

Genetic and epigenetic interactions in adaptive thermogenesis pathways in association with obesity from a Public Health Genomics perspective

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ABSTRACT

BACKGROUND: studying and apprehending the pathways and mechanisms by which overweight and obesity trigger complex disease progression is of prime importance for the development of therapy and prevention measures of this major public health burden. This review describes Single Nucleotide Polymorphisms (SNPs) and epigenetic methylation as well as histone modification in genes with relevance in adaptive thermogenesis and their possible role in the development of obesity. Epigenetic marks are discussed as solid biomarkers for gene-environment interactions.

METHODS: a PubMed search on genetic and epigenetic variation of genes involved in adaptive thermogenesis was performed. The search included English publications between December 1996 and July 2010 reporting associations between SNPs and obesity in Caucasians. The search on epigenetic regulation was limited to DNA methylation and histone modifications. Genes that were found to be associated with the adaptive thermogenesis pathway included beta-3 adrenergic receptor (ADRB₃), uncoupling protein 1 (UCP₁), the transcription factors peroxisome proliferated activator receptor gamma (PPAR γ), peroxisome proliferated activator receptor gamma co-activator 1 alpha (PGC1 α), retinoid acid X receptor alpha (RXR α), CCAAT/enhancer-binding protein alpha (C/EBP α), fatty acid binding protein 4 (FABP₄) and lipoprotein lipase (LPL).

RESULTS: epigenetic studies are mainly discovery orientated and do not test hypotheses. However, SNPs as well as epigenetic mechanisms seem to regulate obesity and adaptive thermogenesis whereas genetic association studies are inconsistent.

CONCLUSIONS: the aim of this work was to confirm evidences on the contribution of genetic variations as well as epigenetic regulatory mechanisms of genes associated to obesity. The integration of epigenetic markers in epidemiologic research could help to unravel multi gene-environment interactions.

With this article we show the importance to introduce aspects of the epigenetic regulation in the assessment of obesity: We also discuss the benefits of including epigenomics as an integrative way to account for an individual's environmental impact in public health policies.

Key words: Genetic; Epigenetic; Thermogenesis; Obesity; Public health genomics

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INTRODUCTION

The World Health Organization (WHO) uses the body mass index (BMI) to define overweight: $BMI = \text{weight (kg)} / \text{height (m}^2\text{)}$ (1).

For adults, a BMI in the range of 18.5 to 24.9 is associated with the lowest risk of mortality and morbidity. Overweight is defined as a BMI of 25.0 to 29.9 and obesity is defined as a BMI of at least 30. Overweight and obesity are associated with increased risks for serious health consequences (1).

Overweight is characterized by an excessive accumulation of lipids in body cells especially in adipose tissue. It develops by the complex interaction of heterogeneous genetic factors and an imbalance in energy intake and expenditure. This health threatening condition is a major risk factor for chronic diseases such as diabetes mellitus 2, cardiovascular and musculoskeletal systems diseases and some types of cancers as for example oesophagus, pancreas, colon, rectum, breast (after menopause), endometrium (lining of the uterus), kidney, thyroid and gallbladder. Estimates suggest a heritability of 50–70% for BMI (2) and of 75–80% for total body fat (3). However, recent scientific results from association studies (4) could not confirm such result and epigenetic mechanisms were proposed to explain this “missing heritability”. Genome wide association studies (GWAs) identified numerous genes and polymorphisms playing a role in the development of obesity. Mostly, these genes regulate food intake and influence nutrient metabolism. For example, the cellular receptor beta-3 adrenergic receptor (ADRB3) responds to noradrenalin and mediates lipolysis in adipocytes. A polymorphism that changes the amino acid sequence of the protein might be able to influence the receptor’s ability in transmitting the signal and thus have an effect on body fat accumulation. The polymorphism Pro12Ala in the ubiquitously expressed gene of the nuclear peroxisome proliferator activated receptor gamma (PPAR γ) is positively associated with the BMI in numerous studies. PPAR γ senses a variety of lipids and lipid like compounds and is an essential transcription factor in adipocytes. Furthermore, polymorphisms in hormones and their receptors such as leptin and adiponectin have been identified as key factors modelling a thrifty genotype (5).

