KUOPION YLIOPISTON JULKAISUJA D. LÄÄKETIEDE 462
KUOPIO UNIVERSITY PUBLICATIONS D. MEDICAL SCIENCES 462

TUOMAS KILPELÄINEN

Physical Activity, Genetic Variation, and Type 2 Diabetes

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium, Mediteknia building, University of Kuopio, on Friday 16th October 2009, at 12 noon

Institute of Biomedicine
Unit of Physiology
University of Kuopio

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KUOPIO 2009

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Professor Markku Tammi, M.D., Ph.D.
Institute of Biomedicine, Department of Anatomy

Author´s address: MRC Epidemiology Unit
Institute of Metabolic Science
Box 285
Addenbrooke's Hospital
Hills Road
Cambridge
CB2 0QQ
UNITED KINGDOM

Supervisors: Professor Timo Lakka, M.D., Ph.D.
Institute of Biomedicine
Unit of Physiology
University of Kuopio

Docent David Laaksonen, M.D., Ph.D.
Department of Medicine
University of Kuopio and Kuopio University Hospital

Professor Markku Laakso, M.D., Ph.D.
Department of Medicine
University of Kuopio and Kuopio University Hospital

Reviewers: Professor Pekka Jousilahti, M.D., Ph.D.
National Institute for Health and Welfare
Helsinki, Finland

Docent Olavi Ukkola, M.D., Ph.D.
Department of Internal Medicine
Institute of Clinical Medicine
University of Oulu

Opponent: Professor Urho Kujala
Department of Health Sciences
University of Jyväskylä

ISBN 978-951-27-1362-2
ISBN 978-951-27-1219-9 (PDF)
ISSN 1235-0303

Kopijyvä
Kuopio 2009
Finland

Kilpeläinen, Tuomas. Physical Activity, Genetic Variation, and Type 2 Diabetes. Kuopio University Publications D. Medical Sciences 462. 2009. 121 p.
ISBN 978-951-27-1362-2
ISBN 978-951-27-1219-9 (PDF)
ISSN 1235-0303

ABSTRACT

Type 2 diabetes results from the interaction between genetic predisposition and unhealthy lifestyle. Increasing physical activity may protect against the development of type 2 diabetes, but genetic factors likely influence the response to physical activity. The interactions between physical activity and genes in the development of type 2 diabetes are poorly understood. The main aim of the present study was to investigate interactions between physical activity and genes in the etiology of type 2 diabetes in the Finnish Diabetes Prevention Study (DPS).

The DPS is a multicentre randomized controlled trial on the effects of a multi-component (physical activity, diet, weight reduction) lifestyle intervention on the risk of developing type 2 diabetes among 522 overweight individuals with impaired glucose tolerance (IGT). In the DPS, changes in physical activity were a strong predictor of the risk of type 2 diabetes independent of changes in diet and body weight. Several genetic polymorphisms have also been associated with the risk of developing type 2 diabetes. For many of the genes, the polymorphisms had effects predominantly in either the intervention or control group, indicating a gene-lifestyle interaction.

In the present study, interactions between physical activity and genes were investigated by secondary analyses of the DPS data. The associations of the rs17036314 and rs1801282 (Pro12Ala) single–nucleotide polymorphisms (SNPs) in the *PPARG* gene with the progression from IGT to type 2 diabetes were modified by changes in physical activity during the intervention. Increased physical activity seemed to remove the harmful effect of the risk alleles ($P_{\text{interaction}}$=0.002 and 0.031 for rs17036314 and rs1801282, respectively). Similarly, changes in physical activity modified the associations of the rs5393, rs5394, and rs5400 SNPs in the *SLC2A2* gene and the association of the rs3758947 SNP in the *ABCC8* gene with the risk of type 2 diabetes. Physical activity attenuated the effect of the risk genotypes on the risk of developing type 2 diabetes ($P_{\text{interaction}}$=0.022-0.027 for SNPs in *SLC2A2* and $P_{\text{interaction}}$=0.008 for rs3758947). Furthermore, the rs696217 (Leu72Met) SNP in the *GHRL* gene modified the effect of physical activity on changes in body weight and waist circumference, the rs26802 SNP in the *GHRL* gene modified the effect of physical activity on changes in serum high density lipoprotein cholesterol, the rs1137100 (Lys109Arg) SNP in the *LEPR* gene modified the effect of physical activity on changes in systolic blood pressure, and the rs1800795 SNP in the *TNF* gene modified the effect of physical activity on changes in serum C-reactive protein. The beneficial effects of increased physical activity were only seen in the carriers of specific genotypes of these SNPs.

These secondary analyses of the Finnish DPS indicate that variation in genes regulating both insulin sensitivity (*PPARG*) and insulin secretion (*SLC2A2*, *ABCC8*) interact with changes in physical activity on the risk of developing type 2 diabetes. Furthermore, variation in the *LEPR* and *GHRL* genes may modify the effects of physical activity on changes in features of metabolic syndrome, and variation in the *TNF* gene may modify the effect of physical activity on changes in serum CRP levels. However, replication in independent study populations is necessary to confirm the findings.

National Library of Medicine Classification: QZ50, WD210, WK810, WK820, QU95, WG106, WE103

Medical Subject Headings: Blood Pressure/genetics; Body Weight Changes; Cholesterol, HDL/genetics; C-reactive Protein; Diabetes Mellitus, Type II/genetics; Finland/epidemiology; Gene Frequency; Genetic Predisposition to Disease; Genetic variation; Ghrelin; Glucose Transporter Type 2; Insulin/secretion; Insulin Resistance/genetics; Intervention Studies; Leptin Receptor; Lifestyle; Metabolic Syndrome X/genetics; Motor Activity; Overweight; Polymorphism, Single nucleotide; PPAR Gamma; Prospective Studies; Risk factors; Sulfonylurea Receptor; Tumor Necrosis Factor-alpha

ACKNOWLEDGEMENTS

This work was carried out in the Unit of Physiology of the Institute of Biomedicine, University of Kuopio. I owe my warmest thanks to all people who have contributed to this work. In particular, I would like to express my special thank you to:

Professor Timo Lakka, my principle supervisor, for his excellent guidance and feedback and for keeping me motivated throughout this study. I also owe Timo great thanks for leading me to exciting projects beyond this thesis.

Docent David Laaksonen, my second supervisor, for his invaluable contribution in planning the studies and statistical expertise, for his encouragement and skills in preparing the manuscripts, and for revising the English language of this thesis.

Academy professor Markku Laakso, my third supervisor, for making this study possible through the use of his genetics laboratory, and for all his crucial comments and suggestions which encouraged me to improve my work.

Professor Matti Uusitupa and professor Jaakko Tuomilehto, the principal investigators of the DPS, for the opportunity to perform the thesis within the Finnish Diabetes Prevention Study, for their essential comments in preparing the manuscripts, and for all the insightful feedback throughout the study.

Professor Pekka Jousilahti and docent Olavi Ukkola, my official reviewers, for giving constructive and valuable critique which helped me to improve this work.

The staff in the Unit of Physiology for pleasant collaboration and company during these years.

The personnel of the Physical Activity and Nutrition in Children –study, for creating such a friendly atmosphere to work in during the last two years of my work.

Docent Heikki Pekkarinen, for his help in finding this PhD project, and for pleasant collaboration in other projects.

Statistician Vesi Kiviniemi for his support in statistical issues, and bioinformatician Teemu Kuulasmaa for his help in handling of the genotype data.

Members of the Tahdistin orchestra and the Paraboloidi vocal group for sharing musical life.

My mum, dad, and all my three brothers for their support and encouragement.

And finally, to all my friends, relatives, and colleagues in Kuopio and elsewhere for their friendship and support. For their special contributions to the PhD work, in a way or another, I would particularly like to thank Atte, Otto, James, Patrick, Jenni, Yannes, and Arietta.

For the financial support of this work, I would like to thank the Ministry of Education of Finland, and the Finnish Academy.

Kuopio, October 2009

Tuomas Kilpeläinen

ABBREVIATIONS

A	adenine
ABCC8	ATP-binding cassette, sub-family C, member 8
ACE	angiotensin I converting enzyme 1
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif 9
ADRA2B	adrenergic receptor alpha-2B
ADRB2	adrenergic receptor beta-2
ADRB3	adrenergic receptor beta-3
Ala	alanine
ANOVA	analysis of variance
Arg	arginine
ATP	adenosine triphosphate
AUC	area under the curve
BMI	body mass index
bp	base pair(s)
C	cytosine
CAMK1D	calcium/calmodulin-dependent protein kinase 1D
CAP1	adenylate cyclase-associated protein 1
CDC123	cell division cycle 123 homologue
CDKAL1	CDK5 regulatory subunit-associated protein 1-like 1
CDKN2B	cyclin-dependent kinase inhibitor 2B
CI	confidence interval
COMT	catechol O-methyltransferase
CRP	C-reactive protein
CYP19	cytochrome P450, family 19, subfamily A
DNA	deoxyribonucleic acid
DPP	Diabetes Prevention Program
DPS	Diabetes Prevention Study
E	glutamic acid
ELISA	enzyme-linked immunosorbent assay
EXT2	exostoses 2
FFA	free fatty acid
FSIGT	frequently sampled intravenous glucose tolerance test
FTO	fat mass and obesity associated
G	guanine
GH	growth hormone
GHRL	ghrelin/obestatin preprohormone
Gln	glutamine
Glu	glutamic acid
GLUT2	glucose transporter isoform 2
GLUT4	glucose transporter isoform 4
Gly	glycine
GNB3	guanine nucleotide binding protein, beta polypeptide 3
HDL	high density lipoprotein
HERITAGE	Health, Risk Factors, Exercise Training, and Genetics
HHEX	haematopoietically expressed homeobox
His	histidine
HNF1B	hepatocyte nuclear factor 1 homeobox B
I/D	insertion/deletion
IDE	insulin degrading enzyme
IFG	impaired fasting glucose
IGF1R	insulin–like growth factor 1 receptor
IGF2BP2	insulin-like growth factor 2 mRNA binding protein 2
IGT	impaired glucose tolerance
IL6	interleukin-6 gene
IL-6	interleukin-6
JAZF1	juxtaposed with another zinc finger gene 1

K	lysine
K_{ATP}	ATP–sensitive potassium channel
KCNJ11	potassium inwardly rectifying channel, subfamily J, member 11
KIHD	Kuopio Ischaemic Heart Disease Risk Factor Study
Kir6.2	inwardly rectifying potassium channel
LDL	low density lipoprotein
LEP	leptin
LEPR	leptin receptor
Leu	leucine
LIPC	hepatic lipase
LPL	lipoprotein lipase
Lys	lysine
MAF	minor allele frequency
Met	metionine
MET	metabolic equivalent of oxygen consumption
NCBI	National Center for Biotechnology Information
NO	nitric oxide
NOS3	nitric oxide synthase 3 (endothelial cell)
OGTT	oral glucose tolerance test
OR	odds ratio
PCR	polymerase chain reaction
PPARG	peroxisome proliferator-activated receptor gamma gene
PPARγ	peroxisome proliferator-activated receptor gamma
Pro	proline
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RR	relative risk
S	serine
SBP	systolic blood pressure
SD	standard deviation
SLC2A2	solute carrier family 2 (facilitated glucose transporter) member 2
SLC30A8	solute carrier family 30 (zinc transporter), member 8
SNP	single nucleotide polymorphism
SSCP	single-strand conformation polymorphism
SUR1	sulfonylurea receptor 1
T	thymine
TCF7L2	transcription factor 7 like 2
THADA	thyroid adenoma associated
TNF	tumor necrosis factor alpha gene
TNF-alpha	tumor necrosis factor-alpha
TNMD	tenomodulin
Trp	tryptophan
TSPAN8	tetraspanin
Val	valine
VDR	vitamin D (1,25-dihydroxyvitamin D3) receptor
VNTR	variable number of tandem repeats
VO_2max	maximal oxygen uptake
WFS1	Wolfram syndrome 1
WHO	World Health Organization
X	undetermined amino acid

LIST OF THE ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, referred to in the text by their Roman numerals.

I Kilpeläinen TO, Lakka TA, Laaksonen DE, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne–Parikka P, Keinänen-Kiukaanniemi S, Lindi V, Tuomilehto J, Uusitupa M, Laakso M, for the Finnish Diabetes Prevention Study Group. SNPs in *PPARG* associate with type 2 diabetes and interact with physical activity. Med Sci Sport Exerc 2008;40:25-33.

II Kilpeläinen TO, Lakka TA, Laaksonen DE, Laukkanen O, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Aunola S, Ilanne–Parikka P, Keinänen–Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M, for the Finnish Diabetes Prevention Study Group. Physical activity modifies the effect of SNPs in the *SLC2A2* (GLUT2) and *ABCC8* (SUR1) genes on the risk of developing type 2 diabetes. Physiol Genomics 2007;31:264-272.

III Kilpeläinen TO, Lakka TA, Laaksonen DE, Mager U, Salopuro T, Kubaszek A, Todorova B, Laukkanen O, Lindström J, Eriksson JG, Hämäläinen H, Aunola S, Ilanne–Parikka P, Keinänen–Kiukaanniemi S, Tuomilehto J, Laakso M, Uusitupa M, for the Finnish Diabetes Prevention Study Group. Interaction of SNPs in *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR* and *GHRL* with physical activity on the risk of type 2 diabetes and changes in characteristics of the metabolic syndrome. The Finnish Diabetes Prevention Study. Metabolism 2008;57:428-436.

IV Kilpeläinen TO, Laaksonen DE, Lakka TA, Herder C, Koenig W, Lindström J, Eriksson JG, Uusitupa M, Kolb H, Laakso M, and Tuomilehto J, for the Finnish Diabetes Prevention Study Group. The rs1800795 polymorphism in the *TNF* gene interacts with physical activity on the changes in C-reactive protein levels in the Finnish Diabetes Prevention Study. Diabetes Care 2009 (submitted).

In addition, some unpublished data are presented.

TABLE OF CONTENTS

I INTRODUCTION

Type 2 diabetes is one of the severest public health problems worldwide [1]. It is a common metabolic disease with a rapidly increasing prevalence in both developed and developing countries [2]. The micro- and macrovascular complications of diabetes, such as renal failure, retinopathy, neuropathy and cardiovascular disease create a large amount of human suffering [3]. Furthermore, while type 2 diabetes was previously considered essentially a disease of middle-aged and older individuals, it is now emerging as a new serious health problem in children [4]. As the worldwide epidemic of type 2 diabetes threatens to increase the burden on health care systems dramatically worldwide, the prevention of type 2 diabetes has become a major challenge for clinicians and public health policy makers all over the world.

To prevent type 2 diabetes, it is important to gain knowledge on the factors that contribute to its development. The strong familial clustering of type 2 diabetes points to the important role of genetic mechanisms [5,6]. However, the recent rapid changes in diabetes prevalence, which could not have emerged for genetic reasons, indicate that environmental and lifestyle factors are also of major relevance [7]. Indeed, type 2 diabetes seems to develop as the result of a complex interaction between genes and lifestyle, where numerous susceptibility genes combined with an unhealthy lifestyle gradually lead to the development of manifest disease [8].

Physical inactivity is an important risk factor for type 2 diabetes, whereas increased physical activity is protective [9]. However, the magnitude of responses to regular physical activity differs considerably among individuals. With regard to any component of health-related fitness such as maximal oxygen uptake (VO_2max), blood pressure, heart rate, and high-density lipoprotein (HDL) cholesterol, there seems to be high-responders, low-responders, and even non-responders to exercise interventions in the population [10]. A large part of such inter-individual variability may be explained by genetic differences [10,11]. Individuals with specific genetic profiles are also expected to be more responsive

to the beneficial effects of physical activity in the prevention of type 2 diabetes. At present, however, it is not known which key genetic factors modify the individual responses to physical activity. Such information would be important, as it would increase understanding of the disease etiology, and might lead to the development of better therapies. Furthermore, such knowledge could make it possible to identify the individuals and groups who are at increased risk of type 2 diabetes, and to identify those individuals who have the potential to benefit most from a targeted lifestyle prevention [12].

The study of gene–physical activity interactions is challenging. Firstly, most gene variants associated with common disease have only modest effects [13,14]. Secondly, lifestyle factors such as physical activity are difficult to quantify precisely [15-17]. Thirdly, several lifestyle and environmental factors confound the analyses. Therefore, the detection of gene–physical activity interactions requires large study populations and careful measurement of lifestyle and environmental exposures.

Because of careful collection of data on lifestyle and phenotype and the genotyping of numerous genetic polymorphisms, the Finnish Diabetes Prevention Study (DPS) provides an excellent possibility to investigate interactions between genes and lifestyle in a prospective study setting. The main aim of the present study was to investigate how physical activity and genes interact in the development of type 2 diabetes in the high-risk population sample of the DPS.

II REVIEW OF THE LITERATURE

TYPE 2 DIABETES

Definition

Type 2 diabetes is a common disease, primarily characterized by an increased level of plasma glucose. In contrast to type 1 diabetes where insulin secretion from the pancreatic β–cells is lost, type 2 diabetes is the result of concomitant defects in insulin secretion and peripheral insulin sensitivity, the latter being typically associated with obesity [1,18]. Because insulin regulates glucose uptake into tissues and the release of stored fatty acids, defects in insulin action and secretion will cause chronic increase in blood glucose levels (hyperglycemia) and impaired lipid and lipoprotein metabolism (dyslipidemia) [19]. These may further impair insulin secretion and action, and in the long run, lead to severe micro- and macrovascular complications such as renal failure, neuropathy, retinopathy, and cardiovascular disease [3,20]. The avoidance of such hyperglycemia-related complications is the underlying rationale behind the diagnostic criteria of type 2 diabetes (Table 1). The diagnosis can be based on the measurement of fasting plasma glucose or the measurement of 2-hour glucose after ingestion of 75 g oral glucose (Table 1). Apart from type 2 diabetes, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are commonly defined (Table 1). Both IGT and IFG are associated with an increased risk of type 2 diabetes, and about 30% of individuals with IGT develop type 2 diabetes in 10 years [21].

Table 1. The World Health Organization (1999) diagnostic criteria of type 2 diabetes, impaired fasting glucose (IFG), and impaired glucose tolerance (IGT) [1,18].

	Fasting plasma glucose (mmol/L)		2-h plasma glucose (mmol/L)[1]
Normoglycemia	<6.1	and	<7.8
IFG	≥6.1 and <7.0	and	<7.8
IGT	<7.0	and	≥7.8 and <11.1
Diabetes	≥7.0	or	≥11.1

[1]2 hours after ingestion of 75 g oral glucose

Prevalence and environmental risk factors

Type 2 diabetes is one of the leading health problems in the developed world, and is becoming increasingly important in the developing world. The worldwide prevalence was 171 million in 2000, but the number is projected to reach 366 million by 2030 [2]. Furthermore, although most cases of type 2 diabetes are diagnosed after the age of 40, diabetes is becoming increasingly common among the younger age groups [4,22,23]. The increasing worldwide prevalence of type 2 diabetes combined with the shift in its age of onset will heavily burden health-care systems in the future.

The causes of the rapid spread of type 2 diabetes are incompletely understood. However, it is known that the genetic pool of human population changes slowly. New genetic polymorphisms fixate gradually and are estimated to become balanced in no less than 5,000 years [24]. Epigenetic modifications where changes in chromatin structure occur without changes in nucleotide sequence, can take place within a relatively short time frame and may result in altered gene expression. Nonetheless, it is still controversial how extensively epigenetic changes are transmitted to future generations [25,26]. It is thus likely that genetic changes do not substantially account for the rapid increase in the prevalence of type 2 diabetes [7], and that the main causes of the diabetes-epidemic are environmental. In particular, the epidemic of type 2 diabetes correlates with the recent explosion in the prevalence of obesity [27]. Indeed, both observational and randomized trial data indicate that overweight and obesity defined as a body mass index (BMI) of greater than 25 kg/m^2

and 30 kg/m^2, respectively, increase the risk of type 2 diabetes [27]**.** The risk rises steadily above low levels of BMI, but exponentially above a BMI of 30 kg/m^2. Women with BMI between 23 and 25 kg/m^2 have an almost three-fold increased risk of developing diabetes compared with women with a BMI below 23 kg/m^2 [28]. This relative risk increases to 20 for women with BMI ≥35 kg/m^2 [28]. In men at BMI >35 kg/m^2, the risk is around 40-fold compared with a BMI <23 kg/m^2 [29].

