Genetic Determination of Serum Levels of Diabetes-Associated Adipokines

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■ Abstract

Adipose tissue secretes an abundance of proteins. Some of these proteins are known as adipokines and adipose-derived hormones which have been linked with metabolic disorders, including type 2 diabetes, and even with cancer. Variance in serum adipokine concentration is often closely associated with an increase (obesity) or decrease (lipodystrophy) in fat tissue mass, and it is affected by age, gender, and localization of the adipose tissue. However, there may be genetic variants which, in consequence, influence the serum concentration of a certain adipokine, and thereby promote metabolic disturbances or, with regard to the “protective”

allele, exert beneficial effects. This review focuses on the genetic determination of serum levels of the following adipokines: adiponectin, chemerin, leptin, progranulin, resistin, retinol binding protein 4, vaspin, adipsin, apelin, and omentin. The article reports on the latest findings from genome-wide association studies (GWAS) and candidate gene studies, showing variants located in/nearby the adipokine genes and other (non-receptor) genes. An extra chapter highlights adipokine-receptor variants. Epigenetic studies on adipokines are also addressed.

Keywords: type 2 diabetes · adipokine · ADIPOQ · SNP · chemerin · leptin · progranulin · resistin · RBP4 · vaspin

1. Introduction

In the last decades, the “static” role of adipose tissue as fat storage and mechanical protector has changed. Today, it is regarded as an endocrine organ secreting a multitude of metabolic active adipokines and adipose-derived hormones. The relationship between obesity and insulin resistance is complex, giving rise to a range of metabolic disorders, including type 2 diabetes (T2D), dyslipidemia, and coagulation disorders [1]. To date, over 300 proteins secreted from adipose tissue are known [2-5]. Multiple roles in metabolic and inflammatory responses are attributed to adipokines, which makes them interesting drug targets for the treatment of obesity or insulin resistance [6].

The secretion of adipokines seems to be tissue-specific, with some of them primarily secreted in

visceral adipose tissue (VAT) and others in subcutaneous adipose tissue (SAT) [5, 7-9]. The difference in gene expression patterns between VAT and SAT points to genetic heterogeneity [10]. In most cases, the dysregulation of these bioactive proteins and their possible adverse effect on metabolism is associated with increasing fat mass at ectopic sites and/or the development of obesity. Also, a number of additional factors that impact on serum adipokine concentrations have to be taken into account. However, based on the known impact factors such as gender, body mass index (BMI), or fat mass there are still considerable large residuals in regression models. These residuals may result from lifestyle or other environmental factors. There may also be underlying genetic factors that promote metabolic disturbances or exert beneficial effects, as shown for retinol binding protein 4 (RBP4) [11-12].

Abbreviations:

ACTR3C	actin-related protein 3 homolog C (yeast)
ADARB2	adenosine deaminase RNA-specific B2
ADIPOQ	adiponectin
ADIPOR1/R2	adiponectin receptor 1/receptor 2
APLN	apelin
ARL15	ADP-ribosylation factor-like 15
ATG7	autophagy related 7
BMI	body mass index
C3AR1	complement component 3a receptor 1
CAP1	CAP, adenylate cyclase-associated protein 1
CDH13	cadherin 13
CFD	complement factor D; adipsin
CMIP	c-Maf-inducing protein
CMKLR1	chemerin chemokine-like receptor 1
CVD	cardiovascular disease
DTC	differentiated thyroid cancer
EDIL3	EGF-like repeats, discoidin I-like domain 3
EMSA	electrophoretic mobility shift assay
eQTL	expression quantitative trait loci
FER	fer (fps/fes related) tyrosine kinase
Glut 4	glucose transporter 4
GNL3	guanine nucleotide binding protein-like 3
GPR109A	hydroxycarboxylic acid receptor 2
GRP78	heat shock protein family A, member 5
GWAS	genome wide association study/studies
hK7	kallikrein 7
HNF1	hepatocyte nuclear factor 1 alpha
HOMA-IR	homeostasis model assessment for insulin resistance
IL-6	interleukin 6
IRS-1 or 2	insulin receptor substrate 1 or 2
ITLN1	omentin
KCNJ11	potassium channel, inwardly rectifying subfamily J, member 11
KNG1	kininogen
LD	linkage disequilibrium
LEP	leptin
LEPR	leptin receptor
LYPLAL1	lysophospholipase-like 1
MCEMP1	mast cell-expressed membrane protein 1
NDST4	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 4
PEPD	peptidase D
PDE3A	phosphodiesterase 3A, cGMP-inhibited
PGRN	progranulin
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
RARRES2	retinoic acid receptor responder (tazarotene induced) 2; chemerin
RBP4	retinol binding protein 4
RETN	resistin
SAT	subcutaneous adipose tissue
SNP	single nucleotide polymorphism
SORT1	sortilin 1
STRA6	stimulated by retinoic acid 6
T2D	type 2 diabetes
TRIB1	tribbles pseudokinase 1
TYW3/CRYZ	tRNA-yW synthesizing protein 3 homolog/zeta-crystallin
VAT	visceral adipose tissue
VEGFA	vascular endothelial growth factor A
WHR	waist-to-hip ratio

Family and twin studies also indicate a strong heritability for adipokines, suggesting that there are genetic factors that account for the variability in serum adipokine concentrations [13-14]. It is thus surprising that VAT and SAT distribution have not been regarded as traits in genome-wide association studies (GWAS) to date, except for the study by Fox *et al.*, and irrespective of waist-to-hip ratio (WHR) [15]. Their inclusion could unravel adipokines that are directly involved in the regulation of fat distribution and subsequently in the development of T2D [16]. To date, it is not clear what comes first in the pathogenesis of T2D, adipose tissue dysfunction leading to imbalanced physiologic concentrations of adipokines or dysregulation of adipokines on a genetic basis promoting obesity and T2D (**Figure 1**). Finally, the genetics of adipokine receptors should not be underestimated as they are responsible for signal transduction in the target tissues.

This review aims to summarize human studies on T2D-related adipokines and genetic variants associated with the serum concentration of specific adipokines. In particular, the review considers the following three aspects: *i)* polymorphisms located in/nearby the adipokine gene and other (non-receptor) variants that associate with serum adipokine concentration, *ii)* genetic variation located in/nearby adipokine receptors that associate with serum adipokine concentrations, and *iii)* epigenetic mechanisms on adipokines discovered to date.

2. Genetic variants associated with serum adipokine concentration

2.1 Adiponectin (ADIPOQ)

Adiponectin is involved in the regulation of insulin sensitivity and glucose metabolism. A decrease in this adipokine is associated with deterioration in virtually all parameters of the metabolic syndrome, including T2D and cardiovascular disease (CVD) [17]. Variants of the *ADIPOQ* gene seem to cause substantial changes in adiponectin levels; this finding was replicated in independent study populations (see detailed review in Breitfeld *et al.*, 2011) [17]. During the years following this discovery the data were supported by further studies (**Table 1**) [18-21]. Besides *ADIPOQ*, several other loci have been described to harbor variants that are associated with adiponectin levels, including:

- *ADP-ribosylation factor-like 15* (*ARL15*, rs4311394 C/T) [22]

- *Cadherin 13* (*CDH13*, rs3865188 A/T)
- Near *kininogen 1* (*KNG1*, rs11924390 C/T) [23]
- *Lysophospholipase-like 1* (*LYPLAL1*, rs3001032 C/T)
- *Guanine nucleotide binding protein-like 3* (*GNL3*, rs1108842 A/C)
- *Vascular endothelial growth factor A* (*VEGFA*, rs998584 C/A)
- *Tribbles pseudokinase 1* (*TRIB1*, rs2980879 T/A)
- *Phosphodiesterase 3A, cGMP-inhibited* (*PDE3A*, rs7955516 A/C)
- *Hydroxycarboxylic acid receptor 2* (*GPR109A*, rs601339 C/T)
- *c-Maf-inducing protein* (*CMIP*, rs2925979 T/C)
- *Peptidase D* (*PEPD*, rs731839 C/T) [21]
- *Fer (fps/fes related) tyrosine kinase* (*FER*, 10447248 A/G) (**Table 1**) [24]