Epigenetics

Monozygotic twins living in different environments can develop different phenotypes (6) whereas unrelated individuals can develop similar health problems when they share the same environment (6).

Observations from the Dutch famine birth cohort (6) or the agouti mouse model (7) showed influences of pre- and postnatal nutritional factors on the “programming” of the genotype for later life, therefore influencing the risk for common diseases. The process is mediated through epigenetic mechanisms such as DNA methylation, histone modifications, chromatin remodelling and miRNAs (8). Methylation of cytosines in CpGs in the promoter region of a gene silences transcription by hindering transcription factors and RNA polymerases to bind to the DNA strand. Promoter methylation is a mechanism by which cells differentiate. For example, the PPAR γ promoter is 7 fold more methylated in T cells (not expressing the gene) than in adipocytes, which carry the PPAR γ receptor (9). Histone modifications result in different transcriptional outcomes depending on type, amount and site of the modification and on its reversibility (reviewed in (10)). miRNAs are a class of non-coding endogenous RNA molecules shown to play critical regulatory roles in a wide range of biological and pathological processes. Compared to other epigenetic modifications, their mechanisms of action are relatively unknown (11).

Epigenetic modifications are cell type specific and can be dynamically influenced during the entire life by dietary components and lifestyle factors (12-15), toxins (16), endocrine disruptors (17), radiation (18), air pollution (16) and social, in particular maternal, behaviour (19). They are passed on over numerous cycles of cell division and sometimes even from one generation to the next (20). In response to different environments, this plasticity makes it possible to develop a range of phenotypes based on a single genotype.

Epigenome analyses might overcome the difficulties associated with studying effects of dietary composition on long-term health or effects of gene-environment interactions in human health and disease.

Energy dissipation and thermogenesis

The physical activity of the skeletal muscles is crucial to maintaining a balance between

energy intake and expenditure. In addition, brown adipose tissue (BAT) is responsible for 1-2% of the overall energy dissipation.

BAT was long thought to exist only in neonate but can prevent a weight gain of 1-2 kg/year by increasing thermogenesis also in adults (21). It produces heat and thereby increases energy expenditure by uncoupling the oxidation of fatty acids from ATP synthesis by the inner mitochondrial membrane protein UCP1 (22). In obese adults, a 50% decreased expression of UCP1 compared to normal weight individuals has been demonstrated (23). In elderly individuals, the amount of BAT correlates inversely with the BMI (24).

BAT's thermogenic activity and therefore its energy dissipation, depends on the degree of stimulation by thyroid hormones and also on stimulation by the sympathetic nervous system through ADRB3 (22). An activation of ADRB3 results in the increased expression of the PPAR γ co-activator 1 alpha (PGC1 α) (25). PGC1 α forms a transcription factor complex together with PPAR γ and retinoid acid X receptor alpha (RXR α) and activates the transcription of UCP1 upon binding to its promoter region. In the absence of ligands, PPAR interacts with co-repressor proteins and builds a complex with histone deacetylases (HDACs) resulting in a locally more compact chromatin packaging (26). The amount of UCP1 determines the degree of heat production and therefore the amount of energy expenditure.

The role of BAT in the development of obesity is well established and its transcriptional network is strongly affected by environmental and genetic influences. Thus, studying epigenetic marks in addition to genetic influences regulating thermogenesis in obesity shows great promise for finding solid biomarkers for gene-environment interactions

METHODS

We performed a literature search in Pubmed on human genome studies in English between December 1996 and July 2010 associating obesity with SNPs or epigenetic markers using the terms "gene" or "genome" and/or "epigenetic" or "methylation" or "histone" in title or abstract. Observational studies associating the polymorphisms with anthropometric measurements and related obesity phenotypes were included in this review. If no ethnicity

was reported in a study conducted in central Europe it was assumed to include merely Caucasians and otherwise excluded. We did not restrict studies by minimal subject number.

