Studies into epigenetic variation and its contribution to cardiovascular disease

Talens, R.P.

Chapter 1
Introduction to epigenetic research on common diseases

BOX1: The Hunger Winter Families Study

We explored the potential for epigenetic changes due to prenatal adversity on participants of the Hunger Winter Families Study [11]. This study investigates individuals who were exposed in utero to the Dutch Famine. The Dutch Famine was the consequence of a German imposed food and fuel embargo for the western part of the Netherlands in the winter of 1944–45, toward the end of World War II. The famine was a 6 month period of severity and extreme stress. Official daily rations contained less than 700 kcal on average, and although the winter was average meteorologically, the fuel shortages would make it tough. As the civil infrastructure was maintained during the war, individuals who were either conceived or born during the Dutch Famine, could be tracked through their birth records at the institutions in which they were born (midwife clinics in Rotterdam and Amsterdam, university hospital in Leiden. These individuals were compared with their same sex older or younger siblings. This design achieves to isolate the prenatal exposure event as best as possible in human population studies, by controlling for childhood environment and social economic status, sex, post-natal experience of the Famine and genetic variation, as siblings still share half of their genome. This study focuses on insults during the important stages of gametogenesis, organ and tissue development and the late gestational period of fetal (and neonatal) growth, characterized by remarkable increase in size.

Coronary heart disease (CHD) is an ageing related common disease that was responsible for 83,941 hospitalizations and 9,720 deaths in the Netherlands in 2012 [1]. It is caused by a narrowing of the coronary blood vessels with a myocardial infarction (MI) being its most common manifestation. CHD is a disease of a complex multifactorial nature. An individual's risk of CHD is determined by the lifelong interaction between genetic background and environmental exposures [2,3]. Genetic and environmental factors contribute equally to CHD etiology [4]. Investigations into genetic sequence variation have been a main focus of the past decade and have revealed many genes behind the heritable components of CHD and its risk factors (e.g. type 2 diabetes (T2D) complications, metabolic syndrome, or hypertension) [5]. Classical environmental risk factors of CHD (e.g. smoking, diet, low physical activity) are well-established as contributors to its clinical pre-stages such as hypertension, lipid profile, and obesity [6]. The risk of CHD is strongly correlated with age [1,7], which may reflect an accumulation of environmental exposures and ageing-related (stochastic) processes that both result in a randomness in onset and etiology of CHD. Geographical and epidemiological

studies showed that poor conditions at the beginning of life also contribute to risk and severity of CHD in later life [8,9]. A survey across studies on famine cohorts, amongst which the Dutch Famine of 1944-1945 [Box 1], reported consistent associations between prenatal famine exposure and a higher BMI, more adiposity, a less favorable glucose metabolism and a more adverse lipid profile [10].

While the identification of genetic risk factors of CHD has contributed insights in molecular pathways involved in its etiology, the molecular mechanisms that mediate the effect of environmental risk factors are less well understood. Intervention studies on animal models present evidence that changes in DNA methylation, a major epigenetic mark, may be mechanistically involved in the association of prenatal adversity and ageing-related disease phenotypes [12,13]. In humans changes in DNA methylation at the *IGF2* locus were associated with exposure to the Dutch Famine during conception [14]. Changes in DNA methylation have been repeatedly found associated with lifestyle and environmental exposures [15]. Ageing has been repeatedly associated with stochastic changes in DNA methylation and other epigenetic marks such as histone modifications [16]. Thus, research on ageing related common diseases such as CHD has partly refocused its attention towards the involvement of epigenetic regulation, as a mediator of the interactions between genome and (prenatal) environment [17,18]. This general introduction will explain the biochemical basis of epigenetic mechanisms, explore current knowledge on its involvement in disease development, and describe the facilities and resources available to epigenetic research, and finally present an outline of this thesis.

