High resolution 1H NMR spectroscopy successfully discriminates fetuses with congenital diaphragmatic hernia from normal pregnancies

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Introduction

High resolution ^{1}H NMR spectroscopy has a high potential in becoming a valuable non-invasive technique for the evaluation of fetal health and development. It was shown to predict fetal lung maturation [1] and normative values were suggested for the second and third trimester [2]. Very limited information is available comparing normal and complicated pregnancies. Lung hypoplasia in congenital diaphragmatic hernia (CDH) is a prenatal condition that can be targeted in utero, hence, precise information on the fetal situation is needed. In this study, we compared amniotic fluid (AF) from fetuses with CDH undergoing temporary tracheal occlusion (TO) with an inflatable balloon to stimulate lung development or its reversal against healthy controls.

Methods and data

51 AF samples from healthy fetuses, 22 from fetuses with CDH at the time-point of TO as well as 9 samples from CDH fetuses at the reversal of TO were evaluated. The samples were obtained during amniocentesis, cesarean section or the prenatal procedures described for CDH. Gestational age ranged from 15-38 weeks, 21-35 weeks and 31-35 weeks in fetuses of the control, CDH at TO and CDH at TO reversal group, respectively. ^{1}H NMR spectra of AF were acquired at 37°C using an Avance 400 MHz NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) after diluting 0.4ml amniotic fluid with 0.1ml D_2O in a 5mm NMR tube. Residual water signal was suppressed by means of selective excitation by using pulse field gradients. One-dimensional spectra were acquired with a spectral width of 12 ppm and using 16 k data points. Free induction decays were averaged over 8 k accumulations. A relaxation delay of 2'' was allowed. An exponential function was applied prior to Fourier transformation, resulting in a line broadening of 0.1 Hz. NMR spectra were phase and baseline corrected using the Topspin software (Bruker Biospin). Frequency alignment and normalization with respect to external sodium 3-(trimethylsiyl) propane sulfonate at 0.0 ppm was performed. The region between 0.0 and 4.2 ppm was selected for further studies. Principle Component Analyses (PCA) [3] and Multidimensional Scaling (MDS) [4] were separately applied to reduce the dimension of spectral patterns and select the most relevant features. Then, the effect of the feature extraction methods and the number of features considered in combination with automated classification techniques for the complicated pregnancies prognosis was studied. Three different classifiers: linear discriminant analysis (LDA), k-nearest-neighbor method (KNN) and support vector machine method (SVM), are considered and compared. To estimate the error of the classifiers 10-fold cross-validation was applied.

Results

^{1}H NMR on AF successfully discriminated the control group from the complicated pregnancy groups, regardless of the gestational age. The highest variance for our data is given when considering the first six most representative features. We observe that MDS in combination with the considered classifiers outperforms PCA in discriminating between the groups. Best accuracy is obtained when considering KNN applied on the features extracted with MDS, see Table 1. Also a significant difference between the two CDH subgroups is observed, see Figure 1. Analysis of ^{1}H NMR spectra based on comparison of peaks between the considered groups reveals higher levels of taurine in controls and additional not yet identified resonances are present at 1.16 and 1.19 ppm for the control group.

	MDS+LDA	MDS+KNN	MDS+SVM
HC vs CDH_TO	0.86	0.94	0.90
HC vs CDH_rTO	0.81	0.94	0.91
CDH_TO vs CDH _rTO	0.86	0.88	0.86

Table 1 Mean classification accuracy over 100 runs repeated random sampling for the differentiation of complicated and control pregnancies. First six most representative features were considered. HC stand for healthy controls, CDH_TO for CDH fetuses at TO and CDH_rTO for CDH fetuses at TO reversal.

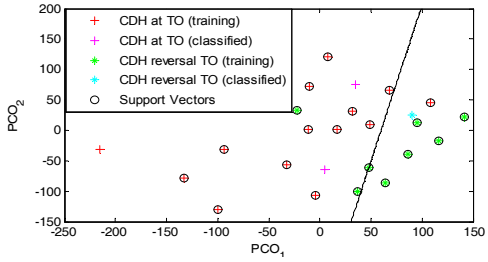

Figure 1 Scatter plot of a SVM fit for classification of CDH fetuses at TO vs CDH fetuses at TO reversal, by highlighting the classes and the support vectors. The PCOs represent the features extracted with MDS.

Conclusions

Using dimensionality reduction methods in combination with automated classifiers when analyzing ^{1}H NMR spectra of human AF, has a high potential in identifying complicated pregnancies. It can represent an attractive tool which in later clinical applications could be combined with routine fetal magnetic resonance imaging and thus help to obtain a more detailed picture of the fetal status.

References

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