Alu elements and human common diseases like obesity

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In the past few years the epigenetic impact on human diseases has been studied extensively. However, a controversial debate remains about the influence of environmental factors on the genetic determination of DNA methylation patterns. Although DNA methylation defects have been described in imprinting diseases and linked to cancer development, its impact on common diseases like obesity has yet to be elucidated. In our study we observed a hypermethylation variant of the *POMC* gene in obese children, which plays a key role in body weight regulation. Phylogenetic analyses reveal a close relationship between the *POMC* DNA methylation at this site and the presence of primate specific *Alu elements*. In this commentary we will extensively discuss our observations, including comments on the current debate about the impact of transposable elements on DNA methylation and on the development of human disease in general.

Since the discovery of transposable elements in the 1940s, new techniques, especially sequencing of whole genomes, have led to an increase in knowledge about the distribution and regulation of transposable elements within the genomes of plants, invertebrates and vertebrates.¹ It has been estimated that more than half of the human genome consists of transposable elements^{2,3} and that these have contributed significantly to the evolution and inter-individual variation of humans.^{4,5} The most prevalent retrotransposons with approximately over 1 million copies² and 200 subfamilies are primate specific *Alu elements*,⁶ accounting for more than 10% of the human genome. *Alu elements* have been introduced into the primate

genome approximately 65 million years ago and several studies have discussed the impact of this dynamic influence on primate evolution.⁷⁻⁹ It has been estimated that one *Alu element* insertion occurs in every twentieth birth in humans.¹⁰ Taking into account the impact of retrotransposition on genetic variation, diversity and its influence on genetic plasticity,^{11,12} insertion of retrotransposons can, in principle, harbour a disease causing effect. Consecutively, more than 60 diseases¹³⁻¹⁵ owing to *Alu element* insertion into the human genome have so far been identified. To counteract the influence of transposable elements like *Alu elements* on the primate organism, defense mechanisms of the host have evolved to induce silencing of the foreign DNA. Apart from RNAi¹⁶ and different histone modifications like H3K9 methylation,¹⁷ DNA methylation plays a key role in silencing of transposable elements.^{18,19} However, as a main drawback of this efficient counter-regulatory reaction, the defense mechanism of hypermethylation itself can cause a disease. Within the vicinity of *Alu element* insertions, the silencing hypermethylation-reaction of the organism may lead to alterations of adjacent gene expressions. This mechanism has initially been described as “position effect variegation” in *Drosophila melanogaster*.^{20,21} Further examples of variegation have been observed at an intracisternal A-particle (IAP) retrotransposon within the agouti locus in *A^y/a* mice²² and the *axin fused* (*Axin^{Fu}*) mice.²³ In both models the expression of the agouti or axin gene depends on the degree of the DNA methylation of the IAP site.^{23,24} Therefore, transposable element induced epigenetic silencing seems to be a potential mechanism to disturb adjacent gene regulation

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leading to a reduced gene dosage and an altered phenotype, e.g., a disease or an increase of an individual risk for a given disease. So far, this very likely disease causing mechanism of transposable element induced hypermethylation has been demonstrated only in one disease in humans: X-linked dystonia-parkinsonism (XDP). In this endemic movement disorder, the genetic insertion of an SVA (short interspersed nuclear element, variable number of tandem repeats and Alu composite) in the vicinity of the disease causing gene *TAF1* leads to the alteration of DNA methylation and expression of the *TAF1* gene.²⁵ Given the high rate of *Alu element* insertions into the human genome, the small number of examples for *Alu element* triggered hypermethylation defects diagnosed so far is surprising. With the advance of whole genome sequencing in patients with unexplained hereditary diseases, which will be available soon, a larger number of *Alu element* insertion events in the 5' or 3' prime vicinity of genes with subsequent hypermethylation of regulatory relevant parts of the genes can be diagnosed in the near future.

