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

Caroline Brettfeld[1], Stephanie Englert[1], Eva Aumueller[1], Alexander G. Haslberger[1]

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 (PPARγ), peroxisome proliferated activator receptor gamma co-activator 1 alpha (PGC1α), retinoid acid X receptor alpha (RXRα), CCAAT/enhancer-binding protein alpha (C/EBPα), 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

(1) Department for Nutritional Sciences, University of Vienna, Austria

CORRESPONDING AUTHOR: *Alexander.G.Haslberger; Department for Nutritional Sciences, University of Vienna, A-1090 Vienna, Althanstrasse 14, Vienna, Austria.*
e-mail: Alexander.haslberger@univie.ac.at
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INTRODUCTION

The World Health Organization (WHO) uses the body mass index (BMI) to define overweight: BMI=weight (kg)/height (m^2) (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 (PPARγ) is positively associated with the BMI in numerous studies. PPARγ 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 PPARγ promoter is 7 fold more methylated in T cells (not expressing the gene) than in adipocytes, which carry the PPARγ 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 PPARγ co-activator 1 alpha (PGC1α) (25). PGC1α forms a transcription factor complex together with PPARγ and retinoid acid X receptor alpha (RXRα) 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/EBPα), a transcription factor essential during adipogenesis (27), fatty acid binding protein 4 (FABP4) and lipoprotein lipase (LPL), two other target genes of PPARγ 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	ADRB3	8p12-p11	rs4994
Peroxisome proliferated activator receptor gamma coactivator 1 alpha	PGC1α	4p15	rs8192678
Peroxisome proliferated activator receptor gamma	PPARγ	3p25	rs1801282
Retinoid acid X receptor alpha	RXRα	9q34	-
Uncoupling protein 1	UCP1	4q31	A-3826G
CCAAT/enhancer-bindingproteinalpha	C/EBPα	19q13	rs12691 rs41490344 rs41504144 rs41344151 rs16967952 rs34508287 rs41428545 rs34529039 rs41367646 rs1049969 rs707656
Fatty acid binding protein 4	FABP4	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 PGC1α

A SNP in PGC1α 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 PGC1α 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 PGC1α 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 weeksdiet 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/m² 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 PGC1α			
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 PPARγ

The Pro12Ala polymorphism (rs1801282) is one of the most frequently investigated SNP of PPARγ 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 PPARγ 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 RXRα

The literature review for RXRα 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 PPARγ			
TISSUE	HUMAN TISSUE	EVIDENCE	REF.
Rat hepatocytes		Due to dietary protein restriction of female pregnant rats PPARγ1 promoter methylation in offspring did not differ	(50)
Human adipose stem cells	✓	Overall CpG hypomethylation of PPARγ2 due to senescence	(51)
wt- mice Lep+/Lep+ mice Diet-induced ob mice		Increased promoter methylation in wt-mice	(52)
3T3-L1		PPARγ2 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 PPARγ2 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 PPARγ 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 (PPARγ) and rs8192678 (PGC1α) SNP (104). Studies observing synergistic effects of the minor variant of the UCP1gene 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 RXRα. 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 PPARγ				
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 PPARγ2 gene during on-going differentiation and hyperacetylation in PPARγ1 gene before induction of differentiation	(56)
H3K4 methylation	Embryonic fibroblast		Adipogenesis: association with 2 methyltransferases	(57)
	Long-term cultured undifferentiated adipose stem cells		Inactive PPARγ2 promoter	(58)
	Differentiated adipose stem cells		No change of trimethylation in PPARγ2 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 (PPARγ2 promoter activation)	(59)
	NIH 3T3 3T3-L1		Adipogenesis: acetylation upon differentiation (PPARγ2 promoter)	(60)
	Long-term cultured undifferentiated adipose stem cells		Inactive PPARγ2 promoter	(58)
	Differentiated adipose stem cells		Hyperacetylation in promoter region	
H3K9 methylation	Mesenchymal stem cells		Osteoblastogensis/adipogenesis: inactivation of PPARγ through methylation	(61)
H3K14 acetylation	Retrovirus infected fibroblasts 3T3-L1		Adipogenesis: Hyperacetylation during differentiation (PPARγ2 promoter activation)	(59)
	NIH 3T3 3T3-L1		Adipogenesis: acetylation upon differentiation (PPARγ2 promoter)	(60)
H3K27 methylation	Embryonic fibroblasts		Adipogenesis: association with H3K27 specific demethylase	(57)
	Long-term cultured undifferentiated adipose stem cells		Inactive PPARγ2 promoter	(58)
	Differentiated adipose stem cells		Demethylation in PPARγ2 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 (PPARγ2 promoter activation)	(59)
H4K16 acetylation	NIH 3T3 3T3-L1		Adipogenesis: acetylation upon differentiation (PPARγ2 promoter)	(60)
H4K20 methylation	3T3-L1		Adipogenesis: PPARγ 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 PPARγ gene. The minor allele is positively associated with the BMI. In the case of the PPARγ 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 PPARγ. 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, PGC1α, ADRB3, or PPARγ. 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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References

