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Epigenetics and methylation in the rheumatic diseases

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ABSTRACT

Objectives: Rheumatic diseases encompass a wide range of conditions of poorly characterized etiopathology, many having both genetic and environmental susceptibility factors. Epigenetic studies are providing new insights into disease pathogenesis. Recent rheumatology literature related to DNA methylation studies—both epigenome-wide and candidate gene—are discussed, as well as methodological issues.

Method: A PubMed search for articles published until April 2013 was conducted using the following keywords: ("methylation" OR "epigenetics") AND ("rheumatoid arthritis" OR "lupus" OR "autoimmune disease" OR "osteoporosis" OR "osteoarthritis" OR "musculoskeletal disorder") and EWAS. The reference lists of identified articles were searched for further articles.

Results: Several genome-wide methylation studies have been reported recently, mostly in autoimmune rheumatic diseases. Overall, these studies have identified methylation signatures in disease, clustering of subgroups as well as new and known epigenetic associations. Methodological issues, small sample sizes and reduced coverage of methylation assays render many results preliminary.

Conclusions: There have been a number of epigenetic advances in rheumatic diseases recently. The new technologies and emerging field of epigenome-wide association will provide novel perspectives in disease etiology, diagnosis, classification, and therapy.

Introduction

The field of epigenetics in medicine has grown exponentially over the last few years, benefiting from the advanced application of high-throughput technologies. Epigenetics has however been harder to define with any consensus [1,2], not least because of the wide variety of regulatory mechanisms including histone modifications, chromatin folding, DNA methylation (DNAm), and miRNA expression (micro-RNAs). Among these, DNA methylation is currently the most well studied because of the availability of suitable technology. DNA methylation forms the subject of this review—for other epigenetic mechanisms in the field, see reviews [3–6].

Rheumatic disease encompasses a wide range of different conditions, from autoimmune, inflammatory diseases to degenerative and mechanical conditions. Disease classification relies mainly on the evolving clinical, radiologic, and biological composite criteria because the etiopathology of few such diseases has been elucidated fully. Although twin and family studies have established genetic risk factors for most, if not all the conditions, genetic variants identified so far explain a small proportion of the total phenotypic variance. Epigenetics has great promise, therefore, in explaining and redefining diseases in the field as well as accounting for the role of some of the environmental factors.

In this review, we aim to provide clinicians with an overview of the current methodological concepts, challenges, and issues in methylation analysis. We present results of methylation studies in the context of rheumatic diseases and discuss future perspectives.

Methylation architecture and distribution

The genome sequence is composed of four nucleotides—A (adenosine), T (thymine), C (cytosine), and G (guanine). DNA methylation (DNAm) refers to the addition of a methyl group to cytosine at the carbon 5 position (5-methylcytosine, 5mC). Other types of methylation marks exist such as hydoxymethylcytosine (5hmC), which has been identified as a modification of developmental importance at least in early life [7]. The global distribution of cytosine modifications across the genome is referred to as the DNA methylome.

In humans, methylation occurs mainly when cytosine is followed by a guanine on the same strand of DNA linked by a phosphate group (called CpGs or CpG dinucleotides), although several other types of methylation, e.g., at CpHpG (H = A, T, or C),

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exist as well [8]. Overall distribution of methylation in the genome is bimodal; the majority of CpGs across the genome are methylated (70–80%), whereas a high density of CpGs enriched in the promoter region of the genes (CpGs islands) are generally unmethylated [9]. It is therefore challenging to summarize these changes over the genome.

Methylation properties and functions in human biology and disease

Methylation plays a key role in physiologic conditions, and alterations in methylation regulation have been identified in pathological processes [10,11]. Current knowledge indicates that the function of methylation and its relationship to gene expression varies according to the position in the genome as well as in regions with poor or rich CpG content [12,13]. High levels of 5mC in CpG-rich promoter regions are usually strongly associated with transcriptional repression [14], whereas gene body methylation may not be and shows a more context-dependant relationship [15]. DMAm does not act alone on gene regulation but is closely interconnected with other genetic and epigenetic mechanisms (e.g., transcription factors and histone-modifying enzymes), acting together on chromatin structure to determine the state of gene accessibility. Methylation is thought to reinforce the maintenance of closed chromatin through subsequent cell divisions and mediate long-lasting changes in gene expression [13]. The aforementioned regulatory mechanisms rely on two central properties of DNAm: stability (mitotic inheritance) and plasticity. Plasticity refers to the constant adaptation of the methylome to specific cell regulatory processes in response to internal and external stimuli.

