Genetic determinants of fat intake and risk factors of chronic diseases

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ABSTRACT

Background: The *CD36* gene is a candidate for sensory detection of fatty acids and has been associated with individual differences in fat preferences and consumption. Excess adiposity may compromise sensory detection, but few studies have examined whether associations between *CD36* SNPs and fat consumption differ between underweight/normal weight (UW/NW) and overweight/obese (OW/OB) individuals.

Methods: Diet (assessed by food frequency questionnaire), genetic (9 variants), body mass index (BMI), lifestyle, and biomarker data were obtained from the CARTaGENE biobank (n=12,065), a Quebec cohort of middle-aged adults. Primary outcome variables included intakes (%kcal/day) of total, saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fat. Secondary outcome variables included consumption (servings/day) of food categories with a high-fat content (added fats and oils, MUFA- and PUFA-rich foods, high-fat foods, and desserts) and biomarkers of chronic disease. Multivariate regression models stratified by BMI category were used to assess associations between 9 *CD36* variants and outcome variables adjusting for age, gender, alcohol intake, income status, education, smoking status, physical activity, total sugar intake, sodium intake, total energy intake and BMI.

Results: Among UW/NW, minor allele homozygotes of rs1049654 were associated with higher intakes of total fat, MUFA, and PUFA (34.8% vs. 30.5%, P=0.0105; 13.9% vs. 12.0%, P=0.0247; 6.8% vs. 5.3%, P=0.0066, respectively) and allele carriers of rs10499859 (GG + AG) had higher consumption of PUFA than non-carriers (AA) (5.9% vs. 5.0%, P=0.0291, 5.8% vs. 5.0%, P=0.0243, respectively), while allele carriers of rs1527483 (AA + AG) and rs3211956 (CA + CC) were associated with higher SFA (11.8% vs. 10.7%, P=0.0278) and lower PUFA (5.3% vs. 6.3%, P=0.0466) intake, respectively. Minor allele carriers of rs1527483 and rs3211956 were also associated with higher consumption of daily servings of high-fat foods and desserts (1.9 vs. 1.4, P=0.0210; 1.3 vs. 1.1, P=0.0209, respectively). Among

OW/OB, rs1054516 heterozygotes (AG) and minor allele carriers (GA + GG) of rs3173798 were associated with higher SFA intake (10.1 vs. 10.4%, *P*=0.0185; 10.6% vs. 10.2%, *P*=0.0223, respectively), and rs1054516 minor allele carriers (AG + GG) were also associated with higher serum triglycerides (0.22 vs. 0.19, *P*=0.0065).

Conclusion: *CD36* variants are associated with habitual fat consumption, which may be responsible for subsequent associations with chronic disease biomarkers. Associations differ by BMI status and dietary fat type.

RÉSUMÉ

Contexte: Le gène CD36 est un candidat de la perception gustative des acides gras provenant des aliments. Il a été associé à la variation interindividuelle de la préférence et la consommation du gras. Un excès d'adiposité peut compromettre la détection orosensorielle. Par contre, peu d'études ont été effectuées sur l'association du gène CD36 et la consommation du gras entre les individus qui sont en sous-poids/ poids normal (SP/PN) et en surpoids / obèses (S/O).

Méthodes: Les données sur la nutrition (évaluées par le questionnaire de fréquence alimentaire), la génétique (9 variantes), l'indice de masse corporelle (IMC), le mode de vie et les biomarqueurs ont été obtenues de la biobanque CARTaGENE (n = 12,065). Celle-ci est une cohorte d'adultes québécois d'âge moyen. Les variables de résultat principales sont constituées des apports (% kcal / jour) des acides gras totaux, saturés (AGS), monoinsaturés (AGMI), et polyinsaturés (AGPI). Les variables de résultat secondaires sont la consommation (portions / jour) de quatre catégories d'aliments riches en lipides (les gras et huiles ajoutés, les aliments riches en AGMI et AGPI, les aliments riches en gras et les desserts) et les biomarqueurs des maladies chroniques. Des modèles de régression multiple stratifiés selon les catégories d'IMC ont été utilisés pour évaluer les associations entre les variantes de CD36 et les variables de résultat. Tous les modèles sont ajustés pour l'âge, le sexe, la consommation d'alcool, la catégorie de revenu, l'éducation, le statut de fumeur, l'activité physique, la consommation de sucres, la consommation de sel, l'apport énergétique et l'indice de masse corporelle.

Résultats: Chez les individus SP/PN, les porteurs de deux allèles mineurs de rs1049654 sont associés avec des apports plus élevés en lipides, en AGMI et en AGPI (34.8% vs. 30.5%, *P*=0.0105; 13.9% vs. 12.0%, *P*=0.0247; 6.8% vs. 5.3%, *P*=0.0066, respectivement). Parmi les porteurs de rs10499859, comparés aux porteurs de deux allèles AA, les porteurs des allèles GG + AG ont consommé plus de AGPI (5.0% vs. 5.9%, *P*=0.0291, 5.0% vs. 5.8%, *P*=0.0243,

respectivement). Les porteurs de rs1527483 (AA + AG) et rs3211956 (CA + CC) sont associés avec des apports plus élevés en AGS (11.8% vs. 10.7%, *P*=0.0278) et moins élevés en AGPI (5.3% vs. 6.3%, *P*=0.0466), respectivement. En plus, les porteurs d 'un allèle mineur de rs1527483 et rs3211956 sont associés avec une consommation plus fréquente en aliments riches en lipides et les desserts (1.9 vs. 1.4, *P*=0.0210; 1.3 vs. 1.1, *P*=0.0209, respectivement). Chez les individus S/O, les porteurs de rs1054516 (hétérozygote AG) et de rs3173798 (GA + GG) sont associés avec un apport alimentaire plus élevé en AGS (10.1 vs. 10.4%, *P*=0.0185; 10.6% vs. 10.2%, *P*=0.0223, respectivement). Ceux qui portent un allèle mineur de rs1054516 (AG +GG) sont associé avec des triglycérides sériques plus élevés (0.22 vs. 0.19, *P*=0.0065).

Conclusion: Les polymorphismes de CD36 sont associés à la consommation habituelle de lipides, qui potentiellement contribue aux associations entre CD36 et des biomarqueurs de maladies chroniques. Ces associations diffèrent selon le statut d'IMC et le type de gras consommé.

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CONTRIBUTION OF AUTHORS

Tongzhu Meng (MSc Candidate) was mainly responsible for conducting the study. The candidate conducted the statistical analyses, wrote this thesis, and prepared all the figures and tables.

Dr. Daiva Nielsen (Supervisor of Candidate, Assistant Professor, School of Human Nutrition, McGill University): Provided the research direction, guidance and feedback for this study, its data access and ethics application, and thesis development. Dr. Nielsen provided critical review and extensive edits on the thesis.

Dr. Stan Kubow (Committee member, Associate Professor, School of Human Nutrition, McGill University): Provided feedback on all aspects of the study direction. Dr. Kubow critically reviewed the thesis and provided valuable feedback.

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LIST OF ABBREVIATIONS

The following abbreviations are used in this thesis:

A	Adenine
A/C	Adenine to cytosine nucleic acid substitution
A/G	Adenine to guanine nucleic acid substitution
BMI	Body mass index
C	Cytosine
C/T	Cytosine to thymine nucleic acid substitution
CD36	Cluster of differentiation 36
DHQ	Diet history questionnaire
DRK	Delayed rectifying K^+
NTPDase2	Nucleoside triphosphate diphosphohydrolase-2
α-ENaC	Epithelial sodium channel subunit α
5-HT	Serotonin
NE	Norepinephrine
GABA	ɤ-aminobutyric acid
IP_3	Inositol trisphosphate
TRPM5	Transient receptor potential cation channel subfamily M member 5
PKD2L1	Polycystic kidney disease 2-like 1 protein
PKD2L3	Polycystic kidney disease 2-like 3 protein
TAS2R38	Taste receptor 2 member 38
FA	Fatty acid
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
ALA	Alpha-linolenic acid
ARA	Arachidonic acid
EPA	Eicosapentaenoic acid
HDL-C	High-density lipoprotein cholesterol
IPAQ	International Physical Activity Questionnaire
LCFA	Long chain fatty acids
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
G	Guanine
G/A	Guanine to adenine nucleic acid substitution
GPCR	G-protein coupled receptors
SNP	Single-nucleotide polymorphism
T	Thymine
T/C	Thymine to cytosine nucleic acid substitution
T/G	Thymine to guanine nucleic acid substitution
T1R	Taste receptor, type 1
T2R	Taste receptor, type 2
T1R1	Taste receptor, type 1, member 1
T1R2	Taste receptor, type 1, member 2
T1R3	Taste receptor, type 1, member 3
TBC	Taste bud cell

TG	Triglycerides
TRC	Taste receptor cell
WC	Waist circumferences
UW/NW	Underweight/Normal weight
OW/OB	Overweight/Obese
MAF	Minor allele frequency
HWE	Hardy-Weinberg equilibrium
MetS	Metabolic syndrome
FRS	Framingham risk score
PAL	Physical activity level
TEE	Total energy expenditure
BMR	Basal metabolic requirement
ACE	Angiotensin-converting enzyme
DLW	Doubly labelled water
DASH	Dietary Approaches to Stop Hypertension

CHAPTER 1:

RATIONALE AND OBJECTIVES

1.1 Rationale

Ingestive behaviours, including eating and drinking behaviours, are critical in maintaining human body energy homeostasis and necessary physiological functions (1). Any decisions made related to ingestive behaviours are key determinants of human health. Physiological, nutritional, environmental and sociocultural factors all exert considerable influences on individual food choices and preferences (2).

For instance, humans derive palatability from sweet foods and a natural dislike for bitter-tasting foods from infancy (3). Through evolution, humans have also developed an innate preference to consume energy-dense foods that are high in fats and sugars in order to store energy for periods of food deficiency (4). However, these innate behaviours are causing prevalent development of obesity and its comorbidities in modern society due to excess energy intake, which is the consequence of noticeably decreasing in physical activity and the readily available access to energy-dense foods (5).

While it is clear that all of the above factors play significant roles in regulating an individual's susceptibility to metabolic diseases, a growing body of knowledge proposes a contribution of genetic predisposition as well. Many recent studies have suggested the influences of common genetic variants on food preferences, dietary habits and cause, and prevention of prevalent chronic diseases, including obesity, cardiovascular disease, type 2 diabetes mellitus and metabolic syndrome (6-8). Among these candidate gene studies, genetic variants implicated in human taste perception are increasingly reported as being associated with food preferences and, consequently, metabolic outcomes (6). The sense of taste is a nutrient and hedonics sensing system. Inter-individual differences in taste perception or variations of taste preferences may be caused by genetic differences in taste receptors, and any abnormalities may exert significant consequences on food selection and health (2, 9).

Fat is referred to as naturally occurring triglycerides in the human diet (10). As the most energy-dense macronutrient, it is an essential and unique part of the diet. It makes the palatability of foods and provides oral sensations of foods such as texture, flavour and aroma (11, 12). People are at risk of a series of health problems such as impaired vision, growth retardation, skin lesions and reduced learning abilities when they are essential fatty acid-deficient (13). Essential fatty acids play nutritional and physiological roles in human health, while overconsumption of fat can result in undesirable medical conditions such as obesity, diabetes and certain types of cancer (14-18).

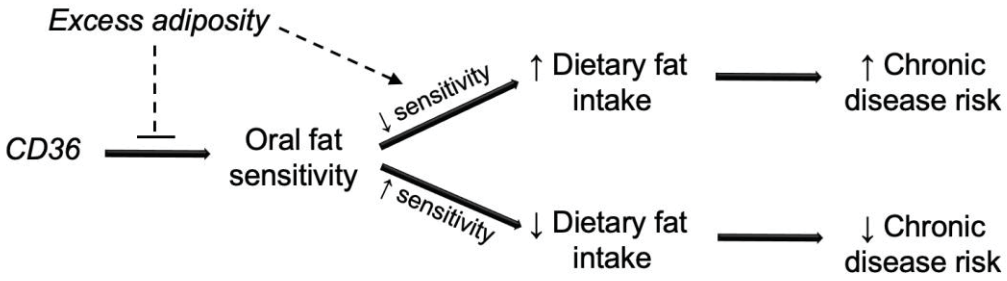
There has been evidence that genetic variants within the putative fat taste receptors have been associated with fatty acid taste sensitivity (19), lipid and glucose metabolism and risks for cardiovascular diseases and metabolic disorders (20-26). Moreover, excess adiposity has been shown to weaken the sense of taste due to a decrease in numbers of taste buds caused by systematic inflammation (27). Oleic acid taste thresholds of lean subjects were found to vary according to their intake of diets that differed in fatty acid percentage (28, 29); however, such relationships were not noted among obese individuals. Hence, it is conceivable that obese individuals might have a reduced ability to detect fatty acids at both the oral cavity and gastrointestinal tract, which could lead to an impaired satiety response and excessive fat intake (30).