Epigenetic mechanisms

The genome of multi-cellular organisms appears in a condensed state within the nucleus, forming a nine to thirty nanometer thick fiber of a DNA – protein complex called chromatin [19,20]. These chromatin fibers are formed by the coiling and packaging of its nucleosomes, which consist of 147 base pair long stretches of DNA wrapped twice around a protein scaffold of eight different histone subunits. Classically the condensation state of chromatin is grouped in three structural categories, open (euchromatin), intermittent (bivalent chromatin), and closed (heterochromatin), although

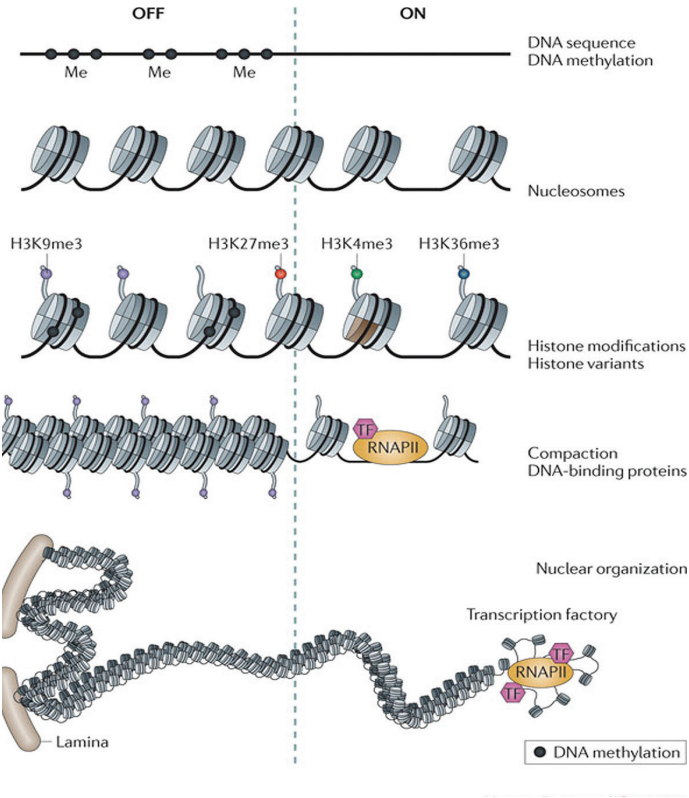

Figure 1.1 The different layers of epigenetic information, exemplified by the two extremes of the spectrum: transcriptionally inactive heterochromatin (left) and transcriptionally active euchromatin (right) with a selection of their associated epigenetic marks. The primary layer of chromatin structure constitutes the methylation of genomic DNA (Me) at cytosine bases in specific contexts, and the packaging of DNA into nucleosomes, which vary in histone composition (differently colored pie slices) and modifications of the histone tails (indicated by colored dots). DNA in chromatin may remain accessible to DNA-binding proteins such as transcription factors (TFs) and RNA polymerase II (RNAPII) or may be further compacted. Chromatin can also organize into higher-order structures such as nuclear lamina-associated domains and transcription factories [21]. *Image reprinted with permission from Macmillan Publishers Ltd: Nature Reviews Genetics Volume 12 (1); Zhou, Goren & Bernstein, pages 7-18; copyright 2011*

more distinct chromatin states were recently defined [21]. The amount of condensation at each genomic area is regulated by epigenetic mechanisms, the molecular basis of which is formed by several correlated layers of biochemical information on and around the nucleosomes (Figure 1.1).

These epigenetic layers include the methylation of DNA at cytosine residues, the addition of small chemical groups (e.g. phosphate, acetyl, or methyl groups) to the various histone subunits, nucleosomal packaging; non coding RNAs; and nuclear localization [22–25].

The capacity of the transcription machinery to access a gene after receiving the correct cues decreases with increasing condensation of its chromatin. A further assortment of epigenetic modifications, mainly on histone tails, results in the attraction or repulsion of transcription factors (i.e. enhancers and repressors) that enables a more or less continuous spectrum of fine tuning for epigenetically regulated gene expression [21,26]. Epigenetic silencing of repetitive DNA is generally thought to protect our genomes against the potentially detrimental effects of random insertion of transposons, mobile elements of parasitic origin [27,28]. Further, through variation of the epigenetic state at a genomic locus, epigenetic mechanisms determine the gene expression potential of a cell without changes to its DNA sequence [29]. Extensive epigenetic remodeling during development and differentiation generates the multitude of our body's cell types, each with its unique cellular phenotype, function, and response repertoire, that nonetheless all contain the same genotype as the zygote they ultimately derived from [30].