(1) Elmadfa I, Ernährungslehre, 2009, Verlag: Utb; Ulmer (Eugen)

(2) Haslam DW, James WP. Obesity. Lancet 2005; 366 (9492): 1197–209

(3) Stunkard AJ, Foch TT, Hrubec Z. A twin study of human obesity. JAMA 1986; 256: 51-4

(4) Neel JV, Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet 1962; 14: 353-62

(5) Manolio TA, et al. Finding the missing heritability of complex diseases. Nature 2009; 461:747–53

(6) Painter RC, et al. Early onset of coronary artery disease after prenatal exposure to the Dutch famine. Am J Clin Nutr 2006; 84(2): 322-7; quiz 466-7

(7) Dolinoy DC, et al. Metastable epialleles, imprinting, and the fetal origins of adult diseases. Pediatr Res 2007; 61(5 Pt 2): 30R-37R

(8) Dolinoy DC, Jirtle RL. Environmental epigenomics in human health and disease. Environ Mol Mutagen 2008; 49(1): 4-8

(9) Noer A, Boquest AC, Collas P. Dynamics of adipogenic promoter DNA methylation during clonal culture of human adipose stem cells to senescence. BMC Cell Biol 2007; 8: 18

(10) He H. Lehming N. Global effects of histone modifications. Brief Funct Genomic Proteomic 2003; 2(3): 234-43

(11) Heneghan HM, Miller N, Kerin MJ. Role of microRNAs in obesity and the metabolic syndrome. Obes Rev. 11(5): 354-61

(12) Thaler R, Aumüller E, Berner C, Haslberger AG. Interaction of Hereditary and Epigenetic Mechanisms in the Regulation of Gene Expression, in Epigenetics and Human Health, A.G. Haslberger, S. Gressler, Editor. 2010, Wiley-VCH Verlag: Weinheim

(13) Misík M, Nersesyan A, Parzefall W, Knasmüller S. Carcinogens in Foods: Occurrence, Modes of Action and Modulation of Human Risks by Genetic Factors and Dietary Constituents, in Epigenetics and Human Health, A.G. Haslberger, S. Gressler, Editor. 2010, WILEY-VCH Verlag: Weinheim

(14) Stefanska BM, Fabianowska-Majewska K. Effects of Dietary Natural Compounds on DNA Methylation Related to Cancer Chemoprevention and Anticancer Epigenetic Therapy, in Epigenetics and Human Health, A.G. Haslberger, S. Gressler, Editor. 2010, WILEY-VCH Verlag: Weinheim

(15) Rust P. Health Determinants Throughout the Life Cycle, in Epigenetics in Human Health, A.G. Haslberger, S. Gressler, Editor. 2010, WILEY-VCH Verlag: Weinheim

