2015).

Periarticular structures can also be involved in PsA, particularly dactylitis and enthesitis. Dactylitis is a result of inflammation of the synovial sheath. This often causes the digit to have limited mobility and become tender and cause a swollen like 'sausage' digit. Typically, this is seen in the feet more than hands, commonly affecting the second and fifth toes. It occurs in about 40 to 50% of PsA patients at some point in their disease progression and is associated with more severe disease progression (Gladman 2015; Brockbank *et al.* 2005).

Enthesitis refers to the inflammation at the insertion of tendons and ligaments into bone. It is seen in about 30 to 50% (Ritchlin *et al.* 2017) of PsA patients. When enthesitis is the sole feature of PsA these individuals are often difficult to diagnose clinically. Ultrasound is helpful in this setting (Gladman 2015).

1.1.3 Diagnosis and Classification Criteria

Originally a case descriptive definition was created by Moll and Wright in 1973 (Moll & Wright, 1973). PsA was defined as inflammatory arthritis in the presence of psoriasis and absence of rheumatoid factor (Leung *et al.* 2018). Over the years, PsA classification criteria have continued to evolve. The most widely used criteria were created by the CLASsification criteria for Psoriatic Arthritis (CASPAR) study group. The classification criteria are displayed in the following table (**Table 1.1**). To be classified as having PsA an individual must have

inflammatory articular disease AND >3 points in other subgroups (Taylor *et al.* 2006). The CASPAR criteria have revealed both high sensitivity and specificity in terms of diagnosis (Taylor *et al.* 2006).

Table 1.1 The CASPAR criteria for psoriatic arthritis.

Criteria	Description	Points
1. Evidence of psoriasis; either current psoriasis, a history of psoriasis or a family history of psoriasis	Present psoriatic skin or scalp disease as judged by rheumatologist of dermatologist, history of previous psoriasis diagnosed by family doctor, rheumatologist, or dermatologist; or family history in first or second degree relative	2 for current presentation 1 for previous history or family history
2. Psoriatic nail dystrophy	Onycholysis, pitting, or hyperkeratosis observed during physical examination	1
3. A negative test for rheumatoid factor	ELISA (enzyme-linked immunosorbent assay) or nephelometry	1
4. Current dactylitis	Current swelling or entire digit or history recorded by rheumatologist	1
5. Radiographical evidence of juxa-articular new bone formation	New bone formation near joint margins (Excluding osteophyte formation) X-rays of the hand or foot	1

1.1.4 Overview of Pathogenesis

PsA is defined as a complex multifactorial disease, meaning multiple genetic, environmental, and immunological factors all play in disease pathogenesis. A combination of both specific genetic factors and environmental factors can trigger aberration of the immunological defence mechanism likely leading to the development of PsA (Veale & Fearon 2018).

1.1.4.1 Environmental

Environmentally, specific infections, injuries, stressors, and occupations could contribute to the development of PsA (Ocampo & Gladman 2019). For example, elevated

levels of the antibody of Streptococcus exotoxin anti-deoxyribonuclease B is seen in some PsA patients but absent in psoriasis patients alone (Vasey *et al.* 1982). Streptococci are thought to produce CD8+ T-cells which lead to polyfunctional, proinflammatory cytokine releasing mediators that leads to an autoimmune response and psoriasis. The microbiome may also play a role in inducing autoimmunity. When dysbiosis occurs, the immune system is activated and recruits T-helper cells. Trauma sites have also been known to develop psoriasis, known as the 'Koebner phenomenon'. This phenomenon refers to skin trauma that induces psoriasis (Thorarensen *et al.* 2017). A similar phenomenon is seen within PsA patients, where trauma at entheseal insertion sites is a potential trigger for PsA (Talotta *et al.* 2019). When mechanical stress is present in genetically susceptible individuals, the immune system is in turn activated.

1.1.4.2 Immunological

Immunologically, PsA is also associated with changes in both the innate and adaptive immune systems with the specific involvement of T-cells (Warren & Menter 2016).

In the skin, when keratinocytes are stressed, they release DNA that binds to cathelicidin, an antibacterial peptide. This forms a DNA-LL37 complex which binds to TLR-9 in plasmacytoid dendritic cells. These cells are stimulated to release IFN-alpha which activates dermal dendritic cells and migrates to draining lymph nodes. This then triggers naïve T-helper cells to differentiate into Th1 and Th17, these then migrate back to the skin. Th1 and Th17 produce IFN-gamma, IL-12, IL-17, IL-22, and TNF-alpha (**Figure 1.1**). Within the skin, CD8+ T cells are also present which also produce IL-17. Many T-cells, including Th17, CD8+, and NK cells, release IL-22, a cytokine that is responsible for keratinocyte proliferation. Dermal dendritic cells release IL-23 which allows for further

survival of Th17 cells. IL-17A, IL-22, and TNF-alpha all work together to produce a large amount of IL-19 within the skin. IL-19 is known to be significantly upregulated in psoriatic plaques when compared to normal skin (Ocampo & Gladman, 2019; Barnas & Ritchlin 2015).

Enthesitis is a key clinical feature of PsA. When trauma or stress is seen at the tendon insertion site, 'danger signals' are released leading to cytokine and growth factor production (**Figure 1.1)**. IL23 is released which activates Th17 cells and cytokines which leads to inflammation, bone erosion, and abnormal bone growth (Ocampo & Gladman, 2019; Barnas & Ritchlin 2015).

PsA generates inflammation within the synovium, entheses, and spine. Within the synovium, an increase has been noted in infiltrating immune cells. Raised levels of proinflammatory cytokines have also been noted, including p40, TNF-ALPHA, IL-1, IL-6, IL-8, and IL-10 (Warren & Menter 2016). Activation of CD8+ T cells and natural killer (NK) cells in psoriatic synovium and the response of the diseases to therapeutic immunomodulation (such as anti-TNF, anti-IL-17A, and anti-IL-23) suggest that the immune system has a large influence on PsA pathogenesis (Ocampo & Gladman, 2019). CD4+, CD8+ lymphocytes, and CD4+ Th17 and the more recently discovered type 3 lymphocytes were all seen to be increased in PsA patient's synovium fluid compared to rheumatoid arthritis patients (Leijten *et al*. 2015).

Bone phenotypes in PsA range wildly from bone degradation to new bone formation. These two extremes can happen in the same joint. The IL-23/Th-17 axis has been noted to be involved in bone remodelling. Within the synovium, regulation of the receptor activator of NF-kappa-B ligand (RANKL) and low expression of its antagonist, osteoprotegerin have

been detected. RANKL binds to RANK on the surface of osteoclast precursors, which triggers proliferation of the osteoclast precursors, and they differentiate into osteoclasts, which resorb bone (Ritchlin *et al.* 2017). Those that have PsA have been noted to have elevated levels of circulating CD14+ monocytes in peripheral blood, these are the precursors that form osteoclasts, compared to healthy controls. Those that are using anti-TNF agents for treatment have seen a decrease of osteoclast precursors in their blood (Anandarajah *et al.* 2007).

The Wnt/beta-catenin signalling pathway is required in osteoblast differentiation. Wnt ligands bind to Lrp5/6 receptors which allows beta-catenin to accumulate in the cell, which regulates gene expression. Osteocytes may produce Wnt signalling inhibitors, sclerostin, and Dickkopf-related protein 1 (DKK-1) (Barnas & Ritchlin, 2017). These both are negative regulators of bone mass and promote bone resorption through increased osteoclastogenesis while inhibiting osteoblastogenesis. Another protein family, bone morphogenetic protein (BMP), has been hypothesized to have a role in additional bone growth seen in PsA, as BMP-2 and BMP-7 were upregulated in areas of new bone formation in mouse models (Lories & Luyten, 2005).

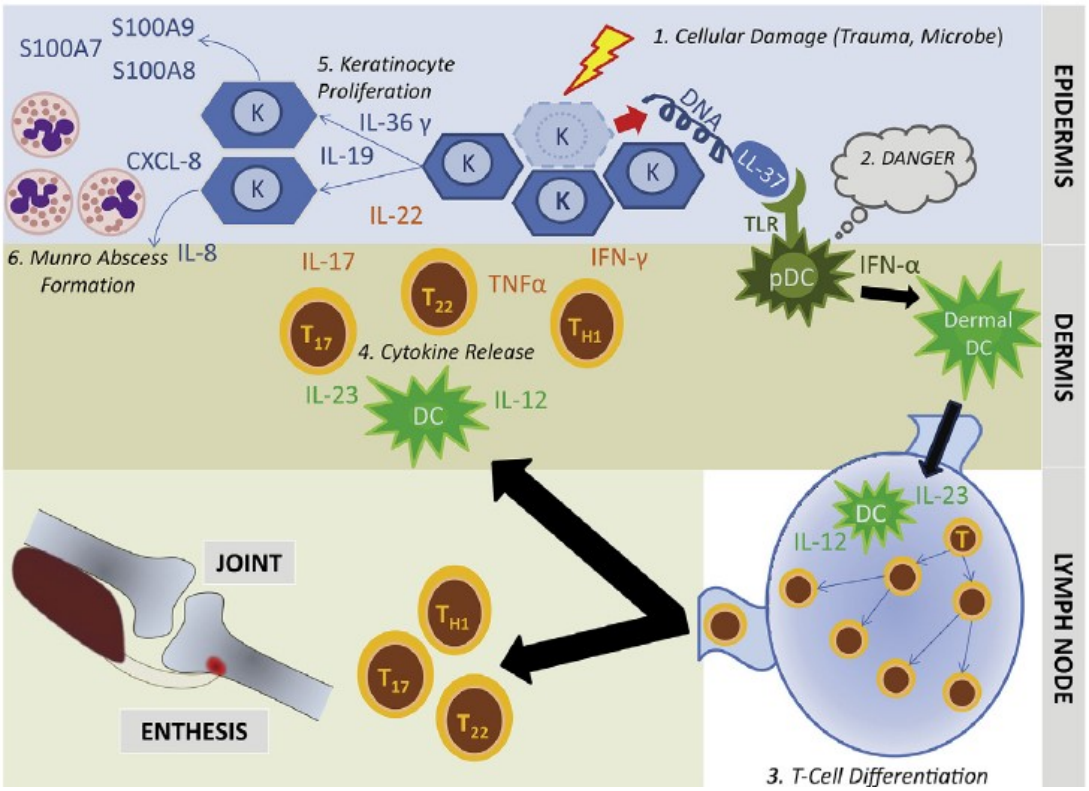


Figure 1.1 Relationship of skin and psoriatic disease. (1) Keratinocytes may be activated by mechanical trauma or pathogens inducing IFN-alpha release which (2) activates dendritic cells releasing cytokines and T-cell differentiation. IL-23 triggers IL-17 producing T-cells and IL-12 promote IFN-gamma (3). T-cell migrate back to the skin and to enthesis and joints which also release cytokines (4). IL-22 stimulates keratinocyte proliferation causing antibacterial S100 proteins and leading to psoriasis (5/6). Reprinted from: Rheumatic Disease Clinics of North America, 41/4, Etiology and Pathogenesis of Psoriatic Arthritis, Barnas JL & Ritchlin CT, 643-663, Copyright (2015), with permission from Elsevier.

1.1.4.3 Genetics

Many PsA studies focus on the disease's strong genetic component. Both psoriasis and PsA are known to be highly heritable diseases. Heritability can be estimated using population-based studies or twin-based studies. From population-based studies, the recurrence ratio in siblings and first-degree relatives is between 30 and 55 (Moll & Wright, 1973; Chandran *et al.* 2009). For psoriasis, first-degree relatives are at a 19-fold increased risk of developing the disease and heritability has been estimated to be between 60-90% (Swanbeck *et al.* 1994; Myers *et al.* 2005). Therefore, both PsA and psoriasis are

considered complex genetic disorders. Twin studies in psoriasis have shown the complexity of psoriatic disease. Monozygotic psoriasis twins were at a threefold increased risk of psoriasis when compared to dizygotic twins (Elder *et al.* 1994). However, concordance is never 100% among monozygotic twins, meaning that other factors apart from genetics are playing a role in pathogenesis (Rahman & Elder, 2005).

PsA and psoriasis are consistently associated with chromosome 6q21.3 within the MHC region. The main associations are in class I MHC alleles, located in a roughly 300kb segment known as psoriasis susceptibility region 1 (*PSORS1*) (Nair *et al.* 2006). A high-risk allele with psoriasis is HLA-C*0602, which is also associated with PsA at a weaker association. This allele was noted at 57.5% in psoriasis, 28.7% in PsA, compared to a control group of 19.7%. Other associations include HLA-B*27 (OR=2.6), HLA-B*38 (OR=1.6), HLA-B*08 (OR=1.6), and HLA-B*39 (OR=3.5), which were all associated with PsA more frequently than psoriasis and controls (Winchester *et al.* 2018). Depending on which HLA alleles are present implicate possible features of PsA (phenotype/genotype correlations) and disease progression. Those with HLA-B*27:05:02 or HLA-B*39:01:01, tend to develop arthritis closer to the onset of psoriasis. While HLA-C*06:02 patients have a much slower onset of arthritis after skin disease appears (Winchester *et al.* 2018). PsA spondylitis has been associated with both HLA-B*27:05:02 and HLA-B*08:01. Enthesitis and dactylitis are associated with HLA-B*27:05. Peripheral polyarthritis in PsA has been associated with HLAB*38 and HLA-B*39. In terms of disease progression, HLA-B*39 has been the most associated (O'Rielly & Rahman, 2014). *MICA* is a non-HLA gene located in the MHC region, which is near HLA-B locus has also been associated with PsA and psoriasis (Korendowych *et al.* 2005; O'Rielly & Rahman 2014).

Other genes relating to immune function outside of the MHC region have also been associated with PsA. Genome-wide association studies (GWAS) and meta-analyses have revealed about 85 genetic loci associated with psoriatic disease (Rahmati *et al.* 2020; Winchester *et al.*2018). This includes *IL-12B, IL-23R, STAT2, TNIP1, TRAF3IP2, TYK2, FBXL19*, and *REL*, most of these genes being associated with both PsA and psoriasis (Winchester *et al.* 2018). *KIR* genes, specifically *KIR2DS2,* have been described as key in the susceptibility and pathogenesis of PsA. KIRs interplay with HLA-B and HLA-C immune response. *KIR2DS2* is more specific to PsA, as it has remained more associated with PsA when compared with psoriasis patients (O'Rielly & Rahman, 2015). *ERAP1* is associated with peptides binding to MHC class I molecules, specifically HLA-C*06:02 and HLA-B*27. Many SNPs that have been associated all play a role in immune function, particularly NF-kB signalling, IFN signalling, IL23 pathway, antigen presentation, or play a role in T helper cells.

Given the overlap between PsA and psoriasis, most associated genes of PsA, have also been associated with psoriasis. They also share many associated genes with other immune-mediated inflammatory disorders, including Crohn's disease, rheumatoid arthritis, ankylosing spondylitis, and systemic lupus erythematosus. Some shared genes include *TNFAIP3, IL23-R, ERAP-1, IL12B, REL,* and *PTPN22* (Winchester *et al.* 2018). Non-MHC genes seem to be more associated with PsA than with psoriasis. Specific PsA only alleles have been noted, such as a specific variant within *IL-23R, PTPN22*, and 5q31 (Winchester *et al.* 2018*).* Other associated genes are associated with both PsA and psoriasis but have an increased PsA effect size. Some examples of PsA associated genes include *TRAF3IP2* and *FBXL19*. *IL12B* and *IL23R* are of special interest as they are independently identified to be associated with PsA, not psoriasis (Chandran 2012).

Although many recent GWAS studies have contributed to understanding the genetics of both psoriasis and PsA, many genes involved are still largely unknown (Winchester *et al.* 2018).

1.1.5 Treatment and Disease Outcome

Measuring disease outcomes in PsA is difficult due to the disease's clinical heterogeneity, waxing and waning symptoms and the difficulty to clinically assess synovitis. Also, many of the treatments mentioned work well for some symptoms of PsA and then exhibit secondary failures but other medications are primary failures, meaning no response. but not for others. Generally, reducing inflammation with treatment leads to decreased symptoms, prevents damage, and reduces comorbidities (Leung *et al.* 2018).

Patient outcomes can be measured in a variety of ways and it's important to evaluate different domains of the disease, including both musculoskeletal and cutaneous domains. Assessment of musculoskeletal features typically includes 66/68 tender and swollen joints exam, spinal range of motion and pain, enthesitis index (measured by Leeds Enthesitis Index or SPARCC Enthesitis Index), and dactylitis (Leeds Dactylitis Index). Psoriasis is assessed using Psoriasis Area and Severity Index (PASI) and nails are examined for pitting and onycholysis (Ritchlin *et al.* 2017). For response to treatment, composite outcome measure instruments are used such as the American College of Rheumatology (ACR). ACR is a commonly used index to measure primary outcomes which defines response at 20%, 50%, or 70% reduction of tender and swollen joints, which is based on a 66/68 tender and swollen joint count, physician's global assessment, acute phase reactant, and three patient reported outcomes (Health Assessment Questionnaire- Disability Index (HAQ-DI), patient pain assessment, and patient global assessment) (Oabai & Ogdie, 2016; Gladman *et al.* 2007). Minimal disease activity (MDA) is also used in evaluating treatment, in which improvement must be seen in 5 of 7 categories of criteria. The criteria

include tender joint count, swollen joint count, PASI, patient pain scale, patient global disease activity, Health Assessment Questionnaire, and tender entheseal points (Ritchlin *et al.* 2017). Other measures include acute phase reactants such as C-reactive protein and erythrocyte sedimentation rate (ESR) (Coates & Helliwell. 2010; Ritchlin *et al.* 2017). More recently, other measures more specific to PsA and psoriasis have been created. These include Psoriatic Arthritis Disease Activity Score (PASDAS), and Composite Psoriatic Disease Activity Index (CPDAI).

Treatment and management for psoriasis and PsA vary depending on the severity of the disease. Treatments are largely on a case-by-case basis due to the heterogeneous nature and associated comorbidities. The goal of all treatments is for the patient to eventually reach minimal-disease activity (MDA), increase the quality of life, prevent structural damage, and avoid complications from the disease and treatment (Ocampo & Gladman 2019). Typically, treatments begin with non-steroidal inflammatory drugs for pain or topical treatments for psoriasis. Then, single disease-modifying anti-rheumatic drugs (DMARDs) are used, typically methotrexate, followed by a combination of DMARDs. Finally, if combination DMARDs are not efficacious or well-tolerated, biologic agents are prescribed (Coates & Helliwell 2017).

Oral corticosteroids are used sparingly in PsA and every attempt is made to look at alternatives. About 40% of joints improve with local corticosteroid injections, but over 30% relapse. For moderate to severe disease, systemic drugs are recommended to aid in arthritis symptom relief. However, many DMARDs do not convincingly slow the progression of the disease, help with axial symptoms, enthesitis, or dactylitis. Methotrexate is the most common DMARD therapy. This drug shows some evidence of decreasing synovitis, enthesitis, and

dactylitis symptoms as well as arthritis (Ricthlin *et al. 2017*). Sulfasalazine, cyclosporine, and leflunomide are other DMARDs that are used in PsA (Ricthlin *et al. 2017*).

Tumour necrosis factor (TNF) inhibitors (TNFi) were the first biologic used to treat PsA patients and have shown success for multiple domains of PsA. The response rate for most TNFis when assessed by the ACR20 score was 50 to 60% as compared to 30 to 40% for the placebo group (Ritchlin *et al.* 2017). TNFis improve synovitis, psoriasis, as well as enthesitis, and dactylitis. However, drug retention does fall over time, but drug switching within and outside the therapeutic class does recapture the disease but at a lower rate. Some examples of common TNFi treatments are etanercept, adalimumab, infliximab, golimumab, and certolizumab pegol (Ocampo & Gladman, 2019; Ritchlin *et al.* 2017). Biologics have become increasingly common in PsA treatment. Ustekinumab is an IL-12/23 inhibitor that shows improvement for psoriasis, arthritis, enthesitis, and dactylitis. IL-17A monoclonal antibodies Secukinumab and ixekizumab are now well established for PsA treatment as well as a monoclonal antibody to IL-23 Guselkumab (Ritchlin *et al.* 2017).

1.1.6 Comorbidities

There are a variety of comorbidities associated with PsA and psoriasis. More than half of PsA patients have at least one comorbidity (Husni 2015). Both diseases have been linked to an increased risk of developing irritable bowel disease (IBD), cardiovascular disease, and depression. PsA has been associated with the development of metabolic syndrome, which includes an increased risk of type 2 diabetes and obesity (Warren & Menter, 2016). For example, atherosclerosis pathogenesis has been associated with low-grade inflammation activity, which is seen in PsA (Husni 2015). Type 2-diabetes has been attributed to the increased risk of obesity as well as potential insulin-resistance associated

with PsA inflammation. Depression and anxiety have been seen in an estimated 36% and 22% of PsA patients respectively (McDonough *et al.* 2014). Those with PsA seem to have significant disease burden and decreased quality of life. PsA patients tend to have higher rates of depression and anxiety when compared to a similar disease like RA. This can potentially be attributed to the physical and visible impairment of psoriasis and not just joint pain alone (Husni 2015). Additional comorbidities include osteoporosis, anterior uveitis, fatty liver disease, and lymphoproliferative associated malignancies.

1.2 Obesity

Obesity is one of the most common diseases worldwide and is defined as a condition of abnormal or excessive accumulation of fat in the adipose tissue (World Health Organization, 2000). About 10-15% of the global population is considered obese (Goodrazi 2018). Obesity can simply be explained by a positive energy balance, meaning energy intake exceeds energy expended (Singh, Kumar, Mahalingam 2017). However, energy balance is not just regulated by environmental factors like food intake and exercise output, it is also affected by genetic and immunological factors. Therefore, obesity is considered a multi-factorial disease, caused by multiple influences.

There are different forms of obesity; syndromic and non-syndromic. Syndromic forms of obesity are caused by a genetic abnormality, most commonly chromosomal rearrangements. Some examples of syndromic obesity include Prader-Willi syndrome and Bardet-Biedl syndrome (Farqooqi & O'Rahilly, 2017). The most common form of obesity is non-syndromic forms which include monogenic and polygenic obesity. Monogenic obesity is caused by a single gene mutation, this is typically rare and usually discovered in early childhood. Genes such as *POMC,*

PC1, NPY, MC4R, and *FTO* are associated with this form of obesity. Polygenic obesity, also known as common obesity, is the other form of non-syndromic obesity. Polygenic obesity is considered complex and caused by the interplay of multiple different factors (Singh *et al.* 2017).

1.2.1 Classification Criteria

Body mass index (BMI), waist circumference, and waist-to-hip ratio are all used to measure and classify obesity. The most common way to classify obesity is using body mass index. BMI is calculated by a formula which uses one's height and weight to determine their index.

$$Body\ mass\ index\ (BMI) = \frac{weight\ (kg)}{height\ (m)^2}$$

BMIs are broken down into ranges, typically into six groups, ranging from underweight (<18 kg/m^2) to morbidly obese (>40 kg/m^2) (**Table 1.2**). Waist circumference, which takes into account abdominal adiposity, is also a common indicator of obesity. Waist circumferences exceeding 94 cm in men and 80 cm in woman of European descent is noted as a risk factor for metabolic syndrome. The waist circumference risk factors vary by sex and ethnicity (Alberti *et al.* 2009).

Table 1.2 Body mass index (BMI) ranges.

Weight Groups	BMI Range (kg/m^2)
Underweight/normal weight	<25
Overweight	25-29.9
Obese Class I	30-34.9
Obese Class II	35-39.9
Obese Class III	>40

1.2.2 Overview of Pathogenesis

Like PsA, obesity is a complex disease, with multiple contributing factors aiding in pathogenesis. This includes environmental, immunological, and genetic factors all being involved in obesity etiology. All of these factors likely play a role together, genes increase susceptibility, working with the hypothalamic homeostatic regulator of energy balance, and environmental factors to cause obesity.

1.2.2.1 Environmental

Multiple social and environmental factors contribute to the development of obesity. Some examples include smoking, socioeconomic status (SES), diet, and medications. The 'built' environment has been noted as a contributor to obesity. This refers to the increased usage of cars, elevators, escalators, video games, TV, etc. that has allowed for less active lifestyles (Meldrum *et al.* 2017). An increase in sugar intake has also been noted within our built environment. A large contributor to obesity is the easy, cheap, accessibility to large portions of unhealthy foods. This also explains why those with lower SES in developed countries are more likely to be obese (Tyrrell *et al.* 2016). Another factor contributing to obesity could be a large decrease in smoking rates. Smoking cessation has been related to weight gain and nicotine has been noted to suppress

weight gain (Mineur*et al.* 2011). Also, prescription drugs and various treatments are much more common than previously. Many drugs, including antipsychotics, antidepressants, antihyperglycemics, antihypertensives, and corticosteroids do cause weight gain as a side-effect. Corticosteroids are a common treatment used for PsA patients, including cortisone, prednisolone, and prednisone, all of which have been associated with significantly increased weight change. Typically, weight gain is noted during longer use (>3 months) (Brown *et al.* 2001). There are many reasons why obesity is increasing worldwide, many of the contributing factors are related to environmental changes.

1.2.2.2 Pathophysiology

Adipocytes store extra fat as triacylglycerol in fat depots within the body and release fatty acids into the bloodstream using proteohormones. Proteohormones (leptin, adiponectin, and visfatin) as well as insulin, regulate body-fat mass with the help of cytokines, growth factors, and complement proteins (TNF-alpha, IL-1, IL-6) (Redinger 2007). IL-6 and TNF-alpha work to promote insulin resistance by inhibiting lipoprotein lipase (LPL). This decreases triglyceride hydrolysis and increases storage in adipocytes resulting in storage. The enlargement of fat depots and increased fat storage is thought to play a role in obesity in adults (Redinger 2007). The adipocytes population increases steadily throughout childhood and only increases in size during adulthood (Spalding *et al.* 2008). Studies have previously shown there was an association between adipose tissue and leptin deficiency. This means that fat storage and increased appetite with lower energy expenditure likely work together to promote obesity (Montague *et al.* 1997).

Leptin, a molecule secreted by white adipose tissues within the central nervous system, is involved in inhibiting food intake and energy expenditure. Leptin communicates with the hypothalamus to determine how much energy is stored in adipose tissues and suppresses appetite

where appropriate (Friedman & Halaas, 1998). Obese individuals typically have high circulating levels of leptin, referred to as leptin resistance (Montague *et al.* 1997). In obese individuals, the brain oftentimes does not respond to leptin, causing an increase in circulating leptin to be produced. Leptin is also involved in immune response where it activates the adaptive immune system, specifically regulatory T cell function. Visfatin, another adipokine, increases pro-inflammatory monocytes promoting T-lymphocyte activation (Porta *et al.* 2021). Adiponectin is secreted by adipose tissue and plays a protective role in obesity, as it lowered fatty acid oxidation and insulin sensitivity. Those who are obese, tend to have low levels of adiponectin (Singh *et al.* 2017; Kong *et al.* 2019).

In obese patients, adipocytes are dysfunctional creating an increase in pro-inflammatory adipokines. This creates chronic inflammation throughout the body. Increased leptin secretion occurs due to the imbalance at the adipose tissue which causes the accumulation of proinflammatory macrophages. IL-6 and TNF-alpha increase along with other pro-inflammatory cells (Th1, B cells) and a decrease in anti-inflammatory cells (Porta *et al.* 2021).

The hypothalamus is responsible for the detection of hunger and feeding behaviours. The hypothalamus works with groups of neurons, agouti-related protein/neuropeptide-gamma (AGRP/NY-gamma), proopiomelanocortin/cocaine amphetamine-regulated transcript (POMC/CART), to circulate metabolites and hormones to signal energy is needed. Leptin, ghrelin, insulin, and glucose all play a role in these signals (Gadde *et al.* 2018).

The melanocortin system is a crucial network for appetite and metabolic response, with several pathways being involved in energy homeostasis control. The melanocortin system is also responsible for stress response, sexual activity, and inflammation. It is a complex system composed of various signalling and biological molecules. It is composed of various signalling

and biological molecules including agonists (cocaine amphetamine, ACTH, and alpha, beta, gamma-MSH), antagonists (agouti, AgRP, and NPY), and accessory proteins (MRAP1 and MRAP2) (Cooray & Clark, 2011). Melanocortin Receptors (MCR) are within G-protein coupled receptor group (GPCR) and consists of five receptors (MCR1R, MCR2R, MCR3R, MCR4R, and MCR5R). All MCR have been shown to be expressed in cells within adipocytes. The melanocortin signalling pathway is regulated in two ways; leptin-associated signalling and GPCR associated signalling. Leptin-associated signalling occurs when leptin binds to receptors within the hypothalamus and enhances POMC/CART which produces alpha, beta, and gamma MSH, which then inhibits the actions of NPY/AgRP to regulate feeding. The GPCR signalling is associated with POMC which binds to MCRs. This activates the cAMP/PKA/ERK1/2 pathway and converts cytoplasmic ATP to cAMP, which activates protein kinase A and phosphorylates CREBS (Singh *et al.* 2017*;* Cooray & Clark 2011).

1.2.2.3 Genetics

Genetic causes of obesity can be broadly classified into different categories, starting with syndromic and non-syndromic forms (**Figure 1.2)**. Non-syndromic forms can then be classified further into monogenic obesity, caused by a single gene mutation, and polygenic obesity which is caused by multiple genes (**Figure 1.2)**.

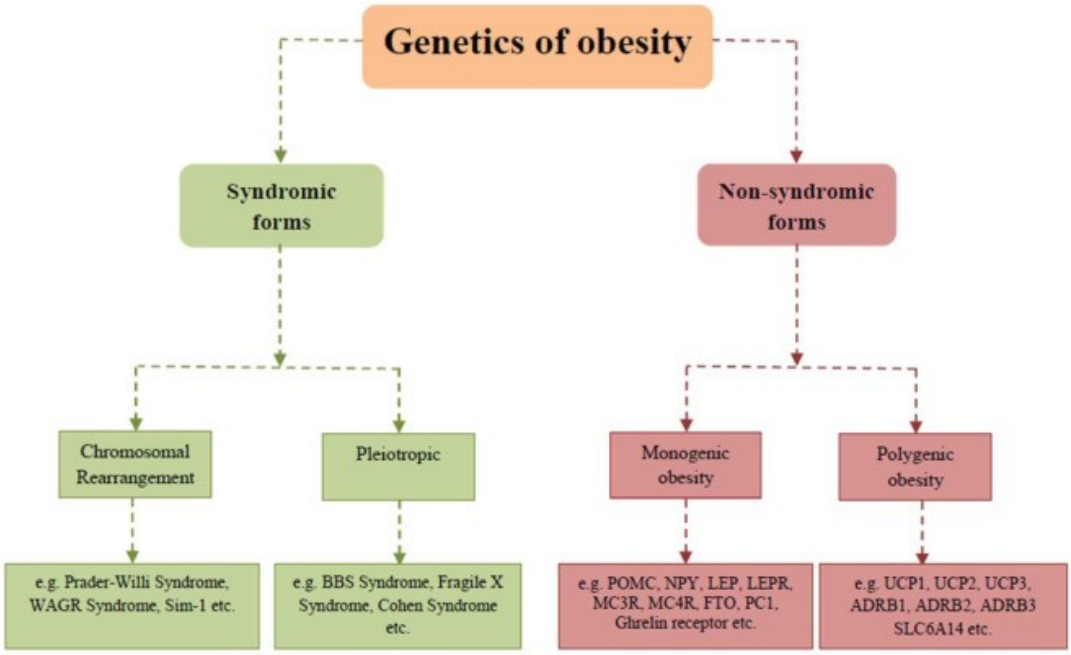


Figure 1.2 Genetics of obesity including syndromic and non-syndromic forms. Reproduced with permission from Singh RK, Kumar P, Mahalingam K. Molecular genetics of human obesity: A comprehensive review. Compte Rendus Biologies. 2017. 340(2):87-108.

Syndromic forms of obesity are associated with various syndromes that cause obesity, which can also be broken down into chromosomal rearrangements like Prader-Willi syndrome and pleiotropic syndromes like Fragile X syndrome and Bardet-Biedl syndrome. Obesity is a feature of over 100 syndromes with a lot of phenotypes that overlap with features like intellectual disability and dysmorphic facies (Thaker 2018). Those with Prader Willi syndrome have an inactivated region on chromosome 15 which has been associated with 5 genes within the region. These genes are expressed in the hypothalamus and control feeding pathways, which is likely what causes an increase in appetite (Thaker *et al.* 2018). A disease like Bardet-Biedl syndrome has great genetic heterogeneity with over 20 genes identified as causative (Kaur *et al.* 2017). Genes associated with Bardet-Biedl syndrome

impair ciliary function which may lead to abnormal trafficking of the leptin receptor and leptin resistance in those with the syndrome, causing obesity (Guo & Rahmouni, 2012).

In terms of non-syndromic obesity and more specifically monogenic obesity, many genes have been discovered. The most commonly associated genes with monogenic obesity include *LEP*, *LEPR*, *POMC*, *MC4R*, PCSK1 and *BDNF* (OMIM, 2021). The first gene discovered was *LEPR,* which was identified using mouse models. It was discovered that leptin deficiency led to obesity, which encoded the leptin receptor gene (*LEPR*) (Chen *et al.* 1996). Also using mouse models, the melanocortin pathway was identified to be associated with body-weight regulation, leading to the discovery of various genes in this pathway to be associated with obesity (*POMC, MC4R, PCSK1*) (Loos & Yeo, 2021; Jackson *et al.* 1997; Huszar *et al.* 1996; Krude *et al.* 1998).

Genome-wide association studies have been a common tool in identifying polygenic obesity related variants over the last number of years. Among one of the first GWAS studies, the *FTO* gene was found to be associated with BMI, with an increased risk of obesity. The gene association was originally noted in type 2 diabetes, but after adjusting for BMI, the gene was no longer associated (Loos & Bouchard, 2008). This established the association with BMI. This is one of the largest effect alleles to date, although only accounting for 1% of total heritability of BMI (Loos & Bouchard, 2008). Each allele within *FTO* has been shown to confer a 20-30% increase in obesity risk (Scruteri *et al.* 2007). Since the first GWAS, nearly 940 variants have been associated with BMI. Other associated genes with polygenic obesity that have been identified through GWAS include *ETV5*, *BDNF*, *PPARG*, *MC4R* and *POMC* (Bouchard, 2021; Loos & Yeo, 2021). The genes identified through GWAS are of modest effect size and only account for a proportion of alleles contributing to increased BMI.

BMI has been attributed to genetic factors. It has been estimated that 40-70% of the variation seen in BMIs can be heritable (Goodrazi 2018). Heritability has been estimated via twin studies as between 70-85% (Silventoinen *et al.* 2008). Genetic predispositions that have been identified often are genes related to energy regulation which play a role in metabolism and appetite. Since polygenic obesity is not caused by a single gene mutation, it's likely that many genetic factors are working together to cause an increased BMI. There are many other genetic associations with obesity that have yet to be discovered and will likely continue to be uncovered through further GWAS studies and increased knowledge of the disease. About two-thirds of BMI heritability can be attributed to common DNA variants. Gene-gene interactions, gene-environment interaction and rare variants likely explain the missing heritability that has not yet been discovered (Yang *et al.* 2015; Ge *et al.* 2017). Most common BMI SNPs have comparable effects among Asian, African, and White European individuals. It's also been noted that many of these SNPs associated with increased BMI exert minimal effects in those with normal BMI but have a larger effect on those who are considered overweight or obese (Monda *et al.* 2013).

1.2.3 Comorbidities

Obesity is associated with many serious health complications, ranging from increased risk of mortality and other adverse health effects (World Health Organization, 2012). The World Health Organization defined the risk of additional obesity related health problems into greatly increased risk, moderately increased risk, and slightly increased risk (World Health Organization, 2012). Greatly increased risk included type 2 diabetes, gallbladder disease, dyslipidemia, insulin resistance, and sleep apnea. Those considered moderate risk were coronary heart disease, hypertension, osteoarthritis, and gout. Lower risk diseases were cancer, hormone abnormalities,

polycystic ovary disease, impaired fertility, low back pain, and fetal defects (World Health Organization, 2012). Obesity is a risk factor for developing cardiovascular disease due to increased metabolic demand and cardiac output needed to deal with excess body weight (Singh *et al*. 2017). The risk of hypertension and type-2 diabetes are also increased if obese. About 60-90% of those with type-2 diabetes were obese at some point in their lifetime (Singh *et al*. 2017). Obesity has also been shown to increase the risk of late-set Alzheimer's disease and certain forms of cancer. Osteoarthritis and PsA have also been known to be associated with obesity (Singh *et al*. 2017). Obesity is associated with a large number of health risk factors and complications.

1.3 Psoriatic Arthritis and Obesity

Patients with PsA tend to have higher BMIs than the general population and those with similar inflammatory joint diseases, such as rheumatoid arthritis (Radner *et al.* 2017; Labitigan *et al.* 2014). PsA patients were found to be heavier than RA patients by an average of 7.7 kg (17 pounds) (Radner *et al.* 2017; Labitigan *et al.* 2014). When being compared to psoriasis, those with PsA were 61% more likely to be obese (Husni 2015; Radner *et al.* 2017). Body weight has also been shown to negatively affect PsA therapy, causing obese individuals to have a lower probability of achieving minimal disease activity when compared to normal weight patients. When weight loss was seen of 5% or more of their body weight, patients were more likely to respond to treatments and were more likely to achieve minimal disease activity (Di Minno *et al.* 2014). The prevalence of obesity in PsA patients has been estimated at 45%, with mean BMI being at 30.6 ± 6.8 kg/m^2 (Labitigan *et al.* 2014).

