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Genome-wide analysis reveals DNA methylation markers that vary with both age and obesity ☆

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ABSTRACT

The combination of the obesity epidemic and an aging population presents growing challenges for the healthcare system. Obesity and aging are major risk factors for a diverse number of diseases and it is of importance to understand their interaction and the underlying molecular mechanisms. Herein the authors examined the methylation levels of 27578 CpG sites in 46 samples from adult peripheral blood. The effect of obesity and aging was ascertained with general linear models. More than one hundred probes were correlated to aging, nine of which belonged to the KEGG group map04080. Additionally, 10 CpG sites had diverse methylation profiles in obese and lean individuals, one of which was the telomerase catalytic subunit (*TERT*). In eight of ten cases the methylation change was reverted between obese and lean individuals. One region proved to be differentially methylated with obesity (LINC00304) independent of age. This study provides evidence that obesity influences age driven epigenetic changes, which provides a molecular link between aging and obesity. This link and the identified markers may prove to be valuable biomarkers for the understanding of the molecular basis of aging, obesity and associated diseases.

1. Introduction

Obesity and its associated co-disorders are some of the largest global health problems and are together with the increasing elderly population in many western countries a major challenge for the healthcare system. The risk for several age related diseases such as cancer, type-2 diabetes and neurodegenerative diseases is increased by obesity and overweight (Beydoun et al., 2008; Calle et al., 2003; Haslam and James, 2005). Hence, it is of utmost importance to understand the mechanisms that underlie these connections and the development of obesity and age related diseases. Recent progress in genetic research has revealed a growing number of genetic variants that predispose carriers to age related diseases, in particular cancers (Hindorff et al., 2009). However, in the last few years epigenetic alterations have been given an increasing amount of attention as important factors in disease (Feinberg and Irizarry, 2010). In contrast to genetic variations, the epigenetic profile is dynamic and varies with both intrinsic and extrinsic factors throughout lifetime. Epigenetics covers a number of cellular mechanisms that alter the information and interpretation of the genome without changing its nucleotide sequence in contrast to classic genetic variations. One of the most studied epigenetic mechanisms is the methylation of cytosine residues, which is maintained and controlled by different DNA-methyltransferases (DNMTs). Cytosines across the genome tend to be methylated (Ehrlich et al., 1982), but in cytosine phosphate guanine (CpG) rich regions in proximity of genes the methylation is dynamic and functions as a gene specific regulatory mechanism of transcription (Bird et al., 1985). Such cytosine enriched regions are called CpG islands and a higher methylation in this type of region is often associated with a reduced expression of the nearby gene, due to chromatin rearrangement, inhibition of transcription activators and/or recruitment of transcription repressors (Campion et al., 2009; Mohn et al., 2008; Stein et al., 1982). Hence, DNA methylation provides a regulatory mechanism of gene transcription and is essential for cell fate, differentiation and tissue integrity.

The methylation status of monozygotic twins diverges with age, which demonstrates that DNA methylation is susceptible to environmental factors (Fraga et al., 2005). This strengthens the notion that the epigenome is an adaptive entity capable of changing an individual's gene expression pattern due to environmental factors. In fact, it has been demonstrated that factors such as diet and nutrient intake affect the methylation status as well as conditions such as inflammation, oxidative stress and hypoxia (Campion et al., 2009). Several studies have

Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes; Go-term, gene ontology term as defined by www.geneontology.org; CpG, CG sequence motif; LGDB, Latvian Genome Data Base.

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reported genomic regions where the methylation level is differentiated in obese individuals and varies with body-weight (Almen et al., 2012; Franks and Ling, 2010; Milagro et al., 2011; Wang et al., 2010). In contrast, methylation levels are also associated with genetic variations and can thus be governed, at least in part, by genetic factors (Heijmans et al., 2007). This is exemplified by a genome-wide longitudinal study of DNA methylation in an Icelandic population that confirms that inter-individual variation in methylation level depends on both genetic and environmental factors and that the extent of their influence differs between regions (Bjornsson et al., 2008). We have previously demonstrated that the obesity associated SNP rs9939609 in the FTO gene is linked to methylation changes in several CpG regions in preadolescents (Almen et al., 2012). FTO was the first gene to be associated with common obesity (Frayling et al., 2007).

