Perspective on the Technical Challenges Involved in the Implementation of Array-CGH in Prenatal Diagnostic Testing

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Abstract Our aim was to construct a streamlined technical workflow to facilitate a prospective, multi-centre evaluation of array comparative genomic hybridisation (array-CGH) in the prenatal diagnostic context. A collection of commercially available DNA extraction and quantification techniques were evaluated and compared using minimal quantities of amniotic fluid, chorionic villi and cultured cells. When prenatal DNA of suitable quality and quantity was obtained, array-CGH was performed using Oxford Gene Technology’s (OGT, Oxford, UK) CytoSure™ ISCA 8 × 60 K oligo array platform. With starting quantities of 2–4 ml amniotic fluid, 2–5 mg chorionic villi or under 150,000 cultured cells the following optimised technical workflow was identified: DNA extraction using the iGENatal™ kit (igenbiotech, Madrid, Spain) and quantification by the Qubit® 2.0 Fluorometer with the Qubit® dsDNA BR assay kit (Invitrogen™, Eugene, OR, USA). In addition, it was elucidated that array-CGH can be successfully performed with as little as 125 ng DNA in the experiment using the OGT CytoSure™ ISCA 8 × 60 K oligo array platform. Amidst an on-going debate on whether array-CGH should be applied in the prenatal diagnostic setting, by following the technical recommendations described here genetics laboratories can now gain exposure to prenatal array-CGH testing without compromising the conventional karyotype result.

Keywords Array-CGH · Chromosomal microarray · Prenatal diagnosis · DNA extraction and quantification · EACH study

Introduction

The introduction of array comparative genomic hybridisation (array-CGH) into postnatal diagnostic testing has facilitated an increase in the detection rate of pathogenic abnormalities which were previously undetectable by conventional G-banded karyotyping. Following the publication of several large-scale clinical studies it is clear that there is an increasing demand for the use of this technology in the prenatal setting [1–4, 6–9]. Several groups have recommended the replacement of karyotyping with array-CGH as a front-line prenatal test. In this context, a multi-centre study in the USA involving more than 4,000 foetuses showed that for samples with a normal karyotype, array-CGH detected clinically relevant copy number changes in 6.0 % of cases with abnormal ultrasound and 1.7 % of those whose indications were advanced maternal age or a positive serum screening result [9]. With the potential for such a marked increase in the detection rate of pathogenic abnormalities utilising this technology, genetics laboratories would now benefit from exposure to optimised technical procedures involved in prenatal array-CGH testing.

In the UK, a National Institute for Health Research (NIHR) funded multi-centre study called ‘evaluation of array-CGH in prenatal diagnosis of foetal anomalies’ (EACH) is currently evaluating the feasibility of
introducing prenatal array-CGH into the UK’s National Health Service. As part of the EACH study’s remit, detailed technical evaluations were initially performed to optimise the laboratory processes—especially DNA extraction, quantification and array quality assurance. This was necessary to ensure that it was possible to obtain sufficient DNA yields and high-quality array-CGH profiles from minimal amounts of amniotic fluid, chorionic villi and cultured cell suspension specimens without compromising the conventional karyotype result. Here, we present a summary of the findings from these technical evaluations including recommendations supplied to the participating EACH laboratories.

Materials and Methods

Technical evaluations were performed by scientists at two laboratories: the Wessex Regional Genetics Laboratory (WRGL) at Salisbury District Hospital and the North East Thames Regional Genetics Laboratory (NETRGL) at Great Ormond Street Hospital, London.

Currently, one of the main technical challenges for prenatal array-CGH is the ability to extract sufficient DNA of good quality from the minimal quantity of prenatal material available for DNA extraction. In the UK, this is especially true for amniotic fluid samples from which typically only 2–4 ml can routinely be spared for this purpose (since no more than 20 ml of amniotic fluid is usually obtained from amniocentesis). Therefore, several commercially available extraction techniques were trialled including: QiAmp® Blood Mini kit (Qiagen, Hilden, Germany); QiAmp® Blood Midi kit (Qiagen, Hilden, Germany); QiAmp® Blood Maxi kit (Qiagen, Hilden, Germany); Puregene® Blood Core kit B (Qiagen, Hilden, Germany); QiAmp® Circulating Nucleic Acid kit (Qiagen, Hilden, Germany); Genomic DNA Mini kit (Geneaid, Taipei, Taiwan); WaxFree™ DNA kit (TrimGen, Sparks, Maryland, USA); DNA Isolation kit (Cat No. 15DDK-3, VH B.O. Ltd., Gateshead, UK); iGenNatal™ kit (igenbio-tech, Madrid, Spain); Maxwell® 16 Cell LEV DNA Purification kit (Promega, Madison, Wisconsin, USA); MagNA Pure™ 96 System (Roche, Mannheim, Germany) and the QuickGene—Mini80 System (FujiFilm, Bedford, UK).

The prenatal material used for DNA extraction and array-CGH experiments was obtained from routine invasive specimens received at both diagnostic laboratories (WRGL and NETRGL). All material was tested on an anonymous basis and neither the conventional karyotyping procedure nor the results of such testing were compromised. The quantity of material used was either 2–4 ml amniotic fluid (clear and unstained), 2–5 mg chorionic villi or approximately 150,000 cultured cells. DNA quality was assessed using a combination of spectrophotometer ratios (260/230 and 260/280), agarose gel electrophoresis and, if deemed acceptable for array-CGH testing, by array probe evaluation.

When performing array-CGH using the low concentrations of DNA frequently obtained from prenatal extractions, the ability to make accurate quantification measurements is essential. Therefore, a selection of commercially available fluorometric quantification techniques were trialled including: QuantiFluor™ dsDNA assay kit (Promega, Madison, WI, USA), Quant-it™ PicoGreen® dsDNA assay kit (Invitrogen™, Eugene, OR, USA) and Qubit® dsDNA BR assay kit (Invitrogen™, Eugene, OR, USA). The quantification results were compared to those obtained using the NanoDrop™ spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

In the EACH study, the array platform used in prenatal testing is, for most participating laboratories, the same as that used routinely in postnatal testing (i.e. evidence-based International Standards for Cytogenomic Arrays (ISCA) 8 x 60 K oligo array). Oxford Gene Technology’s (OGR, Oxford, UK) CytoSure™ ISCA 8 x 60 K oligo array platform is one of the most widely used amongst the EACH consortium and the array-CGH evaluations were, therefore, performed using this platform, according to the manufacturer’s operating protocol. DNA was labelled using CytoSure™ HT Genomic DNA Labelling kit (OGR). Prenatal DNA was matched with an equivalent quantity of male or female Megapoof™ Reference DNA (Kreatech Diagnostics, Amsterdam, The Netherlands).

Results

Evaluation of Prenatal DNA Extraction Techniques

The work undertaken here focused on identifying suitable techniques for obtaining a high yield of good quality DNA from very low quantities of invasive material (as detailed in the “Materials and Methods”). Particular attention was paid to amniotic fluid extractions, since the ability to obtain sufficient DNA for array-CGH using 2–4 ml of this sample type was regarded to pose the greatest challenge. To be readily adoptable into multiple testing centres participating in the EACH study, the chosen techniques also needed to be economically viable without high initial capital outlay for equipment or machinery. Consequently, any technique ruled out here does not preclude their suitability for use in other studies or scenarios with different parameters.

The Maxwell® 16 Cell LEV DNA Purification kit (Promega, Madison, WI, USA) and the QuickGene—Mini80 System (FujiFilm, Bedford, UK) were not considered appropriate for the EACH study, despite producing satisfactory yields of good quality DNA from small