Furthermore, in a Japanese population, the AA (TT) genotype of single nucleotide polymorphism (SNP) rs2805533 (A/G or T/C, respectively) in the *adenosine deaminase RNA-specific B2* (*ADARB2*) gene was associated with lower adiponectin levels, and with higher BMI, greater abdominal circumference, and higher triglyceride levels (**Table 1**) [25]. One of the largest non-GWA studies was conducted by Mather *et al.* who investigated seventy-seven tagging SNPs in *ADIPOQ* and its receptors *ADIPOR1* and *ADIPOR2* [26]. They found 13 *ADIPOQ* SNPs associated with baseline adiponectin concentrations in the Diabetes Prevention Program (DPP) cohort, four of which remained significantly associated with log adiponectin concentrations in a multivariable analysis including all 13 significant *ADIPOQ* SNPs:

- rs17366568 A/G
- rs1648707 A/C
- rs17373414 C/T
- rs1403696 C/T [26]

One year later, Peters *et al.* published a similar study, confirming the result obtained by Mather and colleagues [27]. They found nine tagSNPs significantly associated with serum adiponectin. However, these SNPs explain only <5% of the variance in serum adiponectin concentrations (**Table 1**). SNPs that were associated with adiponectin levels in the meta-analysis were summed up for each risk allele an individual possessed to form an allele score [27]. The allele score of three

Figure 1. Genetics of adipokines and metabolic disease: cause and consequence. The figure illustrates different effect routes (arrows) impacting a disease outcome (e.g. T2D or obesity), including genetic information, adipose tissue mass, and secreted adipokines. These effects are influenced by multiple factors such as age, gender, nutrition, environment, ethnicity, and epigenetic mechanisms (illustrated by the framing space).

SNPs (rs12637534, rs16861209, rs17366568) was associated with T2D independently of serum adiponectin levels. None of the receptor SNPs was associated with serum adiponectin in this study [27]. Furthermore, rare population-specific mutations have been shown to account for ~17% of the variance in serum adiponectin concentrations (**Table 1**) [28-29].

2.2 Chemerin (*RARRES2*)

The initial report describing chemerin as an adipokine and elucidating its role in mouse and human white adipocyte differentiation and function was published by Goralski *et al.* in 2007 [30]. Circulating chemerin levels were positively correlated with HbA1c and HOMA-IR, and associated with metabolic syndrome phenotypes; they were also associated with chemerin-induced insulin resistance in primary human skeletal muscle cells [31-34]. Alterations in chemerin serum concentration are detectable in prediabetic states, and may thus reflect adipose tissue dysfunction as an early pathogenic event in T2D development [31].

Table 1. Variants found to be associated with serum adipokine concentrations

Adipokine/ gene	SNP-ID	SNP	Gene/ nearby gene	EA	ED	% variance	Reported in
Adiponectin/ <i>ADIPOQ</i>	p < 10⁻⁸						
	rs3774261	A/G	<i>ADIPOQ</i>	G	↓	1.2% total, 2% cases, 0.9% controls [133]; < 5% [27]	[133], rev. [17]; [27]
	rs6444175	A/G	<i>ADIPOQ</i>	G	↓	n/a [22]; < 5% [27]	[22], rev. [17]; [27]
	rs266717	C/T	<i>ADIPOQ</i>	T	↑	n/a	[22], rev. [17]
	rs1426810	A/G	<i>ADIPOQ</i>	G	↑	n/a	[22], rev. [17]
	rs1648707	A/C	<i>ADIPOQ</i>	C	↓	n/a [20]; 7% attributed to <i>ADIPOQ</i> SNPs [26]; < 5% [27]	[22], rev. [17]; [26]; [27]
	rs17366568	A/G	<i>ADIPOQ</i>	A	↓	7% attributed to <i>ADIPOQ</i> SNPs [26]; <5% [27]	rev. [17]; [26]; [27]
	rs864265	G/T	<i>ADIPOQ</i>	T	↓	n/a [23], 3.2% offspring, 2.2% Fili- pino mothers	[23], [28], rev. [17]
	rs6810075	C/T	<i>ADIPOQ</i>	T	↑	n/a	[21]
	rs16861209	A/C	<i>ADIPOQ</i>	C	↓	< 5% [27]	[27]
	rs4311394	C/T	<i>ARL15</i>	C	↓	n/a	[22], rev. [17]
	rs3865188	A/T	<i>CDH13</i>	T	↓	n/a	[23]; [134]*, rev. [17]
	rs12922394	C/T	<i>CDH13</i>	T	↓	n/a	[19]
	rs11924390	C/T	<i>KNG1</i>	C	↓	with rs3865188 & rs864265 7.5% Filipino mothers, 8.9% offspring (In- transformed <i>ADIPOQ</i> levels)	[23], rev. [17]
	rs3001032	C/T	<i>LYPLAL1</i>	T	↓	n/a	[21]
	rs1108842	A/C	<i>GNL3</i>	C	↑	n/a	[21]
	rs998584	C/A	<i>VEGFA</i>	C	↑	n/a	[21]
	rs2980879	T/A	<i>TRIB1</i>	T	↑	n/a	[21]
	rs7955516	A/C	<i>PDE3A</i>	C	↑	n/a	[21]
	rs601339	C/T	<i>GPR109A</i>	C	↑	n/a	[21]
	rs2925979	T/C	<i>CMIP</i>	T	↓	n/a	[21]
	rs731839	C/T	<i>PEPD</i>	C	↓	n/a	[21]
	p > 10⁻⁸						
	rs2805533	C/T	<i>ADARB2</i>	T	↓	n/a	[25]
	rs10447248	A/G	<i>FER</i>	A	↓	1.3% NHS sample, 2.0% Italian sam- ple	[24], rev. [17]
	rs822354	A/G	<i>ADIPOQ</i>	A	↑	1.1% NHS sample and 2.5% Italian sample	[24], rev. [17]
	rs17373414	C/T	<i>ADIPOQ</i>	C	↓	7% variability attributed to <i>ADIPOQ</i> SNPs [26]; <5% [27]	[26]; [27]
	rs1403696	C/T	<i>ADIPOQ</i>	T	↓	7% variability attributed to <i>ADIPOQ</i> SNPs	[26]
	rs12637534	A/G	<i>ADIPOQ</i>	G	↑	< 5%	[27]
	rs10937273	A/G	<i>ADIPOQ</i>	G	↓	< 5%	[27]; [137]
	rs822395	A/C	<i>ADIPOQ</i>	A	↓	< 5%	[27]
	Rare variants						
	rs117016164	A/G	<i>ETV5</i>	A	↓	n/a	[28], rev. [17]
	rs138773406	C/A (p.R221S)	<i>ADIPOQ</i>	A	↓	17.1% in offspring, 13.2% in Filipino mothers	[28]
	rs200573126	G/C (p.G45R)	<i>ADIPOQ</i>	C	↓	16-17% complete sample set; 62-63% families of linkage study	[29]
Chemerin/ <i>RARRES2</i>	p < 10⁻⁸						
	rs7806429	A/G	<i>ACTR3C</i>	G	↑	2%	[36]
	p > 10⁻⁸						
	rs2594989	C/T	<i>ATG7</i>	T	↑	n/a	[36]
	rs8027521	C/T	n/a	C	↑	n/a	[36]
	rs347344	C/T	<i>EDIL3</i>	n/a			[35]