However, given the general force of hypermethylation around *Alu elements* in the human genome, one would expect that existing *Alu elements*, having accumulated throughout the human evolution, would already exhibit an impact on diseases. Apparently, based on the known strong genetic influence on DNA methylation, all *Alu element* associated hypermethylation of CpG positions must occur during embryogenesis.²⁶ However, this needs to be restricted in order to spare regulatory relevant regions of genes and thus to avoid an impact on the phenotype, respectively health of the individual. It is tempting to speculate that some inter-individual variations of this highly dynamic process will most likely occur during this critical time of development when hypermethylation of *Alu elements* occurs. So far, these inter-individual, *Alu element* associated variations have been overlooked and a causal link to the individual phenotype has not been established as a molecular cue to the individual risk for e.g., a common disease.

We identified such a transposable element induced inter-individual variation site during our studies on the DNA

methylation pattern of the *proopiomelanocortin* (*POMC*) gene by coincidence. *POMC* plays a key role in body weight regulation. It is embedded in the leptin-melanocortin signaling pathway within the arcuate nucleus. A decade ago we were able to identify the first *POMC* deficient patient.²⁷ The phenotype comprises ACTH (adrenocorticotrophic hormone) deficiency, severe early onset obesity and red hair. The occurrence of overweight also in individuals with a heterozygous *POMC* mutation led to the hypothesis that this gene is dosage sensitive, especially for the body weight phenotype.²⁸ To gain further insights into the epigenetic regulation of body weight we analyzed in a classical candidate gene approach the DNA methylation pattern of two *POMC* CpG islands. DNA was extracted from peripheral blood cells (PBC), in which *POMC* is expressed. The first CpG island is located at the *POMC* promoter region and previous studies have found a DNA methylation sensitivity of its function in ACTH expressing tumor cells.²⁹ The second 3' CpG island is located at the intersection between intron 2 and exon 3. Based on the hypothesis that epigenetic modifications of the *POMC* gene might be tissue specific, we analyzed the DNA methylation pattern in laser-microdissected β -MSH positive cells of human post-mortem brain samples. We found the same DNA methylation pattern as in DNA extracted from peripheral blood cells (Fig. 1A and B). After identification of an obviously non-tissue specific *POMC* DNA methylation pattern in expressing tissues, we compared the *POMC* DNA methylation from normal weight and obese individuals in DNA extracted from peripheral blood cells. We observed an identical pattern at the *POMC* 5' CpG island with identical peaks of methylation intensity. Unexpectedly, we identified a DNA hypermethylation variant at the intersection between the hypermethylated intron 2 and the hypomethylated exon 3 that was more frequent in obese patients (Fig. 1C) at the 3' CpG island. These results were verified by a second independent bisulfite-based method in an independent second cohort of obese and non-obese children. The DNA hypermethylation "overweight-variant" is

shifted toward the coding exon 3 region and affects only few CpG positions that are not methylated in the "normal-weight" variant.³⁰ While having a closer look at the genomic structure of this region, we recognized the presence of *Alu elements* in the human intron2 (Fig. 1A). Based on the primate specificity of *Alu elements* and their non-existence in mice we analyzed the mouse *POMC* DNA methylation pattern. Thereby, we observed a similar DNA methylation pattern of the 5' CpG island, but striking differences were found at the 3' CpG island in which we did not observe the hypermethylation of intron 2 (Fig. 2). These findings suggested a link between the presence of *Alu elements* in intron 2 and the hypermethylation state of intron 2 in humans. To follow this assumption we analyzed different primate DNA samples for their *Alu element* and methylation state. In primates with the same *Alu elements* as in humans (gorilla and chimpanzee) we identified the same distinct DNA methylation pattern of intron 2 hypermethylation. However, in samples of lemurs which have been separated from the main primate lineage already approximately 60 million years ago and which lack these *Alu elements*, intron2 was not hypermethylated. This is similar to the mouse samples. Based on these findings we speculated that hypermethylation of intron 2 is triggered by the adjacent *Alu elements* as a defense mechanism against further activity of these transposable elements. In addition, the sharp border of the *Alu element* triggered intron 2 hypermethylation at the start of exon 3 is a strong argument for a functional relevance of hypomethylation of exon 3. Therefore, it seems that the *Alu element* defense mechanism of hypermethylation at this genomic region needs to be balanced against the hypomethylation of exon 3. How this process of *Alu element*-triggered hypermethylation and at the same time exon 3 hypomethylation sustainability is regulated is largely unknown. Obviously there is a signal for the methylation machinery at the intron-exon intersection to avoid methylation of exon 3. Our results of individual variations at the intron 2-exon 3 methylation border of the *POMC* gene in obese patients suggest that this process is not 100% exact and that

