Investigating the Relationship Between Aging and Epigenetic Differences in Monozygotic Twins

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ABSTRACT
When it comes to monozygotic twins, Mario F. Fraga and colleagues' paper "Epigenetic differences arise during the lifetime of monozygotic twins" focuses on epigenetic modifications in monozygotic twins and what causes monozygotic twins to become more distinct as they age. This study is going to compare epigenetic profiles of monozygotic twin siblings of varying ages and genders. This includes DNA methylation as well as histone acetylation patterns in the H3 and H4 histone regions. Even though monozygotic twins were difficult to distinguish in their early lives, it has been observed that the older twins have shown significant differences in histone acetylation and the genomic distribution of some DNAs, which affects their gene expression and, as a result, makes them appear more distinct. One of the most common causes of monozygotic twin discordance is the presence of a specific disease in only one of the twins. As a result, understanding the role that epigenetic factors play in the development of monozygotic twins is critical because it allows us to identify diseases that can be prevented or treated.

Keywords: *Epigenetics, Monozygotic Twins, DNA Methylation, Histone Acetylation.*

1. INTRODUCTION

The term "epigenetics" refers to the study of changes in gene expression that occur without affecting the DNA sequence that was originally present. This change is heritable and reversible, and it can be influenced by a variety of factors including personal habits and environmental factors [1]. So far, DNA methylation and structural alterations in chromatin caused by histone modification are the epigenetic modifications that have received the most research attention thus far in the field of epigenetics [2]. DNA methylation refers to the transfer of a methyl group to the C5 position of a cytosine to produce 5-methylcytosine (5mC), which changes the activity of a specific DNA segment without changing the sequence of that segment. DNA methylation patterns in humans are dynamic during the process of development and may shift constantly throughout a person's lifetime as the DNA methylates and demethylates [3]. Nucleosomes are formed by the wrapping of DNA molecules around histone proteins, which are then folded further to form chromatin structures. Histone modification is defined as the attachment of methyl or acetyl groups to the N-terminal tails of histones, which has an effect on the organisation and structure of chromatin as well as transcriptional activity [4].

As we know, monozygotic twins and dizygotic twins differ by the number of zygotes. Dizygotic twins are born from different zygotes and monozygotic twins are born from the same zygote, which then undergo mitosis separately and go through independent development and births. The genetic makeup of dizygotic twins is totally different, resulting in their appearance being distinct or even having a different gender depending on their parents' genes. Despite the fact that monozygotic twins have identical genetic information, there are still significant differences in the way these genes are expressed between the twins, particularly as the twins grow older. In 2005 during the author's time, other scientists already observed the fact that it is possible for monozygotic twins to develop different diseases [5]. However, the majority of studies have been conducted on the genetics surface, with the epigenetic perspective remaining unexplored [6]. As a result, only a small number of studies have been conducted in order to determine the cause of this phenomenon.

Since it can be concluded that genetics could not account for the observed differences, Fraga's team took a deeper look into the relationship between epigenetic

factors and phenotypic differences in monozygotic twins [7]. The epigenetic profiles of a group of monozygotic twins of various ages and genders are being compared. These profiles include DNA methylation and H3 and H4 histone acetylation patterns and more. Even though monozygotic twins were difficult to distinguish early in life, older twins have revealed significant differences in histone acetylation and the genomic location of specific DNAs, which influences their gene expression and, as a result, makes them appear more dissimilar. The presence of monozygotic twin discordance is closely associated with the presence of a specific disease in only one of the twins. It is therefore critical to understand how epigenetic variables influence the development of monozygotic twins because doing so will allow us to potentially prevent and treat certain disorders [8].

2. METHOD AND RESULTS

One hundred and eighty Caucasian monozygotic twins from Spain (thirty men and fifty women) with ages ranging from three to seventy-four years participated in Fraga's experiment. In order to study and analyse the volunteers, samples of their lymphocyte cells, muscle tissue, epithelial skin cells, and abdominal tissues are collected and analysed in the laboratory. The first step taken by Fraga's team was to confirm that all of the participants were monozygotic. This is accomplished through the use of microsatellite marker analysis, in which DNA and RNA are extracted from lymphocyte cells and subjected to PCR amplification and capillary electrophoresis before being analysed [9].

