

# CARDIAC GENE EXPRESSION OF GATA-4 TRANSCRIPTION FACTOR IN HUMAN TRISOMY 21 FETUSES WITH INCREASED NUCHAL TRANSLUCENCY

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## SUMMARY

This study examines GATA-4 gene expression in cardiac tissue from fetuses with trisomy 21 presenting with increased nuchal translucency thickness at 10–14 weeks of gestation. mRNA was extracted from cardiac tissue after termination of pregnancy at 10–18 weeks of gestation in ten trisomy 21 fetuses and 29 normal controls. Northern and slot blots were performed and densitometric analysis of slot blots was used to determine the steady-state levels of expression of GATA-4. GATA-4 transcript levels were also compared with ANP and BNP, which have previously been measured in the same panel of samples. GATA-4 expression increased significantly with gestation but there was no significant difference between fetuses with trisomy 21 and controls. There was no significant association between GATA-4 expression and the steady-state level of transcripts for the natriuretic peptides. © 1998 John Wiley & Sons, Ltd.

KEY WORDS: nuchal translucency; trisomy 21; gene expression; transcription factor; GATA-4; natriuretic peptide

## INTRODUCTION

Chromosomal abnormalities are associated with subcutaneous oedema in the nuchal region that is visualized by ultrasonography as increased nuchal translucency thickness at 10–14 weeks of gestation (Nicolaidis *et al.*, 1992; Snijders *et al.*, 1996).

A possible cause for increased nuchal translucency thickness is cardiac dysfunction, because anatomical studies have demonstrated that a high

proportion of both chromosomally normal and abnormal fetuses with increased translucency have defects in the heart and great arteries (Hyett *et al.*, 1997a,b). At present, it is not possible to investigate directly fetal cardiac function in the first trimester of pregnancy. However, indirect evidence for possible heart failure in fetuses with increased nuchal translucency was provided by the finding of increased gene expression of natriuretic peptides in cardiac tissues from trisomy 21 fetuses (Hyett *et al.*, 1996). Animal studies have demonstrated that GATA-4 plays an important role in both cardiac development and function. In the rat, GATA-4 is involved in the regulation of transcription of a number of cardiomyocyte-specific genes including ANP and BNP promoters (Grepin *et al.*, 1994; Thuerauf *et al.*, 1994). The aim of this study was to investigate GATA-4 gene

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expression in the hearts of first-trimester fetuses with trisomy 21.

## MATERIALS AND METHODS

GATA-4 gene expression was investigated in the cardiac tissue of fetuses with trisomy 21 and normal fetuses at 10–18 weeks of gestation. All heart tissues were collected immediately following therapeutic termination of pregnancy. Written informed consent was obtained from the patients. The study was approved by the hospital's ethical committee and tissue collection was made in accordance with the Polkinghorne guidelines on the research use of fetal material (Polkinghorne, 1989).

The diagnosis of trisomy 21 ( $n=10$ ) was made by chorionic villus sampling, which was performed after an ultrasound scan at 10–14 weeks of gestation demonstrated increased fetal nuchal translucency thickness (Nicolaidis *et al.*, 1992). The normal heart tissues were obtained from pregnancies terminated for psychosocial reasons ( $n=29$ ). Cardiac tissue was identified and dissected from the evacuated products and immediately frozen in RNase-free polypropylene tubes in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . The preservation of tissue for mRNA studies precluded anatomical examination.

RNA extraction was performed and Northern and slot blots were prepared as previously described (Brizot *et al.*, 1995). The probe for human GATA-4 was derived from a polymerase chain reaction (PCR) product which had been cloned and confirmed by sequence analysis (Central Molecular Biological Services, King's College School of Medicine and Dentistry, London, U.K.). The primers used were 5'TTTTGGAGTCAGATTTGGTATTAGG3' and 5'CTATGCGTCTCCCGTCAGC3', which amplify a 620 bp fragment of the C terminus and the 3' untranslated region of human GATA-4 (Genbank accession number L34357). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control for loading and transfer. The GAPDH probe consisted of a 360 bp fragment of human GAPDH (Genbank accession number M33197). Autoradiography was then carried out and signals from slot blots were measured. The densitometric scores of GATA-4 were normalized to the signal obtained for GAPDH by dividing the densitometric values of the target gene by the GAPDH

values, thus correcting for any uneven loading of the RNA samples.

## Statistical analysis

Spearman rank correlation analysis was used to determine the possible association between GATA-4 and gestation, and the Mann-Whitney *U*-test was used to compare values in the trisomic group with the controls. Regression analysis was used to examine the significance of the association between GATA-4 expression and fetal nuchal translucency thickness.

## RESULTS

In the Northern blots of RNA extracted from normal and trisomic fetal cardiac tissue, transcripts of approximately 4 kb for GATA-4 and 1.6 kb for GAPDH (as a loading control) were detected (Fig. 1). Normalized densitometric scores were obtained from slot blots. In the normal controls, the scores for GATA-4 increased significantly with gestation (Fig. 2,  $r=0.223$ ,  $P<0.05$ ). The median densitometric score in fetuses with trisomy 21 was not significantly different from normal (Fig. 2,  $t=0.19$ ,  $P=0.86$ ) and there was no significant association between GATA-4 expression and nuchal translucency thickness ( $P<0.05$ ).

## DISCUSSION

The findings suggest that in normal fetal hearts, there is a significant increase in the GAPDH-normalized levels of expression of GATA-4 within the gestational range of 10–18 weeks. Additionally, in this gestational range, there is no significant difference from normal in the expression of GATA-4 cardiac tissue in trisomy 21 fetuses, or a significant association with fetal nuchal translucency thickness. Furthermore, there was no significant correlation between the level of GATA-4 mRNA expression and ANP or BNP transcript levels which had been previously measured in the same panel of samples (Hyett *et al.*, 1996).

