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DNA Methylation in Stroke. Update of Latest Advances

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ABSTRACT

Epigenetic modifications are hereditable and modifiable factors that do not alter the DNA sequence. These epigenetic factors include DNA methylation, acetylation of histones and non-coding RNAs. Epigenetic factors have mainly been associated with cancer but also with other diseases and conditions such as diabetes or obesity. In addition, epigenetic modifications could play an important role in cardiovascular diseases, including stroke. We review the latest advances in stroke epigenetics, focusing on DNA methylation studies and the future perspectives in this field.

1. Introduction

Stroke is the 2nd leading cause of death worldwide. It is also the leading cause of disability in adults, with 20% of stroke survivors needing help to walk and being dependent on others to perform daily tasks. Stroke survivors therefore require significant social and healthcare resources [1].

In the elderly population of 15 European countries during the year 2000, estimates showed 2,700,000 recurrent stroke cases, and 536,000 incident stroke cases per year [2]. The total number of deaths due to stroke in the total European Union (EU) members is estimated at 508,000/year. Given that age is one of most important risk factors for stroke, the aging of the world population implies a growing number of people at risk. An international comparison of stroke cost studies showed that 0.27% of domestic product was spent on stroke care by national health systems, and stroke care accounted for approx. 3% of total healthcare expenditure [3].

1.1. Stroke Recurrence

The risk of ischemic stroke recurrence after a first stroke is high, especially in the early stages, being around 6–12% within the first year of the initial stroke [4]. Moreover, stroke patients also have a high risk of developing other vascular diseases such as acute myocardial infarction and vascular death. Data suggest that within 10 years of having an ischemic stroke or Transient Ischemic Attack (TIA), around 60% of patients will die and 54% will experience a new vascular event.

1.2. Stroke Functional Outcome

The variability in functional status and neurological outcome after stroke can be influenced by many factors, including age, haemorrhagic transformation (HT), infarct size and location, or the efficiency of revascularisation (by thrombolytic drugs or mechanical thrombectomy) [5,6]. Results from previous studies suggest that basal glucose, age, hypertension and arterial revascularisation success accounted for 33% of the variability in neurological outcome during the acute phase of stroke (at 24 h). However, 57% of the neurological variability remains unexplained. In addition, 25% of neurological outcome could be explained by common polymorphisms or single nucleotide polymorphisms (SNPs) [7].

1.3. Stroke Genetics

Stroke is influenced by genetic risk factors. These genetic risk factors can affect stroke occurrence, acute outcome, long-term outcome and vascular recurrence, among others. Interestingly, different molecular

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pathways modulate these processes, suggesting that different genetic risk factors influence these processes.

Different Genome Wide Association studies (GWAs) have been performed in ischemic stroke and different loci have been associated with the risk of suffering an ischemic stroke. Specifically, 3 genes have been associated with atherothrombotic stroke subtype (HDAC9, CDKN (locus 9p21) and TSPAN2), 2 genes with cardioembolic stroke subtype (PITX2 and ZFHX3), and 2 genes with young strokes (ABO and MMP12). Other GWAs analyses have found other genes and loci associated with stroke (PRKCH, NINJ and genetic locus 6p21.1), although further studies are needed to confirm those results [8–17].

In relation to stroke recurrence several studies in Asian populations [18,19] associations of *ANRIL* and *NINJ2* polymorphisms with vascular recurrence. Other studies in Caucasian populations found associations between *CRP* and *MGP* polymorphisms and recurrent stroke [4,20]. However, other studies have not observed those associations [21].

Different polymorphisms in the genes MMP2, COX-2, GPII, and TP53 have been associated with post-stroke outcome [22–24], although these results have not been consistently replicated.

1.4. Epigenetics

The risk associated with the genetic background in stroke is in the order of 37.9% [25,26]. However, the genetic risk associated with the variants found to date only account for 5–10% of that genetic risk [25]. Therefore, there are more genes and heritable risk factors associated with stroke that have not yet been discovered. One of these possible heritable changes could be associated with epigenetic modifications.

Epigenetic mechanisms are known to alter gene expression or cellular phenotype [27].

There are three major components of epigenetic modification: a) methylation, b) histone modifications and c) non-coding ribonucleic acid (RNA) interference. Both methylation and histone modifications join hands to provide a dynamic epigenetic code. Along with non-coding RNAs (ncRNAs) and certain interacting proteins, these modifications regulate the transcription process.