With age the global methylation level of the genome (including non-CpG regions) is decreased, which leads to a global hypomethylation (Wilson and Jones, 1983; Wilson et al., 1987). This may be caused by a lower expression of DNMT1 with age, which would lead to a slower *de novo* and maintenance of methylation. In contrast, several CpG islands in promoter regions are hypermethylated during aging (Kim et al., 2004; Toyota et al., 1999; Tra et al., 2002). While this demonstrates that the genome can be locally hypermethylated during aging, it is unclear if this is a general process for promoter associated CpG islands. These age associated methylation changes could be important in age driven diseases and has received particular attention for its potential important role in cancer (Rando, 2010). Whereas it is well established that genetic factors influence age related diseases, it is now clear that epigenetic factors also play an important role (Liu et al., 2003).

In order to investigate the relation between methylation status, obesity and age we collected peripheral blood samples from 46 individuals and analyzed their methylation profiles. DNA methylation levels were measured with microarrays in 27,578 genomic sites, of which the majority were found in CpG islands in proximity of promoters. Moreover, the influence of the obesity associated FTO genotype (rs9939609), which lies in intron one and is not likely to have any cis effects on the methylation, level was assessed in order to replicate our previous results. Blood samples provide the best way to locate potential diagnostic biomarkers due to the simplicity of the clinical procedures involved. Importantly, it is known that epigenetic changes identified in blood may be representative for other tissues and can therefore be valuable as diagnostic and prognostic biomarkers (Calabrese et al., 2011).

2. Methods

2.1. Ethics statement

Written confirmed consent was acquired from all participants of the study. The study was approved by the Central Medical Ethics Committee of Latvia.

2.2. Subjects

Study samples were acquired from the Latvian Genome Data Base (LGDB), a national biobank of health and genetic information collected for adult residents of Latvia (over 18 years old). Health status of the participants was asserted by health care professionals according to International Classification of Diseases (ICD-10) codes. Information on a familial health status, ethnic and social background, lifestyle and anthropometric measurements were obtained in a questionnaire based interview.

We selected 24 obese and 22 lean female adults from a total group of 934 females, with a known FTO rs9939609 genotype, that were recruited to LGDB from 2003 to May 2009. Selection criteria included rs9939609 genotype, Body Mass Index (BMI) (lean <25 kg/m^2 and obese ≥ 30 kg/m^2) and health status (participants diagnosed with endocrine diseases and malignant tumors before recruiting to LGDB were not included). Middle age females were selected and a comparable age range of the lean (41–69 years old) and obese females (42–70 years old) were ascertained. The individuals were selected so that both the obese and lean groups were composed of equal proportions of homozygous carriers for the normal and risk allele of the rs9939609 SNP. Heterozygous individuals were excluded. Age weight and BMI details of the participants can be found in Table 1.

2.3. Illumina bead array

DNA isolation was performed as we have previously described using the phenol chloroform method (Jacobsson et al., 2008). Bisulfite conversion was performed with the EZ DNA Methylation-Gold™ kit (Zymo Research): 500 ng of DNA was subjected to bisulfite treatment including heating to 98 °C for 10 min followed by conversion at 64 °C for 150 min. The bisulfite conversion converts all (>99%) unmethylated cytosine to uracil, which gives rise to a DNA sequence that can be defined by its initial methylation status. The Illumina Infinium HumanMethylation27 BeadChip array (Illumina) probes 27,578 different CpG sites across the whole genome and has been shown to yield reproducible results in agreement with technologies such as bisulfite sequencing (Bibikova et al., 2009). The BeadChip is designed by the manufacturer to preferentially target CpG sites in proximity to the promoter of 14,475 genes of the consensus coding sequences (CCDS) and known cancer genes as well as the promoter of 110 miRNA promoters. Hence, the array is designed to study CpG sites in proximity to genes and not the methylation of intergenic cytosines or repeat regions. Chip design allowed for 12 samples to be processed on the same chip. The DNA was whole-genome amplified, enzymatically fragmented, precipitated, and resuspended and after hybridization overnight at 48 °C the difference between a C or a T nucleotide was detected by single-base primer extension. The fluorescent detection was done using the Illumina iScan scanner. Preprocessing of the fluorescence signals and calculation of the β-values, the ratio between the signal from the C and the sum of the C and T signals, was performed with the GenomeStudio 2009.2 (Illumina) software. Probes that exhibited low quality (detection p-value >0.01) were discarded from the set. The array data have been deposited at the Gene Expression Omnibus under accession GSE44763.

