Epigenetics 8:8, 775–777; August 2013; © 2013 Landes Bioscience

The promise and challenges of blood spot methylomics

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Epigenome-wide association studies (EWAS) are being extensively performed to identify epigenetic variants associated to complex diseases. However, EWAS may identify variants that are disease-induced rather than disease-causal. Recent studies have highlighted the use of Guthrie cards to profile the methylome at birth, permitting researchers to find epigenetic variants present in patients before they are diagnosed with clinical disease, with the implicit suggestion that these variants are more likely to be disease causal. The use of Guthrie cards for research purposes throws up a number of ethical issues. We review here the promises and pitfalls of Guthrie cards for disease research.

Keywords: longitudinal studies, epigenetics, blood spot, epigenome wide association study, biomarker

Submitted: 05/17/13

Revised: 06/10/13

Accepted: 06/10/13

http://dx.doi.org/10.4161/epi.25357

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Epigenome-wide association studies (EWASs) are currently being conducted to uncover epigenetic variants associated to a range of different diseases.[1] These studies have so far utilized monozygotic twins discordant for disease or affected patients compared with healthy controls.[2-4] Given the dynamic nature of the epigenome, it is plausible that associations thus far identified represent disease-induced epigenetic variation and not disease-causal epigenetic changes.[1] One way of addressing this issue is to determine whether disease associated epigenetic variants are present before clinical diagnosis of disease, but this requires obtaining a significant number of samples taken years before clinical disease onset. For many adult onset complex disorders, this is a challenging hurdle.

In the 1960s, Robert Guthrie devised an ingeniously simple test for the metabolic disease phenylketonuria.[5] Shortly after birth, the heel of a newborn is pricked to collect several drops of blood on filter paper (a so-called Guthrie card). Newborn screening has since been widely adopted and is a regular feature in all developed world maternity wards.[6] Laboratories now test the blood spots for a variety of rare conditions, including congenital hypothyroidism and cystic fibrosis.[6] The hospital or testing service then stores the Guthrie card and a record of the test results. The public health benefits of newborn screening programs are well established.[6] These programs trigger timely treatment and advice to parents and are a highly cost-effective strategy for early identification of genetic disorders. The term Guthrie card refers to the blood spot card taken at birth, whereas blood spot card can mean a Guthrie card or to blood spotted on filter paper at any point in time.

The storage of Guthrie cards after testing opens the possibility of using them for other studies.[7] In the UK, four blood spots are taken; one is used for disease screening, leaving up to three other spots for other uses. We and others recently have shown that DNA extracted from Guthrie cards (up to a decade old) or blood spots can be used to perform whole-genome methylation analysis using MeDIP-seq, MBD-seq and the Illumina 450K array.[8-10] In our study, a comparison between Illumina 450K-based fresh cord blood and 10-y-old Guthrie card methylation profiles revealed an excellent correlation (R^2 = 0.99, Pearson's).[8] This was also seen in the work of Joo and colleagues, in which 3-y-old Guthrie cards and matched frozen buffy coat samples from the same individual showed near perfectly correlated 450K results (r = 0.99, Pearson's).[9] Further, in our investigation, MeDIP-seq profiles of Guthrie cards showed very good agreement (75–93%) with the Illumina

450K data across a range of CpG densities.[9] Aberg and colleagues found that the sequence quality and the enrichment profile for MBD-seq from up to 19-y-old blood spots gave comparable results to DNA extracted from whole blood.[10]

The use of a Guthrie card sample can thus provide details on the individual's methylome at birth, prior to them having a clinical diagnosis of disease. Therefore, Guthrie cards can be used to try to identify disease causal epigenetic variation by allowing targeted retrospective longitudinal studies. The widespread use of Guthrie cards means that this resource offers many more advantages than prospective birth cohorts to identify blood samples taken at birth for a large number of individuals with a complex disease, especially those with relatively rare incidences. Blood spots, as a fairly non-invasive blood collection procedure and being easy to store, ship and handle, can enable large-scale studies of DNA methylation. Guthrie cards and blood spots are also being used for a number of other research studies, including the assessment of metabolites, such as vitamin D,[11] and for gene expression investigations, as it is possible to extract more DNA from a Guthrie card.[12] These additional analyses can also perhaps be combined with epigenetic analyses to assess methylation-metabolome and methylation-expression correlations.