Contractility of cardiac myocytes is determined by the expression of a set of cardiac muscle-specific genes. By analogy to other mammalian developmental systems, it is likely that the coordinate

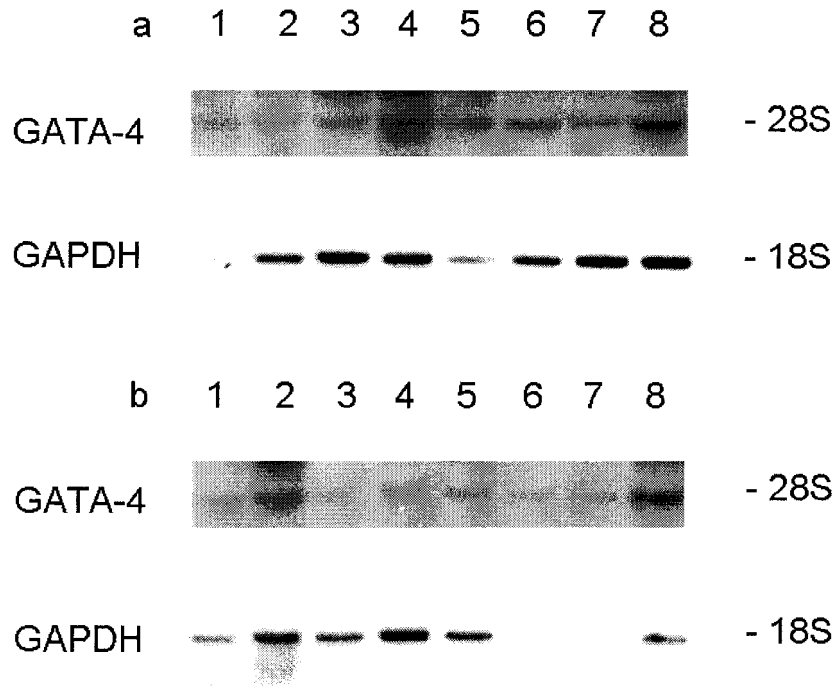


Fig. 1—Representative Northern blot of RNA extracted from normal (a) and trisomic (b) human fetal cardiac tissues (trisomy 21, lane b: 1, 2, 4, 7, 8; trisomy 18, lane b: 3, 5, 6). Bands represent single transcripts for GATA-4 (4.0 kb) and GAPDH (1.6 kb) as a loading control

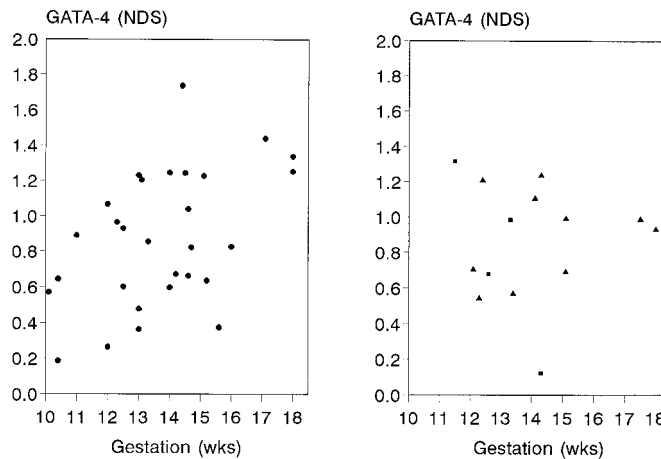


Fig. 2—Normalized densitometric scores for GATA-4 of fetal hearts in relation to gestational age in 29 normal pregnancies (●) (left), and ten fetuses with trisomy 21 (▲) and four fetuses with trisomy 18 (■) (right)

expression of cardiac genes is controlled by lineage-specific transcription factors that interact with promoter and enhancer elements in the transcriptional regulatory regions of these genes.

GATA-4 is actively expressed in cardiac tissue and is involved in the regulation of transcription of a number of cardiomyocyte-specific genes, including cardiac muscle-specific troponin C promoter-

enhancer (Ip *et al.*, 1994), alpha-myosin heavy-chain gene (Molkentin *et al.*, 1994) and ANP and BNP promoters (Grepin *et al.*, 1994, 1995; Thuerauf, 1994). However, alterations in GATA-4 expression have not yet been demonstrated in cardiac disease. In the rat, GATA-4 gene expression has been shown in coelomic epithelial cells of the primitive streak embryo, the endocardium, myocardium, and embryonic structures such as the septum transversum and endocardial cushion tissue of the developing heart (Heikinheimo *et al.*, 1994; Kelley *et al.*, 1993). GATA-4, which is located on 8p23.1 (Huang *et al.*, 1995; White *et al.*, 1995), has not been examined previously in human fetuses.

GATA-4 transcript levels in the hearts of fetuses with trisomy 21 at 10–18 weeks did not correlate with changes in the expression of ANP or BNP. Therefore, either GATA-4 plays no part in the regulation of these genes in this particular context, or the complexity of the regulation is such that it is not reflected by any simple relationship in the steady-state levels of the respective transcripts. Steady-state expression depends on the degradation rate as well as on the rate of transcription. If a decrease in degradation was a mechanism for regulation by GATA-4, this would be represented in the Northern blots. Alteration in the rate of translation of GATA-4 into protein, or stability or activity of that protein, would be consistent with GATA-4 control over ANP or BNP transcription without correlation with the mRNA levels. However, it is unlikely that GATA-4 is involved in the regulation of other genes at post-transcriptional levels, because it is almost certainly a transcription factor.

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