3. Statistics

The collection of site specific β-values was analyzed using the statistical software R (www.r-project.org) in conjunction with the *methylumi* and *limma* packages (Gentleman et al., 2004; Smyth, 2004). Beta values are values ranging from zero to one corresponding to zero and 100% methylation. Downstream analysis was made using the entire dataset (excluding only high detection p-values). General linear models were fitted for each methylation value with the *lmFit* command using its *robust* setting to minimize the influence of deviant samples, with maximum iterations set to 1000. The empirical Bayes model implemented in the *limma* package (*eBayes* command) was used to create moderated t-statistics. p-Values were adjusted for multiple testing using the Bonferroni method and the Benjamini–Hochberg method (Benjamini et al., 2001). Probes that displayed an adjusted p-value of more than 0.05 were considered non-significant. In addition to p-

Table 1
Description of the participants in the obese and lean group.

	Obese	Lean
N	24	22
Age (years)[a]	57 (42–70)	55 (41–69)
Weight (kg)[a]	92 (78–108)	60 (40–75)
BMI (kg/m^2)[a]	35 (30–42)	22 (16–25)

[a] The values are given as the average and the range in parentheses.

values, we estimated the level of false discovery rate (FDR) by calculating Q-values using the *qvalue* package in Bioconductor with default settings. The Q-value is a measurement of the number of expected false positive that is detected among all significant tests for a certain p-value level (Storey and Tibshirani, 2003). If any probe for a specific gene was considered significant, all other probes for that same gene were also investigated. A nominal p-value <0.05 was considered significant for these adjacent probes. Each probe was investigated for potential interaction between age and weight group (obese and lean) using general linear models. Probes that had a significant interaction were also analyzed within the obese and lean group separately to retrieve the group specific effect of aging on the methylation level of the site. Furthermore, the probes with no significant interaction were analyzed without the interaction term to detect the main effect of obesity and age on the methylation level. The influence of the FTO rs9939609 genotype, which lies in intron one and is not likely to have any cis effects, on the methylation pattern was investigated by implementing a linear model controlled for age and weight group. The significant genes were analyzed for enrichment of function using the Consensus database (Kamburov et al., 2009, 2011) with all the 14,446 genes represented in the Illumina Infinium HumanMethylation27 BeadChip array that passed QC used as a background. Both KEGG (Kanehisa et al., 2004), the Kyoto Encyclopedia of Genes and Genomes (which catalogs genes based on the biological pathway they are involved in) and level 4 biological process GO terms (Ashburner et al., 2000) were used for enrichment analysis.

4. Results

We wanted to ascertain whether age, obesity and their interaction could predict the methylation status of specific CpG sites using an array that predominantly targets regions in proximity of promoters. We also sought to replicate our previous findings that a genetic variation within the FTO gene is associated with methylation changes. A linear model was implemented where the methylation of each site was evaluated as a linear function of obesity, age and their interaction term. In the case of the age associated genes, statistical analysis using ConsensusPathDB was used to identify enriched functional clusters among the differentially methylated genes.

