Collecting saliva samples for DNA extraction from children and parents on the fifth wave pilot of the Millennium Cohort Study

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**Background**

- Number of surveys in different countries include the collection of range of different biomarkers.
- Well established that lay interviewers can carry out anthropometric measurements in home setting (e.g. Health Survey for England, English Longitudinal Study of Ageing, US Health and Retirement Study).
- Collection of biological samples e.g. blood, saliva, urine, teeth and hair also well established – often by medically-trained personnel in a clinic setting or by nurse interviewers in a home-visit setting.
- More recently, increasing interest in collection of biological samples using non-medically trained interviewers.
- Advances in genetic research mean we can now extract DNA from saliva, as well as from blood. As collecting saliva is non-invasive technique - co-operation rates are typically much higher than for blood samples.
- In context of widespread interest in genetic influences on health and other outcomes, a number of multi-disciplinary longitudinal studies regard DNA as an important complement to other forms of data (e.g. German Socio-Economic Panel Study, and Health and Retirement Study).
Background

• However, on all these studies, saliva for DNA extraction only collected from adults
• Collection of samples from children alongside biological parents provides particular benefits for research and enhances our understanding of epigenetic processes
• To our knowledge, only major longitudinal study that has done this is Fragile Families and Child Well-being Study in the US
  • Saliva samples collected from children and mothers as part of age 9 sweep
  • Collection administered by lay interviewers
  • Samples collected from 86% of children and 80% of natural mothers
• Suggests collection of saliva from children is both feasible and acceptable, but hadn’t been tested in UK context where both cultural judgments on acceptability and sensitivity of genetics, and surveying practice and norms may be different
• Fragile Families not designed to collect samples from both biological parents
• Therefore, was a need to ascertain feasibility of collecting samples of saliva from a diverse sample of children and natural parents using lay interviewers in a home visit setting in the UK
Millennium Cohort Study (MCS)

- Cohort study of around 19,000 children born 2000/2001
- Home visit-based survey
- Families surveyed when children were aged around 9 months, 3 years, 5 years and 7 years old
- Data collected from main carer (typically mother) and partner (typically father or step-father): interview and self-completion
- Direct assessments of children (cognitive assessments and weight and height)
- Data collected from older siblings in some sweeps via self-completion
- From age 7, data collected from cohort children themselves via a self-completion
- Some bio-measures already collected (e.g. teeth, oral fluid via mouth swabs (not for DNA extraction) and height, weight and body fat percentage, and waist measurement)
- For the fifth wave taking place at age 11, wanted to include collection of saliva samples from children and natural parents for DNA extraction to facilitate research projects relating to the understanding of growth (obesity), and learning and behaviour, and epigenetics and to establish a DNA bank for subsequent research
This paper

• Outlines process developed for and carried out on the pilot study for the fifth wave of the Millennium Cohort Study (MCS)
• Pilot aimed to collect saliva samples from 11-year old children and both resident natural parents using lay interviewers as part of home visit
• Enabled evaluation of:
  • Procedures for gaining informed consent and ensuring co-operation
  • Administration of collection procedure
  • Return of samples
  • Suitability of samples for analysis
• Gave indication of likely response rates
MCS5 pilot sample

• Recruited by Ipsos MORI interviewers in five locations in UK representing diversity of types of region
• None of families had taken part in MCS previously
• Quotas set to ensure cross-section of different family types were included
• Interviews were carried out with 45 families (including one set of twins)
• Interviewers attended three-day, face-to-face briefing – received training on all elements of study including collecting saliva samples
Gaining informed consent and securing co-operation

• Essential for interviewers to gain informed consent from respondents to carry out saliva sample collection
• Ethical requirement for all survey research but particularly on MCS due to its complexity and potentially sensitive elements
• Requirement of Research Ethics Committee (MREC) that reviews each wave of MCS to ensure it complies with recognised ethical standards
• Fully informed consent required from parents – particularly as children may not be able to grasp full implications of participation for themselves
• Informed assent required from children
Ensuring informed consent was gained from parent

- Carried out in stages
- When appointment made, parents and children given letter and leaflets describing each individual survey element
- Interviewers responsible for ensuring respondents had read and understood letters and leaflets, and understood procedure, before attempting to carry it out
- Had to obtain consent from parent and assent from child before attempting collection
- Worked through paper consent form
  - CAPI script to guide them
  - Used leaflet to ensure full understanding of process
- Consent to collect sample from child could only be given by someone with legal parental responsibility – CAPI established this
Developed a specific saliva sample information leaflet for the pilot.