(16) Bursch W. Genotoxic, Non-Genotoxic and Epigenetic Mechanisms in Chemical Hepatocarcinogenesis: Implications for Safety Evaluation, in Epigenetics and Human Health, A.G. Haslberger, S. Gressler, Editor. 2010, WILEY-VCH Verlag: Weinheim

(17) Sato K, et al. Neonatal exposure to diethylstilbestrol alters the expression of DNA methyltransferases and methylation of genomic DNA in the epididymis of mice. Endocr J 2006; 53(3): 331-7

(18) Schofield PN. Impact of genomic imprinting on genomic instability and radiation-induced mutation. Int J Radiat Biol 1998; 74(6): 705-10

(19) Szyf M, Weaver I, Meaney M. Maternal care, the epigenome and phenotypic differences in behavior. Reprod Toxicol 2007; 24(1): 9-19

(20) Kaati G. Case Studies on Epigenetic Inheritance, in Epigenetics and Human Health, A.G. Haslberger, S. Gressler, Editor. 2010, WILEY-VCH Verlag: Weinheim

(21) Grundy SM, H. Bryan Brewer Jr, Cleeman JI, et al, for the Conference Participants. Definition of Metabolic Syndrome Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to DefinitionCirculation 2004; 109: 433-8

(22) Silva JE Thermogenic mechanisms and their hormonal regulation. Physiol Rev 2006; 86(2): 435-64

(23) Oberkofler H, Beer A, Breban D, et al. Human obese gene expression: alternative splicing of mRNA and relation to adipose tissue localization. Obes Surg 1997; 7(5): 390-6

(24) Tiraby C, Langin D. Conversion from white to brown adipocytes: a strategy for the control of fat mass? Trends Endocrinol Metab 2003. 14: 439-41

(25) Cao W, et al. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. Mol Cell Biol 2004; 24(7): 3057-67

(26) Li M, Pascual G, Glass CK. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. Mol Cell Biol 2000; 20(13): 4699-707

(27) Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Mol Biol Cell 2006. 7: 885-96

(28) Pierola J, Barcelo A, et al. Beta3-Adrenergic receptor Trp64Arg polymorphism and increased body mass index in sleep apnoea. Eur Respir J 2007; 30(4): 743-7

(29) Bracale R, Pasanisi F, et al. (2007). Metabolic syndrome and ADRB3 gene polymorphism in severely obese patients from South Italy. Eur J Clin

Nutr 2007. 61(10): 1213-9

(30) de Luis DA, Aller R, et al. Relation of Trp64Arg polymorphism of beta 3-adrenergic receptor gene to adipocytokines and fat distribution in obese patients. Ann Nutr Metab 2008; 52(4): 267-71

(31) Lwow F, Dunajska K, et al. "Post-exercise oxidative stress and obesity in postmenopausal women: the role of beta3-adrenergic receptor polymorphism. Gynecol Endocrinol 2007; 23(10): 597-603

(32) Gjesing AP, Andersen G, et al. Studies of associations between the Arg389Gly polymorphism of the beta1-adrenergic receptor gene (ADRB1) and hypertension and obesity in 7677 Danish white subjects. Diabet Med 2007; 24(4): 392-7

(33) Zafarmand MH, van der Schouw YT, et al. T64A polymorphism in beta3-adrenergic receptor gene (ADRB3) and coronary heart disease: a case-cohort study and meta-analysis. J Intern Med 2008. 263(1): 79-89

(34) Zawodniak-Szalapska M, Stawerska R, et al. Association of Trp64Arg polymorphism of beta3-adrenergic receptor with insulin resistance in Polish children with obesity. J Pediatr Endocrinol Metab 2008; 21(2): 147-54

(35) Dunajska K, Lwow F, et al. Beta(3)-adrenergic receptor polymorphism and metabolic syndrome in postmenopausal women. Gynecol Endocrinol 2008; 24(3): 133-8