Sedentary lifestyle is another important risk factor for type 2 diabetes [9]. Physical inactivity increases the risk of weight gain [30], but has additional effects beyond the regulation of body weight [9]. Similarly, unhealthy diet, including low unsaturated/saturated fat intake ratio [31], high glycemic-index or glycemic load of diet [32-34], and low fiber intake [35] increases the risk. Smoking is also associated with an increased risk [36], whereas consumption of coffee [37] and moderate use of alcohol [38] are protective.

Age, gender, prior gestational diabetes or glucose intolerance, and low-birth weight especially if followed by rapid growth in childhood, are non-mofidiable risk factors for type 2 diabetes [39,40]. The risk of type 2 diabetes increases with age, and the peak age of onset is 60-70 years [22,23]. The risk is slightly higher among men than women, but because women have longer life expectancy there are more diabetic women than men in the world [41].

Pathogenesis

The current evidence suggests that obesity and unhealthy lifestyle exert their deleterious effects on glucose homeostasis mainly by increasing insulin resistance of peripheral tissues [42-45]. Visceral obesity in particular is closely related with insulin resistance, and is thought to be the core feature of the metabolic syndrome, characterized by insulin resistance, dyslipidemia, and hypertension [46-48]. The key role of visceral fat in the development of insulin resistance and related metabolic disturbances may partly be related with its high metabolic activity [43], but also its anatomical location right next to the

hepatic portal circulation is likely to be important [49]. The unhindered entry of fatty acids from visceral fat to the liver leads to an elevation in hepatic triglyceride synthesis, which decreases liver insulin sensitivity and subsequently increases hepatic glucose production [50,51]. Apart from fatty acids, adipocytes in visceral fat and elsewhere are known to excrete a multitude of hormones and cytokines (adipokines), such as adiponectin, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which may modify insulin signaling and contribute to the systemic low-grade chronic inflammation that characterizes the development of insulin resistance and type 2 diabetes [20,52-56].

Similarly with liver, increased circulating levels of fatty acids and adipokines may cause insulin resistance in the skeletal muscle [42]. Skeletal muscle has a central role in the regulation of glucose homeostasis, because it is the main tissue responsible for insulin-stimulated glucose uptake [57]. High levels of circulating fatty acids decrease muscle glucose uptake and increase fatty acid uptake [42]. This imbalance in fat and glucose uptake leads to an accumulation of intramyocellular lipid metabolites, which seems to disrupt normal insulin signaling cascade and may contribute to mitochondrial dysfunction [58-60]. Furthermore, obesity is associated with endothelial dysfunction and impaired muscle microcirculation [61-63], which may impair whole-body insulin sensitivity by hindering the entry of insulin and glucose into skeletal muscle and decreasing their availability to muscle cells [54-56,64-67].

Chronic exposure to glucose and fatty acids is detrimental to pancreatic beta-cells and may gradually lead to beta-cell failure, involving a partial loss of beta-cell mass and a deterioration of beta-cell function [20,43,68-71] Beta-cell failure is the triggering factor for the transition from an obese, insulin-resistant state to full-blown type 2 diabetes [20] Although the mechanisms of the beta-cell failure are still incompletely understood, the current evidence suggest the failure to be caused by a combined consequence of metabolic overload [70], oxidative stress [72], increased rates of beta-cell apoptosis [73], and loss of expression of key components of the insulin secretory machinery [74].

Heritability

Although lifestyle changes generally explain the recent rapid spread of type 2 diabetes, there are large differences in the individual susceptibility to develop glucose abnormalities when environmental risk factors are present. For example, not all obese people develop insulin resistance, and some insulin resistant individuals are able to tolerate insulin resistance by augmenting insulin production to overcome the increased demand. Such differences are likely due to genetic factors [75,76]. Indeed, strong evidence from both family and twin studies indicate that type 2 diabetes is strongly heritable. The risk of type 2 diabetes increases by 40% if one parent has type 2 diabetes, and by 70% if both parents are affected [5,6]. According to twin studies, 60-90% of monozygotic twin pairs become concordant for type 2 diabetes [77]. Altogether, genetic susceptibility seems to account for around half of total disease susceptibility [78].

GENETIC BACKGROUND OF TYPE 2 DIABETES

Despite the strong heritability of type 2 diabetes, the search for diabetes susceptibility genes has long been unsuccessful. The complex etiology of type 2 diabetes has been a challenge to geneticists. Type 2 diabetes is a multigenic disease where numerous genes affect the risk and many combinations of gene defects exist among diabetic patients [79]. Furthermore, the multiple genetic variants interact with various environmental factors in their effect on the risk. Indeed, type 2 diabetes has earlier been described as 'a geneticist's nightmare' [80,81].

Linkage scans and candidate gene studies

Over the past decade human geneticists relied predominantly on two approaches for gene discovery: genome-wide linkage scans and candidate gene studies [82]. In the genome-wide

linkage approach, the variation in the entire human genome is coarsely screened with selected marker variants without a priori assumptions about the importance of specific chromosomal regions or genes. The genomic regions shared between diabetic relatives more often than expected by chance are then analysed with a denser marker map, and the attractive candidate gene is localized [83]. In the candidate gene approach, the understanding of the pathophysiology of type 2 diabetes is first used to identify promising candidate genes, and variations in these genes are then tested for association with type 2 diabetes [84].

Although numerous genome-wide linkage scans and candidate gene studies on type 2 diabetes have been performed, they have produced little unequivocal evidence for common gene variants associated with type 2 diabetes. The few robust findings from these studies include the E23K variant in the potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*) gene [85-87], the Pro12Ala variant in the peroxisome proliferator-activated receptor-gamma (*PPARG2*) gene [88,89], and variants in the hepatocyte nuclear factor 1 homeobox B (*HNF1B*) [90,91] and Wolfram syndrome 1 (*WFS1*) genes [92] (Table 2). However, the vast majority of the detected associations between gene variants and type 2 diabetes have not been replicated. The main problem was that geneticists have an immense number of possible genetic variants to study, but only few are likely to be involved in type 2 diabetes. Multiple testing has led to many false positive findings [93].

Genome–wide association studies

The recent application of the genome-wide association approach in the study of disease genetics represents a revolution in the search of diabetes susceptibility genes. In genome-wide association studies, hundreds of thousands or millions of single nucleotide polymorphisms (SNPs), spread across the entire genome, are studied in a single analysis [94]. The common variation across the human genome can thus be comprehensively covered, allowing the identification of diabetes susceptibility genes at the genome-wide level. The most significant genome-wide associations are confirmed by genotyping in

independent cohorts. Genome–wide association studies are hypothesis-free and unbiased by previous theories concerning candidate genes and pathways. Therefore, they provide the opportunity to identify completely unexpected genes, broadening understanding of disease mechanisms. The genome-wide association studies are, however, limited by the modest effect sizes of common susceptibility variants and multiple testing, which lead to very large sample size requirements. On the other hand, large population samples are difficult to characterize accurately, which complicates the assessment of gene-environment interactions in such studies.

In the past two years, several large genome–wide association analyses for type 2 diabetes have been carried out within case-control studies, uncovering a number of previously unsuspected variants (Table 2). Except for the fat mass and obesity associated (*FTO*) gene which increases body weight [95], all the newly found susceptibility genes may affect insulin secretion (Table 2). At present, only one susceptibility gene, *PPARG2*, is primarily thought to affect insulin resistance [96]. Indeed, an etiological model has now been suggested where type 2 diabetes emerges when environmentally triggered insulin resistance takes place in the context of genetically programmed β–cell dysfunction [44]. However, still many more susceptibility loci have to be identified as only a small fraction of heritability can be explained by the known variants [14]. Genome-wide association studies are limited by the modest effect sizes of common susceptibility variants and multiple testing, and it has not yet been possible to detect rare susceptibility variants for type 2 diabetes [97]. Furthermore, other forms of genetic variation than SNPs, including copy number variation, micro-RNAs, and epigenetic mechanisms, may modify the risk of type 2 diabetes [14]. Future efforts are likely to be directed towards other types of genetic variation and towards rarer variants in general [13].

Table 2. Single nucleotide polymorphisms (SNPs) with confirmed association with the risk of type 2 diabetes in genome-wide association studies, large-scale association studies or robust candidate gene studies

SNP	Nearest Genes	Probable Mechanism	OR	References
rs7901695	*TCF7L2*	β–cell dysfunction	1.37	[98-101]
rs2237892	*KCNQ1*	β–cell dysfunction	1.29	[102,103]
rs10811661	*CDKN2A/2B*	β–cell dysfunction	1.20	[100,104,105]
rs8050136	*FTO*	Altered body mass index	1.17	[100,106]
rs1111875	*HHEX/IDE*	β–cell dysfunction	1.15	[100,101,101,104,105]
rs13266634	*SLC30A8*	β–cell dysfunction	1.15	[100,101,101,104,105]
rs7578597	*THADA*	Unknown	1.15	[107]
rs1801282	*PPARG2*	Insulin resistance	1.14	[89,100]
rs5215	*KCNJ11*	β–cell dysfunction	1.14	[86,100]
rs10946398	*CDKAL1*	β–cell dysfunction	1.14	[99,100,104,105]
rs4402960	*IGF2BP2*	β–cell dysfunction	1.14	[100,104,105]
rs10923931	*NOTCH2*	Unknown	1.13	[107]
rs10010131	*WFS1*	Unknown	1.12	[92,108]
rs12779790	*CDC123/CAMK1D*	Unknown	1.11	[107]
rs757210	*HNF1B*	Unknown	1.10	[91]
rs864745	*JAZF1*	β–cell dysfunction	1.10	[107]
rs7961581	*TSPAN8/LGR5*	Unknown	1.09	[107]
rs4607103	*ADAMTS9*	Unknown	1.09	[107]

Abbreviations: *ADAMTS9*, ADAM metallopeptidase with thrombospondin type 1 motif 9; *CAMK1D*, calcium/calmodulin-dependent protein kinase 1D; *CDC123*, cell division cycle 123 homologue; *CDKAL1*, CDK5 regulatory subunit-associated protein 1-like 1; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *FTO*, fat mass and obesity associated; *HHEX*, haematopoietically expressed homeobox; *HNF1B*, hepatocyte nuclear factor 1 homeobox B; *IDE*, insulin degrading enzyme; *IGF2BP2*, insulin-like growth factor 2 mRNA binding protein 2; *JAZF1*, juxtaposed with another zinc finger gene 1; *KCNJ11*, potassium inwardly rectifying channel, subfamily J, member 11; *LGR5*, leusine-rich repeat-containing G-protein coupled; OR, odds ratio; *PPARG2*, peroxisome proliferator-activated receptor gamma 2; *SLC30A8*, solute carrier family 30 (zinc transporter), member 8; SNP, single-nucleotide polymorphism; *TCF7L2*, transcription factor 7 like 2; *THADA*, thyroid adenoma associated; *TSPAN8*, tetraspanin 8; *WFS1*, Wolfram syndrome 1.

Loci are sorted by descending order of per-allele effect size. ORs are estimated for European-descent samples.

Evidence from diabetes prevention trials

Unlike genome-wide association studies where diabetic cases and their healthy controls are compared, large-scale lifestyle intervention studies allow to assess the association of genetic variants with the prospective risk of progressing from IGT to type 2 diabetes. Furthermore, they allow the investigation of interactions of candidate genes with lifestyle changes. Five lifestyle intervention studies have now demonstrated that a combination of lifestyle modifications, including increased physical activity, dietary changes, and weight reduction, delay the development of type 2 diabetes in individuals with IGT [109-113]. Two of these, the Finnish Diabetes Prevention Study (DPS) and the U.S. Diabetes Prevention Program (DPP), have also included a systematic analysis for the genetic predictors of type 2 diabetes. The DPS enrolled 522 participants and the DPP 3,234 participants with overweight and impaired glucose tolerance who were randomised to an intensive diet and exercise intervention group or a control group. In both DPS and DPP, the risk of developing type 2 diabetes was reduced by 58% in the intervention group compared to the control group during an average follow-up of around three years [111,112]. Since this original finding, several candidate gene studies on the association of gene polymorphisms with the progression from IGT to type 2 diabetes have been carried out (Tables 3 and 4).

The Diabetes Prevention Study

In the DPS, polymorphisms in 14 genes, including *PPARG2*, tumor necrosis factor-α (*TNF*), interleukin-6 (*IL6*), adrenergic receptor beta-2 (*ADRB2*), adrenergic receptor beta-3 (*ADRB3*), hepatic lipase (*LIPC*), insulin-like growth factor 1 receptor (*IGF1R*), adrenergic receptor alpha 2B (*ADRA2B*), ATP-binding cassette, sub-family C, member 8 (*ABCC8*), solute carrier family member 2 (*SLC2A2*), leptin receptor (*LEPR*), ghrelin (*GHRL*), transcription factor 7 like 2 (*TCF7L2*), and tenomodulin (*TNMD*), have been associated with the risk of developing type 2 diabetes (Table 3). Two of these genes, *PPARG2* and *TCF7L2*, have also been confirmed to increase the risk of type 2 diabetes in large-scale case-control studies (Table 2). While the Pro12Ala SNP in the *PPARG2* gene was

associated with an increased risk of developing type 2 diabetes in the DPS [114], the vast majority of other studies suggest a protective effect of the Ala12 allele on the risk.

With many of the genes associated with type 2 diabetes, the polymorphism have had effects predominantly in either the intervention group [115-120] or the control group [114,119,121-123] (Table 3). This may indicate a gene-lifestyle interaction, *i.e.* that the lifestyle intervention has modified the association of the genetic variant with the risk of type 2 diabetes. However, it must be noticed that in the control group the chance of finding a statistically significant association is also *a priori* higher because there were twice as many incident cases of diabetes than in the intervention group of the DPS [111]. A statistically significant interaction term between the genotype and the lifestyle intervention of the DPS was found for the variants in the *TNF*, *LIPC*, and *ADRA2B* genes [115,117,118].

Table 3. Genes and their single-nucleotide polymorphisms (SNPs) associated with the risk of developing type 2 diabetes in the Finnish Diabetes Prevention Study (DPS).

Gene	SNP	OR_{tot}	p_{tot}	OR_{con}	p_{con}	OR_{int}	p_{int}	Reference
PPARG2[1]	Pro12Ala	2.11	*0.010*	2.36	*<0.05*	1.90	*NS*	[114]
TNF	G-308A	1.80	*0.034*	1.12	*0.75*	4.39	*0.006*	[115]
TNF & IL6[2]	C-174G	2.22	*0.045*	1.25	*0.68*	6.19	*0.001*	[115]
ADRB2 & ADRB3[3]	Gln27Glu Trp64Arg	2.34	*0.11*	1.73	*0.10*	1.91	*0.023*	[116]
LIPC	G-250A	2.89	*0.037*	–	*0.51*	–	*0.001*	[117]
IGF1R[4]	GAG1013GAA	–	*0.033*	–	*0.27*	–	*0.083*	[124]
ADRA2B	12Glu9	–	*NS*	5.17	*0.003*	0.09	*0.049*	[118]
ABCC8	G-2886A	2.69	*0.002*	2.42	*0.017*	3.71	*0.037*	[119]
	G-1561A	2.08	*0.009*	1.72	*0.30*	2.30	*0.013*	[119]
	A-1273G	2.27	*0.005*	1.97	*0.055*	3.51	*0.023*	[119]
	AGG1273AGA	2.00	*0.014*	3.01	*0.002*	1.36	*0.55*	[119]

Table 3. Continued

Gene	SNP	OR_{tot}	p_{tot}	OR_{con}	p_{con}	OR_{int}	p_{int}	Reference
SLC2A2	rs5393	3.04	*0.008*	5.56	*0.003*	1.17	*0.82*	[121]
	rs5394	2.54	*0.026*	4.91	*0.007*	0.85	*0.79*	[121]
	rs5400	2.60	*0.009*	3.78	*0.005*	1.40	*0.58*	[121]
	rs5404	2.57	*0.025*	5.07	*0.005*	0.84	*0.77*	[121]
LEPR	Lys109Arg	1.69	*0.069*	2.38	*0.016*	0.88	*0.80*	[122]
	Gln223Arg	2.01	*0.042*	2.33	*0.047*	1.55	*0.47*	[122]
GHRL	Leu72Met	0.47	*0.016*	0.61	*0.20*	0.28	*0.016*	[120]
TCF7L2[5]	rs12255372	1.71	*0.18*	2.85	*0.021*	0.61	*0.62*	[123]
TNMD	rs2073162[6]	–	*NS*	–	*NS*	–	*NS*	[125]
	rs2073163[6]	–	*NS*	–	*NS*	–	*NS*	[125]

Abbreviations: –, not reported; *ABCC8*, ATP-binding cassette, sub-family C (CTFR/MRP), member 8; *ADRAB2*, adrenergic receptor alpha 2B ;*ADRB2*, adrenergic receptor beta-2; *ADRB3*, adrenergic receptor beta-3; DPS, Diabetes Prevention Study; *GHRL*, ghrelin/obestatin prepropeptide; *IGF1R*, insulin-like growth factor 1 receptor; *IL6*, interleukin 6; *LEPR*, leptin receptor; *LIPC*, hepatic lipase; NS, non-significant; *PPARG2*, peroxisome proliferator-activated receptor gamma 2; *SLC2A2*, solute carrier family 2 (facilitated glucose transporter), member 2; SNP, single-nucleotide polymorphism; *TCF7L2*, transcription factor 7 like 2; *TNF*, tumor necrosis factor; *TNMD*, tenomodulin.

The footnotes *tot*, *con* and *int* refer to the total study population, control group and intervention group, respectively. Genes are presented in the chronological order of publication.

[1]The Ala12 allele increased the risk of type 2 diabetes in the DPS, contrasting meta-analyses of case-control studies where Ala12 was found to be protective [89,126].

[2]The combination of *TNF* -308A and *IL6* C-174C genotypes was associated with type 2 diabetes.

[3]The combination of *ADRB2* Gln27Gln genotype and *ADRB3* Arg64 allele was associated with type 2 diabetes.

[4]The heterozygous genotype was associated with type 2 diabetes, but no difference was seen between the rare and common homozygous genotypes.

[5]Relative risks shown instead of ORs.

[6]Associated with type 2 diabetes only among men (rs2073162: OR 2.14, p=0.028; rs2073163: OR 2.11, p=0.035).

The Diabetes Prevention Program

In the DPP, variants in four genes, including *TCF7L2*, *PPARG2*, *KCNJ11*, and *WFS1* have been associated with the progression from IGT to type 2 diabetes (Table 4). Similarly with the DPS, the association of rs7903146 in *TCF7L2* with the risk of developing type 2 diabetes was stronger in the control group than in the lifestyle intervention group,

indicating the possibility of a gene–lifestyle interaction [127]. While the Ala12 allele of the Pro12Ala SNP in *PPARG2* was associated with a decreased risk of type 2 diabetes in the DPP, it was associated with an increased risk in the DPS [114]. In the DPP, the effect was also modified by BMI, and a decreased risk was found only among the carriers of the Ala12 allele with a BMI below 34.5 kg/m^2 [128]. However, similarly to the Ala12 allele in the DPS, the association of the E23K SNP in the *KCNJ11* gene with a lower risk of type 2 diabetes was inverse to the risk-increasing effect of the lysine allele seen in a previous meta-analysis [129]. A SNP in the adjacent *ABCC8* gene that was in a strong linkage disequilibrium with the E23K SNP was also associated with an increased risk of developing type 2 diabetes [128]. In the DPS, the E23K SNP was not associated with the progression to type 2 diabetes [119], but SNPs in *ABCC8* were [119]. It thus remains possible that variants in either one or both genes are required to mediate the effects on diabetes risk [130].

