Age Estimation Science Advisory Committee

AESAC

Interim report on the scientific feasibility of using DNA methylation to assist in assessing the age of unaccompanied asylum-seeking children

May 2024

Contents

1. Executive Summary

1.1. The interim Age Estimation Science Advisory Committee (AESAC) produced a report in 2022 on biological methods of assessing the age of unaccompanied asylum-seeking children.

1.2. That report focused largely on methods of age assessment based on radiological and magnetic resonance imaging (MRI). However, it was recommended that further work be conducted on determining whether emerging age estimation methods, including but not restricted to DNA methylation, might offer a suitable alternative means of assessing age.

1.3. DNA methylation is an epigenetic process whereby molecules, separate from those that dictate the genetic sequence, can become attached to an individual's DNA. This methylation occurs at certain locations in the genome, known as CpG sites, with some of these demonstrating a strong correlation with chronological age.

1.4. These identified methylation sites are the key to estimating chronological age via DNA methylation. If the right selection of methylation sites are incorporated into a model, then it may be possible to estimate the age of an individual within an agreed level of accuracy.

1.5. However, this process is not straightforward. Factors such as the sample tissue type, the demographics of the population being considered, and the environmental conditions they have been exposed to may lead to significant methylation variation. Methylation sites should be carefully selected to minimise these so as to produce an accurate model.

1.6. Most research on using DNA methylation to estimate chronological age has, to date, been conducted on largely white populations in Western Europe and North America. This is not representative of the asylum-seeking population which has different demographics and variable exposure to environmental factors such as heightened stress.

1.7. AESAC recognises that DNA methylation is a viable method of age assessment and acknowledges its potential advantages over current methods of age assessment. However, further research is needed to assess the feasibility of applying it to the age assessment of unaccompanied asylum-seeking children.

1.8. As such, the primary recommendation of this report is for the Home Office to commission a research study that would allow the collection of an appropriate DNA methylation dataset representative of the current asylum-seeking population. Analysis of this dataset should then be tested to determine its efficacy and reliability in the chronological age estimation of unaccompanied asylum-seeking children.

1.9. Only once relevant research has been conducted will AESAC be able to evaluate whether DNA methylation is suitable for assessing the age of unaccompanied asylum-seeking children.

2. Introduction

2.1. AESAC was commissioned to provide advice on whether it would be scientifically feasible to use **DNA methylation** to support the **chronological age** estimation of unaccompanied asylum-seeking children.

2.2. This commission came as a response to Recommendation 14 of the Interim AESAC's 2022 report *Biological methods to assess the age of unaccompanied asylum-seeking children's age* that "*a watching brief should be maintained over the development of emerging age estimation methods including, but not restricted to, facial images and DNA methylation*" [1].

2.3. This report focuses solely on the question of whether the use of DNA methylation could add value to the age estimation of unaccompanied asylum-seeking children, and what further research may be needed for this to be the case. It is acknowledged that, if deemed to add value, this approach could be used in conjunction with Merton-compliant assessments and the likelihood ratio approach outlined in the interim AESAC's report.

2.4. This report begins by reviewing the science underpinning DNA methylation, and how DNA methylation has the potential to predict the chronological age of an individual. The report then examines the scientific rigour and feasibility of using DNA methylation in this way. Finally, the report concludes with AESAC's findings on this matter and recommendations for future work.

2.5. Some aspects of this report focus on relatively technical aspects of DNA methylation. To maintain accessibility, a glossary of key terms is included as Annex A. In addition, the first time each term from the glossary appears in the text, this is highlighted in **bold** to notify the reader that further detail can be found in the glossary.

3. Background

3.1. The Interim AESAC produced a report in 2022 that sought to advise on scientific methods of age assessment that could be deployed within a 12 - 18-month time window in support of the existing Merton-compliant process.

3.2. The recommendations focused predominantly on the use of radiological imaging and MRI to assess skeletal development, and radiological imaging to assess dental development, as methods of age assessment. These methods have already been used internationally in both immigration and criminal justice settings [2] [3].

3.3. The report recognised that the use of radiological imaging in this way presented a risk in the form of ionising radiation.

3.4. As a result, the interim AESAC recommended the Home Office maintain a watching brief over other technologies which may have the potential to compliment or supersede radiological imaging going forward.