(36) de Luis DA, Gonzalez Sagrato M, et al. Influence of Trp64Arg polymorphism of beta 3-adrenoreceptor gene on insulin resistance, adipocytokines and weight loss secondary to two hypocaloric diets. Ann Nutr Metab 2009; 54(2): 104-10

(37) Esterbauer H, Oberkofler H, et al. Peroxisome proliferator-activated receptor-gamma coactivator-1 gene locus: associations with obesity indices in middle-aged women. Diabetes 2002; 51(4): 1281-6

(38) Ambye L, Rasmussen S, et al. Studies of the Gly482Ser polymorphism of the peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC-1alpha) gene in Danish subjects with the metabolic syndrome. Diabetes Res Clin Pract 2005; 67(2): 175-9

(39) Vohl MC, Houde A, et al. Effects of the peroxisome proliferator-activated receptor-gamma co-activator-1 Gly482Ser variant on features of the metabolic syndrome. Mol Genet Metab 2005; 86(1-2): 300-6

(40) Pihlajamaki J, Kinnunen M, et al. Haplotypes of PPARGC1A are associated with glucose tolerance, body mass index and insulin sensitivity in offspring of patients with type 2 diabetes. Diabetologia 2005; 48(7): 1331-4

(41) Barroso I, Luan J, et al. Meta-analysis of the Gly482Ser variant in PPARGC1A in type 2 diabetes and related phenotypes. Diabetologia 2006; 49(3): 501-5

(42) Leipold H, Knoefler M, et al. Peroxisome proliferator-activated receptor gamma coactivator-1alpha gene variations are not associated with gestational diabetes mellitus. J Soc Gynecol Investig 2006; 13(2): 104-7

(43) Franks PW, Ekelund U, et al. PPARGC1A coding variation may initiate impaired NEFA clearance during glucose challenge. Diabetologia 2007; 50(3): 569-73

(44) Stefan N, Thamer C, et al. Genetic variations in PPARD and PPARGC1A determine mitochondrial function and change in aerobic physical fitness and insulin sensitivity during lifestyle intervention. J Clin Endocrinol Metab 2007; 92(5): 1827-33

(45) Deeb SS, Brunzell JD. The Role of the PGC1alpha Gly482Ser Polymorphism in Weight Gain due to Intensive Diabetes Therapy. PPAR Res 2009: 649286

(46) Ling C, Del Guerra S, et al. Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia 2008; 51(4): 615-22

(47) Barres R, Osler ME, et al. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab 2009; 10(3): 189-98

(48) Gemma C, Sookoian S, et al. Maternal pregestational BMI is associated with methylation of the PPARGC1A promoter in newborns. Obesity (Silver Spring) 2009; 17(5): 1032-9

(49) Brons C, Jacobsen S, et al. Deoxyribonucleic acid methylation and gene expression of PPARGC1A in human muscle is influenced by high-fat overfeeding in a birth-weight-dependent manner. J Clin Endocrinol Metab 2010; 95(6): 3048-56

(50) Lillycrop KA, Phillips ES, et al. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr 2005; 135(6): 1382-6

(51) Noer AA, Boquest C, et al. Dynamics of adipogenic promoter DNA methylation during clonal culture of human adipose stem cells to senescence. BMC Cell Biol 2007; 8: 18

(52) Fujiki K, Kano F, et al. Expression of the peroxisome proliferator activated receptor gamma gene is repressed by DNA methylation in visceral adipose tissue of mouse models of diabetes. BMC Biol 2009; 7: 38

(53) Sorensen AL, Timoskainen S, et al. Lineage-Specific Promoter DNA Methylation Patterns Segregate Adult Progenitor Cell Types. Stem Cells Dev 2010; 19(8): 1257-66

(54) Hong SJ, Oh JH, et al. DNA methylation patterns

of ulcer-healing genes associated with the normal gastric mucosa of gastric cancers. J Korean Med Sci 2010; 25(3): 405-17