A textbook example illustrating the importance of epigenetically regulated gene expression is the mechanism of genomic imprinting [31,32]. Imprinted genes are epigenetically marked during gametogenesis so that they are expressed only from the paternally or maternally derived chromosome. Only about 0.3 % of our genes are imprinted, most of such genes reside in clusters, such as the *GNAS* locus which contains silenced genes of both maternal and paternal origin and at least one non coding RNA that regulates imprinting of the adjacent genes [33]. Most imprinted genes code for key players in the signaling pathways that coordinate growth and development with metabolism [34,35]. Their effect covers as diverse processes as the development of extra-embryonic tissues, fetal nutrient acquisition, neonatal suckling, and the control of appetite during life [33,34]. Incorrect establishment of imprinting marks leads to fetal lethality, growth impairments, insatiable appetite, and mental retardation [34], exemplified by imprinting disorders

such as the antagonistic Prader-Willi_Angelman syndrome [36], and Beckwith-Wiederman_Silver-Russel syndrome [37].

In contrast with the genetic sequence, epigenetic marks are not permanently fixed in a differentiated cell, they are, however, usually maintained over longer periods of time, and are faithfully transmitted to its daughter cells. During cell division the DNA separates from its protein scaffold to split the duplex for replication. Although several mechanisms have been proposed [38], mitotic transmission of the various histone modifications is less well understood than that of DNA methylation. The methylation marks are directly transmitted, as the methyl groups are covalently bound to the old strands of DNA, each of which is passed to one daughter cell. DNA methylation is the most studied layer of epigenetic information. A high degree of DNA methylation is generally associated with heterochromatin (Figure 1.1) and was long considered an epigenetic mark specific for the repression of transcription [39], although recent results from genome-wide techniques show that its function depends on context of its location within the transcriptional unit [28].

In humans, most DNA methylation occurs at the cytosine residue of CpG sites (a cytosine directly followed by a guanine residue) [24], but this is not the only form of DNA methylation. In embryonic stem cells and induced pluripotent stem cells substantial DNA methylation has been observed outside a CpG context, which is removed during differentiation [40,41]. Two recent studies have discovered cytosine residues that had a hydroxy-methyl group (hmC) covalently bound instead of a methyl group (mC) in both a-mitotic cell types of the brain [42] and in embryonic stem cells [43]. In stem cells (i.e. embryonic, pluripotent, and induced) high levels of hmC are often observed at epigenetically poised loci involved in pluripotency and maintenance of differentiation potential. The finding that methyl CpG binding proteins have a reduced affinity for hmC compared with mC [44], suggests that during differentiation the conversion of mC to hmC may be a molecular mechanism for rapid epigenetic decisions [43,45]. Its proposed role as biochemical intermediate in the process of active and passive DNA de-methylation [43,45] further supports the importance of hmC in cell differentiation, but also holds that it is likely not retained during multiple cell divisions. In line with this, hmC was found to occur only in low to marginal levels in tissues

relevant for CHD development [46]. Thus, most studies on the epigenetic contribution to CHD and other common ageing related diseases focus on the methylation of DNA at CpG sites in differentiated tissues.

The epigenetic contribution to diseases with an environmental component

The regulatory role of epigenetic mechanisms in cellular gene expression has forwarded research on the epigenetic involvement in common diseases, to both complement and understand their genetic component [47], with promising results. Two recent longitudinal studies reported an association of developing obesity [48] or type 2 diabetes [49] with different DNA methylation levels in leukocytes at established genetic candidate loci. Both studies reported that the epigenetic differences were found to precede disease manifestation and had remained stable throughout disease development during the study, showing that the epigenetic variation was persistent [48,49]. The epigenome has a dynamic capacity to undergo changes in each individual cell. This potential changeability of epigenetic marks is another topic of interest in research on common diseases [50]. The most studied example of epigenetic changes involved in disease are those affecting tumor cells [51]. Tumor cells often display high DNA methylation at promoters of genes controlling cell cycle arrest, while the genes promoting cell division are unmethylated, and both changes have been associated with the uncontrolled growth that is typical of malignant tumors [52–54]. Epigenetic differences have also been observed in ventricular tissue affected by MI compared with unaffected tissue from the same individual [55,56]. Studies on monozygotic twin pairs that are discordant for Beckwith-Wiederman and Silver-Russel syndromes suggest that epigenetic changes can precede the manifestation of metabolic diseases [57], and a recent genome wide study reported associations of DNA methylation with calendar age and ageing related phenotypes [58]. These and other results showcase that epigenetic variation may be an important factor in the process of ageing and risk for its related common diseases [18,59].