The relationship between PsA and obesity is likely complex and potentially bidirectional. Obesity has been known as a risk factor for developing PsA and those with PsA may be obese due to the joint disease affecting their ability to be active (Kumthekar & Ogdie 2020). They may also be obese due to anxiety and depression that are noted to be of higher prevalence in PsA, which are comorbidities also associated with obesity (Zafiriou *et al.* 2021; Husni 2015). There have been hypotheses created to potentially explain the link between the two diseases. It could potentially be caused by shared environmental factors that cause obesity. For example, enthesitis, a feature of PsA, is common in obese individuals (Kumthekar & Ogdie 2020). When excess weight is present, mechanical stress and microdamage are placed on joints (**Figure 1.3**). These joints then undergo an immunological response, through the synovial-entheseal complex that can trigger release of IL-23 cytokines. It is possible that this process triggers PsA, as a similar reaction is seen within joints of PsA patients (Kumthekar & Ogdie 2020). This is a similar hypothesis to explain the relationship between obesity and osteoarthritis.

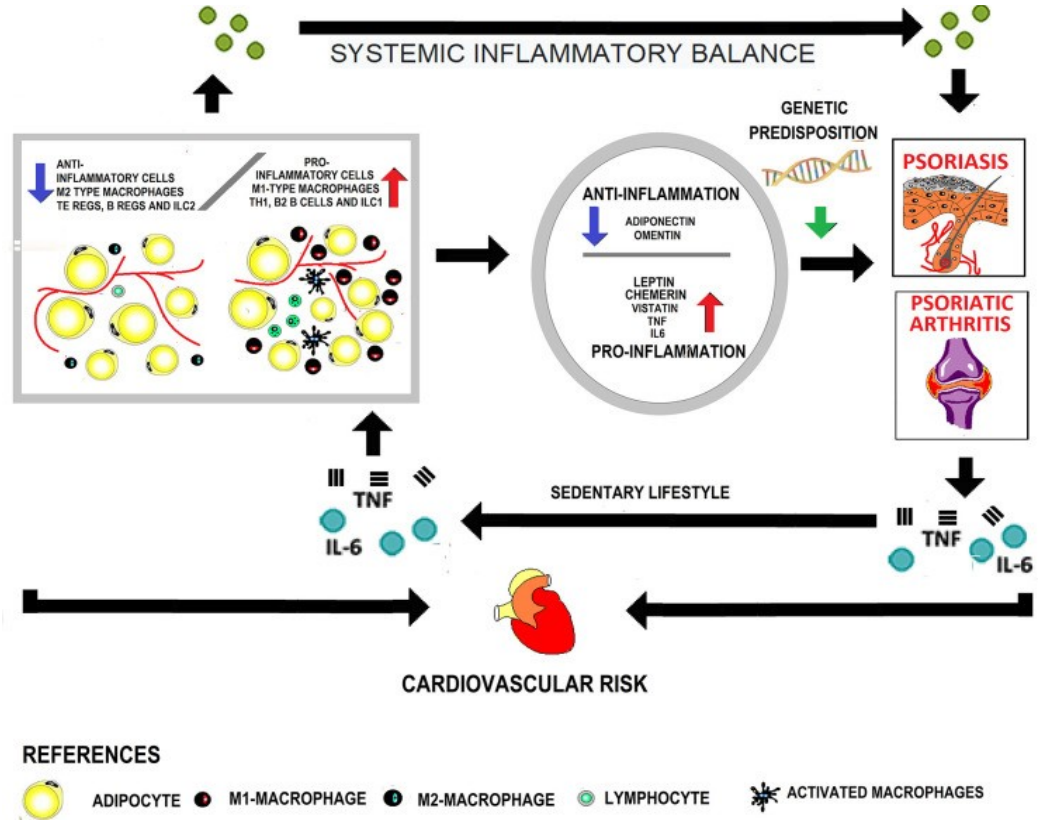


Figure 1.3 Obesity and the development of psoriasis, psoriatic arthritis, and cardiovascular disease. In obese individuals, adipose tissue causes infiltration of pro-inflammatory cells which further increases secretion of pro-inflammatory adipokines. This inflammatory state favours skin psoriasis to form in genetically predisposed individuals. The increased mechanical load at the enthesis sites also contributes to the onset of psoriatic arthritis. The inflammation associated with PsA and the sedentary lifestyle secondary to joint involvement creates adipose tissue growth. This in turn also causes cardiovascular risk to increase. Retrieved from: Porta et al. 2020 Licensed under CC BY.

The link could also be due to obesity and PsA inflammation. Obesity is characterized by a low-grade inflammatory state while PsA is an inflammatory disease. There is evidence suggesting that adipose tissue releases adipocytokines and pro-inflammatory mediators. Adipokines are involved in regulating the immune system and inflammatory response (**Figure 1.3**). Leptin is also known to promote pro-inflammatory cytokines and suppress anti-inflammatory cytokines. An adipokine, adiponectin has anti-inflammatory effects. Patients with psoriasis had lower levels of adiponectin and higher levels of leptin compared to controls (Wang *et al.* 2008). Other pathways, like the c-Jun N-terminal kinase (JNK)

pathway, have also been upregulated in obese individuals which led to immune-inflammatory response (Sharma *et al.* 2008).

The microbiome also plays a role in autoimmunity, working as another potential link between psoriatic arthritis and obesity. In healthy individuals, there's a balance between pathogen and tolerant species within both the gut and skin microbiome. When an increase of pathogen species is seen, this is called dysbiosis. Dysbiosis may cause activation of the immune system, primarily by recruiting T-helper lymphocytes (Eppinga *et al.* 2014). The overgrowth of pathogenic flora may activate TLRs and the release of PAMPs. It's possible that dendritic and macrophage cells recognize microbial substrates and produce cytokines and other mediators which are typically involved in autoinflammation. The microbiome may link genetic disposition with inflammatory activation (Talotta *et al.* 2019). Obesity has also been associated with dysbiosis. Obese individuals are more likely to have unbalanced microbiota compared to those who are not considered obese or those who are obese and actively losing weight (Blottiere, 2017). It is possible that obese individuals are more likely to have dysbiosis then triggers an autoinflammatory response leading to psoriasis and/or psoriatic arthritis.

Lastly, genetics could potentially play a role in the relationship between psoriatic arthritis and obesity. While PsA and obesity pathogenesis are quite complex, they do share similar pathways in relation to inflammation. It is possible that there are shared genetic factors leading to an increased risk of being obese and developing PsA or vice versa. As mentioned, both of the diseases are complex, with many genetic variants being associated with both diseases. It would not be surprising to have some of these genetic variants overlap and contribute to the relationship. A recent study used Mendelian randomization to support a causal relationship between BMI and psoriasis (Budu-Aggrey *et al.* 2019).

Those with PsA who are also obese tend to have higher disease severity (Kumthekar & Ogdie 2020). In a recent study, patients with higher BMI have higher joint counts, C-reactive protein, health assessment quality disability index, and other composite measures. They are also more likely to have comorbidities associated with PsA. Obese PsA patients also have poorer response to treatment. Typically, a poorer response to TNF inhibitors is seen. Obesity was noted in multiple studies as the variable at highest risk of not achieving Minimal Disease Activity (MDA). It has also been noted that weight loss improves response to therapy and increases chances of reaching MDA (Di Minno *et al,* 2013; Kumthekar & Ogdie 2020; Di Minno *et al.* 2014). Overall, obese PsA patients typically have more severe disease paired with lower response to treatment, making it difficult to manage their disease. It is important to understand the link causing PsA patients to be at a higher risk of being obese or why obese patients are more likely to develop PsA. When we better understand the connection between the two diseases, rheumatologists will be able to better advise their patients on treatment options for disease.

1.4 Rationale of the study

There is a clear association between psoriasis/PsA and obesity given that PsA patients tend to have higher BMIs than those with similar diseases and higher BMIs than the general population (Radner *et al.* 2017; Labitigan *et al.* 2014). Epidemiologically, the relative risk for the development of psoriasis is 2.7 in patients with a BMI over 35 kg/m^2 (Setty *et al.* 2007). Also, the incidence of psoriasis almost doubled within the US population between 1970 and 2000, which is parallel to the sharp increase in the prevalence of obesity within the same population (Icen *et al.* 2009). As mentioned previously, weight loss decreases the severity of both psoriasis

and psoriatic arthritis, indicating weight influences the severity of PsA (Alotailbi *et al.* 2018). In terms of molecular function, PsA and obesity share various metabolic and immune pathways. Adipose tissue is associated with increased expression of various immune cells, including T-cells and myeloid cells. These cells, especially myeloid cells are involved in creating an inflammatory phenotype which creates cytokine and adipokines release (Rosen & Spiegelamn 2014; Zhu e*t al.* 2013). As previously mentioned, these adipokines levels are increased in those with PsA (Eder *et al.* 2016). Insulin resistance, which is also noted in obese individuals, is closely related with psoriatic disease and PsA (Toussirot *et al.* 2021; Ferguson *et al.* 2019). Lastly, genetics has been hypothesized to play a role in the relationship of psoriasis and obesity. Through a mendelian randomization study with the UK Biobank and HUNT cohort, a suggestive causative relationship between BMI and psoriasis was noted (Budu-Aggrey *et al.* 2019). Therefore, to better understand the underlying relationship between PsA and obesity, the genetic relationship was explored within this study.

1.4.1 Research Objectives

The objective of my research study is to determine if genetics plays a role in the link between PsA and obesity. To determine this, various SNPs which have been associated with each disease have been investigated and compared to a cohort of the alternate disease. Firstly, a cohort of obesity patients were genotyped on a previously designed PsA SNP panel. Secondly, a novel obesity SNP panel was designed and used to genotype a cohort of PsA patients. This method will hopefully provide insight into any potential variants that connect the two diseases. My specific research objectives are as follows:

1. To determine if PsA-associated variants are overrepresented in an obese population. If so, determine if there is a dose-dependent effect between higher BMI and PsA-associated variant(s).
2. Design and optimize a novel obesity-weight SNP panel.
3. To determine if obesity-associated variants are overrepresented in a PsA cohort. If so, establish if there is any trend in terms of clinical features and PsA variants.

Chapter 2 Methods

2.1 Patient Cohorts

For the obesity cohort, DNA samples, which were isolated from whole blood, were obtained from participants previously recruited for a previous population-based obesity study investigating the genetic basis of obesity in the NL population. Inclusion criteria were any subject residing in Newfoundland at the time of the study and over the age of 18 that would volunteer for this study. As patients with normal and increased BMI were required, there was no specific BMI cut off for this study. All volunteers were assessed using a standardized protocol, which included a detailed history and physical examination. The history not only included demographic information, past medical and surgical history, and medication but also environmental factors, including activity level, typical diet, and smoking history. No autoimmune disease was present at time of exam. A complete physical exam was administrated and height, body weight, hip-waist ratio, skin changes, stretch marks, varicose veins, and acanthosis nigricans were carefully recorded.

For the PsA cohort, DNA samples were identified from two sites: Memorial University and the University of Toronto (UoT). The UoT PsA clinic has been prospectively collecting patients with a standardized protocol for 40 years and the PsA Clinic at MUN has used the UoT protocol to collect patients for 19 years. Ethics approval can be found within **Appendix A**. Overall, 1650 patients are being actively followed in both cohorts, 700 from Toronto and 950 from St. John's. The clinical protocols included a standardized collection of disease features, class, patient reported outcomes (PRO), biochemical and inflammatory markers, radiological assessment, and biospecimens. Whole blood was collected from each patient. Consecutive patients satisfying the CASPAR criteria with BMI recorded were selected for this study.

2.2 Variant Selection for MassARRAY Panels

2.2.1 Obesity Panel

Using recent genome wide-association studies that identified 97 BMI associated SNPs (Locke *et al.* 2015) and 700 BMI associated SNPs (Yengo *et al.* 2018), a list of prioritized genes was created. Locke *et al.* used 339,224 individuals for their meta-analysis from 125 studies including genome-wide association studies (GWAS) and Metabochip which analyzed over 2.5 million SNPs combined. Yengo *et al.* analyzed 456,426 individuals from the UK Biobank of European ancestry. Over 16.6 million SNPs were tested for association with BMI. About 800 strong obesity associated and non-extreme rare variants were selected (MAF >0.1%), and their corresponding genes were selected for gene prioritization.

To create the list of prioritized genes, the web-based tool Phenolyzer was used. Phenolyzer uses phenotype keywords and gene to gene interactions to prioritize the SNPs (Yang *et al.* 2015). The keyword "obesity" was used as the phenotype term. The gene-disease databases were built from Online Mendelian Inheritance in Man (OMIM, 2021), Orphanet (Orphanet, 1997), ClinVar (Landrum *et al.* 2018), Gene Reviews (Adam *et al. 2021)* and GWAS Catalog (Buniello *et al.* 2019). Gene to gene interactions database was built from various tools: Human Reference Protein Database (HPRD) (Peri *et al.* 2003; Prasad *et al.* 2009), Kyoto Encylopedia of Genes and Genomes (KEGG) (Kanehisa & Goto, 2000; Kanehisa 2019; Kanehisa *et al.* 2021), BioCyc (Karp *et al.* 2017), Reactome (Jassal *et al.* 2020), Pathway Interaction Database (Schaefer *et al.* 2009), WikiPathways (Martens *et al.* 2021) and Gene Ontology (Ashburner e*t al, 2000; The Gene Ontology recourse, 2021)*. Gene scores were defined by measures specific to each database and confidence of the gene-disease relationship. The score was weighted and normalized from 0 to 1. The seed genes were generated by collating all the genes related with phenotype and the lists were

grown based on all gene-gene interaction. Scores were also calculated for all genes that connect with seed genes. After the scores were generated, the genes were ranked by their relevance to obesity. All the information was integrated to generate a final weighted score for each gene by a logistic regression model. The scores were renormalized to rank the final prioritized gene list. This gene prioritization method was performed with assistance from Dr. Quan Li.

2.2.2 PsA Panel

A multiplex panel was designed by members of the Rahman/O'Rielly Lab for a previous study on psoriasis and psoriatic arthritis (PsA) (Eder *et al.* 2019). This panel was composed of 42 genetic variants associated with Ps or PsA based on previously published GWA studies (**Table 2.1**). The panel was developed as part of a diagnostic test to identify PsA, thus contained genes enriched for Ps or PsA.

Table 2.1 PsA panel of 42 SNPs associated with PsA/Ps.

Variant	Associated gene	OR	P-value	Minor allele frequency (MAF)	Source
rs12044149	*IL23R*	1.30 (PsA)	$2.5x10^{-12}$	T=0.214617/26949	Budu-Aggrey, 2016
rs4655683	*IL23R*	1.32 (PsA)	$7.8x10^{-14}$	A=0.374108/46976	Stuart, 2015
rs7152885	*5q31*	1.27 (PsA)	$2.04x10^{-4}$	T=0.273541/34348	Bowes, 2015
rs2476601	*PTPN22*	1.32 (PsA)	$1.49x10^{-9}$	A=0.071083/15583	
rs9321623	*TNAFIP3*	1.20 (PsA)	$5.91x10^{-8}$	T=0.427529/53684	Stuart, 2015
rs12189871	*HLA-C*	4.46 (Ps)	$6.6x10^{-248}$	T=0.087737/11017	
rs4908742	*TNFRSF9*	1.22 (Ps)	$2.2x10^{-8}$	A=0.365921/45948	
rs10888503	*LCE3A*	1.25 (Ps)	$1.8x10^{-11}$	C=0.414222/52013	
rs1050414	*HLA-C*	4.02 (Ps)	$6.5x10^{-225}$	G=0.097808/24453	
rs13214872	*HLA-C*	3.23 (Ps)	$1.2x10^{-206}$	G=0.123957/15565	
rs12212594	*HLA-C*	5.00 (Ps)	$3.5x10^{-249}$	A=0.05554/6974	
rs12191877	*HLA-C*	2.64 (Ps)	$<10^{-100}$	T=0.123941/15563	Nair, 2009
rs4349859	*HLA-B*27*	3.05 (PsA)	<0.0001	A=0.027125/3406	Eder, 2012
rs3129944	*HLA-B*38*	5.09 (PsA)	<0.0001	G=0.288131/36180	
rs2734331	*HLA-B*38*	5.09 (PsA)	<0.001	G=0.047327/11844	
rs3131382	*HLA-B*39*	2.51 (PsA)	0.009	T=0.043339/5442	
rs2844603	*HLA-B*39*	2.51 (PsA)	0.009	A=0.347485/43633	
rs9468859	*HLA-B*39*	2.51 (PsA)	0.009	A=0.011592/1449	
rs3869115	*HLA-C*12*	1.29 (PsA)	0.13	G=0.099476/12491	
rs396960	*HLA-C*12*	1.29 (PsA)	0.13	A=0.291922/36656	
rs3130457	*HLA-C*12*	1.29 (PsA)	0.13	C=0.219785/27598	
rs2248901	*HLA-C*12*	1.29 (PsA)	0.13	T=0.204049/25622	
rs6457374	*HLA-B*08*	1.61 (PsA)	0.009	C=0.189722/23823	
rs2844535	*HLA-B*08*	1.61 (PsA)	0.009	G=0.282938/35528	
rs887466	*HLA-C*06*	1.71 (PsA)	0.0001	A=0.391469/49156	

Variant	Associated gene	OR	P-value	Minor allele frequency (MAF)	Source
rs2894207	*HLA-C*06*	1.71 (PsA)	0.0001	C=0.203292/25527	
rs1800925	*IL13*	1.28 (PsA)	$4.5x10^{-2}$	T=0.257621/32349	Eder, 2011
rs848	*IL13*	1.61 (PsA)	$7x10^{-4}$	A=0.315717/39644	
rs67841474	*MICA*	1.34 (PsA with Ps)	0.11	-=0.212116/29748	Pollock, 2011
rs2082412	*IL12B*	1.44 (Ps)	$3x10^{-20}$	A=0.283687/35622	Nair, 2009
rs9304742	*ZNF816A*	1.02 (PsA)	$8.3x10^{-1}$	C=0.418785/52586	Yang, 2013
rs33980500	*TRAF3IP2*	1.60 (PsA)	$1.1x10^{-17}$	T=0.085558/21353	Huffmeier, 2010
rs587560	*KIR2DS2*	1.26 (PsA)	0.03		Chandran, 2014
rs10782001	*FBXL19*	1.16 (PsA)	$1x10^{-5}$	G=0.460595/57836	Stuart, 2010
rs13017559	*REL*	1.35 (PsA)	$4.56x10^{-7}$		Ellinghaus, 2012
rs11209026	*IL23R*	2.51 (PsA)	0.03	A=0.042204/10589	Elris, 2014
rs2201841	*IL23R*	1.75 (PsA)	0.02	G=0.290799/36515	
rs2066808	*IL23A*	3.04 (PsA)	0.02	G=0.208461/26176	
rs4406273	*HLA-C*	3.38 (Ps)	$1.8x10^{-81}$	A=0.088828/11154	Stuart, 2015
rs146571698	*TNIP1*	1.79 (PsA)	$1.9x10^{-23}$	T=0.047918/6017	
rs34536443	*TYK2*	1.93 (PsA)	$2.7x10^{-9}$	C=0.026803/6632	
rs9266242	*HLA-B*	1.2 (Ps)	0.001	T=0.28379/35635	Eder, 2012

2.3 MassARRAY Genotyping Technology

The MassARRAY System was selected as the genotyping platform as it can be multiplexed up to 40 variants per reaction well and we needed to genotype many variants for each cohort. This technology uses matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry for the detection of DNA molecules. Genetic variants are distinguished by analysis of their individual mass which then uses the arrival time of the individual ionized DNA analytes, to display a mass spectrum identifying the different genetic

targets (Ellis & Ong, 2017). Genotyping on the MassARRAY system requires PCR and extend primer adjustments, a PCR reaction, SAP clean-up reaction, extension reaction, and a final clean-up set using resin. The final reaction is then placed in the Nanodispenser which distributes the reaction onto a chip, which is then placed on the MassARRAY and it uses MALDI-TOF to determine the genotype. Each plate reaction were then analyzed using Typer Analyzer software, where additional calling criteria was added to ensure accuracy and confidence in the genotype.

2.4 MassARRAY Panel Design

2.4.1 Obesity Panel

Assay design suite v2.0 by Agena Biosciences was used to design the panel. SNPs corresponding 'rs' numbers were inputted, in order of importance. The SNPs were kept in order of highest scored SNPs from the variant selection process. The organism was set as 'Human' and database was Dec.2013(GRCh38/hg38), chemistry was set as 'iPLEX' and the multiplex level was set at 40, the maximum number of SNPs in one well, was set at 30. The flank size was changed from 100 to 300 to allow a greater area to create the most suitable primer and proximal SNPs with status not validated were excluded. The maximum amplicon length was changed from 120 to 300 to allow a larger amplicon size for easier primer design. Default settings and user settings are listed (**Table 2.2**), all other parameters remained at the default setting.

Table 2.2 Assay design suite settings used to create obesity panel.

Parameter	Default Setting	User Setting
Under Quick Fix Tab:		
Amplicon Primer Potential:		
False Priming	1	100
Hairpin/Dimer Extension	1	100

Extend Primer Potential:		
Hairpin/Dimer Extension	1	0.9
Multiplex Evaluation Potential:		
False Primer	1	0.8
Primer-dimer	1	0.8
Under Amplicon Tab:		
Amplicon (Maximum)	120	300
Under Multiplex Tab:		
Design Iterations	1	10
Best iteration	Highest average multiplex	Fewest rejects by low plex

The design summary gave an overview of the outputted design. The report includes the SNPs, SNPs in each well, PCR and extend primers, primer lengths and directions, uniplex confidence percentage, masses of un-extended primers and extend primers, and warnings. The warnings ranged from primer-dimer potentials, primer hairpin potential, and self-dimer potential. The validation report gave the number of true hits, false hits, and null hits of each SNP (**Table 2.3**). It also showed both the forward and reverse PCR primer hits. True hits were the number of amplicons produced by the PCR primer pair, which ideally would be one. Anything above one would indicate multiple amplicons produced. False hits were the number of amplicons produced that contain an invalid target for the extend primer of the same SNP. The number should be 0 for each SNP to allow for specificity. Null hits were the number of amplicons produced by the primer pair that do not contain a target sequence for the extend primer to bind, this number should be 0 for each SNP. The PCR1 and PCR2 hits determined how many matches are present for each primer to the genome. Although these numbers may be high, they represent both incomplete and complete matches to the genome. Secondary validation of these primers was

completed to double check for specificity. The Cross Array Hits Report summarized the types and number of amplicons that could result from a cross-hybridization reaction. No results should be present in this report.

SNP rs9332817 was consistently displaying as invalid rs number when added to the design and therefore could not be included in the original design. Following the initial design, SNP rs9939609 was added to the panel due to its importance to obesity. Both SNPs were manually added by support at Agena Bioscience. This was to avoid changing the design completely as the newest version of Agena Design Suite does not allow manual changes.

Table 2.3 Validation Hits Report from Agena Design Suite for the obesity panel.

Assay ID	Well ID	True Hits	False Hits	Null hits	PCR1 Hits	PCR2 Hits
rs11208662	W1	1	0	0	8	34
rs12327272	W1	1	0	0	59	66
rs2229616	W1	1	0	0	58	20
rs8087550	W2	1	0	0	71	78
rs881301	W1	1	0	0	75	7
rs6265	W1	1	0	0	43	6
rs1899951	W1	1	0	0	33	30
rs6235	W2	1	0	0	47	37
rs17203016	W1	1	0	0	200	65
rs3753549	W1	1	0	0	87	43
rs7784465	W2	1	0	0	109	80
rs1075901	W2	1	0	0	180	245
rs11792069	W2	1	0	0	18	87
rs4624596	W2	1	0	0	72	57
rs6879326	W1	1	0	0	90	45
rs12042959	W2	1	0	0	23	49
rs3134353	W2	1	0	0	19	120
rs2304607	W1	1	0	0	9	108
rs12458	W1	1	0	0	60	67
rs2124499	W2	1	0	0	47	50
rs11629783	W2	1	0	0	1756	81

Assay ID	Well ID	True Hits	False Hits	Null hits	PCR1 Hits	PCR2 Hits
rs8070454	W2	1	0	0	39	39
rs10182181	W1	1	0	0	53	24
rs879620	W2	1	0	0	14	45
rs12675063	W1	1	0	0	5	73
rs6968554	W2	1	0	0	11	73
rs7777102	W2	1	0	0	35	8
rs4307239	W2	1	0	0	163	73
rs12939549	W1	1	0	0	64	1
rs6804842	W2	1	0	0	5	34
rs573455	W1	1	0	0	47	21
rs13227433	W2	1	0	0	6	113
rs2030342	W1	1	0	0	82	60
rs4372296	W1	1	0	0	39	7
rs9349239	W1	1	0	0	54	99
rs156151	W2	1	0	0	13	58
rs6919443	W1	1	0	0	34	95
rs9816226	W1	1	0	0	91	81
rs11915371	W1	1	0	0	39	33
rs17024393	W1	1	0	0	53	47
rs10499276	W2	1	0	0	9	31
rs3807049	W2	1	0	0	55	39
rs1205106	W1	1	0	0	123	92
rs1394	W1	1	0	0	79	20
rs403656	W2	1	0	0	24	25
rs3751813	W1	1	0	0	200	36
rs11672660	W1	1	0	0	16	20
rs13329567	W2	1	0	0	84	16
rs10745785	W1	1	0	0	41	182

2.4.1.1 Primer Validation

After creation of the panel, all primers that were created by Assay Design Suite were checked to ensure appropriate design. The corresponding SNPs ‘rs’ number and PCR primers were entered into various web-tools to determine specificity. National Center for Biotechnology

Information (NCBI), Basic Local Alignment Search Tool (BLAST) program (Altschul *et al.* 1990), University of California Santa Cruiz (UCSC), BLAST-like alignment tool (Blat) program (Kent 2002), UCSC's In-Silco PCR (Kent *et al. 2002)*, and SNP-Check (Certus Technology & EMQN, 2021) were the programs used.

NCBI's BLAST was used to determine how the primers align to the reference genome. Both forward and reverse primers were copied and pasted from the generated excel sheet into the 'blastn' query box. The database was set at 'Human RefSeqGene Sequences', and 'highly similar sequences (megablast)' was selected under program selection. Then "BLAST" was selected. The result showed specificity, how well the primers matched to the reference genome, and gave a genomic location. The location was double checked to match the location in the Assay Design Suite file.

Genomic alignment was second checked by UCSC's Human Blat Search. The forward and reverse primers for each SNP were copied and pasted into the search box separately. The 'Genome' selection was human, and the 'Assembly' was Dec. 2013 (GRCh38/hg38). The-results show the percent match of the primer to the genome. All primers showed 100% match and the genomic location was checked to ensure Agena Design Suite output was correct.

UCSC's In-Silico PCR was used to determine the amplicon produced by entering a primer pair. The 'Genome' selection was human, the 'Assembly' was Dec. 2013 (GRCh38/hg38), and the 'Target' was set as genome assembly. The amplicon sequence and the genomic position was cross-checked with the ADS report. To determine if there were any SNPs within the primer SNPCheck was used. No SNPs of concern were found within the primer sequences.

Using the extend primers from the generated file from Assay Design Suite and the corresponding amplicon, extend primers were validated. Depending on the direction of the extend primer, forward or reverse, the extend primer was either searchable within the amplicon, or was reverse complemented to find the primer within the amplicon.

2.4.1.2 Obesity Panel Version 1

After secondary validation, a two-well design was finalized. Well 1 consisted of 26 SNPs and Well 2 consisted of 23 SNPs (**Table 2.4**). The PCR and extend primers were ordered from Integrated DNA Technologies (IDT) at a concentration of 25nm for PCR primers (**Appendix B1 and B2)** and 250nm for extend primers (**Appendix C1 and C2)**.

Table 2.4 Obesity Panel Version 1 for Well 1 and Well 2 designed with Agena Assay Design Suite.

SNP W1	Gene	SNP W2	Gene
rs11672660	*GIPR*	rs6804842	*RARB*
rs12458	*GATA4*	rs2124499	*ADCY5*
rs6265	*BDNF*	rs403656	*SCAPER*
rs17203016	*MIR1302-4, CREB1*	rs10499276	*RGS17, OPRM1*
rs11208662	*LEPR*	rs4307239	*NPY, MPP6*
rs2304607	*MEF2C-AS2*	rs6235	*PCSK1*
rs10182181	*ADCY3, DNAJC27*	rs4624596	*GSK3B*
rs6919443	*GRIK2, HACE1*	rs8070454	*PSMD3, CSF3*
rs9349239	*PRPH2*	rs11629783	*MAP2K1*
rs12327272	*PMAIP1, MC4R*	rs11792069	*EHMT1*
rs1394	*TNKS*	rs13329567	*MAP2K5, SKOR1*
rs12939549	*RPTOR*	rs7777102	*MLXIPL, VPS37D*

SNP W1	Gene	SNP W2	Gene
rs2229616	*MC4R*	rs879620	*ADCY9*
rs9816226	*ETV5, DGKG*	rs7784465	*RAC1*
rs573455	*CEP164*	rs3134353	*YWHAZ*
rs12675063	*ADCY8, EFR3A*	rs8087550	*MC4R, CDH20*
rs1205106	*SIPA1L1, RGS6*	rs6968554	*AGR3, AHR*
rs881301	*FGFR1, C8orf86*	rs12042959	*SDCCAG8*
rs17024393	*GNAT2*	rs3807049	*TULP1, FKBP5*
rs11915371	*LINC01212, FOXP1*	rs156151	*NONE, HACE1*
rs4372296	*DPYD*	rs1075901	*NCOR1*
rs6879326	*PDE4D*	rs13227433	*GTF2I*
rs3753549	*AKT3*	rs9939609	*FTO*
rs1899951	*PPARG*		
rs2030342	*PTBP2, DPYD*		
rs3751813	*FTO*		
rs10745785	*NEDD1, RMST*		
rs9332817	*KMT2A*		

2.4.1.3 Obesity Panel Version 2

After initial genotyping, two SNPs were removed from panel due to consistently failing to yield any genotype calls, rs12939549 and rs2030342. The updated version, referred to as version two, consisted of Well 1 containing 26 SNPs and Well 2 containing 23 SNPs. New well 1 PCR primer mixes and extend mixes were created (**Table 2.5**).

Table 2.5 Obesity Panel Version 2.

SNP Well 1	Gene Well 1	SNP Well 2	Gene Well 2
rs11672660	*GIPR*	rs6804842	*RARB*
rs12458	*GATA4*	rs2124499	*ADCY5*
rs6265	*BDNF*	rs403656	*SCAPER*
rs17203016	*MIR1302-4,CREB1*	rs10499276	*RGS17,OPRM1*
rs11208662	*LEPR*	rs4307239	*NPY,MPP6*
rs2304607	*MEF2C-AS2*	rs6235	*PCSK1*
rs10182181	*ADCY3,DNAJC27*	rs4624596	*GSK3B*
rs6919443	*GRIK2,HACE1*	rs8070454	*PSMD3,CSF3*
rs9349239	*PRPH2*	rs11629783	*MAP2K1*
rs12327272	*PMAIP1,MC4R*	rs11792069	*EHMT1*
rs1394	*TNKS*	rs13329567	*MAP2K5,SKOR1*
rs2229616	*MC4R*	rs7777102	*MLXIPL,VPS37D*
rs9816226	*ETV5,DGKG*	rs879620	*ADCY9*
rs573455	*CEP164*	rs7784465	*RAC1*
rs12675063	*ADCY8,EFR3A*	rs3134353	*YWHAZ*
rs1205106	*SIPA1L1,RGS6*	rs8087550	*MC4R,CDH20*
rs881301	*FGFR1,C8orf86*	rs6968554	*AGR3,AHR*
rs17024393	*GNAT2*	rs12042959	*SDCCAG8*
rs11915371	*LINC01212,FOXP1*	rs3807049	*TULP1,FKBP5*
rs4372296	*DPYD*	rs156151	*NONE,HACE1*

SNP Well 1	Gene Well 1	SNP Well 2	Gene Well 2
rs6879326	*PDE4D*	rs1075901	*NCOR1*
rs3753549	*AKT3*	rs13227433	*GTF2I*
rs1899951	*PPARG*	rs9939609	*FTO*
rs3751813	*FTO*		
rs10745785	*NEDD1,RMST*		
rs9332817	*KMT2A*		

2.4.1.4 Validation of Obesity Panel via Sanger Sequencing

Using Typer program and the Plate Analyzer, genotype call plots were used for 176 genotyped samples. Based on the genotype cluster plot, at least three samples that were homozygous were chosen for confirmatory Sanger sequencing for each SNP. At least six samples that were heterozygous were chosen for confirmatory Sanger sequencing for each SNP. The number of samples used per SNP vary depending on the plot. An example plot and samples that were picked for Sanger sequencing is shown (**Figure 2.1**). If plots showed a skew or 'messy' calls, more samples were used to confirm the calls were accurate (**Figure 2.2**).

Sanger primers were designed using various in-silico tools. Firstly, Primer3 was used, and amplicon length was set at >500. The primers designed by Primer3 were then validated using UCSC In-Silico PCR, BLAT, and SNPCheck using the same method as '2.9.1.1 PCR Primer Validation'. The primers were tagged with M13 tails (**Appendix D1**). Lyophilized primers were ordered from Integrated DNA Technologies (Coralville, IA, USA) and primer pairs were reconstituted in molecular grade water to 100 µM. The primers were further diluted in molecular grade water to make 10 µM working dilutions from the 100 µM tube. Two primer sets were

designed and ordered for rs1205106, rs10745785, rs6804842, rs4307239 and rs4624596 due to potential issues with specificity of SNPs within the primer regions. After initial PCR and gel electrophoresis additional primers were ordered for rs9349239, rs879620, and rs10499276 (**Appendix D2**).

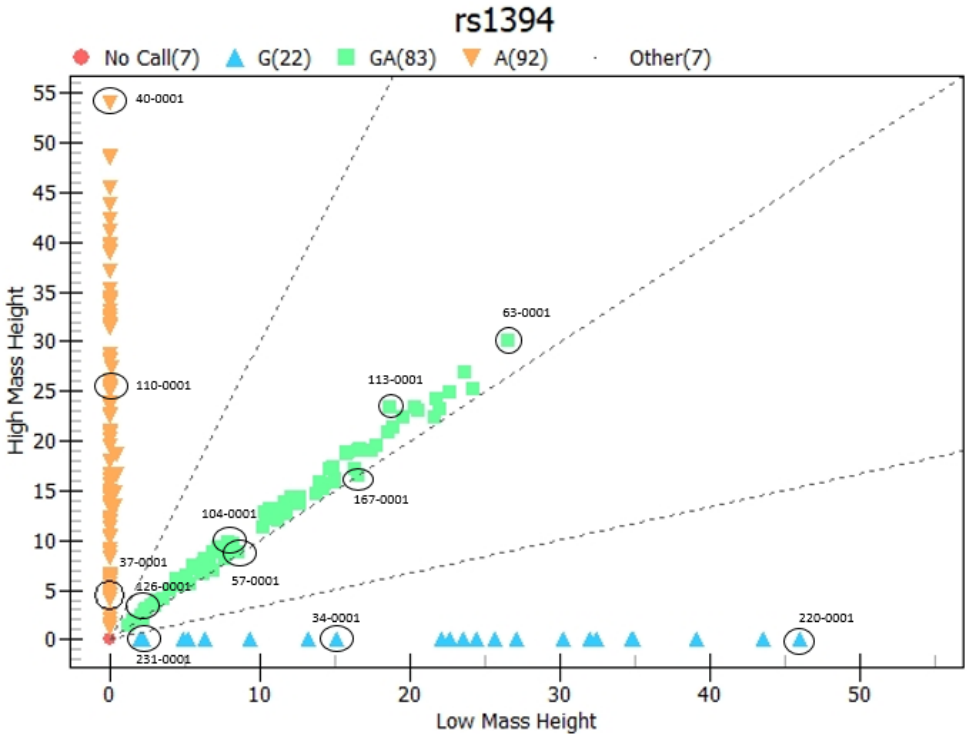


Figure 2.1 Example of a genotype plot used to choose samples for confirmatory Sanger sequencing. The plot has low-mass height calls along the x-axis and high-mass height calls along the y-axis. With 'A' calls being represented with an orange triangle, G calls being a blue triangle and GA calls being a green square. No calls are indicated by a red circle. Dashed lines indicate where sample should fall within for each call. Circled samples are the samples chosen for Sanger sequencing.

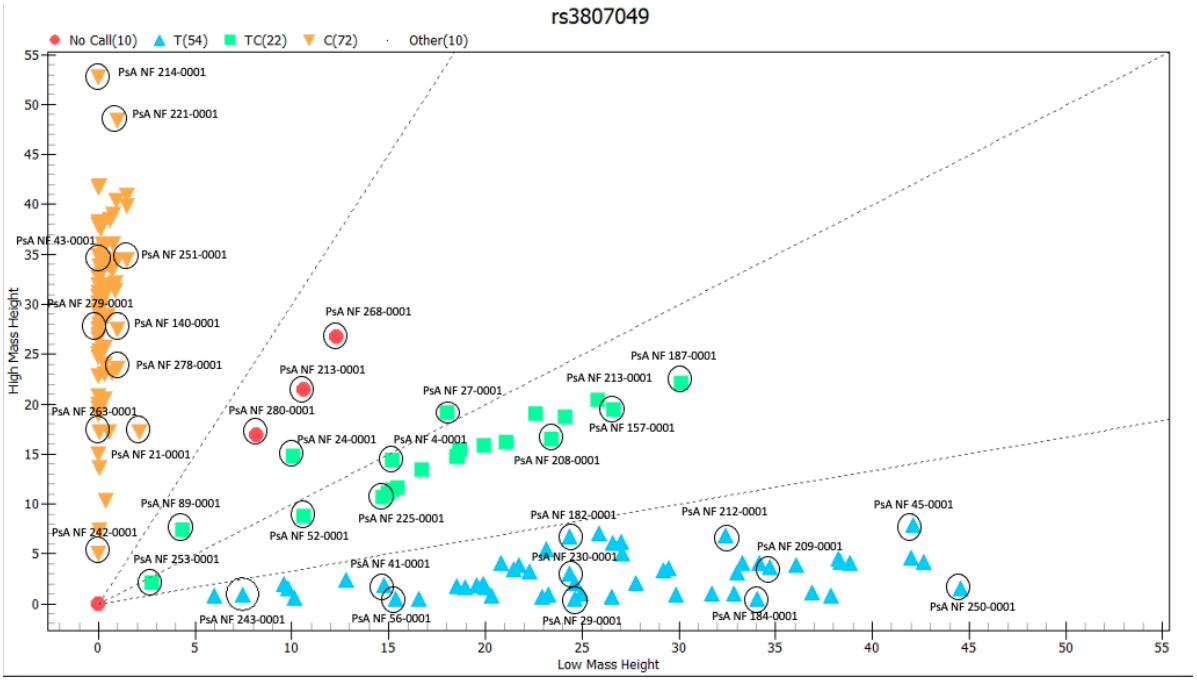


Figure 2.2 Example of a messy genotype plot used to choose samples for confirmatory Sanger sequencing. The plot has low-mass height calls along the x-axis and high-mass height calls along the y-axis. With 'C' calls being represented with an orange triangle, 'T' calls being a blue triangle and TC calls being a green square. No calls are indicated by a red circle. Dashed lines indicate where sample should fall within for each call. Circled samples are the samples chosen for Sanger sequencing.