While previous investigations have provided initial evidence of a relationship between these gene variants implicated in taste receptors and fat consumption and subsequent chronic disease risk factors, they have been limited in statistical power due to smaller sample sizes. Moreover, the relationship between weight status (overweight/obese vs. normal weight), the candidate gene variants and chronic disease risk factors require further investigation.

1.2 Hypotheses

Variation in the *CD36* gene is associated with oral fat sensitivity, influencing the consumption of dietary fat and subsequent risk factors of chronic diseases. Excess adiposity dampens the relationship between *CD36* and oral fat sensitivity (**Figure 1.1**).

Figure 1.1: Proposed relationship between *CD36* variants, adiposity, and dietary fat consumption



Hypotheses include:

a) There are significant associations between the candidate gene variants implicated in fat taste perception and consumption of dietary fat while excess adiposity may dampen the effect.

b) There are significant associations of the gene variants with biomarkers that are reflective of diet-related chronic diseases.

1.3 Objectives

Primary: To evaluate the relationship between the candidate gene variants implicated in fat preference and consumption of dietary fat between under/normal weight and overweight/obese individuals.

Secondary: To investigate the associations of the gene variants with the proxy for fat preference and biomarkers that are reflective of diet-related chronic diseases.

CHAPTER 2:

LITERATURE REVIEW

2.1 General taste

The gustatory system or sense of taste plays a vital role in recognizing and distinguishing the nutritional content or potential toxins, and indigestible materials of food compounds before ingestion (31-33). It is believed to aid in acquiring the intake of essential nutrients while avoiding the consumption of toxins. In addition to guide dietary selection, other essential but often neglected functions of taste include supporting the regulation of satiety, preparing other organs for metabolic adjustments (insulin release) (34), and preparing the digestive tract for the absorption of incoming foods such as releasing digestive enzymes and initiating peristalsis (10, 31). Upon the satisfaction of hunger, the sense of taste also allows humans to experience hedonistic sensations of foods.

By convention, there are only five taste primaries that have been identified in humans: sweet, savoury (umami), sour, salty and bitter — the so-called "basic" tastes (10), until recently a study has provided evidence of the sixth taste modality, oleogustus (35). Each of these tastes is thought to be associated with a particular biologically relevant class of compounds (31, 36, 37). Sweet sensations are associated with carbohydrates that serve as an energy source for humans. The umami taste is generated by amino acids and small peptides that might reflect protein content in foods. The sour taste signals the presence of dietary acids that often come from spoiled foods and may interrupt the acid-base balance of the body. The salty taste is associated with the presence of sodium or other ions that are essential for maintaining the body's water balance and blood circulation. The bitter taste is innately aversive and is thought to protect humans against consuming toxins that often taste bitter. Oleogustus (fat taste) is associated with fatty acids, the most concentrated source of dietary energy and contributes to the texture, flavour, and aroma of a wide variety of foods (11).

The ability to detect and differentiate among different taste primaries is facilitated by the anatomical units of taste perception, taste receptor cells (TRCs), which possess various

functional significance impacted by variations in the receptor genes (6). Emerging research suggests that genetic variations play a significant role in both determinants of food preferences and perceived strength of sensory experiences, both of which have been hypothesized to affect chronic disease risk by influencing what people choose to eat and delaying satiety response (6). Therefore, understanding how these variations contribute to eating behaviour, health and disease allows us to predict individual taste functions and potentially dietary patterns.

2.2 Taste physiology

2.2.1 The taste system and taste bud cell types

Taste, or gustation, is one of the five traditional senses (38), and it is triggered when a chemical stimulus or tastant comes in contact with and being recognized by the taste receptors in the oral cavity (34).

Anywhere from 50 to 150 TRCs, including most elongate epithelial cells and a small number of proliferative basal cells are clustered and form a taste bud (31). Taste bud has a structure similar to that of a garlic bulb, where dozens of taste receptor cells arranged like "garlic cloves" inside (**Figure 2.1**) (38). Between 2000 and 5000 taste buds exist along the surface of the front and the back of the tongue, on the palate, and the epiglottis (31). Taste bud cells (TBCs) experience self-renewal from the local epithelium and the average lifespan of a TBC has been estimated to be about ten days (36). The apical tips of the cells are connected by tight junctions that permeate water and some solutes into their intercellular spaces and protect the harmful chemicals from entering the cells (31, 36). These taste buds are organized within the walls of taste papillae and the grooves surrounding them. Taste papillae (**Figure 2.1**), which are visible small pink protrusions on the tongue, can be categorized into three types according to their topographical representations (31). The fungiform papillae are concentrated on the front of the tongue, the foliate papillae are located at the sides of the tongue, and the circumvallate

papillae are concentrated on the back of the tongue. There are also filiform papillae presented on the tongue. However, those are not associated with taste buds. Even though there are subtle regional differences in sensitivity to different compounds, taste buds across the oral cavity serve similar functions (31).

When tastants are recognized by specific TRCs, gustatory signals are transmitted through three sensory nerves: the chorda tympani (cranial nerve VII), the glossopharyngeal nerve (cranial nerve IX), and the vagus nerve (cranial nerve X) to the nucleus of the solitary tract in the brain stem. After that, the sensory nerves signal the thalamus and forebrain structures, which evokes taste perception and informs the acceptance or rejection of the food based on its intensity, quality and hedonics (39).

It is believed that the taste stimuli are detected through taste receptors, which are located at the apical side of taste bud cells on the surface of the tongue (10). TRCs are classified into four different morphological types: Type I, II, III and Basal (IV) cells (6). Cells from each subtype are contained in all taste buds despite their anatomical location (**Figure 2.1**) (38).

Type I cells are the most abundant cell type in the taste buds, and about 50% of the total number of TRCs are Type I cells (38). It is thought that Type I cells play a role in supporting the structure of the taste buds, similarly to glial cells of the central nervous system (6, 31). Moreover, Type I cells express membrane-bound nucleotidase such as nucleoside triphosphate diphosphohydrolase-2 (NTPDase2) that is capable of degrading neurotransmitters like ATP following a synaptic transmission (6, 31, 38). Thus, Type I cells also restrict the spreading of signalling molecules throughout the taste buds (31, 38). Type I cells also express membrane ion channels such as amiloride-sensitive sodium channel subunit α (commonly known as epithelial sodium channel subunit α [α-ENaC]) that allows for the perception of salty taste, like NaCl or KCl (6). Mice with TRC-specific deletion of α-ENaC had shown completely no interest in salt in behavioural tests (38, 40).

Type II cells, often referred to as “receptor” cells, are a group of cells that do not form conventional synapses with gustatory nerve fibres (31) and can be further categorized based on the expression of sweet, bitter and umami taste receptors (6). These taste receptors are either homodimeric or heterodimeric complexes of seven-transmembrane G-protein coupled receptors (GPCRs), more specifically, from T1R and T2R families (6, 10, 38). It is noticeable that each Type II TRC expresses a combination of GPCR receptors exclusively for only one of sweet, umami, or bitter tastants, not both (31, 38).

Naturally occurring sweet tastants, for example, sucrose, fructose, glucose, sugar alcohols, D-amino acids, glycosides, and artificial sweeteners such as sucralose, aspartame, saccharine sodium are recognized by heterodimeric receptors of taste receptor type 1 member 2 (T1R2) and taste receptor type 1 member 3 (T1R3). Whereas umami tastants, for example, glutamate, broth, mushrooms, meat and L-amino acids, are mediated by heterodimeric receptors of taste receptor type 1 member 1 (T1R1) and T1R3 (6, 38). Bitter tastants, for example, caffeine, quinine and denatonium benzoate, are elicited by several GPCRs from T2R families, depending on the particular bitter substances consumed (6, 38).

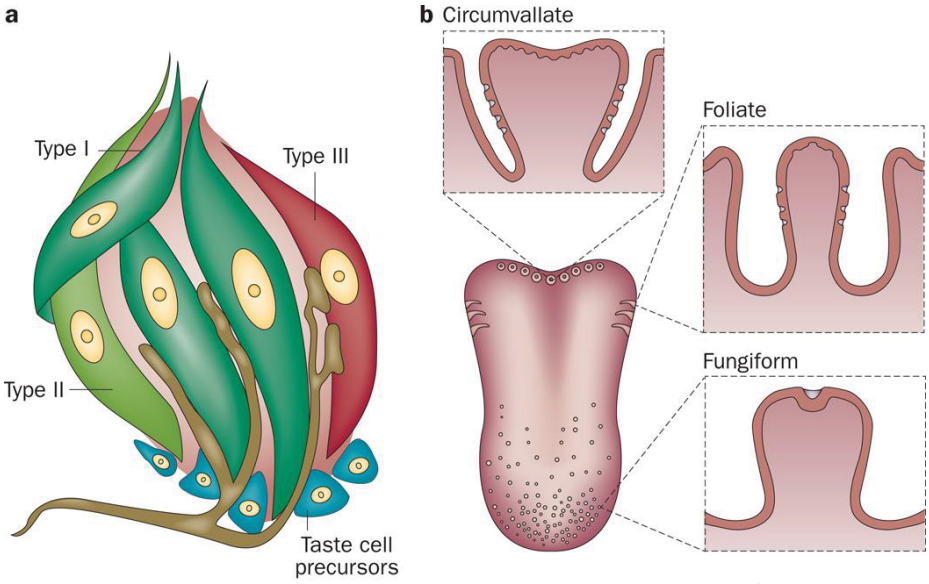
Thus, T1R [taste receptor, type 1] and T2R [taste receptor, type 2] have well-established links with sweet, umami and bitter tastants while some other physiological channels are found to be associated with transductions of sour and salty tastants (40). A growing body of evidence suggests the presence of fat taste sensors, such as fat taste receptor cluster determinant 36 (*CD36*) and *GPR120* that can detect long-chain fatty acids (LCFAs) are expressed in Type II cells.

Type III cells, also known as presynaptic cells, are the only type of TRCs that express synapse-associated proteins and form synaptic junctions with sensory afferent intragemmal nerve terminals (10, 31, 38). They receive input and integrate signals generated from receptor cells. Thus, Type III cells respond broadly to all taste primaries instead of being specific to only

one (31). Presynaptic cells express voltage-gated Ca^{2+} channels and release neurotransmitters such as serotonin (5-HT), acetylcholine, norepinephrine (NE), and ɤ-aminobutyric acid (GABA) in the synaptic vesicles when they are stimulated (41). Sour taste is perceived when proton influx to Type III cells causing changes of intracellular proton concentration and resulting in membrane depolarization. Neurotransmitters are released, and channels like polycystic kidney disease 2-like 1 protein (PKD2L1) and polycystic kidney disease 1-like 3 protein (PKD1L3) are involved in the perception (10, 31). Mice with Type III cells without expressing PKD2L1 have shown no response or reduced sensitivity to acidic stimuli (38).

The basal (Type IV) cells are a group of cells located at the base of the taste bud and are used to be thought of as progenitor cells for the differentiated TRCs (38). However, studies have evidenced that these cells could be categorized into either quiescent precursor cells or immature taste cells (38). Thus, it is not accurate to say that the Type IV cell is another subtype of TRC.

Figure 2.1: Schematic diagram of a taste bud, taste receptor cells and localization of taste papillae (38)

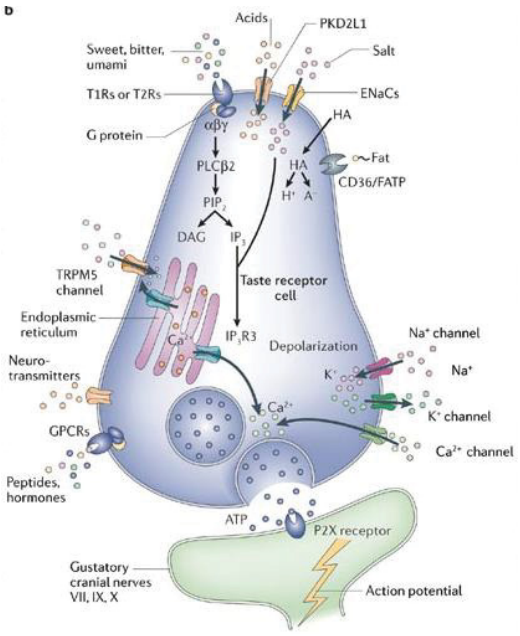


a | Illustration of four subtypes of taste bud cells presented in a taste bud. b | Circumvallate papillae is located on the back of tongue, foliate papillae is located at the sides of tongue, and fungiform papillae is located at the front of tongue.