A significant correlation between age and methylation levels after Benjamini–Hochberg correction (FDR < 0.01) was observed in 125 probes (Supplementary Table 1) of which 13 also proved significant under Bonferroni correction (Table 3). 70 of these 125 probes were annotated to genes that had multiple probes associated to age with a nominal p-value <0.05. Of all 125 probes, 34 showed reduced methylation with age and 91 were hypermethylated. An enrichment analysis of functional and biological terms revealed the KEGG pathway map04080 "Neuroactive ligand–interceptor interaction" to be enriched in this dataset (FDR < 0.01). Nine (PTGDR, MTRN1A, PRLHR, HTR7, MLNR, GRIA2, GRM1, GLRA1, THRB) of the members of this KEGG group were found among our age related sites. We identified an additional 10 regions after Benjamini–Hochberg correction (Table 2, FDR < 0.02) where the methylation levels depended on the interaction between obesity and age and analyzed them separately in the lean and obese group (Fig. 1). In eight (ADCY1, CXADR, KCNS2, LMX1B, FNDC4, NAT8L, AQPEP and FBLIM1) of the ten cases the obese subjects displayed decreased methylation with age when compared to their lean counterparts, whereas the opposite was true for the remaining two sites (RNH1 and NNAT). The gene "Long intergenic non coding RNA 304"-LINC00304 (Illumina ID: cg03819692, position chr16:87753140, located 11 bp from the transcription start site) displayed higher methylation (p = 0.0030, adjusted with Benjamini–Hochberg, FDR < 0.001) in the obese individuals compared to the lean, independent of age (Fig. 2a). No other gene was found to be differentially methylated between obese and lean individuals. Furthermore, no

Table 2
Genes in proximity to sites where the methylation level changes with age dependent on weight status (obese or lean). Each probe is listed along with the other probes that are annotated to the same gene.

Gene symbol	Location	Illumina ID	Avg. %	Control %/10 years[a]	Obese %/10 years[a]	p–value[b]	Q–value[c]
RNH1	Chr11:497970	cg06417962	83.95	−2.36	0.69	0.018	0.0042
	Chr11:496809	cg15796682	5.83	0.86	0.31	0.368	
ADCY1	Chr7:45581245	cg06417962	13.41	3.35	−0.64	0.018	0.0042
	Chr7:45580250	cg13523557	14.18	1.47	−0.97	0.006	
NNAT	Chr20:35582093	cg12862537	76.98	−1.35	2.42	0.028	0.0066
	Chr20:35582535	cg22510412	63.32	−1.01	2.15	0.008	
	Chr20:35582869	cg21588305	79.27	−1.26	0.92	0.012	
	Chr20:35582608	cg18433380	68.39	−0.07	1.63	0.215	
	Chr20:35583164	cg23566503	66.6	−0.45	0.67	0.323	
	Chr20:35582274	cg22298088	68.08	−0.78	0.52	0.361	
	Chr20:35583475	cg10642330	72.48	−0.56	0.43	0.388	
CXADR	Chr21:17807964	cg03167275	10.05	1.88	−0.33	0.029	0.0069
	Chr21:17805938	cg00744433	72.87	−1.35	0.8	0.172	
KCNS2	Chr8:99509124	cg05373457	24.16	5.33	−1.13	0.029	0.0069
LMX1B	Chr9:128415668	cg09660171	9.24	1.7	−0.7	0.029	0.0069
	Chr9:128416725	cg18453621	10.54	1.58	−0.6	0.016	
FNDC4	Chr2:27571213	cg17918501	10.46	3.56	−0.24	0.029	0.0069
	Chr2:27571677	cg20369763	9.63	1.42	−0.24	0.027	
NAT8L	Chr4:2030017	cg25044651	9.94	1.85	−1.33	0.039	0.0093
	Chr4:2031721	cg15489294	11.33	1.77	0.25	0.127	
AQPEP	Chr5:115326619	cg21269934	18.53	3.95	−0.33	0.039	0.0093
	Chr5:115325752	cg08211091	24.68	1.78	−0.43	0.037	
FBLIM1	Chr1:15958229	cg23002761	9.03	2.5	−1.15	0.044	0.01
	Chr1:15957345	cg07846167	22.84	0.08	0.24	0.779	

a — % refers to methylation level of the site where 0 means that no alleles are methylated and 100 means that all alleles are methylated. %/10 years denotes the percentage change over ten years.
b — p-values in shaded rows refer to the interaction term between age and weight status and are adjusted according to the Benjamini–Hochberg method. p-Values in un-shaded rows are the nominal p-values of the probes that are annotated to the reported gene.
c — the Q-value is an estimation of the false discovery rate (FDR) for the unadjusted p-value of a certain test.

