Longitudinal Variation of locus specific DNA Methylation

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Towards Epigenetic Epidemiology of Metabolic Disease

Epigenetic dysregulation is frequently proposed to contribute to complex age-related diseases such as metabolic disease (MD). Empirical data in humans, however, are only just emerging. Epidemiological studies on the epigenetics of MD require the characterization of both the variation in DNA methylation patterns in the general population and their stability across age and between tissues. Here, we present such data on selected loci, implicated in MD, with relevant epigenetic features.

Conclusions

- DNA methylation is a quantitative parameter highly variable between loci
- Variation in DNA methylation correlates into genome wide patterns
- DNA methylation remains stabile in time for at least 20 years
- DNA methylation in blood marks that in other tissues

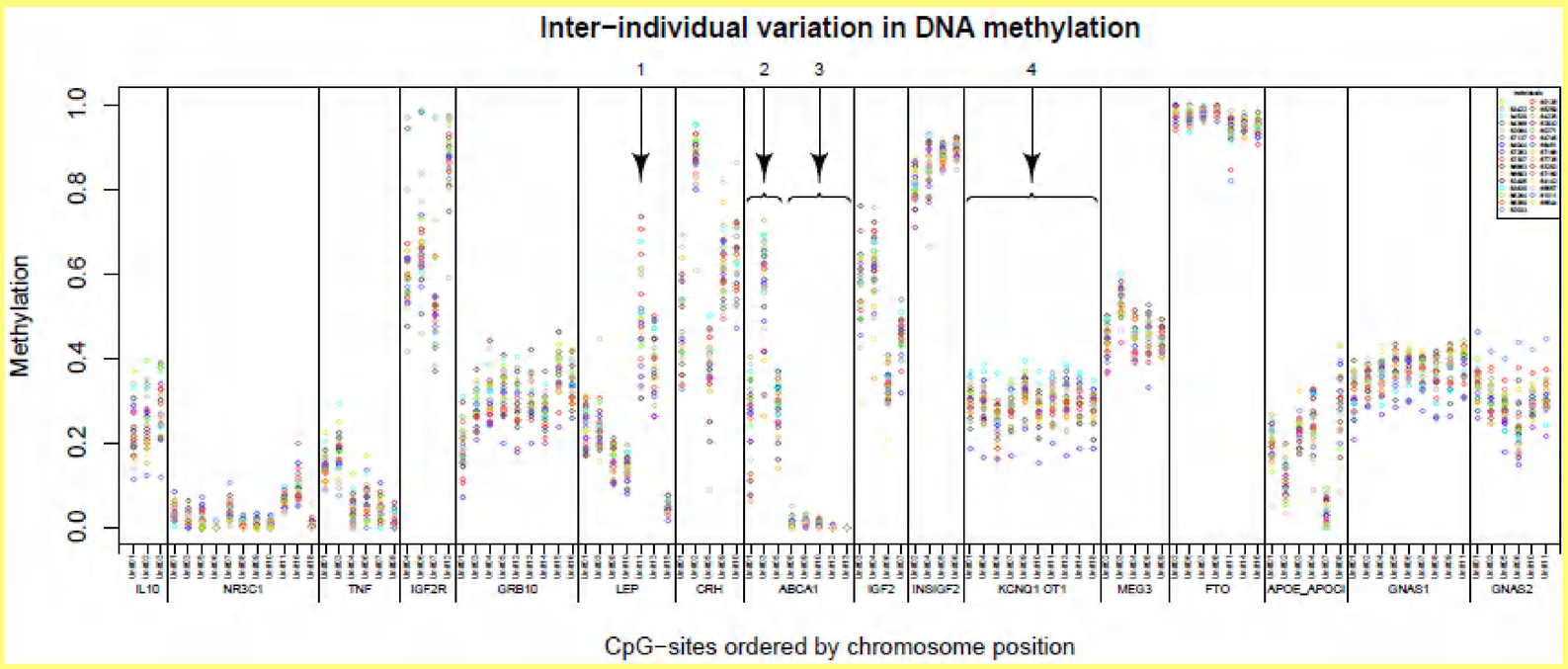

Figure 1: DNA methylation varies between individuals and between CpG-sites Loci can have several methylation patterns (Arrows 2 and 3), or be stabile across all sites (Arrow 4). Individuals also have a distinct methylation pattern spanning these loci (e.g. sky-blue dots).

