Charting the dynamic methylome across the human lifespan

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General discussion

6

SUMMARY OF RESULTS

In this thesis we aimed to get insight in how the methylome is established during development and subsequently degenerates during ageing using an integrative approach to the analysis of DNA methylation in conjunction with other levels of genomics data. The first two empirical chapters of this thesis describe the establishment and the maintenance of the epigenome and the subsequent two chapters the loss of control over the methylome in blood and other tissues.

First, we investigated the normal DNA methylation profiles that define tissue identity in peripheral and internal tissues (**Chapter 2**, **Figure 1**). In **Chapter 2** we developed a new algorithm to identify and annotate tissue-specific differentially methylated regions (tDMRs). tDMRs, in both internal and peripheral tissues, were found to be depleted for CpG island (CGI) promoters and enriched in CpG-poor distal and proximal promoters. tDMRs that overlapped with a CGI-promoter were often near developmental genes, while tDMRs overlapping with non-CGI promoters were often near genes involved in tissue-specific processes. Lastly, we showed that for some CpG sites one tissue may be used as a proxy for another tissue, as individual variation in one tissue was correlated to individual variation in another tissue, but the use of proxy tissues was largely complicated by SNPs.

To study how tDMRs arise during fetal development, we next investigated DNA methylation in four tissues (amnion, muscle, adrenal, pancreas) during first and second trimester of human gestation (**Chapter 3**, **Figure 1**). The extra-embryonic tissue amnion was very distinct in DNA methylation from embryonic tissues. Already at week 9, a tissue-specific signature was seen for each embryonic tissue. During fetal development the tissue-specific signature became more apparent as the differences between the embryonic tissues increased. Differential DNA methylation occurred particularly on enhancers. Gene expression data of dynamically methylated genes indicated that gain of DNA methylation was associated with the progressive repression of developmental programs and loss of DNA methylation during development with the activation of genes involved in tissue-specific processes. In this study we showed for the first time how tissue-specific DNA methylation patterns arise during fetal development in different tissues to form the tissue-specific signature identified in **Chapter 2**. These two studies

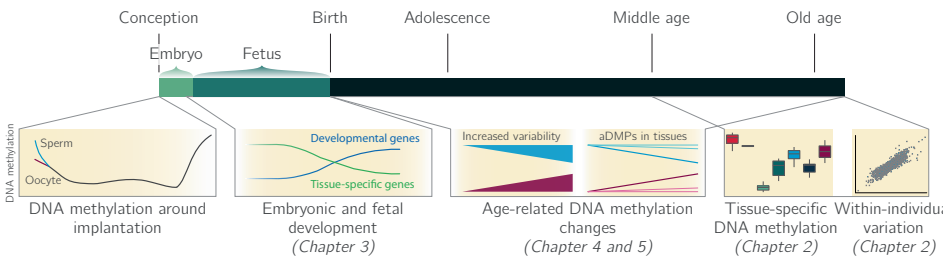

Figure 1. DNA methylation during the human lifespan. Y-axis represents DNA methylation level. From left to right: average change in DNA methylation after conception until around implantation; Average change in DNA methylation of developmental genes (gain) and tissue-specific genes (loss); Example of differences in DNA methylation between tissues; Example of increased variability in DNA methylation with age (aVMPs); Examples of age-related change in DNA methylation (aDMPs).

give more insight into the CpGs and processes that mark or mediate the differences between tissues.

