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Carbohydrate and fatty acid perturbations in the amniotic fluid of the recipient twin of pregnancies complicated by twin-twin transfusion syndrome in relation to treatment and fetal cardiovascular risk

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ABSTRACT

Introduction: Twin-twin transfusion syndrome (TTTS) complicates 15% of monochorionic twin pregnancies, often being associated with recipient cardiac dysfunction. Untreated, it has a fetal mortality rate of at least 90%; although treatment by fetoscopic laser coagulation significantly improves prognosis. Measurement of recipient amniotic fluid metabolites, such as cardiac Troponin T and atrial natriuretic polypeptide, correlate with cardiac function in this fetus. The aim of this study is to describe the amniotic fluid metabolomic profile in TTTS, relate this to fetal recipient cardiac function and assess the metabolomic changes induced by fetoscopic laser coagulation.
Methods: Prospective single centre cohort study. The metabolomics profile of the amniotic fluid from the recipient sac of TTTS pregnancies was assessed using ultra high performance liquid chromatography-mass spectrometry. Profiles were compared pre- and post-laser coagulation and related to fetal recipient cardiac function, as assessed using Doppler ultrasound within 4 h of treatment.
Results: Eleven metabolites had significant associations with recipient fetal right and left ventricular myocardial performance index pre-laser. 200 metabolites in recipient amniotic fluid demonstrated a change in relative concentrations when comparing pre- and post-laser coagulation ($p < 0.005$). The most prominent change is in the balance of carbohydrate and fatty acid metabolic profile contributing to fetal or placental energy metabolism. These changes were also associated with the echocardiographic measures of recipient cardiac function.
Discussion: Changes in carbohydrate and fatty acid metabolic profiles are noted in recipients with cardiac dysfunction, and further changes are noted after treatment. Validation and investigation may identify targets for potential pharmacological treatment.

1. Introduction

Approximately 15% of monochorionic diamniotic (MCDA) twins are complicated by twin-twin transfusion syndrome (TTTS), a condition associated with unidirectional intertwin blood flow through placental arteriovenous anastomoses (AVA) and high perinatal mortality. This leads to the severe haemodynamic imbalances seen within the fetal circulations of this condition with a hypertensive circulation in the "recipient" twin and subsequent cardiac dysfunction [1], noted in up to 70% of pregnancies. There is

Abbreviations: ANF, atrial natriuretic factor; AVA, arteriovenous anastomoses; BNP, brain-type natriuretic factor; DV, ductus venosus; E/A, early passive/atrial; FLC, fetoscopic laser coagulation; IUGR, intrauterine growth restriction; LV, left ventricle; MCDA, monochorionic diamniotic; MPI, myocardial performance index; QC, quality control; RV, right ventricle; TTTS, twin-twin transfusion syndrome; UHPLC-MS, ultra high performance liquid chromatography-mass spectrometry.

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corresponding dysregulation of fetal endocrine systems, including the renin-angiotensin-aldosterone [2], atrial natriuretic factor (ANF) [3] and Endothelin-1 systems [4]. Such changes have been measured in the amniotic fluid of the recipient twin, and related to cardiac dysfunction and overall fetal prognosis [3].

Untreated, fetal mortality is at least 90%, for each fetus [5]. Treatment by fetoscopic laser coagulation (FLC) is effective with survival of two babies approaching 60% [6]. FLC also reduces the risk of neurodevelopmental morbidity compared to other treatments [7]. Improvement in recipient cardiac function is noted within 48 h of FLC in approximately half of recipients [8], which is associated with improved fetal outcome [9].

Metabolomics is the holistic untargeted study of metabolism [10] and investigates the final downstream product of genotype-environment interactions. It provides the identification of a dynamic and sensitive phenotypic signature associated with human health ageing and disease molecular pathophysiology [10]. In human pregnancy, this technique has been used to investigate intrauterine growth restriction (IUGR) [11–14], pre-eclampsia [15–17] and gestational diabetes [18,19]. In non-pregnant adults, it has been used to profile functional and metabolic changes associated with heart failure [20,21].

In MCDA pregnancies affected by TTTS, a small number of studies, targeting specific areas of metabolism have been reported [22,23]. Molecular patho-mechanistic changes have been observed in angiogenic growth factors [24], cytokine levels [25] and gene transcripts in amniotic fluid and maternal plasma which appear to predict fetal outcome [26,27].

We describe the metabolomic profiles in amniotic fluid from the recipient sac of MCDA twin pregnancies complicated by severe TTTS and note associations between fetal echocardiographic recipient right ventricular (RV) and left ventricular (LV) function. In addition, the effects of FLC on the metabolomic profile signatures are described.