However, there are some limitations to the use of Guthrie cards for epigenetic studies. First, it is a sample of whole blood. For many diseases, it is questionable whether blood is the most useful tissue to study; further, blood is a heterogeneous tissue and any DNA methylation difference between patients and controls could be confounded by differences in the cellular composition of the whole blood sample.[13] However, this can be addressed to some extent by adjusting for cellular proportion differences by using reference information on cell-specific methylation signatures to estimate cell proportions present in each sample.[14] Further, it is not always clear how Guthrie cards have been stored and handled—and this will vary between different sites—meaning there is the potential for contamination. Guthrie cards use untreated cellulose paper, which does not offer any protection to the applied blood sample. Degradation can occur over time especially if there are no precautions taken and they are stored in an environment where temperature and humidity can fluctuate. However, other cards used for blood spots can contain impregnated chemicals used to preserve DNA quality. In the study by Joo et al., the authors found approximately 5000 probes on the Illumina 450K array that were consistently different between Guthrie card and buffy coat samples. The authors suggested that these probes are ones that may be the most affected by differences in preparation methods and/or storage conditions between the two sample types, but this requires further study. Despite these limitations, there still remains an enormous potential for using Guthrie cards for epigenetic studies.

A raft of legal and ethical uncertainties surrounds the use of Guthrie cards for research purposes.[15] For example: who owns the cards? Who may access them and under what conditions? What uses are permissible beyond the initial battery of genetic tests?[15] In recent years, controversial incidents have brought these questions to the fore.[16] In the United States, the Texas Civil Rights Project recently sued the state on behalf of five parents over storage and continuing use of Guthrie cards held by the Texas Newborn Screening Program. The state settled the case, agreeing to destroy 5.3 million cards that had been collected before 2009.[16] A more recent controversy has taken place earlier this year in Ireland. Here, until 1988, when the Data Protection Act was enacted, consent to Guthrie testing, and the retention of cards, was based on the principle of "implied consent." Keeping pace with international practice, and respecting data protection law, a process of "explicit consent" was required in 2011, meaning that parents must give written consent to both the test and to storage of the Guthrie card. Recently, a member of the public made a complaint to the Data Protection Commissioner arguing that their Guthrie card was being held by the Health Safety Executive (HSE) without their consent. The advice from the commissioner to the HSE was that continued retention of the cards without explicit consent constitutes a breach of data protection legislation. Following an internal review, the Minister of Health decided to destroy the archive. However, after hearing the opinion of a large proportion of the medical community, who stated that disposing of these cards would be a serious mistake that does not take into account the medical and societal value of the cards, the Health Minister has now informed the HSE that no such destruction will take place until an expert group meets to discuss the issue of how these cards can be maintained without breaking data protection regulations.

A common element in these controversies is the public apprehension that they provoke.[15] Parents are not adequately informed with regards of their rights to refuse to participate in newborn screening programs, nor are they appropriately told about the potential storage and further uses of Guthrie cards. Critics state that the retention of cards, followed by their secondary use without the consent of the child or parents, effectively creates an unauthorized national biobank. The types of legal protections that regulators would normally point to in assuaging such concerns—clear restrictions on access and use, well-defined property rights and preservation of patient autonomy through informed consent—are often missing or lack clear application to newborn screening programs. There are few straightforward answers to address these issues, but they are urgently required before vast collections of Guthrie cards are destroyed. The absence of specific legal regimes governing most Guthrie cards means that rules regarding ownership, storage and use must be drawn from a complicated mix of directly and indirectly related laws. Some countries, for example Denmark, do have specific legislation for Guthrie cards, and these laws can perhaps be examples for other countries to follow.[17] In Denmark, parents are informed that their child's sample will be stored in a Guthrie card biobank, and the card may be used for retesting, quality assurance or research. Parents may choose to opt out of their child's sample being stored in the biobank at any point. The samples are stored in a freezer at –20 °C with access for authorized personnel only. Any research study wishing to use Guthrie cards has to be

approved by a national committee who evaluates the scientific value of the project and also ensures that there is always enough blood left directly related to the original purpose of the Guthrie card (the health of the child).[17]

Residual Guthrie cards represent a valuable scientific resource with the potential to provide important data of benefit to society. It currently represents perhaps the most powered way to appropriately identify disease causal epigenetic variation. However, international guidelines are needed in order to preserve such precious resources. Attempts are currently being made to standardize newborn screening programs within countries, hopefully leading to invaluable health benefits in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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