Intended to explain:

- Importance
- Relevance
- Uses of samples
- What samples WILL NOT be used for
  - Paternity testing and Police
- Process, storage and withdrawal of consent
Ensuring informed assent was gained from child

- Once someone with legal parental responsibility had given their consent, interviewers had to gain child’s assent to the procedure.
- After ensuring child had read leaflet, interviewers ran through series of questions to ensure they were happy to take part, that their participation was voluntary, and that they could stop at any time.
- The child didn’t have to sign anything, but interviewers had to sign to confirm their assent.
- If parent gave their consent for the procedure, but child didn’t give their assent, the procedure couldn’t continue (and this principle applied to all elements of the study).
Information leaflet covered DNA with other elements of study

- Explained process and voluntary nature of participation – so telling children what the process would be like
- Said something about future use, but not in detail
Collection of saliva sample

• Used Oragene DNA self-collection kit
• Widely used on other studies
• Designed for easy administration
What the respondents and interviewers had to do

1. Spit until the amount of saliva (not bubbles) reaches the fill line.
2. Close lid by pushing down hard on the funnel lid.
3. Unscrew the tube from the funnel.
4. Close tube tightly with small cap and mix.

Respondents were asked not to eat, drink, smoke or chew gum within 30 minutes of giving the sample.
Sending samples back to the laboratory

- Interviewers labeled samples with time and data sample was collected
- Provided with set of barcodes for each family, colour coded for each family member
- Told to attach barcode to side of tube
- No identifying information recorded on sample
- Wrote serial number and respondent ID number onto despatch note (to enable laboratory to cross-check samples against it)
- Instructed to place tubes into individual plastic bags
- Placed both tubes and despatch note into padded envelope marked ‘Exempt Human Specimen’
- Returned all samples they had collected to lab twice a week
- Samples stable at room temperature – no need to refrigerate
Reconciliation of consent forms and samples

- Essential only samples for which consent had been obtained had DNA extracted by the lab
- Ipsos MORI Field Department and processing lab designed systems to enable this check to be carried out
- All consent forms booked in by Ipsos MORI Field Department on spreadsheet generated from CAPI data
- Visual check carried out to confirm names on consent forms were names of individuals we knew had been interviewed in each household and were recorded as child’s parent
- When samples arrived in lab, serial numbers recorded, and list sent back at regular intervals to Ipsos MORI Field Department – enabled them to chase up interviewers who hadn’t sent back a consent form
- At end of fieldwork, Ipsos MORI Field Department sent full list to laboratory of all valid consents obtained to refer to before processing samples
Findings - Pilot sample response

- Response was promising
- Families with no existing link to study or history of data collection, therefore lower than hoped for main study (where expected 85% from children)
- Response rates from eligible, responding fathers good, but because large proportion of families do not contain two natural parents, the % of triads was only a bit over 40% of families

<table>
<thead>
<tr>
<th></th>
<th>Number interviewed</th>
<th>Number collected</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>46</td>
<td>34</td>
<td>74</td>
</tr>
<tr>
<td>Mothers</td>
<td>45</td>
<td>33</td>
<td>73</td>
</tr>
<tr>
<td>Fathers</td>
<td>25</td>
<td>19</td>
<td>76</td>
</tr>
</tbody>
</table>
Findings – process of gaining informed consent and co-operation