To assess the presence of epigenetic modification in the UCP1 transcription network, a second literature search that focused on evidence for DNA methylation and histone modification in the same genes was performed (Table 1). We additionally searched the article references for further suitable studies. No information on post transcriptional modifications was included.

In a third step we widened the network for CCAAT/enhancer-binding protein alpha (C/EBP α), a transcription factor essential during adipogenesis (27), fatty acid binding protein 4 (FABP4) and lipoprotein lipase (LPL), two other target genes of PPAR γ whose transcripts are important factors ensuring the steady substrate availability for mitochondria.

Details of the search process are summarized in Figures 1-3 (Supplemental Materials).

RESULTS

Results are summarized in Tables 2-8.

Further detailed results (Tables 9-16) are included in Supplemental Materials.

Results on ADRB3

The transcription process is initiated with an incoming signal from the hypothalamus as a reaction to cold surroundings. In the gene of the mediator ADRB3, the SNP rs4994 changes the amino acid sequence at position 64 from Trypsin to Arginine (Arg) and has been researched in relation to obesity. A meta-analysis looking for an effect of the SNP was published in 2008 by Kurokawa et al (28). 53 studies had been summarized with an overall size of around 23 000 Caucasians. Taken together, no positive association with obesity was found.

Since then, further studies investigated the association between rs4994 and obesity (Table 2). In 284 postmenopausal Polish women, no association was detected either (35). The results from associations in Mediterranean populations are more inconsistent. In a group of obese individuals, carrier of the Arg allele had significantly higher BMIs than

TABLE 1

GENES UNDER OBSERVATION			
GENE NAME	GENE	GENE MAP LOCUS	SNP
Beta-3 adrenergic receptor	ADRB ₃	8p12-p11	rs4994
Peroxisome proliferated activator receptor gamma coactivator 1 alpha	PGC1 α	4p15	rs8192678
Peroxisome proliferated activator receptor gamma	PPAR γ	3p25	rs1801282
Retinoid acid X receptor alpha	RXR α	9q34	-
Uncoupling protein 1	UCP1	4q31	A-3826G
CCAAT/enhancer-bindingproteinalpha	C/EBP α	19q13	rs12691 rs41490344 rs41504144 rs41344151 rs16967952 rs34508287 rs41428545 rs34529039 rs41367646 rs1049969 rs707656
Fatty acid binding protein 4	FABP ₄	8q21	rs1054135
Lipoprotein lipase	LPL	8p22	rs285 rs320 rs328

the control group (28, 29, 30). A study on 264 individuals did not replicate any effect on obese phenotypes at all (36). A study on 387 patients suffering from obstructive sleep apnoea syndrome demonstrated a higher BMI for Arg allele carrier compared to controls (28), while in a group of 5 822 obese patients with type 2 diabetes this association could not be detected (32). In two subgroups of cohort studies with Caucasians, no associations with obesity phenotypes were found either (31- 36).

A literature search for evidences of epigenetic regulation of ADRB₃ did not reveal any study observing DNA methylation or histone modifications.

Results on PGC1 α

A SNP in PGC1 α in some individuals changes the amino acid sequence at position 482 from glycine to serine (Ser) (rs8192678). By now, it is one of the most frequently

studied polymorphism in this gene in relation to obesity as summarized in Table 3. Three studies observed positive associations of the Ser variant and obese phenotypes (37, 43, 45). One of them detected a gender specific effect in 467 women (62). In men participating intensive type 1 diabetes therapy over 6 years, the variant was associated with higher weight gain (302 participants) (45). The third study demonstrated the positive association of non-essential fatty acid concentration and fat mass (410 obese and 281 controls) (43). In a group of 1 147 patients with the metabolic syndrome (and 1 108 controls), no association was detected either (38). Furthermore, neither in a study on morbidly obese (276 cases and controls) (39) nor in a study on 156 healthy persons (40) or in a group of 100 women with gestational diabetes and 100 controls (41) associations of the investigated SNPs with obese phenotypes were detected.