(55) Tikoo K, Kumar P, et al. Rosiglitazone synergizes anticancer activity of cisplatin and reduces its nephrotoxicity in 7, 12-dimethyl benz{a}anthracene (DMBA) induced breast cancer rats. BMC Cancer 2009; 9: 107

(56) Okamura M, Kudo H, et al. COUP-TFII acts downstream of Wnt/beta-catenin signal to silence PPARgamma gene expression and repress adipogenesis. Proc Natl Acad Sci USA 2009; 106(14): 5819-24

(57) Cho YW, Hong S, et al. Histone methylation regulator PTIP is required for PPARgamma and C/EBPalpha expression and adipogenesis. Cell Metab 2009; 10(1): 27-39

(58) Noer A, Lindeman LC, et al. Histone H3 modifications associated with differentiation and long-term culture of mesenchymal adipose stem cells. Stem Cells Dev 2009; 18(5): 725-36

(59) Salma N, Xiao H, et al. Temporal recruitment of transcription factors and SWI/SNF chromatin-remodeling enzymes during adipogenic induction of the peroxisome proliferator-activated receptor gamma nuclear hormone receptor. Mol Cell Biol 2004; 24(11): 4651-63

(60) Badri KR, Zhou Y, et al. Effects of the SANT domain of tension-induced/inhibited proteins (TIPs), novel partners of the histone acetyltransferase p300, on p300 activity and TIP-6-induced adipogenesis. Mol Cell Biol 2008; 28(20): 6358-72

(61) Takada I, Suzawa M, et al. Suppression of PPAR transactivation switches cell fate of bone marrow stem cells from adipocytes into osteoblasts. Ann N Y Acad Sci 2007; 1116: 182-95

(32) Zhu NL, Wang J, et al. The Necdin-Wnt pathway causes epigenetic PPAR{gamma} repression in hepatic stellate cells. J Biol Chem 2010; 285(40): 30463-71

(63) Wakabayashi K, Okamura M, et al. The peroxisome proliferator-activated receptor gamma/retinoid X receptor alpha heterodimer targets the histone modification enzyme PR-Set7/Setd8 gene and regulates adipogenesis through a positive feedback loop. Mol Cell Biol 2009; 29(13): 3544-55

(64) Heilbronn LK, Kind KL, et al. Association of -3826 G variant in uncoupling protein-1 with increased BMI in overweight Australian women." Diabetologia 2000; 43(2): 242-4

(65) Ramis JM, Gonzalez-Sanchez JL, et al. The Arg64 allele of the beta 3-adrenoceptor gene but not the -3826G allele of the uncoupling protein 1 gene is associated with increased leptin levels in the Spanish population. Metabolism 2004; 53(11): 1411-6

(66) Sramkova D, Krejbichova S, et al. The UCP1 gene polymorphism A-3826G in relation to DM2 and body composition in Czech population. Exp Clin Endocrinol Diabetes 2007; 115(5): 303-7

(67) Clement K, Ruiz J, et al. Additive effect of A-->G (-3826) variant of the uncoupling protein gene and the Trp64Arg mutation of the beta 3-adrenergic receptor gene on weight gain in morbid obesity. Int J Obes Relat Metab Disord 1996; 20(12): 1062-6

(68) Urhammer SA, Fridberg M, et al. Studies of genetic variability of the uncoupling protein 1 gene in Caucasian subjects with juvenile-onset obesity. J Clin Endocrinol Metab 1997; 82(12): 4069-74

(69) Olofsson LE, Orho-Melander M, et al. CCAAT/enhancer binding protein alpha (C/EBPalpha) in adipose tissue regulates genes in lipid and glucose metabolism and a genetic variation in C/EBPalpha is associated with serum levels of triglycerides. J Clin Endocrinol Metab 2008; 93(12): 4880-6

(70) Schaffler A, Palitzsch KD, et al. Frequency and significance of the A-->G (-3826) polymorphism in the promoter of the gene for uncoupling protein-1 with regard to metabolic parameters and adipocyte transcription factor binding in a large population-based Caucasian cohort. Eur J Clin Invest 1999; 29(9): 770-9