Physiological variations in the methylome

Gametogenesis and pre-implantation zygotic phases are both associated with extensive methylation resetting and reprogramming [16]. Later, in post-zygotic somatic development, most blocks of genomic methylation patterns are static across tissues and throughout life, changing only in local context as specific cellular processes are activated or repressed [13]. These include, for example, mechanisms sustaining cellular fate and differentiation, responsible for specific methylome signatures across different cell types (cell-specific methylome) [17]. Aging—which plays a role in many rheumatic diseases—seems also to be associated with global reproducible methylation changes with a tendency towards hypermethylation [18].

Epigenetic epidemiology and human diseases

Parental contributions of imprinted genes are currently the clearest examples of how human disease may result from epigenetic deregulation [19]. Examples of monogenic epigenetic diseases are congenital syndromes such as Beckwith–Wiedemann or Prader–Willi/Angelman syndromes. These are recognizable syndromes associated with congenital abnormalities and/or developmental delay. These syndromes are due to the consequence of the loss of normal imprinted gene regulation in specific subdomains due to different underlying genetic mechanisms (microdeletion and uniparental disomy) [19].

In common diseases, it has been suggested more recently that each individual has many “epigenomes” and that these may play a central role in disease pathogenenesis [19,20]. Although there is some evidence of epigenetic change influenced by the environment [21,22], such as smoking in humans [23], it remains largely unknown as to precisely how the environment triggers alterations in the epigenome leading to disease susceptibility [24]. There is also accumulating evidence for genetic influence on DNAm [25–30], which may mediate disease susceptibility.

Table 1
Disease concordance rate within MZ twin pairs and heritability estimates—the proportion of phenotypic variance attributed to additive genetic factors—in several rheumatic diseases based on twin studies

Disease/trait	MZ concordance rate (%)	Heritability estimates (%)	References
RA	12–15	65	[31]
SLE	24–56	69	[76,77]
OA	31–50	≈ 50	[78–80]
Osteoporotic fractures	13–19	27	[81]
Hyperuricemia	53	49	[74]
Gout	42	0[a]	[74]
Psoriasis	4–32	66	[82]
Psoriatic arthritis	10	–	[83]
Ankylosing spondylitis	40–75	94	[84,85]

RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; OA = osteoarthritis.
[a] 95% Confidence interval = 0–61.8%.

Disease modeling using concordance rate between twin pairs or family members helps tease apart the respective contributions of genetics and environment in disease variance. Heritability is an estimate of how much of the variance of a trait may be attributed to genetic factors. Despite known heritable influence in the majority of rheumatic diseases (Table 1), concordance rates between identical (MZ) twin pairs are frequently surprisingly low (e.g., 12–15% in rheumatoid arthritis) [31]. This suggests that a variety of genetic and non-genetic factors as well as their complex interactions contribute to disease susceptibility. Among these, epigenetics has recently emerged as a potential explanatory element as signals vary within MZ pairs [32]. This hypothesis is for example sustained for many rheumatic diseases by the observation of gender bias, geographic differential distribution, remission and relapse as well as age-related prevalence but also decline of clinical symptoms with age, all of which are considered good epidemiological, clinical, and etiopathogenic indicators of epigenetic properties [20].

EWAS

Epigenome-wide association studies (EWAS), which is related to the better known genome-wide association study (GWAS), aim to identify epigenetic marks associated with disease by performing a hypothesis-free testing across the whole genome [33]. Before discussing the application of EWAS in rheumatic diseases, it is important to consider the current methodological aspects to allow interpretation of the results obtained so far [34]. (Fig.) depicts a flowchart on how EWAS are carried out and the following paragraphs discuss the different points in more details.

Research question

Results of epigenetic studies provide insight into disease mechanisms or potential biomarkers. Indeed, although challenging to detect [35], in contrast to genetic polymorphisms, plastic DNAm properties may help identify much-needed biomarkers for disease prognosis, monitoring progression, or predicting response to treatment with potential direct translation into the clinics. Comparison of patients with positive specific autoimmune antibodies but free of clinical symptoms versus clinically affected or in responders versus non-responders for a specific medication may help identify such biomarkers.