Table 3
Genes in proximity to sites that display change in methylation with age.

Gene symbol	Location	Illumina ID	Avg. %[a]	%/10 years[a]	p–value[b]	Q–value[c]
MLNR	Chr13:48692682	cg02620013	27.76	2.9	0.00004	7.44*10-6
	Chr13:48692127	cg07935568	16.85	0.84	0.01858	
AGPAT4	Chr6:161615551	cg07074571	34.4	3.02	0.00130	8.61*10-5
ATP8A2	Chr13:24941066	cg18236477	21.68	2.95	0.00140	8.61*10-5
	Chr13:24941472	cg12111714	36.72	1.18	0.08145	
BRUNOL6	Chr15:70399179	cg21801378	10.64	2.06	0.00250	0.00011
	Chr15:70399621	cg16778903	4.45	0.26	0.17883	
NHLRC1	Chr6:18230698	cg22736354	21.9	2.42	0.00350	0.00013
	Chr6:18231028	cg00772000	19.02	0.51	0.32754	
SERHL	Chr22:41226632	cg12078929	13.74	4.13	0.00540	0.00015
	Chr22:41226049	cg03855656	42.93	0.32	0.75337	
HBQ1	Chr16:170341	cg07703401	14.54	2.28	0.00570	0.00015
	Chr16:169983	cg17714030	80.2	−1.45	0.07096	
PIGC	Chr1:170680235	cg08587864	42.22	−3.87	0.01300	0.00028
	Chr1:170679460	cg11584111	7.94	0.29	0.33264	
NAGS	Chr17:39437481	cg00462994	11.93	1.36	0.01400	0.00028
	Chr17:39437918	cg04032226	10.96	1.76	0.00247	
CECR6	Chr22:15982681	cg18137704	21.97	1.7	0.01900	0.00036
	Chr22:15981381	cg12373771	12.57	1.06	0.05924	
HTR7	Chr10:92607142	cg06291867	17.41	2.38	0.02300	0.00038
	Chr10:92608043	cg26332534	15.56	1.15	0.09341	
FOXE3	Chr1:47654901	cg18815943	11.31	2.31	0.03000	0.00043
	Chr1:47653843	cg18983672	69.48	-1.54	0.08635	
ZNF154	Chr19:62912306	cg21790626	10.71	2.07	0.03000	0.00043
	Chr19:62912474	cg08668790	14.22	3.06	0.00000	

a — % refers to methylation level of the site where 0 means that no alleles are methylated and 100 means that all alleles are methylated. %/10 years denotes the percentage change over ten years.
b — p-values in shaded rows are adjusted for multiple tests using Bonferroni correction. p-Values in un-shaded rows are the nominal p-values of the probes that are annotated to the reported gene.
c — the Q-value is an estimation of the false discovery rate (FDR) for the unadjusted p-value of a certain test.