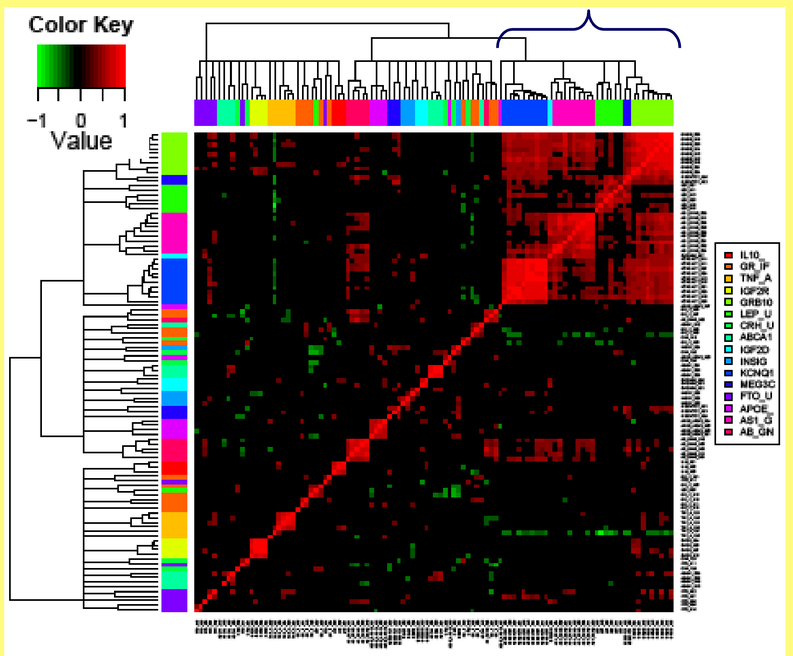

Figure 2: Methylation of CpG-sites correlates within and across loci, irrespective of their chromosomal location.

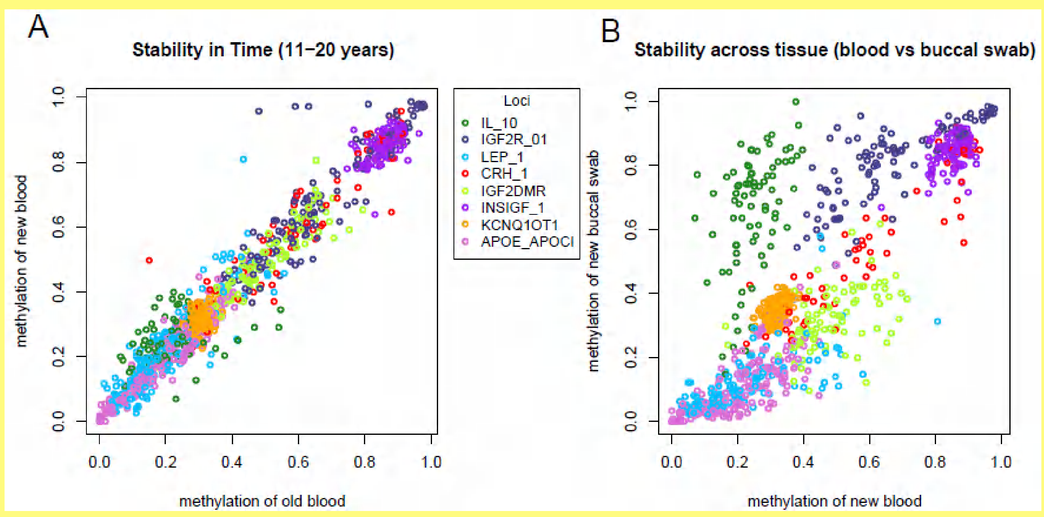

Figure 3: DNA methylation of 8 loci in blood is stable across time (A) marks methylation in buccal cells (B). Tables 1 and 2 give the results for the statistical model with which we tested the validity of these observations.

- Difference in methylation of blood and buccal swabs, which is not consistent for all CpG-sites (Fixed effects; Tissue and CpG-site*Tissue)
- No significant difference in average methylation of individuals
- Strong within individual variation between CpG-sites in the same tissue (+/- 7.2% methylation difference)
- Weak within individual variation between tissues on the same CpG-sites (+/- 1.8%)

Table 1: Type III Tests of Fixed Effects

Source	F	p
Intercept	28159.769	.000
Tissue	7.630	.010
CpG-site	990.986	.000
CpG-site * Tissue	235.231	.000

Table 2: Estimates Random Effects

Parameter	CI of difference to mean	p
Residual	10.59	1.0E-16
Intercept	1.96	0.059
Tissue	1.81	0.012
CpG-site	7.20	1.0E-16

Methods and Materials

First, DNA methylation status of 16 loci (104 CpG-sites, Table 1) was quantified in 30 non-related individuals using mass spectrometry (Sequenom). These individuals were selected from the Netherlands Twin registry (NTR) to represent the range of normal variation in MD risk factors and age. The neutrophil fraction, the major fraction (#%) and highly correlated with fractions of other cell types was used to test for the potential influence of cell heterogeneity on DNA methylation. Little to no influence was found for most loci (data not shown), the exceptions were IGF2R (0.4) and IL-10 (0.6), but we could correct for this.

Secondly, we measured DNA methylation of 8 loci (38 CpG-sites, Table 1 underlined loci) in 34 additional individuals (27 to 67 years of age at first sampling) of whom genomic DNA was available from whole blood, drawn 11 to 20 years apart, and buccal swabs, taken 2 to 8 years apart. In the statistical model used to formulate the stability across time and tissues we tested CpG-site, Time point (old vs. new), Time in years, tissue and the interactions between all these parameters.

Table 3: The 16 Loci of which the Methylation was investigated

Imprinted genes		Non-imprinted genes	
Name	Function	Name	Function
GNAS1	Growth/lypolytic signal	ABCA1	Cholesterol transport
GNAS2	Growth/lypolytic signal	APOC1	Metabolic
GRB10	IIS inhibitor	CRH	HPA axis
IGF2	Early growth	FTO	Fat metabolism
IGF2R	Growth/apoptosis	IL10	Anti-inflammatory
INSIGF2	Growth	LEP	Metabolic
KCNQ1 OT	11p15.5 ICR	NR3C1	HPA axis
MEG3	Growth suppressor	TNF	Pro-inflammatory

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