Individuals may harbor persistent epigenetic differences due to, first of all, the genetic sequence, which may prevent or force the establishment of epigenetic marks [60,61].

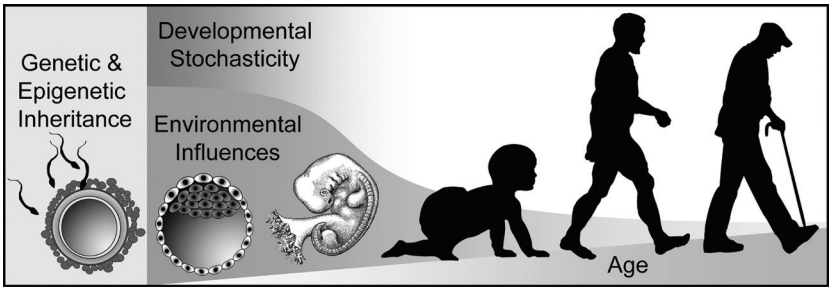

Figure 1.2 Contrary to genetic sequence variation, which is fixed for life after fertilization, epigenetic variation has a more changeable nature that involves different factors during phases of life. Two periods of prenatal life are proposed to be particularly vulnerable to stochastic and environmentally driven changes. First, the conditions in which both parents live prior to fertilization can disturb the extensive epigenetic remodeling during gametogenesis. Genetic sequence variation may form another source of epigenetic variation (e.g. polymorphisms affecting CpG sites) in the zygote. Second, the epigenetic remodeling during tissue development can be shaped by both stochastic factors (e.g. epigenetic differences in MZ twins), and by the intra-uteral environment, shaped by both internal physiological factors (e.g. placental morphology) and external maternal environmental factors (e.g. smoking, nutrition). Cell type differentiation also involves epigenetic remodeling, especially during early post-natal growth, resulting in a potentially still increased susceptibility to childhood environmental exposure like nutrition. During adult life environmental factors (lifestyle, living conditions) are thought to be of equal importance to stochastic changes (due to imperfect epigenetic maintenance), while in old age the efficiency of epigenetic maintenance is thought to decrease [119]. *Immage republished with permission of Annual Reviews from Annual Review of Nutrition, volume 27, pages 363 – 388: Epigenetic Epidemiology of the Developmental Origins Hypothesis; Waterland & Michels, copyright 2007; permission conveyed through Copyright Clearance Center, Inc.*

Next, epigenetic differences between newborn monozygotic twins [62] and isogenic littermates [63] indicated that the extensive developmental program of epigenetic remodeling [30] may induce stochastic epigenetic variation, as has been proposed [64]. Further, a wealth of intervention studies on animal models showed that prenatal adverse conditions can induce epigenetic changes [65,66]. In humans, prenatal famine [14], and smoking during pregnancy [67] were also found associated with persistent epigenetic differences. Early development may provide a period of specific vulnerability to environmental influences (Figure 1.2) after which the epigenetic differences may persist through faithful transmission during mitosis [68,69].

In differentiated cells, epigenetic information can change

over time. Imperfect maintenance of epigenetic marks has long been proposed to result in stochastic changes without any environmental influence [50,70]. A recent study reported solid evidence for this by following two initially identical cell cultures growing separately under the same conditions for 300 generations [51]. Epigenetic information may further change under the influence of environmental exposures related to lifestyle and living conditions [15] (Figure 1.2). For instance, prolonged or repeated exposure in adulthood to tobacco smoke [71,72], traffic exhaust fumes [73] or low doses of arsenic [74] were found associated with differences in DNA methylation. Intrinsic cues such as chronic inflammation [75,76] or stress [77] were also found to induce postnatal epigenetic changes.