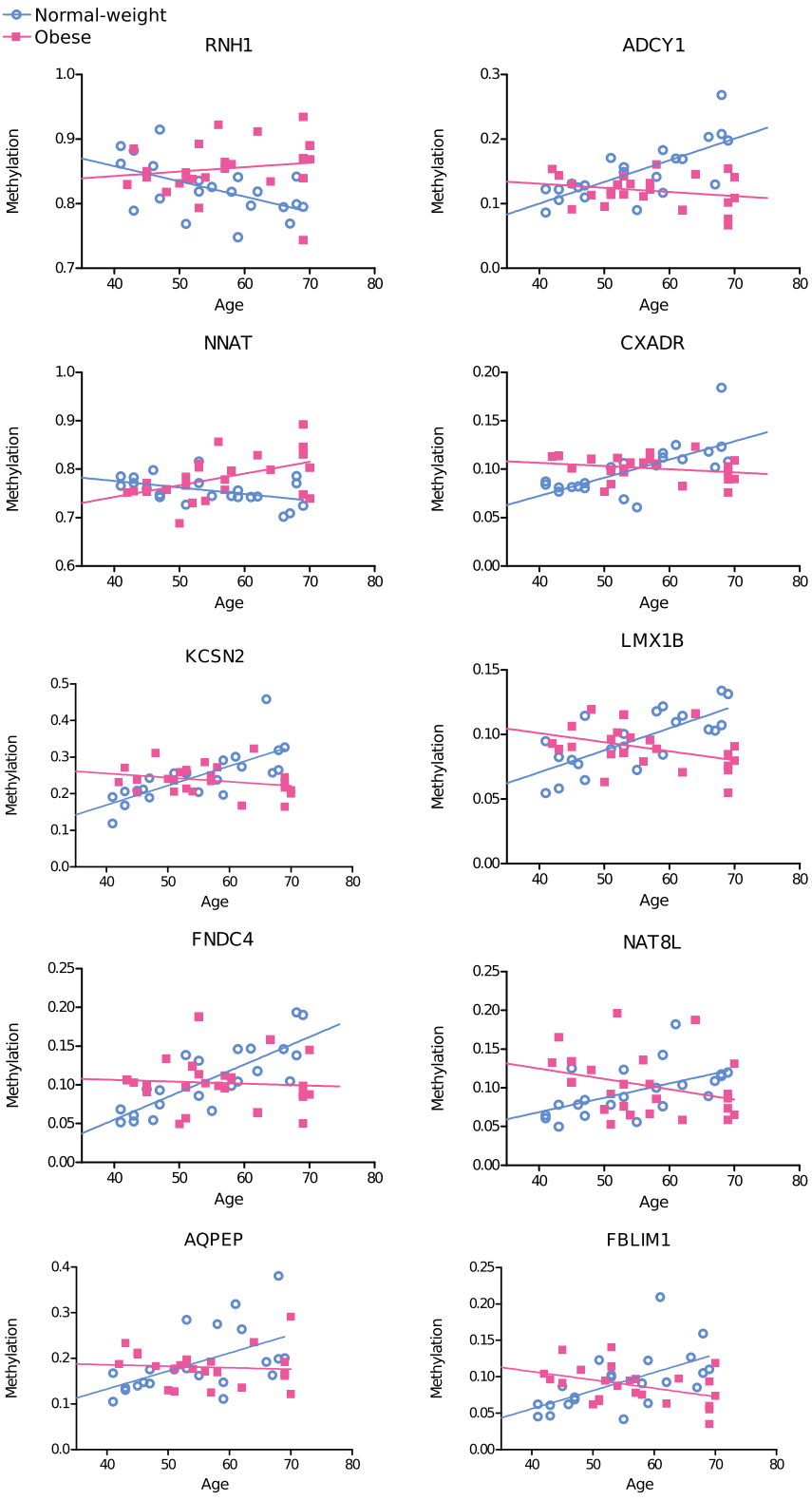

Fig. 1. Methylation changes with age in obese and lean individuals. Regression lines for age dependent methylation changes are analyzed and depicted separately in the obese (pink color) and lean (blue color) groups for the 10 genes that displayed an interaction between age and weight group in the statistical analysis. Methylation level was measured in beta-values (0–100) and age in years.

differential methylation level could be detected between the normal and risk allele carriers of the FTO gene.

The average methylation level of all probes on the array was calculated. This value was fitted to a linear model and investigated for correlation with age and obesity. A trend for the average genome-wide methylation levels to increase ($p = 0.10$) with age was observed (Fig. 2b). No differential global methylation level was detected between the obese and lean individuals ($p > 0.25$). It is important to stress that