2.2.2 Signaling mechanisms of taste perception (Figure 2.2)

When a tastant (sweet, bitter, umami, and LCFAs) binds to its specific taste receptor, GPCRs activate their corresponding GTP-binding proteins (31). Even though receptors are co-expressed with taste-selective GTP-binding proteins, the major pathway is through Gβγ subunits (42). Once the ligand is recognized by the specific receptor, Gβγ subunits are released from the taste GPCRs and interact with a phospholipase, PLCβ2 (43). The activation of PLCβ2 stimulates the synthesis of a second messenger, inositol trisphosphate (IP_3), which opens IP_3R3 ion channels on the endoplasmic reticulum and leads to the releasing of Ca^{2+} into the cytosol (44, 45). The elevated intracellular Ca^{2+} leads to the opening of TRPM5 (transient receptor potential cation channel subfamily M member 5) channels and depolarization of TRCs (46). ATP is released through Panx1 hemichannel into extracellular space surrounding the activated receptor cells (10, 31). Released ATP then targets the purinergic receptors on the gustatory afferent nerve fibres and adjacent Type II and Type III cells (38). In Type II cells, ATP acts as an autocrine manner that activation of purinergic receptors increases the secretion of ATP (31). In adjacent presynaptic cells, ATP stimulates them to release neurotransmitters such as NE and 5-HT (31). ATP released upon the recognition of taste stimuli in Type II cells are degraded by membrane-bound ATPases expressed on type I cells to prevent purinergic receptor desensitization (38). The released 5-HT can inhibit receptor cells and transmit the integrated signals from taste buds to the hindbrain for decision-making at the same time (31).

Figure 2.2: Illustration of a taste receptor cell with an associated neuron (45)



All the transduction pathways and receptors are drawn in a single TRC. The apical membrane of this TRC contains receptors for tasants that are not necessarily presented in the same TRC. These receptors include GPCRs for umami tastants (T1R1/T1R3), sweet tastants (T1R2/T1R3), bitter tastants (T2Rs); ion channels for salt taste (α-ENaCs) and sour taste (PKD2L1) and transmembrane protein for long chain fatty acids (*CD36*). When the taste receptor cell is activated by specific tastant, there is degradation of phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce diacylglycerol (DAG) and IP_3. IP_3 then binds to and activate IP_3R3 receptors on the endoplasmic reticulum that release Ca^{2+}. The increase in Ca^{2+} activates TRPM5 receptors and cause transmitters such as ATP to be released from synaptic vesicles to bind to their receptors on primary neurons.

2.3 Fat taste

Fat is the most energy-dense component of the human diet, and it is a significant contributor to the texture, aroma and flavour for a large variety of foods (11). Foods with high energy and high-fat content are generally perceived as the most palatable (11). The taste, smell and hedonic attributes of fat contribute to its appeal, which leads to the close association between palatability and energy density of foods (11). Previously, fat perception is thought to be sensed through smell/aroma (olfactory), the texture of lipids and post-ingestive signals (11, 47, 48). The initial sensation of fat is through the olfactory perception of volatile, fat-soluble molecules through the nose in a process known as orthonasal olfaction (11). Retronasal

olfaction is the continuing version of orthonasal sensation once the foods are ingested in the mouth (11). The later process may have different brain pathways than the previous one (47). As for the texture attributes, the texture of fat can be measured in some properties, such as viscosity (thickness) and lubricity (slipperiness or oiliness) (11).

Mounting evidence suggested that oral fat may activate the gustatory system as well, in addition to the activation of the trigeminal and olfactory system, because there is still detection of dietary fat when these sensations are negated or masked. In short-term behavioural tests, both intact and anosmic rodents are able to distinguish between oil and xanthan gum (to mimic fat texture), suggesting that gustation plays a role in lipid sensation (49, 50). Moreover, humans can taste LCFAs even when olfaction cues are eliminated with the uses of nose clips, and the specific textural cue of fatty acids is masked (51). In addition, anosmic rats are able to discriminate between oleate and triolein solutions that are suspended in 0.3% xanthan gum (49). Both rodents and humans can still detect the sensation of fat when the olfactory and textual cues are masked, suggesting that there is a detection system of dietary fat or more likely to their fatty acid breakdown products (49, 51). Free fatty acids that occur in small amounts in fatty foods and being released from the hydrolyzation of triglycerides (the main components of oils and fatty foods) by lingual lipases can be detected by the taste system and initiate the perception (52, 53). Indeed, studies have shown that fat can be detected through chemoreception and *CD36*, *GPR40* and *GPR120* are some putative fat receptors (10, 38, 54-57).

2.3.1 Fat taste receptor

Fatty acid translocase, *CD36,* is a heavily glycosylated 88kD transmembrane protein that is widely expressed in multiple cell types, including adipocytes, skeletal muscle cells, monocytes and macrophages and has broad functions in immunity, inflammation and lipoprotein metabolism (58-60).

CD36 is known as a plausible candidate for detecting long-chain fatty acids (LCFAs) since the inactivation of the *CD36* gene in rat models has shown a lack of sensitivity to LCFAs without impacting the detection of sweet or bitter taste (6, 10). Wildtype and *CD36* knockout mice were tested in a 2-bottle preference test over a 48 h period (55). The control bottle contained 0.3 % xanthan solution to mimic the texture of fat, while the treatment solution contained 2% linoleic acid solution emulsified with 0.3% xanthan gum (55). The wild-type mice preferred the bottle containing 2% linoleic acid over the control one that has a similar texture compared with the treatment (55). The *CD36* knockout mice consumed an equal amount of solutions from both bottles indicated the importance of *CD36* receptor in fat taste perception (55). In order to control for the effects of post-ingestive cues, wild-type and *CD36*-null mice were 1 h-water restricted or 12 h fasted. Wild-type mice showed an immediate preference for the fat-containing bottle over the control one, while there was no preference for *CD36*-null mice. This finding is consistent with the previous one (55). Similar tests using solid foods containing 5% linoleic acid or 5% paraffin (control) also showed consistent results with the liquid experiment. In order to eliminate a liquid specific effect from the tests, solid foods were tested, and wild-type mice showed a preference for the linoleic acid-contained diet over the control diet, which had a comparable texture. *CD36*-null mice did not show any preferences (55). In order to ensure it is a fat-taste specific effect, wild-type and *CD36*-null mice were tested for preferences for sweet and quinine. Both groups showed a preference for sweet substances and aversion for bitter substances indicating that the taste function, in general, was intact, and the difference observed between wild-type and *CD36*-null mice was specific due to *CD36* knockout. Moreover, the normal increase in intracellular calcium of taste receptor cells in response to free fatty acids is not presented in *CD36* knockout mice, as well as the activation of brain areas associated with fatty acid stimulation (39). Pharmacological inhibition of lingual lipases significantly decreases the preferences for lipids suggested the likelihood of LCFAs being the

orosensory cue for dietary fat (53).

Additionally, two G-protein coupled receptors (GPCR), *GPCR40* and *GPCR120* may act as candidate fatty acid receptors that respond to medium-chain and long-chain fatty acids according to two gene knockout studies in mice (10, 40). *GPR40* is mainly found in Type I taste cells of mice (54), but there is no evidence to support the expression in the gustatory epithelium in humans (32). On the other hand, the expression of *GPR120* is detectable in gustatory and non-gustatory lingual epithelia in humans (32), which may suggest its role in fatty acid taste perception. *GPR40* knockout mice and *GPR120* knockout mice showed an attenuated preference for linoleic acid and oleic acid, as well as eliminated nerve responses to several fatty acids compared to wildtype mice (54). Expressions of *GPR120* in adipose tissue were significantly higher in obese individuals than in lean controls, and a deleterious non-synonymous mutation (rs116454156) inhibits *GPR120* signalling activity such that increases the risk of obesity in European populations (61).

Other candidates for fat taste receptors are delayed rectifying K^+ channels (DRK channels). DRK channels are found within the apical membrane of lingual taste cells that facilitate the flow of K^+ into the intracellular space (10). *Cis*-polyunsaturated fatty acids are found to inhibit DRK channels, and this may allow the cells to generate an action potential (52). Thus, DRK channels are thought to be another type of signal transduction.

2.3.2 Fat, diet and adiposity

Since taste perceptions are known to differ between individuals, common variations in genes involved in taste perception may account for some of the differences in food preferences and food intake between individuals. For example, individuals who intensely taste bitterness due to variations in the *TAS2R38* gene have been reported to consume fewer vegetables and more sweet foods compared to individuals who are less sensitive to bitter taste due to their

genotype (6). Regarding fatty acid perception, genetic variants within the *CD36* gene have been associated with fatty acid taste sensitivity (19), lipid and glucose metabolism and risks for cardiovascular diseases and metabolic disorders (20-26). Common single nucleotide polymorphisms (SNPs) like rs1527483 (MAF = 0.1018) is associated with oral fat perception and those who carried C/T or T/T genotypes in African American and Malaysian subjects perceived higher fat content in salad dressings regardless of actual fat concentration and gave high rankings of added fat as well (19, 62). African-American subjects homozygous for rs3211938 (MAF=0.094) had significantly lower mean HDL-C levels and decreased *CD36* expressions compared with heterozygous subjects that had higher mean HDL-C levels and lower mean TG levels when compared with non-carriers (24). Moreover, subjects homozygous for rs3211931 were associated with increased metabolic syndrome risk in Puerto Rican adults (25). A summary of evidence of *CD36* SNPs that had been associated with oral fatty acid sensitivity, metabolic syndrome and associated disorders and lipid levels is included in **Table 2.1**. A list of additional SNPs in *CD36* gene that no associations have been previously reported or have not been investigated in the past is included in **Table 2.2**.

An association between oral fatty acid sensitivity with fat consumption and consequently, body weight regulation has been identified in human and animal studies (37, 63). This relationship summarizes the significant difference between obese and lean subjects in relation to fat intake and fatty acid sensitivity (6). Individuals who are hypersensitive to fatty acids consume less fat (on average 21 g / day difference) and have lower BMI compared with hyposensitive individuals (10, 64). Changes of oleic acid taste thresholds in lean subjects are in accord with the consumption of diets that are changing in fatty acid percentage (10). For example, the taste thresholds of lean subjects increase when a high-fat diet (> 45% fat) is consumed and decreases when a low-fat diet (< 20% fat) is consumed. However, this change is not found among obese individuals (28, 29). Furthermore, a recent study suggested that obese

people might have a reduced ability to detect fatty acids at both the oral cavity and gastrointestinal tract leading to an impaired satiety response and resulted in excess fatty acids intake and obesity (30).

Table 2.1: A summary of evidence of *CD36* SNPs that had been previously associated with oral fatty acid sensitivity, metabolic syndrome and associated disorders and lipid levels, table is adapted from Chamoun et al. 2018 (6) and Liu et al, 2016 (10).

SNP ID	MAF	Study	Sample Info	Outcome	Finding
rs1761667	0.4	Pepino et al., 2012 (65)	21 population	Oral fatty acid sensitivities and fat consumption	A allele associated with reduced fatty acid sensitivity ($p = 0.03$)
rs1527483	0.1	Keller et al., 2012 (19)	317 African Americans		T allele associated with increased perceived ratings of fat content ($p < 0.05$)
rs3211867	0.2	Bokor et al., 2010 (20)	646 European adolescents	Metabolic syndrome and associated disorders	AA/CA associated with increased risk of obesity ($p = 0.003$)
rs3211883	0.4				TT/AT associated with increased risk of obesity ($p = 0.007$)
rs3211908	0.1				TT/CT associated with increased risk of obesity ($p = 0.0005$)
rs9784998	0.2	Heni et al., 2011 (66)	1790 European		CC associated with larger BMI and waist circumference ($p \leq 0.004$)
rs3211956	0.1				TT associated with larger BMI and waist circumference ($p \leq 0.004$)
rs3840546	0.1	Keller et al., 2012 (19)	317 African Americans		DD deletions associated with higher BMI ($p < 0.001$)
rs1527479	0.3	Corpeleijin et al., 2006 (21)	675 Dutch		TT associated with T2D ($p = 0.005$) and larger BMI
rs1049673	0.3	Noel et al., 2010 (25)	1178 Puerto Ricans		GG associated with higher likelihood of metabolic syndrome (OR: 1.89 (1.0, 3.5))
rs3211931	0.3				TT associated with higher likelihood of

					metabolic syndrome (OR: 1.77 (1.0, 3.1))
rs3211938	0.09	Love-Gregory et al., 2008 (24)	2020 African Americans		Associated with the MetS ($p < 0.01$)
rs13246513	0.2				Associated with the MetS ($p < 0.01$)
rs1984112	0.3	Ma et al., 2004 (67)	585 Caucasian		GG associated with plasma free fatty acids ($p = 0.008$)
rs2151916	0.3	Ramos-Arellano et al., 2013 (26)	232 adults		TC associated with lower HDL-C ($p = 0.04$)
rs10499859	0.3	Love-Gregory et al., 2008 (24)	2020 African Americans	Lipid levels	Associated with HDL-C levels ($p < 0.0001$)
rs13438282	0.3				Associated with HDL-C levels ($p = 0.0007$)
rs1054516	0.3				Associated with HDL-C levels ($p = 0.0004$)
rs1049654	0.4				Associated with HDL-C levels ($p = 0.0002$)
rs3211909	0.2				Associated with HDL-C levels ($p = 0.021$)
rs3211849	0.5				Associated with HDL-C levels ($p = 0.0027$)
rs3211913	0.4				Associated with HDL-C levels ($p = 0.039$)
rs3173798	0.2				Associated with HDL-C levels (p=0.0062)
rs3211870	0.4				Associated with HDL-C levels (p=0.0029)
rs3211842	0.4				Associated with HDL-C levels (p< 0.0001)
rs3211868	0.2				Associated with HDL-C levels (p=0.0062)

Table 2.2: A list of additional SNPs in *CD36* gene while no associations have been reported or have not been investigated in the past.