In Table 4 findings from literature

TABLE 2

STUDIES ASSOCIATING OBESITY PHENOTYPES AND THE SNP RS4994 IN ADRB3 SINCE THE PUBLICATION OF KUROKAWA, 2008 (28)									
PHENOTYPE	+/-	P	STUDY POPULATION				ADDITIONAL INFORMATION	REF.	
			ETHNICITY	FEMALE	MALE	CHILDREN			
BMI	+	< 0.01	Spanish	50 33 controls	337 104 controls		Patients suffered from obstructive sleep apnoea syndrome	(28)	
		< 0.001	Italian	165 53	100 25 controls		Cases were severely ob. An increased BMI was related to age	(29)	
		< 0.05	Spanish	154	63		Patients were obese but healthy	(30)	
	-			Polish	200			Participants were healthy, postmenopausal obese and lean women	(31)
				Danish	2 919	2 903		Cases were obese and/or suffered from type 2 diabetes	(32)
				Dutch	1 519			Subgroup of the EPIC study cohort	(33)
				Polish			60 33 controls	Case patients were obese	(34)
				Polish	284			Of the postmenopausal women 15 % suffered from the metabolic syndrome	(35)
Lower weight loss	+	< 0.05	Spanish	145	48		The obese but healthy patients received one of two hypocaloric diets and exercise programs for a period of 3 months. No difference between the groups was detected	(36)	

Phenotype: Investigated trait; +/- : association of SNP; Cases: obese patients

search on epigenetic regulations are listed. No information on studies observing histone modifications of PGC1 α were found. Compared to cells from healthy controls, DNA methylation of the promoter region in

skeletal muscle and pancreatic cells from diabetics was increased (46, 49). A high fat diet and high titers of free fatty acids seem to enhance methylation even more. In human umbilical cord, the promoter methylation

correlated positively with maternal BMI but not with characteristics of the new-born (48). In skeletal muscle tissue of male humans

who were born with low birth weight, a 5-day high fat overfeeding results in higher promoter methylation (47) (Table 4).

TABLE 3

STUDIES OBSERVING ASSOCIATIONS BETWEEN THE SNP RS8192678 IN PGC1 α AND OBESITY PHENOTYPES

PHENOTYPE	+/-	P	STUDY DESIGN	STUDY POPULATION			ADDITIONAL INFORMATION	REF.
				ETHNICITY	FEMALE	MALE		
BMI	+	<0.005	Case-control	Austrian	467		The association was only significant in middle-aged women heterozygote for the allele.	(37)
			Case-control	Austrian		591	The association was significant in female heterozygote for the allele.	
	-	Case-control	Danish	1,147	1,108	Subgroup from the DanMONICA cohort Participants suffered from the metabolic syndrome.	(38)	
		Case-control	French-Canadian	197	79	Participants were morbidly obese.	(39)	
		Case-control	Finnish	86	70		(40)	
		Case-control	Austrian	100	100 controls	Women were suffering from gestational diabetes mellitus.	(41)	
		Cohort	Spanish	87	93	8 weeks diet in healthy obese and overweight participants.	(42)	
Non-essential fatty acids clearance	+	<0.02	Case-control		410	281	The association between fat mass on non-essential fatty acid concentrations and genotype is significant in obese individuals.	(43)
Change in body weight		-	Cohort	Germans	73	63	9 months intervention (diet & physical activity). BMI >27 kg/m ² and a family history of type 2 diabetes.	(44)
Higher weight gain	+	0.0045	Cohort			302	Intensive diabetes therapy. The mean weight gain was 17 kg over 6.2 yrs intensive therapy. Patients were suffering from type 1 diabetes. Association was significant only in men.	(45)
	-		Cohort		280		No positive association in female patients with type 1 diabetes on intensive diabetes therapy.	