Leptin/ <i>LEP</i>	rs2167270	A/G	<i>LEP</i>	G	↓	n/a	[49], rev. [48]; [52]
	rs7799039	A/G	<i>LEP</i>	G	↓	n/a	[50], [51], rev. [48]; [52]
	rs1550805	C/T	<i>PI3K</i>	T	↑	n/a	[53]
	rs5219	G/A (p.E23K)	<i>KCNJ11</i>	A	↑	n/a	[54] [*]
Progranulin/ <i>PGRN</i>	rs646776	T/C	<i>CELSR2</i> , <i>PSRC1</i> , <i>SORT1</i>	C	↓	21.4%	[67]
	rs611917	A/G	<i>CELSR2</i> , <i>PSRC1</i> , <i>SORT1</i>	G	↓	n/a	[67]
Resistin/ <i>RETN</i>	$p < 10^{-8}$						
	rs34861192 ^a	A/G	<i>RETN</i>	A	↑	37.9% with rs3745368	[82] ^a
	rs3745368	A/G	<i>RETN</i>	G	↑	37.9% with rs34861192	[82]
	rs3931020	C/T	<i>TYW3/CRYZ</i>	C	↑	n/a	[85]
	rs13144478	A/T	<i>NDST4</i>	T	↑	n/a	[85]
	$p > 10^{-8}$						
	rs1862513 ^{a,b,c}	C/G	<i>RETN</i>	G	↑	9.68 % [76]	[76] ^b ; [77] ^c ; [78]-[81]
	rs34124816 ^b	A/C	<i>RETN</i>	A	↑	1.33 %	[76]
	rs3219177 ^c	C/T	<i>RETN</i>	T	↑	n/a	[77]
	rs3745367 ^c	G/A	<i>RETN</i>	A	↑ [77;85]; ↓ [89]	n/a; 3.01% [89]	[77]; [85]; [89]
	rs4804765	G/T	<i>RETN</i>	T	↑	1.5% with rs1423096	[78]
	rs1423096	C/T	<i>RETN</i>	T	↑ [78]; ↓ [89]	1.5% with rs4804765 [78]; 15.44% [89]	[78]; [89]
	rs10401670	C/T	<i>RETN</i>	T	↑	n/a	[78]; [90]
Retinol binding protein 4/ <i>RBP4</i>	rs3758539	G/A	<i>RBP4</i>	A	↑	n/a	[95]
	rs10882273	A/G	<i>RBP4</i>	G	↓	n/a	[98]
	rs3758538	A/C	<i>RBP4</i>	C	↓	n/a	[98]
	rs17108993	C/G	<i>RBP4</i>	G	↓	n/a	[98]
Vaspin/ <i>SERPINA12</i>	$p < 10^{-8}$ - GWAS						
	rs11160190	C/T	<i>VASPIN/</i> <i>SERPINA4</i>	C	↓	n/a	[103]
	rs6575436	T/A	<i>VASPIN</i>	A	↑	n/a	[103]
	rs4905203	C/G	<i>SERPINA11</i>	C	↓	n/a	[103]
	rs1956713	T/C	<i>SERPINA9</i>	C	↑	n/a	[103]
	rs1956721	C/T	<i>SERPINA11</i>	C	↓	n/a	[103]
	rs11621467	G/T	<i>SERPINA11/</i> <i>SERPINA1</i>	G	↓	n/a	[103]
	$p < 10^{-8}$ - fine mapping of vaspin locus (for further variants please see [103])						
	rs8006968	T/A	<i>VASPIN</i>	T	↓	n/a	[103]
	rs7158068	A/G	<i>VASPIN</i>	A	↓	n/a	[103]
	rs10145558	T/C	<i>VASPIN</i>	C	↑	n/a	[103]
	rs1956709	T/C	<i>VASPIN</i>	C	↑	n/a	[103]
	rs12433651	T/C	<i>VASPIN</i>	C	↑	n/a	[103]
	rs2236240	G/A	<i>VASPIN</i>	A	↑	n/a	[103]
	rs2236242	A/T	<i>VASPIN</i>	T	↓	n/a	[103]
	rs3736803	C/T	<i>VASPIN</i>	C	↓	n/a	[103]
	Rare variants						
	rs61757459	C/T (p.R211X)	<i>VASPIN</i>	T	↓	n/a	[105]

Legend: Data for adiponectin partly adopted from Breitfeld *et al.* [17] and extended. Included are SNPs with the best statistical and/or functional evidence reported in the literature; further (LD) SNPs are discussed in the relevant references. *Abbreviations:* EA - effect allele, ED - effect direction serum level, rev - reviewed in. ^{a/b/c} SNPs in LD based on data in the cited study. SNP given for the genomic strand where the SNP's gene/nearby gene is located. This may deviate from the alleles reported in the publication. Percentage variance of adiponectin concentration belongs to the population cited in the study. * Discovery cohort. ^{*} Discrepancies between text and table in the publication cited.

Chemerin is coded by the *retinoic acid receptor responder (tazarotene induced) 2 (RARRES2)* gene on chromosome 7q36.1. The study by Bozaoglu *et al.* revealed that serum chemerin levels are highly heritable (25%) in the San Antonio Family Heart Study comprising individuals of Mexican-American origin [35]. The GWAS was performed in 523 subjects and detected a SNP in the *EGF-like repeats and discoidin I-like domains 3 (EDIL3, rs347344 C/T)* gene. The authors supported the role of *EDIL3* by showing that chemerin promotes angiogenesis in endothelial cells in a dose-dependent manner. Further loci were described as being associated with serum chemerin levels, with $p < 0.0001$ in that cohort, but the evidence for these associations still needs to be proven in replication or functional studies [35].

A recent genome-wide meta-analysis ($n = 2,791$) supported the high heritability estimates for serum chemerin levels, and revealed genetic variation near *RARRES2* to be involved in the regulation of the circulating serum chemerin concentrations [36]. The strongest evidence of association was for rs7806429 (A/G) in the *actin-related protein 3 homolog C (yeast) (ACTR3C)* gene, representing a cluster of SNPs in pairwise linkage disequilibrium (LD), which in addition associated moderately with *RARRES2* mRNA transcript levels in VAT. Albeit not significant at a genome-wide level, two other lead SNPs, rs2594989 (C/T) in *ATG7* and rs8027521 (C/T), and their LD partners might be worth investigating (**Table 1**) [36]. In this study, serum chemerin concentrations were also positively correlated with BMI and percentage body fat as well as with elevated fasting insulin, HOMA-IR, and AUC_{glucose} which supports the findings of previous studies [36-38].

2.3 Leptin (LEP)

In the early 1970's, initially discovered as a monogenetic determined cause for obesity in mice [39-40], the *ob* gene product leptin was described as one of the first adipokines. It was named adipokine because the blood-circulating factor was found to have its main source in adipose tissue, and its structure and function were predicted to be cytokine-like [41-45]. Among the adipokines known to date, leptin and its receptor exhibit a unique feature: it is the only adipokine/adipokine receptor for which mutations are known to cause monogenic obesity in humans [46-47]. *LEP* genetics have been intensively studied in humans and livestock such as cattle and pigs (reviewed in [48], 2005).

Until 2005, the *LEP* polymorphism G>A at position 19 (now rs2167270 A/G) of the untranslated exon 1, and a variant in the promotor region, G(-2548)A (rs7799039 A/G), were the only variants in/nearby *LEP* which have been described as being associated with serum leptin levels (**Table 1**) [48-51]. Obese subjects, homozygous for the G-allele in one or other SNP, exhibit lower serum leptin levels than AG- or AA-allele carriers. The association of both SNPs with serum leptin levels was confirmed in a very recent study on differentiated thyroid cancer (DTC) [52].

In 2006, a study by Jamshidi *et al.* reported that SNP rs1550805 (C/T) in the *phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PI3K)* locus was significantly associated with serum leptin levels and body fat measures in a UK cohort of twin pairs and singletons [53]. Carriers of the T-allele had higher serum leptin levels. The authors speculated that the site marked by rs1550805 reflect a diminished ability on the part of PI3K to signal via insulin receptor substrate 1 or 2 (IRS-1 or IRS-2) in response to leptin [53]. This study is a good example of how genetic variants in downstream signal transducers might affect adipokine secretion by disturbed feedback mechanisms.