SNP ID	SNP ID	SNP ID
rs17263407	rs1334511	rs3173804
rs3211805	rs3211810	rs3211944
rs3211812	rs3211813	rs7755
rs3211822	rs997906	rs13230419
rs3211834	rs3211850	rs2103134
rs3211885	rs3211886	rs3211917
rs3211890	rs3211892	rs3173801

2.3.3 Measurements of the oral fatty acid threshold, fat perception, fatty food liking

Fatty acids can be detected over a range of concentrations (30), with large interindividual differences in sensitivity (10). The differences in the functionality of oral nutrient receptors influenced individual variability in oral fatty acid detection and are modulated by genetic and environmental factors (68). Since common variants in the *CD36* gene have been associated with oral fat sensitivity and/or dietary consumption of fat, the associations between *CD36* SNPs and dietary fat consumption may have existed through their influences on the functionality of taste receptors. The common SNP rs1761667 AA genotype has been associated with decreasing oral fat perception among human subjects (69). Previous studies have suggested that those who are hyposensitive to fatty acids appear to consume more animal fats and have a higher body mass index (64). As current studies have focused on the associations between *CD36* SNPs and dietary fat consumption, it is meaningful to extend the knowledge of the significance of oral fat sensitivity and its putative impact on fat consumption in order to understand the underlying mechanisms.

Haryono et al. established a set of reliable and reproducible sensory techniques, including determination of oral fatty acid thresholds, oral fatty acid threshold testing, fat ranking test, fatty food liking and tongue photography, to access detection thresholds (68). The detection threshold is defined as the lowest concentration of fatty acids to be detected in solutions (64). The subjects need to pick up the fatty acids sample as an “odd” sample from two other control samples successfully in three consecutive tests under red light and with the nose clips on (68). With the individual detection threshold, the mean threshold can be determined as well as the categorizations of hypersensitive and hyposensitive individuals (68). As fatty foods are more commonly consumed in daily life, putting the fat content back into the foods is another straightforward way to investigate the associations between oral fat sensitivity and fat consumption in “real-life” settings. The taste threshold for the other five traditional tastes can

also be performed in parallel to make sure the integrity of the data. As the evidence for the relationship between fungiform papillae density and oral fatty acid detection is growing, tongue topography can be used to evaluate genetic and physiological differences between individuals (68).

2.4 Types of fat and lipid absorption

Moderate consumption of dietary fat is crucial for human health because it supplies the body with essential fatty acids (FAs) and fat-soluble vitamins and regulates satiety and energy homeostasis (70). Dietary fat is mainly (90–95%) composed of triglycerides (TGs), but also of phospholipids, sterols, and fat-soluble vitamins (57). In order to be absorbed, TG requires to be hydrolyzed by lipases in the intestinal lumen to yield free fatty acid and 2-monoacyl-glycerol (65). The primary fatty acids of dietary TGs are oleate, palmitate, stearate, and linoleate (57).

Saturated fatty acids (SFAs), including myristic acid, palmitic acid, stearic acid and arachidic acid, are fatty acids saturated with hydrogen molecules and contain only one single bond between carbon molecules (71). These are fatty acids found in dairy fat, coconut oil, palm kernel oil, peanut oil and other vegetable oils. Monounsaturated fatty acids (MUFAs), including oleic acid and palmitoleic acid, are found in most animal and vegetable oils, but particularly in olive, canola and safflower oil (72). Polyunsaturated fatty acids (PUFAs), including linoleic acid, alpha-linolenic acid (ALA), arachidonic acid (ARA) and eicosapentaenoic acid (EPA). ALA is a major component of seeds, nuts and some vegetable oils (73). ARA is present in meat, eggs, fish and algae, while EPA is mostly found in oily fish and marine oils.

Lipid absorption in the gastrointestinal tract is influenced by neural and humoral factors at several levels (57). Before food ingestion, events such as seeing, smelling, or thinking of food can prepare and induce modest salivary and gastric secretions via the autonomic nervous

system and pancreatic and biliary secretions via the vagus nerve (57). Then the absorbed lipids are picked up by the TG-rich chylomicrons and very-low-density lipoproteins (VLDLs) (57). Fatty acids are transported and released from the TGs through hydrolysis of lipoprotein lipase in the vasculature are taken up by various tissues (57). In adipose tissue, fatty acids are mainly converted to TG to be stored in lipid droplets, whereas in skeletal muscle, they are used primarily as energy fuel (57). There are also fatty acids taken up by the liver and packaged into VLDL. Lipid utilization is communicated across multiple tissues, and the integrations among them regulate lipid homeostasis (57).

CHAPTER 3:

MANUSCRIPT

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Common variants in the *CD36* gene are associated with dietary fat intake, high fat food consumption, and serum triglycerides in a cohort of Quebec adults

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Short running head: *CD36*, polymorphisms, dietary fat intake

Key messages:

- *CD36* is a candidate gene for fat preference, but excess adiposity may weaken taste perception and thus blunt associations of *CD36* variants with dietary outcomes.
- We observed that *CD36* variants are associated with consumption of different types of dietary fats and foods with high fat content, and that patterns varied according to BMI status.
- Our findings align with previous studies linking certain *CD36* variants with biomarkers of chronic disease, but extend the evidence to suggest that some of these relationships are mediated by links with dietary fat intake.
- *CD36* variants may be useful markers of preferences for different types of dietary fats and offer new targets for prevention of obesity and diet-related chronic diseases.

3.1 Introduction

Through evolution, humans have developed a native preference to consume calorie-dense foods in order to store energy for periods of food deficiency (4). However, this innate behavior has been associated with the obesity epidemic in modern day society due to excessive energy intake, which leads to higher body mass index (BMI), weight gain and subsequent obesity (74-76). In that regard, overweight (OW) and obesity (OB) risk can be attributed to overconsumption of highly palatable, energy dense foods containing saturated fats and added sugars as well as a sedentary lifestyle (5).

In addition to being the most energy-dense macronutrient, dietary fat is a major contributor to the aroma, texture, flavor and palatability of foods (11, 12). Consequently, high-fat foods are generally considered to be highly desirable; however, interindividual differences in taste perception, acceptance, preference and consumption exist (6). Differing dietary habits and preferences related to intake of fatty foods can be attributed to variations in metabolic needs as well as behavioral, emotional and economic factors (11). Chronic disease risk has recently been hypothesized to be affected by food preferences and the perceived strength of food sensory experiences that, in turn, can be strongly modulated by genetic variations in candidate genes (6).

Fat taste or oleogustus has recently been identified as the sixth basic taste modality (35, 77) in addition to the five traditional modalities of sweet, bitter, sour, salty and umami. The taste system can detect the presence of minor amounts of free fatty acids naturally occurring in fatty foods as well as fatty acids released by the hydrolytic action of lingual lipases upon triacylglycerols, which are the main lipid component of oils and fatty foods (52, 53). Oral perception of long-chain fatty acids (LCFAs) has been attributed to the texture of lipids, and to a lesser extent to their associated odors (78, 79). On the other hand, humans and rodent studies demonstrate detection of fat sensation when textural and odor cues are masked (15). Such

findings suggest the presence of a specific detection system to dietary fat, which is likely sensitive to the fatty acid breakdown products of triglycerides (51). Fatty acid translocase *CD36* is a plausible candidate for detecting long-chain fatty acids (LCFAs) since inactivation of the *CD36* gene in rat models led to a lack of sensitivity to LCFAs without impacting detection of sweet or bitter taste (55). *CD36* is a heavily glycosylated 88kD transmembrane protein that has demonstrated high affinity uptake of long-chain LCFAs across cell membranes (57, 80). This protein has broad functions in immunity, inflammation and lipoprotein metabolism and is widely expressed in multiple cell types, including adipocytes, skeletal muscle cells, monocytes and macrophages (58-60).

Common single nucleotide polymorphisms (SNPs) within the *CD36* gene have been associated with variations in fatty acid taste sensitivity (19, 62), lipid and glucose metabolism as well as risks for cardiovascular diseases and metabolic disorders (6, 20-22, 25, 26, 67). For example, an increased risk of metabolic syndrome was observed among Puerto Rican adults who were homozygous (TT) for rs3211931 (25). The rs1527483 genotypes are associated with oral fat taste sensitivity as African American and Malaysian subjects who carried the CT or TT genotypes perceived greater fat content in salad dressings regardless of actual fat concentration and also gave high rankings of added fat (19, 62). While a consistent body of evidence supports a role of *CD36* in fat taste detection, excess adiposity has been shown to weaken the sense of taste due to a decrease in numbers of taste buds caused by systematic inflammation (27). Oleic acid taste thresholds of lean subjects were found to vary according to their intake of diets that differed in fatty acid percentage (28, 29); however, such relationships were not noted among obese individuals. Hence, it is conceivable that obese individuals might have a reduced ability to detect fatty acids at both the oral cavity and gastrointestinal tract, which could lead to an impaired satiety response and excessive fat intake (30).

Few studies have assessed associations between SNPs in *CD36* and dietary fat consumption while considering BMI status. The objective of this study was to investigate the associations of nine common *CD36* SNPs with habitual dietary fat consumption, fat preferences, and biomarkers of diet-related chronic diseases in a Quebec population cohort of normal weight and OW/obese adults. We hypothesized that *CD36* SNP carriers would be associated with higher fat consumption and that these associations would be less pronouced amongst overweight/obese individuals.

3.2 Subjects and Methods

3.2.1 Study population

Existing data from the CARTaGENE (CaG) biobank (www.cartagene.qc.ca) was utilized, which is a Quebec population-based cohort comprised of 43,004 adults aged 40-69 years old from regions of Québec province: Gatineau, Saguenay, Sherbrooke, Québec City, Trois-Rivieres and the Greater Montreal Area (81). The study participants were broadly representative of middle-aged adults in Québec, who are most at risk of developing chronic diseases. Overall concordance in sociodemographic variables between CaG participants and 2006 Canadian Census data for Québec was observed, with the exception that CaG participants were more highly educated (81). Sociodemographic, lifestyle, medical, physiological, measured anthropometric and biological data were collected between 2009-2015 and dietary assessment was conducted in 2012 with the use of the Canadian-adapted Diet History Questionnaire II (DHQ II). Genome-wide genotyping was conducted on a subset of the participants (n=12,065) using the UK Biobank Axiom Array, Illumina Omni and Illumina Infinium Global Screening Array from DNA extracted from blood samples (82). For the present analyses, only participants with genomic data for *CD36* variants were included in. This study was approved by the CaG Sample and Data Access Committee (SDAC) and ethics approval

was obtained from the Faculty of Agriculture and Environmental Sciences Research Ethics Board at McGill University (REB #: 488-0518).

3.2.2 Anthropometrics, Physical Activity, and Chronic Disease Risk Assessment

Height, weight and waist circumference were measured and BMI was calculated by dividing the body weight in kilograms by height in meters squared. We identified under/normal weight as the BMI < 25 kg/m^2 and overweight/obesity as the BMI ≥ 25 kg/m^2 (83). Physical activity data was measured through use of the short and long forms of the International Physical Activity Questionnaire (IPAQ), and was expressed as metabolic equivalent task minutes per week (MET-minutes/week). Both forms measured the specific types of activities undertaken within the domains of leisure time physical activity, domestic and gardening activities, work-related physical activity and transport-related physical activity. One MET equals to 1 kcal expended per kilogram of body weight per hour (84). Physical activity level (PAL) was calculated as the result of total energy intake (TEE) divided by basal metabolic requirement (BMR). A subset of CaG participants had body composition assessment via electrical impedance, which was used to calculate BMR. However, among participants who did not undergo electrical impedance, BMR was calculated from Mifflin-St Jeor equation with participants' height, weight and age. TEE was assessed with the uses of BMR, MET and participants' weight.