3.5. One such methodology that was recommended for monitoring was DNA methylation. With further research and development, DNA methylation was identified as offering the potential to be useful as a non-ionising method of age estimation in the medium-long term.

4. DNA Methylation

4.1. DNA methylation can be defined as a chemical change to the structure of a component of DNA (known as a **nucleotide**) whereby a **methyl group** (a molecule of one carbon and three hydrogen atoms) is added (Figure 1) [4]. The process of DNA methylation does not alter the overall DNA sequence/genetic code, it is an **epigenetic** change, meaning that the expression of a **gene** is changed, while the genetic code itself is not.

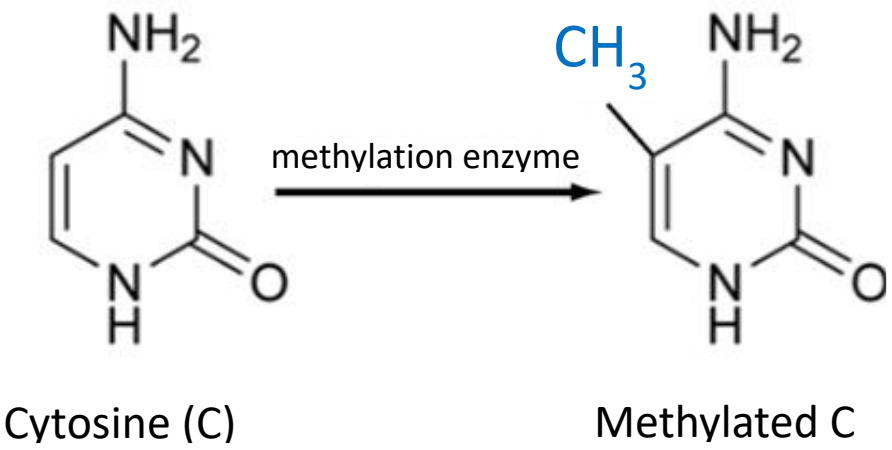

Figure 1: A cytosine molecule (one of the DNA nucleotides) is shown on the left, and its methylated (CH_3) version on the right.

4.2. Within the human genome nucleotides are represented by four molecules (adenine - A, **cytosine** - C, **guanine** – G, and thymine - T). The particular order of these molecules on a DNA strand provide instructions to make proteins. Methylation, in humans, occurs predominantly when a C is followed by a G in the nucleotide sequence of the 'forward' DNA strand.

These are referred to as CpG dinucleotides (where the p refers to the phosphate group in the DNA backbone that separates the nucleotides). Dispersed across the genome, normally most CpGs are methylated. This is shown in Figure 2, where three of the four **CpG sites** are methylated in Figure 2B.