Epigenetic changes due to environmental exposures could result from direct chemical damage, or from the incomplete (e.g. lack of methyl donors) or compromised (e.g. toxic interference) establishment of epigenetic marks. It is even conceivable that environmentally driven epigenetic changes may to some extent constitute an adaptive response [65], as repeated transcription of a locus has been suggested to change its epigenetic state [21,26]. Changing epigenetic marks could thus be an indicator of an active, or activated biological process, although their downstream biological effects remain to be revealed [15]. In conclusion, at birth epigenetic variation may be persistent, semi-heritable, and to a degree soma-wide [69]. Then, as uncorrected stochastic aberrations and environmentally driven epigenetic changes accumulate throughout life, this persistent level gradually changes in each cell individually [15]. It has been hypothesized that a build-up of uncorrected dysfunctional epigenetic perturbations may eventually result in a state called epigenetic dysregulation, which is implicated in ageing and its related diseases [58,59].

Technology, data resources, and biobanks in epigenetic research

The huge advance in DNA sequencing technologies of recent years (reviewed by Harrison & Parle-McDormott (2011) [78]) has enabled measurement of DNA methylation at individual CpG sites on both genome wide and high throughput platforms [Box 2]. Genome wide platforms are best suited for constructing epigenome maps and for

BOX2: Measuring DNA methylation by sequencing bisulphite treated DNA

Most studies assess DNA methylation by sequencing DNA that is treated with bisulphite (BS), although more techniques exist (reviewed by Harrison & Parle-McDormott (2011) [78]). BS treatment transforms unmethylated cytosines into uracils, while retaining the methylated cytosines, creating an artificial sequence variant that genetic sequencing tools and techniques can measure. However, BS based methods cannot distinguish between methyl-cytosine and hydroxy-methyl-cytosine [81], an issue in stem cells and brain, but not in other somatic cells [46]. Determining the relative proportions of the artificial BS sequence variants on a representative number of DNA molecules, which requires a slight alteration of the protocols from genetics, forms a measure for the amount of methylated and unmethylated alleles in the tissue sample. The DNA sequencing technologies developed in recent years have been modified to enable sequencing of BS treated DNA, and thus measurement of DNA methylation at individual CpG sites on whole genome, genome-wide and locus-specific medium throughput platforms (reviewed by Gupta et al. (2010) [82] and by Harrison & Parle-McDormott (2011) [78]). An example of a platform for whole genome BS sequencing is the powerful Illumina HiSeq platform (Illumina, San Diego, USA), which uses next generation sequencing technology to assess the methylation of over 90 % of the genomic total of 28 million CpG sites [83]. An example of a genome-wide platform is the Infinium BeadArray platform (Illumina), which has increased the amount of CpG sites it can interrogate from 1.500 in 2006 [84] to over 450.000 in 2011 [85]. Examples of medium throughput platforms are the sequencing-by-synthesis based Pyrosequencing platform [86] and the mass spectrometry based Sequenom MASSarray platform [87].

uncovering epigenetic candidate loci. However, these platforms still interrogate only a fraction of all CpG sites in the human genome and are biased towards CpG islands and promoter regions [79]. Medium-throughput platforms, like the Sequenom EPITYER platform, enable accurate quantitative measurement of methylation of a limited set of (nearby) CpG sites at any genomic (candidate) locus on large populations [80].

Following the technological aspect of measuring data, epigenetic research will need resources to facilitate interpretation of epigenetic associations. Parallel to genetics, these resources must include epigenome maps charting consensus epigenetic marks (DNA methylation and histone modifications), preferably for multiple cell types [88,89] and epivariome maps describing inter-individual variation in these marks [90–92]. Further, notwithstanding a few notable exceptions [93], the regulatory effect of DNA methylation in a cell population is commonly thought to rely on the status of multiple CpG sites within a genomic region [94], requiring data describing such potentially exploitable patterns within the normal variation (cf. linkage disequilibrium) [60,95]. Epigenetic databases, like those from the International

Human Epigenome Consortium [96] (http://www.ihec-epigenomes.org/) and the National Institute of Health (NIH) Epigenomics Roadmap [97] (http://www.roadmapepigenomics.org/), are under construction, but will require much effort to be as effective as their genetic counterparts.