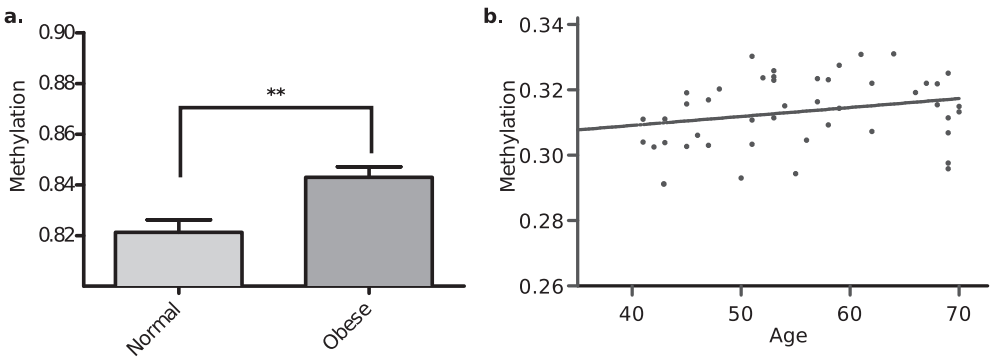

Fig. 2. Methylation change for LINC00304 in obese and lean (a) and regression for genome-wide average methylation changes with age (b). a. The gene LINC0040, which codes for an ncRNA, was the only gene differentially methylated in obese and lean individuals independent of age ($p = 0.0030$, adjusted with Benjamini–Hochberg). b. The average genome-wide methylation displays a trend ($p = 0.10$) to be increased with age. This measurements are mainly calculated from CpG sites in proximity of promoters and do not necessarily reflect changes in intergenic regions.

the genome-wide average methylation does not reflect the global methylation, which includes intergenic sites that are underrepresented on the array.

5. Discussion

In this study we determined that 135 genomic sites are subject to differential methylation during aging and that this process is influenced by weight in a subset of loci, and that genes involved in neuroactive, ligand–receptor interaction are overrepresented among the 125 age-related probes. In both tests, we identified several genes with multiple significant probes. We identified 10 sites with an interaction effect between obesity and aging. 8 of the sites had an interaction effect, i.e., a higher level of hypermethylation during aging in lean individuals, compared to obese individuals (Table 2 and Fig. 1). In contrast, the remaining two sites were hypermethylated with aging in the obese group compared to lean individuals. Hence, DNA methylation is a potential molecular link between obesity and age related diseases, although the mechanisms of this connection remain unclear. A possible explanation is the elevated inflammation status associated with obesity, which is tightly connected with adipokines and hormones that are secreted by excessive adipose tissue and activated immune cells, which alter the hormonal profile of obese individuals (Hotamisligil, 2006). Another interesting aspect is the beneficial effect of caloric restriction, which is known to prolong lifespan and protect against age related disorders such as cancer, diabetes and cardiovascular disease in primates (Colman et al., 2009). Restriction of energy intake has been reported to induce elevated levels of DNMTs, which in turn is thought to increase the methylation of genes that otherwise are upregulated during aging and thought to be involved in cancer and other diseases (Chouliaras et al., 2011). Hence, the different DNA methylation patterns that we observe in obese and lean individuals during aging may be related to higher energy intake in the obese group. Intriguingly, some of the detected genes are known to be involved in age related diseases and obesity: a genetic variation of the transcription factor LMX1B, which is involved in the development and maintenance of dopaminergic neurons, is associated with Parkinson's disease (Bergman et al., 2009); the gene NNAT, is metabolically regulated in the hypothalamus through leptin signaling and the locus is associated with obesity (Vrang et al., 2010). The NNAT gene is also interesting because it is considered an imprinted gene. Our results regarding this gene could reflect a difference at infancy between subjects. Thus, these 10 obesity-susceptible genes are candidates for biomarkers that may improve the understanding of how obesity affects the aging process and determine the underlying molecular mechanisms between obesity and aging. Furthermore, methylated sites associated with the genes CDKN2A, NPTX2, and GRIA2 are associated with aging (Alves et al., 2013; Bocklandt et al., 2011; Koch and Wagner, 2011; Liau et al., 2014). Although past studies differ from ours in terms of analysis/samples/species, the detection of the same genes is strong support of our results.