Phenotype: Investigated trait; +/- : association of SNP; Cases: obese patients

TABLE 4

EVIDENCE ON DNA METHYLATION OF PGC1 α			
TISSUE	HUMAN TISSUE	EVIDENCE	REF.
Human pancreatic islets from non-diabetic and type 2 diabetic subjects	✓	Twofold promoter methylation in diabetic compared to non diabetic subjects.	(46)
Human skeletal muscle tissue from non-diabetic and type 2 diabetic subjects	✓	Promoter hypermethylation in diabetic subjects.	(47)
Myocytes from healthy male humans	✓	Free fatty acids induce cytosine methylation at non-CpG sites. DNMT3b silencing prevented methylation.	
Human umbilical cord	✓	No association among characteristics of newborns and status of promoter methylation. Positive correlation between maternal BMI and promoter methylation.	(48)
Skeletal muscle tissue of male humans	✓	After 5 days high fat overfeeding subjects born with low birth weight had higher promoter methylation.	(49)

Phenotype: Investigated trait; +/- : association of SNP; Cases: obese patients

Results on PPAR γ

The Pro12Ala polymorphism (rs1801282) is one of the most frequently investigated SNP of PPAR γ in the last years. Two meta-analyses on associations between the Alanine variant and obese phenotypes have been published. In 2003, Masud et al (103) detected a positive association for BMI although no distinguishing between ethnicities was done. The latest meta-analysis from Tonjes et al. in 2006 focused solely on healthy Caucasians (in total 19 699 subjects) and indeed detected a positive association (104).

Studies found by the literature search on epigenetic regulation of PPAR γ are summarized in Tables 5 and 6. In human adipose stem cells (3T3-L1 cultures), the hypo-methylated promoter was demonstrated to gradually demethylate upon induction of differentiation while being hypo-methylated due to senescence (9). Furthermore, in lean mice, promoter methylation was higher compared to obese mice (52).

The results from studies observing histone modifications draw a similar picture. In cancer research, increased histone acetylation and phosphorylation were associated with

less expression (55). During adipogenesis, enzymatically coordinated (de)acetylation and (de)methylation events at H3 and H4 facilitate the transcription of the gene (57, 58, 59, 60, 61).

Results on RXR α

The literature review for RXR α variants and association with obese phenotypes revealed one study that identified 3 common variations in Caucasians: -25 A>G, 69 G>A, and 1470 C>T (105). Though this receptor plays a key role in development and metabolism, no study associated any genetic variation with obesity related traits. Also, the literature search for epigenetic regulation of the gene returned only one single study in which the effects of green tea on demethylation of the promoter region in cancerous mice was examined (106).

Results on UCP1

UCP1 can harbour several different polymorphism of which the A-3826G substitution is the most investigated studies associating the polymorphism with obese

TABLE 5

EVIDENCE ON DNA METHYLATION OF PPAR γ			
TISSUE	HUMAN TISSUE	EVIDENCE	REF.
Rat hepatocytes		Due to dietary protein restriction of female pregnant rats PPAR γ 1 promoter methylation in offspring did not differ	(50)
Human adipose stem cells	✓	Overall CpG hypomethylation of PPAR γ 2 due to senescence	(51)
wt- mice Lep+/Lep+ mice Diet-induced ob mice		Increased promoter methylation in wt-mice	(52)
3T3-L1		PPAR γ 2 promoter hypermethylation. Progressive demethylation upon induction of differentiation	
Human umbilical cord	✓	Hypermethylation of selected CpGs in promoter region	(48)
Adipose stem cells	✓	Largely unmethylated in PPAR γ 2 promoter region	(53)
Human gastric mucosa of ulcer patients, cancer patients, and controls	✓	Hypomethylation of promoter region in ulcer patients and cancer patients compared to methylation status in controls	(54)

Phenotype: Investigated trait; +/- : association of SNP; Cases: obese patients

(phenotypes are summarized in Table 7). A gender specific effect observed in women (55 cases and 117 controls) (51) could not be detected in a group of 77 women in a weight reduction program (64). Studies on obese and morbidly obese and controls associated BMI positively with the polymorphism (64, 65). Further, an intervention study on 12 pairs of male twins demonstrated lower weight loss in carriers of the variant (66). On the other hand, a study with obese and/or morbidly obese did not detect an association and a weight reduction program on 282 participants did not show differences (67, 68, 69, 70).

Epigenetic regulation of the UCP1 gene in several (non-human) tissues was observed. Results are summarized in Table 8.