In the last two chapters we investigated the loss of control over the methylome in blood and other tissues, by investigating changes in average DNA methylation and changes in variance with age. First, we investigated loss of control of DNA methylation in an ageing population, by identifying and characterizing CpGs that accrue variability between individuals in DNA methylation with age (**Chapter 4**, **Figure 1**). Age-related variably methylated positions (aVMPs) were not primarily the result of shifts in cell heterogeneity. Increased variability occurred particularly in regions under control of polycomb. A role for polycomb repressive complex 2 (PRC2) was further confirmed as aVMP methylation was associated *in trans* with the expression PRC2 genes. Yet, other genes were also associated to aVMP methylation *in trans*. Trans-associated aVMPs comprised two types: aVMPs that gained DNA methylation at PRC2-marked CpG islands and aVMPs that lost DNA methylation at enhancers. Differential age-associated DNA methylation was not limited to blood as it also extended to other tissues. Furthermore, in multiple cancer types aVMPs showed differential DNA methylation compared to their healthy counterpart. The associated trans-genes linked to pathways that change during ageing: upregulation of apoptosis and DNA repair associated genes and downregulation of cellular metabolism associated genes. This study links for the first time age-related DNA methylation changes to changes in expression of genes that link to well-known ageing pathways.

Lastly, we investigated the tissue-independency or –dependency of age-related differentially methylated positions (aDMPs) in seven tissues (brain, buccal, liver, kidney, monocytes, Th cells and subcutaneous fat) (**Chapter 5**). Differences were found in the number of aDMPs identified in each tissue and the largest number of aDMPs in brain and buccal epithelial cells. The majority of aDMPs were tissue-dependent and the overlapping aDMPs mainly gained DNA methylation with age (gain-aDMPs). Gain-aDMPs almost exclusively mapped to CpG islands and their flanking shores that often overlapped with a PRC2 binding site. Loss-aDMPs, on the contrary, were overrepresented in active regions, such as tissue-specific enhancers. Genes nearby gain-aDMPs were generally lowly expressed and linked to developmental pathways, while genes near loss-aDMPs showed a moderate expression and linked to cell motility and intracellular signaling cascade pathways.

Together, we identified 5 sets of CpGs: tDMPs (**Chapter 2**), hypomethylated CpGs during fetal development (**Chapter 3**), dynamic CpGs during fetal development (**Chapter 3**), CpGs that increase in variability in blood during ageing (**Chapter 4**) and CpGs that gain and lose DNA methylation with age in multiple tissues (**Chapter 5**).

Establishing and maintaining a healthy methylome

In **Chapter 2** and **Chapter 3** we investigated the establishment of the normal methylome. After conception, the methylome is largely erased and re-methylated around implantations (**Figure 1**) (Guo et al., 2014; Okae et al., 2014; Smith et al., 2014). How the methylome is formed during fetal development *in vivo* is largely unknown and much has been derived from *in vitro* experiments (Laurent et al., 2010). In **Chapter 2** and **Chapter 3** we investigated the normal developing methylome during fetal development in multiple tissues and in the adult life. In **Chapter 3** we

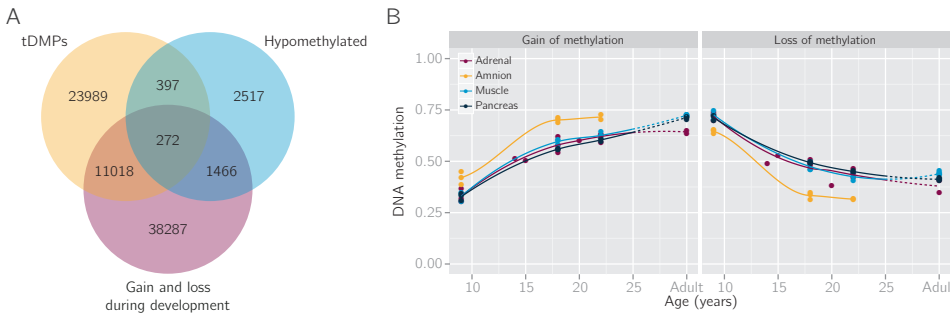

Figure 2. A. Overlap between CpGs identified in Chapter 2 and Chapter 3. tDMPs, tissue-specific differentially methylated positions; hypomethylated, CpGs hypomethylated in one fetal tissue relative to other fetal tissues B. Average DNA methylation of CpGs with gain and loss of DNA methylation during fetal development in the four tissues investigated in Chapter 3 compared to their adult counterpart.