In the DPP, the results of genome–wide association studies were recently extended to evaluate how SNPs in the novel diabetes–associated genes, including exostoses 2 (*EXT2*), CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), cyclin-dependent kinase inhibitor 2B (*CDKN2B*), insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), hematopoietically expressed homeobox (*HHEX*), similar to hCG1816027 (*LOC387761*), and solute carrier family 30, member 8 (*SLC30A8*), affected the incidence of type 2 diabetes. However, none of the SNPs were statistically significantly associated with the incidence of type 2 diabetes [131].

Table 4. Genes and their single nucleotide polymorphisms (SNPs) associated with the risk of developing type 2 diabetes in the Diabetes Prevention Program (DPP).

Gene	SNP	RR_{tot}	p_{tot}	RR_{con}	p_{con}	RR_{int}	p_{int}	Reference
TCF7L2	rs7903146	1.55	*<0.001*	1.81	*0.004*	1.15	*0.60*	[127]
PPARG2[1]	Pro12Ala	1.24	*0.007*	1.28	*0.17*	–	–	[128]
KCNJ11[2]	E23K	–	–	0.71	*0.01*	1.09	*0.61*	[130]
WFS1	rs752854	0.98	*0.90*	1.14	*0.56*	0.41	*0.056*[3]	[132]
	10012946	0.85	*0.026*	–	–	–	–	

Abbrevations: –, not reported; DPP, Diabetes Prevention Program; *KCNJ11*, potassium inwardly rectifying channel; NS, non-significant; *PPARG2*, peroxisome proliferator-activated receptor gamma 2; RR, relative risk; SNP, single-nucleotide polymorphism; *TCF7L2*, transcription factor 7 like 2; *WFS1*, Wolfram syndrome 1.
The footnotes *tot*, *con* and *int* refer to the total study population, control group and lifestyle intervention group, respectively. Genes are presented in the chronological order of publication.
[1]There was an interaction between genotype and body mass index (p=0.03). Ala12 carriers were more susceptible to the deleterious effect of body mass index on diabetes incidence than proline homotsygotes.
[2]The Ala1369Ser SNP in the *ABCC8* gene was also studied with essentially identical results to E23K.
[3]The minor homozygotic genotype protected from diabetes among white participants in the intervention group (RR=0.30, p=0.048).

Considerations and implications

The replication of the association of *TCF7L2*, a relatively powerful genetic factor, with the risk of developing type 2 diabetes in both the DPS and the DPP illustrates that large-scale lifestyle intervention studies are appropriate for studying genetic variants of high frequency and with strong effects on the risk of type 2 diabetes [123,127]. However, these studies seem to lack sufficient power to study the associations of variants with a low or modest effect on the risk of type 2 diabetes, because even the DPP with more than 3,000 participants could not replicate the findings of the recent genome-wide association studies [131]. In the DPS, a large number of associations of gene polymorphisms with the incidence of type 2 diabetes have been reported (Table 3), but it is likely that some of these associations are false positives due to the problem of multiple testing [133]. Some of the positive associations may, however, be weak and could be confirmed in larger-scale genome–wide association studies or in robust meta-analyses of candidate gene studies. Nevertheless, in the light of the many previous unreplicated findings in candidate gene

studies [134], it is obvious that the number of genetic hypotheses that are tested has to be limited to reduce the likelihood of false discovery.

The DPS and DPP are, moreover, not directly comparable to the genome-wide association studies, and they may also provide complementary evidence on diabetes susceptibility genes because of their distinct study design. Whereas the case-control design was used in the genome–wide association studies and in most candidate gene studies, the DPS and DPP are prospective studies where the participants were at a high risk of type 2 diabetes at baseline. Therefore, the participants of the DPS and the DPP are likely to have been at a relatively late stage in the pathogenesis of diabetes in the beginning of the study. The participants of the DPS and DPP were also more homogeneous in characteristics (obese with IGT) than type 2 diabetic and their control subjects in case-control studies. This may have affected the ascertainment of the role of genetic variation as a risk factor for type 2 diabetes. The selection of high-risk individuals for a lifestyle intervention study increases the number of incident cases, but it may also result in the selection of high-risk genotypes that limits the generalisability of the results. Furthermore, half of the participants in the DPS and DPP followed a lifestyle intervention, which allowed the investigation of gene-lifestyle interactions but may have affected the sensitivity of the studies to detect genetic main effects. Nevertheless, because of their unique characteristics, intervention studies on the prevention of type 2 diabetes may give further insights into the etiology of the disease that remain uncovered in case-control studies.

PHYSICAL ACTIVITY IN THE PREVENTION OF TYPE 2 DIABETES

Prospective epidemiological studies

Prospective epidemiological studies strongly suggest a link between higher levels of physical activity and a decreased risk of type 2 diabetes. Individuals with higher levels of leisure-time, occupational and commuting physical activity have had 15-60% lower risk of type 2 diabetes, and most studies showed a 30-50% reduced risk among physically active individuals [135-158]. The benefit has been apparent in both men and women when controlled for age, BMI, and several other confounding factors.

Cardiorespiratory fitness is considered an objective measure of an individual's recent physical activity pattern [143,159-163]. The studies on cardiorespiratory fitness have shown similar results as physical activity studies, but with somewhat stronger magnitude of association [143,159-163]. One reason for the stronger association between cardiorespiratory fitness and the risk of type 2 diabetes may be that measures of cardiorespiratory fitness are more accurate and less prone to misclassification than those of physical activity [164]. Physical fitness has a strong genetic background, however, that may at least partly be independent of physical activity patterns [165-168]. The same genetic factors that are associated with a high level of fitness may also protect from type 2 diabetes [168,169].

Lifestyle intervention studies

No information on the independent effect of physical activity on the incidence of type 2 diabetes from randomized controlled trials is available. Five lifestyle intervention studies on the prevention of type 2 diabetes, including the Malmö Study from Sweden [109], the Da Qing Study from China [110], the Finnish DPS [111], the DPP in the USA [112], and the Indian Diabetes Prevention Study [113] have, however, included physical activity in

their intervention programs. Of these, only the Da Qing Study that was randomised by clinic rather than by individual, has included an exercise-only intervention arm, whereas the four other studies combined physical activity with dietary changes and weight reduction in their intervention schemes. In the Da Qing Study, the cumulative incidence of type 2 diabetes during the 6-year follow-up was similarly low (41%) in the exercise-only group compared with the diet-only (44%) and combined diet and exercise (46%) groups [110]. The study also suggested that the decrease in the development of type 2 diabetes in the exercise-only arm occurred without a substantial change in BMI. However, physical activity was poorly documented in the Da Qing Study, and the apparent success of the exercise-only intervention may be partly attributable to the significantly higher baseline physical activity in the exercise group compared with the control group [110].

Other lifestyle intervention studies have not included an exercise-only arm. However, in the DPS, the independent effects of physical activity have been assessed through statistical adjustments for the other components of the intervention [170]. The subjects who increased their physical activity most (i.e. were in the upper tertile of the change) were 66% less likely to develop type 2 diabetes than those in the lower tertile during 4.1 years of follow-up while adjusting for changes in diet and body weight [170]. In the DPP, there was no independent effect of increased physical activity on diabetes risk after adjustment for weight change [171]. However, among the participants who did not meet the weight loss goal, those who met the activity goal had a 44% reduction in diabetes incidence, independent of the small weight loss (-2.9 kg) that occurred [171]. In the Malmö Feasibility Study, improved cardiovascular fitness and weight loss were equally correlated with improved glucose tolerance [109], a finding that is supported by data from the Study on Lifestyle Intervention and Impaired Glucose Tolerance Maastricht [172]. These secondary analyses of the data suggest a beneficial effect of physical activity on the risk of type 2 diabetes. However, evidence from randomised controlled trials with an exercise-only intervention arm is required to draw definite conclusions.

Mechanisms

The inverse association between physical activity and the reduced risk of type 2 diabetes in epidemiological studies is supported by the evidence of the physiological adaptations that take place in response to exercise training. Regular physical activity leads to a number of beheficial changes that may improve glycemic control, as summarized in Figure 1 and discussed below.

Body composition

Obesity and particularly excess visceral fat increases insulin resistance and predisposes to the development of type 2 diabetes [29,173-180]. Regular physical activity reduces excess total and visceral fat and the risk of developing obesity [181-188]. Some randomized controlled trials have suggested that exercise training decreases total, visceral, and abdominal subcutaneous fat even without weight loss in both normal weight and obese individuals [189,190]. Resistance exercise increases skeletal muscle mass, which increases muscle glucose uptake [191,192]. Physical activity may thus decrease the risk of type 2 diabetes by modifying body composition [193]. However, exercise has other beneficial effects on glycemic control that are not mediated by body composition [194].

Muscle insulin signaling

Skeletal muscle is the major site for insulin-mediated glucose disposal [57], and insulin-stimulated muscle glucose uptake and utilization are largely impaired in patients with type 2 diabetes [195-197] and in the first-degree relatives with normal glucose tolerance [196-198]. Exercise training improves skeletal muscle insulin sensitivity [199,199-204]. The mechanisms work likely through increased expression and activity of key proteins involved in muscle insulin signaling and glucose metabolism [205,206]. Exercise increases the muscle content of glycogen synthase (GS) and glucose transporter isoform 4 (GLUT4) in skeletal muscle [207]. The increased content of GS accelerates non-oxidative glucose disposal as glycogen [191,199,208]. GLUT4 facilitates the passive diffusion of circulating

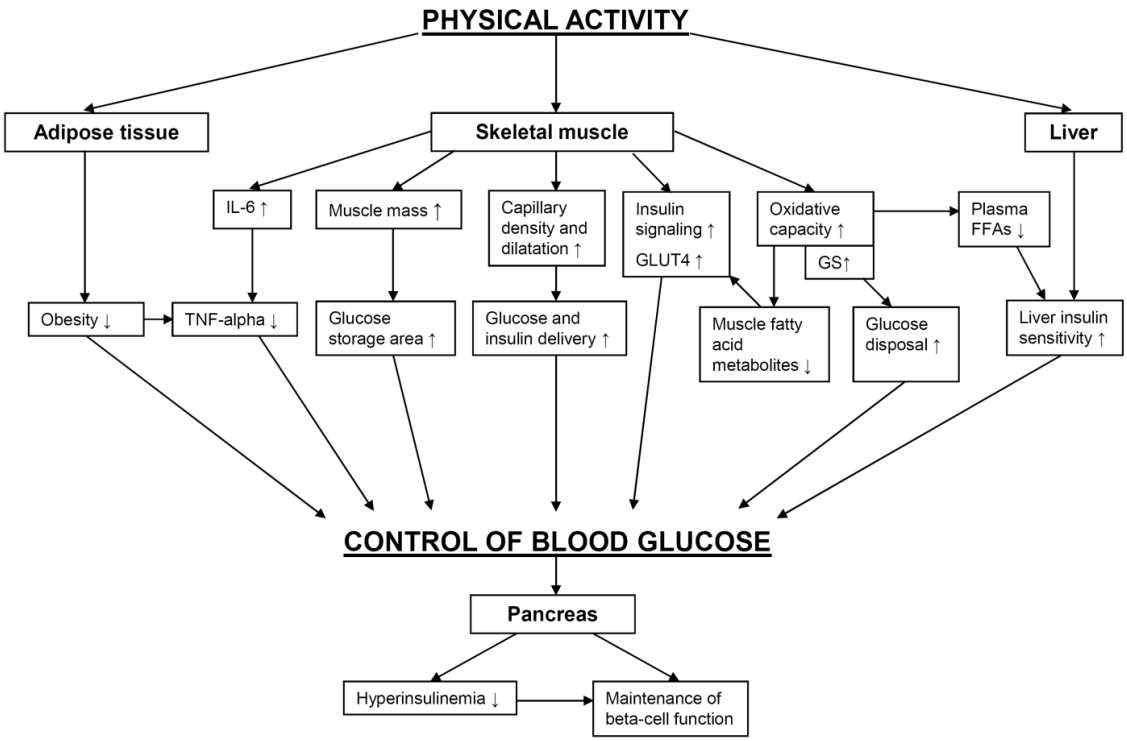

Figure 1. Mechanisms by which regular physical activity may improve glycemic control and prevent type 2 diabetes. Abbreviations: FFA, free fatty acid; GLUT4, glucose transporter 4; GS, glycogen synthase; IL-6, interleukin-6; TNF-alpha, tumor necrosis factor-alpha.

glucose down its concentration gradient into muscle cells, and muscle glucose transport is closely associated with GLUT4 content of the cells [209-211]. However, in type 2 diabetic patients, the expression of GLUT4 is similar to insulin-sensitive control subjects [212,213], suggesting that other mechanisms are involved in the impairment of insulin sensitivity among these individuals. Indeed, some data suggest that exercise may potentiate insulin signaling by upregulating the expression and activity of proteins involved in insulin signal transduction [206,214,215]. At present, the exact mechanisms are incompletely understood. Increased insulin receptor content [191] and autophosphorylation [215], as well as increased expression of downstream signaling components of insulin [191,208] have been reported. These may contribute to enhancements in insulin signaling in response to regular physical activity.

Lipid oxidation capacity

The exercise-induced increase in the oxidative capacity of skeletal muscle results in a higher rate of whole-body fat oxidation at rest and during submaximal exercise [216-218], which may prevent lipid-mediated insulin resistance. Intramuscular accumulation of triglycerides is modestly associated with insulin resistance in sedentary individuals [219,220]. However, athletic skeletal muscle shows high insulin sensitivity despite accumulating large amounts of intramyocellular triglycerides [221]. Futhermore, markers of skeletal muscle oxidative capacity have proven better correlates of insulin sensitivity than muscle lipid content [222]. Indeed, instead of triglycerides themselves, their metabolites such as fatty acyl-CoAs, diacylglycerols and ceramides, may induce insulin resistance [58-60]. Exercise-induced increase in lipid oxidation capacity improves fatty acid turnover and thus prevents the accumulation of fatty acid metabolites in the muscle, leading to improvements in insulin sensitivity [223].

The increased oxidation of lipids may also improve liver insulin sensitivity by affecting plasma free fatty acid (FFA) levels [50]. Increased FFA supply to the liver may directly cause insulin resistance [224]. Exercise decreases the concentration of FFAs in the circulation, which may attenuate liver insulin resistance [50,225,226].

Microvascular function

Insulin is known to control its own, as well as substrate, delivery to muscle by vascular effects [65,227]. Insulin increases the number of perfused muscle capillaries, and at the same time the total amount of insulin and glucose that enters muscle capillaries. Microvascular dysfunction may, however, impair the ability of insulin to increase muscle capillary perfusion, and lead to decreased muscle insulin sensitivity [54-56,66,67]. Regular physical activity may counteract microvascular dysfunction by increasing the number of capillaries surrounding muscle fibers, and by dilating muscle capillaries via increased formation of nitric oxide (NO) in the vascular endothelium [227-230]. Increased capillary perfusion leads to increased amounts of insulin and glucose seen by the insulin receptor [66].

Anti-inflammatory effects

Low-grade chronic inflammation, as reflected by increased systemic levels of proinflammatory cytokines and C-reactive protein (CRP) [231], may play an important role in the pathogenesis of insulin resistance and type 2 diabetes [54-56]. Regular physical activity may reduce inflammation by increasing the systemic levels of a number of anti-inflammatory cytokines [232,233]. In particular, interleukin-6 (IL-6) is produced by muscle fibers during exercise, resulting in an exponential (up to 100-fold) increase in circulating IL-6 [234-236]. Muscle-derived IL-6 stimulates the appearance of other anti-inflammatory cytokines in the circulation, including interleukin-1 receptor antagonist, soluble TNF-α receptor, and interleukin-10 [237-240]. IL-6 also inhibits the production of TNF-α, a proinflammatory cytokine that is produced by adipose tissue and thought to play a mechanistic role in insulin resistance [241-248]. Regular exercise may thus offer protection against TNF-α-induced insulin resistance via increased production of IL-6 [236]. Some data suggest that exercise also suppresses TNF-α via IL-6 independent pathways [244,249].

Beta-cell function

Exercise may, at least indirectly, exert beneficial effects on pancreatic β–cell function. Chronic exposure of pancreatic islets to elevated levels of glucose and fatty acids impairs β–cell function [68-71]. By improving the insulin sensitivity of skeletal muscle, physical activity diminishes glycemic stress that may exhaust β–cells. In addition, some data suggest that physical activity may directly improve the first-phase insulin secretion, which is an indicator of β–cell function [250,251].

GENE–PHYSICAL ACTIVITY INTERACTIONS IN THE DEVELOPMENT OF TYPE 2 DIABETES

Regular physical activity exerts beneficial effects on glycemic control. However, adaptations to exercise differ substantially between the individuals, and some individuals are more responsive to the effects of physical activity interventions than others. For example, the magnitude of changes in VO_{2max}, blood pressure, heart rate or HDL cholesterol in response to exercise training differs dramatically among individuals [10]. Such variability in the responsiveness to physical activity is common for nearly all components of health-related fitness [11]. Furthermore, correlations between the exercise-induced changes in health-related phenotypes are low. In other words, an individual may not experience favourable changes in one risk marker, for example, systolic blood pressure, in response to physical activity, but may still exhibit highly favourable changes in some another trait, such as plasma glucose levels [11].

Three major factors affect phenotype variability: measurement error, environment, and genes [252]. Measurement error is small for most phenotype measurements and is usually not an important source of variability. Environmental differences, such as history of physical activity and diet, are more important but they can, at least partly, be accounted for in the research design or through statistical adjustments. After controlling for measurement

error and environmental factors, there are still large inter-individual differences in physical activity responses that are attributed to genetic variation [10]. Individuals with specific genetic profiles are more responsive to physical activity interventions for specific traits, and perhaps less responsive or even non–responsive with regard to some other phenotypes [11]. Identification of the key genes and alleles that modify the success of physical activity interventions is important, as it would increase our understanding of the etiology of type 2 diabetes and could lead to the development of new therapies. Furthermore, such information could be used in the identification of individuals who have the potential to benefit most from targeted lifestyle prevention in the prevention of insulin resistance and type 2 diabetes [12]. Such predictions would create possibilities for tailoring interventions and therapies individually, making them better targeted and more efficient.

Study designs for investigating gene-physical activity interactions

Several different epidemiological study designs are available to assess gene–physical activity interactions, as summarised in Table 5. In addition, genome-wide linkage studies have been used to detect such chromosomal loci in which genetic variants modifying responses to exercise training could be present (Table 6).

Either a short or long–term approach is possible in the study of gene-physical activity interactions. Studies with a short follow-up (weeks or months) reflect the immediate responses to changes in physical activity, which is important in informing selection of individuals for targeted prevention [253]. Studies with a long follow-up gives information on gene-physical activity interactions over lifetime. A case-control study may be the best design from the lifelong perspective and is efficient in statistical terms, as an equal number of cases and controls are recruited. However, since the report of lifestyle habits may be biased in individuals who know they have diabetes, recall bias affects the results. Furthermore, when investigating traits such as obesity, there is the added problem of inference about the direction of causality, as it cannot be determined whether inactivity precedes the development of disease, or *vice versa* [253]. Therefore, prospective studies are

also needed to investigate the interactions of genes and physical activity in the development of type 2 diabetes.