Two different PCR protocols were used, one using Amplitaq and one using Platinum Taq DNA polymerase. **Table 2.6** describes which reagent was used for each SNP. For Amplitaq Gold Master Mix, a volume of 2 µL of each DNA sample, at a concentration of 10 ng/µL, was mixed with 1.0 µL of 10 µM M13-tagged forward and reverse primers, 12.5 µL of AmpliTaq Gold 360 Master Mix (Applied Biosystems Inc., Foster City, CA, USA), and 8.5 µL of molecular grade water. A non-template control was included with each SNP reaction by adding 2 µL of molecular grade water instead of DNA template. Samples were amplified in a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems Inc.) under the following conditions: initial denaturation of 95 °C for 10 minutes; 35 cycles of 94 °C for 30 seconds, 60 °C for 45 seconds, and 72 °C for 1 minute; and an elongation of 72 °C for 7 minutes; followed by a 4 °C hold.

For the Platinum Taq Master a volume of 1 µL of each DNA sample, at a concentration of 10 ng/µL, was mixed with 0.5 µL of 10 µM M13-tagged forward and reverse primers, 0.1 µL Platinum Taq DNA Polymerase (Invitrogen), 2.5 µL of 10x PCR Buffer, 0.75 µL of 50mM $MgCl_2$, 0.5 µL of dNTP mix, and 19.15 µL of molecular grade water. A non-template control was included with each SNP reaction by adding 1 µL of molecular grade water instead of DNA template. Samples were amplified in a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems Inc.) under the following conditions: initial denaturation of 94 °C for 5 minutes; 5 cycles of 94 °C for 30 seconds, 64 °C for 30 seconds, and 72 °C for 30 seconds; 30 cycles of 94 °C for 30 seconds, 54 °C for 30 seconds, and 72 °C for 30 seconds; and an elongation of 72 °C for 7 minutes; followed by a 4 °C hold. An additional PCR thermocycler method was used in order to troubleshoot double banded and smeared amplicons. The method was as follows: 94 °C for 2 minutes; 30 cycles of 94 °C for 30 seconds, 60°C for 30 seconds, and 72 °C for 30 seconds; followed by a 4 °C hold.

Table 2.6 PCR protocol used for each SNP in Sanger Sequencing.

AmpliTaq Gold 360	Platinum Taq Polymerase
rs1205106	rs9349239
rs1899951	rs3753549
rs881301	rs4372296
rs1075901	rs573455
rs12042959	rs6265
rs1205106	rs6879326
rs403656	rs6919443
rs7777102	rs10499276
rs10182181	rs6804842

AmpliTaq Gold 360	Platinum Taq Polymerase
rs10745785	rs6968554
rs9939609	rs8070454
rs11208662	rs879620
rs12675063	rs13329567
rs17024393	rs156151
rs2229616	rs2124499
rs2304607	rs3134353
rs3751813	rs4307239
rs9332817	rs4624596
rs3807049	rs6235
rs7784465	rs6968554
rs8087550	rs9816226
rs11672660	rs11629783
	rs11792069
	rs12327272
	rs1394
	rs11915371
	rs12458
	rs17023016

The successful amplification of PCR products was confirmed by horizontal electrophoresis using a 1% agarose gel in 1X tris-borate-ethylenediaminetetraacetic acid (TBE) buffer. Gels were comprised of 100 mL of 1X TBE, 1.0 g of UltraPure agarose (Invitrogen), and 3.0 μL of SYBR Safe DNA Gel Stain (Invitrogen). The gel mixture was boiled using a microwave and after cooling, was poured into a gel electrophoresis chamber containing loading

well combs. After the gel solidified, the combs were removed and 5 µL of 100 bp DNA ladder (Invitrogen) or 3 µL of each PCR product mixed with 1 µL of 6X sample buffer were pipetted into the wells of the gel. Gels were electrophoresed at 110 volts for 30 minutes. The PCR products were visualized using a U:Genius GelVue UV transilluminator (302 nm; Syngene, Frederick, MD, USA) to determine successful PCR amplification and appropriate amplicon size.

PCR products were purified by adding 2 µL of ExoSAP-IT (Applied Biosystems Inc.) to 5 µL of the PCR product. The mixture was run on a GeneAmp PCR System 9700 Thermocycler at 37 °C for 15 minutes to degrade the primers and excess nucleotides, followed by 80 °C for 15 minutes to inactivate the ExoSAP-IT reagent, followed by a 4 °C hold.

An aliquot (1µL) of purified PCR product was added to 16.18 µL of molecular grade water, 2 µL of 5X sequencing buffer (Applied Biosystems Inc.), 0.32 µL of 10 µM M13-tagged forward or reverse primers (Integrated DNA Technologies), and 0.5 µL of BigDye Terminator v3.1 Ready Reaction Mix (Applied Biosystems Inc.). Samples were ran on a GeneAmp PCR System 9700 Thermocycler for 25 cycles of 96 °C for 1 minute, 96 °C for 10 seconds, 50 °C for 5 seconds, 60 °C for 4 minutes and a 4 °C hold.

A cocktail of 10 µL of X-Terminator Beads (Applied Biosystems Inc.) and 45 µL of SAM solution (Applied Biosystems Inc.) was added to the cycle sequencing product. The plate was vortexed using an Advanced Vortex Mixer (VWR, Radnor, PA, USA) for 40 minutes at 1700 rpm, after which it was centrifuged for 2 minutes at 1000 g to ensure the beads were pelleted to the bottom of the plate. The purified sequencing products were then sequenced using a capillary electrophoresis instrument (ABI 3130xl).

DNA sequences were analyzed using Mutation Surveyor software version 5.0.0 (SoftGenetics LLC, State College, PA, USA). This software aligns the sequences to the reference

and allows visual inspection of the chromatograms. A representative number of samples for each SNP were inspected for the forward Agena primer, reverse Agena primer, the extend primer sequence used in the genotyping reaction. The genotype of the target SNP was also recorded. All calls were recorded in an excel file and was second checked by a staff member for call confidence. Each SNP plot from Typer Program was analyzed and labelled with the Sanger sequencing calls to ensure full coverage of the plot. If plots had accurate coverage of homozygous calls and heterozygous calls from sequencing, the SNP was determined to be complete, and no further analysis was necessary. The Sanger sequencing calls were then compared to the Agena genotype calls. If Sanger and Agena calls were discordant, the discordant samples were re-sequenced to ensure correct sample was used. If call remained discordant, further analysis of the plot and genotype was necessary as discussed in results **Section 3.2.2.4**.

2.4.2 PsA Panel

This panel was broken down into three different wells, well 1 contained 15 single nucleotide polymorphisms (SNPs), well 2 contained 16 SNPs, and well 3 contained 11 SNPs (**Table 2.7)**. SNP assays from this panel were uploaded into the Typer 4 program within 'Assay Editor' for MassARRAY genotyping.

Table 2.7 PsA Panel Well Breakdown for Agena MassARRAY.

SNPs in Well 1	SNPs in Well 2	SNPs in Well 3
rs12044149	rs10782001	rs1050414
rs12189871	rs11209026	rs10888503
rs12212594	rs12191877	rs13214872

rs13017599	rs146571698	rs1800925
rs2201841	rs2066808	rs2082412
rs2248902	rs2734331	rs2844603
rs2476601	rs3129944	rs2894207
rs2844535	rs34536443	rs4406273
rs3130457	rs396960	rs887466
rs3131382	rs4349859	rs9266242
rs33980500	rs4908742	rs9321623
rs3869115	rs6457374	
rs4655683	rs67841474	
rs587560	rs715285	
rs848	rs9304742	
rs9468859		

2.5 Primer Adjustment for MassARRAY

2.5.1 PCR Primer Mix

Primer mixes were made for both polymerase chain reaction (PCR) (**Appendix E1, E2, and E3**) and extend primers. PCR primers were received from Integrated DNA Technologies (IDT) at a concentration of 100 μM with both forward and reverse primers included in the same mix. The concentration of each forward and reverse primer was 0.5% of the total volume. When making a 1 mL PCR primer mix for example, 5 μL of each primer was added, and molecular grade water was added to bring the primer mix to a final volume of 1 mL. The volume of water

depended on the number of SNPs in the plex: the more SNPs within the plex, less water would be added.

2.5.2 Extend Primer Mix for MassARRAY

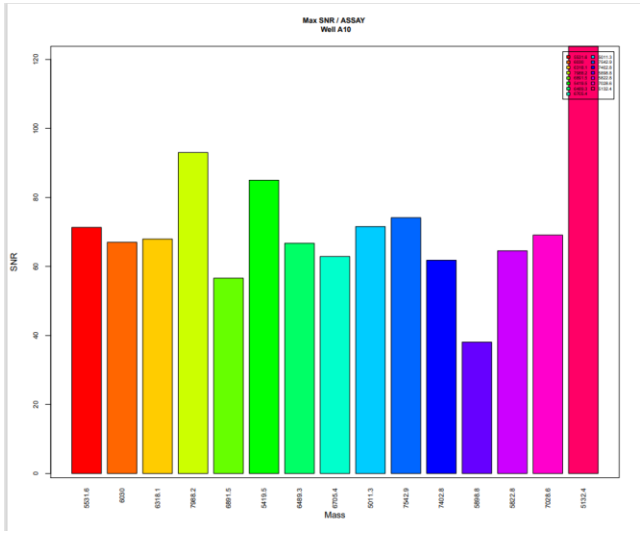
Extend primers were ordered from IDT at a concentration of 500 μM. When making the extend primer mix, different volumes of the primer were added depending on the SNP mass (**Appendix F1, F2, F3**)**.** SNPs with a higher mass tend to have a lower signal-to-noise ratio (SNR). To ensure all SNPs have a similar SNR, more extend primer would be added for higher mass SNPs. To calculate the volume of each SNP added, the lowest mass SNP in each plex would start at a percentage of the final volume. Typically, 1mL mixes were made, and the lowest mass primer would be 1% of the total volume or 10 μL in this case. The subsequent SNPs, listed in order of increasing mass, in the well would then follow a formula to determine the amount to add.

$$\textit{Amount of extend primer to add } (\mu L) = \left(\frac{Mass\ of\ SNP}{Mass\ of\ previous\ SNP}\right)(Volume\ of\ previous\ SNP\ added)(1.02)$$

A quality check was then completed on the mixes to determine if primer volumes required adjustments. This was performed in triplicate, where 1 μL of the mix was added to three tubes containing 49 μL of water. The mix was vortexed and spun down. The mixture was then added to a 'primer adjust' 96-well plate, in three separate wells. The wells were visually inspected to ensure no air bubbles were present and then was spotted using the Nanodispenser using a single pin format. After the samples have been spotted onto the chip, the chip was then transferred to the MassARRAY. A 'Primer Adjust Report' was generated containing a bar graph

of each well (**Figure 2.3**) and an excel sheet. The bar graph gave a graphical visual of the mass of each SNP versus the SNR of the SNP. The excel sheet depicted the specific numbers from the bar graph, with the mass and the SNR for each SNP, and calculated 'percent to add'. If the percent to add was above 50%, the primer needed to be adjusted. To adjust a specific SNP, the original amount of primer added to the mix was multiplied by the 'percent to add' amount, giving the amount of that SNP that needed to be added to the original extend mix. These steps were repeated until all primers percent to add were below 50%.

A.



B.

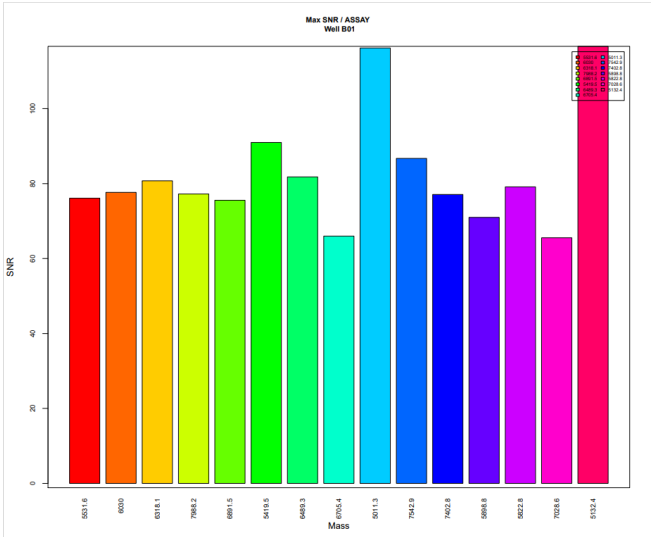


Figure 2.3 Example of bar graph report for PsA panel Well 2 generated by Typer Program during extend primer adjustment. X-axis indicates mass of each SNP while y-axis indicates SNR. A) Report pre-adjustment; and B) Report after final adjustment.

2.6 Reaction for Genotyping on Agena MassARRAY

2.6.1 Polymerase Chain Reaction

This step was required to amplify the regions surrounding the variants of interest.

Samples of DNA were amplified containing the SNPs of interest using PCR. A PCR master mix

(1x) was made which contained: 0.80 µL of High-Performance Liquid Chromatography (HPLC) molecular grade water, 0.50 µL of 10X PCR buffer (Agena Bioscience), 0.40 µL of 2mM $MgCl_2$, 0.10 µL of 25 mM dNTP, 0.20 µL of 5 u/µL PCR Enzyme, and 1 µL of 0.5 µM PCR Primer Mix. The master mix was then vortexed and spun down. The master mix was divided into an 8-well strip and 3 µL was dispensed in each well of a 96 well plate (Veriti Low Profile) using a multichannel pipette. 2 µL of sample DNA was dispensed per well at a concentration of 10ng/µL. 2 µL of water, instead of DNA, were dispensed in two wells per reaction plate and were used as the no template controls (NTCs). The plate was covered with Applied Biosystems MicroAmp clear adhesive film, vortexed, and spun down. The plate was then placed in a ThermoFisher Veriti Thermocycler using a PCR program, as specified by Agena's protocol, as follows: 95°C for 2 minutes, 45 cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minute, followed by 72°C for 5 minutes, and a 4°C hold. If not immediately preceding to the next step, the reaction plate was stored at -20°C.

2.6.2 Shrimp Alkaline Phosphatase (SAP) Step

This step was required to remove incorporated dNTPs after the amplification reaction. A shrimp alkaline phosphatase (SAP) master mix (1x) was prepared containing 1.53 µL of HPLC molecular-grade water, 0.17 µL of 10X SAP buffer, and 0.30 µL of 5 u/µL SAP enzyme (Agena Bioscience) to ensure a clean PCR reaction. The plate was spun down, and the film was removed. The SAP master mix was divided evenly into an 8-well strip and 2µL was then pipetted using a multichannel pipet into the reaction plate. The plate was covered with film, vortexed, and spun down. The plate was then placed in a Thermofisher Veriti Thermocycler on 'SAP' program, as specified by Agena's protocol, as follows: 37°C for 40 minutes, 85°C for 5 minutes, and then a 4°C hold.

2.6.3 Extension Step

This step incorporated a nucleotide as a primer termination extension reaction. An extend master mix was prepared containing a 1x reaction: 0.62 μL of HPLC molecular-grade water, 0.20 μL of iPLEX 10X Buffer Plus, 0.20 μL of iPLEX 10X Terminator Mix, 0.94 μL of Extend Primer Mix, and 0.041 μL of iPLEX Pro Enzyme 32 u/μL (Agena Bioscience). The master mix was vortexed and quickly spun. The previous reaction plate was removed from the thermocycler and spun down quickly. The master mix was equally aliquoted into an 8-well strip. The film from the plate was removed and 2 μL of master mix was added to each well using a multichannel pipet. The plate was sealed, vortexed, and spun down, before being placed in the Thermofisher Veriti thermocycler on the 'Extend' program. The program was as follows: 95°C for 30 seconds, [94°C for 5 seconds, (5°C for 5 seconds, 80°C for 5 seconds for 5 cycles) for 40 cycles], 72°C for 3 minutes and a 4°C hold. The plate was stored at -20°C until the next step was performed.

2.6.4 Resin Step

This step removes impurities from the sample to be tested as the MassARRAY system is very sensitive to contamination. Resin (Agena Bioscience) was spooned out onto a dimple plate. A scraper was used to spread the resin along the dimple plate with a beveled edge. Excess resin was scraped off and the wells were examined to ensure they contained even amounts of resin. The resin plate was then left to dry for 12 minutes. During this time, 41 μL of HPLC molecular-grade water was added to each well of the reaction plate and was centrifuged at 2204 gravitational force (G) for one minute. Once the resin was dried, the reaction plate was inverted, and the wells were aligned with the dimples containing dry resin. This was once again inverted allowing the resin to fall into the wells of the reaction plate. The plate was then sealed and rotated for 30 minutes. After 30 minutes, the plate was centrifuged for 5 minutes at 2204 g.

2.7 MassARRAY Setup

The supply tank of the Agena Nanodispenser was filled with Milli-Q purified water and the waste tank was drained before use. The sonicator was drained and filled with 50% ethanol/50% milli Q H_2O solution and placed back into the machine. The pins were then primed by running a 10x wash cycle using the 50% ethanol. Once the pins have been rinsed, the film was removed from the reaction plate and the plate was placed in the plate holder of the Nanodispenser. A chip was placed in the Nanodispenser using forceps. 3-point calibrant was thawed and brought to room temperature, 60 μL of calibrant was pipetted into the calibrant holder and placed within the Nanodispenser.

Target volume was set at 15 nL with volume check enabled between 5 nL and 25 nL. Aspirate time was set at 8 seconds with an offset at 5.9 mm and speed of 60 mm/seconds. Dispense time was 0.2 seconds, offset of 1 mm and speed of 60 mm/seconds. Dry time was set at 0.5 seconds with a rinse time of 3.5 seconds, and a wash time of 5 seconds. Transfer was selected and 'Run' was then started. Once the rinse station was confirmed to be operating correctly, the spotting was started.

The plate information was uploaded in Typer 4 and 'iPLEX' was selected for the terminator chemistry and 'Genotype + Area' as the process and the dispenser was selected as 96-well plate and 96-spot chip '96-96'. The chip was placed on the carrier in slot 1 and a blank chip was placed in slot 2 using forceps. The chip carrier was placed back on the stage in the machine. The 'Automatic run set-up' option was selected, and a 'Barcode Report' was generated. The barcode report was reviewed to ensure the correct perimeters were selected. Once the green

'ready' light came on, an additional 5 minutes were elapsed to allow the pressure to stabilize, and then 'autorun' was selected.

2.8 Genotyping Call Criteria using Agena MassARRAY

Cluster plots were viewed to see how well the plate performed and NTCs were checked to ensure no genotype calls were seen. The desired minimum passing rate was 95% of samples for each SNP.

A calling tree algorithm, previously developed within the laboratory as a quality control metric to gain confidence in SNP calls, was implemented (**Figure 2.4**). This was an extra measure taken by our laboratory to ensure accurate genotypes. The first decision was 'call description' includes peak height, SNR, call probability, and distribution. Call description could be conservative, moderate, aggressive, or low probability based on a calling algorithm generated by Agena Bioscience. The call description would determine which other metrics need to be assessed to be confident in the call. The next decision was peak height, SNR ratio, and then peak height ratio. While this criterion works for the majority of the SNPs in the panel, some SNPs had additional criteria to ensure confidence in the call. SNR and allele ratios were the most common criterion adjusted, due to skewing of SNP clusters. Any genotypes with allele ratio falling within the specific 'exclusion zone' would be skewed too far from the calling cluster and would be removed regardless of any other criteria. The validity of extra criteria was confirmed by Sanger sequencing and by repeat genotyping previously when the PsA panel was initially designed with a representative number of samples. The SNPs with additional criteria were rs1050414, rs1800925, rs2066808, rs2248902, rs4908742, and rs848, their alternate calling criteria in indicated in **Table 2.8**. The SNP rs2844603 had additional criteria shown in **Table 2.9**.

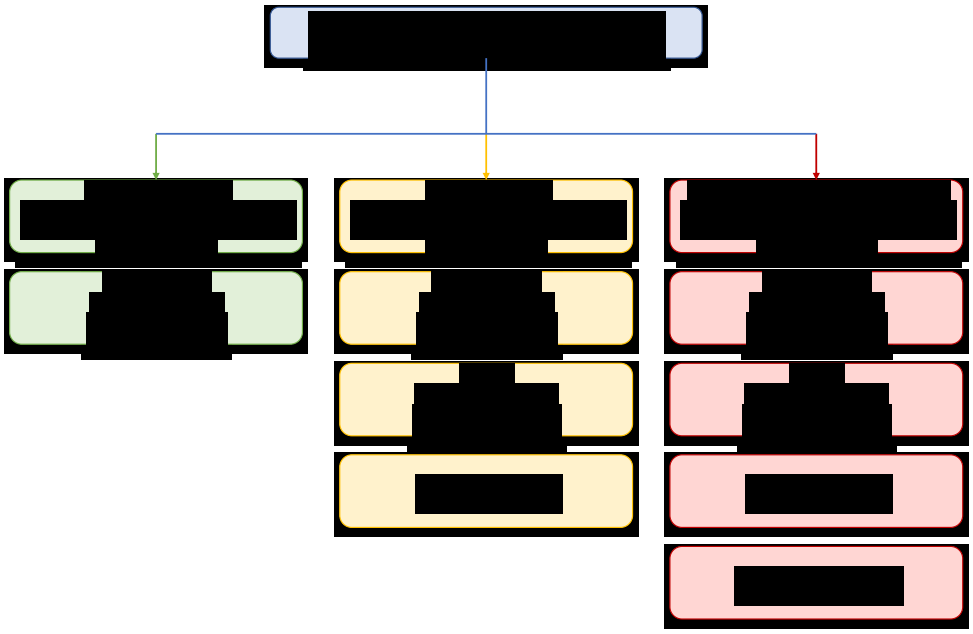


Figure 2.4 Genotype calling criteria used to analyze variants using Typer 4.0. This criterion was designed and implemented within the Rahman-O'Rielly Laboratory to ensure confidence in all genotype calls. If a call was denoted as 'conservative' by the Agena Typer 4.0 program, we are more confident that the call was accurate (i.e. less stringent additional criteria); if the call was considered 'moderate' by the Agena Typer 4.0 program, we would add more criteria to ensure accuracy and so on.

Table 2.8 Alternate calling criteria for rs1050414, rs1800925, rs2066808, rs4908742, and rs848 used to analyze the PsA panel.

SNP	SNR	Allele Ratio	Allele Ratio Exclusion Zone
rs1050414	Reduce to 15 and 7.5		0.2-0.55
rs1800925	Reduce to 15 and 7.5		
rs2066808	Reduce to 15 and 7.5		0.1-0.4
rs2248902			0.1-0.4
rs4908742	Reduce to 10 and 5	≥0.45	
rs848		≥0.45	

Table 2.9 Alternate calling criteria for rs2844603 used to analyze the PsA panel.

Allele Ratio 1/2	Allele Ratio 2/1	Genotype Call
≥1.10	≤0.91	A/A
0.41-0.77	1.3-2.46	A/G
≤0.12	≥8.0	G/G
0.75-1.09	0.95-1.29	Exclude calls (Failed call)

2.9 Data analysis

2.9.1 Frequency Analysis

Genotype frequencies were calculated for each SNP by the number of each genotype divided by the respective number of samples genotyped.

AA frequency= # of AA genotypes/Number of samples genotyped

The allele frequencies were then calculated by dividing the number of alleles present for each SNP by the total number of alleles (number of samples multiplied by 2).

A frequency= # of A alleles/2xNumber of samples genotyped

Minor allele frequencies were recorded and compared to published MAF. Fisher's exact test was performed for each SNP to determine if there was a significant difference between the cohort MAF and the published MAF. The p-value two-tailed and significance was set at <0.05. Hardy-Weinberg equilibrium was calculated for each SNP using the following formula, where p represents the frequency of the major allele and q represents the frequency of the minor allele:

$$p^2 + 2pq + q^2 = 1$$

A comparison of MAF was completed by dividing the PsA cohort into two groups, normal weight (BMI <25 kg/m^2) and obese weight (BMI >30 kg/m^2). MAF of the two groups

were compared to determine if obesity-associated SNPs were more common in both groups and p-value was calculated using chi-square test.

2.9.2 Quantitative Trait Locus Analysis

For the PsA cohort, quantitative trait analysis was performed, by Dr. Quan Li, with BMI as the quantitative trait. Wald test, which was used to determine association between genotype and phenotype, was used under the null hypothesis so that all genotypes have the same mean and variance. Within this analysis, BMI was considered the phenotype (P), which depends on the genetics (G), which would be the genotype. Other residual environmental effects were labelled as E.

$$P= G + E + G \times E$$

A linear regression analysis of BMI as the phenotype versus genotype was performed. For biallelic polymorphisms, beta coefficients (b), genotypes as aa, Aa, and AA, where A was the major allele and a was the minor allele, and the additive effects of allele dosage (ADD) were applied. The simplified model without covariates was:

$$P = b0 + b1.ADD + e$$

Other clinical covariates were also considered, such as age, sex, smoking, and height:

$$P = b0 + b1.ADD + b2.age + b3.sex + b4.smoking + b5.height + e$$

The beta (regression coefficient), p-values, genotype frequency, and mean BMI from these linear models were stratified by genotypes (aa, Aa, and AA). In total, there were 42 SNPs where Bonferroni correction was used to adjust for multiple comparisons, where $p<0.001$.

A similar analysis was performed for the obesity cohort. Clinical covariates differed as follows, age, sex, and height.

$$P = b0 + b1.ADD + b2.age + b3.sex + b4.height + e$$

The beta (regression coefficient), P-values, genotype frequency, mean BMI from these linear models were stratified by genotypes (aa, Aa, and AA). In total, there are 46 SNPs where Bonferroni correction was used to adjust for multiple comparisons.

In order to assess the possible association between two continuous clinical variables or features in our PsA cohort, such as age of onset of PsA, BMI, height, weight, PASI score, number of tender joints, number of swollen joints, depression score, and disease severity score, correlation analysis was performed. In our analysis, we mainly focused the correlations between BMI with other features.

In correlation analysis, which was performed by Dr. Quan Li, the correlation coefficient was used to measure the strength of the association between the features. There are mainly two types of correlations coefficients: Pearson's and Spearman's rank. For Pearson's correlation, it was applied only when both features are normally distributed. We did the tests of normality from Shapiro-Wilk for our features, also look the skewness and kurtosis, most of the features did not follow the normal distribution. Therefore, nonparametric correlation as Spearman's rank was proposed in this association study. Spearman's rank correlation coefficient as ρ (rho) was calculated as

$$\boldsymbol{\rho = 1 - \frac{6 \sum d_i^2}{n\,(n^2 - 1)}}$$

Where d_i was the difference in ranks for two features, n was the number of observations. A correlation coefficient of zero indicates that there were no association between two continuous features, while a correlation coefficient of −1 or +1 indicated a strongest negative or positive relationship respectively.

Chapter 3 Results

3.1 PsA-associated Variants in Obesity Cohort

3.1.1 Clinical Information

The obesity cohort consisted of 696 individuals who were genotyped for 42 psoriatic disease-related SNPs. All individuals within this cohort had information regarding weight, height, age, sex, and smoking-status collected. The cohort consisted of 73% female with an average age of 49 ± 15 years and average BMI of 35 ± 8 kg/m^2. No patients had manifestations of Ps, PsA, spondyloarthritis, or any other immune related disease (**Table 3.1**).

Table 3.1 Clinical Information for Obesity Cohort.

Clinical Parameter	Average	Standard deviation
Height	163.58 cm	8.96 cm
Weight	96.75 kg	22.85 kg
BMI	35 kg/m^2	8 kg/m^2
Age	49 years	15 years
Sex	73% female	N/A
Smoking Status	58% of cohort smoked	N/A

3.1.2 PsA Panel

The PsA panel was previously designed and validated within the laboratory by research staff for a prior project. An initial validation cohort was completed on the panel as shown in **Appendix G1** and **G2** with a summary of peak height averages and signal to noise ratio (SNR) averages. This cohort was previously genotyped by a laboratory staff member when the panel was initially designed.

3.1.3 Agena Quality Control

To ensure accurate calls, quality control metrics were used when analyzing results as described in the methods (**Section 2.8**). These metrics were applied to the three wells of the panel, allowing confidence in the genotypes. The samples that did not meet these cut-offs were removed from the final data. The breakdown of samples genotyped, samples that failed to meet the criteria, and samples that passed the criteria is provided (**Table 3.2**), broken down by each SNP. Ideally, each SNP should have a pass rate of >95%.

Table 3.2 Percentage of Obesity Cohort Samples Passing/Failing Criteria in the PsA Panel SNPs for MassARRAY Genotyping.

SNP	Total	Failed	Passed	% Fail	% Pass
rs1050414	696	106	590	15.23	84.77
rs10782001	696	51	645	7.33	92.67
rs10888503	696	27	669	3.88	96.12
rs11209026	696	5	691	0.72	99.28
rs12044149	696	9	687	1.29	98.71
rs12189871	696	10	686	1.44	98.56
rs12191877	696	5	691	0.72	99.28
rs12212594	696	59	637	8.48	91.52
rs13017599	696	7	689	1.01	98.99
rs13214872	696	6	690	0.86	99.14
rs146571698	696	17	679	2.44	97.56
rs1800925	696	30	666	4.31	95.69
rs2066808	696	15	681	2.16	97.84
rs2082412	696	6	690	0.86	99.14
rs2201841	696	15	681	2.16	97.84
rs2248902	696	111	585	15.95	84.05
rs2476601	696	41	655	5.89	94.11
rs2734331	696	9	687	1.29	98.71
rs2844535	696	6	690	0.86	99.14
rs2844603	696	109	587	15.66	84.34
rs2894207	696	18	678	2.59	97.41
rs3129944	696	24	672	3.45	96.55
rs3130457	696	9	687	1.29	98.71
rs3131382	696	16	680	2.30	97.70

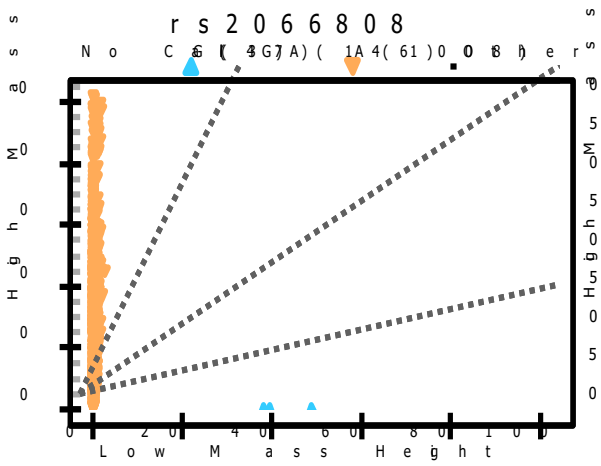
SNP	Total	Failed	Passed	% Fail	% Pass
rs33980500	696	23	673	3.30	96.70
rs34536443	696	5	691	0.72	99.28
rs3869115	696	6	690	0.86	99.14
rs396960	696	17	679	2.44	97.56
rs4349859	696	6	690	0.86	99.14
rs4406273	696	6	690	0.86	99.14
rs4655683	696	8	688	1.15	98.85
rs4908742	696	72	624	10.34	89.66
rs587560	696	8	688	1.15	98.85
rs6457374	696	69	627	9.91	90.09
rs67841474	696	7	689	1.01	98.99
rs715285	696	8	688	1.15	98.85
rs848	696	26	670	3.74	96.26
rs887466	696	7	689	1.01	98.99
rs9266242	696	12	684	1.72	98.28
rs9304742	696	17	679	2.44	97.56
rs9321623	696	13	683	1.87	98.13
rs9468859	696	17	679	2.44	97.56

3.1.4 SNPs with additional criteria

Seven SNPs in the PsA panel were analyzed using additional criteria that was implemented during panel design and optimization which was previously decided by research staff. Examples of the SNPs with additional criteria cluster plot are below (**Figure 3.1)** and their calling criteria changes as previously discussed (**Section 2.8**). SNP rs2066808 had SNR lowered to 15 and 7.5 and the exclusion zone changed to 0.1-0.4. SNP rs2844603, formed two distinct heterozygous clusters which needed its own calling criteria, as shown in methods **Table 2.9**. SNP rs4908742 had the SNR lowered to 10 for homozygous calls and 5 for heterozygous calls and the allele ratio cut-off lowered to 0.45. This SNP was difficult due to low yield of the extend primer as shown in **Figure 3.2**. When yield was low, a low probability call was often made, like seen in this SNP. SNP rs848 had its allele ratio lowered to 0.45. rs1050414 had SNR changed to

15 for homozygous calls and 7.5 for heterozygous calls and the exclusion zone changed to 0.2-0.55. SNP rs1800925 had SNR lowered to 15 for homozygous calls and 7.5 for heterozygous calls. Lastly, rs2248902 had the exclusion zone more stringent at 0.1-0.4.

A.



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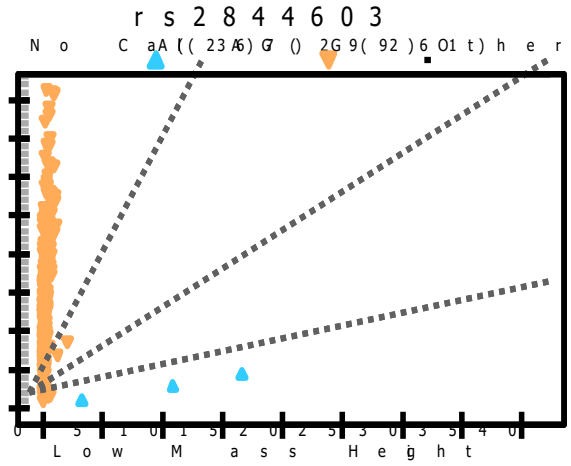
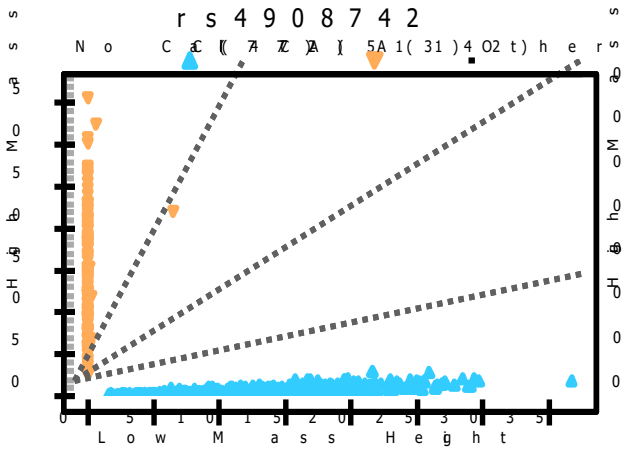
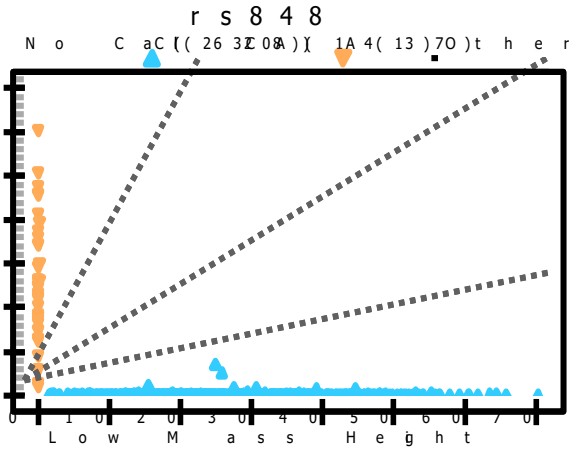


C.



D.