showed that already at week 9 the epigenome of tissues has a tissue-specific signature. We showed that the methylome is very dynamic between week 9 and 22, as about 10% of investigated CpGs showed either gain or loss of DNA methylation in one or more tissues. Gain of DNA methylation at these dynamic CpGs was associated with the downregulated expression of developmental genes, while loss of DNA methylation was associated with upregulated expression of tissue-specific genes. The general shut-down of developmental programs and the gain of function of tissue-specific pathways has also be seen in *in vitro* studies (Gifford et al., 2013; Laurent et al., 2010; Nazor et al., 2012; Xie et al., 2013). Investigating the epigenome all the way through development until birth would give the best insight into the dynamic epigenome, but this is not feasible. To get a good understanding of the epigenome in fully developed organs, we investigated differences between adult tissues in **Chapter 2** in internal and peripheral tissues. Identified tissue-specific DMPs showed a substantial overlap with the hypomethylated and dynamic CpGs identified in **Chapter 3**. This illustrates that many (>11k) of the tissue-specific differences that are found between tissues are formed during the first weeks of fetal development. This is in line with the observation in **Chapter 3** that the DNA methylation at week 22 of human gestation was often similar with the adult counterpart, suggesting that the changes at these CpGs after week 22 of gestation are limited (**Figure 2B**).

Age-related loss of epigenetic control across the human lifespan

In **Chapter 2** and **Chapter 3** we showed that many of the patterns that are formed during fetal development are maintained into the adult life. Yet, in the past years several studies have shown that the epigenome is also dynamic after birth, but both stochastic (epigenetic drift) and orchestrated changes occur. To get a better insight in the epigenetic changes that occur after birth we investigated the postnatal epigenetic changes in **Chapter 4** and **Chapter 5** (**Figure 1**). We explored two types of age-related changes: CpGs whose DNA methylation closely tracks chronological age (aDMPs, **Chapter 5**) and CpGs with an increased variability in DNA methylation irrespective of chronological age (aVMPs, **Chapter 4**). The difference between these types