2.1. Comparison of X chromosome inactivation patterns

The inactivation patterns of the X chromosome in twins are being compared using PCR amplification of the androgen receptor locus after digestion with the DNA methylation restriction enzyme HpaII, which is a DNA methylation restriction enzyme. Only the androgen receptor genes on the inactivated X chromosomes were amplified, allowing Fraga's team to locate and quantify the patterns of X-chromosome inactivation. X-inactivation patterns in female twins should be compared because different skewing of X-chromosome inactivation can be observed for X-linked diseases, so this is an especially important step. The findings revealed that the majority of female monozygotic twins shared the same X chromosome inactivation pattern, which could be skewed or unskewed, according to the findings. X-inactivation patterns that differed between siblings were found in only a small percentage of the population, and examples can be found in all year groups. This demonstrates that differences in X-inactivation patterns are not related to age and, as a result, suggests that epigenetic differences can develop at a very young age as well.

2.2. Comparison of 5mC DNA content and H3 and H4 histone acetylation levels

The acetylation of histone H3 and H4 at the global level was then measured and compared between monozygotic twins. This is accomplished by extracting fragments of H3 and H4 histones from the nuclei of cells, which are then purified using high-performance liquid chromatography and resolved using capillary electrophoresis, respectively. Total DNA methylation can also be determined by capillary electrophoresis, which measures the amounts of cytosine and 5mC present in a DNA sample. The results revealed that the majority of the volunteers had nearly identical 5mC genomic content and histone acetylation levels for H3 and H4 histones as one another. In 35% of the volunteers, all three epigenetic measurements revealed significant discordance between twin pairs across all three measurements. It has been discovered that there is a strong correlation between age and discordance in 5mC genomic content and H3 H4 histone acetylation levels. Additionally, according to the results of questionnaires completed by volunteers, monozygotic twins who spent less of their time together tend to be more different in terms of 5mC genomic content and H3 H4 histone acetylation levels than their more closely related counterparts.

However, it is still possible that the epigenetic changes observed in the monozygotic twin pairs were simply the result of an increase in epigenetic variability in older populations, rather than a combination of both factors. As a result, Fraga's team used ANOVA to rule out the possibility of this happening. To begin, they divided the subjects into two age categories: young and elderly. This allowed them to observe the individual descriptive values of the subjects and compare them to the rest of the subjects. They then compared the ESD (Euclidean Squared Distance) values, which is a measure of heterogeneity within twins and is a measure of genetic variation. In terms of epigenetic variability, the researchers discovered that no matter what age group the individuals belonged to, the descriptive values remained consistently high throughout the study. The difference in ESD between older twin pairs, on the other hand, is significantly greater than the difference between younger twin pairs. Further confirmation came from the ANOVA analysis, which revealed that older twins epigenetically differed more from younger twins. It did, however, demonstrate that this variation is not associated with a generally greater difference in descriptive epigenetic values in the older population, as previously thought.

2.3. Identification of DNA methylation differences

The location in the genomes of monozygotic twins where epigenetic differences arose was then investigated

by Fraga's research team. This is accomplished through the use of amplification of intermethylated sites (AIMS), which produces a methylation fingerprint consisting of bands of DNA fragments that are amplified by polymerase chain reaction (PCR). Using gels, researchers were able to resolve the AIMS bands and observe that the bands appeared differently in different twin pairs. A correlation exists between the number of different AIMS bands and both age and 5mC genomic content and histone acetylation levels, indicating that differences in these epigenetic measurements are all closely associated with increasing age.

For further investigation, Fraga's team selected a set of AIMS bands that were found to be significantly different between the twin pairs. These bands were then cloned and compared to a number of different databases. According to their findings, 43 percent of the clones corresponded to Alu sequences, 34 percent corresponded to ESTs (expressed sequence tags), 13 percent corresponded to single copies of genes, and 9 percent corresponded to other sequences. Using bisulphite genomic sequencing, Fraga's team was able to demonstrate that these sequences have variations in DNA methylation patterns in the twins. It has been demonstrated that the three-year-old twins have very similar methylation status on each CpG dinucleotide in the Alu sequences and single-copy genes that have been chosen. Nevertheless, in a pair of 50-year-old twins, one of the siblings had significant amounts of hypermethylation on the CpG dinucleotide, whereas the other sibling had significant amounts of hypomethylation instead. For both Alus and single-copy genes, the researchers noted that variations in DNA methylation were strongly associated with altered expression of a specific region in the DNA.