Figure 1. (A) *POMC* gene structure with annotated *Alu* element localization. *POMC* DNA methylation pattern of CpG island 1 and 2 in (B) normal weight adolescents, (C) microdissected β -MSH positive cells of human post-mortem hypothalamic samples and (D) normal weight and obese individuals. The CpG position is numbered according to its position to next exon start (modified according to ref. 30).

this fuzziness might predict the individual susceptibility for a given disease. Taking into account the large number of transposable elements within the genome it may be tempting to speculate on the existence of more regions within the DNA methylation landscape, which are as sensitive to individual DNA methylation variations as the *POMC* intron 2-exon 3 border. Many more diseases may be influenced by these *Alu* element associated methylation variants, as it has been observed in cancer tissue.^{31,32}

Despite the observation that *Alu* elements could serve as a genetic cause for

different diseases, including cancer, so far there was only one example of inaccurate epigenetic defensiveness, which led to the development of a certain disease, e.g., the XDP.²⁵ Our results now extend this list and we are able to show that a certain subtle individual variation in an *Alu* element triggered methylation pattern might contribute to the susceptibility for obesity. Due to the frequency of transposable element insertion we assume, that the impact of *Alu* element associated epigenetic mechanisms in human diseases is probably underestimated. This may be due to

the occurrence of only minor changes in the DNA methylation pattern, which are induced by transposable elements comparable to the changes in cancer or imprinting diseases.

Our observation of a *POMC* hypermethylation is an additional example for an induced inter-individual DNA methylation variation, which is assumed to increase the susceptibility to a common disease like obesity.³⁰ This might be comparable to the effect of a single nucleotide polymorphism (SNPs) on the risk for certain diseases, which does not

Figure 2. Species-specific DNA methylation analysis in human, chimpanzee, lemur and mouse DNA extracted from peripheral blood cells reveal an association between the presence of *Alu* elements and *POMC* intron 2 DNA hypermethylation. The CpG position is numbered according to its position to *POMC* exon 3 start (modified according to ref. 30).

lead to a complete loss of gene function, but to an altered gene expression and thereby to an increased risk e.g., gaining body-weight.³³ However, the *POMC* gene methylation variation, which we have identified, shows a much higher probability for obesity as compared with all other previously described genetic SNPs. In the future, improved techniques to analyze the methylome in a tissue-specific and single CpG resolution will potentially lead to the identification of further examples, in

which the defense system of the organism to silence transposable elements increases the risk for a disease.

The field is now open for the discovery of more “auto-epigenetic diseases” where the defense mechanisms against transposable elements are misbalanced and lead to a new pathogenic mechanism as in autoimmune diseases where the immune system is imbalanced, thereby leading to the development of disease like diabetes mellitus type 1 or arthritis.