Table 5. Study designs for the investigation of gene-physical activity interactions

Study design	**Use**	**Strengths**	**Weaknesses**
Case-control cross-sectional	Interactions between gene variants and physical activity on the prevalence of disease or levels of related traits	The same number of cases and controls are recruited, which means efficient utilization of subjects.	Susceptible to recall bias. Inference of causality not possible.
Cohort studies	Interactions between gene variants and physical activity on the incidence of disease or changes in levels of related traits.	Inference of causality possible. Less susceptible to recall bias.	Many individuals have to be studied but only few become informative as incident cases, which means inefficient utilization of subjects.
Nested case-control studies (hybrid design).	Interaction between gene variants and physical activity on the incidence of disease or changes in levels of related traits.	The same number of cases and controls are recruited, which means efficient utilization of subjects. Inference of causality possible. No recall bias.	Large cohort study is required to have a sufficient number of incident cases
Lifestyle intervention, secondary analysis	Interaction between gene variants and physical activity on the incidence of disease or changes in levels of related traits.	Controlled study design. Careful follow-up of subjects.	Adjustment for other lifestyle changes than physical activity required. Only a limited number of subjects can be included. Suitable for common variants only.
Exercise intervention, secondary analysis	The effects of gene variants on the responses to exercise training.	Exercise-only intervention. Controlled study design. Careful follow-up of subjects.	Only a limited number of subjects can be included. Suitable for common variants only.
Exercise intervention with subjects preselected by genotype	Tailored intervention in preselected genetic groups.	Confirms whether there is differential response to physical activity by genotype for the outcome of interest.	Very strong prior likelihood of success required.

The constraints of a case-control study can be removed by nesting the case–control study within a cohort, as the recall of exposure then predates the attribution of the disease label, and the inference of causality becomes possible [253]. Secondary analyses of lifestyle intervention studies are another option. They can only include a limited number of participants, which limits statistical power. The focus is on genetic polymorphisms that are likely to have only a small influence on the investigated phenotype, and identification of true associations thus requires large sample sizes. Power calculations also show that the study of interactions between genes and lifestyle requires larger study populations than the investigation of genetic main effects [254]. Furthermore, the method used for the measurement of physical activity is important, since the precision of this measure is critical to the power to detect interactions [15]. Nonetheless, even in a large-scale study with precise measurement of lifestyle factors, the number of interactions that are tested has to be limited to reduce the likelihood of false discovery. The priority should lie within those genes and gene variants that have the greatest biological plausibility for interaction, or that are supported by evidence of genetic main effects [253].

Ultimately, if robust evidence on gene-physical activity interactions becomes available, a study where individuals are selected on the basis of a risk genotype could be carried out. Such a study would be a stepping stone to the introduction of targeted interventions. However, the prospect of selecting people by genotype for a trial is presently limited, as one would need a very strong prior likelihood of success before carrying out an expensive long-term trial that followed people up to a relevant clinical outcome [17]. Therefore, at present, the retrospective examination of differential response by genotype in existing lifestyle intervention trials is more plausible.

Present evidence on gene-physical activity interactions

The existing evidence on gene–physical activity interactions in the development of type 2 diabetes or related metabolic traits consists of genome–wide linkage scans, cross–sectional association studies of quantitative traits and relatively small lifestyle intervention studies.

Linkage scans

Two genomic scans have been carried out for intermediate phenotypes of type 2 diabetes in the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study [255-257]. These scans have identified loci for the changes in quantitative traits in response to a 20-week endurance training program in sedentary healthy individuals, as summarized in Table 6. Numerous genes located in these genomic regions may modify the effects of endurance training on diabetes–related traits [256,257]. However, the history of linkage scans has shown that the scans are not sensitive enough for the detection of variants for multigenic diseases, such as type 2 diabetes, where each genetic variant has only a low or modest effect on the disease risk [258]. This may be the case with the scans on the exercise-related chromosomal loci as well, since the effects of exercise are likely mediated by many genes, each with only a modest effect on any phenotype of interest [259]. One possibly successful example of a linkage scan has, however, been demonstrated in the HERITAGE Study. The genomic region 7q31 was first linked to fasting insulin response to exercise training [256], and subsequently, two nearby genes, the leptin (*LEP*) and leptin receptor (*LEPR*), were found to modify the effects of exercise training on glucose homeostasis in a candidate gene study [260].

Table 6. Chromosomal loci associated with responses of intermediate phenotypes of type 2 diabetes to exercise training in genome-wide linkage scans.

Phenotype	Subjects	Chromosomal loci	Reference
Fasting insulin	Caucasians	7q31	[256]
Insulin sensitivity	African-Americans	20q13, 22q11-12	[257]
Acute insulin response	African-Americans	15q15, 18q12	[257]
Disposition index	Caucasians	1p35, 3q25, 6p21-22, 7q21, 1p13, 12q24	[257]
Disposition index	African-Americans	6p22, 13q14	[257]
Glucose effectiveness	African-Americans	1p31, 1q44, 2p22-21, 10p12, 10q23, 12q13, 15q26, 19q13	[257]

Candidate gene studies

In candidate gene association studies, several genes have shown an interaction with physical activity on phenotypes of glucose and insulin metabolism or adiposity (Table 7).

Table 7. Interactions between physical activity and genetic polymorphisms on glucose and insulin metabolism phenotypes, body weight and adiposity.

Gene	Polymorphism	Subjects	Study design	Outcome	Reference
ACE	I/D	35 men	Exercise intervention	Insulin sensitivity increased and acute insuiln response decreased more in the I/I homozygotes than in others.	[261]
ADRB2	Gln27Glu	420 men	Cross-sectional population-based	Among those who were physically inactive, body weight, waist circumference, and waist-to-hip ratio were higher in Glu27 carriers than in others.	[262]
ADRB2	Gln27Glu	252 women	Cross-sectional case-control	Among those who were active in their leisure-time, carriers of the Glu27 allele had a higher BMI than others.	[263]
ADRB2	Arg16Gly	251 women	Exercise intervention	Among white women, BMI, fat mass and percent body fat decreased more in Gly16Gly homozygotes than in others.	[264]
COMT	Val108/158Met	173 women	Exercise intervention	Percent body fat decreased more in Val/Val carriers than in individuals with Met/Met genotype.	[265]
CYP19	(TTTA)*n repeat, n=7-13	173 women	Exercise intervention	Total fat and percentage body fat decreased more in carriers of two 11-repeat alleles than in others.	[265]
ENPP1	K121Q	84 women	Exercise intervention	Body weight, BMI, and fasting insulin decreased more in KK carriers than in others.	[266]
FHL1	rs2168002, rs9018	222 men	Exercise intervention	Fasting insulin, insulin sensitivity index, glucose disposition index, and glucose disappearance index improved more in rs2180062 C allele carriers than in others. Fasting insulin decreased more in rs9018 T allele carriers than in others.	[267]
FTO	rs9939609	5,554 men and women	Cross-sectional population-based	Carriers of the rare homozygous genotype had higher BMI than common homozygous genotype carriers only among the individuals who were physically inactive	[268]
FTO	rs1861868, rs1477196	702 men and women	Cross-sectional population-based	Carriers of the rare allele of rs1861868 or rs1477196 had higher BMI only among the individuals with low physical activity scores.	[269]
FTO	rs1421085	4,672 adolescents	Cross-sectional population-based	Carriers of the rare allele had higher BMI only among the individuals who were physically inactive.	[270]
FTO	rs1421085	3,167 men and women	Cross-sectional population-based	Carriers of the rare allele had higher BMI only among the individuals who were physically inactive.	[270]

Table 7. Continued

Gene	Polymorphism	Subjects	Study design	Outcome	Reference
GNB3	C-825T	255 men and women	Exercise intervention	Fat mass and percent body fat mass decreased more in TT homozygotes than in others.	[271]
GNB3	C-825T	3,378 men and women	Cross-sectional population-based	Each T allele was associated with a 20% increased prevalence of obesity in physically active individuals, whereas each T allele was associated with a 23% decreased prevalence of obesity in inactive individuals.	[272]
IL6	C-174G	56 men and women	Exercise intervention	Glucose AUC in OGTT decreased more in GG homozygotes than in others	[273]
INS	VNTR	729 men	Cross-sectional case-control	Among those with low physical activity, 1-hour insulin was higher in class III allele carriers than in others	[274]
LEP	Ala19Gly	397 men and women	Exercise intervention	Gene-gene interaction with the Lys109Arg SNP in LEPR, so that among Ala19 homozygotes, decrease in fasting insulin was greatest in those who were also Arg109carriers for *LEPR*.	[260]
LEPR	Lys109Arg	397 men and women	Exercise intervention	Insulin sensitivity and disposition index increased and fasting glucose decreased only in Arg109 carriers. Glucose disappearance index increased more in Arg109 homozygotes than in others.	[260]
LIPC	-514 C/T	662 men and women	Exercise intervention	Insulin sensitivity increased more in CC homozygotes than in others.	[275]
LPL	S447X	249 white, 171 black women	Exercise intervention	Among white women, BMI, fat mass, and percent body fat decreased more in X447 carriers than in others. Among black women, abdominal visceral fat decreased more in X447 carriers than in others.	[276]
NOS3	Haplotype	706 men and women	Prospective cohort	A haplotype had an interaction with total energy expenditure on changes in 2-hour glucose levels.	[277]
PPARG	Pro12Ala	123 men	Exercise intervention	IRI and HOMA-IR decreased in Ala12 carriers but not in others	[278]
PPARG	Pro12Ala	32 men	Exercise intervention	Insulin AUC decreased more in Ala12 carriers than in others	[279]
PPARG	Pro12Ala	139 men	Exercise intervention	Fasting glucose decreased more in Ala 12 carriers than in others	[280]

Table 7. Continued

Gene	Polymorphism	Subjects	Study design	Outcome	Reference
PPARG	Pro12Ala	29 men and women	Exercise intervention	Body weight decreased more in Ala12 carriers than in others.	[281]
PPARG	Pro12Ala	236 men and women	Cross-sectional population-based	Risk of type 2 diabetes was greater only in Pro12 allele carriers with low physical activity	[282]
VDR	BsmI	1,539 men	Cross-sectional population-based	Among those with low physical activity, fasting glucose was higher in BB carriers than in others	[283]

Abbreviations: AUC, area under the curve; *ACE*, angiotensin I converting enzyme (peptidyl-dipeptidase A) 1; *ADRB2* beta-2 adrenergic receptor; *COMT* catechol O-methyltransferase; *CYP19* cytochrome P450, family 19, subfamily A; *ENPP1,* ectonucleotide pyrophosphatase/phosphodiesterase 1; *FHL1,* four and a half LIM domains 1; *FTO,* fat mass and obesity associated; *GNB3,* guanine nucleotide binding protein (G protein), beta polypeptide 3; I/D, insertion/deletion; *IL6*, interleukin 6 (interferon, beta 2); *INS*, insulin; *LEP*, leptin; *LEPR*, leptin receptor; *LIPC*, hepatic lipase; *LPL,* lipoprotein lipase; *NOS3*, nitric oxide synthase 3 (endothelial cell); OGTT, oral glucose tolerance test; *PPARD,* peroxisome proliferator-activated receptor delta; *PPARG*, peroxisome proliferator-activated receptor gamma; *VDR*, vitamin D (1,25- dihydroxyvitamin D3) receptor; VNTR, variable number tandem repeats.

Only such genes that either had a statistically significant interaction between physical activity and a polymorphism, or that showed a differential genotypic response to an exercise intervention in at least one study are included. Results from intervention studies where a multi-component intervention interacted with gene variant are not included.

Thus far, two main study designs have been utilised to investigate interactions between physical activity and genes in candidate gene studies (Table 7). Firstly, observational analyses have been carried out on the interaction between the habitual level of physical activity and genetic polymorphisms. Secondly, exercise intervention studies have been conducted to compare responses to exercise training among candidate gene genotypes. Most of the detected interactions between genes and physical activity have not been replicated. At present, the *ADRB2* gene; the fat mass and obesity associated gene (*FTO*); the guanine nucleotide binding protein, beta polypeptide 3 gene (*GNB3*); and the *PPARG2* gene are the only genes that have interacted with physical activity on body weight or glucose and insulin metabolism phenotypes in more than one study (Table 7).

The *ADRB2* gene encodes a receptor that binds the endogenous catecholamines epinephrine and norepinephrine, which are released during sympathetic nervous system activity causing increased lipolysis. Physical activity stimulates the sympathetic nervous system [284]. Variation in the *ADRB2* gene could modify the exercise-induced activation of lipolysis via sympathetic nervous system and thereby affect the magnitude of weight changes in response to physical activity. Some reports suggest that fat oxidation rate [285,286] and lipolysis [285] are decreased in Glu27Glu obese women compared with Gln27Gln homozygous individuals after a bout of exercise.

Three studies have detected an association between an SNP in the *ADRB2* gene and physical activity on body weight. In a cross-sectional study among 836 French men and women, body weight, waist circumference, and waist-to-hip ratio were higher in physically inactive men who carried the Glu27 allele of the Glu27Gln SNP in *ADRB2* than in inactive men with other genotypes, whereas no significant difference between the genotypes was found among men who were physically active [262]. No significant difference between the genotypes was, however, found among women [262]. In contrast, a case-control study among 139 obese Spanish women and their 113 healthy controls found that the carriers of the Glu27 allele who were active in their leisure-time, had a higher BMI than other individuals with the same level of activity, whereas no difference between the genotypes was seen among the inactive individuals [263]. In the third report on *ADRB2* and physical activity, the responses of *ADRB2* genotypes to a 20-week endurance training program were followed among 482 white and 260 black

participants [264]. BMI, fat mass, and percent body fat decreased more during the endurance training program in white women who were homozygous for the Gly16 allele of the Arg16Gly SNP in *ADRB2* than in white women with other genotypes [264]. However, no difference in the responses to the training program were found among the genotypes of the Glu27Gln SNP. The findings on variation in the *ADRB2* gene and changes in body weight in response to physical activity are thus inconsistent with regard to the specific variants that have modified the responses, as well as with regard to the effect of gender.

Very recently, four studies have suggested an interaction between SNPs in the *FTO* gene and physical activity. Firstly, the carriers of the rare homozygous genotype of rs9939609 in *FTO* had a higher BMI than the common homozygous individuals among 5,554 Danish men and women, but the difference was only seen in the individuals who were physically inactive [268]. Secondly, the rare alleles of rs1861868 and rs1477196 in *FTO* were associated with BMI only among those with low physical activity scores among 702 Amish individuals [269]. Thirdly, among 4,672 Finnish adolescents, the association of the rare allele of rs1421085 in *FTO* with BMI was strongest in the individuals who were physically inactive [270], and fourthly, the rare allele of the same rs1421085 SNPs was associated with higher BMI among physically inactive individuals, but not in those who were physically active among 3,167 French men and women [270]. Although the findings on the interaction between the *FTO* gene and physical activity are fairly consistent, a recent study among 4,210 individuals from the Singapore National Health Survey did not find an association between the *FTO* gene and physical activity on BMI [287]. Furthermore, the mechanism that would mediate the interaction between the *FTO* gene and physical activity on adiposity remains unresolved.

Two studies have indicated that the C-825T variant in the *GNB3* gene may modify the association between physical activity and fat mass. In the HERITAGE Study, changes in fat mass and body fat percentage differed significantly among 255 black men and women on the completion of the 20-week endurance training program [271]. The TT homozygotic individuals had the largest decrease in fat mass and fat percentage while the CC homozygotes showed little or no decrease. Consistently, in a recent cross-

sectional study among 3,378 men and women, each T allele was associated with a 20% reduced prevalence of obesity in physically active individuals, whereas each T allele was associated with a 23% decreased prevalence in low-active individuals [272]. Physical activity may thus be more effective in reducing obesity among the T allele carriers of the C-825T SNP. It has been suggested that by influencing G protein receptor–mediated signal transduction, variation in the *GNB3* gene may negatively affect the interaction of catecholamines with β-adrenergic receptors during physical activity [288]. This could lead to a decrease in response to catecholamines, and consequently, to altered energy utilization and adipose homeostasis [289].

The *PPARG2* gene encodes PPARγ, a member of the peroxisome proliferator-activated receptor subfamily of nuclear receptors, which regulates the transcription of a number of genes involved in adipocyte differentiation [290-293] and insulin sensitization [294]. Thiazolidinediones, the insulin-sensitizing drugs used in the treatment of type 2 diabetes, target *PPARG* [294]. The most widely studied SNP of *PPARG2*, the Pro12Ala, has been associated with an increased insulin sensitivity and a moderately decreased risk of type 2 diabetes in various populations [295]. Moreover, many studies suggest that physical activity modifies the association of the Pro12Ala SNP with glucose homeostasis and the risk of type 2 diabetes [278-282,296-299].

A statistically significant difference between the Pro12Ala genotypes in insulin sensitivity improvement in response to exercise training has been reported in three different studies (Table 7). Firstly, the Ala12 carriers of the Pro12Ala SNP had a larger decrease in fasting plasma glucose in response to endurance or resistance training in 139 patients with type 2 diabetes [280]. Secondly, the Ala12 allele was associated with an improvement in insulin sensitivity after exercise training in 123 healthy Japanese men [278]. Thirdly, endurance training resulted in a greater improvement in insulin area under the curve during an oral glucose tolerance test in the Ala12 allele carriers among 32 sedentary men [279]. Furthermore, in a study among 29 normoglycaemic first-degree relatives of type 2 diabetic patients, insulin sensitivity tended to improve more in Ala12 carriers than in Pro12 homozygotes [281]. Physical activity may thus be more effective in improving insulin sensitivity in carriers of the Ala12 allele than among others. In a recent cross-sectional study among 236 non-Hispanic whites from Colorado, however,

the Ala12 allele was associated with a lower risk of type 2 diabetes among the individuals with a lower level of physical activity, whereas no difference between the genotypes was found among those with a higher level of physical activity [282]. It thus remains unclear whether the exercise-induced improvements in insulin sensitivity among the Ala12 carriers translate to benefits in the prevention of type 2 diabetes.

Overall, the present evidence concerning the details of gene-physical activity interactions in the development of type 2 diabetes is weak. The cross-sectional observational studies and various intervention studies have mostly included only small numbers of participants. No nested case-control studies (hybrid design) on the interaction between genes and physical activity have yet been performed. So far, only one cross-sectional study [282] and no prospective studies have looked at an interaction between a genetic variant and physical activity on the risk of type 2 diabetes. Moreover, as most of the detected interactions have not been replicated, there is a strong possibility that they represent false positive findings.

III AIMS OF THE STUDY

The main aim of the study was to detect gene variants that contribute to individual differences in the magnitude of the effects of physical activity on the incidence of type 2 diabetes in the Finnish DPS.

The following sub-themes were covered:

1. The associations of SNPs in the *PPARG2* gene with the incidence of type 2 diabetes and their interactions with physical activity (*Study I*).

2. The interactions of SNPs in the *SLC2A2, ABCC8,* and *KCNJ11* genes with physical activity on the incidence of type 2 diabetes (*Study II*).

3. The interactions of SNPs in the *ADRB2, ADRB3, TNF, IL6, IGF1R, LIPC, LEPR,* and *GHRL* genes with physical activity on the incidence of type 2 diabetes and on the changes in characteristics of the metabolic syndrome (*Study III*).

4. The interactions of SNPs in the *TNF* and *IL6* genes with physical activity on the changes in serum levels of CRP and IL-6 (*Study IV*).

IV METHODS

STUDY POPULATION AND DESIGN

The DPS is a randomized controlled trial with the main aim to investigate the effects of intensive lifestyle counselling on the incidence of type 2 diabetes in high-risk (overweight, glucose intolerant) individuals. Altogether 522 subjects (172 men, 350 women) aged 40–64 years were recruited, and were randomized into the intervention group (n = 265) or the control group (n = 257) [111,300].