Individual chronic disease risk was assessed with the use of two measures: metabolic syndrome (MetS) and Framingham Risk Score (FRS). Individual components of these measures were also evaluated as a subanalysis that included total cholesterol, serum glucose, triglycerides, HDL-cholesterol, waist circumference, systolic blood pressure, and diastolic blood pressure. The presence of metabolic syndrome was considered as a dichotomous trait based on the definition of MetS from the National Cholesterol Education Program Adult Treatment Panel

III, which requires having at least three of the following criteria: waist circumference > 102 cm for men or > 88 cm for women, triglycerides ≥ 150 mg/dl, HDL-C < 40 mg/dl for men and < 50 mg/dl for women, high blood pressure (systolic ≥ 130 mm Hg or diastolic ≥ 85 mm Hg), fasting glucose ≥ 110 mg/dl (85). Drug treatment for elevated blood triglycerides, blood pressure, blood glucose and low HDL-C were each considered positive for individual components of MetS. FRS was calculated according to adapted FRS worksheet from Canadian Cardiovascular Society and provides an estimate of an individual's 10-year risk of developing cardiovascular disease (86).

3.2.3 Food Frequency Questionnaire (FFQ)

The Canadian adaptation of the DHQ II is a modification of the US National Institutes of Health DHQ II to better reflect the Canadian food market and fortification standards. Among our investigative cohort, this instrument was used to assess habitual dietary intake of food and beverages over the previous 12 months. Information on both frequency of intake and serving size was obtained. The DHQ II comprised of 164 food and beverage items, 12 vitamins, 7 minerals and dietary supplement items. Dietary fat intake was presented according to the specific types of dietary fat including energy from total fat intake, energy from total saturated fatty acids (SFA), energy from total monounsaturated fatty acids (MUFA), energy from total polyunsaturated fatty acids (PUFA). Similar to previous research examining the associations between variations in *CD36* gene and fat preferences (19), four food groups were created to capture the type of foods consumed that contribute to a large amount of fat in the diet including added fats and oils, MUFA- and PUFA-rich foods, high-fat foods, and desserts. A full list of specific items included in each food group are presented in **Supplementary Table S1**. The food items included high fat foods (>15 g fat/100 g serving) and foods were separated according to fat type, texture, and flavour. For example, fats in sweet-flavoured foods ("hidden fats") were

grouped as desserts to disentangle confounding with sweet taste. Fats present in a solid food form (e.g., sausages, bacon) or added as a liquid/semi-solid (e.g., oils, butter) were grouped separately to account for textural effects. Foods containing unsaturated fats associated with health benefits such as nuts and seeds, avocado and fatty fish were grouped in a separate category. To our knowledge, this is first time that MUFA and PUFA consumption is considered in terms of assessment of fat preference. Each food item DHQ II consumption response was first converted into daily servings based on the information on consumption frequency and portion size. The scores for the individual food items were then added into their respective food group category and summed to create a continuous score that served as a proxy for fat preference (i.e., a higher score indicating higher habitual consumption).

3.2.4 Genotyping

Genome-wide genotyping was performed using a combination of the UK Biobank Axiom Array, Illumina Omni as well as Illumina Infinium Global Screening Array. Ten CD36 variants were available from the genome-wide assays; however, due to a limited sample size for one variant (rs3211938), nine *CD36* SNPs were assessed in the present analysis. Genotyping data was extracted using PLINK 1.7 software. Orthogonal contrast tests were conducted to test for the mode of inheritance. When sample size permitted, no assumption regarding mode of inheritance was made and three genotype groups were treated independently for analyses. Among SNPs with a minor allele frequency (MAF) < 0.10, heterozygous and minor allele homozygous subjects were combined into one group (minor allele carriers) in order to preserve statistical power for analyses.

3.2.5 Statistical analyses

Statistical analyses were conducted using SAS software (version 9.4, SAS Institute, Cary, North Carolina, USA). The Chi-square test with one degree of freedom was used to evaluate Hardy-Weinberg equilibrium (HWE) among the genetic variants of interest. Implausible values of continuous variables were excluded (BMI > 52 (n=5); energy from total fat < 10% of daily energy intake (n=60); triglycerides > 20 mmol/L (n=1); glucose > 23 mmol/L (n=2); waist circumference < 40.5 cm (n=2)). A crude method described by Willet (87) and used by others (88) was applied to exclude participants with extreme self-reported energy intakes that were considered implausible. Female participants who reported less than an average of 500 kcal/d or greater than 3500 kcal/d were excluded. Male participants who reported less than an average of 800 kcal/d or greater than 4200 kcal/d were excluded.

Sample means and frequencies for subject characteristics of the CaG participants were evaluated with t-tests and Pearson's chi-square tests. Quantitative outcome measures of interest were tested for normality and variables that did not conform to a normal distribution were transformed. Since adiposity has been shown to affect sense of taste among obese individuals (27) all analyses were stratified by BMI. General linear models were used for quantitative traits and analysis of covariance (ANCOVA) were performed to determine differences in dietary fat intake between genotypes adjusting for age, gender, alcohol intake, income status, education, smoking status, physical activity, total sugar intake, sodium intake, total energy intake and BMI (Model 1). Since dietary fat intake was our primary outcome variable of interest, subsequent statistical models evaluating food group consumption and chronic disease risk were only conducted for SNPs that were observed to have a statistically significant relationship in Model 1. Model 2 was used to determine differences in consumption of the high fat food group categories (as a proxy for fat preference) between genotypes adjusted for variables in model 1 without total energy intake. Model 3 was used to compare the Framingham risk score (FRS), its individual components, and individual metabolic syndrome components among genotype

groups adjusting for alcohol intake, income status, smoking status, education, physical activity and BMI. Since blood triglycerides have been shown to influence HDL-C concentrations (89), the HDL-C-associated analyses were adjusted for triglyceride concentrations. Lastly, logistic regression models were used to examine the associations between SNPs and the prevalence of MetS (as a dichotomous trait) adjusting for age and gender.

Significant differences as determined by the general linear model were analyzed with a post-hoc Tukey test in order to determine which genotypes significantly differed from each other. The error term of the ANOVA model was checked for normality to assure the distribution assumptions were met. All reported p-values are two-sided and the alpha level for statistical significance was 0.05.

3.3 Results

The MAF of the 9 *CD36* SNPs ranged from 0.055 to 0.519 (**Table 1**). All SNPs were in Hardy-Weinberg Equilibrium (all $P > 0.5$, data not shown). Demographic and clinical characteristics are shown in **Table 2**. Participants were approximately 55 years old on average with 44% males and mean BMI of 27.3 ± 5.1 kg/m^2. Approximately 75% of males and 55% of females were in the overweight and obese BMI range. While mean daily energy intake was higher among the OW/OB group compared to under/normal weight (UW/NW) individuals (1775 ± 643 vs. 1854 ± 705, $P<0.001$), there were no significant differences in dietary fat intakes between the two BMI groups. Allele frequencies and associations between *CD36* variants and BMI are presented in **Table 3**. Rs1054516 minor allele homozygotes (GG) had a higher BMI compared to major allele homozygotes (AA) (22.7 ± 0.1 vs. 22.4 ± 0.1, $P=0.0228$).

Significant associations between *CD36* variants and dietary fat intake were observed for six of the nine SNPs, with associations differing between BMI categories (**Table 4**). Among UW/NW individuals, minor allele homozygotes (CC) of rs1049654 consumed more energy

from total fat, MUFA and PUFA compared to major allele homozygotes (AA) (34.8% vs. 30.5%, *P*=0.0105; 13.9% vs. 12.0%, *P*=0.0247; 6.8% vs. 5.3%, *P*=0.0066, respectively). Minor allele homozygotes (GG) and heterozygotes (AG) of SNP rs10499859 had higher consumption of PUFA than non-carriers (AA) (5.9% vs. 5.0%, *P*=0.0291, 5.8% vs. 5.0%, *P*=0.0243). Minor allele carriers of rs1527483 (AA + AG) consumed more energy from SFA than non-carriers (GG) (11.8% vs. 10.7%, *P*=0.0278) and also had higher consumption (servings/day) of high fat foods (1.9 vs. 1.4, *P*=0.0210) and desserts (1.3 vs. 1.1, *P*=0.0209) compared to non-carriers (GG) (**Table 5**). Minor allele carriers of rs3211956 (CA + CC) consumed less energy from PUFA than non-carriers (AA) (5.3% vs. 6.3%, *P*=0.0466) and also had higher intake of high fat foods (1.8 vs. 1.4, *P*=0.0184) compared to non-carriers (AA). A subanalysis was conducted among genotypes that had significant associations with MUFA and PUFA to examine intakes of n-6 and n-3 fatty acids. There is increasing intake of n-6 PUFA with the number of minor allele presents. The CC genotype group of rs1049654 had significantly higher intake of n-6 PUFA than AA (3.1 vs. 2.8, *P*=0.0060). For rs10499859, carriers of the minor allele had significantly higher intake of n-6 and n-3 fatty acids than AA (3.0 vs. 2.8, *P*=0.0072; 1.4 vs. 1.3, *P*=0.0042).

Amongst OW/OB individuals, rs1054516 minor allele homozygotes (GG) consumed less saturated fat than heterozygotes (AG) (10.1 vs. 10.4%, *P*=0.0185), and minor allele carriers (GA + GG) of rs3173798 consumed more saturated fat than non-carriers (AA) (10.6% vs. 10.2%, *P*=0.0223). The SNPs that had significant associations with dietary outcomes were further examined for associations with FRS and MetS, but no significant associations were observed (**Table 6**). One significant association was observed between the SNP rs1054516 and an individual component of MetS among OW/OB subjects as heterozygotes (AG) of the minor allele had higher triglycerides concentrations (mmol/L) than non-carriers (AA) (0.23 vs. 0.19, *P*=0.0042) (**Figure 3.1**). This association remained statistically significant in a sensitivity

analysis where AG and GG genotypes were grouped together, with minor allele carriers (AG + GG) having higher triglycerides concentrations than non-carriers (AA) (0.22 vs. 0.19, *P*=0.0065).

3.4 Discussion

Our findings suggest that associations between *CD36* variants and dietary outcomes differ according to BMI status and type of dietary fat. Among UW/NW individuals, four SNPs in the *CD36* gene (rs1049654, rs10499859, rs1527483, rs3211956) were associated with dietary outcomes, while two different SNPs (rs1054516, rs3173798) were associated with dietary outcomes among OW/OB individuals. It is noteworthy that most of the associations were seen with the UW/NW group. Obesity is associated with inflammation and has been demonstrated to negatively impact the abundance and renewal of taste buds (27). It is thus possible that the inflammatory effect of obesity can result in blunted fat taste sensitivity mechanisms of certain SNPs in the *CD36* gene. It is conceivable that the attenuated fat taste sensitivity could result in higher fat consumption from the delayed satiety response.

Among UW/NW individuals, those who had the CC genotype at rs1049654 consumed more total fat as well as health promoting MUFA and PUFA than the AA genotypes. The higher PUFA intake with the rs1049654 CC genotype is noteworthy as this coincides with the positive association of rs1049654 with HDL-C concentrations (24) as omega-3s PUFA intake can increase HDL-C (90). Love-Gregory *et al.* showed that the magnitude of the increase in HDL-C in African Americans was positively associated with the number of minor alleles present (24). While we did not observe a significantly higher omega-3 intake for CC genotype in our subanalysis, FFQs tend to lack precision to accurately measure intake of specific fatty acids contibuting to MUFA and PUFA (91). In terms of rs10499859, individuals who had AG or GG genotypes consumed more PUFA than AA individuals. The minor allele of rs10499859 has

also been associated with HDL-C concentrations in Love-Gregory *et al.* (24) and our result is in accordance with the previous finding as well due to the positive impact of omega-3s PUFA on HDL-C concentrations. At rs1527483, minor allele carriers (AA + AG) consumed more SFA than GG individuals. Similarly, the AA + AG group had higher consumption of high fat foods and desserts than GG genotypes. As higher fat taste sensitivity would be assumed to be associated with decreased fat intake, the above findings are apparently contradictory to observations that the minor allele of rs1527483 is positively associated with oral fat perception. Studies performed by Keller *et al.* and Ong *et al.*, respectively, showed minor allele carriers of rs1527483 perceived greater fat content in salad dressings (19, 62) and cream crackers (19, 62) than subjects homozygous for the major allele, regardless of actual fat concentration of the food items. In the present study, however, the presence of confounding factors such as sweet and salt taste modalities in the tested food items that could have masked the perceived fat content in high fat foods and desserts. Moreover, our investigative cohort was comprised predominantly of Caucasians, while Keller *et al.* and Ong *et al.* studies involved African American and Malaysian individuals, respectively. Accordingly, further studies are needed to assess how the combination of taste modalities present in foods could affect the sensory perception of fat taste among *CD36* genotypes.