A significant association between plasma leptin and rs5219 (G/A, missense variant p.E23K) of *potassium channel, inwardly rectifying subfamily J, member 11 (KCNJ11)* was found in a population from Yucatan, Mexico [54]. Carriers of the A-allele exhibited higher serum leptin levels. *KCNJ11* is coding for a K_{ATP} channel KIR6.2 which is known to play a role in insulin secretion in pancreatic β -cells, and insulin and leptin signal transduction in the brain (**Table 1**) [55-59]. This polymorphism has been thoroughly investigated with regard to T2D and obesity in many populations (reviewed in detail in [60]). From a functional point of view, the Lys amino acid residue was shown to increase the probability of spontaneous K_{ATP} opening and decrease the potassium channel's sensitivity towards ATP-inhibitory activity, resulting in an inhibited insulin secretion in pancreatic beta-cells [60-61].

In the brain, in consequence, the A-allele of rs5219 may contribute to leptin resistance via feedback mechanisms that might be reflected in elevated leptin levels. However, this would be rather an indirect/downstream effect on serum leptin levels than an effect of polymorphisms directly acting on *LEP* gene transcription and protein expression, and the hypothesis is not yet proven. In contrast, in a recent but small study,

leptin levels were shown to be significantly lower in an adolescent group with T2D compared to a BMI-, age-, and gender-matched control group without T2D (74 in each group), with equal adiponectin levels in both groups [62].

2.4 Progranulin (PGRN)

Beside adipose tissue, progranulin is widely expressed in many epithelial cells, and impacts disease states in tumorigenesis, neurodegeneration, wound inflammatory response, and metabolic diseases [63-66]. It has been shown that progranulin levels are elevated in individuals with T2D, and correlate with visceral fat accumulation and macrophage infiltration into adipose tissue [66]. In 2010, Carrasquillo *et al.* identified rs646776 (T/C) near *sortilin 1* (*SORT1*) as a regulator of progranulin levels in human plasma of non-*GRN* mutation carriers [67]. They found that each copy of the minor C-allele decreased PGRN levels by ~15 % (**Table 1**). An association of the C-allele of this SNP with increased *SORT1* mRNA levels was detected in the liver, but not in the brain, and the link between sortilin and progranulin levels was investigated in a HeLa cell culture model [67-68]. *SORT1*-overexpressing cells showed a reduction in PGRN levels in the cell culture media. Knockdown of *SORT1* led to a significant increase in PGRN levels. A number of loss-of-function mutations in *PGRN* have also been described in families with dementia [69].

In case of tissue-specific mutations, it would be interesting to learn whether PGRN expressed from other sources such as adipose tissue could attenuate the deficits. However, it is necessary to consider that PGRN has multiple sources when investigating its genetics in relation to obesity or T2D. Therefore, the cell culture model for functional analyses should be chosen very carefully. In mice, ablation of *PGRN* prevented the animals from high-fat diet-induced insulin resistance, adipocyte hypertrophy, and obesity through blockade of IL-6, and insulin resistance induced by chronic administration of PGRN was suppressed by neutralizing IL-6 *in vivo* [70]. However, *PGRN* is a good example for an antagonistic, pleiotropic gene: excessive PGRN may favor the development of obesity and T2D, but insufficient PGRN levels may be harmful to the brain.

2.5 Resistin (RETN)

It has been proposed that resistin links obesity and diabetes, but the link is controversially dis-

cussed [71-74]. In 2003, Smith *et al.* showed that the G/G genotype of the *RETN* promoter SNP -180C/G (rs1862513) was associated with increased basal promoter activity in adipocytes and higher *RETN* mRNA levels in human SAT *in vivo* [75]. Also, *RETN* mRNA correlated positively with insulin resistance and liver fat. One year later, rs1862513 and a second promoter SNP, -537A/C (rs34124816) were investigated in a Korean population; it was shown that the A-allele of rs34124816 and the G-allele of rs1862513 were associated with higher plasma resistin concentrations [76]. The SNPs are in LD, and in functional studies, it was shown that the rs1862513 G-allele is specific for the binding of nuclear proteins from adipocytes and monocytes.

The association of the rs1862513 SNP and plasma resistin concentrations was replicated in a Finnish population and in a meta-analysis showing the same direction of association (**Table 1**) [77-78]. The putative causal role of the rs1862513 polymorphism has been confirmed by others, and further variants have been found (rs34861192 G/A, rs3745368 G/A) to associate with plasma resistin levels in an aged Japanese population [79-82]. These two variants accounted for 37.9% of the variance in plasma resistin level among all study participants (n = 3,133) [82]. Rs34861192 is in LD with rs1862513. However, in this study, plasma resistin levels appear to depend more on the rs34861192 genotype. The effect of rs1862513 seems to be mediated at the level of binding of the transcription factors (TF) Sp1 and Sp3 and promoter activity [79]. Based on a previous publication [83], the authors suggested that other transcription factors (such as SREBP1c) that bind to the resistin gene promoter in the vicinity of the rs34861192 polymorphism may play a greater role [82]. This may be a population-specific effect as rs34861192 is monomorphic in the Environmental Gene Project Centre d'Etude du Polymorphisme Humain (EGP-CEPH) Panel of the SNP database (dbSNP) [82].

Hivert *et al.* found a strong association of four tag SNPs (rs1477341 A/T, rs4804765 G/T, rs1423096 C/T, and rs10401670 C/T) on the 3' side of *RETN* with resistin levels, with all minor alleles associating with higher levels in the Framingham Offspring Study [78]. Rs4804765 and rs1423096 had independent associations with plasma resistin concentrations (1.5% of the variance in resistin levels in best-fitting model). Rs4804765 explained the association of the two other SNPs (rs1477341 and rs10401670) (**Table 1**). Interestingly, as stated by the authors, rs10401670 is located in the

second intron of the *mast cell-expressed membrane protein 1 (MCEMP1)* gene that is mainly expressed by monocytes and mast cell lines [78, 84]. Since resistin is mainly expressed by macrophages that evolve from monocytes in adipose tissue, it would be interesting to know whether *MCEMP1* and its product are functionally influenced by rs10401670 or the SNPs in its 5' region, and whether this protein is involved in resistin and/or glucose metabolism [78].

A GWAS confirmed the association with the *RETN* locus (represented by rs3745367 G/A), and identified an association of the *tRNA-yW synthesizing protein 3 homolog/zeta-crystallin (TYW3/CRYZ*; represented by rs3931020 C/T) and *N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 4 (NDST4*; represented by rs13144478 A/T) loci with circulating resistin levels [85]. In expression quantitative trait loci (eQTL) analyses, rs3931020 and its LD partner rs277369 T/C showed a significant *cis* association with *CRYZ* gene expression. Likewise, rs3931020 showed a significant but weaker *cis* association with *TYW3* gene expression. The authors speculated that the association of the SNP rs3931020 with circulating and mRNA resistin levels may be mediated by its effect in increasing *CRYZ* expression, which in turn, by stabilizing *RETN* mRNA, increases circulating resistin levels [85].

Zeta-crystallin is an NADPH-dependent quinone reductase that binds to adenine-uracil-rich elements in 3'-UTR of mRNAs. It may act as *trans*-acting factor in mRNA regulation [85-88]. The role of *TYW3* and *NDST4* is not yet clear [85]. Rs3745367 and rs1423096 have been replicated in a smaller GWAS in 2014 by Chung and colleagues. However, in contrast to Ukkola *et al.* and Qi *et al.*, they found the relation with serum resistin concentrations to be inverse for the alleles [77, 85, 89]. A relationship between rs10401670 and serum resistin levels could be detected in 6- to 8-year-old boys (**Table 1**) [90].