2. Methods

This study had ethical approval from Birmingham Black Country Local Research Ethics Committee (No: 06/Q2702/71 accepted in 2006) with written consent obtained from all subjects.

2.1. Patient selection

The cohort consisted of MCDA twins complicated by severe TTTS (presenting before 24 weeks) treated between August 2011–June 2012 and TTTS was defined as polyhydramnios (>8 cm in the deepest vertical pocket of the recipient at <20 weeks of gestation or >10 cm from 20 weeks of gestation onwards) in combination with oligohydramnios in the donor (<2 cm deepest vertical pool depth). All cases were prospectively staged using the Quintero system [28].

2.2. Cardiac function assessment

High-resolution fetal ultrasound and echocardiography were performed in the recipient with curvilinear array transducers (7–3.5 MHz) on a Siemens S3000 ultrasound machine (Siemens Ltd, Erlangan, Germany) by a single operator (MDK) and the myocardial performance index (MPI) calculated for each ventricle [1,29] as previously described. Cardiac dysfunction was indicated by the presence of tricuspid regurgitation, reversed flow in the DV during atrial contraction, and a tricuspid early passive/atrial contraction (E/A) ratio of >95% CI outside the normal limits. This was performed within 4 h of starting the laser procedure and repeated within 4 h post-FLC.

2.3. Fetoscopic laser coagulation (FLC)

FLC was performed using local anaesthesia (1% lignocaine skin/myometrial infiltration) and maternal Remifentanil sedation as previously described [30]. A selective sequential FLC technique was used, with an additional “Solomon” procedure in some cases [31]. No amnio-infusion was performed.

2.4. Non-targeted Ultra high performance liquid chromatography-mass spectrometry (UHPLC-MS) metabolomics analysis

All solvents and chemicals applied were of HPLC analytical grade (J.T. Baker, U.K.).

2.5. Sample collection and preparation

A 10 ml sample of amniotic fluid was taken at insertion of the fetoscope into the recipient amniotic sac and then a further 10 ml sample withdrawn at the end of the laser coagulation treatment. The median duration of the laser procedure and amniodrainage was 34 (range 21–45) minutes. Samples were stored at −80 °C before preparation and analysis. All samples were randomised to ensure no correlation between order of preparation and subject, disease grade or date of sample collection. Deproteinisation was performed as described below. 250 μL of amniotic fluid was vortex-mixed with 1000 μL of methanol for 15 s to precipitate proteins and DNA followed by centrifugation (15 min, 13,000 *g*) drying to induce metabolite stability and then stored at −80 °C prior to analysis. A pooled quality control (QC) sample was prepared by combining 80 μL aliquots of each of the 38 samples [32].

2.6. Ultra high performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis

UHPLC-MS analysis of amniotic fluid extracts and QC samples was performed applying a Dionex U3000 coupled to an electrospray LTQ-FT-MS Ultra mass spectrometer (Thermo Scientific Ltd. UK). Samples were reconstituted in 100 μL of 50:50 methanol:-water, vortex-mixed for 15 s, centrifuged (15 min, 13,000 *g*) and transferred to vials with 200 μL fixed inserts (Thermo-Fisher Ltd. U.K.). All samples were stored in the autosampler at 5 °C and analysed separately in negative and positive electrospray ionisation (ESI) modes within 72 h of reconstitution. UHPLC separations were performed applying a Hypersil Gold C_{18} reversed phase column (100 × 2.1 mm, 1.9 μm) at a flow rate of 400 μL min^{-1}, column temperature of 40 °C and with two solvents: solvent A (HPLC grade water + 0.1% formic acid) and solvent B (HPLC grade methanol + 0.1% formic acid). A gradient elution was performed as follows: hold 100% A 0–1.5 min, 100% A - 100% B 1.5–6 min curve 3, hold 100% B 6–12 min, 100% B – 100% A 12–13 min curve 3, hold 100% A 13–15 min. Injection volume was 5 μL. UHPLC eluent was introduced directly in to the electrospray LTQ-FT Ultra mass spectrometer with source conditions as follows: spray voltage −4.5 kV (ESI-) and +5 kV (ESI+), sheath gas 30 arbitrary units, aux gas 15 arbitrary units, capillary voltage 35 V, tube lens voltage −100 V (ESI-) and +90 V (ESI+), capillary temperature 280 °C, ESI heater temperature 300 °C. Data were acquired in the FT mass spectrometer in the *m/z* range 100–1000 at a mass resolution of 50,000 (FWHM defined at *m/z* 400), with a scan speed of 0.4 s and an AGC setting of 1×10^6. Analysis order was composed of 10 QC sample injections for system conditioning followed by a QC sample injection every 6th injection with two QC sample injections at the end of the analytical run. Amniotic fluid extracts for each subject were analysed in a random order; the two samples for each subject were analysed sequentially.