The main goals of the lifestyle intervention were selected on the basis of knowledge on the major risk factors for type 2 diabetes [173,301-303]. The following goals were chosen: moderate-to-vigorous exercise ≥30 minutes per day, ≥5% reduction in body weight, intake of total fat ≤30% and saturated fat ≤10% of daily energy consumed, and intake of dietary fiber ≥15 grams per 1000 kilocalories of energy intake [300,304]. To reach the lifestyle goals, the participants in the intervention group received individualized lifestyle counseling via face-to-face consultation sessions with the study nutritionist. The sessions were organized seven times during the first year, and every three months thereafter. The goal was to look for individual ways for each participant to reduce weight, improve dietary habits and increase physical activity and, importantly, to equip the participants with the necessary knowledge and skills to achieve gradual but permanent behavioural changes [304].

In the counseling sessions, the dietary advice was based on 3-day food records which were completed four times yearly [304]. The level of physical activity was followed through annual physical activity questionnaires. Participants were encouraged to increase endurance exercise, such as walking, jogging, swimming, aerobic ball games, and skiing. Lifestyle physical activity was also promoted. In three of five centers supervised, progressive, individually tailored circuit-type resistance training was also offered to the participants of the intervention group free of charge.

The participants of the control group received general oral and written information about diet and exercise at baseline. The message to reduce weight, increase physical

activity, and make healthy changes in diet was the same as for the participants in the intervention group, but no specific individualised programs were offered [304].

ASSESSMENTS

Assessment of physical activity

Physical activity was quantified at baseline and during yearly follow-up visits by the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) 12-Month Leisure-Time Physical Activity Questionnaire (Appendix I) [305]. The questionnaire is designed to give detailed information on the most common conditioning and lifestyle leisure-time physical activities among middle-aged Finnish men. The duration, frequency and mean intensity of leisure-time physical activity are logged into the questionnaire as recalled over the previous year. Throughout the follow-up, 89-98% of those remaining in the study completed the forms [306].

The original physical activity estimates were adjusted by reducing the MET values assigned to different subjective intensities for given forms of physical activity by 30%. The adjustment was based on a bicycle exercise test among a sample of DPS participants where maximal oxygen uptake was on average ~30% lower than in the younger and leaner men who participated in the population-based KIHD cohort study [305].

For the physical activity analyses of the DPS, the average duration (minutes per week) of total leisure–time physical activity, moderate-to-vigorous physical activity, and low–intensity physical activity were calculated. Moderate-to-vigorous physical activity was defined as ≥3.5 METs, and low-intensity physical activity as <3.5 METs (1 MET is defined as metabolic expenditure at rest, corresponding to an oxygen uptake of 3.5 ml O_2/kg). The usual moderate-to-vigorous physical activities included moderate-to-high intensity walking, bicycling, swimming, resistance training, cross-country skiing, jogging, ball games, and lifestyle activities, such as chopping wood or clearing brush [306]. The common low-intensity activities included low-intensity walking or bicycling, yard work and gardening, and picking berries and mushrooms.

Assessment of diet

All study participants were asked to complete a 3-day food record at baseline and before every annual visit [304]. The nutrient intakes were calculated using a dietary analysis programme and database developed in the National Public Health Institute [307]. Due to the time-consuming and therefore costly dietary data management, only the data from baseline and years 1, 2, and 3 of the intervention period were included into the final database [307].

Assessment of overweight and obesity

Body weight and height were measured annually, and BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured midway between the lowest rib and iliac crest and hip circumference over the great trochanters, with 0.5 cm precision while the subject was standing. Overweight was defined as BMI 25.0-29.9 kg/m^2 and obesity was defined as BMI $\geq$30 kg/m^2 [300].

Assessment of glucose homeostasis and type 2 diabetes

Glucose homeostasis was assessed by 2-hour oral glucose tolerance tests (OGTT) annually in all subjects. The subjects were asked to fast and to refrain from strenuous exercise for 12 hours before the OGTT. Diabetes was defined according to the 1985 criteria of the World Health Organization as either a fasting plasma glucose concentration $\geq$7.8 mmol/l or a plasma glucose concentration $\geq$11.1 mmol/l two hours after a 75-g oral glucose challenge in an OGTT [308]. If type 2 diabetes was diagnosed in the first OGTT, a second OGTT was performed to confirm the diagnosis [300]. Plasma glucose levels were measured by the glucose oxidase method (Daiichi, Kyoto, Japan).

Other assessments

Blood pressure was measured twice from the right arm using a standard sphygmomanometer after 10 minutes of rest with the subject sitting. The mean of the

two measurements was used in the calculations. Serum insulin concentrations were measured with the two-site monoclonal antibody radioimmunoassay method (Pharmacia Diagnostica, Uppsala, Sweden). Serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured by an enzymatic assay (Boehringer Mannheim, Mannheim, Germany). Serum concentration of high-sensitivity CRP (hs-CRP) was assessed by an immunonephelometric assay (Dade Behring, Marburg, Germany). Serum interleukin-6 (IL-6) was assessed by an enzyme-linked immunosorbant assay (ELISA) using recombinant IL-6 and an antibody pair from Sanquin (Amsterdam, the Netherlands) [309].

Genotyping

The genotyping for the SNPs rs3856806 (His477His) in *PPARG2*, and –174C/G (rs1800795) in *IL6* was performed in 490 subjects by the PCR–SSCP (single–strand conformation polymorphism) method [115,310]. The SNPs in the *LEPR* and *GHRL* genes were genotyped for 507 subjects, and the SNPs in the *ADRB2*, *ADRB3*, *TNF*, *IGF1R*, and *LIPC* genes for 490 subjects by the RFLP (restriction fragment length polymorphism) method [115-117,120,122,124,311]. The rs1801282 in *PPARG2* was genotyped by the PCR–SSCP method for 490 subjects [310], and with the TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, California, USA) for 17 subjects. All other SNPs in *PPARG2*, and the SNPs in *TCF7L2* [123] were genotyped for 507 subjects using the TaqMan assays.

Statistical methods

The SPSS-software (Chicago, IL) was used for all statistical analyses. Haplotypes were reconstructed with SNPHAP, version 1.3 [312], which estimates haplotype frequencies using the expectation-maximization algorithm [313]. The genetic analyses were performed using either the dominant or the additive model. The dominant model was preferred in most cases because the number of individuals carrying the rare homozygous genotype was small.

Baseline differences in continuous variables among the genotypes were evaluated by the univariate ANOVA, general linear model. Logarithmic and square root transformations were used to transform variables that were not normally distributed to approximate normality before further statistical analyses. When the variance and normality assumptions were not met, the non-parametric Mann-Whitney U test or Kruskal-Wallis test was used. Baseline differences in categorical variables among the genotypes, and the deviations of genotype frequencies from the Hardy-Weinberg equilibrium were tested with the chi square test.

Cox regression analysis was used to assess whether the SNPs and haplotypes were associated with the progression from IGT to type 2 diabetes. Cox regression was also used to analyse the interactions of the SNPs and haplotypes with the lifestyle intervention, physical activity, or anthropometric characteristics on the progression from IGT to type 2 diabetes during the follow-up. The univariate ANOVA, general linear model, was used to assess interactions between physical activity and SNPs on characteristics of the metabolic syndrome or on the serum levels of hs-CRP and IL-6.

The original DPS ended after an average follow-up of 3.2 years when a total of 86 incident cases of type 2 diabetes were diagnosed [111]. The associations of SNPs with the risk of type 2 diabetes have therefore been analyzed using the first 3 years of the follow-up only. To increase statistical power for detecting interactions on the risk of type 2 diabetes, follow-up was extended by one year (average follow-up 4.1 years). During the 4.1-year follow-up, 116 of the 522 participants of the DPS developed type 2 diabetes. Of all the 522 subjects, 487 completed physical activity questionnaires at baseline and at least once during the intervention so that the average changes in physical activity could be calculated. These subjects were included in the present analyses on the interactions of SNPs with changes in physical activity. (Table 8). Of the 487 subjects, 107 developed type 2 diabetes during the follow-up.

The 1-year follow-up time was used in the analyses of interactions between polymorphisms and physical activity on the changes in characteristics of metabolic syndrome in *Study III*, as well on the changes in concentrations of serum hs-CRP and IL-6 in *Study IV* (Table 8). Only the 469 subjects who had 1-year data on physical activity available were included in these analyses. Of these individuals, 390 had 1-year

data on serum hs-CRP and IL-6, and thus only these subjects were included in *Study IV* (Table 8). In *Study IV* we also excluded all the 25 individuals who had hs-CRP levels >10 mg/L at baseline or at the 1-year examination to avoid the impact of acute infections or other proinflammatory conditions on the results. Furthermore, as it was previously found that regular exercise reduced hs-CRP levels only among individuals with high (≥3 mg/L) baseline levels of hs-CRP [314], the interactions between the polymorphisms and lifestyle changes on hs-CRP levels were analysed separately among those with high (≥3 mg/L) or lower (<3 mg/L) baseline levels of hs-CRP.

The changes in physical activity during the total length of the intervention were calculated by subtracting the baseline physical activity values (in hours per week) from averaged annual physical activity values. The changes in physical activity during the first year of the follow-up were calculated by subtracting the baseline physical activity values from the first-year physical activity values. Changes in anthropometric, dietary, and biochemical measures were calculated similarly, but the dietary intakes were available from the first 3 years of the follow-up only [307]. The intakes of total and saturated fat and fiber were adjusted by daily energy intake with linear regression analysis before further statistical analyses [306].

Table 8. Sample sizes, follow-up times, and studied genes, PA variables and outcomes in *Studies I-IV*.

Study	*N*	Genes	PA variables[1]	Outcomes	Follow-up times
Study I	487	*PPARG*	Total PA	Incidence of type 2 diabetes.	Average 4.1 years.
Study II	487	*SLC2A2* *ABCC8* *KCNJ11*	Total PA Moderate-to-vigorous PA	Incidence of type 2 diabetes. Changes in fasting and 2-hour glucose.	Average 4.1 years.
Study III	487	*ADRB2* *ADRB3* *TNF* *IL6* *IGF1R* *LIPC* *LEPR* *GHRL*	Total PA Moderate-to-vigorous PA	Incidence of type 2 diabetes. Changes in body mass index, waist circumference, systolic blood pressure, HDL cholesterol and triglycerides.	Average 4.1 years for the incidence of type 2 diabetes. 1 year for changes in continuous traits.
Study IV	390	*IL6* *TNF*	Moderate-to-vigorous PA	Changes in serum interleukin-6 and C-reactive protein.	1 year.

Abbreviations: *ABCC8*, ATP-binding cassette, sub-family C (CTFR/MRP), member 8; *ADRB2*, adrenergic receptor beta-2; *ADRB3*, adrenergic receptor beta-3; *GHRL*, ghrelin/obestatin prepropeptide; HDL, high-density lipoprotein; *IGF1R*, insulin-like growth factor 1 receptor; *IL6*, interleukin 6; *KCNJ11*, potassium inwardly rectifying channel; *LEPR*, leptin receptor; *LIPC*, hepatic lipase; PA, physical activity; *PPARG2*, peroxisome proliferator-activated receptor gamma 2; *SLC2A2*, solute carrier family 2 (facilitated glucose transporter), member 2; *TNF*, tumor necrosis factor.

[1]PA was measured in hours per week. Total PA refers to the total amount of PA and moderate-to-vigorous PA to the amount of moderate-to-vigorous intensity PA (a subcategory of total PA).

V RESULTS

The *PPARG* gene (*Study I*)

Seven SNPs in the *PPARG* gene were genotyped. Two of them, the rs1801282 and rs3856806 SNPs, were selected because of their associations with insulin resistance and type 2 diabetes in other studies [315]. The five other SNPs were chosen from the HapMap data [316], and from the study investigating the association of SNPs in *PPARG* with the response to troglitazone [317]. To investigate the effects of SNP combinations on the risk of type 2 diabetes, we reconstructed haplotypes of the seven SNPs.

Altogether the rs17036314, rs1801282 (Pro12Ala), rs4135263, rs2972162, rs2938395, and rs3856806 (His477His) SNPs covered 74% (with $r^2 \geq 0.8$ and MAF $\geq 5\%$) of the variation in the *PPARG* gene in the HapMap CEU (Utah residents with ancestry from northern and western Europe, NCBI build 35). In addition, the rs1152003 SNP covered the 3' flanking region of *PPARG*. The schematic locations of the SNPs along the *PPARG* gene are presented in Figure 2.

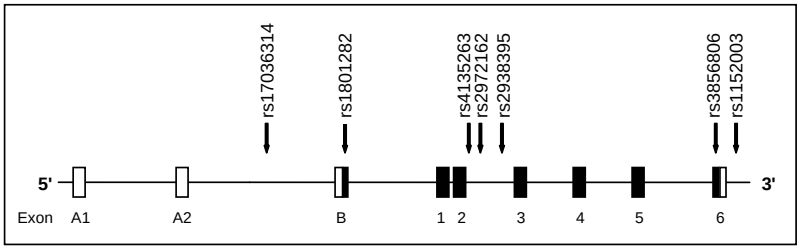

Figure 2. Positions of the SNPs in the *PPARG* gene. Exons are marked by black (coding region) or white (untranslated region) boxes. The coding region of the PPARγ1 is contained in the exons 1 to 6. The coding region of the PPARγ2 is contained in the exons B, and 1 to 6. Exons A1 and A2 encode the untranslated region of the PPARγ.

Baseline differences in fasting glucose

The carriers of the rare allele of rs17036314, rs1801282 (Pro12Ala), or rs3856806 (His477His), and the carriers of the common homozygous genotype of rs1152003, had higher fasting plasma glucose concentrations than subjects with the other genotypes (p = 0.029, p = 0.016, p = 0.004 or p = 0.035, respectively, adjusting for age, gender and BMI). Similarly, the carriers of the haplotype CGTCATC that contains the rare alleles of rs17036314, rs1801282 and rs3856806, and the common allele of rs1152003, had an increased fasting glucose compared with the non-carriers (p = 0.017).

Associations with the risk of type 2 diabetes

The rare alleles of rs17036314 and rs1801282 (Pro12Ala) were associated with the risk of type 2 diabetes in the DPS, but after the adjustment for the baseline fasting glucose, the association was significant only for rs17036314 (Figure 3) (p = 0.037 for rs1801282 without the adjustment for fasting glucose). The CGTCATC haplotype, containing the risk alleles of rs17036314 and rs1801282, had a stronger association with the risk of type 2 diabetes than the individual SNPs before adjustment for baseline fasting glucose (p = 0.022), but not after (p = 0.088).

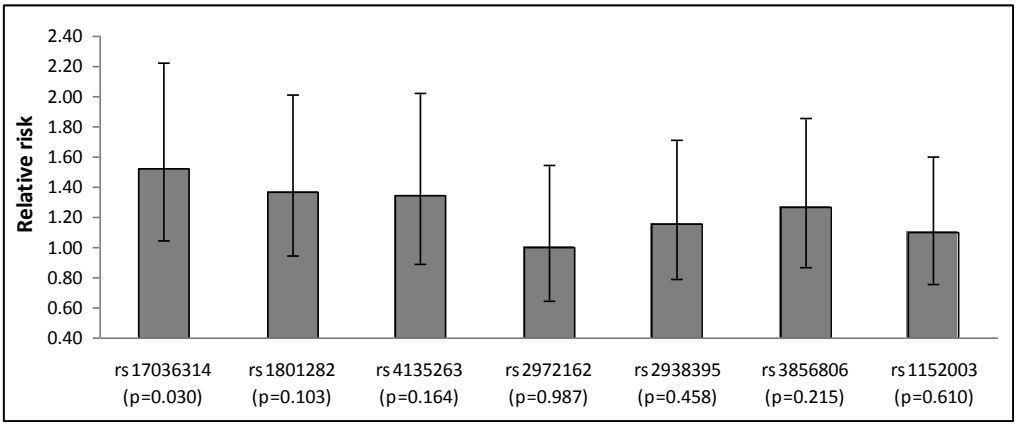

Figure 3. Relative risk (±95% CI) of progressing from impaired glucose tolerance to type 2 diabetes among genotypes (rare allele vs. common homozygous genotype) of SNPs in the *PPARG* gene. The values are adjusted for age, gender, intervention, fasting glucose and weight at baseline, as well as weight change during follow-up.

Although rs1152003 in the 3' flanking region of *PPARG* was not directly associated with the risk of type 2 diabetes, it had a significant interaction with the lifestyle intervention and tended to increase the risk of type 2 diabetes in the intervention group (Figure 4). Adjustment for rs1801282 (Pro12Ala) genotype did not affect the associations.

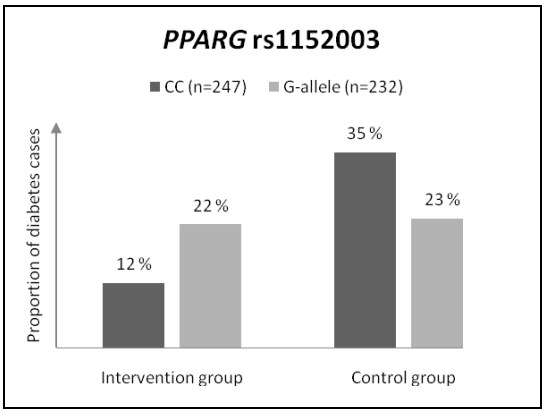

Figure 4. New cases of type 2 diabetes in the intervention and control groups according to genotypes of rs1152003 in *PPARG*. Percentages show the proportion of genotype carriers who progressed to type 2 diabetes. Relative risk for G-allele carriers was 1.9-fold (95% CI 1.0-3.6; p=0.05) in the intervention group and 0.8-fold (95% CI 0.5-1.3; p=0.29) in the control group, adjusting for age, gender, fasting glucose and weight at baseline, and weight change. p=0.027 for the adjusted interaction between group and rs1152003 genotype.

Interactions with physical activity

Apart from their direct associations with type 2 diabetes, rs17036314 and rs1801282 (Pro12Ala) interacted with changes in the total amount of physical activity, categorized by median (0.6 h/wk), on the risk of type 2 diabetes during the follow-up (Figure 5). The risk alleles were associated with type 2 diabetes only in the lower half of the change in physical activity, whereas no association was seen in the upper half.

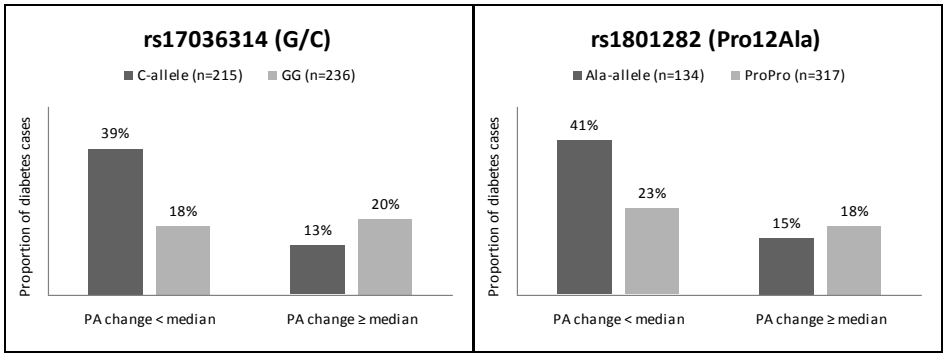

Figure 5. New cases of type 2 diabetes among genotypes of the rs17036314 and rs1801282 SNPs in *PPARG* according to the change in physical activity (PA) during 4.1-years of follow-up. Relative risks for rare allele carriers were 2.5-fold (95% CI 1.4-4.4) and 0.6-fold (95% CI 0.3-1.2) for rs17036314, and 2.2-fold (95% CI 1.3-3.7) and 0.7-fold (95% CI 0.3-1.4) for rs1801282 in the upper and lower half of the change in physical activity, respectively, adjusting for age, gender, intervention, weight, dietary risk factors, baseline physical activity, and baseline fasting glucose. p = 0.002 and p = 0.031 for the adjusted interaction between the change in physical activity and rs17036314 or rs1801282 genotype, respectively.