Distinct to OW/OB individuals, differences in SFA intake were seen according to rs1054516 and rs3173798 genotypes. The minor allele of rs3173798 has been inconsistently associated with chronic disease risk factors (24, 92, 93). The present investigation is the first to demonstrate the association of the above SNP with SFA consumption. It is conceivable that the inconsistent association among previous studies is the lack of consideration of the dietary and BMI status; however, this aspect requires further study. Heterozygotes (AG) of rs1054516 had the highest intake of SFA, which was significantly greater than that of minor allele homozygotes (GG). In a sensitivity analysis where mutation carriers (AG + GG) were combined,

the significant association was attenuated (data not shown). Although the above results are indicative of a partial dominance mode of inheritance, minor allele carriers of this SNP (AG + GG) had higher serum triglycerides than the AA group, which may be linked to excessive dietary SFA intake. It is possible that misreporting of dietary fat intake affected our analysis of rs1054516 and SFA intake and so contribute to a null result in our mutation carrier (AG + GG) sensitivity analysis. Previous studies have shown that the minor allele of rs1054516 was associated significantly with biomarkers of chronic disease, such as lower serum HDL-C (24, 94). The UW/NW subjects who were homozygous for the minor allele of this SNP had a higher BMI compared to major allele homozygotes. Altogether, the present findings replicate the association of rs1054516 with chronic disease risk factors, but also extend previous evidence to suggest that the mutation may impact serum biomarkers by influencing intake of SFA.

It is interesting to note that different SNPs in *CD36* associate with consumption of different types of dietary fat and the observed patterns were consistent within BMI groups. For example, among UW/NW individuals, rs1049654 and rs10499859 were associated with increased intake of MUFA and PUFA, which have known health benefits. On the other hand, rs1527483 and rs3211956 among UW/NW individuals were associated with higher SFA and lower PUFA intake, respectively. Additionally, the latter two SNPs were associated with increased consumption of food groups with a high fat content. Among OW/OB individuals, rs3173798 and rs1054516 were associated with increased SFA intake. Thus, it appears that different *CD36* SNPs modify preferences for different types of dietary fat and that excess adiposity may dampen preferences for dietary MUFA and PUFA with beneficial metabolic properties.

The present study has a number of limitations. The study population used was comprised of highly educated individuals who are mostly Caucasian and so the results cannot be generalized to other population groups. Secondly, dietary fat intake was measured via a self-

reported FFQ, which is prone to measurement error particularly among OW/OB individuals (95). Moreover, consumption frequency of food groups with a high fat content was used as a proxy for fat preference, but fat taste threshold testing would more conclusively illustrate the relationship between oral fat perception and fat preferences. The use of BMI may have misclassified some participants as OW/OB, however, we repeated our analyses using waist circumference to classify OW/OB status and results were not materially altered (data not shown). In addition, the results were not corrected for multiple testing due to the exploratory nature of the investigation although several of the study findings did align with the results from previous investigations.

3.5 Conclusions

This study adds to a body of evidence that implicates *CD36* variants in dietary fat consumption, fat preferences and subsequent biomarkers of chronic disease. Strengths of the investigation included the availability of several *CD36* variants, assessment of both diet and blood biomarkers, and sufficient sample size to stratify analyses by BMI status. The study results extend upon previous findings to indicate that: (a) associations of *CD36* variants with dietary fat consumption differ according to BMI status; and (b) associations of certain *CD36* variants with biomarkers of chronic disease can be linked to dietary fat intake. The *CD36* gene can thus be a potential genetic marker of fat preference, with different SNPs affecting preferences for different types of dietary fat. The variants within this gene may thus have utility as potential intervention targets to attenuate obesity risk and related morbidity as related to dietary fat intake.

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DISCLOSURE

The authors declared no conflict of interest.

Table 3.1: Characteristics of 9 common *CD36* SNPs

SNP ID	NCBI dbSNP	Position	Major/minor allele	MAF	Location
1	rs1049654	80275455	A/C	0.513	5'utr
2	rs10499859	80258810	A/G	0.511	5'utr
3	rs1054516	80284942	A/G	0.519	Intron
4	rs1527483	80301500	G/A	0.089	Intron
5	rs2232169	172165757	G/C	0.026	7q11.2
6	rs3173798	80285850	A/G	0.080	Intron
7	rs3211908	80293916	G/A	0.055	Intron
8	rs3211931	80298173	G/A	0.463	Intron
9	rs3211956	80303762	A/C	0.087	Intron

Table 3.2: Sample characteristics for 5152 participants

Sample Characteristic	UW/NW (n=1872)	OW/OB (n=3280)	*P*-value
Age (years)	53.8 ± 7.5	55.7 ± 7.7	**< 0.0001**
Male (%)	30.4	51.1	**< 0.0001**
BMI (kg/m^2)	22.6 ± 1.7	30.0 ± 4.4	**< 0.0001**
Waist circumference (cm)	80.3 ± 7.6	99.8 ± 11.8	**< 0.0001**
Serum glucose (mmol/L)	5.4 ± 1.2	5.8 ± 1.8	**< 0.0001**
Total cholesterol (mmol/L)	5.1 ± 0.9	5.1 ± 1.0	0.1531
LDL-C (mmol/L)	3.0 ± 0.8	3.0 ± 0.9	0.7730
HDL-C (mmol/L)	1.5 ± 0.4	1.2 ± 0.4	**< 0.0001**
Triglycerides (mmol/L)	1.4 ± 0.8	2.0 ± 1.3	**< 0.0001**
TC/HDL-C	3.7 ± 1.2	4.7 ± 1.5	**<0.0001**
Systolic blood pressure (mm Hg)	120 ± 15.3	127 ± 15.1	**< 0.0001**
Diastolic blood pressure (mm Hg)	70 ± 9.4	76 ± 9.7	**< 0.0001**
Energy intake (kcal/day)	1776 ± 643.2	1855 ± 705.6	**< 0.0001**
Total fat (%kcal/day)	32 ± 6.9	33 ± 6.8	0.5265
SFA (%kcal/day)	10 ± 2.9	11 ± 2.9	0.2003
MUFA (%kcal/day)	13 ± 3.2	13 ± 3.2	0.1231
PUFA (%kcal/day)	6 ± 2.0	6 ± 1.8	0.1697
Smoking			
Never	836	1341	**< 0.0001**
Past/Occasional	792	1610	

Daily	240	326	
Alcohol Use			
Monthly	550	1196	**< 0.0001**
Weekly or more	1248	1967	
Education			
High school or less	324	795	**< 0.0001**
Technical school or college	598	1070	
University and graduate studies	947	1411	

UW/NW, underweight/normal weight; OW/OB, overweight/obese; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Data are mean ± SD or mean (n%)

Sample sizes varied due to missing data, actual sample sizes are as follows: sex (n=2246 men, 2296 women), waist circumference (n=1857 UW/NW, 3252 OW/OB), serum glucose (n=1831 UW/NW, 3218 OW/OB), total cholesterol (n=1863 UW/NW, 3263 OW/OB), LDL-cholesterol (n=1851 NW, 3149 OW/OB), HDL-cholesterol, triglycerides and TC/HDL (n=1863 UW/NW, 3262 OW/OB), systolic and diastolic blood pressure (n=1871 UW/NW, 3274 OW/OB), smoking (n=1868 UW/NW, 3277 OW/OB), alcohol use (n=1798 UW/NW, 3163 OW/OB) and education (n=1869 UW/NW, 3276 OW/OB).

Table 3.3: Associations between *CD36* SNPs and BMI

		UW/NW		OW/OB	
CD36 SNP	Genotype	Number of subjects (*n*)	BMI (kg/m^2)	Number of subjects (*n*)	BMI (kg/m^2)
rs1527483	GG	170	22.7 ± 0.1	290	29.6 ± 0.2
	GA + AA	36	22.8 ± 0.3	69	29.9 ± 0.5
	P-value		0.9056		0.5467
rs1054516	AA	326	22.4 ± 0.1^{a}	636	30.1 ± 0.2
	GA	765	22.6 ± 0.1^{ab}	1360	30.0 ± 0.1
	GG	402	22.8 ± 0.1^{b}	711	30.3 ± 0.2
	P-value		0.0228		0.3876
rs10499859	AA	65	23.1 ± 0.2	79	30.3 ± 0.5
	GA	106	22.6 ± 0.2	195	29.5 ± 0.3
	GG	47	22.5 ± 0.3	100	29.3 ± 0.4
	P-value		0.1058		0.1186
rs1049654	AA	42	22.2 ± 0.3	54	30.6 ± 0.6
	AC	78	22.6 ± 0.2	100	30.0 ± 0.5
	CC	44	22.7 ± 0.3	44	29.5 ± 0.7
	P-value		0.3257		0.4770
rs2232169	GG	209	22.7 ± 0.1	356	29.2 ± 1.0
	GC + CC	10	23.3 ± 0.6	18	29.6 ± 0.2
	P-value		0.3289		0.7293
rs3173798	AA	1414	22.6 ± 0.1	2451	30.0 ± 0.2
	GA + GG	241	22.6 ± 0.1	452	30.1 ± 0.1
	P-value		0.9651		0.7750
rs3211908	GG	1670	22.6 ± 0.04	2912	30.0 ± 0.1
	AG + AA	201	22.5 ± 0.1	365	30.1 ± 0.2
	P-value		0.5007		0.5323
rs3211931	GG	531	22.6 ± 0.1	951	30.3 ± 0.1
	AG	917	22.7 ± 0.1	1635	29.9 ± 0.1
	AA	422	22.5 ± 0.1	690	29.9 ± 0.2
	P-value		0.4246		0.1337
rs3211956	AA	136	22.4 ± 0.2	169	30.1 ± 0.4
	CA + CC	28	23.1 ± 0.3	28	29.7 ± 0.9
	P-value		0.0539		0.7317

Data are mean ± SD

Table 3.4: Associations between *CD36* SNPs and dietary fat intake

	Total Fat (%/kcal/day)		SFA (%/kcal/day)		MUFA (%/kcal/day)		PUFA (%/kcal/day)	
	UW/NW	OW/OB	UW/NW	OW/OB	UW/NW	OW/OB	UW/NW	OW/OB
SNP ID								
rs1049654								
AA	30.5±1.3[a]	31.3±1.2	10.6±0.6	10.3±0.6	12.0±0.6[a]	12.7±0.6	5.3±0.4[a]	5.5±0.3
AC	33.2±1.1[ab]	31.6±1.2	10.4±0.5	10.1±0.6	13.4±0.5[ab]	12.7±0.6	6.5±0.4[b]	6.0±0.3
CC	34.8±1.3[b]	31.3±1.2	11.0±0.6	9.7±0.6	13.9±0.6[b]	12.8±0.6	6.8±0.4[b]	6.1±0.3
P-value	**0.0132**	0.9626	0.5749	0.5287	**0.0219**	0.9621	**0.0039**	0.1882
rs10499859								
AA	31.2±1.0	33.0±1.0	11.2±0.5	10.3±0.5	12.3±0.5	13.5±0.4	5.0±0.3[a]	6.2±0.3
AG	31.9±0.9	33.6±0.8	10.6±0.5	11.0±0.4	12.8±0.4	13.5±0.4	5.8±0.3[b]	6.1±0.2
GG	31.8±1.0	33.9±0.9	10.6±0.5	10.9±0.5	12.6±0.5	13.7±0.4	5.9±0.3[b]	6.3±0.3
P-value	0.7255	0.5781	0.3932	0.2459	0.5533	0.8414	**0.0128**	0.7967
rs1054516								
AA	31.6±0.5	31.7±0.3	10.1±0.2	10.2±0.2[ab]	12.6±0.2	12.8±0.2	6.1±0.1	5.8±0.1
AG	31.7±0.4	31.9±0.3	10.2±0.2	10.4±0.1[a]	12.7±0.2	12.9±0.1	6.0±0.1	5.8±0.1
GG	32.2±0.4	31.5±0.3	10.3±0.2	10.1±0.2[b]	12.9±0.2	12.8±0.2	6.0±0.1	5.9±0.1
P-value	0.3344	0.2873	0.5017	**0.0229**	0.1967	0.7449	0.7383	0.7934
rs1527483								
GG	31.5±0.8	32.5±1.0	10.7±0.4	10.8±0.4	12.5±0.4	13.3±0.4	5.6±0.3	6.2±0.2
GA + AA	33.3±1.2	32.4±0.8	11.8±0.6	10.3±0.5	13.0±0.6	13.5±0.4	5.6±0.4	6.1±0.3
P-value	0.0763	0.2452	**0.0278**	0.2590	0.2464	0.6724	0.8708	0.5916