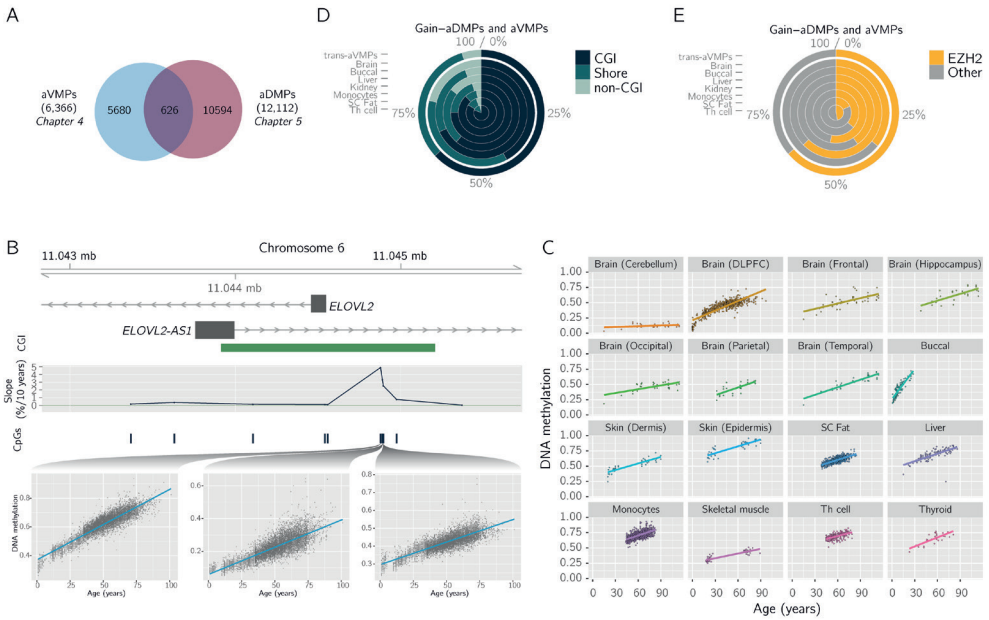

Figure 3.A. Overlap between aVMPs and aDMPs in blood and tissues. Figure 4.A. The ELOVL2 locus (top) with the three aDMPs frequently identified in blood in 7,969 blood samples (bottom). B/C. DNA methylation of the strongest age-associated ELOVL2 CpG site (cg16867657) in multiple tissues. D. Fraction of aDMPs and trans-aVMPs overlapping with CpG islands, shore and non-CGI regions. E. Fraction of aDMPs that overlapped with an EZH2 binding site.

of age-related changes was also reflected in the overlap between these two groups of CpGs, as only 626 CpGs were found in both studies, while 6,366 aVMPs were found and 12,112 aDMPs in multiple tissues (**Figure 3A**). In **Chapter 5** we also showed that aDMPs are tissue-specific and only a small fraction of the aDMPs are tissue-independent. The only locus that we identified in **Chapter 5** to be tissue-independent in all 7 tissues was the *ELOVL2* locus. This locus has been identified before in blood, but also in other tissues (Bekaert et al., 2015; Garagnani et al., 2012; Steegenga et al., 2014) and even mice (Spiers et al., 2016). The CpG island that spans the promoter of *ELOVL2* and *ELOVL2-AS1* contains three subsequent CpG sites which DNA methylation very closely tracks chronological age in blood (**Figure 3B**), but also in many other tissues (**Figure 3C**). *ELOVL2* is an enzyme in the elongase family that plays a role in the metabolism of fatty acids. Yet, its function does not increase the understanding of the tissue-independent age-related DNA methylation changes (Jakobsson et al., 2006).

The results from **Chapter 4** and **Chapter 5** illustrate that the age-related changes that occur to CpG islands can be both independent and dependent of individual and tissue. In **Chapter 4** we illustrated the individual-dependency of age-related changes at aVMPs, where some aged individuals have differential DNA methylation at a subset of the CpG sites, while others retain the DNA methylation profile of that of a young individual. In **Chapter 5** we showed that all individuals show age-related gain or loss of DNA methylation at aDMPs, irrespective

of the individual studied, but dependent of the tissue-studied. While the changes to the ageing methylome are both tissue- and individual-specific in many cases, the genomic features of loci that harbor the CpGs with age-related changes, that is aDMPs and aVMPs, showed a strong resemblance. In both **Chapter 4** and **Chapter 5** we showed that age-related gain of methylation almost exclusively occurred at CpG islands and their shores (**Figure 3D**) that were often bound by the repressive protein EZH2, which is part of the polycomb repressive complex (**Figure 3E**). Other studies investigating blood have also found that age-related gain of DNA methylation was strongly enriched for polycomb-repressed CpG islands (Reynolds et al., 2014; Steegenga et al., 2014). Yet, the way CpG islands in **Chapter 4** and **Chapter 5** gain DNA methylation is different: aDMPs gain DNA methylation in all individuals in several tissues in a tissue-specific fashion, whereas aVMPs only gain DNA methylation in some individuals in blood. Whether aVMPs and aDMPs are the result of different processes remains to be seen.

Mechanisms driving age-related changes and their consequences

While the age-related changes are interesting as they may mark or mediate the ageing processes seen at the organismal level, it remains unclear what molecular mechanisms underlie the age-related epigenetic changes. Both aDMPs and aVMPs are overrepresented at (EZH2-repressed) CpG islands and there are at least two plausible explanations why age-related changes target these features. The first hypothesis attributes the change in DNA methylation to the erosion of repressive marks from CpG islands. Promoters-CGIs near developmental genes are kept in a repressive state during life by the repressive complex PRC2 and EHZ2 is part of this complex. Loss of repression would allow DNA methyltransferases (DNMTs) *de novo* methylate the CGIs (Jung and Pfeifer, 2015) and the repressive mark H3K27me3 would be replaced by an active mark. This hypothesis would explain the differences in the existence of aDMPs between cell type, where the erosion of polycomb takes place at different genomic locations depending on the cell type. It would also explain the differences seen in **Chapter 5** where some individuals show erosion at some CpG sites while others do not.