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References

- McClintock B. Controlling elements and the gene. *Cold Spring Harb Symp Quant Biol* 1956; 21:197-216; PMID:13433592; <http://dx.doi.org/10.1101/SQB.1956.021.01.017>
- Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al.; International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001; 409:860-921; PMID:11237011; <http://dx.doi.org/10.1038/35057062>
- de Koning AP, Gu W, Castoe TA, Batzer MA, Pollock DD. Repetitive elements may comprise over two-thirds of the human genome. *PLoS Genet* 2011; 7:e1002384; PMID:22144907; <http://dx.doi.org/10.1371/journal.pgen.1002384>
- Beck CR, Collier P, Macfarlane C, Malig M, Kidd JM, Eichler EE, et al. LINE-1 retrotransposition activity in human genomes. *Cell* 2010; 141:1159-70; PMID:20602998; <http://dx.doi.org/10.1016/j.cell.2010.05.021>
- Batzer MA, Deininger PL. Alu repeats and human genomic diversity. *Nat Rev Genet* 2002; 3:370-9; PMID:11988762; <http://dx.doi.org/10.1038/nrg798>
- Price AL, Eskin E, Pevzner PA. Whole-genome analysis of Alu repeat elements reveals complex evolutionary history. *Genome Res* 2004; 14:2245-52; PMID:15520288; <http://dx.doi.org/10.1101/gr.2693004>
- Cordaux R, Batzer MA. The impact of retrotransposons on human genome evolution. *Nat Rev Genet* 2009; 10:691-703; PMID:19763152; <http://dx.doi.org/10.1038/nrg2640>
- Xing J, Witherspoon DJ, Ray DA, Batzer MA, Jorde LB. Mobile DNA elements in primate and human evolution. *Am J Phys Anthropol* 2007; (Suppl 45): 2-19; PMID:18046749; <http://dx.doi.org/10.1002/ajpa.20722>
- Kazazian HH Jr. Mobile elements: drivers of genome evolution. *Science* 2004; 303:1626-32; PMID:15016989; <http://dx.doi.org/10.1126/science.1089670>
- Cordaux R, Hedges DJ, Herke SW, Batzer MA. Estimating the retrotransposition rate of human Alu elements. *Gene* 2006; 373:134-7; PMID:16522357; <http://dx.doi.org/10.1016/j.gene.2006.01.019>
- Baillie JK, Barnett MW, Upton KR, Gerhardt DJ, Richmond TA, De Sapio F, et al. Somatic retrotransposition alters the genetic landscape of the human brain. *Nature* 2011; 479:534-7; PMID:22037309; <http://dx.doi.org/10.1038/nature10531>
- Franchini LF, López-Leal R, Nasif S, Beati P, Gelman DM, Low MJ, et al. Convergent evolution of two mammalian neuronal enhancers by sequential exaptation of unrelated retrotransposons. *Proc Natl Acad Sci U S A* 2011; 108:15270-5; PMID:21876128; <http://dx.doi.org/10.1073/pnas.1104997108>
- Callinan PA, Batzer MA. Retrotransposable elements and human disease. *Genome Dyn* 2006; 1:104-15; PMID:18724056; <http://dx.doi.org/10.1159/000092503>
- Miki Y, Katagiri T, Kasumi F, Yoshimoto T, Nakamura Y. Mutation analysis in the BRCA2 gene in primary breast cancers. *Nat Genet* 1996; 13:245-7; PMID:8640237; <http://dx.doi.org/10.1038/ng0696-245>
- Deininger PL, Batzer MA. Alu repeats and human disease. *Mol Genet Metab* 1999; 67:183-93; PMID:10381326; <http://dx.doi.org/10.1006/mgme.1999.2864>
- Sijen T, Plasterk RH. Transposon silencing in the *Caenorhabditis elegans* germ line by natural RNAi. *Nature* 2003; 426:310-4; PMID:14628056; <http://dx.doi.org/10.1038/nature02107>
- Gendrel AV, Lippman Z, Yordan C, Colot V, Martienssen RA. Dependence of heterochromatic histone H3 methylation patterns on the *Arabidopsis* gene DDM1. *Science* 2002; 297:1871-3; PMID:12077425; <http://dx.doi.org/10.1126/science.1074950>