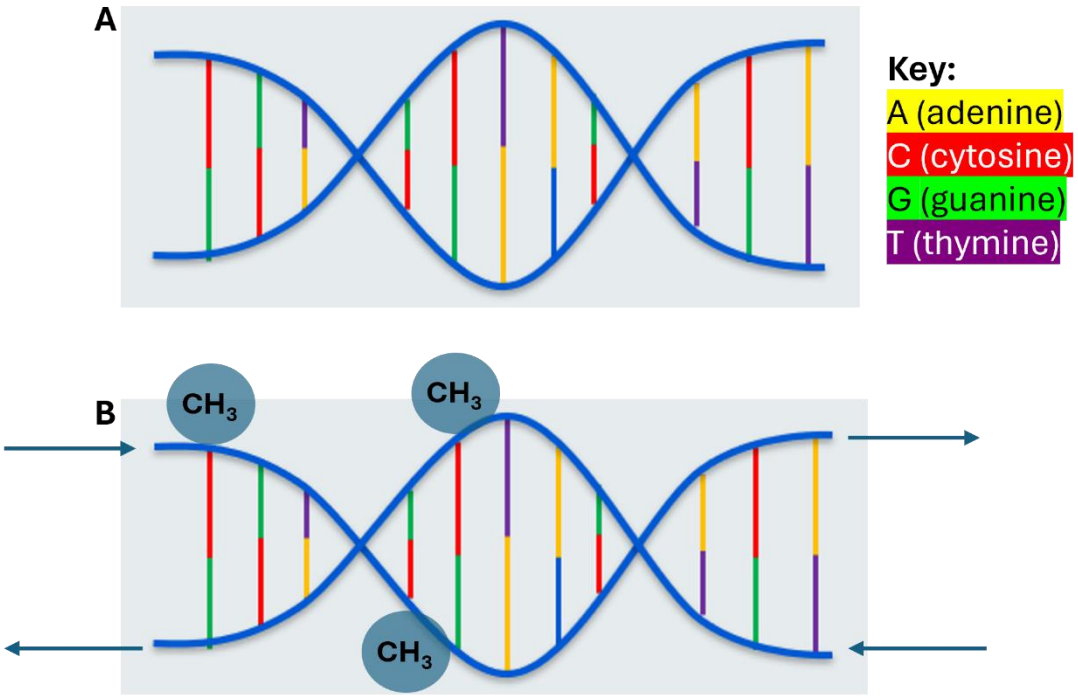

Figure 2A: The DNA double helix molecule with its sugar-phosphate backbone (blue) and the nucleotides (coloured) that join the strands.

Figure 2B: A cytosine (C) nucleotide in the direction of the arrows may or may not be methylated, but can only be if followed by a guanine (G) in the sequence.

4.3. The distribution of CpGs is different in areas of the DNA molecule that regulate whether a gene is expressed [5]. Here, they tend to gather in clusters known as CpG islands where the Cs are mostly unmethylated [6]. CpG islands are found in most human genes, promoting **transcription** of the associated sequence into a protein.

4.4. Areas of methylation throughout the genome can therefore allow or prevent transcription, and can also vary over time. Breakdown of these controls is thought to be a key process in the development of cancer [7], for example, where the CpG islands within a **gene promoter** become methylated, and there is a general loss of methylation elsewhere in the genome that destabilises many genetic mechanisms.

4.5. The amount of methylation in an individual's genome is not fixed, it varies throughout an individual's lifetime as a result of environmental and inherited factors.

4.6. While aberrant DNA methylation is associated with many common diseases, there is also an association between methylation and chronological age. Whole genome estimates of methylation provide a measure of **biological age** and the health of an individual. Separating out subsets of CpG sites that are not disease-associated from those that are will limit the observed correlation to that associated with chronological age.

4.7. CpG sites that display a strong correlation with age form the basis of an epigenetic clock that will estimate chronological age. The original clock described by Horvath was developed from a selection of CpG markers associated with age-related genes across all tissue types [8]. Since then, 'clocks' have developed and improved in their ability to predict chronological age through the selection of CpG sites that are more strongly associated with age and are as independent as possible of other methylation-associated factors that increase the error of chronological age prediction [9] [10] [11].

4.8. It is important to note that the epigenetic clock is different from epigenetic drift. Epigenetic drift refers to changes over time in the total volume of DNA methylation within an individual's genome, but not all methylation sites are strongly associated with age, so the level of epigenetic drift experienced by different people of the same age will vary. In contrast, the epigenetic clock refers to DNA methylation levels at a defined set of CpG sites which are thought to correlate with age across a population of individuals. Limiting the clock to estimation of age in a specific tissue can also reduce error.

4.9. The key to unlocking an appropriate epigenetic clock for age estimation is identifying a suitable subset of CpG sites within a specific tissue that can be reliably expected to have a strong association with chronological age across a large population of individuals.

5. Developing a Model to Estimate Age Using DNA Methylation

5.1. Identifying a set of CpG sites correlated with chronological age is not straightforward. Information to select suitable CpG sites generally relies on research undertaken in clinical medicine, making use of control sets. Research is often carried out using DNA extracted from blood samples, but data from DNA extracted from saliva or **buccal cells** is more limited. Once age-associated CpG sites have been chosen and validated, the markers must also be shown to be strong predictors when analysed together in a multiplex.

5.2. Methylation levels at each site are affected by factors such as (but not limited to) tissue type [12], stress levels, and smoking habits [13]. Protected characteristics including sex and ethnicity may also have an impact. Stress

may produce epigenetic changes that, if ongoing, may also be inherited down generations [14] [15]. These factors must all be considered and, where possible, built into prediction models.

