SHORT REPORT

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Heart morphogenesis is not affected by overexpression of the *Sh3bgr* gene mapping to the Down syndrome heart critical region

Abstract Congenital heart disease (CHD) is the most common birth defect in humans and is present in 40% of newborns affected by Down syndrome (DS). The *SH3BGR* gene maps to the DS-CHD region and is a potential candidate for the pathogenesis of CHD, since it is selectively expressed in cardiac and skeletal muscle. To determine whether overexpression of *Sh3bgr* in the murine heart may cause abnormal cardiac development, we have generated transgenic mice using a cardiac- and skeletal-muscle-specific promoter to drive the expression of a *Sh3bgr* transgene. We report here that heart morphogenesis is not affected by overexpression of *Sh3bgr*.

Introduction

A 5-Mb region in 21q22.2 encompassing *D21S55* to *MX1* has been associated with congenital heart disease (CHD) present in 40% of newborns affected by Down syndrome (DS). The complete sequencing of human chromosome 21 has recently shown that this region contains less than 30 genes (Hattori et al. 2000). The functional characterization of these genes is a crucial step towards the identification of genes involved in the pathogenesis of DS-CHD. The *SH3BGR* gene maps to the DS-CHD critical region and has been previously isolated by cDNA selection in an effort to identify genes that are located in this region and

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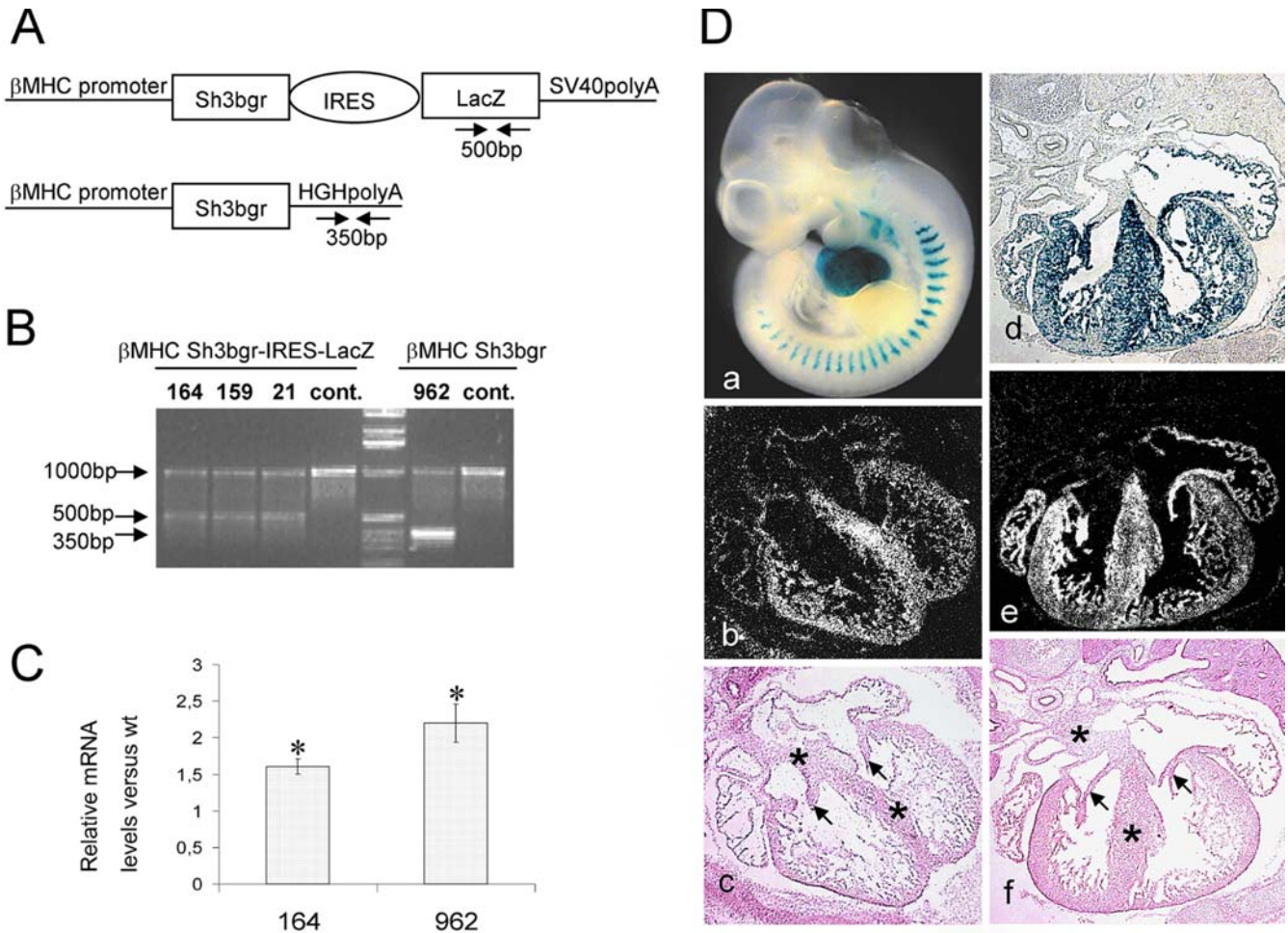Ospedale Galliera Mura delle Cappuccine 14, 16128 Genoa, Italy

that are expressed in the developing heart (Scartezzini et al. 1997). An expression study in the mouse has revealed that the corresponding murine gene is expressed in the heart from early stages of embryonic development (Egeo et al. 2000).