- Bourc'his D, Bestor TH. Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* 2004; 431:96-9; PMID:15318244; <http://dx.doi.org/10.1038/nature02886>
- Hollister JD, Gaut BS. Epigenetic silencing of transposable elements: a trade-off between reduced transposition and deleterious effects on neighboring gene expression. *Genome Res* 2009; 19:1419-28; PMID:19478138; <http://dx.doi.org/10.1101/gr.091678.109>
- Muller HJ, Altenburg E. The Frequency of Translocations Produced by X-Rays in *Drosophila*. *Genetics* 1930; 15:283-311; PMID:17246601
- Sun FL, Haynes K, Simpson CL, Lee SD, Collins L, Wuller J, et al. cis-Acting determinants of heterochromatin formation on *Drosophila melanogaster* chromosome four. *Mol Cell Biol* 2004; 24:8210-20; PMID:15340080; <http://dx.doi.org/10.1128/MCB.24.18.8210-8220.2004>
- Morgan HD, Sutherland HG, Martin DI, Whitelaw E. Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 1999; 23:314-8; PMID:10545949; <http://dx.doi.org/10.1038/15490>
- Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG. Maternal methyl supplements increase offspring DNA methylation at Axin Fused. *Genesis* 2006; 44:401-6; PMID:16868943; <http://dx.doi.org/10.1002/dvg.20230>
- Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 2003; 23:5293-300; PMID:12861015; <http://dx.doi.org/10.1128/MCB.23.15.5293-5300.2003>
- Makino S, Kaji R, Ando S, Tomizawa M, Yasuno K, Goto S, et al. Reduced neuron-specific expression of the TAF1 gene is associated with X-linked dystonia-parkinsonism. *Am J Hum Genet* 2007; 80:393-406; PMID:17273961; <http://dx.doi.org/10.1086/512129>
- Lienert F, Wirbelauer C, Som I, Dean A, Mohn F, Schübeler D. Identification of genetic elements that autonomously determine DNA methylation states. *Nat Genet* 2011; 43:1091-7; PMID:21964573; <http://dx.doi.org/10.1038/ng.946>
- Krude H, Biebermann H, Luck W, Horn R, Brabant G, Grüters A. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet* 1998; 19:155-7; PMID:9620771; <http://dx.doi.org/10.1038/509>
- Farooqi IS, Drop S, Clements A, Keogh JM, Biernacka J, Lowenbein S, et al. Heterozygosity for a POMC-null mutation and increased obesity risk in humans. *Diabetes* 2006; 55:2549-53; PMID:16936203; <http://dx.doi.org/10.2337/db06-0214>
- Newell-Price J, King P, Clark AJ. The CpG island promoter of the human proopiomelanocortin gene is methylated in nonexpressing normal tissue and tumors and represses expression. *Mol Endocrinol* 2001; 15:338-48; PMID:11158338; <http://dx.doi.org/10.1210/me.15.2.338>
- Kuehnen P, Mischke M, Wiegand S, Sers C, Horsthemke B, Lau S, et al. An Alu element-associated hypermethylation variant of the POMC gene is associated with childhood obesity. *PLoS Genet* 2012; 8:e1002543; PMID:22438814; <http://dx.doi.org/10.1371/journal.pgen.1002543>
- Doi A, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet* 2009; 41:1350-3; PMID:19881528; <http://dx.doi.org/10.1038/ng.471>
- Hansen KD, Timp W, Bravo HC, Sabunciyan S, Langmead B, McDonald OG, et al. Increased methylation variation in epigenetic domains across cancer types. *Nat Genet* 2011; 43:768-75; PMID:21706001; <http://dx.doi.org/10.1038/ng.865>
- Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al.; MAGIC; Procardis Consortium. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 2010; 42:937-48; PMID:20935630; <http://dx.doi.org/10.1038/ng.686>