5.3. While it is acknowledged that the above factors all have an impact on methylation levels, the extent of these remains the topic of current research. Avoiding the selection of CpG sites that are associated with genes involved in the hypothalamic-pituitary-adrenal (HPA) axis, a physiological system of the body important in stress response, would be an obvious exclusion criterion when selecting markers to analyse for age assessment purposes, for example.

5.4. Environmental impacts that may change methylation status, whether a history of smoking [16], or significant exposure to toxic fumes in an environmental disaster [17], for example, are difficult to control for. Research to create models to overcome or account for the various environmental impacts will require significant effort.

5.5. Examples of different models produced through varying marker selection and number, and/or the statistical approach, can be seen in the existing literature with different studies showing varying degrees of success in estimating age.

5.6. *McEwen et al. (2019)* demonstrated the use of a model based on 94 marker sites that was able to predict age in a test sample of individuals aged 0-20 years with a **median absolute deviation** of 0.35 years [9]. This reflects the reduced error that can be achieved when models are built in younger age groups, although this error grows with increasing chronological age.

5.7. This study indicates some promise in accurately estimating age using DNA methylation, however there are some caveats which must be noted.

5.8. The McEwen model was helpfully developed using samples taken from buccal cells which is a less invasive sample type than blood, but different markers could be needed if samples were taken from other cellular sources (see section 6). In addition, in the reported research, no consideration was given to the ethnicity of the participants or to environmental factors they had been exposed to, such as smoking or stress levels. It would be imperative for models that are proposed for use in young people seeking asylum to have considered these, and other potentially relevant variables.

6. Sample Type

6.1. Methylation levels vary depending on the tissue type being sampled such that, even when considering the same individual and looking at identical CpG sites, the proportion of DNA methylation seen will vary based on the tissue the DNA sample is taken from, whether blood, buccal cells or saliva.

6.2. Much of the existing research has been undertaken using DNA collected from blood samples, however collecting blood is invasive and issues may arise concerning consent. As a result, AESAC advise against the use of blood samples and instead recommend a model is developed using buccal or saliva cells.

6.3. Although the use of buccal or saliva cells is more acceptable, there is considerably less research available identifying the best CpG sites to build into a model.

6.4. Further research therefore needs to be conducted to determine whether it is possible to develop a sufficiently reliable model that can be used to estimate age in this setting using samples taken from buccal or saliva samples.

7. Protected Characteristics and Environmental Factors

7.1. It is not known whether or not protected characteristics including sex, gender reassignment, sexual orientation, and ethnicity may result in DNA methylation level differences. The recognition of environmental factors such as stress and smoking habits on age estimation have also been discussed in section 5.

7.2. People seeking asylum and refugees experience a multiplicity of stresses associated with political and structural disadvantages from their country of origin, and this will be exacerbated by their actions in trying to escape these [18].

7.3. The impact of different life stressors (behavioural, emotional, mental or physical) on epigenetic age determination may be significant [19], but their exact significance is currently unclear. It is vital that further research is conducted to investigate these important uncertainties. Markers that show a strong association with these variables should be excluded before a method is employed to estimate the age of people seeking asylum and refugees [20] [21].

7.4. The importance of assessing the impact of lifestyle and stressors on asylum-seeking populations is particularly acute because they bear little resemblance to the population samples used in most academic studies. AESAC anecdotally note that these groups likely also have experienced higher levels of smoking, and more trauma, including **intergenerational trauma**, compared to a sample of individuals taken from the UK.

7.5. Further work is needed to determine these effects, which will necessitate working with a population sample representative of the asylum-seeking population.

7.6. AESAC is very aware of the difficulties that are likely to be met if seeking ethical approval for such a study; yet not attempting to address the impact of

such a population on scientific age assessment can only undermine an equitable assessment outcome.

7.7. Any such work involving children or refugees needs to be conducted in line with strong ethical principles, including consideration of the World Medical Association's Declaration of Helsinki [22].

7.8. Investigations conducted among refugees require additional diligence to ensure respect for and welfare of the participants [23]. Significant structural, institutional, bureaucratic, and ethical barriers to conducting research in refugees have been identified [24]. These include, but are not limited to, concerns around power hierarchies impacting on a person's ability to consent to participation [25].