2.6 Retinol binding protein 4 (RBP4)

Rbp4 was identified in adipocyte-specific *glucose transporter 4 (Glut4)* knockout mice as an adipocyte-derived protein contributing to insulin resistance in obesity and T2D [91]. The liver was regarded as the main source for RBP4 until then, but in the insulin-resistant state, RBP4 production in adipose tissue is almost equivalent to that in the liver. RBP4 levels correlated with the degree of insulin resistance in subjects with obesity, impaired glucose tolerance, and T2D, and were also

associated with anthropometric and metabolic measures such as BMI, WHR, serum triglyceride levels, and others [92].

RBP4 is a transporter for retinol in the circulation. It is important for a multitude of (metabolic) functions, including vision, gene transcription, immune system, bone metabolism, and others. *RBP4* is located at the chromosomal region 10q23-q24 which has been linked to the risk for T2D [93, 94]. The role of *RBP4* as a T2D candidate gene has been confirmed in several studies [11, 12]. A haplotype of 4 *RBP4* genetic variants (G-T-G-C; rs3758539 G/A, c.248+44T/C, rs12265684 G/C, and rs10882273 T/C) was shown to associate with higher visceral *RBP4* mRNA expression [12]. Munkhtulga *et al.* demonstrated in a Mongolian population that rs3758539 (-803 G/A) is associated with an increased risk of T2D, which supports the hypothesis that rs3758539 may be a causal variant (**Table 1**) [95]. It could be shown that diabetic patients who are homozygote for the A-allele exhibit significantly higher RBP4 serum levels. For the A-allele, the transcription efficiency of *RBP4* and the motif-binding efficiency of hepatocyte nuclear factor 1 alpha (HNF1) were increased in a hepatocarcinoma cell line [95].

These results are in contrast to the results obtained by Craig *et al.* and Kovacs *et al.*, as in these studies the G-allele of the SNP was included in the haplotype [11-12]. However, the effect on transcription could not be observed in 3T3L1 cells. Therefore, the authors suggested clarifying the SNP function in clinical adipose tissue specimens or insulin-resistant 3T3L1 cells [95]. In 2010, a second study by Munkhtulga *et al.* carried out in adipocytes revealed that in a *RBP4* promotor assay in 3T3L1 cells the minor A-allele increased the promotor activity two to three times [96].

Associations of three more SNPs (+2333G/A (rs28383574), +5388C/T (rs7091052), and +8201T/A (rs386746636/rs7079946)) with serum RBP4 levels were found in a study performed in a Chinese population, but these associations were in strong LD to the rs3758539 variant, suggesting that they may simply tag the functional variant [97]. Furthermore, in a study population of 3,210 unrelated Han Chinese, reduced plasma RBP4 levels were significantly associated with the G-allele of rs10882273 A/G and the C-allele of rs3758538 A/C in the total cohort, and with the G-allele of rs17108993 C/G in a subgroup from Shanghai [98]. However, the genetic influence on plasma RBP4 levels seems to decline with age, suggesting that plasma RBP4 in the elderly may be regulated by environmental factors [99].

2.7 Vaspin (*SERPINA12*)

The visceral adipose tissue-derived serine protease inhibitor (vaspin, *serpinA12*) was detected in a rat model of T2D, and exhibits insulin-sensitizing properties [100]. *VASPIN* mRNA and protein expression have been associated with obesity and measures of insulin resistance/sensitivity [101]. Elevated vaspin levels were postulated to represent a compensatory mechanism protecting from insulin resistance in the context of obesity [102].

A GWAS was performed in 826 Sorbs from Germany, following the hypothesis that variability in serum vaspin concentrations has genetic components [103]. The following 6 SNPs were significantly associated with serum vaspin:

- rs11160190 C/T (between *VASPIN* and *SERPINA4*)
- rs6575436 T/A in *VASPIN*
- rs4905203 C/G
- rs1956713 T/C
- rs1956721 C/T
- rs11621467 G/T

The latter four are downstream polymorphisms, all of which could be replicated *in silico* in the KORA cohort (**Table 1**).

Fine mapping of the *VASPIN* locus by genotyping 26 tagging SNPs revealed several SNPs that were significantly associated ($p < 10^{-8}$) with serum *VASPIN* concentrations in a combined analysis including the Sorbs and the KORA cohorts (**Table 1**) [103]. However, none of the SNPs showing association with serum vaspin concentration with $p < 10^{-8}$ was associated with quantitative anthropometric and metabolic traits. Given that the SNP rs11160190 showing the strongest association with circulating vaspin maps 5' upstream of *VASPIN*, and that it is located within various transcription factor binding sites, there is an inevitable need for functional studies to prove the causality of this variant [103].

A functional study for vaspin SNPs was performed by Teshigawara *et al.* who showed that the A-allele of SNP rs77060950 C/A, predicting higher serum vaspin level in a Japanese population, was associated with higher luciferase activity [104]. Indeed, the transcription factor GATA, predicted for the site containing rs77060950, demonstrated a much stronger DNA protein complex by using the A-oligo instead of the C-oligo in the electrophoretic mobility shift assay (EMSA). Furthermore, serum vaspin concentrations tend to be lower in adults

carrying a rare variant in *VASPIN*, rs61757459 C/T, predicting a premature stop codon (p.R211X) [105]. In consequence, this stop codon results in a truncated vaspin protein lacking four helices, multiple β -strands, and the integral reactive center loop.

2.8 Further adipokines

Adipsin (also called complement factor D (CFD)) is a serine protease with complement factor D activity. Its mRNA is highly induced during adipocyte differentiation, and its gene expression is profoundly altered in genetic and acquired obesity in mice studies [106-109]. In *adipsin*^{-/-} mice, Lo *et al.* showed that this protein plays an important role in insulin secretion by the pancreatic β -cells. They also showed that adipsin is decreased in patients with T2D and β -cell failure [110]. The data by Lo *et al.* suggest that adipsin stimulates insulin secretion via actions of C3a on its receptor to improve glucose homeostasis *in vivo*. However, the dysregulation of adipsin expression may arise from certain genetic (like in *db/db* mice) or metabolic defects [110]. In this context, overfeeding studies in rats showed that the change in adipsin expression upon feeding a cafeteria diet was only minor. Similarly, human studies have shown that subjects with mild to moderate obesity did not have reduced levels of circulating adipsin [110-113].

Despite the fact that adipsin is an "old" adipokine, there are only a few studies linking genetic polymorphisms in any gene with serum adipsin levels. One study was conducted by Ukkola *et al.*, but it should be interpreted with caution. The authors performed an overfeeding experiment in 12 pairs of young male monozygotic twins, and investigated a restriction fragment length polymorphism (RFLP) in *adipsin* (6.1 kb *Hinc II* polymorphism) [114]. The variant was associated with changes in body fat composition in response to the caloric surplus, but not with adipsin levels. However, the investigated sample size was low and participants were monozygotic twins, which resulted in a limited statistical power. Another publication reported on SNPs in CFD in age-related macular degeneration, but no association of rs3826945 (C/T) with plasma CFD concentrations was found [115].

Apelin (*APLN*) has pleiotropic effects on numerous organs and tissues, and appears to be a beneficial adipokine with anti-obesity and anti-diabetic features (reviewed in [116] and [117]). It exerts effects on the whole body metabolism at a level similar to adiponectin and leptin. The *APLN*

gene is located on the X chromosome. SNPs in/nearby *APLN* have been tested only in a few genotype-phenotype association studies, mainly in Asian populations [118-121]. In human primary adipocyte culture derived from 10 women (2 x CC, 4 x GC, 4 x GG), it was shown that carriers of the CC genotype from SNP rs3115757 C/G exhibit higher *APLN* mRNA and protein concentrations when cells were exposed to high glucose plus insulin [120]. However, this result was not evaluated statistically because of the small sample size. A recent study showed that rs3115758, which is in LD with rs3115757, is located in a miR-765 binding site in the 3'-UTR of *APLN* [122]. A reporter assay confirmed that the different alleles of rs3115758 interfered with miR-765 binding and modified *APLN* expression [122]. This was investigated in context with arterial stiffness, but remains to be investigated in adipocytes.