An alternative interpretation comes from studies investigating DNA damage in cancer cells. Cancer cells with H_2O_2-induced oxidative DNA damage leading to double strand breaks, show a translocation from non-GC rich to GC-rich regions of a complex containing DNMT1, DNMT3A, PRC4 (O'Hagan et al., 2011). PRC4 is identical to PRC2, except that it contains the EED2 isoform instead of EED. The oxidative damage has persistent changes, that are different for lowly and highly expressed genes. Highly expressed genes show loss of active marks (H3K4me3), gain of repressive marks (H3K27me3) and loss of expression, without a change in DNA methylation (O'Hagan et al., 2011). In contrast, lowly expressed genes gain DNA methylation at CpG islands, without specific changes in histone modifications (O'Hagan et al., 2011). The latter observation is in line with the changes seen here, where gain of DNA methylation is seen at lowly expressed genes, such as developmental genes. In addition to these *in vitro* studies, *in vivo* studies have also found support for the second hypothesis. For example, a study comparing young to old satellite cells saw an accumulation of H3K27me3 (Liu et al., 2013a) that may be linked to a redistribution of PRC1 and PRC2 seen for example after DNA damage (Liu et al., 2013a; O'Hagan et al., 2011; Parker, 2015).

Functional consequences of age-related changes on the molecular and organismal level

aDMPs and aVMPs differ in terms of functional consequences on the molecular level. While only few aDMPs have been associated with gene expression changes (Steegenga et al., 2014; Yuan et al., 2015), aVMPs in blood are associated with gene expression *in cis* and *in trans* as shown in **Chapter 4.** Especially the genes that were associated to altered expression of genes *in trans* were of interest, as they were enriched for classical ageing pathways, such as DNA repair, apoptosis, immune activation and metabolism changes, with in each category key genes. So while the understanding of aDMPs is limited with respect to molecular consequences, aVMPs are associated with changes in expression that link to loci in pathways known to be relevant for ageing.

In addition to changes on the molecular level, a better understanding is required how the epigenetic changes contribute to the ageing process on the organismal level. Future work should show to what extent the aVMPs identified in **Chapter 4** mark or contribute to morbidity and mortality. Evidence is emerging that there is a link between age-related epigenetic changes and health, at least for the 'clocks' and particularly Horvath's DNA methylation age clock (Horvath, 2013), which has been shown to be predictive for mortality (Marioni et al., 2015) and morbidity (Horvath et al., 2014).

Age-related changes in the light of cancer and senescence

The epigenetic changes associated here with chronological age show resemblance with the changes seen in cancer and cellular senescence at the epigenome and transcriptome level. Long term growth of cells is associated with epigenetic changes that track the number of passages, is associated with hypermethylation of CGIs (lung fibroblasts) and CGI shores (human mesenchymal stem cells), while hypomethylation occurs on non-CGI features (Cruickshanks et al., 2013; Koch et al., 2013; Schellenberg et al., 2011). Cancer, however is also characterized by the global loss of DNA methylation, the loss of control over CGI-shores and the hypermethylation of CpG islands (Akalin et al., 2012; Irizarry et al., 2009; Schlesinger et al., 2007; Timp and Feinberg, 2013). In **Chapter 4** we found that the same CpGs that show age-related increased variability in blood are also differentially methylated in various cancer types. At the transcriptional level, we also found similarities between senescence and age-related changes. For example, in senescent cells there is an accumulation of $p16^{INK4a}$ (*CDKN2A* locus) and/or *p53*, where the latter is associated with for example upregulation of apoptosis, DNA repair, metabolic reprogramming pathways (Bieging et al., 2014; Chandler and Peters, 2013; van Deursen, 2014). In **Chapter 4** we showed that increased variability was also associated with the upregulated expression the pathways that link to the *p53* induced response with key genes involved, including DNA repair (*FANCG*, *DDB2*), cell cycle (*CDKN2A*, *PCNA*) and cell death (*CASP7*). The changes in **Chapter 4** may be a reflection of clonal outgrowth of an early senescent cells that show activation of the $p16^{INK4A}$ and *p53* pathways (van Deursen, 2014). Although cause and consequence cannot be distinguished, the loss of control over the epigenome and transcriptome may predispose cells to become senescent and subsequently cancerous whether or not together with SNPs in for example *DNMT3A*, *TET2* and *CREBBP* (Timp and Feinberg, 2013; Wagner et al., 2015; Xie et al., 2014). Yet, in healthy individuals an increased number of somatic mutations in *DNMT3A* and