(71) Kiec-Wilk B, Wybranska I, et al. Correlation of the -3826A >G polymorphism in the promoter of the uncoupling protein 1 gene with obesity and metabolic disorders in obese families from southern Poland. J Physiol Pharmacol 2002; 53(3): 477-90

(72) Nieters A, Becker N, et al. Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a sub-sample of the EPIC-Heidelberg cohort. Eur J Nutr 2002; 41(5): 210-21

(73) Herrmann SM, Wang JG, et al. Uncoupling protein 1 and 3 polymorphisms are associated with waist-to-hip ratio. J Mol Med 2003; 81(5): 327-32

(74) Valve R, Heikkinen S, et al. Synergistic effect of polymorphisms in uncoupling protein 1 and beta3-adrenergic receptor genes on basal metabolic rate in obese Finns. Diabetologia 1998; 41(3): 357-61

(75) Fogelholm M, Valve R, et al. Additive effects of the mutations in the beta3-adrenergic receptor and uncoupling protein-1 genes on weight loss and weight maintenance in Finnish women. J Clin Endocrinol Metab 1998; 83(12): 4246-50

(76) Ukkola O, Tremblay A, et al. Genetic variation at the uncoupling protein 1, 2 and 3 loci and the response

to long-term overfeeding. Eur J Clin Nutr 2001; 55(11): 1008-15.

(77) Kiskinis E, Hallberg M, et al. RIP140 directs histone and DNA methylation to silence Ucp1 expression in white adipocytes. EMBO J 2007; 26(23): 4831-40

(78) Shore A, Karamitri A, et al. "Role of Ucp1 enhancer methylation and chromatin remodelling in the control of Ucp1 expression in murine adipose tissue. Diabetologia 2010; 53(6): 1164-73

(79) Tateishi K, Okada Y, et al. Role of Jhdm2a in regulating metabolic gene expression and obesity resistance. Nature 2009; 458(7239): 757-61

(80) Bennett CE, Nsengimana J, et al. CCAAT/enhancer binding protein alpha, beta and delta gene variants: associations with obesity related phenotypes in the Leeds Family Study. Diab Vasc Dis Res 2010; 7(3): 195-203.

(81) Chim CS, Wong AS, et al. Infrequent hypermethylation of CEBPA promotor in acute myeloid leukaemia. Br J Haematol 2002; 119(4): 988-90

(82) Takai N, Kawamata N, et al. (2005). "Discovery of epigenetically masked tumor suppressor genes in endometrial cancer. Mol Cancer Res 2005; 3(5): 261-9

(83) Dannenberg LO, Edenberg HJ. Epigenetics of gene expression in human hepatoma cells: expression profiling the response to inhibition of DNA methylation and histone deacetylation. BMC Genomics 2006; 7: 181

(84) Tada Y, Brena RM, et al. Epigenetic modulation of tumor suppressor CCAAT/enhancer binding protein alpha activity in lung cancer. J Natl Cancer Inst 2006; 98(6): 396-406

(85) Hackanson B, Bennett KL, et al. Epigenetic modification of CCAAT/enhancer binding protein alpha expression in acute myeloid leukemia. Cancer Res 2008; 68(9): 3142-51

(86) Katari S, Turan N, et al. DNA methylation and gene expression differences in children conceived in vitro or in vivo. Hum Mol Genet 2009; 18(20): 3769-78

(87) Guibal FC, Alberich-Jorda M, et al. Identification of a myeloid committed progenitor as the cancer-initiating cell in acute promyelocytic leukemia. Blood 2009; 114(27): 5415-25

(88) Bennett KL, Romigh T, et al. Activator protein 2 alpha (AP2alpha) suppresses 42 kDa C/CAAT enhancer binding protein alpha (p42(C/EBPalpha)) in head and neck squamous cell carcinoma. Int J Cancer 2009; 124(6): 1285-92

(89) Kumagai T, Wakimoto N, et al. Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (Vorinostat, SAHA) profoundly inhibits the growth of human pancreatic cancer cells Int J Cancer 2007; 121(3): 656-65.