Results on joint effects of polymorphisms

We also identified studies that observed joint effects of polymorphisms. A case study on 185 obese with sex- and age-matched controls revealed that carrier of both, the Pro12Ala variant in the PPAR γ and the Trp64Arg variant in the ADRB3 gene had a higher BMI (103). An association study on patients suffering from type 2 diabetes (cases and controls in total 1 193) did not detect a joint effect of the

Pro12Ala (PPAR γ) and rs8192678 (PGC1 α) SNP (104). Studies observing synergistic effects of the minor variant of the UCP1 gene and the Arg variant in the ADRB3 variant are summarized in Table 9. A combined effect of higher weight gain was demonstrated in obese and morbidly obese individuals (total 410 subjects). Higher metabolic rates were detected in 170 obese and 112 controls when carrying both variants but a synergistic effect on BMI could not be demonstrated (total 410 subjects).

DISCUSSION

The aim of this work was to identify genetic and epigenetic variations in adaptive thermogenesis contributing to the development of obesity. We chose the thermogenic pathway as this pathway is a more self contained component in the otherwise very complex aspects of obesity.

The literature review revealed studies observing polymorphisms in all investigated genes except for the transcriptional co-activator RXR α . In addition, despite for ADRB3, observations on epigenetic mechanisms exist for all genes. So far, much more effort has gone into finding associations between genetic variations and obesity than in observations of

TABLE 6

EVIDENCE ON HISTONE MODIFICATIONS AT PPAR γ				
MODIFICATION	TISSUE	HUMAN TISSUE	EVIDENCE	REF.
H3K4 acetylation	Mammary rat tumours		Cancerogenesis: in cancer-rats increased acetylation and phosphorylation associated with less expression	(55)
	3T3-L1		Adipogenesis: hyperacetylation in PPAR γ 2 gene during on-going differentiation and hyperacetylation in PPAR γ 1 gene before induction of differentiation	(56)
H3K4 methylation	Embryonic fibroblast		Adipogenesis: association with 2 methyltransferases	(57)
	Long-term cultured undifferentiated adipose stem cells		Inactive PPAR γ 2 promoter	(58)
	Differentiated adipose stem cells		No change of trimethylation in PPAR γ 2 promoter region when differentiated	
H3K4 phosphorylation	Mammary rat tumors		Cancerogenesis: in cancer rats increased acetylation and phosphorylation associated with less expression	(55)
H3K9 acetylation	Retrovirus infected fibroblasts 3T3-L1		Adipogenesis: Hyperacetylation during differentiation (PPAR γ 2 promoter activation)	(59)
	NIH 3T3 3T3-L1		Adipogenesis: acetylation upon differentiation (PPAR γ 2 promoter)	(60)
	Long-term cultured undifferentiated adipose stem cells		Inactive PPAR γ 2 promoter	(58)
	Differentiated adipose stem cells		Hyperacetylation in promoter region	
H3K9 methylation	Mesenchymal stem cells		Osteoblastogenesis/adipogenesis: inactivation of PPAR γ through methylation	(61)
H3K14 acetylation	Retrovirus infected fibroblasts 3T3-L1		Adipogenesis: Hyperacetylation during differentiation (PPAR γ 2 promoter activation)	(59)
	NIH 3T3 3T3-L1		Adipogenesis: acetylation upon differentiation (PPAR γ 2 promoter)	(60)
H3K27 methylation	Embryonic fibroblasts		Adipogenesis: association with H3K27 specific demethylase	(57)
	Long-term cultured undifferentiated adipose stem cells		Inactive PPAR γ 2 promoter	(58)
	Differentiated adipose stem cells		Demethylation in PPAR γ 2 promoter region	
	Hepatic stellate cells		Cell differentiation: repression mediates anti-adipogenic transdifferentiation	(62)
H4 acetylation	Retrovirus infected fibroblasts 3T3-L1		Adipogenesis: tetra-acetylated before onset of differentiation (PPAR γ 2 promoter activation)	(59)
H4K16 acetylation	NIH 3T3 3T3-L1		Adipogenesis: acetylation upon differentiation (PPAR γ 2 promoter)	(60)
H4K20 methylation	3T3-L1		Adipogenesis: PPAR γ self upregulation	(63)