TET2 do not necessary lead to health issues (van den Akker et al., 2016).

Crossing lines: overlap between development, tissue-specific patterns and ageing

It is of interest whether the CpGs identified in each of the studies show overlap. In this thesis, we identified 5 sets of CpGs: tDMPs (**Chapter 2**), hypomethylated CpGs during fetal development (**Chapter 3**), dynamic CpGs during fetal development (**Chapter 3**), CpGs that increase in variability in blood during ageing (**Chapter 4**) and CpGs that gain and lose DNA methylation with age in multiple tissues (**Chapter 5**). In the sections above we showed the similarities within the CpG sets identified in (fetal) tissues and within the CpGs sets that change during ageing. In **Figure 4** the overlap between all described sets is shown. For each of the sets of CpGs the largest fraction was unique for that set. As we showed above, there was a large overlap between CpGs dynamic during fetal development and tDMPs. Interestingly, a high overlap was also found between aDMPs and tDMPs (3,023, OR=4.3, $P<0.0001$) and CpGs that are dynamic during development (2,941, OR=2.6, $P<0.0001$). The overlap between aVMPs and tDMPs (824, OR=1.8, $P<0.0001$) and CpGs dynamic during development (1,064, OR=1.5, $P<0.0001$) was also substantial. So, CpG sites that are dynamic during fetal development and harbor tissue-specific DNA methylation profiles in adult tissues, are also the CpGs in the same or other tissues that show linear age-related changes. This suggests that the regions that change are, or have been, important regions for normal tissue-specification, that may become active as a result of other changes in the cell as suggested in the dysdifferentiation theory (Cutler, 1985). In case of the aVMPs, the overlap with tissue-specific methylated regions was lower compared to aDMPs. The lower overlap fits with the observation that aDMPs are different from aVMPs, and in contrast to aDMPs, aVMPs are commonly associated with gene expression changes both *in cis* and *in trans*.

The added value of integrative genomics

The publication of reference epigenomes and transcriptomes in projects such as ENCODE, Epigenomics Roadmap and GTEX accelerates the application of integrated genomics. In this thesis we integrated DNA methylation data with whole genome DNA methylation data (WGBS), genomic annotations (promoter, CpG islands etc), expression data (RNA-seq, CAGE), histone modification data (ChIP-seq), chromatin state segmentations (based on histone modifications), DNaseI hypersensitivity sites (DNase-seq) and transcription factor binding sites (ChIP-seq). Without this integration, the interpretation of DNA methylation data would have been impossible, as the role of DNA methylation is context dependent. For example, the use of chromatin state segmentations links changes in DNA methylation to functional elements, such as in **Chapter 3** where we showed that loss of methylation during fetal development particularly occurred on enhancers. Similarly, the transcription factor binding sites give information on the pathways involved: in **Chapter 3** loss of DNA methylation in muscle often overlapped with the muscle specific MYOD binding sites. Altogether, in this thesis we illustrated the use of additional information layers greatly improves the understanding of the observed changes in DNA methylation and without integrative omics the interpretation of DNA methylation may even be impossible.