Epigenetic research will also require access to diverse biomaterial of human population or patient based studies that investigate ageing and its common diseases. Many of these studies have collected blood samples at baseline, subsequently storing the extracted DNA in biobanks for future investigation. Epigenetic studies on these biobanks are technically possible since DNA methylation is not affected by standard methods for DNA extraction and storage. However, these biobanks were originally set up for genetic research and often contain only DNA from blood obtained during a single round of sampling. Their use in epigenetic research may thus be limited, since their construction did not consider resampling to allow studies on a dynamic epigenome [94]. First, epigenetic marks are susceptible to environmental and physiological influences. This may impede cross-sectional epigenetic studies in distinguishing cause from consequence of disease [98,99], while prospective epigenetic research will require either longitudinal sampling [49] or another form of evidence that the epigenetic variation remained stable during the study. Second, the frequently described tissue differences in (genome wide) epigenetic patterns [100–102] may limit the relevance of epigenetic variation to the tissue that was assessed, which may confine most epigenetic studies into disease causes to the search for potential epigenetic markers of disease risk, and limit implicating an epigenetic mechanism in disease etiology to those diseases for which the relevant tissues are available. Third, and more pressing is the fact that inter-individual variation in the composition of the heterogeneous leukocyte population may confound the epigenetic variation in blood, since each different cell type may carry its own specific epigenetic marks [103]. Although not relevant for candidate gene studies, methods that address this issue through mathematical correction are being developed for genome wide investigations [104].

Unfortunately there will never be biobanks that contain repeated longitudinal DNA samples from disease related internal tissues (e.g. heart, brain), thereby dismissing the direct analysis of epigenetic marks on other than biosamples

Table 1.1: Candidate loci investigated in the various studies of this thesis

Locus	Chromosome	Gene function	Studied in chapter				
			2	3	4	5	6
IL10	01q32.1	Anti-inflammation	x	x		x	
NR3C1	05q31.3	Stress response	x	x			
TNF	06p21.33	Pro-inflammatory	x	x			
IGF2R	06q25.3	Growth/Apoptosis	x	x			
GRB10	07p12.2	IIS inhibitor	x	x			
LEP	07q32.1	Metabolism	x	x	x	x	
CRH	08q13.1	Stress response	x	x	x		
ABCA1	09q31.1	Cholesterol transport	x	x	x	x	
IGF2[a]	11p15.5	Early growth	x		x	x	
INSIGF	11p15.5	(Embryonic) Growth	x	x	x	x	
KCNQ1OT1	11p15.5	Imprinting control region	x	x	x		
MEG3	14q32.2	Growth suppressor	x	x			
FTO	16q12.2	Development	x	x			
APOC1	19q13.32	Metabolism	x	x			
GNASAS	20q13.32	Growth/Lypolytic signal	x	x	x	x	
GNAS A/B	20q13.32	Growth/Lypolytic signal	x	x			

a: The susceptibility of DNA methylation to prenatal conditions was observed previously at the *IGF2* locus [14,120].

obtained during post mortem examinations [105] or repeated sampling of peripheral tissues. However, loci at which epigenetic marks are set early in development, for instance imprinted loci, are commonly thought to harbour a similar epigenetic information throughout the soma. Thus, at such loci epigenetic variation in peripheral tissues is proposed to also inform on internal tissues [106]. Although not often collected, other peripheral tissues than blood can be obtained with minimal (e.g. buccal cells, or epidermis) [107,108] or moderate invasiveness (e.g. fat, or muscle biopsies) [109,110], which may be useful in elucidating similarities of epigenetic variation across tissues. In recent years a few projects have been launched to establish biobanks designed for epigenetic research that for now mostly contain cross-sectional samples from multiple peripheral tissues [62,111]. However promising the emergence of such projects, it will take time for many more of them to emerge. Thus, if the clinical biobanks with all their limitations can still provide meaningful answers to some epigenetic research questions,

they will present an unmatched source of patient based DNA samples, due to their combined size, disease repertoire, and detail of participant information gathered during the (long) follow up period.