The *SLC2A2*, *ABCC8*, and *KCNJ11* genes (*Study II*)

Four SNPs from both the *SLC2A2* (rs5393, rs5394, rs5400, and rs5404) and the *ABCC8* (rs3758947, rs2188966, rs3758953, and rs1799859) genes, and one SNP (rs5219) from the *KCNJ11* gene were investigated. However, as the genotype distribution of *ABCC8* rs2188966 deviated from the Hardy-Weinberg equilibrium ($p < 0.001$), it was excluded from all further analyses. The schematic positions of the SNPs in *SLC2A2* and *ABCC8* are presented in Figure 6. The E23K SNP (rs5219) was located in exon 1 of *KCNJ11*.

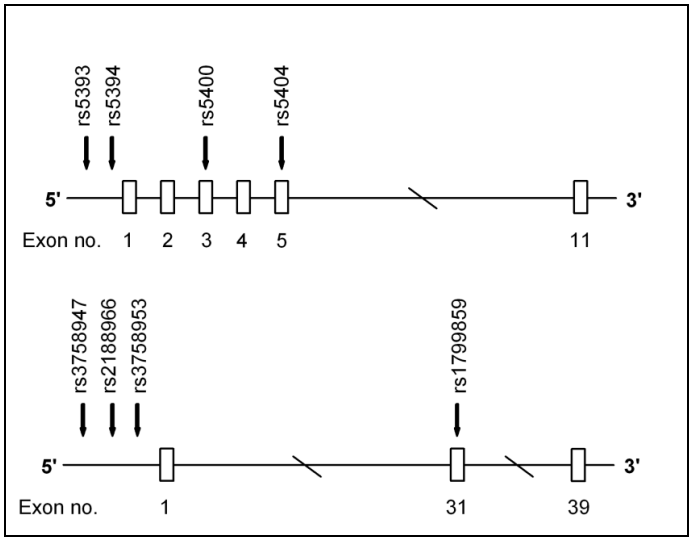

Figure 6. Positions of the SNPs in the *SLC2A2* (upper) and *ABCC8* (lower) genes. Exons are marked with white boxes.

In addition to the individual SNPs, we reconstructed haplotypes based on the four SNPs in *SLC2A2* that were in close linkage disequilibrium with each other (r^2>0.6 between each pair of SNPs). Seven haplotypes (reconstructed of rs5393, rs5394, rs5400, and rs5404) were found: ACCG, CTTA, ACTG, CCTG, CCTA, CTTG, and ACTA.

Interactions with physical activity

A significant interaction with the change in moderate-to-vigorous physical activity on the risk of type 2 diabetes was found for rs5393, rs5394, and rs5404 in *SLC2A2* (p = 0.027, p = 0.022, and p = 0.022 for the interaction, respectively, adjusting for age, gender, intervention, body weight and diet). Similarly, the CTTA haplotype that contained the rare alleles of all four genotyped SNPs had an interaction with physical activity (p = 0.021) (Figure 7). Those who did not carry the CTTA haplotype tended to have a higher risk of type 2 diabetes than the CTTA carriers in the lower tertile of the change in physical activity, but tended to have a lower risk in the upper tertile. Consistently, there was an interaction between the CTTA haplotype and moderate-to-vigorous physical activity on the change in 2-h plasma glucose during the follow-up

(Figure 8). Those who did not carry the CTTA haplotype had an increase in 2-h glucose levels compared to the CTTA carriers in the lower tertile of physical activity, whereas no significant difference between genotypes was seen in the upper tertile.

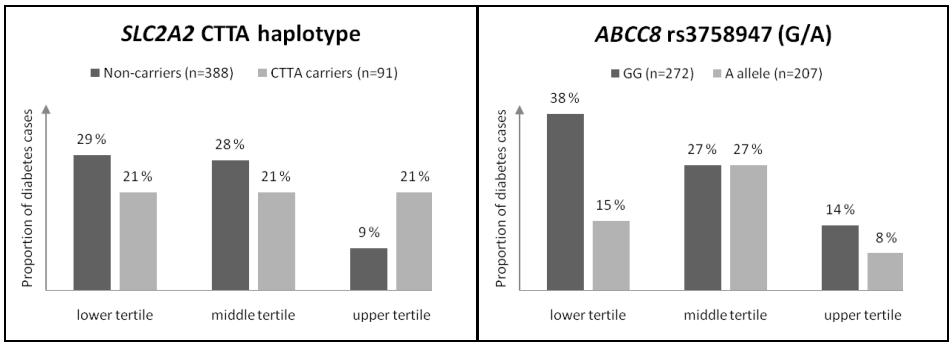

Figure 7. New cases of type 2 diabetes among carriers and non-carriers of *SLC2A2* CTTA haplotype or A-allele of *ABCC8* rs3758947 SNP in tertiles of change in moderate-to-vigorous physical activity during 4.1-years of follow-up. Relative risks in the upper, middle, and lower tertile of physical activity were 2.2-fold (95% CI 0.9-5.2), 1.6-fold (95% CI 0.7-3.5) and 0.4-fold (95% CI 0.1-1.3) for non-carriers of CTTA, and 5.6-fold (95% CI 2.5-1.3), 1.0-fold (95% CI 0.5-1.8) and 1.2-fold (95% CI 0.4-3.7) for non-carriers of A-allele of rs3758947, respectively, adjusting for age, gender, intervention, weight, and dietary risk factors. p = 0.021 and p = 0.007 for the adjusted interaction between change in moderate-to-vigorous physical activity and the carrier status of *SLC2A2* CTTA or rs3758947 A-allele, respectively.

Similarly with the CTTA haplotype of *SLC2A2*, rs3758947 of *ABCC8* interacted with moderate-to-vigorous physical activity on the conversion to type 2 diabetes (p = 0.007, adjusting for age, gender, intervention, weight and diet) (Figure 7). The carriers of the common homozygous genotype of rs3758947 had an increased risk of type 2 diabetes in the lower tertile of the change in moderate-to-vigorous physical activity, whereas no statistical difference between the genotypes was seen in the middle or upper tertiles. Consistently, the rs3758947 also interacted with physical activity on the change in 2-hour plasma glucose (Figure 8). Those who carried the common homozygous genotype had an increase in 2-h glucose levels compared to rare allele carriers in the lower tertile of physical activity, whereas no significant difference between genotypes was seen in the middle or upper tertiles.

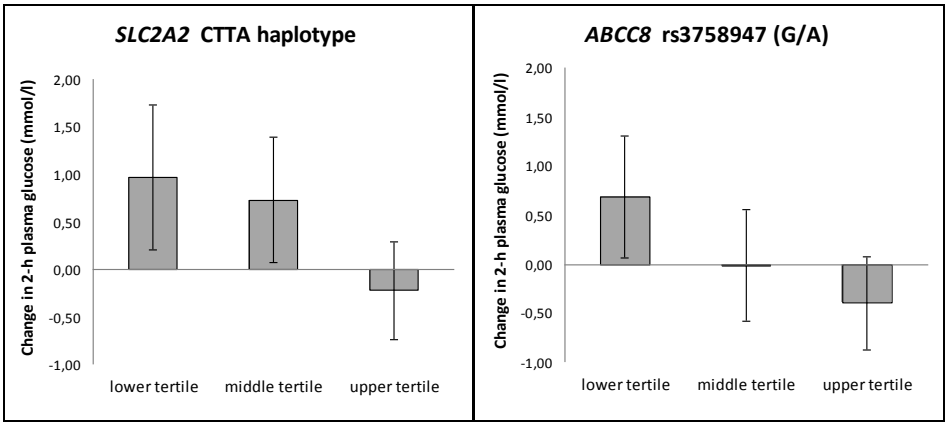

Figure 8. Difference (± 95% CI) in the change in 2-h plasma glucose between non-carriers vs. carriers of *SCL2A2* CTTA haplotype and between non-carriers vs. carriers of A-allele of *ABCC8* rs3758947 SNP in tertiles of change in moderate-to-vigorous physical activity during 4.1-years of follow-up. The values are adjusted for age, gender, intervention, weight, dietary risk factors, and baseline 2-h glucose. $p = 0.022$ and $p = 0.015$ for the adjusted interaction between change in moderate-to-vigorous physical activity and the carrier status of the CTTA haplotype or the rs3758947 A-allele, respectively.

No interaction with physical activity was found for the E23K SNP (rs5219) in *KCNJ11* (data not shown).

The *ADRB2*, *ADRB3*, *IGF1R*, *LIPC*, *LEPR*, *GHRL*, and *TCF7L2* genes (*Study III*)

One SNP from each of the *ADRB2*, *ADRB3*, *IGF1R*, and *LIPC* genes, three SNPs of the *LEPR* gene, six SNPs of the *GHRL* gene, and two SNPs of the *TCF7L2* gene were investigated [92-94,97,99-101]. The genotypes of the SNPs in *ADRB2*, *ADRB3*, *IGF1R*, and *LIPC* were available for 490 subjects, and the genotypes of the SNPs in *LEPR*, *GHRL*, and *TCF7L2* were available for 507 subjects.

Baseline differences in physical activity

The baseline level of total physical activity differed among two SNPs in *GHRL*. The level of physical activity was higher in the carriers of the Met allele of rs696217

(Leu72Met) and in the carriers of the A allele of rs26802 (–501A/C) in *GHRL* than in those carrying the common homozygous genotypes (p = 0.032 and p = 0.034, respectively).

Interactions with physical activity

There were no significant interactions between the SNPs in the *ADRB2, ADRB3, IGF1R, LIPC, LEPR, GHRL,* or *TCF7L2* genes and the change in total or moderate-to-vigorous physical activity on the risk of type 2 diabetes. However, interactions of SNPs in *LEPR* and *GHRL* with physical activity on changes in some characteristics of the metabolic syndrome were found.

Rs696217 (Leu72Met) in *GHRL* modified the effect of moderate–to–vigorous physical activity on changes in BMI and waist circumference during the first year of the intervention of the DPS (p = 0.001 and p = 0.006 for the interaction on BMI and waist circumference, respectively) (Figure 9). BMI and waist circumference decreased more with increasing tertiles of physical activity in the carriers of the Met allele than among the carriers of the common homozygous genotype.

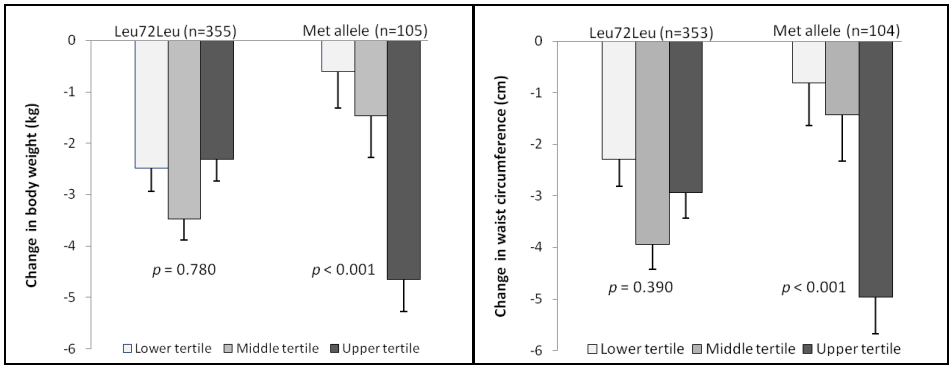

Figure 9. 1-year changes (mean and standard error of mean) in body weight and waist circumference among the genotypes of *GHRL* rs696217 (Leu72Met) SNP in tertiles of change in moderate-to-vigorous physical activity. *p*-values are for the linear trend. The values are adjusted for age, gender, intervention, energy intake, and the baseline values of the dependent variable and physical activity. p = 0.001, and p = 0.006 for the adjusted interactions on body weight and waist circumference, respectively.

Rs26802 (–501A/C) in *GHRL* interacted with the change in total physical activity on the change in serum HDL cholesterol concentrations (p = 0.005 for the interaction) (Figure 10). The HDL cholesterol concentration increased in the middle and upper tertiles of physical activity among the carriers of the common homozygous genotype, but no increase in HDL cholesterol was seen in the carriers of the C allele.

Rs1137100 (Lys109Arg) in *LEPR* modified the effect of change in total physical activity on change in systolic blood pressure (p = 0.017) (Figure 10). Systolic blood pressure decreased only in individuals with the Lys109Lys genotype who increased their physical activity, whereas the carriers of the Arg109 allele seemed unresponsive to changes in physical activity.

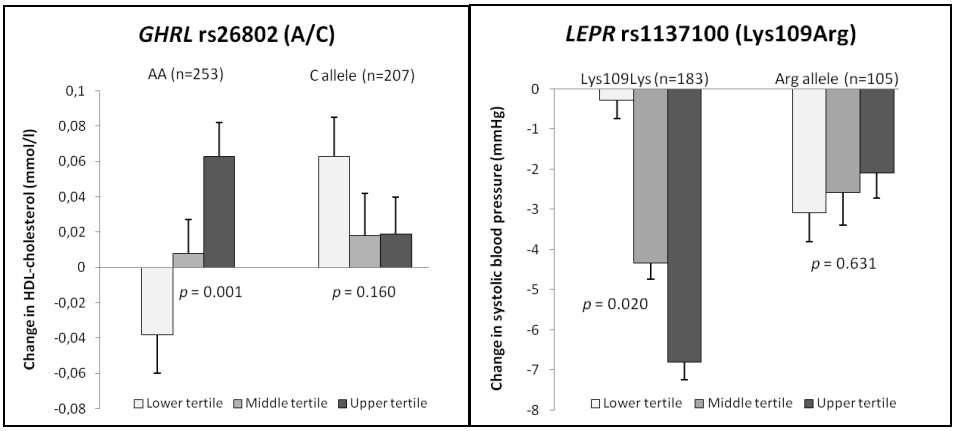

Figure 10. 1-year changes (mean and standard error of mean) in high-density lipoprotein (HDL) cholesterol or systolic blood pressure among the genotypes of *GHRL* rs26802 or *LEPR* rs1137100 (Lys109Arg) SNP, respectively, in tertiles of change in total physical activity. *p*-values are for the linear trend. The values are adjusted for age, gender, intervention, body mass index, and the baseline values of the dependent variable and physical activity. The rs26802 was additionally adjusted for fat intake. p = 0.005, or p = 0.017 for the adjusted interaction between physical activity and *GHRL* rs26802 or *LEPR* rs1137100 SNP, respectively.

The *TNF* and *IL6* genes (*Studies III & IV*)

Rs1800629 (G-308A) in *TNF* and rs1800795 (C-174G) in *IL6* were genotyped from 490 participants of the DPS.

Interactions with physical activity

Rs1800629 in *TNF* and rs1800795 in *IL6* were not directly associated with changes in the levels of hs-CRP or IL–6 during the first year of the DPS. However, rs1800629 in *TNF* interacted with the 1-year change in moderate-to-vigorous physical activity on the 1-year change in serum concentration of hs-CRP among those with high (≥3 mg/L) baseline levels of hs-CRP (p = 0.019, adjusting for age, gender, study group, baseline BMI and 1-year change in BMI). Those who carried the GG genotype of rs1800629 decreased their serum levels of hs-CRP by increasing physical activity, whereas no such effect was seen in the carriers of the A-allele (Figure 11). There was no interaction between rs1800629 and moderate-to-vigorous physical activity among those with lower (<3 mg/L) baseline levels of hs-CRP (p = 0.973). No interaction with physical activity was found between rs1800795 in *IL6* and changes in physical activity on the 1-year changes in serum hs-CRP or IL–6.

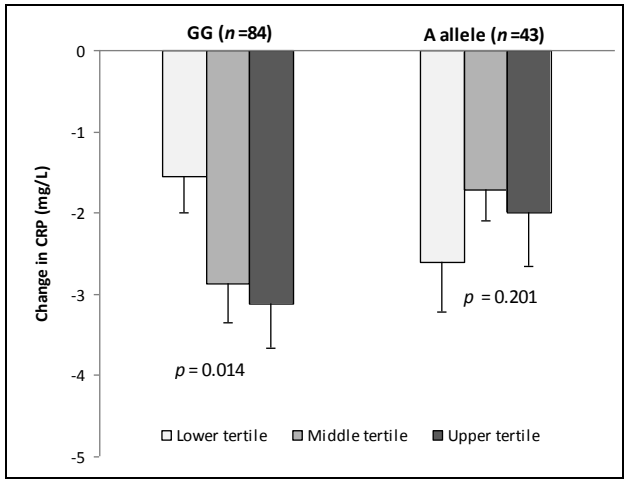

Figure 11. Changes of serum hs-CRP among genotypes of rs1800629 in *TNF* according to tertiles of change in moderate-to-vigorous physical activity during the first year of the Finnish Diabetes Prevention Study. Only the individuals with high (≥3 mg/L) baseline levels of hs-CRP are included (n = 127). Bars indicate adjusted mean of 1-year change in serum hs-CRP in each tertile of 1-year change in moderate-to-vigorous physical activity. Error bars indicate standard error of the mean. The values are adjusted for age, gender, study group, baseline hs-CRP, baseline moderate-to-vigorous physical activity, baseline body mass index (BMI), and 1-year changes in BMI. *P*-values are for the linear trend. p = 0.034 for the adjusted interaction between the change in moderate-to-vigorous physical activity and rs1800629.

In *Study III*, no interaction between the SNPs in *TNF* and *IL6* and physical activity on the risk of type 2 diabetes, or changes in the components of metabolic syndrome (body weight, waist circumference, HDL cholesterol, triglycerides, or systolic blood pressure) was found.

VI DISCUSSION

STUDY DESIGN AND SUBJECTS

The identification and characterization of genes influencing the risk of complex multifactorial disease such as type 2 diabetes is a challenging task. However, unravelling the interactions between genes and lifestyle in the development of these conditions may be even more challenging. Firstly, most gene variants associated with common disease have only low or modest effects [13,14]. Secondly, lifestyle factors such as physical activity or dietary intake are difficult to quantify precisely [15-17]. Thirdly, several lifestyle and environmental factors confound the analyses. The detection of gene-lifestyle interactions thus requires large study populations and careful measurement of various lifestyle and environmental exposures.

In the present study, the interactions of physical activity with gene variants were analysed prospectively with type 2 diabetes as the clinical outcome. To our knowledge, no other studies have yet been performed with a similar analysis strategy. The study was based on the data of the Finnish DPS, a randomized controlled trial on the prevention of type 2 diabetes. The subjects were recruited from five participating centers, including Helsinki, Kuopio, Oulu, Tampere, and Turku [111,300]. All participants were overweight and met the WHO criteria for IGT twice, and were thus at a high risk of developing type 2 diabetes. The selection of high-risk individuals increases the statistical power for detecting interactions. However, the disadvantage is that there may be covert selection by genotype, limiting the generalisability of the results. The results also reflect fairly short-term metabolic responses in individuals who have already developed glucose abnormalities, and thus do not give information on how genetic factors increase the risk of type 2 diabetes over lifetime.

Sample size is a key determinant of the quality of a genetic association study [318]. The population in the present study included 507 participants of the DPS for whom genotype data were available. The size of the study was modest for an interaction study [254] and especially for SNPs with low minor allele frequencies [134]. The precision of

the methods used for measuring physical activity also plays an important role in detecting gene-physical activity interactions [15]. We assessed physical activity with a self-administered questionnaire, which is a fairly imprecise measure of physical activity. The statistical power of the present study is thus limited for investigating interactions. Furthermore, the original DPS used a multi-component intervention where physical activity, dietary changes, and weight loss were combined in a single intervention arm [300,304]. The yearly measurement of changes in diet, physical activity and body weight allowed statistical disentanglement for the other interventions [306]. Statistical adjustments do not, however, allow the evaluation of the independent role of physical activity to the same certainty as a randomised controlled trial containing an exercise-only intervention.

Despite its limitations, the DPS provides a more powerful framework for the assessment of gene-physical activity interactions than cross-sectional epidemiological studies and many other prospective studies. The strengths of the present study lie on the randomized and controlled design of the DPS, the detailed assessment of physical activity using a validated questionnaire, and the repeated measurement of physical activity and other lifestyle exposures. As the present studies investigate, however, association and do not test cause and effect, the replication of genetic associations in independent large-scale study populations is critical.