Omentin (ITLN1) was described in 2003 as a new adipokine secreted from omental adipose tissue (mRNA primarily expressed in the stromal vascular fraction) which enhances insulin-mediated glucose uptake in adipocytes and activates protein kinase Akt/PKB [123-125]. Decreased omentin levels are associated with increasing obesity and insulin resistance [126]. A missense variant Val109Asp (rs2274907 A>T, T representing the amino acid Val) was found by random sequencing, and investigated in patients with T2D and inflammatory bowel diseases [127]. However, no significant associations with clinical parameters could be detected. In patients suffering from psoriasis, serum omentin levels have been investigated in relation to the Val109Asp polymorphism, but no association was found, despite the fact that these patients exhibited lower serum omentin levels than the controls [128].

3. Adipokine receptors

Adipokines either act as autocrine or paracrine factors, or they circulate in the blood to be distributed to the target tissues. The study of their receptors is important as they can be targets for pharmacological treatment in obesity and T2D, and genetic variations in these receptors might codetermine the responsiveness and strength of adipokine signal transduction in the target tissues. For this purpose, pharmacogenomic studies are becoming more important [129]. Genetic variants in adipokine receptors may contribute to the regulation of serum adipokine levels through a feedback mechanism of downstream components which

sense insufficient signal transduction activities or an overshoot in these activities. However, the identification of adipokine receptors is no minor undertaking; the receptors for many adipokines are not yet known, and depending on the receptor, they have been little investigated in genotype-phenotype association studies.

3.1 Adiponectin receptor 1 and 2 (*ADIPOR1*, *ADIPOR2*), cadherin 13 (*CDH13*)

Three receptors have been identified for adiponectin or high-molecular-weight (HMW) species of adiponectin so far: adiponectin receptor (*ADIPOR*) 1 and 2, located on chromosome 1 and 12, respectively, and cadherin 13 (*CDH13*) on chromosome 16 [130, 131]. All receptors have been thoroughly investigated in genetic association studies for T2D, insulin resistance, and related traits, but in most studies serum adiponectin measurements were not available for *ADIPOR1* and 2. In 2009, Ling *et al.* found a SNP within *cadherin 13 (CDH13)*, rs7195409 A/G) to be associated with adiponectin levels [132]. However, the associated allele and the effect direction were not reported in the paper.

The *CDH13* SNP rs3865188 T/A was detected in a GWAS in Filipino women, with the T-allele associated with lower adiponectin levels [23]. It has been recently shown in male mice that there is a feedback regulation between adiponectin and T-cadherin which impacts on adiponectin levels in tissue and plasma via the regulation of the phosphatidylinositol-specific phospholipase C (PI-PLC) mediated T-cadherin cleavage [133].

In the GWAS by Ling *et al.*, adiponectin levels were not associated with *ADIPOR1* and *ADIPOR2* SNPs [132]. The association of SNPs in the *CDH13* locus with plasma adiponectin levels was supported by further studies showing several signals depending on the investigated population, mainly Chinese and Japanese (**Table 2**) [134-137]. The *CDH13* promotor SNP rs12444338 G-allele was associated with increased promoter activity in a luciferase assay [134].

Cohen *et al.* investigated *ADIPOR1* and *ADIPOR2* polymorphisms in relation to serum adiponectin levels in a comparatively large study group of women [18]. None of the receptor SNPs was found to associate with adiponectin levels. *ADIPOR* SNPs have also been investigated in relation to cancer, but likewise in these cohorts, *ADIPOR* SNPs were not associated with circulating adiponectin levels [138].

Table 2. Adipokine receptor variants found to be associated with serum adipokine concentrations

Adipokine receptor / gene	SNP-ID	SNP	EA	ED	% variance	Reported in
Cadherin 13 / <i>CHD13</i>	rs3865188 ^a	A/T	T	↓	n/a	[23]; [134] [*] , rev. [17]
	rs12596316	A/G	Minor (G) [*]	↓	n/a	[134] [*] , rev. [17]
	rs7193788 ^c	A/G	Minor (G) [*] [134]; G [136]	↓ [134]; ↑ [136]	n/a	[134] [*] , rev. [17]; [136]
	rs3865185	A/T	Minor (T) [*]	↑	n/a	[134] [*] , rev. [17]
	rs3865186	A/G	Minor (A) [*]	↑	n/a	[134] [*] , rev. [17]
	rs3852724	A/C	Minor (C) [*]	↑	n/a	[134] [*] , rev. [17]
	rs12922394	C/T	T	↓	n/a	[21]
	rs12444338 ^{a,b}	G/T	G	↑	n/a	[134] ^a
	rs12051272 ^b	G/T	G	↑	3.28% with rs4783244	[135] ^b
	rs4783244 ^{a,c}	G/T	G	↑	3.28 with rs12051272 [135]; 9.28% [136]; >4 % adiponectin & >6% HMW-adiponectin [137]	[135]; [136] ^c ; [137]
	rs8047711 ^c	A/G	G	↑	n/a	[136]
Leptin receptor / <i>LEPR</i>	rs1137101	A/G p.Q223R	G	↓	n/a	[142]; [143]
	rs1137101	A/G p.Q223R	G	↑	41% (together with age, gender, cigarette smoking, CAD & obesity) [144]	[144]; [145]; [146]; [147]; [148] _{men}
	rs1137100	A/G p.K109R	G	↑ [147] ↓ [151]	n/a	[147]; [151] _{women}
	rs1805094	G/C p.K656N	C	↑	n/a	[148] _{men}
Apelin receptor / <i>APLN</i> or <i>APJ</i>	rs11544374	G/A	A	↑	n/a	[174] ^s

Legend: Included are SNPs with the best statistical and/or functional evidence reported in the literature; further (LD) SNPs are discussed in the relevant references. ^{a/b/c} SNPs in LD based on data in the cited study. SNP given for the genomic strand where the SNP's gene/nearby gene is located: this might deviate from the alleles reported in the publication. Percentage variance of adiponectin concentration belongs to the population cited in the study. ^{*} Discovery cohort. ^{*} Assumed for investigated population in publication [134] based on NCBI SNP-database entries. ^s Limited by sample size. **Abbreviations:** EA – effect allele, ED – effect direction serum level, rev – reviewed in.

3.2 Chemerin chemokine-like receptor 1 (*CMKLR1*)

In contrast to the mechanisms previously proposed, recent data from rodents suggest that an agonist of the chemerin chemokine-like receptor 1 (*CMKLR1*) may be beneficial in the treatment of T2D. This suggestion is based on the observation that treatment with chemerin or its C-15 and C-19 terminal peptides causes an increased insulin-stimulated glucose uptake in wild-type rat and mouse epididymal adipocytes [139]. It seems that increased chemerin levels represent a compensatory mechanism in metabolic diseases. It has also been shown in mice that a disruption of the *CMKLR1* gene is associated with reduced adiposity, but the mice were glucose-intolerant and had a decreased glucose-stimulated insulin secretion [140]. Unfortunately, except for the *CMKLR1* in-

tron variant rs1878022 A/G, which was found in a GWAS for the survival rate of patients with non-small cell lung cancer [141], no other “common” *CMKLR1* genetic variant has been investigated.

3.3 Leptin receptor (*LEPR*)

For the leptin receptor (*LEPR*), data exist on the association of polymorphisms in/nearby the gene with serum leptin levels in several populations, although they are contradictory. The p.Q223R (rs1137101 A/G) polymorphism in *LEPR* is the most investigated. While some studies reported an association of the variant coding for the glutamine (“Q”) allele with higher plasma leptin serum levels [142, 143], others found that the arginine (“R”) allele was associated with increased leptin levels (**Table 2**) [144–148]. At the amino acid level, this polymorphism leads to a change in

electric charge from neutral to positive in this position, and may thereby affect the functionality of the receptor and alter its signaling capacity. These changes need to be further clarified (summarized in [149]).