(90) Jost E, Do ON, et al. Aberrant DNA methylation of the transcription factor C/EBPalpha in acute myelogenous leukemia. Leuk Res 2009; 33(3): 443-9

(91) Lu GD, Leung CH, et al. C/EBPalpha is up-regulated in a subset of hepatocellular carcinomas and plays a role in cell growth and proliferation. Gastroenterology 2010; 139(2): 632-43, 643.e1-4

(92) Sertic J, Juricic L, et al. Variants of ESR1, APOE, LPL and IL-6 loci in young healthy subjects: association with lipid status and obesity. BMC Res Notes 2009; 2: 203

(93) Corella D, Guillen M, et al. Gender specific associations of the Trp64Arg mutation in the beta3-adrenergic receptor gene with obesity-related phenotypes in a Mediterranean population: interaction with a common lipoprotein lipase gene variation. J Intern Med 2001; 250(4): 348-60

(94) Garenc, C, Perusse L, et al. Evidence of LPL gene-exercise interaction for body fat and LPL activity: the HERITAGE Family Study. J Appl Physiol 2001; 91(3): 1334-40

(95) Noer A, Sorensen AL, et al. Stable CpG hypomethylation of adipogenic promoters in freshly isolated, cultured, and differentiated mesenchymal stem cells from adipose tissue. Mol Biol Cell 2006; 17(8): 3543-56

(96) Kim JW, Cheng Y, et al. Genetic and epigenetic inactivation of LPL gene in human prostate cancer. Int J Cancer 2009; 124(3): 734-8

(97) Lee JH, Kim KA, et al. Diallyl disulfide accelerates adipogenesis in 3T3-L1 cells. Int J Mol Med 2007; 20(1): 59-64

(98) Fu M, Rao M, et al. Cyclin D1 inhibits peroxisome proliferator-activated receptor gamma-mediated adipogenesis through histone deacetylase recruitment. J Biol Chem 2005; 280(17): 16934-41

(99) Lee J, Saha PK, et al. Targeted inactivation of MLL3 histone H3-Lys-4 methyltransferase activity in the mouse reveals vital roles for MLL3 in adipogenesis. Proc Natl Acad Sci USA 2008; 105(49): 19229-34

(100) Ochoa MC, Marti A, et al. Gene-gene interaction between PPAR gamma 2 and ADR beta 3 increases obesity risk in children and adolescents. Int J Obes Relat Metab Disord 2004; 28 Suppl 3: S37-41

(101) Ek J, Andersen G, et al. Mutation analysis of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to Type II diabetes mellitus. Diabetologia 2001; 44(12): 2220-6

(102) Kurokawa N, et al. The ADRB3 Trp64Arg variant and BMI: a meta-analysis of 44 833 individuals. Int J Obes (Lond) 2008; 32(8): 1240-9

(103) Masud S, Ye S. Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. J Med Genet 2003; 40(10): 773-80

(104) Tonjes A, et al. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with Pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. Diabetes Care 2006; 29(11): 2489-97

(105) Hegele RA, Cao H. Single nucleotide polymorphisms of RXRA encoding retinoid X receptor alpha. J Hum Genet 2001; 46(7): 423-5

(106) Volate, SR, et al. Epigenetic modulation of the retinoid X receptor alpha by green tea in the azoxymethane-Apc Min/+ mouse model of intestinal cancer. Mol Carcinog 2009; 48(10): 920-33

(107) Barak Y et al. PPAR gamma is required for placental, cardiac, and adipose tissue development. Mol Cell 1999. 4(4): 585-95

(108) Khoury MJ, et al. Genome-wide association studies, field synopses, and the development of the knowledge base on genetic variation and human diseases. Am J Epidemiol 2009; 170(3): 269-79