2.4. Chromosomal regions with DNA methylation differences

Chromosomes 1, 3, 12 and 17 were all being studied at the time, and DNA methylation differences were being discovered in their respective chromosome regions. This is accomplished by hybridising the AIMS PCR products of one of the twins with the AIMS PCR products of the other twin in a competitive manner. This has demonstrated that twin pairs with greater differences in 5mC DNA content, histone H3 and H4 acetylation levels, and DNA methylation patterns also tend to have more regions on their chromosomes with distinct profiles of DNA methylation signals than those with fewer differences. In a similar vein to the previous measures, twins who are younger and spend more time together have a smaller number of chromosome regions with DNA methylation differences than twins who are older and spend less time with each other. However, older twins who had lived different lifestyles and spent less time together had more chromosome regions with DNA methylation differences than their younger counterparts.

2.5. Portraits of gene expression

At the end of the study, Fraga's team looked into the impact that previously investigated epigenetic factors had on the actual expression of genes. Extracting RNA from the two most distinct twin pairs and performing gene expression microarray analysis are the methods used to accomplish this. While the younger twin pair had very similar gene expression profiles, with nearly the same number of overexpressed and repressed genes, the older twin pair had significantly different gene expression profiles.

Overall, Fraga's investigation has established that discordance in epigenetic measurements between monozygotic twins is strongly correlated with increasing age, which is consistent with previous findings. The patterns of DNA methylation and the levels of H3 and H4 histone acetylation are also demonstrated to be mechanically connected, as demonstrated by the strong link demonstrated in the results.

3. DISCUSSION

Based on Fraga's findings, it is concluded that epigenetic markers such as DNA methylation and H3 and H4 histone acetylation have a significant impact on the expression of specific genes, affecting phenotypes and explaining why some monozygotic twins appear different and develop different diseases despite having the same genetic makeup. Individual behaviours, such as diet, smoking habits, and medical histories, can have a significant impact on differences in DNA methylation and histone acetylation at the H3 and H4 histone acetyltransferases loci [10]. To wit, twins who live in different places and spend less time together tend to have more distinct epigenetic profiles than twins who live in the same place and spend more time together, as the authors point out. A further finding of the study was that as monozygotic twin pairs get older, the twin pairs' epigenetic changes become more and more dissimilar from one another [11]. Another point raised by the authors is that accumulated errors in the process of transmitting epigenetic information throughout the process of ageing. Also it known as "epigenetic drift," may be responsible for phenotype changes that occur as people grow older, as previously stated.

In this experiment, only Caucasians from Spain were used as test subjects, and they were all from the same ethnic group. It was unable to demonstrate the impact that race and ethnicity may have on epigenetic differences between individuals as a result of this, despite the fact that it helped to maintain consistency in the experimental results. In order to improve the reliability of the investigation's results, Fraga's team will expand

the number of ethnicities participating in the experiment and examine whether or not results differ between races.

4. CONCLUSION

Fraga's study published in Nature Genetics demonstrates that differences in epigenetic factors such as DNA methylation and histone modification patterns between monozygotic twins are directly associated with the discordance. Because they have different lifestyles, living environments, and habits, it is possible that these differences can be explained by these differences.

However, there are some limitations to Fraga's paper that must be considered. It has been demonstrated in the findings that there is discordance in the skewness of X-inactivation patterns across a variety of different age groups. Because all of the other epigenetic markers tested in the experiment showed a close relationship with increasing age, the authors did not provide enough potential explanations in the discussion section to explain why the X-inactivation patterns were independent of age, which was disappointing. X chromosome inactivation patterns and their relationships with other epigenetic marks may become the focus of future research. A deeper understanding of the concept of epigenetic drift is also required, particularly in terms of the mechanisms that human bodies employ to counteract accumulated errors in the transmission of epigenetic information to the next generation. As a result, correcting these epigenetic errors may prove beneficial in the prevention and treatment of specific diseases.

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