Phenotype: Investigated trait; +/- : association of SNP; Cases: obese patients

TABLE 7

STUDIES OBSERVING THE POLYMORPHISM AT -3826 (A->G) IN UCP1 AND OBESITY PHENOTYPES									
PHENOTYPE	+/-	P	STUDY DESIGN	STUDY POPULATION			ADDITIONAL INFORMATION	REF.	
				ETHNICITY	FEMALE	MALE			
BMI	+	0.02	Cohort	Australian	526	-	Participants were overweight and obese and with/without type 2 diabetes	(64)	
		0.008	Case-control	Spanish	55 117 controls	38 160 controls	Association only on female participants	(65)	
		n.a.	Case-control	Czech	408 120 controls		295 cases with type 2 diabetes and 113 offspring. Effect could only be demonstrated in diabetics	(66)	
	-			Case-control	French	238 91 controls		Participants were morbidly obese	(67)
				Cohort with follow-up	Danish	379		Association with juvenile-onset obesity	(68)
				Case-control	Swedish	365 257 controls	310 54 controls	SOS cohort Cases were obese	(69)
				Population based cohort	German	1 020		Diabetomobile stud	(70)
				Cohort	Southern Poland	80	38	Participants were overweight or obese with a family history of obesity	(71)
				Case-control	German	96 96 controls	58 58 controls	Participants were a subsample of the EPIC-Heidelberg cohort No correlation with fatty acid intake	(72)
				Case-control	n.a.	117	45		(73)
				Case-control	Finish	141 59	29 53	The obese subjects were participating in a weight reduction program	(74)
	Higher weight gain	+	0.02	Case-control	French	238 91 controls		Participants were morbidly obese Association occurred only in the morbidly obese participants	(67)
-			Cohort	n.a.	77	-	The obese subjects were participating in a weight reduction program	(75)	
			Population based cohort	German	1,020		Diabetomobile study	(70)	
Lower weight loss	+	n.a.	(twin study)	n.a.	-	24	Dietary intervention (overfeeding)	(76)	
	-		Case-control	Finish	141 59 control	29 53 controls	The obese participants were participating in a weight reduction program	(74)	

Phenotype: Investigated trait; +/- : association of SNP; Cases: obese patients

TABLE 8

EVIDENCE ON EPIGENETIC REGULATIONS OF UCP1				
MODIFICATION	TISSUE	HUMAN TISSUE	EVIDENCE	REF.
CpG methylation	3T3-L1		72-80 % enhancer methylation 40 % promoter methylation	(77)
	3T3-L1 HIB-1B Ex vivo mouse tissue (BAT, WAT, liver)		Tissue specific DNA methylation pattern in enhancer and promoter	(78)
Histone modification (unspecified)	3T3-L1		Repressive histone modifications in enhancer and promoter region	(77)
Histone methylation	3T3-L1 HIB-1B Ex vivo mouse tissue (BAT, WAT, liver)		Tissue specific methylation of histones in enhancer and promoter region	(78)
H3K9 methylation	ex vivo, mouse tissue		Dynamical methylation state of H3K9 regulates UCP1 activation	(79)

Phenotype: Investigated trait; +/- : association of SNP; Cases: obese patients

epigenetic variation. The majority of genetic polymorphisms have not shown qualitative alterations. Solid evidence only exists for the rs1801282 in the PPAR γ gene. The minor allele is positively associated with the BMI. In the case of the PPAR γ gene, epigenetic modifications of its promoter region seem to be essential during cell differentiation and for the maintenance of the adult state of the adipocyte. It is likely that obesity is a trait of genetic canalization (4) and has become quite robust against mutational perturbations. Susceptibility might predominantly be determined by epigenetic variations and, according to the “common disease common variant hypothesis”, some polymorphisms such as the rs1801282 in the PPAR γ . Nonetheless, yet no study observed SNP effects and epigenetic programming simultaneously.