7.9. Refugees are likely to be classed as "participants at risk" and/ or "people whose ability to give free and informed consent is in question" [26]. Therefore, research involving refugees normally requires a significantly higher level of application scrutiny before approval.

7.10. Research involving unaccompanied asylum-seeking children must consist of methodology that proactively acknowledges and accommodates for the experience and impact of trauma [27].

8. Further Requirements of a DNA Methylation Model to Estimate Age

8.1. The cost of developing a method that uses DNA methylation to estimate age should also be considered. Even if scientifically feasible, DNA methylation will need to be implemented in a way that is financially viable.

8.2. The turnaround time for a result also needs to be considered. Current reporting times are between one or two weeks, although this will depend on sample throughput.

8.3. Current technology to estimate the amount of methylation involves chemical manipulation of the sample with bisulphite treatment to change the DNA sequence of unmethylated residues that enables differential sequencing to estimate the amount of methylation [28]. However, this chemical is destructive of DNA and methods are being developed to avoid this processing. Being able to target and enrich selected areas of the genome (adaptive sequencing) could also lead to improved precision in the estimation. It would also considerably reduce the requirement for large quantities of DNA, offering hope in the future for methods to be developed for use in hand-held sequencers, such as the Oxford Nanopore MinION, enabling direct measurement on site [29]. Considerable research efforts will be needed to bring this to reality.

8.4. Given the assumption that the use of DNA methylation would be done in conjunction with a likelihood ratio approach involving other methods of scientific age estimation and a Merton assessment, it is likely to only offer a benefit if a model of sufficient accuracy can be proven.

8.5. An age range of application that extends up to 30 years would be more suitable than having an upper limit of 20 years as applied in the model developed by McEwen in order to address ages across the 18-year-old threshold.

8.6. Many of the publications on age estimation report median absolute deviation levels to illustrate the accuracy of the prediction. The value is the average distance between each data point and the mean in a data set, however there will be individual estimates outside that range. This can be appreciated when looking at Figure 1 in *McEwen et al. (2020)* where the median absolute deviation is given as 0.35 [9]. The dataset has almost 2,000 datapoints, and many will give a predicted age close to the given age, but the plot reveals a proportion that fall between 1 and 2 years away from the predicted, highlighting the importance of having a very low median absolute deviation. It is anticipated that such a model would only be beneficial if a median absolute deviation of less than 1 year could be achieved.

9. Findings

9.1. AESAC has reviewed current research and applications of this emerging field of DNA methylation in the age assessment process.

9.2. At the time of writing, AESAC advise against the use of DNA methylation for this purpose.

9.3. There are significant gaps in current research which fail to investigate the effects of ethnicity, stress, and sample type on the predicted age of an individual. It is critical that these effects are understood before DNA methylation can be considered for use in the age assessment process for unaccompanied asylum-seeking children.

9.4. AESAC recognise that there are existing studies which indicate that age prediction using DNA methylation may hold some promise in being able to predict age, especially given the method is non-ionising and may be significantly less invasive than radiological imaging or MRI.

9.5. As a result, AESAC recommend the commissioning of a study to look at the impact of variables present in different population groups seeking access to the UK on estimates of DNA methylation.

9.6. There will be significant challenges, both ethical and methodological, to conducting such research. These will need to be carefully considered to

meet the necessary requirements for robust age assessment in a group of individuals seeking asylum.

9.7. AESAC recommend that this research be commissioned by the Home Office to ensure that the necessary specifics and requirements are met to determine the effectiveness of using DNA methylation for age assessment purposes.

9.8. AESAC will not be able to give further advice as to whether DNA methylation could be usefully operationalised as a method of scientific age estimation until such research has taken place.

10. Conclusion

10.1. AESAC was asked to review the feasibility of using DNA methylation as a method of assessing the ages of unaccompanied asylum-seeking children.

10.2. AESAC recognise that DNA methylation is a relatively new method of assessing age and remains the subject of much research and debate at present.

10.3. AESAC acknowledge that DNA methylation offers the promise of being less invasive and presenting a lower risk to individuals when compared to radiological and MRI methods.

