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The Emerging Field of Forensic Epigenetics

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DNA profiling characterises small sections of the genome referred to as Short Tandem Repeats. This DNA profile is robust and stable in that the DNA profile will not change over the lifetime of an individual and is in general the same DNA profile regardless of what body fluid is sampled (except in cases of rare chimerism). The value of DNA profiling is undeniably immense and with increasing sensitivity and specificity it is far and beyond the gold standard of forensic science. However, the amount of investment and research has advanced the field to the extent that significant capability gaps are existing elsewhere. For example, the sensitivity of DNA profiling is greater than that of traditional body fluid identification (BFID) testing; which means that DNA profiles are being used as evidence without understanding which body fluid or tissue it came from. For example, not being able to say that the DNA profile was obtained from the semen can be detrimental to resolving rape cases. There is a lot of effort exploring much more sensitive BFID techniques, some of which are through genetic strategies. These involve characterising messenger RNA, microRNA and DNA methylation. Both miRNA and DNA methylation relate to the biological process called epigenetics. Epigenetics refers to a second layer of information found within the genome and is mainly responsible for the regulation and control of the flow of information from the genome to the proteome. For example, the DNA in each of the nucleated cells in the body are the same. Which means that the genes for blood is present in every cell, so why are we not producing such blood-related

proteins from each cell. Simply put, the blood genes are turned off via a 'genetic switch' or DNA methylation. MicroRNA is a post-transcriptional, pre-translation regulator of protein synthesis in the intracellular environment, thus has a strong role in regulating the production of such proteins. Therefore, if a cell produced a blood-specific protein, then there must be a blood-specific miRNA molecule.

Both of these areas, particularly DNA methylation are making significant grounds in research – coming to the point where there is a sub-discipline of Forensic Epigenetics. This would allow the investigator to go beyond human identification and provide additional information from a crime scene stain and offer a more robust evaluation in a court of law.

Body Fluid Identification

Potentially the first application of forensic epigenetics was to the question of the body fluid identity. Working on the principle that for a body fluid protein to be expressed, the relevant gene must be 'switched on', the technique is fundamentally identifying and characterising tDMRs or Tissue Differentially Methylated Regions (1) as well as the use of microRNA (2) that should be present to control the production of body fluid specific proteins.

Differentiating between monozygotic twins

Monozygotic twins have the same DNA profile, but it has been shown that such twins can have differing epigenomes, due to their environmental sensitivity (3). Thus the epigenetic profile can be used to differentiate between the two. Preliminary research so far has indicated that the age of the twin can be critical in whether this is successful or not as well as whether they were

raised in suitably different environments. For example, if one takes up smoking and the other one does not.

Age Prediction

One of the intelligence aspect of the forensic sciences is the ability to form an opinion on the age of an individual, both alive and deceased. This information not only provides intelligence pertaining to the identity of a deceased individual, it can also provide intelligence regarding the offender, where there is no DNA profile on a database. In addition to the criminal element, there is also a civil application in the case of child refugees or asylum seekers, where adults may claim to be a child in order to obtain favourable treatment. As an individual ages, their epigenome changes, due to environmental exposure. A study by Vidaki *et al* (4) showed that this change is relatively predictable. Of the various techniques proposed to predict the age of an adult individual, this one is the most accurate so far.

Challenges in forensic epigenetics

One of the perceived advantages of targeting DNA methylation patterns is that the DNA molecule is relatively stable; however, the same principle may not necessarily apply to the secondary structure of the DNA methylation – especially when it is known to be environmentally influenced. Taking in to consideration that crime scene stains can be exposed to a wide range of different environment, it is crucial to understand if the epigenome may drift over time. Not only in crime scene stains, but also in individuals. Garnering an effective understanding of how the epigenome is influenced environmentally is crucial.

One of the key issues relating to DNA profile is the one of body fluid concordancy, where by the same DNA profile can be obtained from different body fluids and tissues. Currently, unless the test is specifically for tDMRs, it is not known if the epigenetic profile would be the same

regardless of tissue type. This is an area that could be a real stumbling block if the epigenome from a suspect reference cannot be compared with, for example a semen stain from a rape case. In this situation, a semen sample would need to be obtained from the suspect, which raises legal and ethical issues.

A significant challenge is identifying the most appropriate way to characterise the epigenome.

Characterising the epigenome

Currently, most research in to this area is utilising Next Generation Sequencing (NGS) including pyrosequencing to analyse the product, which is essential in order to gain a fundamental understanding of the biology. However, this can be very expensive now and is in general beyond the budget of police forces and state laboratories. In addition, such techniques are also beyond the technical capability of most forensic laboratories, so there is a real need to develop more cost-effective techniques. Stewart *et al* (3) proposed using High Resolution Melt Curve Analysis.

Not only is the end-point analysis is widely varying, there are various upstream processes which can significantly influence the use and viability of the product. For example, one crucial step utilised is bisulphite treatment. This is a process where unmethylated region undergo conversion to physically change the sequence. The sequence can then be characterised – and the extent of the difference between the treated and untreated samples represents the difference in the epigenome. However, for bisulphite treatment to be effective, there needs to be a large sample - generally larger than that recovered from crime scene stains or even from reference samples. In general, there is a great deal of exploratory work required before this can be used in casework.

The field of forensic epigenetics is a very promising field and is one that is currently in its infancy. With a bit of effort, this could significantly enhance the capability of the justice systems to achieve correct outcomes.

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