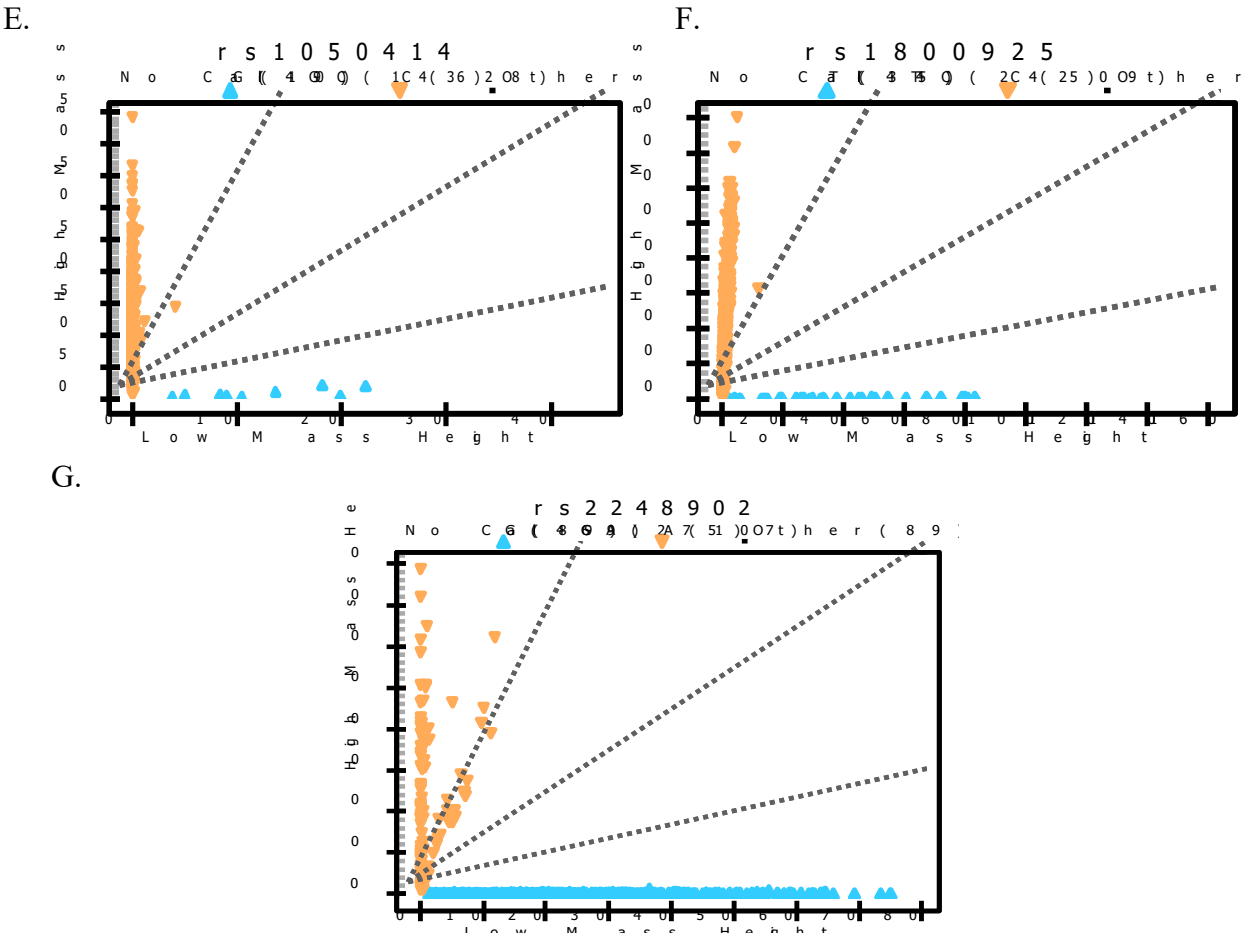


Figure 3.1 Typer genotyping plots for SNPs in the PsA panel with additional calling criteria. A) rs2066808 which has SNR reduced to 15 and 7.5 and exclusion zone set ratio at 0.1-0.4; B) rs2864603 which uses allele ratios to make calls; C) rs4908742 which has SNR reduced to 10 and 5 and allele height ratio >0.45; D) rs848 has allele ratio set at >0.45; E) rs1050414 which has SNR reduced to 15 and 7.5 and exclusion zone set at 0.2-0.55; F) rs1800925 which has SNR reduced to 15 and 7.5; and G) rs2248902 which had exclusion zone 0.1-0.4. Each plot has low-mass height calls on the x-axis and high-mass height calls on the y-axis. Dotted lines indicate 'calling zones' for associated calls. Blue triangles indicate low-mass height calls, orange triangles represent high mass height calls, and green squares indicate heterozygous calls. Red circles indicate no calls.

A. B.

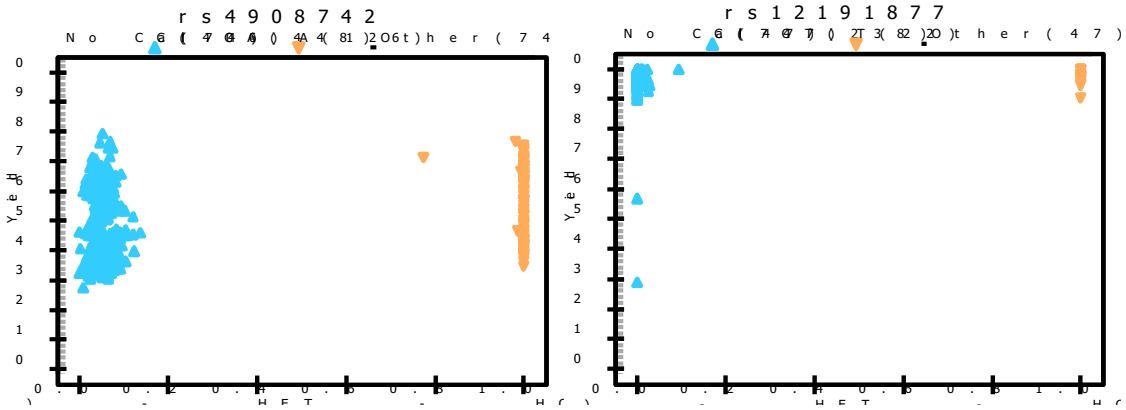


Figure 3.2 Yield versus genotype plots for rs4908742 and rs12191877. A) rs4908742 had low yield for a lot of samples, meaning not all of the extend primer was being used in the reaction; and B) rs12191877 was an example of what good yield looks like on a plot, meaning most of the extend primer was being used in the reaction for all samples. Yield is displayed on the y-axis, ranging from 0-1 (0=no extend primer was used, while 1=all extend primer was used). X-axis displays the three different genotypes depending on mass. Low mass genotype calls are indicated by a blue triangle, heterozygous calls are indicated by a green square, and high mass homozygous calls are indicated by an orange triangle. Red circles indicate samples that were not assigned a call.

SNPs that did not meet >95% pass rate or needed extra criteria within the PsA panel are summarized in **Table 3.3**, which further explains the potential reason for the complication if found and any modifications to address the issues.

Table 3.3 PsA Panel SNPs with genotyping issues and modifications required.

SNP	Issue Observed	Reason for Issue (if found)	Modification (if possible)
rs10782001	Low sample pass rate	One SNP within reverse primer with low MAF (0.0302%)	
rs12212594	Low sample pass rate	MHC region; one SNP with very low MAF (0.105%)	

SNP	Issue Observed	Reason for Issue (if found)	Modification (if possible)
rs4908742	Low sample pass rate	SNP in extend primer and leftover UEP causing low extend yield; very difficult to design SNP with many unspecific areas	
rs2476601	Low sample pass rate	One SNP in froward primer with low MAF (0.005%)	Allele height ratio relaxed <0.45
rs206608	Low signal to noise ratio	Unknown	SNR lowered to 10 and 5; no skewing or clustering on genotyping plot
rs2844603	Low sample pass rate, two heterozygous clusters noted on genotyping plot causing many low probability calls	Located within MHC region; unspecific primer set (2 100% matches using SNPCheck; multiple results in UCSC in-silico PCR)	Unique calling criteria was designed to ensure limited sample fails
rs1050414	Low sample pass rate, dispersed cluster of heterozygous calls due to SNP in extend primer	12 SNPs within the forward and reverse primers; 2 100% hits for forward primer; MHC region; multiple SNPs in extend primer	SNR relaxed to 15 and 7.5, more stringent allele ratio exclusion zone 0.2-0.55
rs2248902	Low sample pass rate Skewing on heterozygous caused by SNP in reverse and extend primer	SNP in reverse and extend primers; located with MHC region	More stringent allele height ratio exclusion zone between 0.1-0.4
rs4908742	Many low probability and aggressive calls	Low yield was noted (leftover UEP)	SNR reduced to 10 and 5, allele ratio height cut off relaxed to <0.45
rs848	Many low probability calls	Skewing on genotyping plot	Allele height ratio relaxed <0.45
rs1800925	Many low probability calls	No skewing was noted on plot, reason unknown	SNR reduced to 15 and 7.5

3.1.5 Genotype Frequencies

Genotype frequencies for the obesity cohort were calculated for each SNP within the PsA panel. The minor allele frequencies (MAF) of the cohort were compared to published MAF using dbSNP (NCBI) in **Table 3.4**. Hardy-Weinberg Equilibrium was also calculated for each SNP and each SNP was in equilibrium. Most of the published MAFs matched very closely with the

cohort's MAF. There were two SNPs that had a statistically significant difference in published and cohort MAF, rs3129944 and rs67841474.

Table 3.4 Published Minor allele frequencies (MAF) for the PsA Panel SNPs versus cohort MAF in the obesity cohort.

SNP	Gene	Published MAF	Homozygous Reference Genotype (n)	Heterozygous Genotype (n)	Homozygous Alternate Genotype (n)	Total (n)	Cohort MAF	P-value of Fisher's
rs1050414	*HLA-C*	G=0.097808	476	107	7	590	0.1025	1
rs10782001	*FBXL19*	G=0.460595	246	324	75	645	0.3674	0.2509
rs10888503	*LCE3A*	C=0.414222	322	274	73	669	0.3139	0.1857
rs11209026	*IL23R*	A=0.042204	594	93	4	691	0.07308	0.5371
rs12044149	*IL23R*	T= 0.21461	365	267	55	687	0.2744	0.4079
rs12189871	*HLA-C*	T=0.087737	570	112	4	686	0.08746	1
rs12191877	*HLA-C*	T=0.123941	518	158	15	691	0.136	0.8339
rs12212594	*HLA-C*	C=0.055348	562	70	5	637	0.06279	1
rs13017599	*REL*	A=0.14715	284	318	87	689	0.357	0.0892
rs13214872	*HLA-C*	G=0.123957	518	158	14	690	0.1348	1
rs146571698	*TNIP1*	T=0.047918	592	83	4	679	0.06701	0.7673
rs1800925	*IL13*	T=0.257621	435	201	30	666	0.1959	0.401
rs2066808	*STAT2*	G=0.082660	594	84	3	681	0.06608	1
rs2082412	*IL12B*	A=0.283687	439	208	43	690	0.213	0.3239
rs2201841	*IL23R*	G=0.308155	308	305	68	681	0.3238	1
rs2248902	*HLA-C*12:03*	A=0.198307	352	186	47	585	0.2393	0.6089
rs2476601	*PTPN22*	A=0.071083	538	114	3	655	0.0916	0.7953
rs2734331	*SKIV2L*	G=0.047327	644	43	0	687	0.0313	0.7209
rs2844535	*HLA-B*08:01*	G=0.282938	358	285	47	690	0.2746	1
rs2844603	*HLA-C*12:03*	A=0.347485	218	347	22	587	0.333	0.8814
rs2894207	*HLA-C*06:02*	C=0.203292	499	152	27	678	0.1519	0.457
rs3129944	*HLA-B*38:01*	G=0.288131	531	120	21	672	0.1205	0.0047*
rs3130457	*PSORS1C3*	C=0.219785	377	265	45	687	0.2584	0.6197
rs3131382	*HLA-B*39:05*	T=0.043339	575	99	6	680	0.08162	0.3727
rs33980500	*TRAF3IP2*	T=0.085558	592	79	2	673	0.06166	0.5928
rs34536443	*TYK2*	C=0.026803	625	65	1	691	0.04848	0.7209
rs3869115	*HLA-C1202*	G=0.099476	653	37	0	690	0.02681	0.0818
rs396960	*NOTCH4*	A=0.291922	386	256	37	679	0.243	0.5218
rs4349859	*MICA*	A=0.027125	647	43	0	690	0.03116	1
rs4406273	*HLA-C*	A=0.088828	571	112	7	690	0.0913	1
rs4655683	*IL23R*	A=0.356082	281	319	88	688	0.3597	1
rs4908742	*TNFRSF9*	A= 0.365921	254	295	75	624	0.3566	1
rs587560	*KIR2DS2*	T=0.30230	332	301	55	688	0.2987	1

SNP	Gene	Published MAF	Homozygous Reference Genotype (n)	Heterozygous Genotype (n)	Homozygous Alternate Genotype (n)	Total (n)	Cohort MAF	P-value of Fisher's
rs6457374	*HLA-B*08:01*	C=0.189722	361	207	59	627	0.2592	0.3096
rs67841474	*MICA*	delG= 0.212116	205	339	145	689	0.4565	0.0003*
rs715285	*5q31*	G=0.342476	198	321	169	688	0.4789	0.0613
rs848	*IL13*	A=0.315717	426	215	29	670	0.2037	0.0756
rs887466	*PSORS1C3*	A=0.391468	268	309	112	689	0.3868	1
rs9266242	*HLA-B*	T=0.283790	291	312	81	684	0.3465	0.3611
rs9304742	*ZNF816*	C=0.418785	265	334	80	679	0.3638	0.4686
rs9321623	*TNFAIP3*	T=0.427529	200	308	175	683	0.4817	0.5702
rs9468859	*HLA-B*	A=0.011592	663	14	2	679	0.01325	1

**Statistically significant p-value <0.05*

3.1.6 Genotype versus BMI

Quantitative trait analysis (QTL) was performed with the assistance of Dr. Quan Li, to explore the association between BMI and genotypes of the 42 SNPs using a multivariate linear regression model. Bonferroni correction was used to adjust for multiple comparisons (**Table 3.5)**. Two SNPs were statistically significant, rs3131382 and rs10782001 (**Table 3.6** and **Figure 3.3**). For rs3131382 variant, those with a BMI with the TT genotype, was 47.1 kg/m^2 vs 35.4 kg/m^2 for the CC genotype, (p=0.00005). For rs10728001, individuals with the GG genotype had a mean BMI of 37.2 kg/m^2 versus 34.3 kg/m^2 for individuals with the AA genotype (p=0.00075).

Table 3.5 Genotypes for 42 SNPs in the PsA Panel versus BMI for all samples in obesity cohort for QTL analysis.

SNP	Gene	BETA	R2	P (adjusted)*
rs4908742	TNFRSF9	0.1154	0.000114	0.8265
rs12044149	IL23R	-0.0411	1.30E-05	0.6742
rs4655683	IL23R	0.02981	7.66E-06	0.4776
rs2201841	IL23R	0.7673	0.004815	0.09776
rs11209026	IL23R	0.5938	0.000907	0.7036
rs2476601	PTPN22	-0.2029	0.000128	0.01069
rs10888503	LCE3A	1.037	0.009118	0.0278

SNP	Gene	BETA	R2	P (adjusted)*
rs13017599	REL	-0.2525	0.000549	0.808
rs715285	5q31	-0.2842	0.000817	0.5867
rs1800925	IL13	-0.128	0.0001	0.8574
rs848	IL13	-0.8686	0.004634	0.2062
rs146571698	TNIP1	0.7291	0.001284	0.6846
rs2082412	IL12B	-0.3583	0.000893	0.1909
rs9468859	HLA-B	0.03394	6.83E-07	0.9198
rs887466	PSORS1C3	0.2099	0.000416	0.8517
rs3130457	PSORS1C3	-0.316	0.000715	0.44
rs3869115	HLA-C*12	-0.4343	0.000181	NA
rs2248902	HLA-C*12:03	-0.4719	0.001775	0.3438
rs1050414	HLA-C	0.7468	0.001866	0.07648
rs2844603	HLA-B*08:01	-0.4548	0.001158	0.1916
rs12189871	HLA-C	0.6648	0.001296	0.6343
rs12191877	HLA-C	0.03932	7.04E-06	0.9883
rs13214872	HLA-C	-0.1413	9.00E-05	0.7764
rs2894207	HLA-C*06:02	-0.2726	0.000404	0.382
rs4406273	HLA-C	0.467	0.000697	0.6306
rs6457374	HLA-B*08:01	0.4683	0.001774	0.7607
rs12212594	HLA-C	0.9062	0.001923	0.56
rs9266242	HLA-B	0.2787	0.000659	0.8752
rs2844535	HLA-B*08:01	-0.6369	0.002937	0.2486
rs4349859	MICA	0.8412	0.000779	NA
rs67841474	MICA	0.2828	0.000753	0.2745
rs3131382	HLA-B*39:05	2.227	0.0146	0.000054**
rs2734331	SKIV2L	-0.9486	0.000993	NA
rs396960	NOTCH4	-0.1155	8.97E-05	0.9537
rs3129944	HLA-B*38:01	0.1428	9.45E-05	0.7326
rs33980500	TRAF3IP2	-1.065	0.002451	0.174
rs9321623	TNFAIP3	0.2265	0.000528	0.4077
rs2066808	STAT2	-1.643	0.006282	0.1374
rs10782001	FBXL19	1.584	0.02023	0.0007524**
rs34536443	TYK2	0.03596	2.21E-06	0.9771
rs9304742	ZNF816	0.0961	7.55E-05	0.4853

SNP	Gene	BETA	R2	P (adjusted)*
rs587560	KIR3DL3	0.5311	0.002128	0.4394

*P-adjusted for age, sex, height, smoking status
**Significant at <0.001
R2=coefficient of determination

Table 3.6 SNPs with significant difference between genotypes and mean BMI.

SNP	rs3131382			rs10782001		
Genotype	TT	TC	CC	GG	GA	AA
Number of samples	6	99	575	75	324	246
Frequency	0.008824	0.1456	0.8456	0.1163	0.5023	0.3814
BMI MEAN (kg/m^2)	47.17	36.73	35.46	37.29	36.36	34.43
SD (kg/m^2)	16.07	6.641	7.12	7.684	7.396	6.829

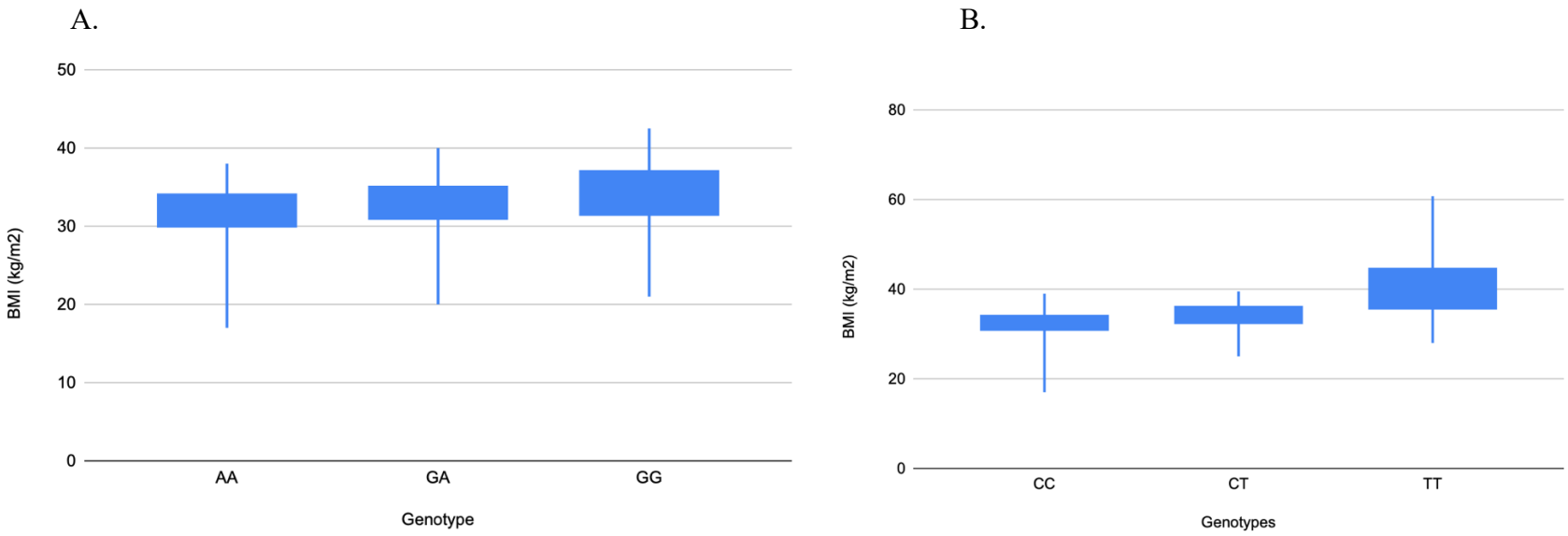


Figure 3.3 Difference in average BMI per genotype of each SNP is shown using box plots. A) rs10782001 with an increase of mean BMI when the 'GG' genotype is present; and B) rs3131382 with an increase of mean BMI when the 'TT' genotype is present. Genotypes per each SNP represented on the x-axis and BMI (kg/m^2) on the y-axis.

3.2 Obesity-associated Variants in PsA Cohort

3.2.1 Clinical information

The PsA cohort consisted of 678 individuals from both Ontario and Newfoundland. 495 samples were from Ontario and 183 were from Newfoundland. The average BMI of the cohort was 29.3 kg/m^2 with a standard deviation of 6.8 kg/m^2. 57% of the patients were male with an average age at visit of 57.3 years. The average age of onset of psoriasis was between 28.44 years and the average age of diagnosis of PsA was about 37 year. The majority of the cohorts had psoriasis, 97.1%. Both patient cohorts had over half of the cohort employed (60%) with the majority also being high school graduates or having college or university level education (**Table 3.7**). Metrics pertaining to PsA was obtained during the patient assessment by research team involving rheumatologist and rheumatology research nurse. A summary of the cohort is in **Table 3.7** and **3.8.**

Table 3.7 Basic Clinical Information for PsA Cohort.

Clinical Parameter	Average	Standard deviation
Height	169.2 cm	9.9
Weight	84.0 kg	19.0 kg
BMI	29.3	6.10
Age	57.3 years	14.1
Sex	56.7% male	N/A

Table 3.8 Newfoundland (NL) and Ontario (ON) PsA Samples Demographic Information.

Demographics	**Results**
Age of Diagnosis of Psoriasis (n=410)	
Mean	28.44
SD	13.56

Age of Diagnosis of PsA (n=424)	
Mean	37.64
SD	11.9
Iritis	14.9%
Psoriasis	97.1%
IBD	32.9%
Work Status	
Employed	60.0%
Retired	18.7%
Homemaker	5.4%
Sick Leave	4.0%
Other	11.9%
Education	
< Grade 8	1.0%
High School (Incomplete)	5.4%
High School Graduate	27.0%
College	28.8%
University	37.8%

3.2.2 Obesity Panel

3.2.2.1 Gene Prioritization

After gene prioritization was completed, which was completed in consultation with Dr. Quan Li, the panel consisted of 50 obesity associated SNPs based on gene-disease relationship score (**Table 3.9**). An additional SNP, rs9939609, was added to the panel at a later date due to relevance with obesity. This resulted in 51 SNPs in the final prioritized list.

Table 3.9 Gene prioritization list for SNPs associated with obesity.

Chromosome	Reference allele	Alternate allele	Location of SNP	Gene-Disease Relationship Score	Gene^	SNP
1	C	G	intronic	0.6608	*LEPR*	rs11208662

Chromosome	Reference allele	Alternate allele	Location of SNP	Gene-Disease Relationship Score	Gene^	SNP
18	A	G	intergenic	0.6583	*PMAIP1,MC4R*	rs12327272
18	T	C	exonic	0.6583	*MC4R*	rs2229616
18	A	C	intergenic	0.6583	*MC4R,CDH20*	rs8087550
8	T	C	intergenic	0.6197	*FGFR1,C8orf86*	rs881301
11	T	C	exonic	0.5614	*BDNF*	rs6265
3	T	C	intronic	0.5601	*PPARG*	rs1899951
5	C	G	exonic	0.505	*PCSK1*	rs6235
2	A	G	intergenic	0.5023	*MIR1302-4,CREB1*	rs17203016
1	T	C	intronic	0.4951	*AKT3*	rs3753549
7	T	C	intronic	0.494	*RAC1*	rs7784465
17	T	C	intronic	0.4884	*NCOR1*	rs1075901
9	A	G	intronic	0.4813	*EHMT1*	rs11792069
3	T	C	intronic	0.4659	*tv*	rs4624596
5	T	C	intronic	0.4493	*PDE4D*	rs6879326
1	A	G	intronic	0.4483	*SDCCAG8*	rs12042959
8	A	T	intronic	0.441	*YWHAZ*	rs3134353
5	A	G	intergenic	0.4392	*LINC00461,MEF2C*	rs2304607
8	A	T	UTR3	0.439	*GATA4*	rs12458
3	C	G	intronic	0.4292	*ADCY5*	rs2124499
15	C	G	intronic	0.4201	*MAP2K1*	rs11629783
17	T	C	intergenic	0.4181	*PSMD3,CSF3*	rs8070454
2	A	G	intergenic	0.4166	*ADCY3,DNAJC27*	rs10182181
16	T	C	UTR3	0.4159	*ADCY9*	rs879620
8	A	T	intergenic	0.4157	*ADCY8,EFR3A*	rs12675063
7	A	G	intergenic	0.3783	*AGR3,AHR*	rs6968554
7	A	G	intergenic	0.3653	*MLXIPL,VPS37D*	rs7777102
7	A	G	intergenic	0.3392	*NPY,MPP6*	rs4307239
17	A	G	intronic	0.3281	*RPTOR*	rs12939549
3	A	G	intergenic	0.3269	*MIR4792,RARB*	rs6804842
11	A	G	exonic	0.3159	*CEP164*	rs573455
7	T	G	intronic	0.2961	*GTF2I*	rs13227433
1	T	C	intergenic	0.2801	*PTBP2,DPYD*	rs2030342
1	A	C	intronic	0.2801	*DPYD*	rs4372296

Chromosome	Reference allele	Alternate allele	Location of SNP	Gene-Disease Relationship Score	Gene^	SNP
6	A	G	intronic	0.2728	*PRPH2*	rs9349239
6	C	G	intergenic	0.2649	*NONE,HACE1*	rs156151
6	A	G	intergenic	0.2649	*GRIK2,HACE1*	rs6919443
3	A	T	intergenic	0.2592	*ETV5,DGKG*	rs9816226
3	A	C	intergenic	0.2567	*LINC01212,FOXP1*	rs11915371
1	T	C	intronic	0.2539	*GNAT2*	rs17024393
6	T	C	intergenic	0.2443	*MIR7641-2,OPRM1*	rs10499276
6	T	C	intergenic	0.2375	*TULP1,FKBP5*	rs3807049
14	A	G	intergenic	0.2327	*SIPA1L1,RGS6*	rs1205106
8	A	G	intronic	0.2317	*TNKS*	rs1394
15	A	G	intronic	0.2302	*SCAPER*	rs403656
16	T	G	intronic	0.2269	*FTO*	rs3751813
19	T	C	intronic	0.2222	*GIPR*	rs11672660
15	T	C	intergenic	0.2213	*MAP2K5,SKOR1*	rs13329567
11	C	G	intronic	0.2111	*KMT2A*	rs9332817
12	T	C	intergenic	0.1949	*NEDD1,RMST*	rs10745785

^Two genes indicate an intergenic region

3.2.2.2 Obesity Panel Validation

A portion of the PsA cohort was used to validate the obesity panel. Twenty-three (23) samples and a negative template control (NTC) were initially genotyped for each well of the obesity panel. A statistical summary of the peak heights and SNR are in **Appendix H1** and **H2.** To ensure the panels genotype was accurate, peak height averages above 6 for homozygotes and above 3 for heterozygotes and SNR averages above 30 for homozygotes and above 10 for heterozygotes should be observed. The results for two SNPs, rs12939549 and rs203034, which were both in well 1, produced low peak heights, low SNR and 'low probability' calls. A new PCR primer mix was created for well 1 to remove the possibility of initial human error when the

PCR mix was made. After genotyping the same 23 samples again, the same results were produced, and these two SNPs were removed from the panel.

3.2.2.3 Obesity Panel Version 2

The new version of the panel, referred to as 'Obesity Panel V2' consisted of 26 SNPs in well one and 23 SNPs in well 2 (**Table 3.10**).

Table 3.10 Obesity Panel Version 2.

SNPs in Well 1	SNPs in Well 2
rs10182181	s10499276
rs10745785	rs1075901
rs11208662	rs11629783
rs11672660	rs11792069
rs11915371	rs12042959
rs1205106	rs13227433
rs12327272	rs13329567
rs12458	rs156151
rs12675063	rs2124499
rs1394	rs3134353
rs17024393	rs3807049
rs17203016	rs403656
rs1899951	rs4307239
rs2229616	rs4624596
rs2304607	rs6235
rs3751813	rs6804842
rs3753549	rs6968554

SNPs in Well 1	SNPs in Well 2
rs4372296	rs7777102
rs573455	rs7784465
rs6265	rs8070454
rs6879326	rs8087550
rs6919443	rs879620
rs881301	rs9939609
rs9332817	
rs9349239	
rs9816226	

3.2.2.4 Sanger Results/Panel Optimization

Confirmatory Sanger sequencing was completed on all 49 SNPs to determine accurate genotype calls and to further optimize the panel. Out of the 49 SNPs, 37 (75.5%) were successfully confirmed using Sanger sequencing without issue, whereas the remaining 12 SNPs (rs10182181, rs3134353, rs17024393, rs2304607, rs11672660, rs1205106, rs6804842, rs6879326, rs9349239, rs12675063, rs3807049, rs3751813) showing discordant calls required additional investigation as noted below to discern the discrepancy in genotype (**Table 3.11**).

For SNP rs10182181, sample PsA_NF_58_0001 gave an AA genotype using Agena but an AG genotype using Sanger sequencing (**Figure 3.4 and 3.4B**), which was due to a SNP (rs148485981) in the extend primer. SNP rs3134353 for sample PsA_NF_009_0001 gave a TT call using Agena and an AT using Sanger sequencing (**Figure 3.4 C and 3.4D**), which was due to a SNP (rs960833561) within the genotyping forward PCR primer causing the skew for the sample. The MAF was low for both SNPs (rs148485981, and rs960833561), found within the primers for these samples (**Table 3.12).** SNP, rs12675063, which showed no heterozygous calls

from genotyping on the Agena MassARRAY (**Figure 3.5**) was due to a SNP in the Agena genotyping forward primer. As there was no way to be confident with the genotyping results, this SNP was removed from the obesity panel. The other two SNPs (rs3807049 and rs3751813; **Figure 3.6**) with discordant calls had SNPs in the primer-binding sequence. The SNP located in the reverse primer of rs3807049 was rs10947556 (MAF of 0.14) and the SNP located in the forward primer of rs3751813 was rs3751814 (MAF of 0.40). As there was no way to be confident in the calls using the Agena platform, these SNPs were removed from the panel. Discordant results for six SNPs (rs17024393, rs2304607, rs11672660, rs1205106, rs6879326, rs9349239) were determined to be due to sample mix-up confined to a single column on one DNA plate ('PsA NF 1'). The original genotype call, the Sanger call, and the new genotype call is shown in **Table 3.13** below.

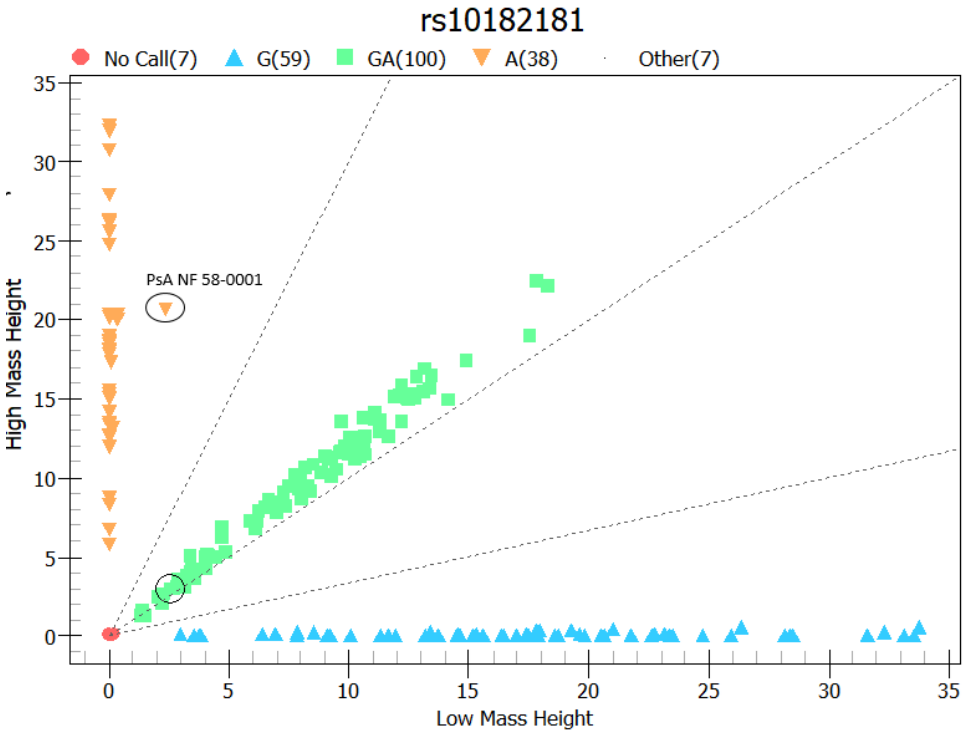
Table 3.11 Obesity Panel SNPs with genotyping issues and modifications required.

SNP	Issue Observed	Reason for Issue	Modification (if possible)
rs11672660	1. No visible PCR amplicon 2. Discordant calls between genotyping and Sanger sequencing	2. Human pipetting error	1. Repeat with same PCR primers 2. Removed inaccurate calls
rs6804842	No visible PCR amplicon		Repeat with new PCR primers
rs1205105	1. No visible PCR amplicon 2. Discordant calls between genotyping and Sanger sequencing	2. Human pipetting error	2. Repeat with new PCR primers 2. Removed inaccurate calls

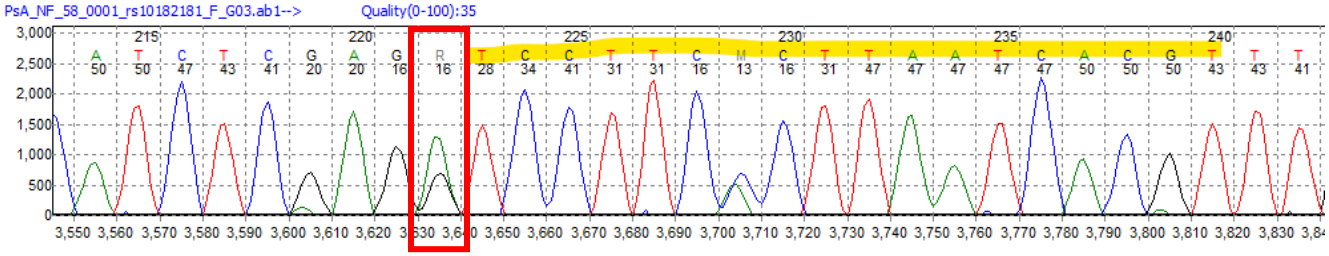
SNP	Issue Observed	Reason for Issue	Modification (if possible)
rs9349239	1. No visible PCR amplicon 2. Discordant calls between genotyping and Sanger sequencing	2. Human pipetting error	1. Repeat with new PCR primers 2. Removed inaccurate calls
rs10499276	No visible PCR amplicon		Repeat with new PCR primers
rs3134353	1. Double bands on gel electrophoresis 2. Single sample skewed	2. SNP within forward PCR of single sample	1. Changed thermocycler parameters 2. Any skewed samples were removed
rs4624596	Double bands on gel electrophoresis		Changed thermocycler parameters
rs11629783	Smeared bands on gel electrophoresis		Changed thermocycler parameters
rs879620		SNP of interest was within the first 12bp of start of sequence	New PCR primers were designed
rs17024393	Discordant calls between genotyping and Sanger sequencing	Human pipetting error	Removed inaccurate calls

SNP	Issue Observed	Reason for Issue	Modification (if possible)
rs2304607	Discordant calls between genotyping and Sanger sequencing	Human pipetting error	Removed inaccurate calls
rs6879326	Discordant calls between genotyping and Sanger sequencing	Human pipetting error	Removed inaccurate calls
rs808550	Many aggressive calls	Skewing on genotyping plot	Allele height ratio cut off was relaxed to >0.45 due to concordant calls during Sanger sequencing
rs10182181	Single sample skewed	SNP within extend primer of single sample	Any skewed samples were removed
rs12675063	No heterozygous calls, discordant genotyping calls with Sanger sequencing	SNP within forward primer (MAF=0.18) producing inaccurate genotyping calls	Removed from the obesity panel
rs3807049	Messy genotyping plot, discordant genotyping calls with Sanger sequencing	SNP within reverse PCR primer (MAF=0.14)	Removed from the obesity panel
rs3751813	Messy genotyping plot, discordant genotyping calls with Sanger sequencing	SNP within forward PCR primer (MAF=0.40)	Removed from the obesity panel

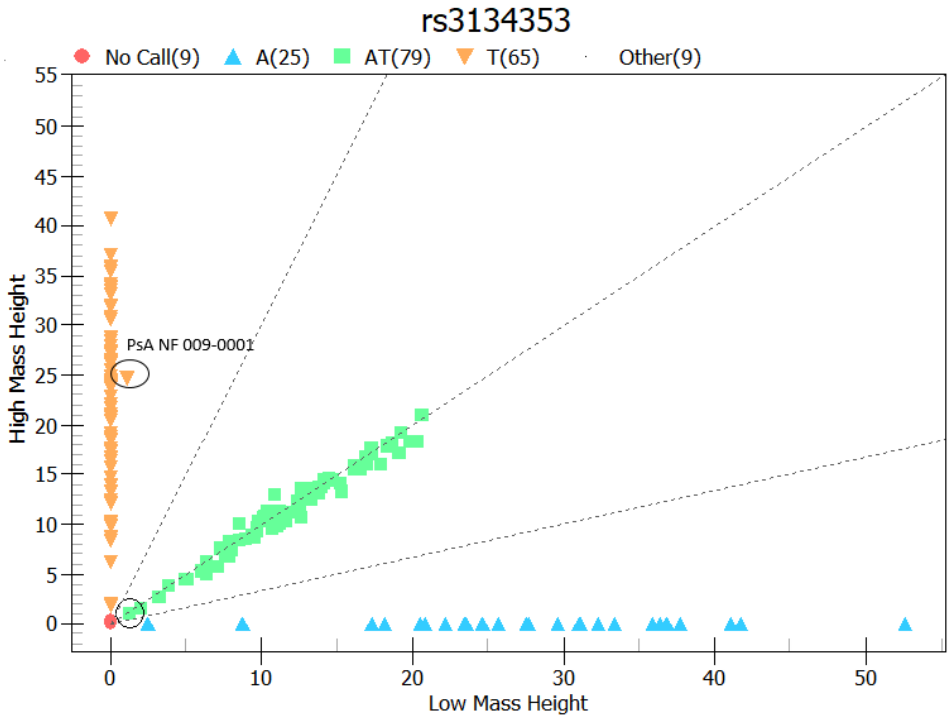
A.


rs10182181
No Call(7)
G(59)
GA(100)
A(38)
Other(7)
PsA NF 58-0001
High Mass Height
Low Mass Height
0
5
10
15
20
25
30
35

B.