10.4. In addition, there is preliminary evidence to suggest that DNA methylation may be able to provide the foundations of a reliable and effective method of age assessment.

10.5. However, it is vital to note that this technology is still maturing and there is currently a significant research gap that exists around using DNA methylation to accurately assess the age of the asylum-seeking population.

10.6. There is evidence that demographic and environmental factors such as age, sex, ethnicity, stress levels, and smoking habits can all have an effect on age estimates made using DNA methylation.

10.7. Consequently, AESAC recommend against the use of DNA methylation in the age assessment process at present.

10.8. AESAC instead recommend that the Home Office commission a full study to determine a DNA methylation model of marker sites that could be used in the asylum-seeking population, and conduct rigorous testing to determine the accuracy and reliability of this. Any such study must comply with established standards of ethical approval in vulnerable populations and maintain a trauma-informed approach at all times.

10.9. Only once this work has been completed will AESAC be in a position to determine whether DNA methylation is suitable to be used as a part of the age assessment process.

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12. Authors

This report has been authored by a working group of the Age Estimation Science Advisory Committee (AESAC) which is an independent expert committee established to advise the Home Office Chief Scientific Adviser on biological methods of age estimation.

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The report was ratified by all members of the Age Estimation Science Advisory Committee (membership at Annex B).

Acknowledgement is also given to the AESAC Secretariat, in particular Oliver Coughlan who provided dedicated support and administration with this piece of work.

Annex A - Glossary

Biological age	The physiological state of a person's cells, tissues and organs which can be affected by factors such as disease, stress, health, and genetics. A measure of lifespan of an individual that is influenced by genetic, lifestyle, and environmental factors.
Buccal cells	Buccal cells are cells that form the lining of the inside of the cheek and other parts of the mouth that are often collected on a swab to provide a DNA sample from an individual.
Chronological age	The time elapsed between a person's birth and the current time.
CpG site	When a cytosine nucleotide is followed by a guanine nucleotide in a DNA sequence. There are more than 28 million CpG sites in the human genome and about 70-80% are methylated.
Cytosine	One of the four nucleotides that form the basis of DNA.
Deoxyribonucleic acid (DNA)	Deoxyribonucleic acid (DNA) is the chemical in the cells of an organism that comprises its heritable material, used in the development, functioning, and reproduction of all known living beings. In humans, DNA is composed of two strands of nucleotides, joined by a chemical backbone, that link through the bases to form a double helical chain. DNA is found in the nucleus of cells and is gathered in chromosome pairs inherited from the parents.
DNA methylation	A chemical change to the structure of a nucleotide component of DNA whereby a methyl group bonds to a cytosine molecule. The process of DNA methylation does not alter the overall DNA sequence/genetic code; it is therefore an epigenetic change.
Epigenetic	A change to the function of an individual's genetics that is caused by environmental and hereditable factors. The DNA sequence remains unaltered, instead it is the way in which this DNA sequence is interpreted that changes.
Gene	An area in the DNA molecule in which the particular sequence of the nucleotides forms an instruction that can be copied and translated by molecular processes into a specific protein.
Gene promoter	A region of DNA upstream of a gene that initiates the process of transcription for that gene.

Guanine	One of the four nucleotides that form the basis of DNA.
Intergenerational trauma	Occurs when the experience of trauma on an individual is passed down to subsequent generations. While it is acknowledged that transmission could be through generational 'stories', there is evidence of both genetic and epigenetic inheritance of a cellular stress memory response.
Median absolute deviation	A measure of the spread, or variability, of a variable. The median absolute deviation is the median absolute difference between a set of datapoints from the median. This measure is less affected by extreme values than standard deviation.
Methyl group	A compound comprised of one carbon and three hydrogen atoms, which can link to a partner molecule.
Nucleotide	An organic molecule which is a building block of DNA, forming a single DNA base, e.g. A, C, G, or T.
Transcription	Copying a sequence of DNA into an RNA molecule by an enzyme that is part of the process of gene expression.
Ribonucleic acid (RNA)	A molecule that carries instructions from DNA to help synthesise protein molecules.

Annex B – AESAC Membership

The members of AESAC, at the time of publication, were:

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- **Mr Liam Way**, Policy and Practice Improvement Consultant & Registered Social Worker