METHODS

Diagnosis of diabetes

The 1985 WHO criteria for diabetes [308] were used in all analyses of the present study despite newer criteria have been published in 1999 [1]. In the new criteria, the cut point of fasting glucose for the diagnosis of diabetes was lowered from 7.8 to 7.0 mmol/L, which could slightly affect our results. The 1985 criteria were, however, in use when the Finnish DPS was planned and through most of the trial itself, and all data on the DPS

have thus been published using them. Therefore, we used the same diagnostic criteria to maintain comparability with other published data in the DPS.

Measurement of physical activity

The self-reported data on physical activity was collected through the KIHD questionnaire (Appendix I) [305]. The questionnaire has provided valuable information on physical activity and health outcomes in the KIHD Study [319-322]. The validity of the questionnaire has been tested against VO_{2max} [323], and the questionnaire is also quite repeatable [305]. Nonetheless, measurement error is still large. The yearly measurement of physical activity and the use of averaged physical activity levels during the follow–up, however, reduce measurement variability. Average physical activity values also reflect the actual levels of physical activity throughout the study better than a single follow-up measurement. The disadvantage is that there may be differential measurement error, as those individuals who did not develop diabetes were followed-up longer and were more likely to have multiple measurements. Physical activity is thus more precisely measured in these individuals, which may lead to slight underestimation or overestimation of our results [324].

Objective measurement of physical activity would have been desirable in the Finnish DPS, because it increases the precision of physical activity estimates [325]. However, it was not feasible at the time the DPS was carried out, and it would have been exceedingly expensive to assess changes in physical activity of individuals over time in a way that also accurately takes into account the substantial seasonal changes in physical activity patterns that occur in countries such as Finland that have four seasons. The rapid development of physical activity monitors will make objective measurement of physical activity viable in future trials.

Measurement of dietary intake

The intakes of total energy, fat, saturated fat, and fiber were used as covariates in the gene-physical activity interaction analyses, and they were measured by the 3-day food records. Although the precision of the food record method is likely sufficient for the

estimation of energy-adjusted macronutrient intakes [326], the dietary data was unfortunately available only from baseline and years 1, 2, and 3 of the intervention period, whereas physical activity data were available from all six years of the intervention. However, it has been shown that the dietary changes achieved during the first three years remained fairly constant even three years after the intervention period ended [327]. Therefore, the lack of dietary data from the latter years of the intervention should only slightly impair the accuracy of the analyses.

Genotyping

Three different methods were used for the genotyping of the candidate gene polymorphisms: PCR-SSCP, RFLP, and TaqMan assays. Although each method is widely used and reliable, the chance of genotyping errors cannot be neglected. A genotyping error rate of between 0.5% and 1% has been usual in many laboratories [328,329]. To avoid substantial genotyping errors and to calculate genotyping error rate, it has been recommended that 5-10% randomly chosen samples should be regenotyped [330,331]. In the DPS, we confirmed the accuracy of the genotyping of SNPs in the *SCL2A2*, *ABCC8*, *TCF7L2*, and *GHRL* genes by replicating a randomly selected subset of samples. Regenotyping of 6.0% of samples in the *SLC2A2* and *ABCC8* genes (*Study II*), 7.1% of the samples in the *TCF7L2* gene [123], and approximately 10% of the samples in the *GHRL* gene [332] gave 100% identical results. With regard to rs1801282 (Pro12Ala) of *PPARG2*, all the samples that were hetero- or homozygous for the Ala12 allele were reanalysed to confirm the genotype [333]. However, there is no information on the genotyping errors or genotyping error rates for the other genes. A test for Hardy-Weinberg equilibrium was, however, performed for each polymorphism, and a significant deviation from the equilibrium is often the result of genotyping errors [334]. Only one of the studied SNPs (rs2188966 in *ABCC8*) differed from Hardy-Weinberg equilibrium, and was thus omitted from all statistical analyses in *Study II*.

Selection of SNPs

Although it is possible that interaction effects exist in the absence of detectable main effects, strong associations of both the measured lifestyle factor and the genetic variant with the outcome increases the likelihood of detecting interactions [254]. As the statistical power of the DPS was limited, we focused on physical activity that was strongly associated with the risk of developing type 2 diabetes in the DPS [170]. With regard to candidate genes, we mainly investigated such genetic variants which had been detected to be associated with the risk of type 2 diabetes in the DPS. This approach was supported by the finding that, for most of the genes associated with type 2 diabetes in the DPS, the association with the risk was more pronounced in either the intervention group [115-120] or the control group [114,119,121-123] of the DPS. This indicated that lifestyle changes modified the association of the polymorphisms with the incidence of type 2 diabetes. In *Study IV*, to avoid multiple testing, we performed a replication study on two SNPs in the *TNF* and *IL6* genes that were shown to interact with physical activity on the serum levels of hs-CRP and IL-6 in previous studies [335,336]. Replication studies provide insurance against errors and biases that can afflict any individual study, and amplify confidence that the associations reflect processes that are biologically interesting rather than methodological inadequacies [134,337].

Statistical analyses

In the Finnish DPS, participants in the intervention group achieved significantly greater lifestyle changes than individuals in the control group [304]. This was also seen as a 58% reduction in the risk of developing type 2 diabetes [111]. However, in the physical activity analyses of the DPS we combined the intervention and control groups instead of analysing the groups separately. Combining study groups was necessary, as it gave a larger sample size and more power to detect interaction effects than a sub-group analysis. Combining the groups was also statistically plausible, as the difference between the intervention and control groups in the changes in physical activity was modest compared with the overall changes in physical activity, and changes in physical activity were associated with a lower risk of type 2 diabetes in both groups [306]. To

adjust for differences between two study groups we used the group assignment as a factor in the statistical models.

Absolute changes in physical activity were used in all our analyses instead of relative (%) changes in physical activity, and differences in the baseline level of physical activity were accounted for by adding baseline physical activity as a covariate in statistical models. The use of relative changes in physical activity would have had the advantage that it directly accounts for baseline differences in physical activity. However, the use of relative changes was not feasible in the present analyses, as individuals with very low physical activity values would, in some cases, have shown very large relative changes in physical activity, whereas the relative changes would have been small in the individuals with high baseline physical activity. Therefore, using absolute changes in physical activity with adjustment for the baseline physical activity was a more favourable option.

In the DPS, changes in total physical activity, but also in moderate-to-vigorous intensity physical activity and low-intensity physical activity, were associated with the incidence of type 2 diabetes [306]. In the present analyses, we mainly chose to study the interactions of gene variants with the changes in total and moderate-to-vigorous physical activity on the risk of type 2 diabetes or changes in metabolic outcomes. The low-intensity physical activity category was of less interest, as it is physiologically unlikely that an interaction with low-intensity physical activity would be apparent without an interaction with the total or moderate-to-vigorous physical activity that more likely has metabolic and other health effects [144,320,323,338]. Furthermore, the amount of low-intensity physical activity did not change in either study group of the DPS, indicating that changes in low-intensity physical activity did not explain the decrease in the incidence of type 2 diabetes brought about by the intervention [306]. The size of the DPS was also not sufficient to study the associations between different forms of physical activity [306] and genetic polymorphisms on clinical outcomes.

We used two different follow-up times in our analyses on gene-physical activity interactions. In particular, 1-year changes in physical activity were used to study interactions on the changes in the characteristics of metabolic syndrome and serum levels of hs-CRP and IL-6, whereas the total length of the follow-up was exploited with

regard to the analyses on the risk of type 2 diabetes. These follow-up times were chosen to increase statistical power for detecting interactions. Changes in the components of metabolic syndrome and in lifestyle were greatest during the first-year of the intervention of the DPS, and a slight relapse occurred towards the later years [327,339]. On the other hand, diabetes cases accumulated steadily over the whole length of the study [327]. Therefore, genotypic differences in the changes in characteristics of metabolic syndrome were most likely to be found during the first year of the intervention, whereas with regard to type 2 diabetes, the increase in the number of cases achieved during the whole length of follow-up was beneficial.

As the sample size of the DPS was modest, the power of the present study to detect differences in additive genotypic models (i.e. three genotypic groups of an SNP), and especially for SNPs with low minor allele frequencies, was limited. Therefore, in nearly all analyses, the subjects who were hetero- or homozygous for the rare allele were combined and compared with subjects who were homozygous for the common allele. This approach (the dominant model) may have led to the loss of some biological information, if the effect of the minor allele on the outcome was additive.

Finally, a large number of statistical tests were performed in the present study. The positive association of a genetic variant with the disease or the trait does not necessarily mean a causal relationship since the association can represent a false positive finding either randomly or, for example, due to selection bias. On the other hand, the absence of association does not necessarily indicate a true negative finding, but may result from the lack of power to detect an association. Therefore, to confirm that the observed associations are true, the results should be repeated in independent study samples.

MAIN FINDINGS

The main findings of the studies on the interaction of physical activity with genetic variants in the DPS are summarised in the Table 9.

Table 9. Summary of interactions of candidate genes with physical activity in the Finnish Diabetes Prevention Study.

Gene	Main finding	Reference
ADRA2B	Increased physical activity did not decrease the risk of type 2 diabetes in the carriers of the risk genotype	[340]
PPARG	Increased physical activity removed the effect of the risk genotypes on the risk of type 2 diabetes	*Study I*
SLC2A2, ABCC8	Increased physical activity removed the effect of the risk genotypes on the risk of type 2 diabetes	*Study II*
GHRL, LEPR	The responses of BMI and HDL cholesterol (*GHRL)* or systolic blood pressure (*LEPR)* to changes in physical activity differed among the genotypes	*Study III*
TNF	Physical activity lowered serum CRP only in the carriers of the common homozygous genotype	*Study IV*

Abbreviations: *ABCC8*, ATP-binding cassette, sub-family C (CFTR/MRP), member 8; *ADRA2B,* adrenergic receptor alpha-2B; BMI, body mass index; CRP, C-reactive protein; *GHRL*, ghrelin/obestatin prepropeptide; HDL, high-density lipoprotein; *LEPR*, leptin receptor; *PPARG*, peroxisome proliferator-activated receptor gamma; *SLC2A2*, solute carrier family 2 (facilitated glucose transporter), member 2; *TNF*, tumor necrosis factor (TNF superfamily, member 2)

The PPARG gene (Study I)

In the *Study I*, we extended the previous analyses of the DPS on the Pro12Ala SNP in *PPARG2* [114] by genotyping six additional SNPs from selected regions of *PPARG*. We showed that the carriers of the rare allele of rs17036314 or rs1801282 (Pro12Ala), which were part of the same haplotype block, had an increased risk of conversion from IGT to type 2 diabetes compared with the carriers of the common homozygous genotype. Furthermore, we showed that changes in physical activity during the intervention modified the effects of rs17036314 and rs1801282 on the risk of type 2 diabetes. We also showed that the distinct rs1152003 SNP in the 3' flanking sequence of *PPARG* had an interaction with the lifestyle intervention of the DPS, and it was associated with a lower risk of type 2 diabetes in the intervention group, independent of rs1801282 (Pro12Ala) genotype.

Associations with type 2 diabetes

Unexpectedly, carriers of the Ala12 allele had an increased risk of type 2 diabetes compared with Pro12 homozygotes in the DPS (Study I) [114]. In contrast, the Ala12 allele has been associated with increased insulin sensitivity and a moderately decreased

risk of type 2 diabetes in various populations [295]. In the largest meta-analysis among 42,910 individuals, the Ala12 carriers had a 19% reduction in the risk of type 2 diabetes [341].

Several factors may have modified the association of the Pro12Ala SNP with the risk of type 2 diabetes in the DPS. Apart from increasing the risk of type 2 diabetes, the Ala12 allele increases the risk of obesity, which may compromise its beneficial effects on insulin sensitivity [342]. Other sequence variants in *PPARG2* may also modify the effect of the Pro12Ala SNP on these metabolic traits [343-346]. Variation in more than one haplotype block of *PPARG* is associated with improvements in insulin sensitivity with the *PPARG* agonist troglitazone [317], and it is logical to expect similar diversity with respect to *PPARG* and the risk of type 2 diabetes. Furthermore, particular coactivators or corepressors may modulate the effects of variants in *PPARG* [347]. Accumulating evidence also supports the role of *PPARG2* as a mediator between lifestyle and the regulation of metabolism [278-281,296-299].

The benefits of the Ala12 allele may also have been compromised by existing defects in insulin secretion among the participants of the DPS. Firstly, the earlier analyses of the DPS on the Pro12Ala SNP showed that the Ala12 allele was associated with the risk of type 2 diabetes particularly in the less obese half of the subjects (mean BMI 27.7 ± 1.6 kg/m^2), in whom the reason for IGT might more be related to impairments in insulin secretion than in insulin action [114]. Secondly, in *Study II* we also found that the Ala12 allele was associated with higher fasting glucose at baseline, whereas a meta-analysis indicated a significantly lower fasting glucose concentration in the Ala allele carriers than in the Pro12 homozygotes among obese nondiabetic subjects [342]. Therefore, the association of the Ala12 allele with an increased risk of type 2 diabetes could be due to the selection of high-risk individuals with IGT, who already have a decreased insulin secretion capacity. *PPARG* has been shown to modify glucose-induced insulin secretion by activating the expression of *GLUT2* and *CAP1* genes in pancreatic β-cells [348], and a functional PPAR response element has been identified in the *GLUT2* gene promoter [349]. The SNPs in the promoter region of *GLUT2* were strongly associated with the risk of type 2 diabetes in the DPS [121].

Interactions with physical activity

In *Study I*, we showed that the carriers of the Ala12 allele were more responsive to changes in physical activity than the non-carriers in the DPS. The result is consistent with most of earlier studies. Altogether four studies have assessed the relationship between the Pro12Ala SNP and exercise-induced changes on insulin action [278-281]. All four studies indicated that the Ala12 allele carriers are more responsive to the insulin-sensitizing effect of exercise training. In sedentary Japanese men, three months of supervised exercise training improved insulin action more in six Ala12 allele carriers than in 117 Pro12 homozygotes [278]. Similarly, six months of supervised endurance training in sedentary men resulted in significantly greater decreases in fasting insulin and insulin area under the curve in an OGTT in Pro12Ala heterozygotes (n=8) compared with Pro12 homozygotes (n=24) [279]. In a study among 29 healthy first-degree relatives of patients with type 2 diabetes, insulin sensitivity tended to improve more in the Ala12 carriers than in the Pro12 homozygotes in response to a 10-week training program, but not in 17 individuals without a family history of type 2 diabetes [281]. Three months of supervised exercise training was also associated with greater decreases in fasting plasma glucose levels in 139 patients with type 2 diabetes carrying the Ala12 allele in comparison to the Pro12 homozygotes [280]. However, in contrast to *Study I*, a recent cross-sectional study among 236 non-Hispanic whites from Colorado reported that the Ala12 allele was associated with a lower risk of type 2 diabetes only among the individuals with a lower level of physical activity [282], whereas in the DPS, the Ala12 allele was associated with a higher risk in the less active individuals.

The Ala12 allele of the Pro12Ala SNP replaces an amino acid in PPARγ2, a key transcription factor in the regulation of adipocyte differentiation. PPARγ2 is particularly expressed in white adipose tissue, and thus its effects on glucose homeostasis may be due to changes in adipose tissue metabolism [315]. Indeed, [350,351], although not all [352] studies have indicated that the Ala12 allele reduces the transcriptional activity of *PPARG2*, leading to an enhanced ability of insulin to suppress lipolysis in adipocytes. Lower amounts of FFAs are thus liberated into the circulation in Ala12 carriers [353]. The reduced availability of FFAs permits glucose to be preferentially oxidized as a fuel source in the skeletal muscle, thereby improving overall glucose homeostasis [354].

Consistent with this hypothesis, a study among type 2 diabetics reported lower plasma FFA levels at baseline and after exercise training in the Ala12 carriers compared with Pro12 homozygotes [280].

PPARG may also enhance glucose homeostasis through other mechanisms. Agonists of the *PPARG* gene increase the expression and plasma levels of adiponectin, a putative insulin-sensitizing hormone [355]. In a study among Finnish servicemen, significant increases in plasma adiponectin were seen in the carriers of the Ala12 allele after weight loss induced by heavy exercise [356]. However, increased adiponectin levels were detected only in individuals with a large weight loss (> 10%). The two studies that have examined the direct association between the Pro12Ala SNP and plasma adiponectin levels have produced inconsistent results [357,358].

The functional PPAR response elements in the promoter of *SLCA2* [349], and the interaction of both the *PPARG* and the *SLC2A2* genes (*Study II*) with physical activity in the DPS support the notion that the interaction of both genes with physical activity could be mediated by effects on pancreatic insulin secretion [348]. Smaller amounts of PPARγ are present in extra-adipose tissues that are central to glucose homeostasis, including skeletal muscle and liver [359]. Furthermore, the expression of PPARγ in macrophages may be important in the development of obesity-related insulin resistance [360]. The pathophysiological importance of PPARγ in these tissues is currently unknown.

Interaction with lifestyle intervention

Rs1152003 in the 3' flanking region of *PPARG* is distinct from any other haplotype block in *PPARG*, and has been associated with troglitazone response in 93 non-diabetic Hispanic women [317]. In the present study, rs1152003 was associated with a decreased risk of type 2 diabetes in the intervention group, independently of rs1801282 (Pro12Ala) genotype. Rs1152003 was also associated with fasting glucose concentration at baseline. This SNP may thus be an important mediator of the effect of *PPARG* agonist treatment or lifestyle modification on glucose homeostasis and the risk of type 2 diabetes. However, in the DPP, no difference between rs1152003 genotypes in the response to troglitazone treatment was found [361]. More studies on the association

of rs1152003 with type 2 diabetes and its possible interactions with lifestyle and troglitazone treatment are required to draw more reliable conclusions.

The SLC2A2, ABCC8, and KCNJ11 genes (Study II)

In *Study II*, we analyzed the interactions of SNPs in three genes regulating insulin secretion, including *SLC2A2*, *ABCC8*, and *KCNJ11* with physical activity on the incidence of type 2 diabetes. In previous analyses of the DPS, SNPs in *SLC2A2* and *ABCC8*, but not in *KCNJ11*, were associated with the risk of progression from IGT to type 2 diabetes [119,121]. However, the lifestyle intervention decreased the risk associated with the specific genotypes [119,121]. In the present study, we detected significant interactions between SNPs in *SLC2A2* and *ABCC8* with moderate-to-vigorous physical activity on the conversion from IGT to type 2 diabetes in the DPS.

Interactions with physical activity

No other studies on the interaction of the *SLC2A2* and *ABCC8* genes with physical activity on the risk of type 2 diabetes have been published. Both genes have an essential role in glucose-induced insulin secretion from the pancreatic β–cells [362,363]. *SLC2A2* encodes glucose transporter 2 (GLUT2), a facilitative glucose transporter that affects insulin secretion by regulating the entry of glucose into the pancreatic β–cell. *ABCC8* encodes sulfonylurea 1 receptor (SUR1), and *KCNJ11* encodes K^+ inward rectifier (Kir6.2), the two subunits of the pancreatic ATP-sensitive potassium (K_{ATP}) channels. K_{ATP} channels regulate insulin secretion by coupling β–cell glucose metabolism to membrane potential. The activity of the channels is inhibited by ATP, and as the ATP concentration in the β–cells increases due to increased glucose metabolism, the cell membrane becomes depolarized thereby triggering insulin exocytosis.