The study by Quinton *et al.* showed that in postmenopausal women carrying the R223-allele, the leptin-binding capacity to the soluble LEPR in plasma was lowered. It is unclear how the soluble form of the LEPR reflects the function of the transmembrane LEPR in various tissues, but it is speculated that subjects having this variant also have a form of leptin resistance [149-150].

Two other variants in *LEPR* are of interest, p.K109R (rs1137100 A/G) and p.K656N (rs1805094 G/C). In a young Dutch adult cohort with recorded weight gain over a period of ~7 years, leptin levels were increased in carriers of the p.K109R R-allele [147]. In contrast, leptin levels were found to be higher in *LEPR* p.K109K homozygote postmenopausal women who were overweight or obese, but with 82 study subjects in this group, the statistical power was limited [151].

In a Caucasian male population, carriers of the Asn656-allele had significantly higher levels of plasma leptin (**Table 2**) [148]. Another study revealed that in 132 men with obesity undergoing a polyunsaturated fatty acid-enriched hypocaloric diet, leptin levels were significantly decreased in the group homozygote for the Lys656-allele after 2 months of intervention [152]. However, this seems to be a long-term mediated effect as no effect of these three *LEPR* polymorphisms on the acute decline in leptin after 4 days of energy restriction was observed [153]. Interestingly, the *LEPR* genotype-serum leptin level association is often found in “diseased” groups (subjects with obesity or T2D) [142, 143, 151]. Unfortunately, many of the studies are statistically underpowered or not properly adjusted.

3.4 Sortilin 1 (*SORT1*)

SORT1 was described as a neuronal PGRN receptor that mediates PGRN endocytosis and delivery to lysosomes [154-156]. The receptor has been implicated in coronary artery disease via LDL metabolism in the liver [157, 158] (reviewed in [159]). It seems that the effect of *SORT1*/PGRN signal transduction in the brain is independent of the effects in the periphery. In the brain, Prudencio *et al.* identified a specific splicing enhancer element that regulates the inclusion of a sortilin exon cassette (Ex17b) which is normally not present in the mature *SORT1* mRNA [156]. The enhancer ele-

ment is present in *Sort1* mRNA of mice and other species, but absent in primates that carry a premature stop codon within the Ex17b sequence. In the absence of the regulatory inhibitor, transactivation response region (TAR) DNA-binding protein-43 (TDP-43), Ex17b is included in the *SORT1* mRNA [156]. In humans, a truncated, non-functional, extracellularly released protein that binds to but does not internalize PGRN is generated, which is due to the stop codon in the Ex17b sequence [156]. Therefore, the authors proposed a potential mechanism linking mis-regulation of sortilin splicing with altered PGRN metabolism. Despite the association of the rs646776 variant near *SORT1* with serum PGRN levels (see section 2.4 above; **Table 1**) [67], no further variants in/nearby the receptor gene have been described as being associated with PGRN levels in the periphery to date.

3.5 CAP, adenylate cyclase-associated protein 1 (*CAP1*)

In 2014, Lee *et al.* discovered that adenylyl cyclase-associated protein 1 (CAP1) acts as receptor for resistin [160]. It could be shown that human resistin directly binds to CAP1 in monocytes and upregulates intracellular cAMP concentrations, PKA activity, and NF- κ B-related transcription of inflammatory cytokines. No candidate gene or GWA study investigating *CAP1* polymorphisms and resistin serum levels has been performed to date.

3.6 Stimulated by retinoic acid 6 (*STRA6*)

The *stimulated by retinoic acid 6 (STRA6)* encodes the receptor for the retinol/RBP4 complex, but it is not expressed in the liver [161]. These observations initiated studies leading to the identification of the novel RBP4 receptor-2 (RBPR2) in 2013 [162]. Polymorphisms in *RBPR2* have not been investigated so far. In *STRA6*, mutations have been identified. These mutations have two effects on vitamin A uptake: they either abolish vitamin A uptake activity of STRA6 from holo-RBP completely because STRA6 cell surface expression is lost, or vitamin A uptake activity is influenced by reduced STRA6 RBP binding activity [163].

In a South Indian population, three SNPs (rs974456 G/A, rs736118 G/A, rs4886578 G/A) were found to be associated with T2D, with the minor alleles appearing to be protective [164]. For rs736118, a polymorphism predicting an amino acid exchange from Met to Ile in the C-terminus of

STRA6 has been suggested to alter the trafficking and cell surface expression of the protein. However, the consequences of the receptor SNPs on serum RBP4 levels cannot be estimated as RBP4 was not measured in this study.

3.7 Kallikrein 7 (hK7), heat shock protein family A (Hsp70) member 5 (GRP78)

It is still not well understood how the extracellular vaspin signal is transduced into the cell [165]. Recently, circulating human kallikrein 7 (hK7) was identified as a protease target of vaspin. In an *in vitro* study, vaspin inhibited hK7 by the serpin mechanism with high specificity, suggesting that vaspin protects insulin from hK7-mediated degradation [166]. In mice, vaspin was shown to bind to cell-surface 78-kDa glucose-regulated protein (Grp78)/murine tumor cell DnaJ-like protein 1 (MTJ-1) complex under conditions of ER stress, causing vaspin transgenic mice to be protected from diet-induced obesity, glucose intolerance, and hepatic steatosis [167]. Furthermore, in endothelial cells vaspin was shown to bind to a GRP78/voltage-dependent anion channel complex after induction of ER stress, and to promote proliferation, to inhibit apoptosis, and to protect from vascular injuries in diabetes [168].

A *GRP78* polymorphism has been investigated in terms of risk for hepatocellular carcinoma (HCC) and onset of T2D peripheral neuropathy, but not in relation to vaspin concentration [169, 170]. However, the promoter SNP rs391957 C/T C-allele was associated with HCC risk and increased levels of *GRP78* mRNA and protein [169].

3.8 Complement component 3a receptor 1 (C3AR1), apelin receptor (APLNR) & omentin (ITLN1)

As mentioned in section 2, the data by Lo *et al.* suggest that adipsin stimulates insulin secretion via actions of C3a on its receptor C3aR1 [110]. In a candidate pathway-based GWAS in a Chinese population, a variant in the 3'-UTR of *C3AR1* (rs7842 A/G) was identified in an eQTL analysis to associate with C3AR1 expression levels and coronary artery disease [171]. In this study, carriers of the minor G-allele exhibited higher *C3AR1* expression levels in leukocytes. However, C3AR1-related measures, e.g. those of C3a and adipsin, have not been collected in this study.

The apelin receptor (APLNR or APJ) is a G protein-coupled receptor; its signaling is implicated in several pathologies, including heart disease, diabe-

tes, obesity, and cancer (reviewed in [172]). A genetic variant (rs9943582 G/A) has been described to affect *APJ* mRNA transcription via modification of a specificity protein 1 (Sp1) transcription factor binding site [173]. It could also be shown that *APLN* is a transcriptional target of Sp1 via a Sp1 binding site in the 5'-flanking region of *APLN*. However, the relationship between the receptor SNP and serum apelin levels was not investigated [173]. To date, the rs11544374 G/A *APJ* polymorphism only was found to exert an effect on circulating apelin levels in childhood obesity (**Table 2**) [174]. Interestingly, obese subjects with impaired glucose metabolism only had lower apelin levels than controls. In the obese subgroup, carriers of the major G-allele had lower apelin levels [174]. However, the investigated sample size and so the statistical power was low (90 obese vs. 90 controls).