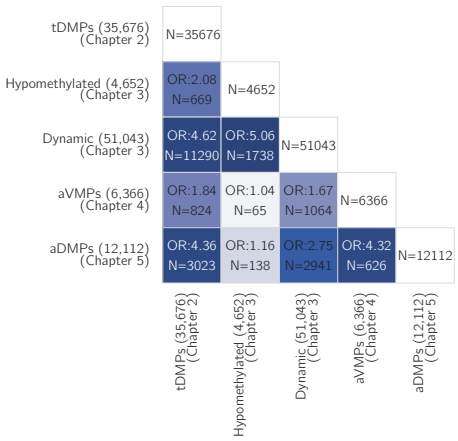

Figure 4 Overlap between sets of CpG sites identified in each of the chapters.

Catalogues of regulatory regions can facilitate in understanding disease variants

A catalogue of regions displaying tissue-specific and dynamic DNA methylation in development and mature organs may become an important tool facilitating the interpretation of GWAS studies in addition to atlases of active enhancers in differentiated cells and tissues (Andersson et al., 2014). For example, in **Chapter 3** we showed that regions near *HNF1A* and *HFN4A* are hypomethylated in the pancreas (relative to other tissues studied). *HNF1A* and *HFN4A* have been shown to play key roles in the development of the pancreas (Brink, 2003; Oliver-Krasinski and Stoffers, 2008). The hypomethylated region identified in the proximal promoter of *HNF1A* overlaps with the GWAS SNP rs1169288 (**Figure 4**). The SNP rs1169288 has been associated with a higher predisposition to develop type 2 diabetes (T2D) (Giannini et al., 2014; Holmkvist et al., 2008; Morita et al., 2015). Similarly, the hypomethylated region on the first exon of *HNF4A* overlaps with a LD block, which contains the SNP rs4812829. This SNP has also been associated in GWASs with a higher T2D risk in Asian populations (D. IAbetes Genetics Replication et al., 2014; Kooner et al., 2011). It remains to be seen if SNP directly influences DNA methylation (methylation Quantitative Trait Loci, meQTL), but it illustrates that catalogues of regulatory elements can give clues in explaining the effects of SNPs identified in GWASs. After all, if the SNP would influence the expression of genes relevant for organ development it may predispose an individual for disease as a result of less well developed organs.

Limitations and opportunities of methylation profiling technologies

The development of new technologies to measure DNA methylation, such as array-based technologies (27k, 450k, EPIC array) and bisulfite sequencing (RRBS, WGBS), has greatly accelerated epigenetic epidemiology. While sequencing based technologies have been proven to be a valuable tool in measuring lower number of samples at a larger number of CpG sites, the array-based technologies are a useful tool for high throughput measurements. In this thesis, we used the successor of the 27k array, the Illumina 450k array, to profile DNA methylation. The 450k array has been shown to be a valuable method to find associations between DNA methylation and a wide variety of diseases and phenotypes, including but not limited to multiple

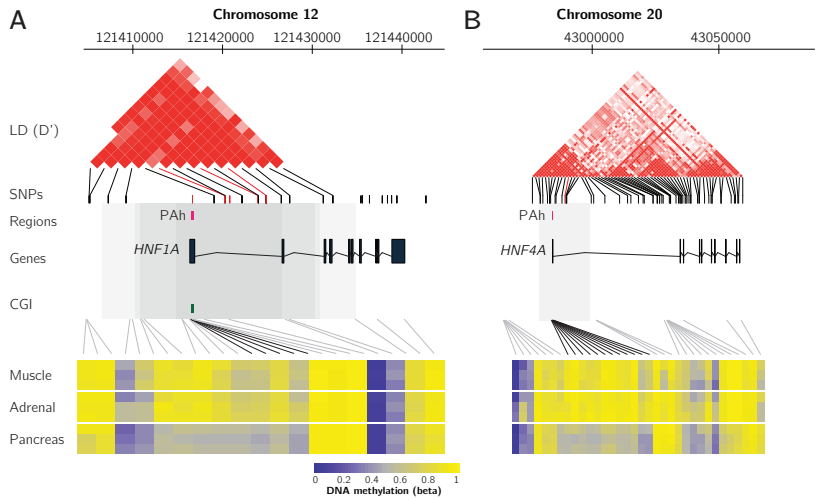

Figure 5. Hypomethylation in the promoter of HNF1A and HNF4A overlap with LDs blocks