PsA_NF_58_0001_rs10182181_F_G03.ab1-->
Quality(0-100):35

C.



D.

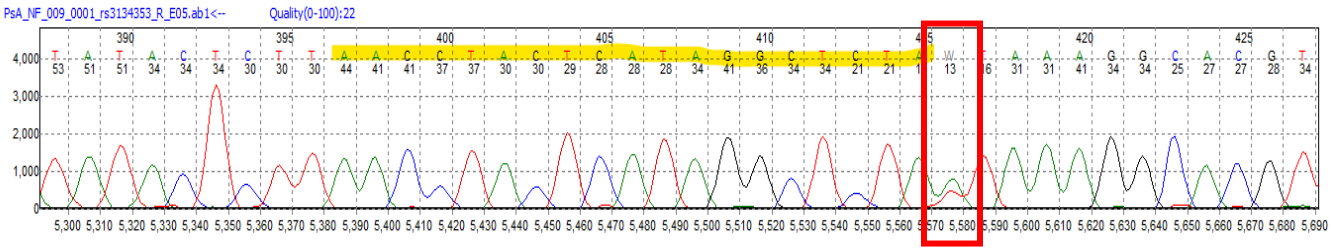


Figure 3.4 Genotyping plots of two SNPs with one discordant call due to a low MAF SNP in primer-binding site. A) rs10182181 genotyping plot with sample PsA NF 58-0001 labelled. This sample was skewed to the right compared to the other samples with genotype AA. B) Chromatogram from Mutation Surveyor of rs10182181 for sample PsA_NF_58_0001 with extend primer region highlighted and 'GA' genotype shown within the red box. C) rs3134353 genotyping plot with sample PsA NF 009-0001 labelled. This sample was skewed to the right compared to other samples with genotype TT. D) Chromatogram from Mutation Surveyor of rs31343531 for sample PsA_NF_9_0001 with extend region highlighted and 'AT' genotype shown within the red box.

Table 3.12 SNPs with discordant calls with variant found within primers of one sample during Sanger confirmatory sequencing.

PsA Panel SNP	Sample ID	Agena Genotype	Sanger Sequencing Genotype	SNP identified in primer using Sanger sequencing	Location of SNP	MAF of SNP in primer-binding siter
rs10182181	PsA NF 58-0001	AA	AG	rs148485981	Extend genotyping primer A>C	C=0.000972
rs3134353	PsA NF 009-0001	TT	AT	rs960833561	Forward genotyping primer C>CT	T=0.000016

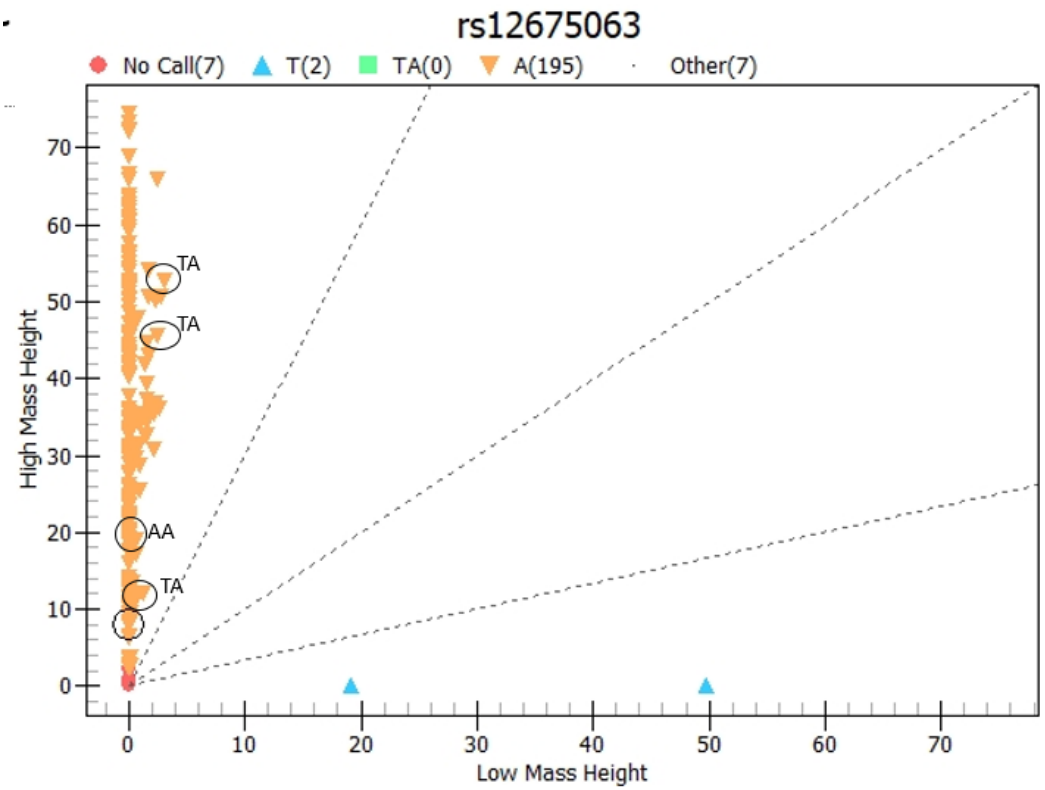
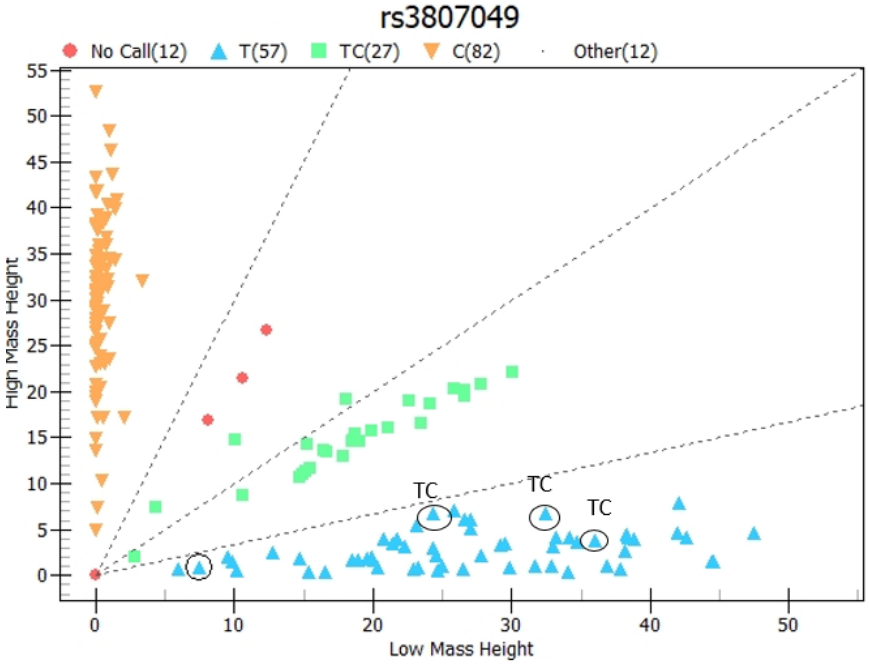


Figure 3.5 rs12675063 Typer Genotype Plot showing discordant calls within the 'AA' cluster. Each plot has low-mass height calls on the x-axis and high-mass height calls on the y-axis. Dotted lines indicate 'calling zones' for associated calls. Blue triangles indicate low-mass height calls, orange triangles represent high mass height calls, and green squares indicate heterozygous calls. Red circles indicate no calls. Agena genotype plot shows only homozygous calls (AA), however the samples that are circled were identified as heterozygotes on Sanger.

A.



B.

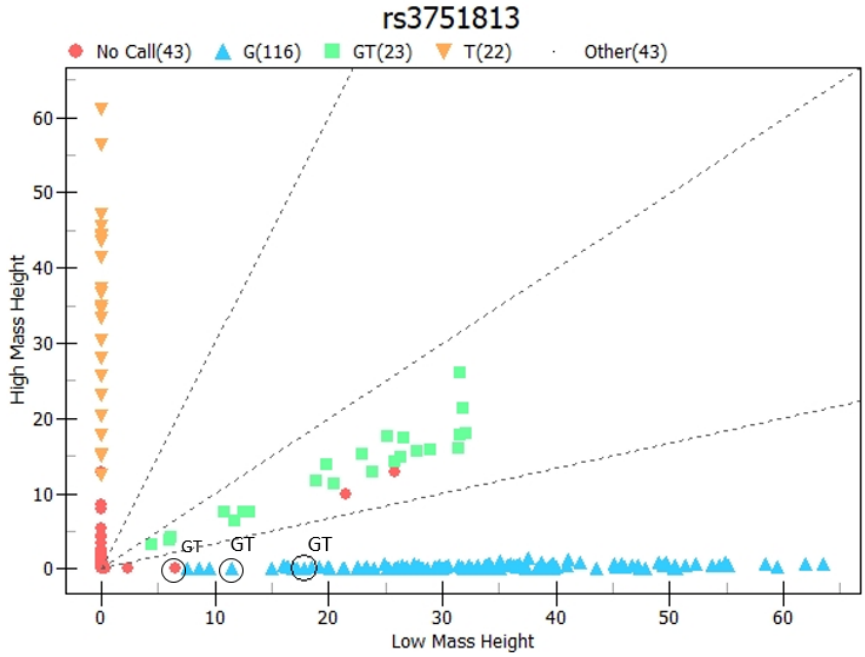


Figure 3.6 Typer Genotype Plot showing multiple discordant calls for two SNPs (rs3807049 and rs3751813) that were removed from the panel. A) rs3751813 Typer Genotype Plot showing discordant calls within the 'GG' cluster; and B) rs3807049 Typer Genotype Plot showing discordant calls within the 'TT' cluster. Each plot has low-mass height calls on the x-axis and high-mass height calls on the y-axis. Dotted lines indicate 'calling zones' for associated calls. Blue triangles indicate low-mass height calls, orange triangles represent high mass height calls, and green squares indicate heterozygous calls. Red circles indicate no calls.

Table 3.13 SNPs with discordant calls between genotyping and Sanger sequencing due to sample mix up.

SNP	Sample ID	Initial Agena Genotype Call	Sanger Call	Repeated Agena Genotype Call
rs17024393	PsA NF 169-0001	CT	AA (TT)*	TT
rs2304607	PsA NF 177-0001	AG	AA	AA
rs11672660	PsA NF 169-0001	TT	CT	CT
rs1205106	PsA NF 171-0001	GG	GA	GA
rs6879326	PsA NF 175-0001	TT	AG (TC)*	TC
rs9349239	PsA NF 177-0001	GG	CT (GA)*	GA

*Two genotypes present, A-T/G-C are base pairs; depending on what strand of DNA is being sequenced, will depend on which base-pair is present.

3.2.2.5 Final Obesity Panel Version 3

After initial genotyping and confirmatory Sanger sequencing, the panel consisted of a two-well design with 46 obesity associated SNPs (**Table 3.14**).

Table 3.14 Final Obesity Panel Design Version 3.

SNPs in Well 1	SNPs in Well 2
rs10182181	s10499276
rs10745785	rs1075901
rs11208662	rs11629783
rs11672660	rs11792069
rs11915371	rs12042959
rs1205106	rs13227433
rs12327272	rs13329567
rs12458	rs156151

SNPs in Well 1	SNPs in Well 2
rs1394	rs2124499
rs17024393	rs3134353
rs17203016	rs403656
rs1899951	rs4307239
rs2229616	rs4624596
rs2304607	rs6235
rs3753549	rs6804842
rs4372296	rs6968554
rs573455	rs7777102
rs6265	rs7784465
rs6879326	rs8070454
rs6919443	rs8087550
rs881301	rs879620
rs9332817	rs9939609
rs9349239	
rs9816226	

3.2.3 Agena Quality Control

To ensure accurate calls, quality control metrics were used when analyzing results as described in the methods **Section 2.8**. 45 SNPs followed the same quality control metrics while one SNP, rs8087550, had its allele ratio set at <0.45 cut-off. Below is a summary of the total number of samples genotyped and the total number of samples that failed and passed quality control metrics for each SNP (**Table 3.15).** Each SNP should have a percent pass rate of >95%.

Table 3.15 Percentage of PsA Cohort Samples Passing/Failing per SNP for the Obesity Panel.

SNP	Total #	Total fail	Total pass	% Fail	%Pass
rs10182181	650	7	643	1.077	98.92
rs10499276	650	12	638	1.846	98.15
rs10745785	650	12	638	1.846	98.15
rs1075901	650	9	641	1.385	98.62
rs11208662	650	15	635	2.308	97.69
rs11629783	650	1	649	0.154	99.85
rs11672660	650	4	646	0.615	99.38
rs11792069	650	1	649	0.154	99.85
rs11915371	650	0	650	0.000	100.00
rs12042959	650	0	649	0.154	99.85
rs1205106	650	28	622	4.308	95.69
rs12327272	650	0	650	0.000	100.00
rs12458	650	11	639	1.692	98.31
rs13227433	650	4	646	0.615	99.38
rs13329567	650	6	644	0.923	99.08
rs1394	650	9	641	1.385	98.62
rs156151	650	2	648	0.308	99.69
rs17024393	650	16	634	2.462	97.54
rs17203016	650	14	636	2.154	97.85
rs1899951	650	8	642	1.231	98.77
rs2124499	650	5	645	0.769	99.23
rs2229616	650	1	649	0.154	99.85
rs2304607	650	14	636	2.154	97.85
rs3134353	650	7	643	1.077	98.92
rs3753549	650	1	649	0.154	99.85
rs403656	650	8	642	1.231	98.77
rs4307239	650	3	647	0.462	99.54
rs4372296	650	3	647	0.462	99.54
rs4624596	650	7	643	1.077	98.92
rs573455	650	4	646	0.615	99.38
rs6235	650	2	648	0.308	99.69
rs6265	650	11	639	1.692	98.31
rs6804842	650	3	647	0.462	99.54
rs6879326	650	2	648	0.308	99.69
rs6919443	650	0	650	0.000	100.00
rs6968554	650	1	649	0.154	99.85
rs7777102	650	3	647	0.462	99.54

SNP	**Total #**	**Total fail**	**Total pass**	**% Fail**	**%Pass**
rs7784465	650	5	645	0.769	99.23
rs8070454	650	7	643	1.077	98.92
rs8087550	650	14	636	2.154	97.85
rs879620	650	1	649	0.154	99.85
rs881301	650	2	648	0.308	99.69
rs9332817	650	0	650	0.000	100.00
rs9349239	650	8	642	1.231	98.77
rs9816226	650	6	644	0.923	99.08
rs9939609	650	14	634	2.154	97.54

3.2.4 SNP with additional calling criteria

SNP rs8087550 was successfully confirmed by Sanger sequencing. Due to a skew in the plot, many genotyping calls did not pass our calling criteria. Many of the heterozygous calls were labelled ‘aggressive’ calls. When aggressive calls are present, stricter criteria is put in place: both allele heights >3, SNR >10, a probability >0.99, and an allele height ratio of >0.55 to pass the calling criteria. Most of the calls that did not meet criteria were just below the allele height ratio of 0.45-0.55. As you can see in the genotyping plot (**Figure 3.7**)**,** the entire heterozygous cluster is skewed downwards towards the homozygous calls. Typically, the heterozygous cluster should be centered towards the middle line of the plot. To ensure confidence, extra samples were Sanger sequenced, specifically in the heterozygous cluster. Sanger sequencing confirmed all calls to be concordant, therefore the allele ratio for this SNP was adjusted to >0.45.

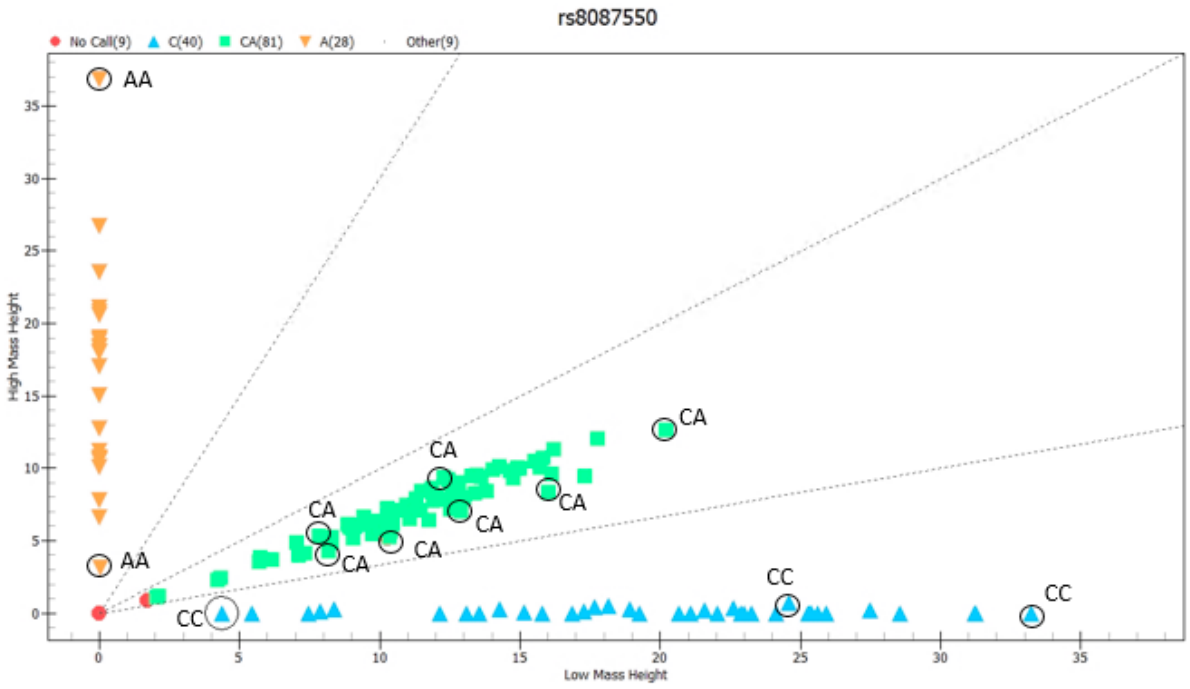


Figure 3.7 Genotyping plot for rs8087550 showing skew of heterozygous samples. Each plot has low-mass height calls on the x-axis and high-mass height calls on the y-axis. Dotted lines indicate 'calling zones' for associated calls. Blue triangles indicate low-mass height calls, orange triangles represent high mass height calls, and green squares indicate heterozygous calls. Red circles indicate no calls.

3.2.5 Genotype Frequencies

Genotype frequencies were calculated for each SNP on the obesity panel for the PsA cohort. The minor allele frequencies of the cohort were compared to published minor allele frequencies on dbSNP (NCBI) in **Table 3.16**. The minor allele frequencies of the cohort were calculated using the homozygous major allele, homozygous minor allele, and the heterozygous allele counts for each SNP. As you can see, most of the published MAF's matched very closely with the cohorts MAF. There was one SNP that had a statistically significant difference between published MAF and cohort MAF. Hardy-Weinberg Equilibrium was also calculated for each SNP and all SNPs were at equilibrium.

Table 3.16 Minor allele frequencies (MAF) for the Obesity Panel and PsA cohort showing the published MAF and the cohort MAF.

SNP	Gene^	Published MAF	Homozygous major	Heterozygous	Homozygous minor	Total	Cohort MAF	P-Value Fisher's
rs10182181	*ADCY3,DNAJC27*	A=0.426613	171	328	144	643	A=0.52099	0.2573
rs10499276	*MIR7641-2,OPRM1*	T=0.117708	496	133	9	638	T=0.11834	1
rs10745785	*NEDD1,RMST*	C=0.285582	291	286	61	638	C=0.31975	0.7589
rs1075901	*NCOR1*	T=0.420760	219	295	127	641	C=0.57176	1
rs11208662	*LEPR*	C=0.116694	519	110	6	635	C=0.09606	0.8217
rs11629783	*MAP2K1*	G=0.165807	372	244	33	649	G=0.23883	0.2933
rs11672660	*GIPR*	T=0.183485	394	229	23	646	T=0.21285	0.7215
rs11792069	*EHMT1*	G=0.115985	480	157	12	649	G=0.139445	0.8339
rs11915371	*LINC01212,FOXP1*	C=0.210953	400	220	30	650	C=0.215385	1
rs12042959	*SDCCAG8*	G=0.122547	471	166	13	650	G=0.14769	0.6796
rs1205106	*SIPA1L1,RGS6*	G=0.439587	203	322	97	622	G=0.414791	0.7749
rs12327272	*PMAIP1,MC4R*	A=0.104071	515	215	10	650	A=0.111538	1
rs12458	*GATA4*	T=0.356221	277	289	73	639	T=0.340376	0.8822
rs13227433	*GTF2I*	G=0.204853	370	246	30	646	G=0.236842	0.6089
rs13329567	*MAP2K5,SKOR1*	T=0.317461	387	222	35	644	T=0.226708	0.205
rs1394	*TNKS*	G=0.411291	294	265	82	641	G=0.334633	0.3053
rs156151	*NONE,HACE1*	C=0.140967	420	203	25	648	C=0.195216	0.3467
rs17024393	*GNAT2*	C-0.043968	605	29	0	634	C=0.022871	0.6827
rs17203016	*MIR13024,CREB1*	G=0.183867	422	198	16	636	G=0.181081	1
rs1899951	*PPARG*	T=0.261133	504	128	10	642	T=0.115265	0.0183*
rs2124499	*ADCY5*	C=0.266899	269	283	93	645	C=0.363566	0.2232
rs2229616	*MC4R*	T=0.016135	634	15	0	649	T=0.001156	0.4975
rs2304607	*MEF2C-AS2*	G=0.153572	457	171	8	636	G=0.147013	1
rs3134353	*YWHAZ*	A=0.328573	245	307	91	643	A=0.380249	0.5546
rs3753549	*AKT2*	C=0.170872	448	181	20	649	C=0.170262	1
rs403656	*SCAPER*	G=0.211296	476	151	15	642	G=0.140966	0.264
rs4307239	*NPY,MPP6*	A=0.452408	176	335	136	647	A=0.469088	0.8872
rs4372296	*DPYD*	C=0.268460	415	209	23	647	C=0.197063	0.317
rs4624596	*GSK3B*	C=0.246201	418	199	26	643	C=0.195179	0.4985
rs573455	*CEP164*	G=0.472489	155	302	189	646	G=0.526316	0.4796

SNP	Gene^	Published MAF	Homozygous major	Heterozygous	Homozygous minor	Total	Cohort MAF	P-Value Fisher's
rs6235	*PCSK1*	G=0.262602	350	252	46	648	G=0.265432	1
rs6265	*BDNF*	T=0.194877	425	187	27	639	T=0.188576	1
rs6804842	*RARB*	A=0.430822	215	288	144	647	A=0.445131	0.8868
rs6879326	*PDE4D*	T=0.465963	130	342	176	648	T=0.464506	1
rs6919443	*GRIK2,HACE1*	A=0.432586	217	305	128	650	A=0.431538	1
rs6968554	*AGR3,AHR*	A=0.386237	243	311	95	649	A=0.385978	1
rs7777102	*MLXIPL,VPS37D*	G=0.164325	486	155	6	647	G=0.129057	0.6885
rs7784465	*RAC1*	C=0.142735	501	134	10	645	C=0.11938	0.8339
rs8070454	*PSMD3,CSF3*	T=0.373347	252	311	80	643	T=0.366252	1
rs8087550	*MC4R,CDH20*	A=0.403805	169	319	148	636	A=0.483491	0.3187
rs879620	*ADCY9*	T=0.488804	112	308	229	649	T=0.590139	0.2015
rs881301	*FGFR1,C8orf86*	C=0.399905	226	311	111	648	C=0.411165	1
rs9332817	*KMT2A*	C=0.016374	611	39	0	650	C=0.03000	1
rs9349239	*PRPH2*	A=0.444572	164	308	170	642	A=0.504673	0.4788
rs9816226	*ETV5,DGKG*	A=0.173141	397	225	22	644	A=0.208851	0.5891
rs9939609	*FTO*	A=0.402730	215	310	109	634	A=0.416404	0.8857

*Statistically significant p-value (<0.05)

^Two gene names indicate an intergenic region

The PsA cohort was divided into two groups, BMI <25 and BMI >30, to determine if MAF differed between the two sub-cohorts (**Table 3.17**). One SNP had significantly different (p-value <0.05) MAFs between the two groups. The SNP rs10499276 minor allele was more common in the obese population (T=0.1339) when compared to the normal weight cohort (T=0.07483).

Table 3.17 MAF of Obesity Panel and PsA Cohort Divided into Normal Weight (<25 kg/m2) and Obese Weight (>30 kg/m2).

SNP	Gene^	MAF <25 kg/m^2	MAF >30 kg/m^2	P-value
rs10182181	*ADCY3,DNAJC27*	0.4628	0.4922	0.4195
rs10499276	*MIR7641-2,OPRM1*	0.07483	0.1339	0.01071*
rs10745785	*NEDD1,RMST*	0.3681	0.3105	0.09714
rs1075901	*NCOR1*	0.4228	0.4606	0.2973
rs11208662	*LEPR*	0.104	0.08661	0.4114
rs11629783	*MAP2K1*	0.2282	0.2327	0.8831
rs11672660	*GIPR*	0.2114	0.2181	0.8217
rs11792069	*EHMT1*	0.1367	0.1448	0.7483
rs11915371	*LINC01212,FOXP1*	0.2033	0.2115	0.7806
rs12042959	*SDCCAG8*	0.1267	0.1519	0.3192
rs1205106	*SIPA1L1,RGS6*	0.4271	0.4116	0.6724
rs12327272	*PMAIP1,MC4R*	0.1367	0.09423	0.06114
rs12458	*GATA4*	0.3074	0.3463	0.258
rs13227433	*GTF2I*	0.2383	0.2442	0.8491
rs13329567	*MAP2K5,SKOR1*	0.2297	0.195	0.2397
rs1394	*TNKS*	0.3221	0.3288	0.8456
rs156151	*NONE,HACE1*	0.2167	0.1795	0.1949

SNP	Gene^	MAF <25 kg/m^2	MAF >30 kg/m^2	P-value
rs17024393	*GNAT2*	0.03401	0.02745	0.5984
rs17203016	*MIR13024,CREB1*	0.1689	0.1798	0.695
rs1899951	*PPARG*	0.1318	0.1167	0.5295
rs2124499	*ADCY5*	0.3277	0.3638	0.2997
rs2229616	*MC4R*	0.006667	0.009615	0.6584
rs2304607	*MEF2C-AS2*	0.1233	0.1602	0.1554
rs3134353	*YWHAZ*	0.4252	0.3624	0.07754
rs3753549	*AKT2*	0.1733	0.1692	0.8805
rs403656	*SCAPER*	0.1318	0.1465	0.5623
rs4307239	*NPY,MPP6*	0.49	0.4595	0.399
rs4372296	*DPYD*	0.1933	0.1853	0.7778
rs4624596	*GSK3B*	0.1565	0.1764	0.4677
rs573455	*CEP164*	0.4764	0.473	0.926
rs6235	*PCSK1*	0.2617	0.2683	0.8373
rs6265	*BDNF*	0.2027	0.1784	0.3946
rs6804842	*RARB*	0.4799	0.4498	0.4069
rs6879326	*PDE4D*	0.47	0.4614	0.8119
rs6919443	*GRIK2,HACE1*	0.4633	0.4096	0.1344
rs6968554	*AGR3,AHR*	0.4233	0.3764	0.1859
rs7777102	*MLXIPL,VPS37D*	0.1453	0.1004	0.05503
rs7784465	*RAC1*	0.1174	0.1206	0.8931
rs8070454	*PSMD3,CSF3*	0.3615	0.3508	0.7588
rs8087550	*MC4R,CDH20*	0.5	0.5	1
rs879620	*ADCY9*	0.4033	0.4154	0.7355
rs881301	*FGFR1,C8orf86*	0.4295	0.3846	0.2072
rs9332817	*KMT2A*	0.01667	0.03462	0.1338

SNP	Gene^	MAF <25 kg/m^2	MAF >30 kg/m^2	P-value
rs9349239	*PRPH2*	0.5338	0.4806	0.1448
rs9816226	*ETV5,DGKG*	0.2067	0.2016	0.861
rs9939609	*FTO*	0.3938	0.4257	0.38

*Statistically significant at p-value <0.05
^Two gene names indicate an intergenic region

3.3.6 Genotypes versus BMI

Quantitative trait analysis was performed, with Dr Quan Li's assistance, to investigate the association between BMI and genotypes of the 46 obesity SNPs using a multivariate linear regression model. Bonferroni correction was used to adjust for multiple comparisons. The results can be found in **Table 3.18** below. There was one SNP that was statically significant with a p-value <0.01, rs11915371 (**Figure 3.8**). If the p-value is relaxed to <0.05, there were four additional SNPs that were significant, rs10499276, rs4307239, rs7777102, and rs13329576 (**Table 3.19**). For rs11915371, individuals with CC genotype had a mean BMI 32.42 kg/m^2 vs 29.77 kg/m^2 for the AA genotype. For the SNPs that were significant with <0.05 p-value; rs10499276, individuals with the TT genotype had a mean BMI of 28.48 kg/m^2 versus 29.13 kg/m^2 CC genotype, and 30.66 kg/m^2 for the heterozygous genotype (p=0.03589). SNP rs4307239, individuals with GG genotype had a mean BMI of 29.46 kg/m^2 versus 30.24 kg/m^2 for the AA genotype and heterozygous genotype had a mean BMI of 28.56 kg/m^2 (p=0.0476). For rs7777102, individuals with GG genotype had a mean BMI of 25.68 kg/m^2, mean BMI of 28.56 kg/m^2 with the heterozygous genotype, and 29.82 kg/m^2 with the AA genotype (p=0.02145). Lastly, rs13329576, those with TT genotype had a mean BMI 28.09 kg/m^2, the mean BMI for the heterozygous allele, CT, was 28.88 kg/m^2 and mean BMI for the CC genotype was 29.97 kg/m^2 (p=0.06036).

Table 3.18 Genotype for 46 SNPs in the Obesity Panel versus BMI for all samples in the PsA cohort.

SNP	Gene^	BETA	STAT	P-value (adjusted)*
rs11208662	*LEPR*	-0.2496	-0.4282	0.2086
rs4372296	*SDCCAG8*	-0.04709	-0.1089	0.3199
rs17024393	*GNAT2*	1.419	1.219	NA
rs12042959	*AKT3*	0.918	1.911	0.1396
rs3753549	*DPYD*	0.0908	0.2022	0.4471
rs10182181	*ADCY3;DNAJC27*	0.5614	1.633	0.4248
rs17203016	*MIR1302-4;CREB1*	-0.2072	-0.454	0.1647
rs1899951	*LINC01212;FOXP1*	-0.7069	-1.336	0.4752
rs6804842	*PPARG*	-0.218	-0.6677	0.3007
rs11915371	*ADCY5*	-0.1546	-0.3744	0.0009424***
rs4624596	*GSK3B*	-0.3527	-0.8271	0.614
rs2124499	*MIR4792;RARB*	-0.1772	-0.5208	0.204
rs9816226	*ETV5;DGKG*	-0.1465	-0.3386	0.6685
rs6879326	*LINC00461;MEF2C*	0.09592	0.2726	0.8766
rs2304607	*PCSK1*	0.3728	0.7522	0.2075
rs6235	*PDE4D*	-0.244	-0.6356	0.5884
rs9349239	*MIR7641-2;OPRM1*	-0.5935	-1.78	0.1859
rs6919443	*NONE;HACE1*	-0.4786	-1.43	0.2523
rs156151	*GRIK2;HACE1*	-0.6213	-1.453	0.3829
rs10499276	*PRPH2*	1.092	2.07	0.03589**
rs7784465	*GTF2I*	0.4342	0.8427	0.5481
rs6968554	*NPY;MPP6*	-0.431	-1.247	0.2301
rs4307239	*AGR3;AHR*	-0.4467	-1.302	0.04766**
rs7777102	*MLXIPL;VPS37D*	-1.378	-2.646	0.02145**
rs13227433	*RAC1*	-0.05001	-0.1215	0.1849
rs1394	*GATA4*	0.1317	0.3761	0.9773
rs12458	*TNKS*	0.131	0.3601	0.9078
rs881301	*YWHAZ*	-0.5844	-1.703	0.2714
rs3134353	*FGFR1;C8orf86*	-0.5293	-1.519	0.2232
rs11792069	*EHMT1*	0.1428	0.2904	0.3543
rs6265	*CEP164*	0.04037	0.09413	0.6826
rs573455	*BDNF*	-0.2488	-0.7549	0.1686
rs9332817	*KMT2A*	0.7263	0.7193	NA
rs10745785	*NEDD1;RMST*	-0.5412	-1.457	0.1692
rs1205106	*SIPA1L1;RGS6*	0.2445	0.6761	0.7811

SNP	Gene^	BETA	STAT	P-value (adjusted)*
rs11629783	*MAP2K1*	-0.05597	-0.1381	0.6879
rs13329567	*MAP2K5;SKOR1*	-1.019	-2.54	0.06036
rs403656	*SCAPER*	-0.09919	-0.2077	0.9947
rs879620	*ADCY9*	-0.226	-0.6604	0.4635
rs9939609	*FTO*	0.1614	0.463	0.4936
rs1075901	*NCOR1*	0.4721	1.411	0.2899
rs8070454	*PSMD3;CSF3*	-0.435	-1.201	0.3926
rs12327272	*PMAIP1;MC4R*	-0.7603	-1.434	0.2438
rs2229616	*MC4R*	1.548	0.9693	NA
rs8087550	*MC4R;CDH20*	-0.05579	-0.1611	0.9155
rs11672660	*GIPR*	0.1868	0.4425	0.5142

**P-value adjusted for age, sex, height*
***Significant <0.05*
****Significant <0.01*
^Two genes indicate an intergenic region

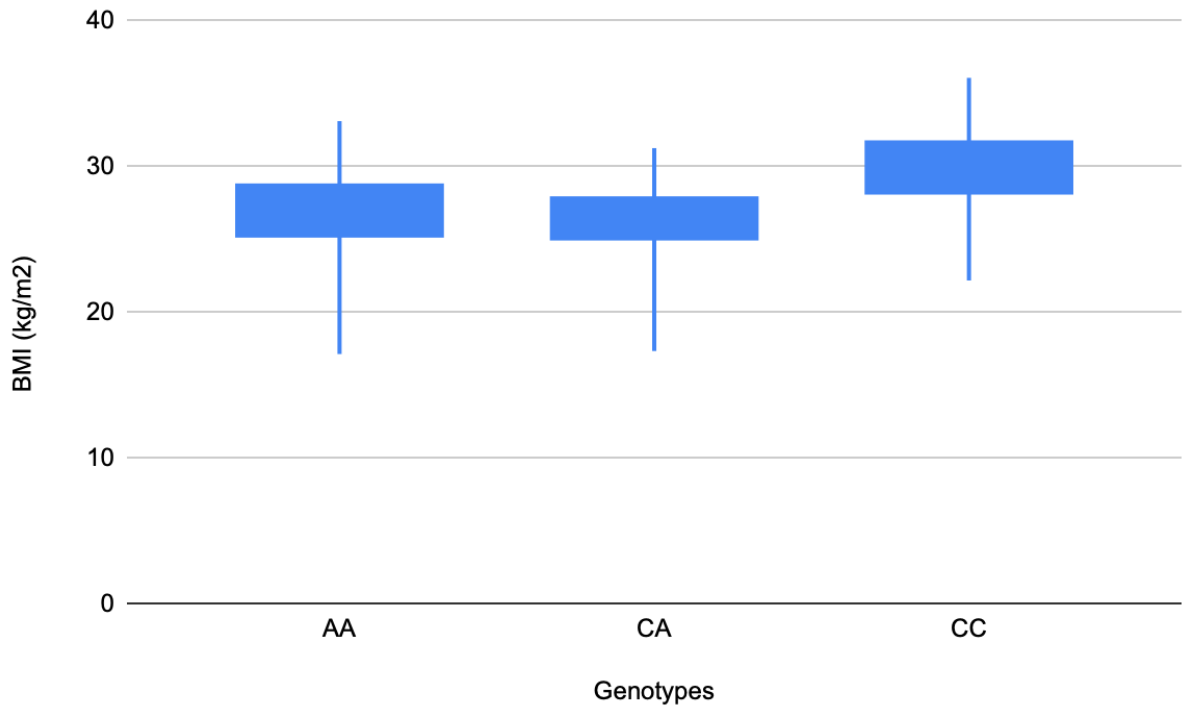


Figure 3.8 Difference of BMI per genotype for rs11915371 (p=0.0009). X-axis represents the three genotypes, and the y-axis shows BMI (kg/m^2). BMI increases in the presence of the 'CC' genotype.

Table 3.19 Significant obesity-associated SNPs showing difference between genotypes and mean BMI, including number of samples genotyped, frequency, and standard deviation (SD). rs11915371 with an increase of mean BMI when the 'CC' genotype is present; rs10499276 with an increase of mean BMI when the 'CT' genotype is present; rs4307239 with an increase of mean BMI when the 'AA' genotype is present; rs7777102 with an increase of mean BMI when the 'AA' genotype is present; rs13329567 with an increase of mean BMI when the 'CC' genotype is present.