- Majority of respondents happy to participate
- Clear that leaflets reassured majority of respondents, and training received by interviewers enabled them to reassure respondents and answer most questions
- For some respondents, providing sample not viewed as concerning, or onerous – just another element of the survey
- But was the element that caused most controversy and refusals from significant minority of respondents
- Due to worries about what would be done with samples, or distaste for process involved
  - Uncertainty about uses data would be put to – concern about police or government databases/ability to identify those engaged in criminal activities
  - Some found process uncomfortable – didn’t like idea of spitting and would have preferred swab
- No issues for interviewers in defining legal parental responsibility, but question from one respondent about definition of natural/biological parents – she had conceived via egg donor but considered herself natural parent
Findings – collection of saliva samples

• Order in which samples were collected varied from household to household and interviewer to interviewer
  • Some reported it helpful to introduce this later in the appointment once rapport established
  • Led to some initial refusals being converted into successful outcomes
• Took 5-10 minutes per sample to complete
• Number of children found process awkward – hard to spit into tube
• Some parents found it hard to produce enough saliva, particularly after CAPI interview
• All those who agreed to sample managed to complete it
• Interviewers didn’t report problems with kits, other than saliva being present on tubes when they handled samples – requested that disposable gloves provided
Findings – reconciliation of consent forms and samples

- 86 samples collected and all received by lab
- Reconciliation carried out four times during fieldwork
- Checks revealed one interviewer had got confused with barcode labels – the correct label was stuck on the sample, but an incorrect one was stuck on the consent form
- Labeling of barcodes did cause some confusion generally
- When samples arrived at lab, samples from mothers and children were scanned successfully, but those of fathers (with a blue background) could not be scanned and were logged manually
Findings – quality of the samples

<table>
<thead>
<tr>
<th>DNA yield</th>
<th>Number collected</th>
<th>Mean total yield (µg)</th>
<th>Range (µg)</th>
<th>% with total yield &gt; 20 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>34</td>
<td>58.81</td>
<td>0.03-238.2</td>
<td>82.9%</td>
</tr>
<tr>
<td>Mothers</td>
<td>33</td>
<td>119.6</td>
<td>0.1-390.4</td>
<td>81.8%</td>
</tr>
<tr>
<td>Fathers</td>
<td>19</td>
<td>99.6</td>
<td>4.0 – 254.7</td>
<td>89.5%</td>
</tr>
</tbody>
</table>

- Pilot yields were generally high - over 81% of samples gave yields of at least 20 micrograms, sufficient DNA for a range of genetic studies
- Was variation in amount obtained from individuals
- Majority of small volume samples collected from one interviewer in one day. Confirmed at debrief that they had misunderstood how to seal tube, and needed additional guidance.
- At analysis stage, discovered some samples turbid and discoloured – indication that instructions regarding not eating, drinking or smoking weren’t adhered to
Conclusions

• Findings were very positive overall
• Clearly feasible to get good quality, analytical samples of saliva from young children and parents using simple kits administered by interviewers in household setting
• Although levels of consent not as high as in other studies, reasonable to expect a large proportion of families will co-operate
• DNA yields from such samples are, for most part, sufficient for analysis
• Consent process was commensurate with best practice in obtaining informed consent from adult respondents/parents, and from children in line with their age and understanding
• Aside from the “icky” factor, collection generally acceptable for and to children of this age
• *Nevertheless, some elements could be improved to enhance collection and extraction*
Conclusions

• Areas for improvement were relatively minor and could be straightforwardly implemented:
  • Providing parents with more information on research purposes (through FAQ document)
  • Encouraging spitting to occur privately
  • Reinforcing interviewer instructions on mixing and sealing of samples
  • Reinforcing interviewer instructions to ensure respondents didn’t eat, drink or chew gum for 30 minutes before collection
  • Issuing interviewers with gloves
  • Reinforcing importance of collection
  • Ensuring barcode labels are applied properly (clear instructions) and that they can be scanned properly (i.e. must not use blue ones)

This has not been taken forward on the mainstage of MCS5 as funding wasn’t secured – it’s hoped that it will be included in the next wave of the survey at age 14
Thank you

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