1.4.1. Methylation

DNA methylation and modifications in histone proteins are the most intensively studied among the major epigenetic modifications. DNA methylation occurs when a methyl group is added to a cytosine nucleotide that precedes guanines (so-called CpG islands or CpG sites).

A CpG island may be defined as the DNA region of at least 500 base pairs with a CG content of >55% [28]. Methylation of CpG islands is catalysed by a family of enzymes, the DNA methyl transferases (DNMTs). DNMT1 maintains cytosine methylation through mitotic and meiotic cell divisions. Methylation of a CpG island within gene promoters is commonly associated with repressed gene expression, as it impedes the binding of transcription factors.

1.4.2. Other Epigenetic Modifications

Post-translational histone modifications, such as methylation and acetylation of lysine residues on histone tails, affect gene expression mainly by altering chromatin structure [27]. Acetylation is brought about by histone acetyltransferase (HATs) enzymes and deacetylation by histone deacetylases (HDACs) [29,30].

Small non-coding RNAs (sncRNAs) are epigenetic elements (<30 nucleotides) with a post-transcriptional biological function. The major components of the sncRNA family, the microRNAs (miRNAs), generally interact specifically with the 3′ untranslated region of a target mRNA to induce its cleavage and degradation, or via a translational repression of gene expression [31,32].

Epigenetic mechanisms are known to alter gene expression [27]. However, other underlying mechanisms such as genetic variations could modify DNA CpG sites modifying the epigenetic regulation of genes [33]. Knowing these mechanisms could be important in finding new treatments for stroke and other cardiovascular diseases [34].

1.5. Epigenome-Wide Association Studies (EWAS). Technical Aspects of the Methylation Chips

Genome-wide association studies (GWAS) have been powerful tools in the identification of the most common genetic variants associated with a multitude of complex traits including common diseases. In contrast, the systematic assessment of epigenetic variation has lagged behind. Technological advances in high-throughput DNA analysis have facilitated the genome-wide examination of epigenetic modifications, primarily DNA methylation. Epigenome-wide association studies (EWAS) have provided systematic, large-scale association testing with disease phenotypes. The latest EWAS arrays (the Infinium EPIC HumanMethylation BeadChip (Illumina)) can detect the methylation levels of >800,000 CpG sites across the genome.

Numerous diseases, exposures and lifestyle factors have been investigated by EWAS, with several significant associations now identified. However, much like the GWAS studies, EWAS are likely to require large international consortium-based approaches to reach the numbers of subjects, and statistical and scientific rigour, required for robust findings.

1.5.1. Tissue-Specific Methylation

DNA methylation is strongly influenced by the tissue analysed and the environment. In fact, epigenetics is one of the metabolic factors that regulate the different expression pattern of the cells and tissues. Consequently, epigenetic studies should be performed in the key tissue for the disease or the condition. In addition, in the case of blood samples there are different cell types with different DNA methylation pattern. This should be taken into consideration before EWAs analysis in order to normalise the results.

Taking into consideration the role of genetics in the risk of stroke but also the outcome after a stroke, DNA methylation could be associated with the occurrence of stroke, with stroke recurrence and with functional outcome after stroke.

2. DNA Methylation in Stroke

2.1. Methods

An extensive literature search was performed, up to October 2017, on PubMed with the following combination of key words: “Epigenetics and stroke” and “EWAs and stroke”. For the combination of “Epigenetics and stroke” we found 128 papers and 4 for the combination of “EWAs and stroke”. Three papers were common between the two combinations of words (Fig. 1). Two researchers independently check the papers. We selected the papers that 1) were performed using human samples and an EWAs approach, and 2) papers that analysed the global methylation pattern of stroke patients. Finally, eight papers were included in the current revision (Fig. 1).

In addition, we included examples of other diseases or studies in animal models that support the results observed in stroke.

2.2. Stroke DNA Methylation Risk Factor

Epigenetic modifications, specifically DNA methylation, are influenced by environmental factors and are heritable modifications. It has been observed that different levels of DNA methylation are associated with the risk of diseases such as cancer, diabetes, obesity, atherosclerosis or arterial hypertension [35].

Human and mouse studies have observed global DNA hypermethylation of cytosines in CpGs as an accompanying feature of atherosclerosis [35–37]. Indeed, a positive correlation between DNA methylation and atherosclerotic lesion grade was discovered by using genome-