Int J Clin Exp Pathol 2016;9(1):293-295
www.ijcep.com /ISSN:1936-2625/IJCEP0017160

Original Article

Expression of *COX7A1* as a blood marker of congenital heart defect

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Received September 30, 2015; Accepted November 22, 2015; Epub January 1, 2016; Published January 15, 2016

Abstract: Congenital heart defects occur in approximately 50% of children with Down syndrome and in 1% of general population. We aimed at defining a molecular blood marker, which could allow for early detection of such defects in case of prenatal testing, performed due to suspected congenital malformation. We conducted a whole genome expression analysis in 21 children with Down syndrome, with or without congenital heart defect. We found upregulation of the *COX7A1* gene in patients born with heart defect. The results of our preliminary study show potential value of assessment of *COX7A1* expressionin blood as a screening test for prenatal detection of congenital heart defects.

Keywords: Down syndrome, COX7A1, congenital heart defect, VSD, ASD

Introduction

Down syndrome is associated with typical facial dysmorphisms, intellectual disability and a spectrum of congenital malformations. Congenital heart defects are among the most severe malformations and occur in nearly 50% of children with this chromosomal abnormality [1]. The most common defects include ventricular septal defect (VSD), common atrioventricular channel (CAVC) and atrial septal defect (ASD) [2], similar to the general population. These defects are usually detected by means of fetal sonographic examination performed during pregnancy [3]. However, only large defects are diagnosed early. Down syndrome is usually diagnosed earlier than the small heart defects, based on prenatal testing of fetal cells, obtained by means of amniotic fluid sampling or cordocentesis. Unfortunately, no good biochemical or molecular markers exist, which could be used for early detection of congenital heart defects during routine prenatal diagnostic procedures. Such marker could be of use in order to increase the precision of genetic consulting of the mother, with regard to continuation of the pregnancy. In addition, as common pathologic mechanisms are probable in the process of development of major types of heart defects in embryos with or without Down syndrome, early molecular markers of heart defect can be potentially useful in prenatal testing in general population.

Several genes playing central roles in ubiquitous metabolic pathways follow similar expression patterns in various tissues [4]. As the tissues of the heart are in a close contact with blood, it could be assumed that the alteration of genome expression in the heart structures in case of heart defect might be partially detectable in the blood.

In light of the above facts, we aimed to find potential genomic blood markers of congenital heart defects in children with Down syndrome.

Materials and methods

A group of 21 children with Down syndrome, aged 7-16 years participated in our study. In all participants classic trisomy 21 was confirmed by means of cytogenetic analysis (mosaicism or translocation were excluded). The children were recruited at the Department of Medical Genetics, Jagiellonian University Medical College in Krakow, Poland. Patients and/or their parents signed an informed consent form (as required by the Jagiellonian University Ethics Committee).

Table 1. Data analysis of real-time PCR-validation of microarray results

Sample	COX7A1 Average Ct	GAPDH Average Ct	dCt (COX7A1-GAPDH)	ddCt (CHD+ vs. CHD-)	Fold difference
CHD+	32.10 ± 0.83	17.98 ± 0.26	14.12 ± 0.87	-0.99	1.99 (1.09-3.64)
CHD-	33.21 ± 0.64	18.10 ± 0.41	15.11 ± 0.76	0 ± 0.87	1.0

The patients were assigned to subgroups with a congenital heart defect (VSD, ASD, CAVC) or without such defect. Microarray technology was used to compare the whole genome expression in blood mononuclears between both subgroups.

Total RNA was extracted from blood samples (1 ml) using RiboPure Kit (Ambion). Subsequently, whole genome expression was assessed by means of Agilent SurePrint Human Gene Expression 8×60 K v2 Microarrays according to the manufacturer's protocol (single color technology). Next, GeneSpring software was used for data analysis. Moderated t-test was applied with the Westfall-Young correction for multiple comparisons to detect significant differences between compared groups (corrected $P<0.05$, fold change >2.0).

Validation of the microarray results was performed with use of real-time PCR (comparative quantitation). Samples of total RNA (500 ng) were used to perform reverse transcription by means of Superscript II reverse transcriptase (Life Technologies). Gene expression was measured with use of commercial TaqMan assays (Life Technologies) with *GAPDH* serving as a control gene.

Results

The group of children with congenital heart defect (CHD+) consisted of 13 patients (six girls and seven boys aged 7-16, mean age 9.6 years). VSD, CAVC and ASD were diagnosed in four, five and four children, respectively. The heart defects were surgically closed in all children in early infancy. The patients did not present with clinical signs of heart insufficiency at the study entry.

The group of children without heart defect (CHD-) consisted of 8 patients (two girls and six boys aged 7-15, mean age 9.6 years).

Genomic analysis revealed significant differences with regard to two transcripts: *COX7A1* (fold change 2.83) and *LOC102725407* (fold change 2.38). Both were upregulated in children with a history of congenital heart defect. The transcript *LOC102725407* seems not to be involved in development of congenital heart defects. Interestingly, the COX7A1 protein is one of the nuclear-coded polypeptide chains of cytochrome c oxidase, the terminal oxidase in mitochondrial electron transport in the muscle cell, and can play a role in heart muscle pathology.

The validation analysis confirmed the above findings (fold change 1.99). **Table 1** presents data analysis on real-time PCR validation.

Discussion

Recently, Chinese authors reported expression differences between healthy children and children with Down syndrome having a congenital heart defect [5]. However, alteration of genome expression resulting from the presence of additional genetic material in patients with Down syndrome might influence the results of such comparisons. Therefore we focused exclusively on patients with Down syndrome.

Our study shows that *COX7A1* gene is differently expressed in children with Down syndrome and with congenital heart defect in comparison to those with Down syndrome but without such defect. Previous studies of cytochrome c indicated the presence of expression of cytochrome VIa, VIIa, VIIIin all muscle tissues (heart, skeletal, smooth muscle) [6, 7]. It was demonstrated that *COX7A1* gene is primarily active in cardiac and skeletal muscle and probably responsible for tissue-specific energy demand in these muscles. Other investigators found that the overall activity of cyclooxygenase decreases in the heart of mice in case of alteration of *COX7A1* gene function. Cytochrome c oxidase is the proposed rate-limiting enzyme of aerobic metabolism in mammals [8].

We found upregulation of *COX7A1* gene in children with congenital heart defect. The patho-

physiological mechanism of this finding remains unclear. The potential role of the *COX7A1* gene in development of heart malformation cannot be excluded, but needs further studies. On the other hand, it seems probable that upregulation of *COX7A1* leads to increased energy production in response to heart defect and that increased expression of this gene could persist in our patients, despite successful surgical treatment of their hearts. This indicates the presence of a subtle, subclinical dysfunction of the heart muscle, not detectable during routine clinical examination but responsible for stimulation of *COX7A1* expression. Consequently, the up-regulation of *COX7A1* could be a marker of heart muscle exertion. It could also have potential for use as a diagnostic test, such as a screening test for early, prenatal detection of congenital heart defects.

In summary, our preliminary results suggest usefulness of expression assessment of *COX-7A1* as a blood marker of presence of most common types of congenital heart defects even many years after their surgical correction. The potential of this marker as a diagnostic test needs to be evaluated.

Acknowledgements

The study was sponsored by the Polish National Science Centre (DEC-2011/03/B/NZ5/01328). The publication was financed by KNOW (Leading National Research Centre).

Disclosure of conflict of interest

None.

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