rs2232169								
GG	31.7±0.8	33.5±0.8	10.8±0.4	10.8±0.4	12.5±0.4	13.5±0.3	5.6±0.3	6.2±0.2
GC + CC	31.9±1.9	34.9±1.5	10.3±1.0	11.9±0.8	13.1±1.0	13.9±0.7	5.6±0.6	6.1±0.4
P-value	0.8805	0.3423	0.6077	0.1560	0.5495	0.5522	0.9895	0.9003
rs3173798								
AA	32.0±0.3	31.8±0.3	10.3±0.2	10.2±0.1	12.8±0.2	12.9±0.1	6.0±0.1	5.9±0.1
GA + GG	31.3±0.5	32.0±0.4	10.1±0.2	10.6±0.2	12.5±0.2	12.8±0.2	6.0±0.2	5.8±0.1
P-value	0.1463	0.4891	0.2267	**0.0223**	0.2132	0.6937	0.7057	0.4715
rs3211908								
GG	31.9±0.3	32.0±0.3	10.3±0.1	10.3±0.1	12.8±0.1	13.0±0.1	6.0±0.1	5.9±0.1
GA + AA	31.3±0.5	31.9±0.4	10.3±0.2	10.5±0.2	12.4±0.2	12.8±0.2	5.8±0.2	5.8±0.1
P-value	0.1882	0.7699	0.9128	0.2162	0.0700	0.3105	0.2093	0.1689
rs3211931								
GG	31.9±0.4	32.0±0.3	10.3±0.2	10.3±0.1	12.8±0.2	13.0±0.1	5.9±0.1	6.0±0.1
GA	32.0±0.3	32.0±0.3	10.3±0.2	10.4±0.1	12.8±0.2	12.0±0.1	6.0±0.1	5.9±0.1
AA	31.4±0.4	31.8±0.3	10.2±0.2	10.3±0.2	12.5±0.2	12.8±0.2	5.9±0.1	5.8±0.1
P-value	0.1926	0.6912	0.6568	0.5302	0.2842	0.58	0.3115	0.2351
rs3211956								
AA	33.0±1.0	30.9±1.5	10.6±0.5	10.0±0.5	13.2±0.5	12.7±0.5	6.3±0.3	5.9±0.3
AC + CC	30.5±1.6	31.4±1.0	10.5±0.7	10.0±0.7	12.0±0.8	12.5±0.7	5.3±0.5	5.7±0.4
P-value	0.0834	0.6975	0.8757	0.9445	0.1069	0.7426	**0.0466**	0.6127

Table 3.5: Associations between *CD36* SNPs and food group consumption†

	Added fats and oils†		MUFA and PUFA†		High fat		Desserts	
	UW/NW	OW/OB	UW/NW	OW/OB	UW/NW	OW/OB	UW/NW	OW/OB
SNP ID								
rs1049654 AA AC CC	-	-	-	-	1.5±0.2 1.4±0.1 1.4±0.2	1.9±0.2 1.9±0.2 2.0±0.2	1.0±0.1 0.9±0.1 0.9±0.1	0.9±0.1 0.9±0.1 1.1±0.1
P-value	-	-	-	-	0.7390	0.7439	0.6711	0.3281
rs10499859 AA AG GG	1.3±0.4 2.3±0.8 1.5±1.0	1.1±0.3 1.0±0.2 1.1±0.3	2.4±0.4 2.4±0.4 2.1±0.4	2.4±0.5 2.0±0.3 1.6±0.5	1.4±0.2 1.5±0.2 1.6±0.2	2.0±0.2 1.9±0.1 2.1±0.1	1.1±0.1 1.1±0.1 1.2±0.1	1.2±0.1 1.2±0.1 1.2±0.1
P-value	0.3849	0.5832	0.6952	0.2686	0.6135	0.5503	0.8031	0.5135
rs1054516 AA AG GG	1.2±0.1 1.3±0.1 1.4±0.1	1.2±0.1 1.4±0.1 1.3±0.1	2.5±0.2 2.3±0.1 2.1±0.1	2.1±0.2 2.0±0.1 2.2±0.1	1.7±0.1 1.6±0.1 1.7±0.1	1.7±0.1 1.8±0.1 1.7±0.1	1.1±0.05 1.0±0.04 1.1±0.05	1.0±0.04 1.1±0.03 1.1±0.04
P-value	0.2445	0.2924	0.0738	0.3331	0.4101	0.3481	0.6877	0.3410
rs1527483 GG GA + AA	-0.1±1.9 -0.03±2.9	1.1±0.2 0.7±0.3	2.3±0.2 2.8±0.5	1.9±0.4 2.0±0.6	1.4±0.2 1.9±0.2	2.0±0.1 1.8±0.2	1.1±0.4 1.3±0.1	1.2±0.1 1.1±0.1
P-value	0.9783	0.0750	0.1458	0.9096	**0.0210**	0.2425	**0.0209**	0.1372

rs3173798 AA GA + GG	1.4±0.1 1.3±0.1	1.3±0.1 1.3±0.1	2.3±0.1 2.5±0.2	2.2±0.1 2.2±0.2	1.6±0.1 1.7±0.1	1.8±0.04 1.8±0.06	1.04±0.03 1.05±0.05	1.05±0.03 1.08±0.04
P-value	0.4217	0.4422	0.3687	0.9995	0.1667	0.6589	0.9546	0.4366
rs3211956 AA AC + CC	-	-	-	-	1.4±0.1 1.8±0.2	2.0±0.2 1.7±0.3	0.9±0.1 0.9±0.1	0.97±0.1 0.97±0.2
P-value	-	-	-	-	**0.0184**	0.2011	0.7732	0.9831

†hyphen (-) indicates no data available.

Table 3.6: Associations between *CD36* SNPs and Framingham Risk Score (FRS)

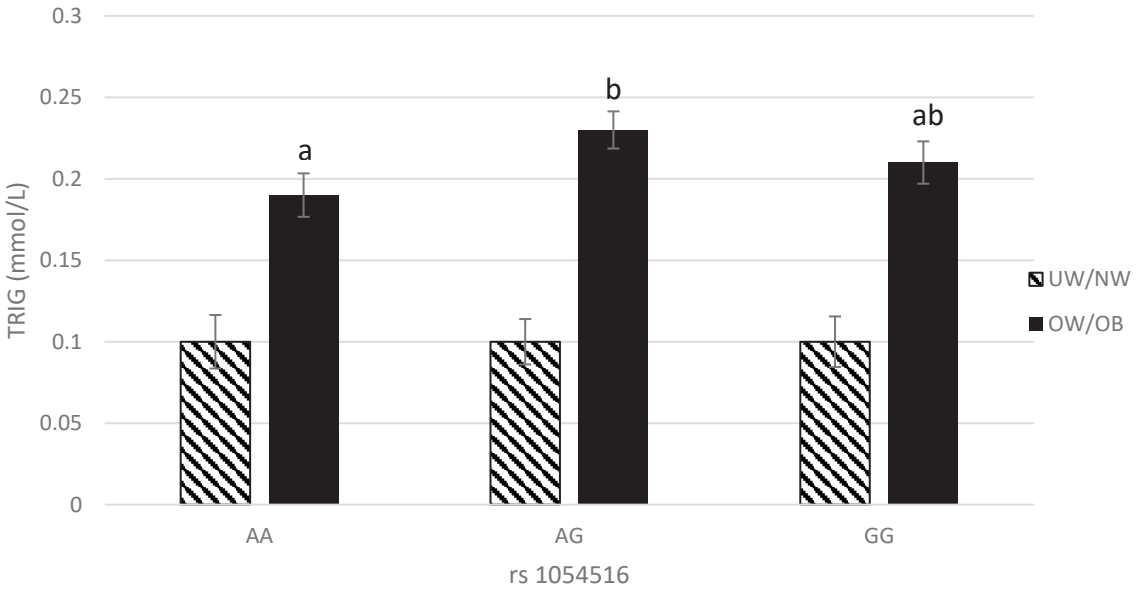
	Men		Women	
	UW/NW	OW/OB	UW/NW	OW/OB
SNP ID				
rs1049654 AA AC CC	 0.9±0.1 0.9±0.1 0.9±0.1	 1.2±0.1 1.3±0.1 1.3±0.1	 0.5±0.1 0.5±0.1 0.5±0.1	 0.9±0.1 1.0±0.1 1.1±0.1
P-value	0.8156	0.8297	0.5789	0.2569
rs10499859 AA AG GG	1.1±0.1 1.0±0.1 1.0±0.1	1.2±0.1 1.2±0.04 1.2±0.1	0.7±0.1 0.6±0.1 0.6±0.1	0.7±0.1 0.8±0.1 0.8±0.1
P-value	0.6130	0.4739	0.2566	0.4267
rs1054516 AA AG GG	1.1±0.03 1.1±0.03 1.1±0.03	1.2±0.02 1.2±0.01 1.2±0.02	0.7±0.02 0.7±0.02 0.7±0.02	0.8±0.03 0.8±0.02 0.8±0.02
P-value	0.4651	0.7814	0.7988	0.5510
rs1527483 GG GA + AA	 1.1±0.1 1.1±0.1	 1.2±0.04 1.2±0.05	 0.6±0.1 0.6±0.1	 0.7±0.04 0.7±0.06
P-value	0.4374	0.8255	0.9671	0.6017
rs3173798 AA GA + GG	 1.1±0.02 1.1±0.04	 1.2±0.01 1.2±0.02	 0.7±0.02 0.7±0.03	 0.8±0.02 0.8±0.03
P-value	0.5146	0.5037	0.1602	0.7876
rs3211956 AA AC + CC	 0.5±0.1 0.4±0.1	 1.3±0.1 1.3±0.1	 0.5±0.1 0.4±0.1	 1.0±0.1 1.0±0.1
P-value	0.2290	0.4153	0.2290	0.7999

Table 3.7: Associations between *CD36* SNPs and the presence of metabolic syndrome (MetS)

SNP	Carriers of major allele	Heterozygous for minor allele	Homozygous for minor allele	*P*-value
rs1049654	AA	AC	CC	
UW/NW	1.0	0.53 (0.15, 1.82)	1.37 (0.37, 5.05)	0.279
OW/OB	1.0	0.82 (0.36, 1.84)	0.73 (0.28, 1.87)	0.801
rs10499859	AA	AG	GG	
UW/NW	1.0	0.67 (0.22, 2.00)	1.68(0.54, 5.25)	0.283
OW/OB	1.0	0.76 (0.42, 1.38)	0.60 (0.31, 1.16)	0.315
rs1054516	AA	AG	GG	
UW/NW	1.0	1.01 (0.68, 1.50)	0.90 (0.58, 1.40)	0.807
OW/OB	1.0	1.16 (0.94, 1.42)	1.03 (0.81, 1.30)	0.289
rs1527483	GG	AG + AA		
UW/NW	1.0	1.59 (0.52, 4.89)		0.419
OW/OB	1.0	1.10 (0.61, 1.97)		0.751
rs3173798	AA	GA + GG		
UW/NW	1.0	1.24 (0.84, 1.83)		0.281
OW/OB	1.0	0.91 (0.73, 1.14)		0.413
rs3211956	AA	AC + CC		
UW/NW	1.0	0.76 (0.22, 2.68)		0.672
OW/OB	1.0	1.16 (0.46, 2.91)		0.748

Values are odds ratio (95% confidence interval)

Figure 3.1: Heterozygotes for SNP rs1054516 had higher serum triglycerides than non-carriers



Appendix

Table S1: Food groups

Added fats and oils (non-MUFA/PUFA)