Evidence on joint effects of genetic variation is not sufficiently robust to determine positive effects of SNPs in UCP1, PGC1 α , ADRB3, or PPAR γ . Small study sizes, population stratification by ethnicity, as well as the heterogeneity of disease aetiology result in a

massive amount of errors, bias and confounders decreasing statistical power (108-110). The meta-analysis found in our literature search reported heterogeneity between studies which can be a main problem when trying to merge information in an appropriate way. Often, individual genome association studies report inconsistent associations and have limited power to detect modest genetic effects (111). Indeed, in the field of genetic epidemiology these are well recognized problems, along with a general lack of reproducibility (111-115). In addition to that, the more factors are involved, the less likely it is to validly describe interactions by commonly used simple multiplicative or additive models (115).

To understand joint effects of genes and environmental factors, information on dietary intake, medications, physical activity or tobacco use are collected. In order to maintain statistical power when increasing the number of investigated factors, sample size has to increase as well (116).

Epigenetic markers might be useful to account for pre-, postnatal and long-time

dietary and lifestyle habits (8). Long-term environmental factors and life style choices become manifest in metabolic networks relevant for energy balance and may contribute to the development of obesity. Dynamical changes in DNA methylation patterns because of the restriction or supplementation with different nutrients during the perinatal period have been reported earlier, and also in the adult state some examples of diet-induced epigenetic changes exist (8, 9). Although, none of the studies on epigenetic markers were conducted in BAT itself, the involvement of epigenetic mechanisms in the transcriptional regulation of selected genes has been demonstrated in other tissues. Some of them are prone to dietary (30) and disease (58) related changes or to treatment with DNA methyltransferase inhibitors or HDAC inhibitors. In general, epigenetic epidemiologic approaches tended to be discovery-oriented rather than designed for testing specific hypotheses. In addition to that, at the moment, no consensus to an appropriate way to model and present data in epigenetic epidemiology exists as discussed previously (118).

The Human Epigenome Project (www.epigenome.org) aims to identify tissue specific genome wide DNA methylation patterns, and to catalogue and interpret them (119). Marks are already classified by type of variation (e.g. heritable) and by intra- and inter-individual variations (120). In the following step, information of correlations (e.g. epigenotype-haplotype association) are going to be examined (121). Similar efforts will have to be made for the histone code and non-coding RNAs.

The integration of epigenetic markers into databases will facilitate their validation as new biomarkers of environmental factors in research.

Validated epigenetic markers can further benefit future public health interventions. By now, no evidence-based platform integrating genomics and epigenomics into practice exists in order to develop public health models. This is one of the reasons why physicians, decision-makers and stakeholders find it difficult to cope with the amount and complexity of new evidence from human genome epidemiology (122). This hampers the development of personalized prevention programs based on genomic testing to susceptible subgroups in the population (114). Indeed, the scientific, policymaking and Public Health communities are now urged, more

than ever, to collaborate, in order to realize the vision of Public Health in order to find the “right” intervention for the “right” person, patient group or sub-population at the “right” time in the “right” setting (125, 126).

CONCLUSIONS

It is unlikely that genetic variance alone explains obesity. Though, studies in human genome epidemiology carry only limited information on environmental exposures or other, non-genetic risk factors so far. Furthermore, biased data on environmental factors decreases the chance to understand complex aetiologies. The more factors are involved, the less likely it is that interactions are described by commonly used models. Indeed, the epigenome probably accounts for some of the heritability not explained by previous and current human genome epidemiology approaches.

In the future, researchers might focus first on standardizing the way we measure, analyse and report environmental factors with respect to gene-environment interactions.

It will be interesting to show epigenetic involvement in and potential environmental contribution to adaptive thermogenesis and especially the trans-differentiation processes from white to brown adipose tissue (127). This will witness the advances and technologies of the genomic and epigenomic sciences responding to human health needs and being translated into practical solutions for the benefit of population health (128-129).

Finally, the Integration of the present and yet to come evidence on the interaction between genetic and environmental factors will benefit future health strategies, and will guarantee accuracy in the decision making process (124,125). In conclusion, epigenetic knowledge has the potential to expand the scientific frontiers of diseases aetiology, risk prediction and prevention, and bears the promise of contributing to population health improvement.

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