cancers (Cahill et al., 2013; Shen et al., 2013), ageing (Garagnani et al., 2012; Hannum et al., 2013), undernutrition (Tobi et al., 2015), rheumatoid arthritis (Liu et al., 2013b), osteoarthritis (den Hollander et al., 2015) and diabetes (Dayeh et al., 2014). The Illumina 450k platform has a high reproducibility and data can be easily compared to external 450k data. We did this in **Chapter 3** where we used external 450k data of fetal and adult samples to compare to our own data. In **Chapter 4** we used external data to validated the identified aVMPs in whole blood and purified monocytes. In **Chapter 5** we used DNA methylation exclusively from public repositories to identify age-related changes in 7 tissues. Second, it measures a representative subset of the CpG sites, although it covers only a fraction of all CpGs in the genome (<2%). Third, the technology is suitable for high-throughput measurement.

Weaknesses of the 450k array

The Illumina 450k array also knows some weaknesses. First, the 450 array covers only a small percentage of the CpGs in the genome (<2%). Particularly distal elements are sparsely covered with about 11k CpGs in enhancers (Phantom 4). The limited coverage of enhancers has been resolved to some extent on the 850k array that covers a much larger number of enhancers. Second, the 450k technology uses of two assay types (Type I/II probes), where type II probes have a different distribution compared to type I probes (Pidsley et al., 2013). Methods have been developed to adjust for this difference and we applied one of these methods (BMIQ) in **Chapter 3** (Dedeurwaerder et al., 2011; Teschendorff et al., 2013). In **Chapter 1** we tested first per CpG site and subsequently merged CpGs to regions to minimalize this effect. In other chapters, the confounding effect of the Type I/II bias is limited as the effect sizes were relative large. Third, subsets of probes ambiguously map to the genome or overlap with SNPs making measurement these CpG sites unreliable (Chen et al., 2013). In **Chapter 2, 3 and 4** we removed ambiguously mapped probes from the data. In **Chapter 1** we did not exclude these ambiguously mapped

probes, but as we only used regions in this chapter the confounding effect will be limited and only a small part of the CpGs were earlier identified as ambiguously mapped (1.6%).

Conclusions and future prospects

The human epigenome is highly dynamic during development. Here we illustrated how dynamic the epigenome likely is during fetal development and after birth. The changes of the epigenome during fetal development are required to shape the different organs with their specific functionalities, while the developmental programs generally seem to shut down. In the adult counterpart, tissue-specific methylation differences mark or contribute to the integrity of the tissue. After birth, the epigenome is not only kept in a steady-state, but also changes in response to intrinsic and extrinsic factors. We showed that age-related epigenetic changes can be both tissue and individual dependent and independent. In every tissue, CpGs closely track the chronological age, like a clock, but we showed that they often differ within tissues. Other CpG sites diverge in the population, where some individual diverge from a 'youthful' methylome, while others retain this 'youthful' profile. The CpGs that diverge are the most interesting CpGs as they might reflect the increased variability in health in the population and be a marker of (prospective) mortality as has been shown for the Framingham risk score (Levine and Crimmins, 2014), but also for molecular scores based on DNA methylation (Horvath clock) (Horvath, 2013; Marioni et al., 2015). Future studies should focus on the mechanistic origin of age-related epigenetic changes, that is why age-related changes occur *in vivo*. Also, when there are larger datasets of other tissues than blood available, it would be valuable to confirm the existence of aVMPs in other tissues. In addition to mechanistic studies, the relation between aVMPs and the health of an individual should be studied to get an insight to what extent the epigenetic changes mark or mediate the physiological changes during the ageing process of the individual.

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