The situation is somehow different for omentin, also known as intelectin 1, as it is expressed as a lectin that binds galactofuranose, but also acts as a receptor for lactoferrin [175]. Human intelectin 1 is speculated to be attached to the epithelial cell membrane via glycosylphosphatidylinositol (GPI) as it has no putative transmembrane region [175, 176]. To date, no other molecules are known to act as a receptor for the adipokine omentin. The rs2274907 polymorphism remains the only genetic variant which has been investigated in the context of serum omentin levels.

4. Epigenetics

Evidently, adipokine epigenetic changes, which are not only limited to adipose tissue and are tightly controlled by nutrient factors, are involved in the development of obesity and related complications beginning early in life [177-180]. Genetic variants may contribute to this scenario as epigenetic mechanisms are influenced by insertion or removal of CpG/non-CpG sites. Also, SNPs may modify binding sites for DNA methyl transferases, histone packaging may be affected, and chromatin organization may be influenced [181, 182].

Methylated (me-)cytidines are susceptible to deamination. While deaminated non-methylated cytidines become uracils and are efficiently detected by DNA proofreading mechanisms, deamination of me-cytidine leads to the formation of thymine that is a regular DNA base [183]. In a recent paper investigating *LEP* and *ADIPOQ* methylation in paired SAT, VAT, and blood samples of 73 severely obese patients, fasting LDL-C levels were found to be positively correlated with *LEP* DNA methylation levels at certain CpG sites in blood

and SAT, and with *ADIPOQ* DNA methylation levels in SAT and VAT [178]. Furthermore, the authors reported that the association between LDL-C and *LEP* mRNA levels were partially dependent on *LEP* rs2167270 G/A genotype and DNA methylation level at CpG17. LDL-C levels were found to be negatively correlated with *LEP* mRNA levels in rs2167270 A-allele carriers only, and the correlation between *LEP* expression and LDL-C levels was no longer significant after adjusting Pearson's correlation coefficient for *LEP*-CpG17 DNA methylation levels [178].

A recent study by Putku *et al.* showed that the *CDH13* promoter harbors methylation quantitative trait loci (meQTLs) associated with cardiometabolic traits [184]. Four common variants (rs12443878 C/A, rs12444338 G/T, rs62040565 C/T, and rs8060301 A/T) exhibited effects on methylation levels of up to 3 CpG sites located 19-167,744 bp from the respective SNPs. SNP rs8060301 was also associated with high-molecular-weight adiponectin levels [184]. Rs8060301 is in LD with the previously described SNP rs4783244. Since rs8060301 is located at the strongest binding site of RNA polymerase II (Pol2) within the *CDH13* promoter, it is speculated that this SNP acts as a causal variant that directly affects gene expression driving the associations with metabolic phenotypes [184]. Finally, two rare *CDH13* promoter meQTLs were associated with HMW adiponectin (rs2239857 C/G, rs77068073 C/T).

Studies on epigenetic modifications in combination with SNP and mRNA expression data of other adipokines are sparse or limited by sample size. This may be due to the facts that environmental factors (including nutrition) across individuals are very heterogeneous, and target tissues (such as adipose tissue) are not easy to access. Furthermore, recent data by Rönn *et al.* suggests that the human DNA methylome in SAT changes considerably with age [185]. They also found BMI exerts effects on the degree of DNA methylation in human adipose tissue, proposing that obesity can mediate some of its effect through changes in the epigenome. Also, CpG sites showed differential DNA methylation in adipose tissue from subjects with T2D compared to controls [186]. Somewhat surprisingly, well-known adipokine/receptor genes were not found among the differentially methylated genes.

The relationship between genotype, methylation, and the mechanisms involved in the regulation of DNA methylation under certain conditions is still not clear [180]. In any case, the potential

epigenetic modification should be taken into account when investigating the genetics of adipokines and adipokine receptors.

5. Concluding remarks

Surprisingly, despite a multitude of studies reporting statistical associations of genetic variants in adipokine genes with T2D, obesity, and related traits, little is known about the genetic determinants of serum adipokine concentrations. On the other hand, there are a lot of studies reporting correlations between serum adipokine concentrations and metabolic traits in small sample sets without investigating a genetic component. Likewise, a SNP is shown to either associate with the metabolic trait/outcome or the serum adipokine concentration, but not with both simultaneously, which would allow a causative chain to be generated. However, when reporting negative results for genetic association studies, it would be helpful to provide power calculations for the trait investigated in the cohort. This would enable that the plausibility of the study could be assessed. In any case, effective strategies will be necessary to study the genetics of adipokines.

GWAS are sparse in the field of the genetics of adipokines. This is mainly due to the lack of effective assays (e.g. ELISA) providing accurate and reliable measurements of circulating adipokines. Yet, there is good evidence from the recent reports that GWAS may indeed uncover novel adipokine regulators. Also, candidate gene studies may play an important role in the investigation of adipokine receptors or downstream signaling components. However, candidate gene strategies combined with deep sequencing of the tested genes/loci are more likely to identify rare variants affecting circulating adipokine levels. Either way, it will be necessary to have well-defined and well-characterized populations as well as valid adipokine measurements.

Another question is to what extent a genetic effect would be expected. In some cases, we may overestimate the genetic influence on serum adipokine levels compared to other factors such as increasing adipose tissue mass or aging. While reviewing the percentage variability in serum adipokine levels for which the genetic variants are responsible, it is obvious that in some cases (such as *ADIPOQ*), age, gender, and BMI have a higher proportion than the SNPs themselves (as shown in [26, 27]). In contrast to these considerations, data on chemerin and resistin suggest that serum adipokine levels have a high heritable component which supports the need to look for genetic vari-

ants determining serum adipokine levels [35, 36, 78].

Reviewing exact data was occasionally hampered by flaws in some studies, where SNP alleles were not reported, or tables and text have not been in agreement [54, 134]. Determining DNA strand specificity and reporting the respective minor and major alleles is easy for A/G, A/C, T/G, and T/C base exchanges. However, for A/T and G/C SNPs, it is sometimes difficult to define the correct direction of the effect as decisive information with regard to the assignment of DNA strand and alleles is frequently missing. Also, minor and major alleles can flip within and even between populations.

The strength of an association of a genetic variant with a trait also depends on the other genetic architecture of the individuals. The investigation of large admixed populations may result in the detection of variants with small effects because the scale of the population delivers sufficient statistical power. In contrast, comparatively large signals may be specific for a certain well-defined population, as shown in the paper by Asano *et al.* [82]. They found an effect of rs34861192 on serum resistin levels specific for the investigated Japanese population as this SNP is monomorphic in the EGP-CEPH Panel of dbSNP. This is also nicely demonstrated in the paper by Grarup *et al.* which is included in this RDS Special Edition [187].

There is a need for functional studies which will help to clarify the consequence of genetic polymorphisms that are statistically associated with serum adipokine levels and with the metabolic features of T2D. New techniques such as genome editing with the CRISPR/Cas system will enable efficient protocols to introduce specific genetic variants, monitor the consequence on gene and protein levels, and explain epigenetic effects [188]. There is no doubt

that our knowledge of the genetic background of adipokines would lead to a better understanding of their regulatory mechanism. Considering the potential of adipokines as treatment agents, alternative, population-specific treatment strategies may be developed, targeting downstream components of the signaling pathways or other regulatory components, which may reduce side effects.

The pleiotropy of adipokines may add another aspect of complexity. In this regard, genetic variants associated with serum adipokine levels have been shown to be associated with numerous metabolic traits such as waist-to-hip ratio. This provides a unique opportunity to detect novel physiological relationships which help to understand the physiology of adipose tissue distribution and eventually the complex etiologies of metabolic diseases. The development of metabolic diseases is driven by a combination of multiple factors, including increased adipose tissue mass, genetic/epigenetic predisposition for elevated/reduced adipokine levels, and modified signal transduction at the receptors in the target tissues. The main challenge in the near future will be to uncover the causative chains behind the genetic associations.

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