We found 125 sites that are differentially methylated during aging, but not interacting with obesity. A majority (75%) of these sites undergoes an increase in cytosine methylation with age, in conjunction with previous reports (Kim et al., 2004; Toyota et al., 1999; Tra et al., 2002). The age dependent genome-wide hypermethylation of promoter associated CpG islands is also supported by the observed trend ($p = 0.10$) of the average of all available markers, which increases with age (Fig. 2b). Hence, our results suggest that increased methylation of CpG islands in proximity of genes during aging is a more common process than hypomethylation, although our results also indicates that most in proximity of genes sites are not affected by age. The design of the array, which targets promoter associated CpG islands, does not allow us to draw any conclusions on the global methylation pattern that includes repeat regions and intergenic cytosines. The genes associated with the investigated sites where the DNA methylation was hypermethylated by age include the telomerase catalytic subunit (TERT, Supplementary information 1), which plays an important role in aging, development and cancer. Also, TERT is known to be down regulated in lymphocytes during aging, which is in concert with our observation of increased methylation of the gene (Weng et al., 1996). Hence, based on our results we speculate that DNA methylation may be an important regulatory mechanism of TERT during aging and thereby indirectly of telomere maintenance and cellular senescence. Several of the other genes that are associated with age dependent methylation changes are related with disorders that are known to be associated with aging. The gene that was observed to have the strongest age dependent methylation changes, MLNR, is a receptor for motilin. Interestingly, MLNR regulates gastrointestinal activity and it is known that dysregulation of this gene leads to constipation and diarrhea (Feighner et al., 1999). The gene BRUNOL6/CELF6, which regulates the activity of troponin T (TNNT2), is hypermethylated during aging and defects in TNNT2 regulation are proposed to cause cardiomyopathy (Ladd et al., 2004). Disorders related to bowel function and cardiovascular functions are known to increase with age and the epigenetic links to these two genes may provide insight into these processes. The serotonin receptor 7 (HTR7) is suggested to be associated with late onset Alzheimer's disease (Liu et al., 2007). Also, detection of differential methylation between obese and lean individuals revealed a novel epigenetic marker that is in proximity to the gene LINC00304 (Fig. 2a). Interestingly, LINC00304 is a long intergenic non-protein coding RNA, a molecule type that often is associated with transcriptional regulation. However, the exact function of LINC00304 is unknown. Albeit, it is unknown whether the observed changes can be translated to other tissues, previous studies have shown that DNA methylation changes measured in peripheral blood

are, in several cases, disease specific for neurodegenerative diseases and also bipolar disorder (Calabrese et al., 2011, Dempster et al., 2011). Disease and gene specific methylation in whole blood has also been shown in Alzheimer's disease (Bollati et al., 2011). This suggests that epigenetic alterations in blood cells can represent biomarkers for disorders specific for other tissues.

A limitation of the study is the size and specificity of the sample. Hence, the results cannot be generalized to a population level or to other groups, such as children or individuals of advanced age. Moreover, the relatively small changes reported here is cause for caution; technical replicates in 5% of probes display a difference of 13.6% (Calabrese et al., 2011) and the biggest change reported in this article is that of SERHL which changes 4.13% every ten years adding up to a total of ~12% change between the youngest and the oldest individuals. Peripheral blood is an available biomaterial and therefore of clinical importance. However, the use of peripheral blood may be a confounding factor as the observed DNA methylation changes may reflect a systematic shift in cell subpopulations with aging. Although changes in peripheral blood have recently been shown to correlate well with that of different brain regions (Horvath et al., 2012) we cannot be sure that this applies for the genes reported here. Further studies that replicate our findings in other groups and tissues and also investigate the function of the observed DNA methylation changes are needed to ascertain the extent and physiological role of our results. Although our results are generally confirmed by past studies, the authors could not replicate the findings in regions reported by Heyn et al. (Heyn et al., 2012). Future studies with larger sample sizes will likely detect differential methylation in sites not reported here, due to limitations in statistical power. Nonetheless, our estimated FDR <2% is a clear indication that the observed changes in methylation are reliable.

Herein, we present evidence that obesity interferes with age induced epigenetic changes. Moreover, we have identified a large number of genes that are susceptible to aging with respect to their DNA methylation profile and a gene that is differentially methylated in obese individuals. Although, the exact mechanisms behind the observed differences remain unclear they emphasize that age has a large impact on epigenetic programming and that this is influenced by lifestyle factors, which may have great implications for the understanding of age and lifestyle diseases.

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Author contributions

MSA and EN conceived and designed the study, analyzed the results and wrote the manuscript. JAJ, IK and JK conceived the study and administrated the selection of subjects. RF conceived the study. HBS conceived and designed the study and wrote the manuscript. All authors have read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2014.07.009.

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