The mechanisms by which an extra copy of chromosome 21 results in the various clinical aspects of DS are still unknown. It remains to be established whether the defects seen in DS are attributable to the overexpression of specific individual genes or small groups of genes located on the triplicated chromosome (Chrast et al. 2000) or to a general stochastic deregulation of transcription when three copies of the chromosome are present (Saran et al. 2003). The use of both microarray approaches and single gene overexpression will be necessary to dissect the molecular mechanisms that underlie the development of DS phenotype. We have used a transgenic approach to explore the functional role of the *Sh3bgr* gene in heart development and its possible involvement in DS-CHD.

Materials and methods

Two different constructs, one containing only the mouse *Sh3bgr* gene and another also containing a *lacZ* gene placed after an internal ribosomal entry site element, were used for the production of the transgenic lines on an FVB genetic background (Fig. 1A). Gene expression in both constructs was driven by the beta/slow MyHC promoter that, in mouse embryos, is expressed exclusively in heart and skeletal muscle (Knotts et al. 1996). We obtained several transgenic lines (Fig. 1B); however, only two lines (164 and 192) were found to express the transgene (not shown). The *Sh3bgr* level of expression in the two transgenic lines was analyzed by real-time polymerase chain reaction (PCR).

Results and discussion

As shown in Fig. 1C, a moderate but significant overexpression of *Sh3bgr* gene (1.6 for line 164 and 2.1 for line 962) was observed at day 17 of embryonic development (E17) in the hearts of transgenic embryos compared with wild-type littermates.

Fig. 1A–D A Schematic representation of the two constructs used to overexpress the *Sh3bgr* gene. The relative position of the primers used for genotyping is indicated. **B** Transgenic positive lines are identified by PCR from genomic DNA. The 1-kb PCR product corresponds to the *α-5 integrin* gene, amplified as a control. **C** Level of expression of *Sh3bgr* gene determined in E17 transgenic hearts by quantitative real-time PCR. Values are expressed as the fold-increase over wild-type (*wt*) littermates and are normalized for *β-actin* (n=5–8 for each group; *$P<0.05$ versus wt). **D** Expression pattern of *Sh3bgr* transgenes. *Part a* E10 embryo, line 164. Whole mount *LacZ* staining; β-gal positive cells are present exclusively in heart and skeletal muscle. *Parts b, c* E14 embryo, line 962. Sections processed for in situ hybridisation with ^{35}S-labelled cRNA probes specific for the *HGHpolyA* gene, counterstained with eosin and viewed with darkfield (*Part b*) or brightfield (*Part c*) optics. Note that both septa (*asterisks*) and atrioventricular valves (*arrows*) are normal. *Parts d–f* E16 embryo, line 164. Serial sections of the heart processed for β-gal staining (*Part d*) or in situ hybridisation with probes specific for *lacZ* gene viewed with darkfield (*Part e*) or brightfield (*Part f*) optics. Note that both septa (*asterisks*) and atrioventricular valves (*arrows*) are normal

In human trisomy 21, the most frequent and specific heart abnormalities are endocardial cushion defects (atrioventricular canal defects and atrioventricular septal defects). In the mouse, endocardial cushion development starts at E10 and septation is almost completed by E14. The transgenes were expressed in cardiac muscle at E10, as determined by reverse transcription/PCR in both lines (not shown) and by whole mount *lacZ* staining for line 164 (Fig. 1D, part a) and was also observed at subsequent developmental stages, as determined by *lacZ* staining or by in situ hybridization with specific radioactive probes (Fig. 1D, parts b–f). Heart morphology was not altered in transgenic hearts at E14 and E16. In particular, the four chambers were correctly separated by valves and septa (Figs. 1D, parts c, f); the outflow tract, which is also frequently altered in the murine model for DS (Ts16), was normally developed in both lines (data not shown). The transgene was expressed at birth and in the adult; heart morphology was also normal at these stages in both transgenic lines (not shown).

In conclusion, we have produced two transgenic lines that moderately overexpress the *Sh3bgr* gene with an expression profile that resembles endogenous *Sh3bgr* gene expression throughout cardiac development. These lines therefore provide an appropriate model to study the contribution of *SH3BGR* gene to the heart phenotype of DS. We have not observed any gross difference in heart morphology between the transgenic animals and their wild-type littermates and, hence, *Sh3bgr* gene overexpression per se is not accompanied by congenital heart defects in the mouse, at least not in those with an FVB genetic background. Our results do not rule out the possibility that

modifier genes are involved in this phenotype; indeed, only a proportion of the patients with Down syndrome develop heart malformations. Furthermore, overexpression of the *Sh3bgr* gene together with other genes of the critical region may be necessary to induce the heart phenotype.

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