- Butter
- Butter (including low-fat) on sandwich bread
- Butter (including light) on bread not in sandwiches
- Butter (including light) on bagels/English muffins
- Butter (including light) on pancakes, etc
- Butter (including light) on potatoes
- Butter (including light) used to cook vegetables during cooking
- Butter (including light) added to vegetables after cooking or at the table
- Butter used to fry/saute/baste/marinate meat, poultry or fish
- Margarine
- Margarine (including light) on sandwich bread
- Margarine (including light) on bread not in sandwiches
- Margarine (including light) on pancakes, etc
- Margarine (including light) on potatoes
- Margarine (including light) on bagels/English muffins
- Margarine (including light) used to cook vegetables during cooking &
- Margarine (including light) added to vegetables after cooking or at the table
- Margarine used to fry/saute/baste/marinate meat, poultry or fish
- Butter or margarine added to oatmeal
- Butter, margarine or oil added to rice or other cooked grains in cooking or at the table
- Butter, margarine, oil or cream sauce added to pasta, spaghetti or other noodles
- Cream/Half-and-half added to coffee or tea
- Mayonnaise
- Mayonnaise or other dressing added to Tuna
- Mayonnaise or mayonnaise-type dressing on sandwich bread
- Cream cheese
- Cream cheese (including low-fat) on bagels/English muffins
- Cream cheese (including low-fat) added to breads, rolls or flatbreads
- Whipped cream, regular
- Whipped cream, substitute
- Lard, fatback or bacon fat used to cook vegetables during cooking
- Lard, fatback or bacon fat added to vegetables after cooking or at the table
- Lard, fatback or bacon fat used to fry/saute/baste/marinate meat, poultry or fish
- Corn oil used to cook vegetables during cooking
- Corn oil used to fry/saute/baste/marinate meat, poultry or fish
- Oil spray, such as Pam or others used to cook vegetables during cooking
- Oil spray, such as Pam or others used to fry/saute/baste/marinate meat, poultry or fish
- Salad dressings
- Salad dressing on salads
- Salad dressing added to cooked vegetables after cooking or at the table

- Vegetable oil added to cooked vegetables after cooking or at the table
- Cheese sauces added to cooked vegetables after cooking or at the table
- White sauce added to cooked vegetables after cooking or at the table
- Gravy on meat, chicken, potatoes
- Sour cream
- Sour cream on potatoes

High-fat foods

- Bacon
- Ground beef in mixtures (meatballs, casseroles, chili, meatloaf)
- Steak
- Fried chicken (including deep fried) or chicken nuggets
- Hot dogs, wieners, frankfurters
- Pork/Beef spareribs
- Pork neck bones, hock, head, feet
- Beef hamburgers/cheeseburgers from a fast food or at a restaurant
- Beef jerky
- Other cold cuts/luncheon meats (bologna, salami, corned beef, pastrami)
- Sausage
- Veal, lamb
- Liver (all kinds)/Liverwurst
- Cheese (including low-fat/on cheeseburgers/in sandwiches/subs, not including cream cheese)
- Cheese/Cheese sauce on potatoes
- Potato chips (low-fat, baked, low-salt)
- French fries, home fries, hash browned potatoes, tater tots
- Poutine
- Corn/Tortilla chips (low-fat, baked, low-salt)
- Crackers
- Pretzels
- Popcorn (including low-fat)

Desserts

- Cake
- Brownies/Cookies
- Doughnuts/Sweet rolls/Danish/Pop Tarts
- Corn bread/muffins
- Sweet muffins/Dessert breads (low-fat/fat-free: banana bread, blueberry muffins, lemon loaf)
- Pies
- Frozen yogurt, Ices/Sorbet
- Ice cream/Ice cream bars (including low-fat or fat-free)
- Cheesecake
- Pudding or custard
- Jell-O, gelatin

MUFA- and PUFA-rich foods

- Shellfish, not fried
- Dark or oily salmon, fresh tuna, trout or mackerel
- White or lean fish (cod, sole, perch or pike)
- Tuna (canned, including in salads, sandwiches or casseroles)
- Fish oil/Omega-3's
- Flaxseed oil
- Flaxseeds
- Other seeds (sunflower or pumpkin seeds)
- Peanuts/Walnuts/Almonds/Other nuts
- Avocado, guacamole
- Peanut butter/Other nut butter
- Canola or rapeseed oil used to cook vegetables during cooking
- Canola or rapeseed oil used to fry/saute/baste/marinate meat, poultry or fish
- Olive oil used to cook vegetables during cooking
- Olive oil used to fry/saute/baste/marinate meat, poultry or fish

CHAPTER 4:

DISCUSSION, LIMITATIONS AND FUTURE DIRECTIONS

4.1 General Summary

In the present thesis project, existing data from the CaG biobank was utilized to evaluate associations of *CD36* SNPs with dietary fat outcomes according to BMI status. Dietary fat consumption was evaluated in two ways. First, it was presented as energy intake from the specific types of fat found in foods, including energy from total fat intake, energy from saturated fatty acids (SFA), energy from monounsaturated fatty acids (MUFA) and energy from polyunsaturated fatty acids (PUFA). Second, four food groups were created from foods that are the main contributors to fat intake: added fats and oils, MUFA and PUFA, high-fat foods and desserts. These categorizations enabled the separation of fat-containing foods according to fat type, texture, and flavour (to control for confounding from other taste modalities such as sweet) and served as a proxy measure for fat preference. Metabolic syndrome (MetS) and Framingham Risk Score (FRS), as well as individual components of these measures, were used as biomarkers to represent individual chronic disease risk.

Six *CD36* SNPs were associated with dietary fat intake, and associations differed between BMI categories. Among the under/normal weight group, four SNPs (rs1049654, rs10499859, rs1527483, rs3211956) were associated with fat consumption, while rs1054516 and rs3173798 were associated with fat consumption among overweight/obese individuals. Among under/normal weight individuals, minor allele homozygotes of rs1049654 consumed more energy from total fat, MUFA and PUFA compared to major allele homozygotes. Minor allele homozygotes and heterozygotes of rs10499859 consumed more PUFA than non-carriers. Minor allele carriers of rs1527483 consumed more energy from SFA than non-carriers. They also had a higher consumption of high-fat foods and desserts than non-carriers. Minor allele carriers of rs3211956 consumed less energy from PUFA than non-carriers, and also higher high fat food consumption. Among overweight/obese individuals, minor allele homozygotes of rs1054516 consumed less SFA than heterozygotes, whereas minor allele carriers of rs3173798

consumed more SFA than non-carriers. The SNPs that had significant associations with dietary outcomes were not associated with FRS or MetS. In a subanalysis of individual components of MetS, heterozygotes of rs1054516 had higher serum triglycerides levels than non-carriers.

Overall, it appears that different *CD36* SNPs modify preferences for different types of dietary fat and foods with high-fat content, and that excess adiposity may dampen preferences for dietary fat with beneficial metabolic properties (i.e. MUFA and PUFA).

4.2 Strengths and Limitations

This thesis presents the first study to investigate the associations between *CD36* variants and dietary fat consumption while considering BMI status. A major strength of this study is that it is closer to a real-life milieu with the detailed assessment of habitual consumption of dietary fats and actual fat-containing food items commonly consumed in the population rather than assessing single fat stimuli as is the case in controlled experimental settings. It is novel to separately measure different types of fats that are presented in diets with a food group score that mainly focuses on one category of dietary fat. In this way, it adds additional confidence to the findings such that the consumption of specific types of fats could be traced back from the diet which is likely representable by the food group scrore. Moreover, as fat-containing foods typically possess combinations of sensory properties, e.g. fat and salty or fat and sweet, targeting actual food items is an advantage to account for the potential impact coming from other modalities that are generally not considered in experimental settings evaluating taste perceptions. Other strengths included the large sample size available to evaluate the genetic associations separately according to BMI status, as well as the assessment of several *CD36* SNPs.

There are also some study limitations. Dietary intake was assessed through the use of a self-administrated FFQ that is prone to measurement error and is vulnerable to social

desirability bias, an individual's intentional misreporting of their consumption on a particular type of foods due to fears of judgement (96, 97). Moreover, the particular instrument used in CaG is quite long and detailed, which may have resulted in inaccurate reporting due to participants experiencing a response burden (97). To better address this issue, future studies may be strengthened by including doubly labelled water (DLW) as a measurement, which is used to measure total energy expenditure (97). Previous studies have confirmed that the measurement error of self-reported energy intake was reduced after using DLW (98, 99). Moreover, estimates of self-reported dietary intake have been shown to improve after total energy intake adjustment (98, 99). Accordingly, energy adjustment was applied in the present analysis to reduce measurement error.

In addition, new technologies such as computer-based questionnaires and mobile phone applications may be integrated with traditional dietary assessment methods to provide faster and more convenient services (100). Since computer-based questionnaires are more intelligent on the skip patterns and mobile applications can record and analyze consumption through digital photos and voice recordings, the response burden can be alleviated (97). However, as any new methodology requires cautious inspections, integration of such novel dietary assessment technologies also requires studies to investigate whether their implementation can improve current methods.

4.3 Future Directions

The obesity epidemic is recognized as the largest and fastest-growing public health problem that increases health-care expenditure burden, and its prevalence is rising worldwide (101). According to the data from Statistics Canada in 2018, 26.8% of Canadians aged 18 and older were classified as obese. Another 36.3% of adults were considered overweight based on their BMI. In total, 63.1% of the total population in Canada are living with increased health

risks due to excess body weight. Excessive consumption of dietary fat, the most energy-dense macronutrient, has been attributed to weight gain and obesity (75). In the Western diet, approximately 40% of dietary caloric intakes come from lipids, even though the recommendation level is 20-35% (55, 102). As fat determines the overall sensory properties of foods and contributes to the palatability of foods, many individuals find that it is difficult to manage portion control of high-fat foods (75). The findings from this investigation, as well as previous studies, provide consistent evidence of a relationship between variation in taste receptor genes and the perceived strength of sensory experiences, subsequently impacting food preferences (19).

While the present study assessed several SNPs in *CD36*, other genes may also be relevant to this relationship. Among animal and human studies, other potential receptors include *GPR40* and *GPR120,* require more further investigation. DRK channel is another potential target, but currently, few genetic studies have evaluated these targets.

Moreover, few controlled experimental studies have focused on fat taste thresholds when other combinations of taste modalities are also present in the test item, such as sweet and salty. Considering that highly palatable foods are often intertwined with sweet, salty and fatty tastes, it would be advantageous for future experimental studies to evaluate fat taste perceptions and preferences of fat-containing foods that possess other taste modalities.

The findings of this research project also contribute to the advancement of precision nutrition. As a result of the rapid development of the consumer genetic testing industry, individuals can have their personal genetic information tested and receive feedback regarding their traits and disease susceptibility at an affordable cost (103). The implication of this genetic-based information on health behaviours is of special interest to clinicians and health researchers (104, 105). A previous study suggested that individuals enjoyed learning about their genotype-based dietary recommendations and found this kind of information to be more useful and

understandable than the general population-based dietary recommendations (106). A separate study also reported positive dietary behaviour changes in response to dietary recommendations that are tailored to an individual's genetic profile compared to standard diet recommendations. Participants that carried risk alleles of a variant in the *ACE* gene (associated with salt-sensitive hypertension) and received genetic-based advice for sodium intake significantly reduced their sodium intake compared to those who received general sodium intake advice with no genetic information (107). In line with this finding, other large-scale studies utilizing research participants (i.e. Food4Me European cohort) or actual genetic testing consumers (i.e. the company 23andMe) demonstrated that genetic-based diet/lifestyle recommendations could facilitate diet and health behaviour changes (108). Taken together, it appears that a personalized genetic-based approach to nutrition recommendations is effective in motivating positive dietary changes. Since the *CD36* gene may be a potential genetic marker of fat preference, variants within this gene may be employed as a potential intervention tool to motivate dietary change and ultimately form a component of precision nutrition.

It is important to recognize that genetics plays a role in susceptibility to obesity. However, another area of research that requires attention is to investigate gene-environment interaction based on the idea that beneficial or adverse environmental exposures may modify individual genetic risk (109). A prior study found that individuals who were carriers of a gene variant involved in dopamine signalling (*Taq1A*) had a poorer dietary quality score when they were exposed to a more unhealthful food environment (110). Separately, Wang et al. showed that adherence to healthy dietary patterns (e.g. DASH diet) could counteract the genetic predisposition to weight gain over time (111). Similarly, Khera et al. demonstrated that a healthy lifestyle (including diet) could result in a ~50% reduction in relative risk of coronary artery disease among those with high genetic risk of heart disease (112). Studies such as these indicate that a substantial knowledge gap in the field of nutritional genomics is how gene-

environment interactions may influence genetic associations with diet and health outcomes. Indeed, 32 common genetic variants have been identified that associate with BMI and subsequently enable the calculation of a polygenic risk score for obesity and those with the highest genetic risk have been reported to have a mean BMI 2.7 kg/m^2 greater than those with the lowest genetic risk (113). Evaluation of how environmental exposures may modify the genetic risk to obesity is an important target for future research. Relevant to this thesis project, consideration of individual exposure to high-fat foods in the food environment would be an important next step in better understanding the genetic and environmental underpinnings of fat preferences and consumption.

4.4 Conclusion

CD36 is associated with the consumption of different types of dietary fats and food items containing high-fat content, and patterns varied according to BMI status. Findings from this project extend previous work to suggest that excess adiposity may impact *CD36* to dampen preferences for dietary fats with beneficial metabolic effects, which may have downstream impacts on biomarkers of chronic disease risk.

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