SNP	rs11915371			rs10499276			rs4307239			rs7777102			rs13329567		
Genotype	C/C	C/A	A/A	T/T	T/C	C/C	G/G	G/A	A/A	G/G	G/A	A/A	T/T	T/C	C/C
Number of samples	30	220	400	155	133	496	136	335	176	6	155	486	35	222	387
Frequency	0.04615	0.3385	0.6154	0.2396	0.2085	0.7774	0.2102	0.5178	0.272	0.009274	0.2396	0.7512	0.05435	0.3447	0.6009
BMI MEAN (kg/m^2)	32.42	28.5	29.77	28.56	30.66	29.13	29.46	28.99	30.24	25.68	28.56	29.82	28.09	28.88	29.97
SD (kg/m^2)	6.122	5.277	6.441	5.352	6.032	6.106	6.155	5.719	6.477	3.353	5.352	6.305	4.78	5.537	6.476

3.3.7 PsA Clinical Feature Analysis

Spearman correlation test was performed, with Dr. Quan Li's assistance, to determine if there was a correlation between any of the clinical features of the PsA cohort. The analysis showed there was a positive correlation between BMI and weight (0.840), PASI score (0.100), number of tender joints (0.141), number of swollen joints (0.103), and disease severity (0.186), shown in **Table 3.20**.

Table 3.20 Spearman's Correlation analysis for clinical parameters of the PsA cohort measured in spearman rho from -1 to 1.

		Age of onset of PsA	BMI	Height	Weight	PASI score	# of tender joints	# of swollen joints	Depression index (sf-mcs)	Disease severity (das28)
Age of onset of PsA	Correlation Coefficient	1.000	-.004	-.187**	-.105	-.046	-.026	-.079	.098	.038
	Sig. (2-tailed)	.	.951	.004	.106	.486	.694	.241	.133	.575
	N	240	240	240	240	235	225	225	236	224
BMI	Correlation Coefficient	-.004	1.000	-.078	.840**	.100*	.141**	.103*	-.030	.186**
	Sig. (2-tailed)	.951	.	.087	.000	.028	.004	.037	.519	.000
	N	240	489	489	489	482	406	406	480	405
Height	Correlation Coefficient	-.187**	-.078	1.000	.428**	.053	-.017	.017	.071	-.182**
	Sig. (2-tailed)	.004	.087	.	.000	.247	.736	.729	.118	.000
	N	240	489	489	489	482	406	406	480	405
Weight	Correlation Coefficient	-.105	.840**	.428**	1.000	.120**	.118*	.116*	.010	.074
	Sig. (2-tailed)	.106	.000	.000	.	.008	.017	.019	.822	.135
	N	240	489	489	489	482	406	406	480	405
PASI score	Correlation Coefficient	-.046	.100*	.053	.120**	1.000	.098*	.123*	-.037	.043
	Sig. (2-tailed)	.486	.028	.247	.008	.	.050	.014	.425	.389
	N	235	482	482	482	482	402	402	476	401
	Correlation Coefficient	-.026	.141**	-.017	.118*	.098*	1.000	.593**	-.120*	.629**

		Age of onset of PsA	BMI	Height	Weight	PASI score	# of tender joints	# of swollen joints	Depression index (sf-mcs)	Disease severity (das28)
# of tender joints	Sig. (2-tailed)	.694	.004	.736	.017	.050	.	.000	.015	.000
	N	225	406	406	406	402	406	406	406	405
# of swollen joints	Correlation Coefficient	-.079	.103*	.017	.116*	.123*	.593**	1.000	-.109*	.449**
	Sig. (2-tailed)	.241	.037	.729	.019	.014	.000	.	.027	.000
	N	225	406	406	406	402	406	406	406	405
Depression index (sf-mcs)	Correlation Coefficient	.098	-.030	.071	.010	-.037	-.120*	-.109*	1.000	-.251**
	Sig. (2-tailed)	.133	.519	.118	.822	.425	.015	.027	.	.000
	N	236	480	480	480	476	406	406	480	405
Disease severity (das28)	Correlation Coefficient	.038	.186**	-.182**	.074	.043	.629**	.449**	-.251**	1.000
	Sig. (2-tailed)	.575	.000	.000	.135	.389	.000	.000	.000	.
	N	224	405	405	405	401	405	405	405	405

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed)

Chapter 4 Discussion

4.1 Summary

Psoriatic arthritis (PsA) is a multifactorial disease with articular and extra-articular features. The disease has many comorbidities, one being obesity. There is a clear association with PsA and obesity, with obese PsA patients having higher BMIs than individuals with other rheumatic diseases and higher BMIs than the general population (Kumthekar & Ogdie 2020). Obese PsA patients often have more severe disease and a decreased response to treatment, including biologics. Weight loss in obese PsA patients typically shows an improvement in PsA symptoms and increased response to treatment. The association between PsA and obesity is complex and potentially bi-directional, meaning obesity is possibly contributing to PsA pathogenesis and PsA is possibly contributing to obesity pathogenesis.

There are multiple explanations of how obesity potentially leads to PsA. Obese individuals typically experience low-grade inflammation, which is also seen in PsA patients. This includes an increase in pro-inflammatory cytokines (IL-6 and TNF-alpha), which are components in both diseases (Kumthekar & Ogdie, 2020). Increased adipokines within obese patients is also common. An increase in adipokines plays a role in osteoclastogenesis, which is a key component in PsA patients (Kumthekar & Ogdie, 2020). Obese individuals also place increased mechanical loading on their joints, this extra stress creates micro-damage. Also, this increased stress can cause enthesitis, a common feature of PsA. Lastly, the c-Jun-N-terminal kinase (JNK) pathway has been upregulated in adipose tissue of obese patients, this same pathway has also been shown to be induced in spondyloarthritis mouse models (SpA) (Kumthekar & Ogdie, 2020).

It's possible that PsA is causing obesity. A hypothesis of decreased mobility in PsA patients, leading to obesity has been discussed (Kumthekar & Ogdie, 2020). However, PsA patients tend to have higher BMI's even when compared to similar rheumatic diseases like rheumatoid arthritis (Kumthekar & Ogdie, 2020). Rheumatoid arthritis typically has more severe articular disease burden, thus has a greater impact on mobility. If mobility issues were the main cause of association, a similar BMI trend would likely be more prominent in RA patients. Depression has been associated with PsA as well, which has been known to cause weight change in individuals (Zafiriou *et al.* 2021). Lastly, PsA treatments, such as corticosteroids have been noted to increase weight, potentially leading to obesity (Brown & Chandler, 2001).

Through previous studies, genetics has been proven to be important in the role of both psoriasis and obesity. A study by Lonnberg *et al* .2016 determined that 74% of variance in BMI was due to genetic factors. They also determined that mean BMI was increased in psoriasis patients when compared to those with no psoriasis, this was also seen in twins, where a twin with psoriasis was more likely to be obese than a twin without. Also, prevalence of psoriasis increased with increasing BMI, those with a BMI of 35 kg/m^2 or more had a 2-fold risk of psoriasis. Another recent study explored the relationship between psoriasis and obesity, which highlighted a causal relationship of BMI on psoriasis (Budu-Aggrey *et al.* 2019). The study analyzed 753,421 individuals from two large population-based studies and GWASs and Mendelian randomization was used to test for casual relationship with BMI. It was determined that risk of psoriasis increased 9% for 1 unit (kg/m^2) increase in BMI. The opposite suggested less influence of psoriasis on an individual's BMI. This study concluded that excess adiposity is part of the reason for individuals developing psoriasis (Budu-Aggrey *et al.* 2019). Since psoriasis and PsA

are so closely linked, its likely similar trends and relationships would be seen with PsA patients and obesity.

It is important to fully understand the cause of the relationship between PsA and obesity to allow for better outcomes and guidance for patients. This study has attempted to determine if genetics plays a role in the relationship between PsA and obesity. To determine how psoriatic disease-associated SNPs affect BMI, an obesity cohort with the absence of immune mediated inflammatory disease (in particular psoriasis, PsA, axSpA, RA, IBD and uveitis) was genotyped using psoriatic disease-weighted SNPs. This analysis revealed two genes of specific interest, *FBXL19* and HLA-B*39. To determine how obesity-associated SNPs affect PsA, a PsA cohort was genotyped on a designed obesity-weighted panel. This analysis revealed one SNP of statistical significance rs11915371 (*SAMMSON/FOXP1*) with multiple SNPs of interest, with nominal significance. Further, the PsA cohort was broken into two groups, normal weight (<25) and obese (>30) to determine if the MAFs differed between the two groups. One obesity-associated SNP, rs10499276 (*OPRM1/RGS17*), had a significantly different frequency between normal weight PsA patients and obese PsA patients.

4.2 Study Design

Case-control studies are the traditional design to compare differences between two groups. In these studies, individuals with a disease are compared to a cohort without the disease of interest. However, access to an appropriate control group is often not readily available. An alternate approach is to use a case-only design. This is where patients with a particular disease are further stratified into groups and then these groups are compared. This is the type of design used within this study. The PsA cohort was stratified based on obesity genotypes (homozygous

major allele, heterozygous, homozygous minor allele) and the mean BMI of these groups was compared. For the obesity cohort, we assessed the prevalence of PsA associated genes and the cohort was also stratified based on PsA susceptible genotypes and compared with mean BMIs. This design makes the study feasible and provides efficient estimates when a control population is not available.

4.2.1 MassARRAY Genotyping

For this project, the genotyping method used was MassARRAY technology by Agena Biosciences. This technology is a cost friendly genotyping method that has been well-established within the laboratory. The cost of genotyping one sample for a three well multiplex panel is $32.33 and for a two well multiplex panel is $21.56. Compared to another method, like Sanger sequencing, where sequencing a sample for all 46 SNPs would cost $203.78. Using multiplex technology is also a benefit as multiple SNPs can be tested within one reaction. It also allows a custom panel to be designed, which was convenient for this project as a novel obesity panel was proposed. The MassARRAY technology was the most cost effective and logical method for this project.

4.2.2 SNP-based Panels

A PsA panel consisting of 42 SNPs associated with PsA and psoriasis was previously designed, validated, and implemented within the laboratory (Eder *et al*. 2019). Using this panel to determine if there is any relationship between SNPs and BMI was decided as the best method for multiple reasons. As previously mentioned, this panel had already been established within the laboratory and was currently being used for research purposes. It was a convenient tool to have

available for the scope of my project. Since obesity also has many genetic variants, I designed and implemented a novel, obesity SNP panel, which was completed in this project.

4.2.3 Cohorts

Two cohorts were interrogated for this thesis, an obesity cohort and PsA cohort. The obesity cohort consisted of individuals from Newfoundland and Labrador (NL) and had clinical information related to obesity, as well as smoking status, age, and sex. It is important to note that these individuals would have been examined for potential inflammatory diseases at time of visit. However, the cohort is from the early 2000's, so it is possible that individuals may have developed inflammatory disease since their clinic visit. The cohort was collected to study the genetic basis of obesity in the Newfoundland population. All individuals with obesity were screened for immune-mediated disease, particularly psoriasis, PsA, IBD, RA, and uveitis. It is conceivable that some patients may have subsequently developed PsA. Given the incidence of PsA, which is less than 1%, there would be minimal patients with PsA within the obesity cohort. The PsA cohort consisted of individuals from both NL and Ontario, with differing clinical information available. Both cohorts had basic clinical information, such as BMI, age, and sex. The Toronto cohort had additional clinical information recorded such as age of onset of PsA, PASI score, depression index, disease severity scores, and number of swollen and tender joints. It's also important to note that samples from both NL and Ontario would have different genetic backgrounds. NL's population would likely be mainly European descent, while Ontario's population for this particular study are Caucasian, they are more diverse with respect to their ancestries.

4.3 MassARRAY Genotyping

4.3.1 PsA Panel Additional Criteria

Seven SNPs within the PsA panel had additional calling criteria for quality metric control. These seven SNPs rs2066808, rs2844603, rs4908742, rs848, rs1050414, rs1800925, and rs2248902 had modified criteria applied for each SNP. Additional criteria are applied when a portion of the calls are failing to meet the first two categories of our calling algorithm, or if we are not confident in all of the genotype calls, more stringent criteria may also be applied. Sanger sequencing is completed to confirm calls from genotyping and investigate calls on the Typer genotyping plots. Quality control metrics used can be found in **Figure 2.4**, with additional criteria used noted in **Table 2.8 and 2.9.** A summary of the SNPs with modified criteria and the reasoning why the SNP may have needed modified criteria is explored in **Table 3.3**.

4.3.2 Agena Quality Control of PsA Panel

Out of 42 SNPs within the panel, there were seven SNPs that had less than a 95% sample pass rate. These SNPs included, rs1050414, rs10782001, rs12212594, rs2248902, rs2476601, rs2844603 and rs4908742. Using SNPCheck, each SNP's genotyping primers were reviewed to investigate the lower pass rate. A summary of the SNPs with a low pass rate and the reasoning why the SNP may have been performing poorly can be found in **Table 3.3**.

4.3.3 Obesity Panel Additional Criteria

Another optimization was implemented into the final version of the obesity panel. This was a change to the Agena Quality Control Metrics. The heterozygous calls for SNP rs808550

were skewed towards the homozygous calls on the genotyping plot. This skewing resulted in many calls being labelled as 'aggressive' which has stricter calling criteria, which in turn caused many of the samples to fail the quality control metric. Through Sanger sequencing, it was confirmed that the calls were all concordant. Extra samples were sequenced along the heterozygous outside boundary to be sure that these calls were all accurate. Since we could be confident in these calls, it was proposed to lower the allele height ratio from >0.55 to >0.45, which is the cut-off many of the 'aggressive' calls were just below. Criteria was changed because of the consistent pattern of aggressive calls and sequencing confirmed all genotyping calls were correct **(Table 3.11)**.

4.3.4 Agena Quality Control of Obesity Panel

When applying the quality control metrics to the obesity panel, high pass rates were seen throughout the two wells as shown in **Table 3.15**. All SNPs passed our ideal percent pass rate of ≥95%, with passing rates ranging from 95.69-100%. The panel performed very well overall and supports the use of the technology for designing custom SNP panels and genotyping. Since the obesity panel did not contain SNPs within the MHC region, like the PsA panel, the panel was much easier to design and produced very good genotyping results with little sample fails.

4.4 Designing a Novel Panel

4.4.1 Gene Prioritization

The obesity panel was designed using gene prioritization, which scored SNPs from two recent GWAS's. Gene prioritization is becoming increasingly popular with the use of larger scale

studies, like GWAS, producing thousands of potential associations. Gene prioritization typically uses two parts, firstly, evidence sources, such as databases of gene-gene interactions, genes function, and disease is compiled. Next, phenotype data and candidate gene lists are inputted. Then, the evidence sources are used to calculate a score to determine the likelihood of each gene to be responsible for the phenotype (Zolotareva & Kleine, 2019). Gene prioritization method was chosen, as obesity has a large number of associated SNPs within the literature and it would be very difficult to choose the most associated SNPs to build a novel obesity panel. Using two recent GWAS (Locke *et al.* 2015; Yengo *et al,* 2018), in-silico tools to build an evidence source, and generating a score for each SNP, a list of prioritized SNPs was created. The top 50 scored genes were chosen to design the initial panel. Fifty was chosen as a panel of similar size to the PsA panel, which consists of 42 SNPs. From previous experience within the laboratory designing panels, it was noted that some SNPs may be lost through the design, validation, and Sanger sequencing confirmation. So, it was decided to start with 50 SNPs for the initial design, with the possibility of removing some SNPs as the panel was optimized. SNPs were scored on gene-disease relationship, which was built from many in-silico tools to score the SNPs. The scores ranged from 0-1, with 1 being the most relevant to obesity. The 50 SNPs on the final list had scores ranging from 0.6608-0.1949. Multiple commonly associated obesity genes were represented on the list, including the highest scored SNP, rs11208662, in gene *LEPR*. This gene is involved in leptin receptor and has previously been associated with obesity (Stelzer *et al.* 2016). Other notable associated genes within the panel included *MC4R, BDNF, PPARG, PCSK1, EHMTI* and *FTO.* An additional SNP was added to the final list, rs9939609. This SNP is within *FTO* and has been significantly associated with obesity phenotypes, specifically within the NL

population (Payne *et al.* 2014). Therefore, it was added to the final list resulting in an obesity panel of 51 SNPs.

4.4.2 Obesity Panel Validation

It is very important to validate a new panel design with another method. In this case, Sanger sequencing was used to determine that the obesity SNP panel was working properly. Initially 176 samples of NL PsA patients, were genotyped using the obesity panel. Then Typer Analyzer genotyping plots were used to randomly pick samples for confirmation. Each plot had a minimum of at least 3 samples confirmed for the homozygous calls and 6 confirmed for the heterozygous calls. If the plot was messy or skewed, then more samples were selected for Sanger sequencing.

As mentioned in results **Section 3.2.2.4**, discordant calls were identified between genotyping and Sanger sequencing. Out of the 49 SNPs sequenced, 37 SNPs were successfully confirmed without issue. Six SNPs were discordant between Sanger sequencing and genotyping which were noted to be close together on sample plates. This included two SNPs for PsA_NF_169_0001 samples, two SNPs for PsA_NF_177_0001, one SNP for PsA_NF_171_0001, and one SNP for PsA_NF_175_0001 as shown in **Table 3.12**. We noticed six samples within the same row of the genotyping plate had discordant SNPs. All SNPs, rs17024393, rs2304607, rs11672660, rs1205106, rs6879326, and rs9349239, were within well one of the obesity panel. It was suggested that it was possibly due to human error during the initial PCR setup since this would have been the same reaction. The samples were re-genotyped on the MassARRAY system using both the row of samples from the DNA plate used for genotyping, as well as the original stock-DNA tube to confirm. After a second round of genotyping, the samples matched the Sanger sequencing calls. It was likely that the wrong DNA

row was multichannel pipetted into the genotyping plate initially, resulting in the discordant calls. This finding further highlights the importance of confirming results using another method and how easy it is for human error to occur, affecting the final results.

Various SNPs including rs10182181, rs3134353, rs12675063, rs3807049, and rs3751813, had discordant results between genotyping and Sanger sequencing validation. A summary of the reason for discordant calls and the action taken for each SNP is explored in **Table 3.11**.

The importance of validating a new design with a different technology was highlighted in this portion of the project. Even after secondary validation and initial genotyping, there were changes to the panel that were necessary after confirming with Sanger sequencing. Without this confirmatory sequencing, I would not have picked up on a sample mix up during genotyping, I would not have known about a SNP causing a skew in a sample for rs10182181 and rs3134353, and I would not have known about multiple discordant calls in three SNPs that were later removed. It's also important to note that the three SNPs that were removed from the obesity panel after they failed Sanger sequencing validation, were three of the poorest performing SNPs of the entire panel. Two of the three, rs12675063 and rs3751813, had very messy genotyping plots. This may point at a potential SNP in a primer, causing a low pass rate and a messy plot. This is an important point to keep in mind when designing and validating other panels in the future.

4.5 PsA Panel with Obesity Cohort

4.5.1 Minor Allele Frequencies of PsA Panel

All SNPs within the panel met Hardy Weinberg Equilibrium (HWE). This is important to determine as it indicates the genetic structure is as expected and no major genotyping errors would be likely. Some SNPs within the panel did not have similar MAF for this obesity cohort when compared to the published MAF. Their p-values were calculated using Fisher's exact test and two were determined to have a statistically significant difference, rs3129944 and rs67841474. The SNP rs3129944 had a published MAF of 28.81% for the G allele, while our cohort had an MAF of 12.05% (p=0.0047). When looking at the MAF of European ancestry from gnomAD browser (Karczewski *et al.* 2020), the published MAF was 20.3%. Due to NL being largely from European descent, the NL population fits best with European ancestry (Zhai *et al.* 2016). When comparing the European MAF and the cohort MAF using Fishers exact test, the association was considered not statistically significant (P=0.1763).

Rs67841474 for this cohort also had a statistically significant difference in MAF when compared to the published MAF (P=0.0003). The published MAF was 21.21% and our cohorts MAF was significantly higher at 45.65%. When looking at the European ancestry, the MAF is lower at 14.07% from gnomAD Browser (Karczewski *et al.* 2020). The presence of delG, the minor allele for this SNP, is much higher in the NL population than the general population and the European population. This could possibly be attributed to NL's unique founder population. NL is considered a series of genetic isolates. Genetic isolates tend to have less genetic diversity, with increased inbreeding coefficient, increased homozygosity, and decreased heterozygosity (Zhai *et al*. 2015; Rahman *et al.* 2003). Differing minor allele frequencies from the global MAF,

as well as European ancestry is not surprising given NL's population ancestry. However, this will be interpreted with a degree of caution, as common SNPs (those with MAF > 5%) usually have similar frequency to a North European population (Rahman *et al.* 2003).

4.5.2 PsA SNPs versus BMI

Within the analysis, two SNPs showed significance when comparing the genotypes to BMI. These two SNPs were rs10782001 and rs3131382. SNP rs10782001 is within gene *FBXL19.* The mean BMI increased with the 'GG' genotype where mean BMI was 37.29 kg/m^2, when compared to the heterozygous GA genotype, 36.36 kg/$m^{2,}$ and the AA genotype, 34.43 kg/m^2. BMI seems to increase in the presence of the G allele. *FBXL19* is involved in proteasome-mediated degradation of protein targets involved in apoptosis, cell migration, and inflammation (Stelzer *et al.* 2016; Acharya *et al.* 2019). The SNP has shown association with both psoriasis and PsA and has been associated with inhibition of the NFkB signalling pathway in both diseases (Llyod *et al.* 2012; O'Rielly & Rahman, 2014). The NFkB complex is activated cytokine stimulation occurs, including TNF-alpha and IL-17. Unbound NFkB can enter the nucleus to initiate transcription of genes that control inflammatory processes, including proinflammatory cytokines. This pathway is also involved in the differentiation of osteoclast precursor cells, leading to an increase in activated osteoclasts. An increase in osteoclasts cause bone resorption, which is a clinical feature of PsA (Llyod *et al.* 2012). A significant increase in *FBXL19* expression is noted in psoriasis skin when compared to normal skin (O'Rielly & Rahman, 2014). A study by Acharya *et al.* 2019 found that microRNA-26 was a suppressor of adipogenesis and when microRNA-26 was knocked-out, *FBXL19* expression was increased by 1.5-fold. When *FBXL19* was knocked out, adipogenic gene expression was greatly reduced.

When *FBXL19* was overexpressed, adipogenesis was strongly stimulated. This indicates a potential biologic function in obesity for *FBLX19.* Since both PsA and obesity have potential biological function related to *FBXL19,* this gene has potential to be a link between the two diseases. *FBXL19* expression has been noted to be increased in the presence of psoriasis and its increase in expression has been noted to cause an increase in adipogenesis, leading to potential obesity.

SNP rs3131382 is a marker for allele HLA-B*39:05. When the 'TT' genotype was present, the average BMI was 47.17 kg/m^2. When the heterozygous genotype was present the mean BMI of the cohort was 37.73 kg/m^2. When the 'CC' genotype was present, the mean BMI was much lower at 35.46 kg/m^2. This is a large increase in mean BMI between the different genotypes, and the presence of the T allele seems to increase the mean BMI. *HLA-B*39* plays an essential role in immune response and has a high odds ratio of causing PsA. (OR=3.5). *HLA-B*39* has been most associated with peripheral polyarthritis and faster disease progression (Rahman & O'Rielly, 2014). *HLA-B*39:05* has been previously associated with the development of type 1 diabetes at an early age (Mikk *et al.2014)*. Interestingly, about 50% of type 1 diabetics fall into the overweight or obese category (Mottalib *et al.* 2017). Also, the HLA-region has been associated with obesity previously through multiple studies. Presence of HLA-DRB1*15 and absence of HLA-A*02 has been associated with obesity and multiple sclerosis (MS) development. MS is another autoimmune disease, which has seen an association with obesity previously, which is hypothesized to be due to an increase in proinflammatory cytokines (Hedstorm *et al.* 2014). An association between high-risk HLA genotypes (HLA-DRB1 and HLA-DQB1), obesity and latent autoimmune diabetes in adults (LADA) was also found (Hjort *et al.* 2019). The relative risk of HLA genotypes on with LADA was 7.59. The HLA region being

associated with multiple autoimmune diseases and obesity gives a potential for HLA-B*39 to also play a role in obesity and PsA pathogenesis.

4.6 Obesity Panel with PsA Cohort

4.6.1 Minor Allele Frequencies of Obesity Panel

All SNPs within the panel met HWE. When comparing the cohorts MAF to that of the published MAF, there was one SNP that showed a statistically significant difference, rs1899951. This SNP had a published MAF of 26.11% and the cohort's MAF was 11.53% (P=0.0183). When looking at just the European MAF on gnomAD Browser (Karczewski *et al.* 2020), it was much closer to the cohorts MAF at 12.9%. When comparing these two using Fishers exact test, the result was not statistically significant. Since NL's population has arisen from natural expansion of descendants of Northern European ancestry, the similarity in MAF of relatively common variants is expected (Rahman *et al.* 2003)

4.6.2 MAF of Normal Weight PsA versus Obese PsA

An analysis comparing MAF's with the PsA cohort was broken down into obese (BMI >30) and non-overweight (BMI <25) groups. One SNP, rs10499276, showed a significant difference in MAF between those with BMI's <25 and those with BMIs >30 in the PsA cohort. Those with normal weight BMIs had a lower allele frequency of the minor (T=0.075) than those with obese BMI's (T=0.134) (p=0.01). Since this SNP's minor allele is less common in the normal weight individuals and more common in obese PsA patients, this could indicate a genetic cause for obesity in PsA. Since these SNPs were chosen for obesity, it would have been expected

that more of the SNPs would have been increased in the obese PsA patients than was shown in the analysis. Most SNPs in obese PsA patients had very similar MAF to the European population. This possibly indicates that obesity in PsA is different than obesity in the general population. And having the SNP rs104499276 in an individual with PsA could indicate genetic susceptibility for obesity.

4.6.3 Obesity SNPs versus BMI

One SNP showed to be significant when p-values were corrected for multiple testing (<0.001), rs11915371. When the p-value was relaxed to <0.05, four obesity-weighted SNPs were significant when comparing the genotypes to BMI: these included rs10499276, rs4307239, rs7777102, and rs13329567.

SNP rs11915371 is an intergenic variant on chromosome 3 between *SAMMSON* and *FOXP1*. In the presence of the 'CC' genotype the average BMI was 32.42 kg/m^2, in the presence of 'CA' genotype the average BMI was 28.5 kg/m^2, and in the presence of the 'AA' genotype the average BMI was 29.77 kg/m^2. In the presence of CC genotype BMI increased. *SAMMSON* is a long non-coding RNA (lncRNA), which are molecules that regulate gene expression and are involved in immune and inflammatory pathways (Dolcino *et al.* 2018). This lncRNA, as well as others, have increasingly been associated with skin pathways, as *SAMMSON* has been noted to reduce survival rates within melanoma patients (Song *et al.* 2021). It has also been associated with neurosarcoidosis, which is an inflammatory disease affecting the central nervous system. Recently, lncRNAs have been noted to be potentially associated with PsA (Dolcino *et al.* 2018). A functional analysis was completed that revealed that lncRNAs target genes involved in biological processes that play an important role within PsA pathogenesis. This includes immune

response, inflammatory response, TNF, Wnt and type I interferon signalling, bone resorption, bone mineralization, and metabolic processes (Dolcino *et al.* 2018). While this specific lncRNA has not been associated with PsA, there has not been much research pertaining to specific lncRNA and PsA pathogenesis. It's possible that this lncRNA, *SAMMSON*, is playing a role in PsA pathogenesis and is also associated with BMI in GWAS.

The next SNP of interest was rs10499276. This is an intergenic variant located on chromosome 6 between *RGS17* and *OPRM1.* When the 'TT' genotype was present the mean BMI was 28.48 kg/m^2, when the heterozygous genotype was seen the mean BMI was 30.66 kg/m^2, and when 'CC' was present the mean BMI was 29.13 kg/m^2. The BMI increased while in the presence of the homozygous 'CC' genotype but also increased with the heterozygous genotype. This may be caused by variability in the BMI's due to small sample size as 63% of the samples had the CC genotype versus about 17% having the heterozygous genotype. *OPRM1* is a receptor for endogenous opioids such as beta-endorphin and endomorphin. This gene has been associated with drug dependence. Through GWAS, the gene has been associated with BMI, blood pressure, and chronic lymphocytic leukemia (Stelzer *et al.* 2016). In a recent study, *OPRM1* was associated with pain within IBD patients (Grossi *et al.* 2020). *RGS17* regulates G-protein-coupled receptor signalling cascades. It has been associated with drug dependence, lung cancer, high density lipoprotein cholesterol, and type II diabetes (Stelzer *et al.* 2016). *RGS17* expression was increased in type II diabetes patients (Chang *et al.* 2015). This may be of interest considering type II diabetes is often considered an autoimmune disease with a large component of the immune system being involved in pathogenesis, similarly to PsA.

SNP rs4307239 was also a SNP of interest, with a change in BMI between genotypes. When the 'GG' genotype was observed the mean BMI was 29.46 kg/m^2, when 'GA' was

observed the mean BMI was 28.99 kg/m^2, and when 'AA' was observed the mean BMI was 30.23 kg/m^2. The BMI increased when in the presence of the 'AA' genotype, decreased with the 'GG' genotype, and further decreased in the presence of the 'GA' genotype. This may be due to the sample distribution, as about 52% of samples had the heterozygous genotype compared to 21% having the 'GG' genotype. SNP rs4307239 is an intergenic variant found on chromosome 7 between *NPY* and *MPP6. NPY* is involved in the control of feeding and also plays a role in the secretion of gonadotrophin-release hormone (Stelzer *et al.* 2016). *NPY* also functions to activate mast cells, induce phagocytosis, and stimulate antibodies and cytokines (Vidal Yucha *et al.* 2019). A new concept, neuro-immuno-cutaneous system, states that these three systems are interconnected and play a role with each other. This is interesting considering *NPY* is a neural peptide that is part of the interconnected system by acting locally by causing inflammation. Another study also noted a positive energy balance and chronic inflammation when a prolonged high-fat diet was given to mice, which was mediated through the increased expression of appetite-stimulating neuropeptide, *NPY* (Dalvi *et al.* 2017). This gene has been associated with BMI and eating disorders. *NPY* seems to play a role in appetite control but also is involved in creating an inflammatory state, which is seen in both PsA and obesity (Dalvi *et al.* 2017).

Another SNP of interest, rs7777102, is an intergenic variant on chromosome 7 between *MLXIPL* and *VPS37D.* This SNP has most commonly been associated with high lipoprotein cholesterol levels. When in the presence of the 'GG' genotype, the mean BMI was 25.68 kg/m^2, and when in the presence of the heterozygous genotype the mean BMI was 28.56 kg/m^2, and the mean BMI for 'AA' genotype was 29.82 kg/m^2. The BMI increased when in the presence of the A allele which is the major allele. *VPS7D* is part of a complex, involved in regulating vesicular

trafficking. The gene has been associated with high lipoprotein cholesterol and high blood pressure (Stelzer *et al.* 2016). This gene had little information available within the literature. *MLXIPL* encodes a helix-loop leucine zipper transcription factor, and the protein activates carbohydrate response element to promote triglyceride synthesis (Stelzer *et al.* 2016). This gene is noted to play a role in adipogenesis differentiation and maturation (Ambele *et al.* 2016). As mentioned previously, adipogenesis and an increase in adipokines typically play a role in PsA pathogenesis as well as obesity.

Lastly, rs13329567 was another SNP of interest. This is an intergenic variant located on chromosome 15 between *MAP2K5* and *SKOR1.* The mean BMI increased when in the presence of major allele, C, with a difference of 2.1 kg/m^2 from the minor allele. *MAP2K5* acts as a scaffold to form the MAP3K2/MAP3K3-MAP3K5 complex that plays a critical role in protecting cells from stress-induced apoptosis, neuronal survival, and cardiac development and angiogenesis. *SKOR1* acts as a transcription repressor of LBX1 and inhibits BMP signalling. Both genes have been associated with BMI, height, and restless leg syndrome (Stelzer *et al.* 2016). There was little information within the literature connecting both of the gene's pathogenesis to PsA.

4.6.4 PsA Clinical Feature Analysis

Spearmen's correlation test was utilized to determine if there was significant correlation between any of the clinical features for PsA within this cohort. Multiple significant correlations were determined, with many of these correlations being expected.

BMI being significantly associated with weight, was an expected result. BMI being associated with increased number of tender and swollen joints, as well as, disease severity, is also

not surprising. As mentioned, obese patients tend to have more severe disease and more difficulty reaching minimal disease activity (Kumthekar & Ogdie 2020). This finding further supports the relationship of BMI and disease severity in PsA.

PASI score, which measures psoriasis severity, being associated with BMI and weight, has been previously noted in the literature. Typically, obese patients tend to have more severe psoriasis with a more difficult time finding effective treatments (Kumthekar & Ogdie 2020). PASI scores being associated with number of tender and swollen joints is also not surprising as an increased PASI score likely means more severe disease and more severe arthritis.

Although many of the associations were expected, it further supports the relationship between multiple clinical features within PsA. Most notably for the purpose of this project, BMI increased with increase in disease severity.

4.7 Limitations

4.7.1 Measure of Obesity

This study has multiple limitations. Firstly, in this project BMI was used as the main indicator of obesity, which has not been regarded as the most accurate measure of obesity. However, BMI is a simple, non-invasive, and inexpensive way to measure body fat. BMI has proven to be correlated with body fat measures, but it cannot distinguish between muscle mass and fat mass or fat distribution (Abad *et al.,* 2018). Other clinical measures that may be more efficient in measuring obesity are, waist-circumference, hip-waist ratio, and waist-height ratio measurements. These measurements are better at taking fat mass and distribution into account.

However, they are about equal to BMI when determining overall health outcomes and mortality. The gold standard of measuring body composition would be dual X-ray absorptiometry (DEXA). DEXA uses X-ray beams which pass through different body tissues at different rates, to distinguish between fat-mass, fat-free mass, and bone mineral density. While it is definitely a better measure of obesity than BMI, it requires expensive equipment that is not available to many. The cohorts were from samples stored within the laboratory and did not have access to DEXA or other measures of obesity. Although BMI may not be the most efficient measure of obesity, it is regarded as a good indicator of body fat measure and overall health outcomes of obese individuals, which was sufficient for this study.

4.7.2 Cohorts

The obesity cohort is a large cohort consisting of almost 700 samples who were clinically examined by an internist. Anyone with autoimmune disease was excluded from the cohort. However, type 2 diabetes was not considered an autoimmune disease during the initial assessment. It's possible that some of the younger patients could have developed psoriasis or PsA in the future. However, given the prevalence of psoriasis in the general population, about 3%, and psoriatic arthritis, about 1%, the overall number of patients within the cohort that would possibly develop psoriasis and PsA would be minimal.

The PsA cohort consisted of patients who were examined and diagnosed using CASPAR criteria by a rheumatologist. The presence of rheumatoid arthritis was ruled out, as anyone who was positive for rheumatoid factor was excluded. However, since these patients were diagnosed by a rheumatologist it is possible that the cohort was weighted towards polyarticular PsA, which affects five or more joints. Other forms of PsA that may be less joint involvement, like distal

interphalangeal and oligoarthritic, are less frequently assessed and diagnosed by a rheumatologist.

The sample size of the two cohorts was modest, with about 700 samples in each cohort. Conversely, when looking at SNPs with small minor allele frequencies the sample counts for these genotypes are very small. An example would be rs3131382, where the genotype 'TT' only had a sample size of 6. The standard deviation was also large at 16.07 kg/m^2. While the results point at some potential significance to this SNP replication is important in this case with a larger sample size.

4.7.3 SNP-based testing

The methodology of SNP-based testing comes with several limitations. With complex diseases, such as PsA and obesity, there are a large number of variants that have been associated with them. So, it is difficult to determine the most appropriate SNPs to create a comprehensive SNP panel. New SNP associations are also discovered often, so panels quickly become outdated if highly associated SNPs are not added. The SNPs provide information about possible association but not causation.

Another limitation of the usage of SNP-based testing is the issue of generalizability to other populations. Both cohorts within my study consist of mainly European descent and both SNP panels were designed based on European ancestry. While that is fitting for a NL study, as it represents most of our population, it may not be suitable for an admixed Canadian population or other ethnicities. The SNPs that I have discussed that may be related to PsA and obesity may only be significant in a European population.

Lastly, SNP-based genotyping does have the potential for allelic dropout, which is what was seen to cause two inaccurate genotypes. Allelic dropout, the phenomenon of the presence of a variant in the primer sequence resulting in one allele failing to amplify, does have the potential to cause inaccurate genotypes. However, Sanger sequencing was completed to confirm accurate calling on the novel-obesity panel, which should have picked up on inaccurate calls, but only a small portion was sequenced for confirmation.

The PsA panel was previously designed within the laboratory and the SNP selection for this panel is potentially a limitation of this study. SNPs were selected based on previously validated genetic associations of PsA and related comorbidities and not based on a systematic gene prioritization method. Also, multiple SNPs within the PsA panel are within the MHC region. This region has been notoriously difficult to genotype due to its highly polymorphic nature. Therefore, some SNPs are in linkage disequilibrium (LD) with the actual allele that's being targeted. While this isn't a perfect method, it allows for important alleles to be present within the panel.

The obesity panel had a more structured way of choosing SNPs to include within the panel. Since obesity has many SNP associations, more SNPs could have been included to create a more comprehensive panel. Fifty SNPs were selected for the panel, which was an arbitrary number that was chosen to be a similar size of the PsA panel. However, the more SNPs present within the panel, the greater the probability of a false positive and thus the greater stringency would be required for the statistical significance cut off value.

4.7.4 Gene Prioritization Methods

Although gene prioritization methods compute genes rank in terms of association with a phenotype, there are limitations to its ability to determine the most associated genes. This

includes challenges of measuring performance and validating data (Zolotareva et al. 2019). In this case, gene prioritization was completed using two recent GWAS pertaining to BMI and obesity. However, there are other GWAS studies published and other identified SNPs that were not included. Further, there is no unified approach identified to determine the performance of a gene prioritization method. This makes gene prioritization methods difficult since much of the input data is coming from various in-silico tools. It's possible there's a highly associated SNP with a low score due to poor understanding of gene function, or highly associated SNPs that did not make the list. Within this study, extreme rare variants were also excluded from the gene prioritization methods. It's unlikely that an extremely rare variant is causing the link between PsA and obesity, since there are both not considered rare diseases. However, it is possible that extreme rare variants could be playing a role, making this a limitation of the study. While there are limitations to gene prioritization methods, they are an important tool to help with ranking candidate genes and SNPs.