(109) Rothman KJ, Greenland S, Lash TL. Modern Epidemiology 2008, Philadelphia: Lippincott Williams & Wilkins

(110) Moonesinghe R, et al. Required sample size and nonreplicability thresholds for heterogeneous genetic associations. Proc Natl Acad Sci USA 2008; 105(2): 617-22

(111) Kitsios GD, Zintzaras E. Genomic convergence of genome-wide investigations for complex traits. Ann Hum Genet 2009; 73(Pt 5): 514-9

(112) Ioannidis JP. Why most published research findings are false. PLoS Med 2005; 2(8): e124

(113) Janssens AC, Gwinn M, Bradley LA, et al. A critical appraisal of the scientific basis of commercial genomic profiles used to assess health risks and personalize health interventions. Am J Hum Genet 2008; 82: 593-9

(114) van der Net JB, et al. Usefulness of genetic polymorphisms and conventional risk factors to predict coronary heart disease in patients with familial hypercholesterolemia. Am J Cardiol 2009; 103(3): 375-80

(115) Botto LD, Khoury MJ. Facing the Challenge of Complex Genotypes and Gene-Environment Interaction: The Basic Epidemiologic Units in Case-Control and Case-Only Designs, in Human genome epidemiology: a scientific foundation for using genetic information to improve health and prevent disease, M.J. Khoury, J. Little, and W. Burke, Editors 2004, Oxford University Press: New York: 111-26

(116) Rothman N, et al. The use of common genetic polymorphisms to enhance the epidemiologic study of environmental carcinogens. Biochim Biophys Acta 2001; 1471(2): C1-10

(117) Palla L, et al. Challenges in the use of literature-based meta-analysis to examine gene-environment interactions. Am J Epidemiol 2010; 171(11): 1225-32

(118) Foley DL, et al. Prospects for epigenetic epidemiology. Am J Epidemiol 2009; 169(4): 389-400

(119) Eckhardt F, et al. DNA methylation profiling of human chromosomes 6, 20 and 22. Nat Genet 2006; 38(12): 1378-85

(120) Tal O, Kisdi E, Jablonka E. Epigenetic contribution to covariance between relatives. Genetics 184(4): 1037-50

(121) Bjornsson HT, Fallin MD, Feinberg AP. An integrated epigenetic and genetic approach to common human disease. Trends Genet 2004; 20(8): 350-8

(122) Khoury MJ, Milikan R, Gwinn M. Genetic and Molecular Epidemiology, in Modern epidemiology, K.J. Rothman, S. Greenland, and T.L. Lash, Editors. 2008, LIPPINCOTT WILLIAMS & WILKINS: Philadelphia. p. 564-579

(123) Figueroa ME, et al. An integrative genomic and epigenomic approach for the study of transcriptional regulation. PLoS One 2008; 3(3): e1882

(124) Zhang Y, et al. Systematic analysis, comparison, and integration of disease based human genetic association data and mouse genetic phenotypic information. BMC Med Genomics 2010; 3: 1

(125) Boccia S, Brand A, Brand H, Ricciardi G. The integration of genome-based information for common diseases into health policy and healthcare as a major challenge for Public Health Genomics: The example of the methylenetetrahydrofolate reductase gene in non-cancer diseases. Mutat Res 2009; 667: 27-34

(126) Brand A, Brand H, Schulte in den Baumen T. The impact of genetics and genomics on public health. Eur J Hum Genet 2008; 16(1): 5-13

(127) Barbatelli G, et al. The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am J Physiol Endocrinol Metab 2010; 298(6): E1244-53

(128) Gagnon J, Lago F, et al. DNA polymorphism in the

uncoupling protein 1 (UCP1) gene has no effect on obesity related phenotypes in the Swedish Obese Subjects cohorts. Int J Obes Relat Metab Disord 1998; 22(6): 500-5

(129) Malczewska-Malec M, Wybranska I, et al. Analysis of candidate genes in Polish families with obesity. Clin Chem Lab Med 2004; 42(5): 487-93

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