Outline of thesis

Epigenetic marks can be persistently or dynamically altered from gametogenesis until death by a combination of influences from exposure to the external environment, from internal physiological processes, and from uncorrected stochastic perturbations. These sources of epigenetic variation cannot be readily isolated for investigation, particularly not in humans, nor will it be possible to capture a lifetime's accumulation of causes and consequences of epigenetic variation in one study. However, careful formulation of specific research question and subsequent selection of appropriate study designs, may still promise meaningful epigenetic research on ageing and its related diseases [112]. In this thesis we will therefore apply a sequence of studies to investigate the relation between epigenetic mechanisms, ageing, and CHD.

Chapter 2 investigates properties of epigenetic variation at 16 candidate loci, that were selected from the domain of cardiovascular and metabolic diseases (Table 1.1), pertaining to the suitability of DNA samples from existing biobanks for epigenetic studies on these loci. First we describe the inter-individual variation and patterns of DNA methylation at 104 individual CpG sites of the loci on recent blood samples from unrelated, healthy participants (14 – 72 years old) of the Netherlands Twin Register (NTR) [113,114]. We also assess the influence of leukocyte population heterogeneity on this variation and present a method to account for its potential effect on epigenetic variation. Then, we determine the temporal stability of methylation variation on longitudinal DNA samples from blood (mesoderm) and buccal cells (ectoderm) over a time span of up to two decades and we survey its correlation between the recent samples of both tissues.

Chapter 3 investigates the effect of prenatal exposure to the Dutch Famine of 1945 on epigenetic marks in individuals from the Hunger Winter Families Study [Box 1] [11]. We compare DNA methylation at 15 of the candidate loci (Table 1.1) between individuals (57 – 59 years old) who were prenatally exposed to the Dutch Famine and their same sex younger or older siblings

(43 – 70 years old), who were not exposed in utero. By measuring middle aged individuals we dismiss transient epigenetic changes that would reverse with improving conditions, thus focusing on lasting effects. The uncovered associations between prenatal famine and persistent DNA methylation are further explored for the role of the timing of the exposure (around conception vs. last trimester) and sex of the exposed.

Chapter 4 investigates the extent to which genomic DNA methylation, approximated with the LINE1 assay [115], and DNA methylation at 7 of the candidate loci (Table 1.1) changes stochastically or due to environmental influences during the adult life span. We assess inter-individual and within-pair variation in DNA methylation with increasing calendar age in a combined cross-sectional and longitudinal study on a large population of Dutch and Danish monozygotic twin pairs (18 – 88 years old) [116,117]. We also evaluate the influence of familial versus individual factors on age-related epigenetic variability. The twins pairs are solely selected on their age in order to randomize potential differences in adult living environments, thus focusing the study on the amount of epigenetic changeability across the full adult lifespan due to the combined effects of stochastic and environmentally driven epigenetic changes.

Chapter 5 investigates whether DNA methylation at the 6 loci at which it marks prenatal environmental conditions (Table 1.1**)** is associated with the risk for developing a myocardial infarction (MI) in a population of individuals (70 – 82 years old) from the PROSPER study [118]. The individuals selected from this study had not experienced a previous CHD event in order to exclude potential epigenetic differences as a consequence of the disease.

Chapter 6 explores the extent to which the variation of DNA methylation in blood may inform on the methylation variation in internal tissues using a genome wide survey of post mortem samples of blood, subcutaneous fat (SC fat), muscle, visceral fat (VS fat), liver, spleen, and pancreas obtained from six individuals (58 – 79 years old) during autopsy. DNA methylation is assessed at 378,239 CpG sites distributed throughout the autosomal chromosomes. Besides comparing the tissues on genome wide patterns, we assess differences in the methylation level and the amount of variation at individual CpG sites and we inspect correlation coefficients between the tissues. Pooled blood from healthy middle aged regular blood donors is included in the study, to ascertain that no overhauling of DNA methylation patterns occurs within the first 24 hours after death.