4.7.5 Environmental Factors

Both PsA and obesity are considered complex diseases that are influenced by a variety of factors including the environment. Physical inactivity, smoking, depression, and the presence of metabolic diseases are common environmental features in PsA and obesity and likely partially contribute to the co-existence of these two entities. Social factors and employment are also compromised in both conditions and thus can be confounding factors. I acknowledge that environmental factors may result in an association between PsA and obesity. However, both these entities have a strong genetic basis, and the possibility of a genetic link is also plausible. Since the Mendelian randomization study mentioned previously that showed BMI causally

increases the odds of psoriasis (Budu-Aggrey *et al.* 2019), this study wanted to assess this for PsA.

4.8 Conclusion and Future Directions

In summary, there is an association between both obesity and PsA, causing PsA patients to have higher BMI's than both the general population, other inflammatory forms of arthritis, and psoriasis patients. A recent Mendelian randomization study appears to support the notion that obesity genes were causally associated with psoriasis but did not find evidence that psoriasis genes were casually related to obesity. We entertained the possibility that the relationship between obesity and PsA may be bi-directional. The first part of the study aimed to identify if there were PsA-associated variants which were overrepresented in an obese population and if so, was there a dose-dependent effect between a higher BMI and PsA-associated variants. If large effect sizes were noted, we planned to further explore the association by assessing a dose effect of PsA genes based on the degree of obesity. However, the two SNPs associations of interest were of modest effect size, so a further subset analysis was not performed.

Secondly, the study aimed to determine if obesity-associated variants were overrepresented in a PsA cohort and if so, were there any trends in relation to clinical features and PsA variants. Again, multiple SNPs were identified to increase BMI within the PsA cohort, and further genotype-phenotype correlations with subsets of PsA would have been performed if a more robust association were noted. As it was difficult to establish a dose relationship and some of the SNPs had a low number of patients with the homozygous MAF, further stratification based on the PsA phenotypes was not conducted. This study has identified multiple possible SNPs that

may be contributing to higher BMI's in PsA patients using a SNP-based testing approach. This includes SNPs within genes *HLA-B*39* (rs3131382) and *FBXL19 (*rs10782001), PsA related SNPs, and rs11915371, an obesity related SNP. The PsA genes within the identified SNPs*, HLA-B*39 and FBXL19*, have some biologic function to obesity. And the obesity genes, *SAMMSON/FOXOP1* have an inflammatory and immune role that may play a role in PsA pathogenesis. Lastly, the SNP, rs10499279 between *OPRM1/RGS17* showed an increase in allele frequency in obese PsA patients when compared to normal weight PsA patients. Due to low counts of minor alleles, these SNPs should be replicated using a larger cohort to ensure the same trend is exhibited. It would also be beneficial to genotype the PsA cohort for the identified significant PsA SNPs for further support of this SNP-association. SNPs and genes identified should also be further explored for function in relation to obesity and/or PsA to further support the potential relationship. Gene expression studies would give a better idea of the function of identified SNPs and the role they play in both PsA and obesity. Lastly, prospective studies to determine if any identified obesity-related SNPs can predict the onset of PsA in psoriasis patients, would be an extremely interesting future direction. More investigation is needed to determine the consistent association between PsA and obesity, and this study offers some insight that genetic determinants may partly contribute to this association.

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Appendix

Appendix A: PsA Ethics Approval (**HIC-99.042**)

Memorial
University of Newfoundland

Office of Research and Graduate Studies (Medicine)
Faculty of Medicine
The Health Sciences Centre

July 27, 1999

TO: Dr. Proton Rahman

FROM: Dr. Verna M. Skanes, Assistant Dean
Research & Graduate Studies (Medicine)

SUBJECT: Application to the Human Investigation Committee - #99.42

The Human Investigation Committee of the Faculty of Medicine has reviewed your proposal for the study entitled **"Disease Related Genes in Psoriatic Arthritis"**.

Full approval has been granted for one year, from point of view of ethics as defined in the terms of reference of this Faculty Committee. For a hospital-based study, it is your responsibility to seek necessary approval from the Health Care Corporation of St. John's.

Notwithstanding the approval of the HIC, the primary responsibility for the ethical conduct of the investigation remains with you.

Verna M. Skanes, PhD
Assistant Dean

cc: Dr. K.M.W. Keough, Vice-President (Research)
Dr. R. Williams, Vice-President, Medical Services, HCC

St. John's, NF, Canada A1B 3V6 • Tel [illegible] • Fax [illegible]

SUPPORT

Appendix B: PCR Primers for Obesity Panel

Appendix B1: Agena Genotyping PCR Primers for Well 1 of Obesity Panel.

SNP	Forward	Reverse
rs11672660	**ACGTTGGATG**TGGAAGGGCGATCAAAGCTG	**ACGTTGGATG**CAGTACTGGGTCACGATCTG
rs12458	**ACGTTGGATG**TGCTGTTTCTGCCCCTGATG	**ACGTTGGATG**GTAATGCCACAGTCAGCTAC
rs6265	**ACGTTGGATG**CTTCATTGGGCCGAACTTTC	**ACGTTGGATG**GCTTGACATCATTGGCTGAC
rs17203016	**ACGTTGGATG**TCTCAGGCTACCTTTCCTTG	**ACGTTGGATG**TGATGCCATCTTTTTCTCCC
rs11208662	**ACGTTGGATG**GATGTATCAGTATGGCTAGG	**ACGTTGGATG**ATGACATCTAGCGACTCCTG
rs2304607	**ACGTTGGATG**TCTTTGGTTTCCCTGGGTTC	**ACGTTGGATG**GGAATAAGGCTGACAGTTCG
rs10182181	**ACGTTGGATG**CTGTGACTATGATGCCTTAC	**ACGTTGGATG**TCTGGCTCACTGGGATGTTC
rs6919443	**ACGTTGGATG**GTGTTTAGCCAAATGGAGAG	**ACGTTGGATG**ATCGTGTCCCGCACATTTTC
rs9349239	**ACGTTGGATG**TTTAGGACTCAGTGGTGTGC	**ACGTTGGATG**TTCCCCTCAGTACAGTGTCC
rs12327272	**ACGTTGGATG**CCAGTTCTCAATATGGTATG	**ACGTTGGATG**ATCTGAGGAGAGCTGTCTAC
rs1394	**ACGTTGGATG**TTCTGGTGCAGTTCCCAATC	**ACGTTGGATG**AGTCACTGTTCTGCAGATAG
rs12939549	**ACGTTGGATG**AATAGTCGTGATCGCCCTTG	**ACGTTGGATG**GAATCCCAAATGGTTGCAAG
rs2229616	**ACGTTGGATG**GTGCATCCGTATCTGTACTG	**ACGTTGGATG**GTGGCTGATATGCTGGTGAG
rs9816226	**ACGTTGGATG**GGCTTCTGACATCACTGTTC	**ACGTTGGATG**GAAAAGAAGCCAGATACCAC
rs573455	**ACGTTGGATG**AGCTCATGGCGCCAATGCTG	**ACGTTGGATG**TCCGTAGTGCCAAGGAGTTC
rs12675063	**ACGTTGGATG**TGACAATCCAGTTTCCCTAC	**ACGTTGGATG**TTTTGGCCAATCAAAAAGCG
rs1205106	**ACGTTGGATG**TATGGAAGCACAAATGAGCC	**ACGTTGGATG**TCCATAAGACTTTCTGAGAC
rs881301	**ACGTTGGATG**ACTAAACCGAAGTATAAGCC	**ACGTTGGATG**GGTTAAATTTCCCCCTAACTG
rs17024393	**ACGTTGGATG**GCCACGTGTCTCAAATGTTC	**ACGTTGGATG**TCCTAAGCAGATCACTGGTC
rs11915371	**ACGTTGGATG**GATTATGCGTCCCACTTCTC	**ACGTTGGATG**GATATTCCCCTTGTAGGCAC
rs4372296	**ACGTTGGATG**AGGAGATGCAAATCCAACG	**ACGTTGGATG**GATGGGATCAAGGTCTTGTC
rs6879326	**ACGTTGGATG**CATTGCCAGACTACTCTTTG	**ACGTTGGATG**AGACTGCACTTGGTGATGAG
rs3753549	**ACGTTGGATG**CCATGTATCTGACATTGGAC	**ACGTTGGATG**GACAGGTGAAGGGTATTTTG
rs1899951	**ACGTTGGATG**AACAATCATAGGTCTTGGGC	**ACGTTGGATG**ATAGGGTCTCTGGCTACTAC
rs2030342	**ACGTTGGATG**ATGTCCTGTTCAGAAGATTG	**ACGTTGGATG**TGTTAACTTGGTCTTGCAAC
rs3751813	**ACGTTGGATG**CATTACCAGTCATTGCATAG	**ACGTTGGATG**GAACATATCTGCAATATCTG
rs10745785	**ACGTTGGATG**GCAGGATTGGAAGTTGTTCT	**ACGTTGGATG**GTCTGTAGCAGCATACAGTA
rs9332817	**ACGTTGGATG**TTCCTCTCAAAGAATCAGGG	**ACGTTGGATG**TGAACTTGTTCTCTTCTAC

Bolded sequence refers to M13 tail

Appendix B2: Agena Genotyping PCR Primers for Well 2 of Obesity Panel.

SNP	Forward	Reverse
rs6804842	**ACGTTGGATG**GTGATGCAAATGCCCTACAC	**ACGTTGGATG**TTGCCTTACTCCTATGCACG
rs2124499	**ACGTTGGATG**ATTCCTAGTCTGCAGACCCA	**ACGTTGGATG**AATCACTGTGCTACACTGGC
rs403656	**ACGTTGGATG**GGCAACGTGTCCTAGATATG	**ACGTTGGATG**CCCTCAACACTACAGGTAAC
rs10499276	**ACGTTGGATG**AGAAGCTGAGGACGCTAAAG	**ACGTTGGATG**TGGAACAAGCAGTAGAAGCG
rs4307239	**ACGTTGGATG**TGGATGCAGCAGCTCATGG	**ACGTTGGATG**AGTCTCACTATTGACCAGGC
rs6235	**ACGTTGGATG**AACTCACCGCCAAAGCAATC	**ACGTTGGATG**AGGGCTTCGTAGAAGTTTTC
rs4624596	**ACGTTGGATG**CCCAGCCCAGAACTCTATTC	**ACGTTGGATG**AGTGGGTGAAGAGCAGTATT
rs8070454	**ACGTTGGATG**CCAAATATTGTGACCAGGGC	**ACGTTGGATG**TGCAATATCTCCGAGGTCTG
rs11629783	**ACGTTGGATG**GTTTTCCAAAATGGTTGTCC	**ACGTTGGATG**ACTCTAGCCCAGGCAACAG
rs11792069	**ACGTTGGATG**TTCTTTCCACACCAGAACAG	**ACGTTGGATG**ACGTCTATTTGCGTATTGCC
rs13329567	**ACGTTGGATG**AGGGAAAGACGGCCCCAAGT	**ACGTTGGATG**CTGCTGTGAGCCATTGGTG
rs7777102	**ACGTTGGATG**AAGGATGACCCGTCCCTTAG	**ACGTTGGATG**ACCTGCTGATTAGCTCAGTC
rs879620	**ACGTTGGATG**TGGCGCTTGGAAAGCACAAC	**ACGTTGGATG**TTCAAAGAGTGTGTGAGGCG
rs7784465	**ACGTTGGATG**GCAATTTAATTGAGATTGGC	**ACGTTGGATG**TAGACCCCATGCTCAGAAAG
rs3134353	**ACGTTGGATG**GCACCTTTAACTTTTCACAC	**ACGTTGGATG**GTGCTTAAAATGTGTAGAACG
rs8087550	**ACGTTGGATG**CAGGAAGTCATTATGTCAAC	**ACGTTGGATG**GCTGATTCAGTGATCTACAG
rs6968554	**ACGTTGGATG**CAAGTCCAGGCCTCTGAAC	**ACGTTGGATG**TTGACTACGCTTAAAGAGGG
rs12042959	**ACGTTGGATG**TATCGCCAAGAGTAACTGGG	**ACGTTGGATG**ACTCACAATTTCGGGTTGTC
rs3807049	**ACGTTGGATG**CCTGCGGGTGAAAAAAGAG	**ACGTTGGATG**TCAGGCAAGTCTTTACTGG
rs156151	**ACGTTGGATG**GGGAGGGGTTTATATAGAG	**ACGTTGGATG**GCCTAGGCCATAGTATTGTC
rs1075901	**ACGTTGGATG**GGGTGAGAAATAAAGGTTGAG	**ACGTTGGATG**GCTGAGCAGCTGCTATATTT
rs13227433	**ACGTTGGATG**GTATCTCAGAGAACAGCATC	**ACGTTGGATG**TGAAAGGAAGAACGCGCTTG
rs9939609	**ACGTTGGATG**GTAACAGAGACTATCCAAGTG	**ACGTTGGATG**ACACTAACATCAGTTATGC

Bolded sequence refers to M13 tail

Appendix C: Extend Primers for Obesity Panel

Appendix C1: Agena Genotyping Extend Primers for Well 1 of Obesity Panel.

SNP	Extend Primer Sequence	Mass
rs11672660	CAGCGCTGACTACCCCT	5091.3
rs12458	CCTGATGCTGGAGCTCA	5186.4
rs6265	CCAACAGCTCTTCTATCA	5378.5
rs17203016	cGCTCACTTATCCTGTCA	5385.5
rs11208662	GTATGGCTAGGCTGCAGT	5570.6
rs2304607	TCCCTGGGTTCATTTTCAT	5735.7
rs10182181	gACGTGATTAAGTGAAGGA	5940.9
rs6919443	gGGAGAGCTGTTTAGTGAA	5947.9
rs9349239	ACTCAGTGGTGTGCTTTCAA	6123
rs12327272	aGCTTCAGAAATGTAGCAAA	6158
rs1394	agccCCTTGCTCCACTTCTGG	6309.1
rs12939549	cgccCTTGCACAGACTTCGTC	6318.1
rs2229616	TACTGTTTAATAGGGTGATGA	6515.3
rs9816226	cGTTTTGCCTGTTTTTGTACTT	6679.3
rs573455	ggtaCGCATGGAGCGTGTCTGC	6807.4
rs12675063	CCTACATACTTACCAACACTATA	6895.5
rs1205106	ggaTCTTGCATGATAAAACTGAT	7086.6
rs881301	CCGAAGTATAAGCCTCTTACAATC	7280.8
rs17024393	GGTTTTATTTACAGCTGTGATCAA	7372.8
rs11915371	gaGTCCCACTTCTCATTCTCAATCA	7511.9
rs4372296	acaggTTATGCTTCCCAACAGAGAA	7659
rs6879326	cagcCTACTCTTTGATAAACCTGTCA	7865.1
rs3753549	gtcCATTGGACATTTAATAAACTGGC	7969.2
rs1899951	ccATCATAGGTCTTGGGCCTTTAGGA	7977.2
rs2030342	gaagcTTGTAATTTTCTGGGTAATGA	8055.3
rs3751813	GAAAAAAAATTGGACTATATTTGGAG	8090.3
rs10745785	ggaAGTCAGTGAGTGAGTGTTGAGTG	8186.3
rs9332817	AAATTAAACCAAAGATGTTTTATACACA	8571.6

Lower case letters indicate extra sequence to bring mass to required mass. Upper case letters indicate extend primer sequence.

Appendix C2: Agena Genotyping Extend Primers for Well 2 of Obesity Panel.

Assay_ID	Extend Primer Sequence	UEP Mass
rs6804842	ATGCCCTACACTAACCC	5059.3
rs2124499	ACAGATGCTGCTGCCCC	5131.3
rs403656	AGATATGCTGAGCAAGT	5258.4
rs10499276	AAGTCGTCTGCCCATTAT	5449.6
rs4307239	AAGGTGGGATGATTGTTC	5609.7
rs6235	TCACCAAAGAAGTCCCCAA	5734.8
rs4624596	TAAATGAGGCATTACCAAG	5844.8
rs8070454	AGGGCTGGAGTATGATCAG	5932.9
rs11629783	AAAATGGTTGTCCTCTCTCT	6058
rs11792069	gggACCAGAACAGACACATC	6129
rs13329567	CCAGCAGGTGGGCAGAAGGG	6257.1
rs7777102	cccaTTAGGCCTCCTTGCAAAC	6615.3
rs879620	CAACAGCCAAATACAAATATTA	6688.4
rs7784465	gcTTGAGATTGGCCAAGAAATA	6807.5
rs3134353	gctgAACCTACTCATAGGCTCTA	6983.6
rs8087550	ggagTTCGGCAGCAGATTTTTTT	7100.6
rs6968554	gagtaCAGGCCTCTGAACTTCTGA	7352.8
rs12042959	GAGAGGTAGATTCAGATTACAGAT	7464.9
rs3807049	ttctAGGGAGGGAATAGTTCAGGC	7472.9
rs156151	ATCTGCATCACAAATTGAATATAAA	7641
rs1075901	ggGAGAAATAAAGGTTGAGATCATG	7843.1
rs13227433	gAAATATTAAGAACCTCAGTTTTTAT	7967.2
rs9939609	cTAACAGAGACTATCCAAGTGCATCAC	8221.4

Lower case letters indicate extra sequence to bring mass to required mass. Upper case letters indicate extend primer sequence.

Appendix D: Sanger Sequencing Primers for Obesity Panel Validation

Appendix D1: Sanger Primers for Obesity Panel.

SNP	Forward	Reverse
rs11672660	**TGTAAAACGACGGCCAGT**GTGGAAG GGCGATCAAAGCTG	AGGAAACAGCTATGACCCTGACGATCAC CCAGGGAATGAC
rs12458	**TGTAAAACGACGGCCAGT**CCGCGTTC TCCTCTGCACATTG	**CAGGAAACAGCTATGACC**GGCTGGGAT GAGTGTGCTTTTC
rs6265	**TGTAAAACGACGGCCAGT**TAACCCAT GGGATTGCACTT	**CAGGAAACAGCTATGACC**CAAACATCC GAGGACAAGGT
rs17203016	**TGTAAAACGACGGCCAGT**GCTGCCAC TGAACCATGTAA	**CAGGAAACAGCTATGACC**CCCCAACTG TAATCCTTCATTT
rs11208662	**TGTAAAACGACGGCCAGT**TGACTCAA AGGCCTGACAGA	**CAGGAAACAGCTATGACC**CGCCCAGCCA GCAATAGACTA
rs2304607	**TGTAAAACGACGGCCAGT**AGCTCCGC CGAAAGAGTTAG	**CAGGAAACAGCTATGACC**CTCTGGGGT TACGGGGAACT
rs10182181	**TGTAAAACGACGGCCAGT**CGGGGAA TAGGAGAGAGCAG	**CAGGAAACAGCTATGACC**GCAGAATCA TGTCCACGCAA
rs6919443	**TGTAAAACGACGGCCAGT**TAGGGCA GGACCTTGAAAAA	**CAGGAAACAGCTATGACC**TGTGTCCTTG TGCCCTGATA
rs9349239	**TGTAAAACGACGGCCAGT**TGGGGAA GGCAGAAACTGTG	**CAGGAAACAGCTATGACC**CGTGAGCTG GGAGTGATAGC
rs12327272	**TGTAAAACGACGGCCAGT**CCTCCTCT GTGTCCCTCTCTT	**CAGGAAACAGCTATGACC**CCATGACAA CACAGAAGTTGG
rs1394	**TGTAAAACGACGGCCAGT**AAACCAC ATCCAGCCCATAA	**CAGGAAACAGCTATGACC**TCTGCCAAA TGCCTGTTAGA
rs2229616	**TGTAAAACGACGGCCAGT**CTTGGCGG ATGGCACCAGT	**CAGGAAACAGCTATGACC**AGCCAAGAA CAAGAATCTGCATTCA
rs9816226	**TGTAAAACGACGGCCAGT**TCTAGGA GGCTGGAAGTTCG	**CAGGAAACAGCTATGACC**CTGGAAACT GCCCAAATGTT
rs573455	**TGTAAAACGACGGCCAGT**CAAGCCA AGAAACTGCCCAC	**CAGGAAACAGCTATGACC**GGGAGCCCA AAGGACTGTTT
rs12675063	**TGTAAAACGACGGCCAGT**GGCACAT GCTCAAGACTCAA	**CAGGAAACAGCTATGACC**TAGCCCCAA AGTAACCATTG
rs1205106 1	**TGTAAAACGACGGCCAGT**AAGAAGT TGGCAAATCAGCACA	**CAGGAAACAGCTATGACC**CCATAACAG TAAAGCCCAGGTT
rs1205106_ 2	**TGTAAAACGACGGCCAGT**GCTTGGCT GTCTCTTCCTGA	**CAGGAAACAGCTATGACC**AGACCACGC CACTACACTC
rs881301	**TGTAAAACGACGGCCAGT**GCAGTGG CAGAACAGTGAAA	**CAGGAAACAGCTATGACC**CCATAGTGG CATGGACCTGT
rs17024393	**TGTAAAACGACGGCCAGT**AACCCAG CTCAGGCATCTAA	**CAGGAAACAGCTATGACC**ACCCTGGAC TTGCAGACTGT
rs11915371	**TGTAAAACGACGGCCAGT**TGGCTTGA ATCATTTGTGTGA	**CAGGAAACAGCTATGACC**TGGCCTTAA AAGCCCTCATT
rs4372296	**TGTAAAACGACGGCCAGT**ACTCTTGG TTCAGCCTTCCA	**CAGGAAACAGCTATGACC**CAGGACCAG CTTGACACAGA
rs6879326	**TGTAAAACGACGGCCAGT**GCTGGCA GTGTGAGTCTGAA	**CAGGAAACAGCTATGACC**ATGAGGGGG CCTAAGAATGT
rs3753549	**TGTAAAACGACGGCCAGT**CCCAAGTT GGAAAACTGCAT	**CAGGAAACAGCTATGACC**GAAGCCAGC CTCTTCCTTTT
rs1899951	**TGTAAAACGACGGCCAGT**TGTTTGTG GTAAGGGATGGTT	**CAGGAAACAGCTATGACC**CCCCAATTTC ACTTTGCCTA

SNP	Forward	Reverse
rs3751813	**TGTAAAACGACGGCCAGT**GCATCCTACCAGCGAAAAAG	**CAGGAAACAGCTATGACC**TCTATTGAGGCTGGTCACACA
rs107457851	**TGTAAAACGACGGCCAGT**TGACCTTGTGTAGACCTAGGC	**CAGGAAACAGCTATGACC**CTGTACAGGGCACTTTCCAT
rs107457852	**TGTAAAACGACGGCCAGT**ACTCAATGGCTAGAATGTAGATGT	**CAGGAAACAGCTATGACC**CACTCCTGGTAAGAGTATGTTTCT
rs9332817	**TGTAAAACGACGGCCAGT**GCCTGGCATCTCTTAATTGCCAC	**CAGGAAACAGCTATGACC**TCCACTCTGGCAACCTCACCA
rs6804842 1	**TGTAAAACGACGGCCAGT**CCCAGGCCTCAGAAGTGTTT	**CAGGAAACAGCTATGACC**TCCTTGCGAACAACCTGAGA
rs6804842 2	**TGTAAAACGACGGCCAGT**CCATGAAAGGGGTCAACTTC	**CAGGAAACAGCTATGACC**AAAAATCCCGAGGTGTACCA
rs2124499	**TGTAAAACGACGGCCAGT**GGTCCCACAAGCAATAAGGA	**CAGGAAACAGCTATGACC**ATGTGCCCCATAATTGGTGT
rs403656	**TGTAAAACGACGGCCAGT**TCCCATCCCTCTCCTCTTTT	**CAGGAAACAGCTATGACC**TGTATTTTTGGGAGCCAAGG
rs10499276	**TGTAAAACGACGGCCAGT**CTTTAGGGACAGGGGCCAGA	**CAGGAAACAGCTATGACC**TCCCCAAGTGAAAAGTCCTGC
rs4307239 1	**TGTAAAACGACGGCCAGT**TCCCTCCAGTCTCTCTCTCT	**CAGGAAACAGCTATGACC**CTACAGGCACATGACACAGC
rs4307239 2	**TGTAAAACGACGGCCAGT**TCCCTAGCACCTGAAAGGAG	**CAGGAAACAGCTATGACC**CCCATTTTGTTTGGGTTTTG
rs6235	**TGTAAAACGACGGCCAGT**ACCTATGATCAATTCTGGAAGTTGAAC	**CAGGAAACAGCTATGACC**CGGAGGGATGAGTTGGAGGAG
rs4624596 1	**TGTAAAACGACGGCCAGT**GACGGGTTTTCGCCATGTT	**CAGGAAACAGCTATGACC**TCTGCACAGGAAAAGAAGCT
rs4624596 2	**TGTAAAACGACGGCCAGT**AGCCTCCTGAGTAGTGGGAT	**CAGGAAACAGCTATGACC**TGCAATCTACCCATCTGACAA
rs8070454	**TGTAAAACGACGGCCAGT**GCACCTCTGTGACACCCTTT	**CAGGAAACAGCTATGACC**TATCAGGTCAGCCTGGGTTC
rs11629783	**TGTAAAACGACGGCCAGT**AGAGACTATTGGCTGGGCAC	**CAGGAAACAGCTATGACC**CAGGGCTTCTTCCACTTAGC
rs11792069	**TGTAAAACGACGGCCAGT**CCGCAGAGTACACCCTTGAT	**CAGGAAACAGCTATGACC**GACTGAGGCAAGAGCTCACC
rs13329567	**TGTAAAACGACGGCCAGT**GTCCATGAGAAAGAGGGGCA	**CAGGAAACAGCTATGACC**GCAGACATCCTGAGCGGTTT
rs7777102	**TGTAAAACGACGGCCAGT**AGCCCAAATTTGAACCCAGT	**CAGGAAACAGCTATGACC**CGCTGCACTGAAGTCTAAGC
rs879620	**TGTAAAACGACGGCCAGT**ATTCATGAATGCAAGTGTGTGTGCG	**CAGGAAACAGCTATGACC**CCGAGGTGCTCTGTTTGTCGAA
rs7784465	**TGTAAAACGACGGCCAGT**AGACTGATAATACACCCGTATGAA	**CAGGAAACAGCTATGACC**AAAAGGTGGGGCAGCAGTAA
rs3134353	**TGTAAAACGACGGCCAGT**GGCAGGATGAGGTTACAGCAG	**CAGGAAACAGCTATGACC**TAGGAGGTGCTTAGCCTGCC
rs8087550	**TGTAAAACGACGGCCAGT**GCTTGGGGTAAAGGGCCAAA	**CAGGAAACAGCTATGACC**GGGAAGCACTCACAGTTGGA
rs6968554	**TGTAAAACGACGGCCAGT**CATAAGACGCCCAAAGAGGA	**CAGGAAACAGCTATGACC**ACCACAGCAATCAACACAGC
rs12042959	**TGTAAAACGACGGCCAGT**AGAGCAGAAACAGGGGACCT	**CAGGAAACAGCTATGACC**CGCTGATGCTAGGAGCAGACA
rs3807049	**TGTAAAACGACGGCCAGT**GCCCAGGACCATGTTTAAGA	**CAGGAAACAGCTATGACC**CACCTTCACCCCCATATGACA

SNP	Forward	Reverse
rs156151	**TGTAAAACGACGGCCAGT**AAGGAATGGGCATTTCTGAG	**CAGGAAACAGCTATGACC**AACCCTCATGCCTCACACTC
rs1075901	**TGTAAAACGACGGCCAGT**GAGCAGTCACTCCCCTGGTA	**CAGGAAACAGCTATGACC**CGCTGGCCTCAAATTCCTATG
rs13227433	**TGTAAAACGACGGCCAGT**ACTGGAACCTTGTTTTGAAGCA	**CAGGAAACAGCTATGACC**CTACAGACTTGCAGCACCAC
rs9939609	**TGTAAAACGACGGCCAGT**GGTGGTACGCTGCTATGGTT	**CAGGAAACAGCTATGACC**TCCCAAAGTCCTGGAAACAC

Bolded sequence indicates M13 Tail

Appendix D2: Additional Sanger Primers Designed after initial PCR

SNP	Forward	Reverse
rs9349239_2	**TGTAAAACGACGGCCAGT**TGTCCCACCTCCTGACCTAC	**CAGGAAACAGCTATGACC**ATCCAATGGCTCTAAGCGGA
rs879620_2	**TGTAAAACGACGGCCAGT**CGAAAGGGAAGAATGGATGA	**CAGGAAACAGCTATGACC**CTGTCCATCTCCCCAGACAT
rs10499276_2	**TGTAAAACGACGGCCAGT**GCCCCACCACACCCATTTAT	**CAGGAAACAGCTATGACC**ATGCTCTCAGTGCTCTGATGTT
rs10499276_3	**TGTAAAACGACGGCCAGT**TTTGCTATGCCCCACCACAC	**CAGGAAACAGCTATGACC**CCCTTTGGCCAATTTCAGAATGT

Appendix E: PCR Primers for PsA Panel

Appendix E1: PCR Primers for Well 1 of PsA Panel.

SNP	Primer Forward	Primer Reverse
rs2476601	**ACGTTGGATG**ACTGAACTGTACTCACCAGC	**ACGTTGGATG**AGATGATGAAATCCCCCCTC
rs587560	**ACGTTGGATG**CGGTGAGTGATAGGAATTGG	**ACGTTGGATG**GCTGTGTATCAATCCCAATC
rs3130457	**ACGTTGGATG**TAGACAAGTGGTAGGGTGAG	**ACGTTGGATG**TCAAACCATCTACCACGCTG
rs12212594	**ACGTTGGATG**GATGACTCAATTAATCCAGC	**ACGTTGGATG**CTAAACATCTGTGCTCTGGG
rs3869115	**ACGTTGGATG**CTACCATGATTTAGCTTAG	**ACGTTGGATG**ATCTGAGAGAAGCTGTTGTG
rs33980500	**ACGTTGGATG**TGAACCGAAGCATTCCTGTG	**ACGTTGGATG**CTGGGATTGGTTTCAGCAAC
rs2248902	**ACGTTGGATG**TTTTCAGCCTGGTTCTGTGG	**ACGTTGGATG**TGACCCTGGTCCATAGATTC
rs3131382	**ACGTTGGATG**CACGAGCCTGCAAAAGGAG	**ACGTTGGATG**ATCCGGGCCTCTCCAATCAA
rs13017599	**ACGTTGGATG**TCCAGTTGCCGTCGTTTTTG	**ACGTTGGATG**GCAGGATTTGATGAGGTGTC
rs848	**ACGTTGGATG**CAGTACTTATTACCAGGGAC	**ACGTTGGATG**TTGGAGCCAAGGGTTCAGAG
rs12189871	**ACGTTGGATG**CATGCAGAGAGGAGGCTATG	**ACGTTGGATG**TCTGTAGGCTCTTGGCCATC
rs4655683	**ACGTTGGATGC**TCTGTGGTTGGTTTCAATC	**ACGTTGGATG**AAAGGAGAGAAGTTGGAAGC
rs2844535	**ACGTTGGATG**ATACCGTGCAGTTGGTTCAG	**ACGTTGGATG**TTGGACCCCAGAGAAGTTTC
rs2201841	**ACGTTGGATG**TGCCTGGCCTATGATTATGC	**ACGTTGGATG**GTGATGATTTGTGACAGTAG
rs12044149	**ACGTTGGATG**GCAACCGGTTTCTACGGAC	**ACGTTGGATG**TTCCGCACCCTGAAACAAAC
rs9468859	**ACGTTGGATGT**AGAGGGTGTGATGGTTTCC	**ACGTTGGATG**CCTATACTGCAGATTCTGAG

Bolded sequence refers to M13 tail

Appendix E2: PCR Primers for Well 2 of PsA Panel.

SNP	Primer Forward	Primer Reverse
rs396960	**ACGTTGGATG**AGACCCAGAGGTGAGGCAT	**ACGTTGGATG**AATCTCCTCCATCCAGCATC
rs9304742	**ACGTTGGATG**AATGTCACCCCTATCTGGAG	**ACGTTGGATG**ATTTATTACCGAACCTAGCC
rs2734331	**ACGTTGGATG**ATGTACAGCTGCATCCGGAG	**ACGTTGGATG**CCCTGTATTGAGTGTCCATC
rs10782001	**ACGTTGGATG**TGTTCCCCTCATAGAGCAAG	**ACGTTGGATG**ACACAGTTATCTGCTCCCAC
rs67841474	**ACGTTGGATG**GAGTCATTGGCAGACATTCC	**ACGTTGGATG**CCTCTGCAGCTGATGTTTTC
rs6457374	**ACGTTGGATG**TTTCAAACCTCCTGCATCTG	**ACGTTGGATG**GGATTCTGCATGAAAACAAGC
rs11209026	**ACGTTGGATG**GACAACAGAGGAGACATTGG	**ACGTTGGATG**AAATTCTGCAAAAACCTAC
rs12191877	**ACGTTGGATG**ACTAAGAGTGCTATTGCTGG	**ACGTTGGATG**TCACCCTCCTCTACTGTTTG
rs3129944	**ACGTTGGATG**TGTGCTTATAAGGTACCCAC	**ACGTTGGATG**TGGGATCTCATAAGCCACTG
rs34536443	**ACGTTGGATG**ATTGCTCTAGCAAACTCCCG	**ACGTTGGATG**TATGAGCTGCTGACGCACTG
rs2066808	**ACGTTGGATG**ACCTGTCTGGATACCCAAAG	**ACGTTGGATG**CCTTCTTAGACTAGCTTCTG
rs715285	**ACGTTGGATG**GTCGCTTAGAGAAGCTCTAC	**ACGTTGGATG**GGACAGTGTTCAAATTCTAC
rs4908742	**ACGTTGGATG**CCACGTGAAGAAGGACGTG	**ACGTTGGATG**TGAGACCTTGCCTTAGTCCA
rs4349859	**ACGTTGGATG**GATCAAAGGTAAACATGCTC	**ACGTTGGATG**AAGCAGCCTAATCCCCTTAC
rs146571698	**ACGTTGGATG**TTGTGATCTGCCTGCCTCG	**ACGTTGGATG**AGCCTTGAACACTGGGCTAA

Bolded sequence refers to M13 tail

Appendix E3: PCR Primers for Well 3 of PsA Panel.

SNP	Primer Forward	Primer Reverse
rs1800925	**ACGTTGGATG**CAACACCCAACAGGCAAATG	**ACGTTGGATG**AGCCATGTCGCCTTTTCCTG
rs887466	**ACGTTGGATG**TCCGCACCTATCACACCTAC	**ACGTTGGATG**GGAAACAATCCTTCCTGACC
rs2844603	**ACGTTGGATG**GTCATGCCCAGCTTGTAATG	**ACGTTGGATG**AAGAGACAAGGGGTGTCTGC
rs13214872	**ACGTTGGATG**ACGCTCTTTTCAGGACGATG	**ACGTTGGATG**GCATAGAATATCATGCTGCAC
rs9321623	**ACGTTGGATG**GCTCTAAGCAGCATGAAAGC	**ACGTTGGATG**ATTTTCAGTGCTCTTCTGCC
rs4406273	**ACGTTGGATG**TAGAGCCTCAGAAGAAATGC	**ACGTTGGATG**GTTCTGGAGGTAAGAAGTC
rs2082412	**ACGTTGGATG**TTCACACATGGACAACCAAG	**ACGTTGGATG**CAGCCCCCATTAGGTACTG
rs10888503	**ACGTTGGATG**TCTTGGCCTGCACACTATTC	**ACGTTGGATG**CTGCCCTTTGAGAGAAGTTG
rs9266242	**ACGTTGGATG**TGCTGTTTTGACACAGGTCT	**ACGTTGGATG**ACTCCTTACCTCTCCTCTTG
rs1050414	**ACGTTGGATG**TCTCCCGGTCCCAATACTC	**ACGTTGGATG**TACGTGGACGACACGCAGTT
rs2894207	**ACGTTGGATG**GATCCCTACTTCACACCACA	**ACGTTGGATG**CCTAATCCAAAGTCATGAAG

Bolded sequence refers to M13 tail

Appendix F: Extend Primers for PsA Panel

Appendix F1: Extend Primers and Primer Mix for Well 1 of PsA Panel.

SNP	Extend Primer Sequence	Mass	Vol to Add (500 µM Primer stock)
rs2476601	CCCCTCCACTTCCTGTA	5017.3	10.0
rs587560	AAACACCTCCTGGAATG	5163.4	10.5
rs3130457	CAGAGACGGCTCTGACT	5195.4	10.8
rs12212594	CTGTGGGTTGGGGTTTCC	5568.6	11.8
rs3869115	AAGCTGTTGTGAAAACTTA	5850.8	12.6
rs33980500	TGGGTATGGTTCTGATTCAT	6169	13.6
rs2248902	ATAGATTCATTTCTCACTCAC	6315.1	14.2
rs3131382	GGGCCTCTCCAATCAACAGCGG	6705.4	15.4
rs13017599	GGAGGATGGAAAAGCTGAACAA	6890.5	16.1
rs848	GGCCCCAGCACTAAAGCAGTGGAC	7356.8	17.5
rs12189871	CTTGGCCATCAGTGTAGAGGGAGC	7433.8	18.1
rs4655683	AGGAGAGAAGTTGGAAGCTGTAGA	7554.9	18.7
rs2844535	CCAGAGAAGTTTCCAAAACTGTGAC	7659	19.4
rs2201841	GGAAACTAATATAGAAGATGATGAC	7771.1	20.0
rs12044149	GCCCCCACCCTTCAGTCCCTTAGCAAC	8061.2	21.2
rs9468859	CACTGCAGCCTCTACCACAGGCTCTGA	8165.3	21.9
Total primer vol µl			251.7
Water to add µl			748.3
Total volume µl			1000.0

Appendix F2: Extend Primers and Primer Mix for Well 2 of PsA Panel.