SNPs in *SLC2A2* and *ABCC8* modify insulin secretion [364-368]. Therefore, a differential genotypic response in β–cell function to changes in physical activity is the most likely explanation for their interaction with physical activity on the incidence of type 2 diabetes. It is now known that the transition from normal glucose tolerance through IGT to full-blown diabetes is characterized by a progressive decline of β–cell

function [369]. Only few studies have investigated the effects of lifestyle interventions on insulin sensitivity and insulin secretion in persons with IGT [45,370]. In the DPS, based on a repeated frequently sampled intravenous glucose tolerance test (FSIGT), insulin sensitivity improved along with lifestyle changes while insulin secretion remained virtually unchanged [45]. Most other data also indicate that lifestyle changes primarily increase insulin sensitivity. However, the glycemic stress that is associated with insulin resistance may exhaust β–cells and impair their function. By improving insulin sensitivity, regular physical activity reduces glycemic stress and thus indirectly protects β–cells [20,371]. Furthermore, some studies suggest that physical activity [250,251], diet [372,373], weight loss [374], or their combination [370] may directly improve the first-phase insulin secretion, which is an indicator of the β–cell function.

We found no interaction between the changes in physical activity and the SNPs in *SLC2A2* and *ABCC8* on changes in fasting or 2-hour insulin levels. However, insulin levels are strongly regulated by insulin resistance and are thus not reliable indicators of early insulin secretion. Because 30 min insulin, the insulinogenic index, or other more precise measures of early insulin secretion were not available in *Study II*, differences in the changes in β–cell function between genotypes could not be assessed.

As *SLC2A2* and *ABCC8* are not exclusively expressed in pancreas, it is possible that their interaction with physical activity could be mediated by effects in extra-pancreatic tissues. *SLC2A2* is expressed in hepatocytes where GLUT2 participates in liver glucose uptake and release [362]. In addition, it is expressed in intestinal and renal cells, where it is involved in intestinal glucose absorption and renal glucose reabsorption. SUR1, encoded by *ABCC8*, is abundant in K_{ATP} channels in many regions in the brain, and particularly in the hypothalamus [375]. The activation of K_{ATP} channels in the mediobasal hypothalamus inhibits hepatic gluconeogenesis, suggesting that the effects of *ABCC8* are partly centrally mediated [376]. Interestingly, in a genome-wide linkage scan, based on the HERITAGE Family Study, a marker within *ABCC8* exhibited one of the most significant linkages to maximal oxygen uptake in the sedentary state [377].

The E23K SNP of *KCNJ11* was not associated with the risk of type 2 diabetes in the DPS [92], and did not interact significantly with physical activity in *Study II*. Consistently, the DPP study did not detect an interaction between the E23K SNP and

lifestyle changes on the progression to type 2 diabetes [128]. It is thus likely that the association of the E23K SNP with type 2 diabetes is not modified by lifestyle changes.

The interaction of physical activity with genes regulating insulin secretion in the DPS suggests that physical activity not only improves insulin sensitivity, but may also preserve β–cell function. There are, however, limited data on direct effects of physical activity on β–cell function. Therefore, the interaction of *SLC2A2* and *ABCC8* with physical activity may be due to an indirect effect. Physical activity improves insulin sensitivity, which slows the progressive decrease in β–cell functioning in the progression from IGT to type 2 diabetes.

The ADRB2, ADRB3, IGF1R, LIPC, LEPR, GHRL, and TCF7L2 genes (Study III)

In the *Study III*, we showed that the Leu72Met (rs696217) SNP of *GHRL* modified the effect of moderate-to-vigorous physical activity on the changes in weight and waist circumference, the –501A/C (rs26802) SNP of *GHRL* modified the effect of total and moderate-to-vigorous physical activity on the change in HDL cholesterol concentration, and the Lys109Arg (rs1137100) SNP of *LEPR* modified the effect of total physical activity on the change in systolic blood pressure. No interaction was found between variants in *ADRB2*, *ADRB3*, *IGF1R*, *LIPC*, or *TCF7L2* and physical activity on the risk of type 2 diabetes or changes in characteristics of the metabolic syndrome.

GHRL Leu72Met and body weight

Increased physical activity resulted in a decrease in body weight and waist circumference in the carriers of the Met72 allele but not in the Leu72 homozygotes of *GHRL*. There are no earlier reports available on the interaction between variants in *GHRL* and physical activity on changes in body weight. Ghrelin binds to the growth hormone (GH) segretagogue receptor, releasing GH into the systemic circulation [378]. Increased GH release through ghrelin action could increase lipolysis and thus lead to decreased adiposity [379]. Ghrelin is also known to stimulate appetite and increase food intake, and thereby affect energy balance [380]. Ghrelin levels increase in relation to a

decrease in body weight, acting possible as a compensatory signal to restore body weight.

Similarly as ghrelin, physical activity of moderate-to-vigorous intensity stimulates GH release [381]. However, acute exercise does not seem to affect ghrelin concentrations [382], and long-term aerobic exercise training increases ghrelin levels only when weight loss is produced [383,384]. Although there was no effect on total ghrelin levels, a 5-day aerobic exercise program was reported to increase the proportion of biologically active, acylated ghrelin in blood [385]. Ghrelin circulates in both acylated and deacyl forms, but only the acylated ghrelin binds to the GH segretagogue receptor, affecting GH release and energy balance [386]. Furthermore, two studies have reported that changes in ghrelin levels were associated with changes in fat-free mass, but not in fat mass during weight loss [387,388]. By maintaining or increasing fat-free mass, physical activity could indirectly affect total ghrelin levels. The Leu72Met SNP has been associated with ghrelin levels in some studies with Met72 allele carriers showing the highest total [389] or acylated ghrelin concentrations [390] and other showing a trend for high total ghrelin levels with Met72 allele carrier status [391,392] or no association [393,394]. Unfortunately, ghrelin levels were not measured in the DPS.

Interestingly, the baseline level of total physical activity differed among the genotypes of the Leu72Met SNP of *GHRL*, suggesting that the carriers of the Met72 allele may adopt a higher level of activity than those homozygous for the Leu72 genotype. Indeed, studies in rats indicate that centrally administered ghrelin decreases spontaneous physical activity [395]. The Leu72Met SNP did not, however, have a significant effect on the change in physical activity during the follow-up.

GHRL -501A/C and HDL-cholesterol

Increased physical activity led to an increase in serum HDL cholesterol in the AA homozygotes of the -501A/C SNP of *GHRL*, whereas no such changes were found among the carriers of the C allele. Physical activity is known to increase serum levels of HDL cholesterol, but there is a large inter-individual variability in the response [396].

Gene variants that modify the effect of physical activity on HDL cholesterol are currently unknown.

The interaction between the -501 A/C SNP and physical activity on serum HDL cholesterol is a novel finding. Three studies have, however, reported a positive correlation between plasma ghrelin and HDL cholesterol [397-399], and one study reported an association between the -1062 G/C SNP in the promoter of *GHRL* and HDL cholesterol [400]. The -501A/C SNP has been associated with the rate of ghrelin mRNA expression [401,402]. It has been suggested that HDL particles may have a role as circulating ghrelin transporters [403]. Recent studies also indicate that ghrelin analogs may affect cholesterol metabolism through binding CD36 and GH segretagogue receptors on macrophages, leading to cholesterol efflux into the HDL reverse pathway [404]. Further studies are needed to elucidate the mechanisms behind the association of ghrelin with HDL cholesterol.

LEPR Lys109Arg and blood pressure

Increase in physical activity led to a decrease in blood pressure only in carriers of the Lys109Lys genotype of *LEPR*, whereas the carriers of the Arg109 allele did not respond to physical activity. No other reports exist on the interaction between variants in *LEPR* and physical activity on blood pressure. However, a differential response to a 3-month lifestyle modification of caloric restriction and moderate physical activity among the genotypes of the Lys656Asn SNP of *LEPR* has been reported [405]. Systolic blood pressure decreased significantly in the Lys656 homozygotes, but not in the carriers of the Asn656 allele [405]. The Lys109Arg SNP has also reported to modify the effect of a 20-week endurance training program on measures of glucose homeostasis in the HERITAGE Family Study [260].

Leptin is an adipocyte-secreted hormone that is mainly involved in the regulation of energy homeostasis [406]. However, it may also affect blood pressure by stimulating sympathetic outflow [407,408]. High circulating levels of leptin may partly explain the increase in renal sympathetic tone observed in obese people [409]. Exercise training alone or in combination with dietary modification has been reported to decrease serum leptin levels independently of weight loss [410,411]. A sequence variation in *LEPR*

might impair the effect of leptin on its receptor, attenuating the favourable effect of physical activity on blood pressure. Indeed, polymorphisms in *LEPR* have been associated with high plasma leptin levels indicating leptin resistance, and a lower whole-body plasma norepinephrine spillover, an index of blunted sympathetic nerve activity [412].

In the HERITAGE Study, the Lys109Arg SNP of *LEPR* has also been shown to modify the effect of 20 weeks of aerobic exercise training on glucose homeostasis among 397 nondiabetic whites [260]. We did not detect interactions between SNPs in *LEPR* and changes in physical activity on the risk of progressing from IGT to type 2 diabetes. However, the interaction of physical activity with the *LEPR* gene could be observed only in healthy individuals [260], and not in persons with IGT who are at a relatively late stage in the pathogenesis of type 2 diabetes. In the HERITAGE, the effect of the *LEPR* gene was also modified by variation in the *LEP* gene [260]. We did not investigate the effects of SNPs in *LEP* on the incidence of type 2 diabetes in the DPS.

The LIPC and ADRB2 genes

No interaction between the G-250A SNP of *LIPC* and physical activity was observed, although the -514C/T variant of *LIPC* has been associated with training-induced improvements in insulin sensitivity in 443 white men and women participating in the HERITAGE Study [275]. We did not genotype the -514 C/T SNP in the DPS. The G-250A SNP was, however, strongly associated with the conversion from IGT to type 2 diabetes in the DPS, and there was an interaction with the lifestyle intervention. This indicates that *LIPC* may interact with lifestyle changes. Our study may have been underpowered to detect an interaction with physical activity on the incidence of type 2 diabetes. Therefore, further studies are required to show whether variation in *LIPC* modifies the responses of glucose homeostasis to lifestyle changes.

Variants in *ADRB2* have reported to modify changes in body weight in response to physical activity in three separate studies [262-264]. In the DPS, we did not find an interaction between the Gln27Glu of *ADRB2* and physical activity with regard to changes in BMI. The results from the earlier studies are, however, also inconsistent with respect to specific variants that have modified the responses, and with regard to the

effect of gender. In the initial cross-sectional study of 836 French men and women, body weight, waist circumference, and waist-to-hip ratio were higher in physically inactive men carrying the Glu27 allele of *ADRB2* than in inactive men carrying other genotypes, whereas no significant difference between the genotypes was found among men who were physically active [262]. No similar interaction was found among women [262]. In the following case-control study among 139 obese Spanish women and 113 healthy controls, the carriers of the Glu27 allele who were active in their leisure-time, had a higher BMI than other individuals with the same level of activity, whereas no difference between the genotypes was seen among the inactive individuals [263]. In the third study, the responses of *ADRB2* genotypes to a 20-week endurance training program were followed among 482 white and 260 black participants [264]. The white women who were homozygous for the Gly16 allele of *ADRB2* decreased their BMI, fat mass, and percent body fat more during the endurance training program than individuals with other genotypes [264]. However, no difference in the responses to the training program were found among the genotypes of the Glu27Gln SNP. These conflicting results between the three earlier studies and the negative result from the DPS leave it unsettled as to whether variation in the *ADRB2* gene modifies the effect of physical activity on BMI.

The TNF and IL6 genes (Studies III & IV)

In *Study IV*, we found that rs1800629 (G-308A) SNP of *TNF* modified the effect of moderate-to-vigorous physical activity on the serum levels of hs-CRP among the individuals with high (≥3 mg/l) baseline levels of hs-CRP. The carriers of the GG genotype of rs1800629 achieved greater decreases in hs-CRP by increasing their moderate-to-vigorous physical activity than the carriers of the A allele. We did not find an interaction between rs1800795 (C-174G) of *IL6* and physical activity on changes in serum IL-6. In *Study III*, there was no interaction between the SNPs of the *TNF* and *IL6* genes and physical activity on the risk of type 2 diabetes, or on characteristics of the metabolic syndrome.

The interaction of rs1800629 with physical activity in the DPS replicates the finding from the HERITAGE Study, where only the carriers of the G allele were able to decrease their serum levels of hs-CRP in response to a 20-week exercise program [335]. In both studies, the gene-physical activity interaction was found among the individuals with high baseline levels of hs-CRP. In the HERITAGE Study, exercise was effective in reducing hs-CRP levels only among those with high baseline levels of hs-CRP [314].
Increased circulating levels of markers of low-grade inflammation, such as hs-CRP and IL-6, are associated with an increased risk of type 2 diabetes [413,414]. Modulation of the levels of inflammatory mediators may be one important approach to prevent type 2 diabetes. In the DPS, moderate-to-vigorous intensity physical activity was significantly correlated with decreases in hs-CRP and IL-6 [415]. The interaction of *TNF* with physical activity on levels of hs-CRP in *Study IV* suggests that the anti-inflammatory effect of physical activity in the DPS may have depended on the genetic variation. The *TNF* gene encodes TNF-α, a proinflammatory cytokine that stimulates CRP production [232]. Rs1800629 is located in the promoter region of *TNF*, and has been shown to modify the transcription-rate of *TNF*. Compared with the A allele, the GG genotype of rs1800629 halves the transcription-rate of *TNF* [416]. Physical activity may also suppress TNF-α production by increasing the synthesis of IL-6 in the skeletal muscle [236], and via other, IL-6 independent mechanisms [244,249]. The interaction between rs1800629 and physical activity on serum hs-CRP may thus be mediated by their combined effect on the production of TNF-α. However, since we did not measure circulating TNF-α due to the limited availability of serum in the DPS, further studies are necessary to elucidate the mechanism.

In *Study III*, we found no interaction between physical activity and rs1800629 on the incidence of type 2 diabetes or changes in components of the metabolic syndrome. The combined evidence from *Studies III* and *IV* thus suggest that changes in hs-CRP do not clearly seem to turn into benefits with regard to components of the metabolic syndrome and type 2 diabetes. The studies do not, however, exclude the relationship between hs-CRP and the metabolic syndrome or type 2 diabetes, because the interaction on hs-CRP levels was only found in individuals with high baseline levels of hs-CRP. An earlier report on the effects of rs1800629 among 56 sedentary 50- to 75-year old men and

women revealed interactions with 24 weeks of exercise training on glucose area under the curve and serum HDL cholesterol [273,417].

We did not replicate the earlier finding on the interaction between rs1800795 in *IL6* and physical activity on the serum levels of IL-6 in individuals with IGT [336]. The most likely reasons for this are that earlier findings may be false positive, because the study was based on a very small sample of individuals (n = 24). The changes in physical activity in the DPS may also not compare well with the 20-week aerobic exercise training program (2 x 60 min of exercise per week) used in the previous study, or the simultaneous lifestyle changes (diet, weight reduction) in the DPS may modify our results. Further studies on the interaction between rs1800795 and physical activity are required to confirm these findings.

CONCLUDING REMARKS

These secondary analyses of the Finnish DPS indicate that variation in genes regulating both insulin sensitivity (*PPARG2*) and insulin secretion (*SLC2A2*, *ABCC8*) interact with changes in physical activity on the risk of developing type 2 diabetes. Furthermore, variation in *LEPR*, and *GHRL* may modify the effects of physical activity on changes in features of the metabolic syndrome, and variation in *TNF* may modify the effect of physical activity on changes in serum hs-CRP levels. These findings give novel information on the interaction between physical activity and genetic variants in the etiology of type 2 diabetes and related traits in a prospective study setting. The results may be important in the ongoing effort to identify individuals at increased risk of developing type 2 diabetes and the metabolic syndrome, and those who are most likely to have enhanced health benefits from regular physical activity. However, the study was limited by its observational study design, a relatively low number of subjects, multiple statistical testing, and the non-objective measurement of physical activity.

During recent years, the genetics of type 2 diabetes has taken a leap forward, and the first robust evidence of diabetes susceptibility genes has been generated through genome-wide association studies. Studies investigating interactions between genes and

lifestyle are, however, generally even more challenging than the studies of genetic main effects, and reliable evidence is yet to be obtained. In the coming years, robustness of gene-physical activity interaction analyses can be improved by performing larger-scale studies with objective measurement of physical activity and by combining data from several studies into a meta-analysis. Sophisticated analytical approaches may be necessary to avoid false discoveries. The use of Bayesian approaches, based on the clear specification of prior probabilities of interaction, may be advantageous [17,337,418]. Increasing knowledge on gene function will help to identify the causal variants. With regard to the Finnish DPS, important steps have already been taken, as the DPS data will be combined with other data from intervention trials within the ongoing Integrated Project InterAct (www.inter-act.eu). The InterAct is a collaborative effort of 35 partners in 10 countries, aiming to discover how diet and physical activity interact on the risk of developing type 2 diabetes. By pooling the data, the InterAct may be the first study to achieve sufficient statistical power to generate robust evidence on gene-lifestyle interactions in the development of type 2 diabetes.

VII SUMMARY

The main findings in Studies I-IV were:

Study I

The haplotype block containing the Pro12Ala SNP of *PPARG2* increased the risk of type 2 diabetes, but an increase in physical activity removed the effect of the risk alleles. Furthermore, the lifestyle intervention of the DPS modified the association of an SNP in the 3' flanking region of *PPARG* with the risk of type 2 diabetes. The interaction of *PPARG2* with physical activity is supported by previous literature, but the mechanisms remain unclear. The interaction of rs1152003 with lifestyle changes has not been previously reported, but is consistent with an earlier study on the association of rs1152003 with responsiveness to rosiglitazone treatment.

Study II

Moderate-to-vigorous physical activity modified the risk of developing type 2 diabetes associated with *SLC2A2* and *ABCC8*. The interaction of physical activity with genes regulating insulin secretion suggests that physical activity not only improves insulin sensitivity but also preserves β–cell function. However, physical activity has not been shown to modify the function of *SLC2A2* and *ABCC8*, and there is limited evidence of a direct effect of physical activity on β–cell function. Therefore, the interaction between *SLCA2* and *ABCC8* and physical activity may reflect the relationship between insulin sensitivity and β–cell function in the development of type 2 diabetes. Physical activity improves insulin sensitivity and thus also slows the progressive decrease in β–cell function.

Study III

No interaction between SNPs in *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, and *GHRL* with physical activity on the risk of developing type 2 diabetes was found. However, SNPs in *LEPR* and *GHRL* modified the effect of physical activity on changes

in systolic blood pressure (*LEPR*), and on changes in body weight, waist circumference, and concentration of HDL cholesterol (*GHRL*). The results indicate that genetic variation in *LEPR* and *GHRL* may modify the magnitude of beneficial effects of physical activity on the characteristics of the metabolic syndrome in individuals with IGT. Nonetheless, multiple statistical tests were performed and the detected interactions were not strongly suggested by previous literature.

Study IV

Physical activity modified the effect of rs1800629 (G-308A) in *TNF* on changes in serum levels of hs-CRP. The finding is consistent with a previous exercise training study. The interaction between rs1800629 and physical activity may be mediated by their combined suppressing effect on the production of TNF-α in the adipose tissue.

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Appendix I: KIHD 12-Month Leisure-Time Physical Activity Questionnaire

Class	Activity	Breathlessness	Sweating
0	recreational, outdoor activities	no	no
1	conditioning exercise	yes	no
2	brisk conditioning exercise	yes	some
3	competitive, strenuous exercise	yes	a lot

	How many times per month?												Duration per occasion (h, min)	Intensity class (0-3)
	January	February	March	April	May	June	July	August	September	October	November	December		
Walking on work trips														
Conditioning walking														
Jogging														
Skiing														
Bicycling														
Bicycling on work trips														
Swimming														
Gymnastics, dancing														
Ball games														
Gardening and snow shoveling														
Hunting, picking berries, gathering mushrooms														
Fishing														
Hobby crafts and repairs														
Rowing														
Forest work, wood cutting														
Other, what:														

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