Assay	Extend Primers	UEP Mass	Vol to add (500 µM Primer stock)
rs396960	TTCCCCCACCCCACTGA	5011.3	10.0
rs9304742	AACCTAGCCAACCAATG	5132.4	10.4
rs2734331	CTCTCACCGAAGGATCTC	5419.5	11.3
rs10782001	AATGAAGGCTTGTCAACA	5531.6	11.7
rs67841474	TAACAAAAATAGCAGCAGC	5822.8	12.6
rs6457374	CCAGATAGGTTTAGTGGTG	5898.8	13.0
rs11209026	CTGCAAAAACCTACCCAGTT	6030	13.6
rs12191877	ACTAAGCCAATCATCTACTCA	6318.1	14.5
rs3129944	AACAAGGAAGAGTAATAACAC	6489.3	15.2
rs34536443	GCACTGTGACTCCAGCCAGAGC	6705.4	16.0
rs2066808	GCTTCTGCTTATTCTACCATTCC	6891.5	16.8
rs715285	AGTGTTCAAATTCTACACGGTTT	7028.6	17.4
rs4908742	CTGAGGAGGCCTCAGGAAACTTGC	7402.8	18.7
rs4349859	CCCCTTACCTTGAGTTAATAGCTTC	7542.9	19.5
rs146571698	CCTCTGGCCGGGCACAGTGAGTCATG	7988.2	21.0
Total primer vol µl			221.6
Water to add µl			778.4
Total pool volume µl			1000.0

Appendix F3: Extend Primers and Primer Mix for Well 3 of PsA Panel.

Assay	Extend Primers	UEP Mass	Vol to add (500 µM Primer stock)
rs1800925	TTTCCTGCTCTTCCCTC	5014.3	10.0
rs887466	TCTACCCTCTCCGGAAA	5090.3	10.6
rs2844603	AGGGGTGTCTGCTGCTGT	5577.6	12.0
rs13214872	TGCTGCACATGAAGAAATAG	6174	13.9
rs9321623	TGCTCTTCTGCCACAGGCTGC	6349.1	14.8
rs4406273	CTATGGGCTAAAATTAACATGT	6757.4	16.4
rs2082412	GAAATTGTACAAATGCAAAATG	6799.5	17.2
rs10888503	CCTCCCATTATCTTAGCTTGCTTC	7180.7	18.8
rs9266242	TCCCTGAATGATTAGAATTCCAAT	7310.8	20.0
rs1050414	ACGCCGCGAGTCCGAGAGGGGAGCC	7743	22.0
rs2894207	ATGTTTTCTTCTAAGAGTTCTCTAAT	7916.2	23.4
Total primer volume µl			179.0
Water to add µl			821.0
Total pool volume µl			1000.0

Appendix G: MassARRAY genotyping statistics (peak height and SNR) for initial PsA panel validation

Appendix G1: Peak height statistics of initial PsA panel validation completed within the laboratory previously using MassARRAY genotyping.

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	Avg	Max	Min	SD	Count	Avg 1	Max 1	Min 1	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs1050414	13.5	15.2	12.3	1.3	3	13.1	24.9	4.4	5.1	25	7.2	13.4	0.7	3.6	6.5	16.3	0.7	3.9	13.0
rs10782001	9.79	9.79	9.79	0	1	18.24	23.74	13.38	3.12568	7	7.82	10.1	5.71	1.414	8.17	11.51	5.9	1.74	6
rs10888503	11.2	16.73	3.81	5.437	3	12.1	27.67	4.17	6.212	18	8.52	22.02	1.86	6.197	8.45	20.57	1.99	6.1749	10
rs11209026	20.9	30.76	11.25	5.921	11	20.01	20.01	20.01	0	1	7.79	8.11	7.47	0.32	8.57	9.38	7.76	0.812	2
rs12044149	14.18	21.31	9.54	3.8163	10	14.19	14.69	13.69	0.501	2	5.33	5.9	4.77	0.56129	6.06	6.84	5.29	0.7715	2
rs12189871	14.22	22.56	10.3	3.74	8	14.06	14.06	14.06	0	1	8.01	16.65	6.51	1.4	8.37	12.37	6.26	2.125	5
rs12191877	16.21	21.29	9.59	3.586	7	18.23	21.59	14.87	3.36	2	8.18	10.9	5.2	1.805	8.19	11.01	5.49	1.75777	5
rs12212594	14.06	22.95	9.1	4.32766	8	17	17	17	0	1	7.53	9.09	5.73	1.40556	9.8	16.9	6.7	4.177	4
rs13017599	16.31	26.18	10.86	5.041	8	14.3	14.66	13.93	0.36665	2	5.51	6.91	3.15	1.43358	6.38	8.14	3.8	1.67086	4
rs13214872	16.88	35.85	2.24	7.70765	24	17.33	19.57	15.19	1.78858	3	7.83	18.31	2.55	3.9777	8.21	18.03	2.91	4.1854	16
rs146571698	9.32	14.39	5.52	3.06576	11	0	0	0	0	0	3.36	5.39	2.34	1.4397	5.19	8.43	3.35	2.2952	3
rs1800925	11.52	30.59	1.42	7.761	33	11.58	22.34	5.32	6.83	4	9.89	20.08	2.35	6.005	7.98	14.8	2.18	4.4277	8
rs2066808	16.8	24.35	10.57	4.15421	11	11.64	11.64	11.64	0	1	6.28	7.53	5.03	1.25075	6.66	8.32	5	1.66043	2
rs2082412	23.53	58.34	2.34	12.7519	32	41.48	64.86	18.15	19.0678	3	14.87	30.51	6.89	7.44709	16.32	33.92	7.56	7.9287	9
rs2201841	14.97	21.49	8.86	5.16432	3	17.91	17.91	17.91	0	1	7.31	12.72	5.4	2.12056	7.19	11.34	5.11	1.70963	10
rs2248902	16.84	17.72	15.96	0.881	2	18.41	27.14	14.1	5.198	4	8.19	11.06	5.45	1.8756	8.54	12.02	6.18	1.9548	6
rs2476601	14.42	17.25	10.72	2.16311	9	11.69	11.69	11.69	0	1	7.92	10.1	5.17	1.8935	8.51	10.94	4.95	2.38884	4
rs2734331	13.98	20.7	7.39	3.41363	13	0	0	0	0	0	5.13	5.13	5.13	0	6.19	6.19	6.19	0	1
rs2844535	15.02	24.08	8.63	4.75203	6	12.36	12.36	12.36	0	1	7.53	11.7	5.32	2.00546	8.14	9.95	5.32	1.7468	7
rs2844603	0	0	0	0	0	11.82	24.03	2.34	6.53811	11	6.86	16.89	1.72	3.6211	7.22	13.62	2.68	3.3212	20
rs2894207	11.34	29.84	1.64	7.9306	19	9.07	9.83	8.32	0.75611	2	5.45	12.86	2.11	3.53288	5.1	12.53	2.22	3.1869	10
rs3129944	12.66	12.66	12.66	0	1	14.68	23.12	7.74	4.237	12	11.83	11.83	11.83	0	11.82	11.82	11.82	0	1
rs3130457	20.87	20.87	20.87	0	1	17.77	25.78	12.93	4.82838	4	8.38	11.99	5.69	1.8042	9.4	13.32	6.23	2.055	9

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	Avg	Max	Min	SD	Count	Avg 1	Max 1	Min 1	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs3131382	0	0	0	0	0	13.32	20.93	9.22	3.46676	9	6.52	8.58	5.19	1.1749	6.58	8.73	5.57	1.1887	5
rs33980500	21.09	33.7	12.43	6.4465	13	0	0	0	0	0	8	8	8	0	10.15	10.15	10.15	0	1
rs34536443	0	0	0	0	0	15.26	20.66	8.9	3.3056	13	7.56	7.56	7.56	0	8.47	8.47	8.47	0	1
rs3869115	19.7	28.82	13.05	4.5911	13	0	0	0	0	0	7.01	7.01	7.01	0	8.05	8.05	8.05	0	1
rs396960	18.62	25.97	12.52	5.5614	3	15.16	20.31	9.43	3.0715	9	9.85	11.03	8.66	1.182	8.64	9.73	7.55	1.0855	2
rs4349859	17.92	22.57	12.75	3.05758	11	15.35	15.35	15.35	0	1	5.7	5.84	5.57	0.1343	5.36	5.48	5.24	0.1205	2
rs4406273	28.75	75.72	2.73	16.477	28	22.84	28	17.67	5.16855	2	12.13	30.25	3.97	7.1275	14.14	34.13	5.67	8.34927	14
rs4655683	16.25	18.17	12.56	2.1189	6	16.07	20.9	12.54	3.5323	3	6.09	10.36	4.15	2.1865	7.2	11.93	4.9	2.4478	5
rs4908742	3.23	3.23	3.23	0	1	4.36	6.95	2.25	1.612	4	2.5	3.25	1.74	0.756	2.86	3.42	2.31	0.555	2
rs587560	20.09	29.76	14.21	1.9019	8	13.5	15.61	11.38	2.11345	2	7.24	8.75	5.03	1.3985	8.72	10.41	6.42	1.45922	4
rs6457374	26.51	26.51	26.51	0	1	19.44	26.79	12.35	4.65127	10	6.56	8.85	4.85	1.682	8.99	11.06	6.9	1.6982	3
rs67841474	19.78	24.02	17.46	3.003	3	19.23	30.98	12.67	6.408	5	10.43	17.09	3.82	4.12355	10.46	15	6.22	2.7986	6
rs715285	15.6	18.09	12.86	2.1416	3	21.29	27.73	12.69	5.8286	5	7.59	10.12	5.43	1.3921	9.5	13.19	5.75	2.1869	6
rs848	14.01	14.01	14.01	0	1	13.54	17.67	9.33	2.54859	8	7.21	8.91	5.52	1.69642	13.02	15.62	10.42	2.5986	2
rs887466	16.22	31.59	3.75	7.5376	16	10.5	18.55	6.05	4.858	5	6.3	10.86	1.58	2.8226	6.12	10.61	1.98	2.84244	20
rs9266242	18.54	31.31	8.27	9.929	3	15.7	28.45	6.09	7.2965	13	6.83	16.14	1.19	4.04907	6.59	14.87	1.39	4.144	13
rs9304742	14.33	22.22	8.01	3.7736	10	22.39	25.37	19.42	2.9741	2	6.14	6.9	5.39	0.756	8.29	9.12	7.46	0.831	2
rs9321623	19.79	41.05	6.9	13.3111	8	14.44	28.7	1.92	9.145	7	6.17	14.53	1.85	3.4102	7.01	16.99	2.33	4.06246	14
rs9468859	12.66	20.09	2.7	4.0269	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Average, maximum, minimum, standard deviation, and number of samples (count) is shown for each SNPs genotype.

Appendix G2: SNR statistics for initial PsA panel validation completed within the laboratory previously using MassARRAY genotyping.

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	MAX	MIN	SD	COUNT	AVG	MAX	MIN	SD	COUNT	Avg 1	MAX 1	MIN 1	SD 1	Avg 2	MAX 2	MIN 2	SD 2	COUNT
rs1050414	25.7	31.7	15.6	7.2	3	26.4	48.0	13.6	9.1	25	14.3	26.5	1.8	6.6	12.8	29.8	1.7	6.9	13.0
rs10782001	38.06	38.06	38.06	0	1	38.81	43.42	35.07	2.374	7	23.91	26.52	20.34	2.0289	23.48	26.7	18.83	2.4267	6
rs10888503	17.92	26.31	8.95	7.1	3	16.25	24.34	7.65	4.64	18	12.74	20.26	5.02	4.9931	10.59	19.56	4.3	4.43	10
rs11209026	45.96	52.35	38.78	3.8669	11	49.92	49.92	49.92	0	1	21.03	22.26	19.8	1.23	22.93	25.11	20.75	2.18	2
rs12044149	36.71	42.93	27.07	4.319	10	38.06	38.08	38.03	0.0232	2	14.48	17.43	11.52	2.952	17.07	21.01	13.13	3.9405	2
rs12189871	38.08	43.2	35.25	2.24	8	6.54	6.54	6.54	0	1	21.28	26.91	17.59	3.22	21.78	30.63	16.57	4.913	5
rs12191877	40.73	43.17	38.95	1.533	7	44.3	46.6	42	2.3037	2	20.13	22.42	17.64	1.8864	22.16	24.54	19.82	1.7474	5
rs12212594	34.95	43.9	28.37	4.588	8	31.79	31.79	31.79	0	1	17.7	21.46	11.89	3.5454	21.79	34.78	13.24	7.9563	4
rs13017599	46.61	55.14	39.01	5.40077	8	44.21	44.86	43.57	0.6467	2	18.04	19.61	14.73	1.938	20.73	22.46	19.73	1.095	4
rs13214872	29.11	42.74	4.18	8.66137	24	28.09	38.98	22.54	7.6967	3	16.47	27.27	6.15	5.59	17.51	26.43	7.45	5.5375	16
rs146571698	27.52	40.61	18.86	6.5167	11	0	0	0	0	0	10.54	11.86	8.45	1.4924	16.46	18.97	12.68	2.7222	3
rs1800925	30.77	74.11	4.6	20.183	33	35.25	56.37	13.54	18.016	4	23.98	48.05	8.14	14.4037	19.38	37.1	8	10.698	8
rs2066808	34.83	42.5	26.37	4.7065	11	42.35	42.35	42.35	0	1	18.49	19.22	17.77	0.724	18.93	20.28	17.59	1.3452	2
rs2082412	34.57	58.14	5.11	12.7453	32	44.19	61.82	20.95	17.153	3	18.21	24.8	10.79	5.37482	19.3	25.92	8.89	6.00253	9
rs2201841	37.38	42.59	31.41	4.59582	3	41.8	41.8	41.8	0	1	20.01	24.58	14.47	3.44197	20.31	23.53	15.72	2.4092	10
rs2248902	42.27	46.16	38.38	3.89065	2	42.42	44.5	40.1	1.5847	4	21.42	28.24	11.73	4.95	23.53	27.32	15.66	4.0906	6
rs2476601	49.33	58.47	36.92	7.89044	9	35.47	35.47	35.47	0	1	24.05	25.8	19.91	2.41983	21.58	24.21	16.98	2.74388	4
rs2734331	36.59	41.47	30.02	3.804	13	0	0	0	0	0	19.74	19.74	19.74	0	22.68	22.68	22.68	0	1
rs2844535	39.43	46.32	30.85	5.52639	6	35.9	35.9	35.9	0	1	18.81	23.84	13.25	3.2687	21.32	25.26	15.83	3.0753	7
rs2844603	0	0	0	0	0	28.49	41.96	5	11.249	11	16.97	30.59	8.21	5.7491	18.07	34.01	8.36	6.1827	20
rs2894207	28.08	50.11	4.72	14.1145	19	23.1	25.49	21.74	1.8776	2	16.35	26.24	7.78	6.1789	15.78	26.53	8.3	5.3408	10
rs3129944	37.37	37.37	37.37	0	1	35.29	46.64	28.27	4.4282	12	25.64	25.64	25.64	0	24.44	24.44	24.44	0	1
rs3130457	47.8	47.8	47.8	0	1	43.91	51.32	37.47	4.9241	4	23.77	27.37	18.77	2.5177	23.93	27.25	17.62	3.37011	9
rs3131382	0	0	0	0	0	40.82	46.04	34.65	4.09119	9	20.71	25.14	16.92	3.5354	19.37	22.25	16.64	2.0392	5
rs33980500	48.7	61.71	33.03	9.21	13	0	0	0	0	0	28.41	28.41	28.41	0	33.23	33.23	33.23	0	1

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	MAX	MIN	SD	COUNT	AVG	MAX	MIN	SD	COUNT	Avg 1	MAX 1	MIN 1	SD 1	Avg 2	MAX 2	MIN 2	SD 2	COUNT
rs34536443	0	0	0	0	0	35.75	38.55	30.69	2.1717	13	17.92	17.92	17.92	0	19.05	19.05	19.05	0	1
rs3869115	50.37	60.95	34.31	7.96	13	0	0	0	0	0	25.04	25.04	25.04	0	29.33	29.33	29.33	0	1
rs396960	59.31	62.91	57.22	2.5563	3	51.9	56.06	47.06	3.46833	9	34.81	37.01	32.62	2.19875	27.59	29.14	26.05	1.54655	2
rs4349859	45.32	55.99	36.21	5.1196	11	42.95	42.95	42.95	0	1	22.05	24.29	19.81	2.23745	20.72	21.92	19.53	1.1976	2
rs4406273	37.11	57.32	5.95	12.936	28	30.16	37.26	23.06	7.09995	2	16.87	27.17	8.97	6.24	19.84	32.64	7.85	8.4756	14
rs4655683	41.27	49.12	35.84	4.0666	6	37.72	42.86	31.56	4.6692	3	16.8	19.74	14.87	1.71	19.75	20.58	18.24	0.816	5
rs4908742	11.52	11.52	11.52	0	1	13.83	16.24	12.54	1.4986	4	5.18	5.51	4.86	0.3203	6.17	6.39	5.94	0.226	2
rs587560	51.6	59.54	44.93	4.7466	8	40.37	44.25	36.48	3.8886	2	20.72	23.19	16.53	2.56119	24.59	28.24	20.98	2.96909	4
rs6457374	48.21	48.21	48.21	0	1	42.66	54.41	36.04	5.1229	10	18.6	21.77	15.86	2.435	26.52	27.83	24.66	1.3536	3
rs67841474	43.28	45.84	40.29	2.2868	3	46.81	57.54	37.62	6.65818	5	25.46	30.56	14.73	5.49844	24.877	27.74	22.39	1.8371	6
rs715285	36.42	37.97	33.72	1.91468	3	44.01	50.66	39.44	3.673	5	19.98	20.71	17.69	1.157	23.97	25.33	22.74	0.902	6
rs848	40.85	40.85	40.85	0	1	37.37	45.07	32.24	4.8475	8	17.29	18.25	16.32	0.96635	32.81	33.33	32.29	0.5221	2
rs887466	39.95	61.61	13.79	13.631	16	29.92	44.43	15.2	11.33	5	16.27	26	7.19	5.89909	14.99	24.1	5.47	5.446	20
rs9266242	29.58	42.58	12.47	12.632	3	33.54	51.8	22.52	8.84	13	15.1	29.53	2.62	6.914	14.17	26.22	3.24	6.56744	13
rs9304742	47.13	58.52	39.72	5.36286	10	52.28	54.59	49.97	2.3105	2	20.12	21.64	18.59	1.5275	26.54	28.27	24.81	1.7337	2
rs9321623	34.87	49.33	24.35	9.4259	8	25.55	38.93	5.18	12.055	7	12.7	19.26	6.9	3.66	15.16	25.31	8.62	5.40065	14
rs9468859	34.31	4.031	12.45	7.5003	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Average, maximum, minimum, standard deviation, and number of samples (count) is shown for each SNPs genotype

Appendix H: MassARRAY genotyping statistics (peak height and SNR) for initial obesity panel validation

Appendix H1: Peak heights statistics for initial obesity panel validation completed with MassARRAY genotyping.

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	Avg	Max	Min	SD	Count	Avg 1	Maz 1	Min 1	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs10182181	9.33	11.52	8.01	1.56	3	10.35	19.25	3.77	4.46	7.00	5.49	8.27	1.27	2.25	6.18	9.44	1.49	2.57	13
rs10499276	0.00	0.00	0.00	0.00	0	36.02	68.28	17.11	12.26	17	10.84	21.38	6.33	5.13	14.43	29.05	8.41	7.06	6
rs10745785	31.26	40.72	16.57	7.47	9	26.59	40.52	15.31	8.72	5	15.93	23.90	4.56	5.94	17.72	27.01	4.53	6.86	9
rs1075901	43.81	57.83	22.90	11.33	6	34.99	44.78	20.56	9.99	6	20.55	33.78	9.52	7.97	21.66	35.18	10.24	8.45	11
rs11208662	0.00	0.00	0.00	0.00	0	13.39	23.86	2.45	6.35	19	4.36	6.24	2.56	1.30	5.60	7.66	3.80	1.38	4
rs11629783	41.91	72.19	17.03	14.93	11	0.00	0.00	0.00	0.00	0	16.07	26.71	9.28	5.04	16.49	27.18	8.78	5.26	12
rs11672660	22.53	22.53	22.53	0.00	1	15.39	28.44	3.49	7.69	16	7.02	11.64	2.32	3.08	6.43	9.32	1.81	2.60	6
rs11792069	26.90	39.82	10.28	8.46	18	47.06	47.06	47.06	0.00	1	11.87	16.83	5.35	4.28	10.38	13.37	7.36	2.44	4
rs11915371	21.31	36.74	5.32	10.18	14	14.82	21.08	8.56	6.26	2	11.42	19.10	2.83	4.75	11.59	17.30	3.21	4.38	7
rs12042959	33.23	57.94	15.50	11.88	20	0.00	0.00	0.00	0.00	0	19.74	27.70	14.65	5.70	22.25	30.53	16.49	6.01	3
rs1205106	14.47	24.34	5.92	7.19	7	14.07	22.66	4.72	6.16	5	6.29	9.78	1.96	2.13	8.47	13.68	2.81	2.80	11
rs12327272	23.72	23.72	23.72	0.00	1	18.20	27.19	4.66	6.66	17	7.82	10.94	3.68	2.42	6.69	9.11	2.90	2.27	5
rs12458	11.29	20.96	2.72	5.82	9	7.79	11.71	3.87	3.92	2	7.67	11.60	2.64	2.98	7.53	12.22	2.32	3.26	12
rs12675063	14.88	23.84	4.68	5.88	23	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
rs12939549	0.90	1.64	0.09	0.50	8	0.67	1.06	0.25	0.33	3	1.02	2.08	0.14	0.60	0.66	2.08	0.12	0.51	12
rs13227433	26.85	48.68	13.57	9.94	14	15.09	15.09	15.09	0.00	1	16.76	26.81	7.25	5.38	13.14	22.65	5.36	4.71	8
rs13329567	14.59	20.78	7.84	5.29	3	18.68	29.49	7.90	7.68	12	9.78	13.52	5.67	2.58	7.57	10.38	4.73	1.91	8
rs1394	14.03	24.58	3.49	6.26	11	14.25	24.96	6.60	7.80	3	4.94	10.41	0.80	2.86	5.89	12.67	1.04	3.50	9
rs156151	49.31	58.53	40.10	9.22	2	50.38	86.82	30.51	14.35	12	22.94	41.58	10.51	11.41	23.96	44.05	11.73	11.03	9
rs17024393	15.75	27.33	3.98	6.65	23	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
rs17203016	20.66	48.86	1.67	12.69	16	39.32	43.64	35.00	4.32	2	9.73	28.28	1.45	10.02	9.14	25.86	1.78	8.89	5
rs1899951	0.00	0.00	0.00	0.00	0	30.02	46.00	11.38	10.26	15	18.29	25.39	11.32	5.08	17.27	23.47	11.24	4.03	8

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	Avg	Max	Min	SD	Count	Avg 1	Maz 1	Min 1	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs2030342	1.85	3.94	0.29	1.09	9	0.00	0.00	0.00	0.00	0	2.16	3.92	0.44	1.04	1.17	3.09	0.19	0.68	15
rs2124499	39.56	68.65	23.63	18.02	5	33.18	50.35	21.82	8.67	7	15.27	28.14	6.83	6.27	17.28	30.55	7.17	7.07	11
rs2229616	0.00	0.00	0.00	0.00	0	10.36	18.32	3.20	4.29	21	4.90	6.90	2.90	2.00	4.97	7.01	2.92	2.04	2
rs2304607	17.04	30.57	2.50	8.44	14	10.38	10.38	10.38	0.00	1	6.65	12.97	1.78	3.75	9.48	17.98	2.99	5.12	8
rs3134353	17.42	19.61	15.23	1.79	3	26.53	47.87	11.12	10.63	13	13.41	19.96	7.01	3.78	12.55	18.06	6.73	3.52	7
rs3751813	13.30	31.78	1.47	9.42	6	32.87	47.56	20.23	9.57	10	20.10	26.53	11.74	4.42	13.41	18.05	8.33	3.09	7
rs3753549	25.49	42.32	8.84	10.39	18	0.00	0.00	0.00	0.00	0	12.87	15.86	9.23	2.55	14.76	19.88	10.21	3.23	5
rs3807049	27.89	55.50	14.93	12.97	8	32.09	50.16	12.36	10.26	13	12.46	18.10	6.82	5.64	12.25	12.92	11.59	0.66	2
rs403656	29.39	49.01	15.34	9.64	16	35.10	57.09	13.11	21.99	2	16.91	25.33	9.66	5.51	14.30	19.87	7.91	4.37	5
rs4307239	25.95	40.67	18.74	7.11	6	28.33	37.54	14.60	7.27	6	14.65	27.22	6.39	6.68	12.30	22.45	5.41	5.60	11
rs4372296	15.30	25.70	3.88	5.95	16	0.00	0.00	0.00	0.00	0	9.21	13.37	6.19	2.21	8.66	13.21	5.55	2.50	7
rs4624596	20.09	38.29	9.27	8.26	15	0.00	0.00	0.00	0.00	0	11.84	16.32	7.03	3.55	11.14	16.16	6.68	3.41	8
rs573455	13.52	17.62	7.36	3.03	8	14.89	20.39	7.80	5.12	4	5.24	8.38	2.07	2.07	5.74	9.90	1.98	2.44	11
rs6235	29.22	53.53	11.70	13.01	11	41.97	52.07	31.88	10.09	2	14.46	20.74	10.04	3.92	16.84	23.45	12.09	4.07	10
rs6265	0.00	0.00	0.00	0.00	0	11.86	19.80	2.98	5.15	13	5.05	8.16	1.39	2.23	5.17	9.51	1.71	2.46	10
rs6804842	40.77	53.12	30.45	9.37	3	36.97	66.55	22.27	13.55	10	15.30	23.97	7.80	5.52	12.57	20.82	5.96	4.89	10
rs6879326	32.71	46.44	24.95	9.74	3	30.42	49.82	10.95	13.02	8	16.34	24.63	5.60	5.78	19.45	30.43	6.84	6.83	12
rs6919443	16.25	25.19	6.80	5.30	8	15.19	24.70	9.44	5.71	4	8.61	12.82	2.83	3.31	8.12	12.41	2.61	3.06	11
rs6968554	38.14	45.53	30.75	7.39	2	31.77	58.82	14.49	13.63	10	16.00	27.31	8.19	5.37	16.53	28.47	8.57	5.66	11
rs7777102	25.95	42.19	11.48	8.86	17	0.00	0.00	0.00	0.00	0	13.80	25.96	5.74	6.16	13.51	24.61	5.39	5.86	6
rs7784465	23.41	41.67	10.61	9.25	20	27.55	27.74	27.37	0.19	2	16.08	16.08	16.08	0.00	15.25	15.25	15.25	0.00	1
rs8070454	23.85	32.11	17.56	5.20	6	17.37	28.71	9.57	5.63	11	6.83	12.99	3.78	3.19	6.37	12.25	3.52	2.99	6
rs8087550	16.39	24.40	10.50	5.56	4	18.71	25.55	8.80	5.93	8	12.53	21.35	5.68	4.59	8.26	14.08	3.01	3.13	11
rs879620	34.80	51.11	20.02	11.57	9	22.79	33.72	12.62	9.44	4	15.48	22.19	6.52	4.74	14.66	20.88	6.52	4.16	10
rs881301	21.33	31.64	10.22	8.16	5	23.22	28.64	16.08	5.27	3	9.98	15.71	3.05	3.71	11.29	17.75	3.03	4.17	15
rs9332817	0.00	0.00	0.00	0.00	0	26.45	42.70	12.47	8.15	19	11.28	19.94	2.49	6.28	11.28	19.37	2.95	6.05	4
rs9349239	14.80	21.81	7.02	4.70	8.00	12.47	24.67	3.49	8.03	7	6.35	10.42	4.18	2.14	6.67	10.75	3.92	2.20	8

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	Avg	Max	Min	SD	Count	Avg 1	Maz 1	Min 1	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs9816226	26.64	26.64	26.64	0.00	1	18.11	30.64	3.81	8.14	17	9.69	12.49	6.17	2.10	9.88	11.57	7.02	1.59	5
rs9939609	28.42	42.92	11.88	9.46	8	28.89	38.72	22.78	7.02	3	17.19	28.13	7.29	6.40	12.04	20.72	5.37	4.69	12

Average, maximum, minimum, standard deviation, and number of samples (count) is shown for each SNPs genotype.

Appendix H2: SNR statistics for initial obesity panel validation completed with MassARRAY genotyping.

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	AVG	Maz	Min	SD	Count	Avg 1	Max 1	Min 2	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs10182181	26.52	29.36	22.75	2.78	3	28.72	34.05	18.89	4.68	7.00	12.87	16.73	6.15	3.00	13.43	16.39	6.76	2.53	13
rs10499276	0.00	0.00	0.00	0.00	0	60.00	71.31	48.74	7.42	17	22.22	26.05	17.95	2.56	34.88	47.62	29.95	6.11	6
rs10745785	41.33	49.98	31.74	6.50	9	38.29	45.26	29.56	5.02	5	20.90	24.56	14.85	3.11	23.77	30.36	15.44	4.31	9
rs1075901	54.60	66.02	46.33	6.09	6	59.95	75.25	48.97	8.43	6	27.42	34.55	20.18	4.61	28.98	34.90	21.30	4.34	11
rs11208662	0.00	0.00	0.00	0.00	0	35.76	51.78	12.85	9.88	19	17.91	22.15	12.44	4.04	22.82	28.05	18.76	3.60	4
rs11629783	64.29	73.79	50.94	7.09	11	0.00	0.00	0.00	0.00	0	33.35	41.29	27.61	4.15	34.77	43.22	27.85	4.53	12
rs11672660	61.30	61.30	61.30	0.00	1	53.55	83.97	18.34	18.67	16	26.13	41.45	10.79	9.37	22.80	28.75	8.02	7.59	6
rs11792069	50.24	64.97	37.92	6.59	18	47.14	47.14	47.14	0.00	1	24.54	31.77	12.99	7.49	22.23	25.97	18.77	3.01	4
rs11915371	44.86	63.56	21.81	10.73	14	43.48	56.43	30.54	12.95	2	24.21	31.86	11.71	6.38	23.90	28.57	12.55	5.05	7
rs12042959	40.56	50.61	31.37	5.44	20	0.00	0.00	0.00	0.00	0	24.03	25.24	22.63	1.08	23.35	25.66	21.12	1.85	3
rs1205106	36.23	47.13	27.73	6.27	7	36.37	43.66	23.24	6.93	5	16.79	20.58	11.49	2.74	22.39	27.71	16.40	3.10	11
rs12327272	38.06	38.06	38.06	0.00	1	44.64	58.69	21.22	8.10	17	21.55	24.40	17.35	2.72	19.90	25.65	13.73	3.82	5
rs12458	36.86	57.24	14.80	13.05	9	29.23	40.44	18.02	11.21	2	22.50	29.91	14.14	4.58	21.46	29.70	11.76	5.51	12
rs12675063	35.52	47.36	20.74	6.97	23	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
rs12939549	2.47	4.10	0.46	1.14	8	2.15	2.96	1.31	0.68	3	2.90	5.73	0.29	1.52	1.73	4.31	0.52	1.05	12
rs13227433	46.38	63.63	34.23	9.11	14	35.43	35.43	35.43	0.00	1	28.35	32.25	24.95	2.39	23.66	27.78	20.06	2.96	8
rs13329567	31.25	40.17	23.81	6.76	3	35.19	42.38	28.81	4.69	12	18.81	22.99	13.63	2.85	15.34	18.43	11.36	2.02	8
rs1394	35.70	50.66	15.34	8.80	11	36.45	44.30	23.35	9.32	3	14.92	22.83	3.98	5.74	17.72	26.41	5.33	6.49	9
rs156151	59.49	67.39	51.60	7.90	2	55.45	66.90	39.84	6.00	12	30.82	40.53	22.98	5.83	31.98	39.58	23.43	4.89	9

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	AVG	Maz	Min	SD	Count	Avg 1	Max 1	Min 2	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs17024393	35.38	46.96	15.89	7.37	23	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
rs17203016	57.48	89.97	8.46	23.45	16	101.98	110.45	93.52	8.47	2	29.05	68.46	7.05	22.92	27.54	61.72	8.67	19.28	5
rs1899951	0.00	0.00	0.00	0.00	0	30.72	37.95	25.10	3.38	15	19.41	22.67	16.97	1.81	17.42	20.53	13.80	2.14	8
rs2030342	1.91	3.44	0.24	1.09	9	0.00	0.00	0.00	0.00	0	2.32	3.53	1.11	0.71	1.29	2.90	0.51	0.66	15
rs2124499	65.09	71.92	58.42	4.31	5	57.06	66.63	49.34	5.02	7	31.09	41.62	23.17	4.90	34.32	43.44	23.94	5.59	11
rs2229616	0.00	0.00	0.00	0.00	0	29.57	37.49	15.55	5.50	21	11.88	13.70	10.06	1.82	12.36	14.57	10.14	2.22	2
rs2304607	43.29	60.74	13.68	13.10	14	31.26	31.26	31.26	0.00	1	18.56	29.15	8.84	6.27	26.00	37.59	13.95	7.10	8
rs3134353	34.94	37.22	33.75	1.61	3	36.59	48.41	29.47	4.81	13	19.01	22.31	15.64	1.94	17.62	19.10	15.07	1.29	7
rs3751813	19.35	34.57	3.11	11.27	6	38.69	44.45	32.77	3.40	10	21.19	24.44	18.88	2.06	14.86	16.61	14.20	0.76	7
rs3753549	26.67	33.76	19.12	4.10	18	0.00	0.00	0.00	0.00	0	13.06	15.25	10.88	1.71	12.94	14.33	11.02	1.17	5
rs3807049	42.07	49.12	30.17	6.27	8	35.53	42.44	26.81	5.57	13	19.62	24.68	14.55	5.06	18.69	20.85	16.52	2.16	2
rs403656	52.14	61.54	40.19	6.15	16	48.47	54.09	42.84	5.62	2	31.18	37.93	24.16	4.56	26.76	34.14	19.81	5.42	5
rs4307239	50.82	65.61	43.75	6.97	6	64.97	78.53	45.34	11.83	6	31.37	38.63	22.45	6.27	26.42	33.35	17.61	5.82	11
rs4372296	27.43	37.27	16.42	4.52	16	0.00	0.00	0.00	0.00	0	14.11	16.21	10.63	1.96	12.94	15.80	9.64	1.68	7
rs4624596	38.79	52.85	32.28	4.97	15	0.00	0.00	0.00	0.00	0	25.54	33.65	20.91	3.55	23.08	31.92	19.08	3.94	8
rs573455	30.64	40.41	20.64	5.57	8	32.01	37.36	24.70	4.58	4	15.09	21.15	9.19	3.33	15.91	20.82	9.59	3.45	11
rs6235	65.14	82.27	43.80	13.31	11	65.81	66.45	65.16	0.65	2	33.09	44.02	26.07	4.86	38.70	50.79	33.09	5.33	10
rs6265	0.00	0.00	0.00	0.00	0	35.34	48.81	15.85	9.12	13	15.79	23.82	6.92	4.99	16.06	27.66	8.41	5.22	10
rs6804842	68.30	74.12	61.53	5.18	3	68.83	81.27	56.37	8.66	10	36.44	44.16	28.77	4.33	29.07	35.43	21.56	4.08	10
rs6879326	40.49	42.78	39.29	1.62	3	39.02	56.72	30.81	8.85	8	19.93	25.11	13.83	3.19	23.75	31.02	16.90	3.70	12
rs6919443	45.22	51.72	33.69	5.84	8	36.17	39.11	29.69	3.79	4	21.38	25.55	13.78	3.43	20.20	23.71	12.64	3.25	11
rs6968554	46.95	51.60	42.30	4.65	2	42.54	50.11	33.78	5.35	10	20.75	25.37	15.15	3.11	22.29	25.34	15.59	3.04	11
rs7777102	61.07	76.27	47.98	7.27	17	0.00	0.00	0.00	0.00	0	30.27	34.16	23.26	3.79	29.44	32.18	21.32	3.83	6
rs7784465	37.22	51.04	30.15	5.00	20	41.29	42.86	39.73	1.56	2	23.64	23.64	23.64	0.00	22.13	22.13	22.13	0.00	1
rs8070454	40.30	46.24	33.21	5.12	6	34.43	40.34	28.32	4.59	11	17.07	25.00	13.01	3.98	15.57	23.25	12.68	3.58	6
rs8087550	24.14	27.29	19.85	2.69	4	29.98	34.40	22.70	3.99	8	18.51	20.77	14.99	1.79	12.09	13.84	8.51	1.50	11

	Homozygous reference genotype					Homozygous alternate genotype					Heterozygous genotype								
SNP	Avg	Max	Min	SD	Count	AVG	Maz	Min	SD	Count	Avg 1	Max 1	Min 2	SD 1	Avg 2	Max 2	Min 2	SD 2	Count
rs879620	49.86	56.45	43.96	3.95	9	53.84	63.84	42.75	8.55	4	34.06	45.38	27.42	5.82	27.21	33.40	24.06	3.12	10
rs881301	54.81	62.41	47.63	5.92	5	52.09	57.90	44.68	5.51	3	26.84	38.16	17.60	4.66	30.31	42.82	17.48	5.57	15
rs9332817	0.00	0.00	0.00	0.00	0	59.92	75.91	48.83	6.90	19	27.59	39.19	15.26	8.57	28.31	38.31	18.84	6.89	4
rs9349239	31.20	46.31	16.54	9.85	7.00	33.65	41.02	28.04	4.1	8	16.67	20.25	13.02	2.00	18.43	25.41	14.17	3.32	8
rs9816226	62.58	62.58	62.58	0.00	1	43.40	63.42	16.81	10.37	17	26.63	29.75	24.07	2.11	27.98	33.45	25.19	3.06	5
rs9939609	60.18	79.60	48.15	8.71	8	52.57	55.12	50.67	1.88	3	37.18	42.58	21.54	6.23	27.57	32.63	19.81	3.95	12

Average, maximum, minimum, standard deviation, and number of samples (count) is shown for each SNPs