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Mapping of Chromosomal Breakpoints Associated with Congenital Heart Defects Using Fluorescence *In-situ* Hybridization (FISH):A Bypass for Isolation of Candidate Disease Genes

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Introduction

Congenital heart defects (CHD) is the most common group of inborn malformations. The incidence of moderate to severe CHD is 6/1000 [1]. The etiology of CHD is complex and involves both environmental and genetic factors. Studies of model organisms and isolation of human genes involved in syndromes, which include CHD in the phenotype, have given some insight in the molecular mechanisms behind. However, the number of identified genes is less than 20 and the genetic basis of CHD is presently far from understood.

The Mendelian Cytogenetic Network (MCN) was initiated in 1995 as a worldwide collaborative study to identify disease associated balanced chromosome rearrangements (DBCRs). Mapping of breakpoints in DBCRs has been instrumental in the isolation of many disease genes and may be used as a shortcut for identification of genes involved in CHD.

The MCN database (MCN*db*, www.mcndb.org) contains data on 125 patients with CHD and ba-lanced chromosome rearrangements. Interestingly, many of the breakpoints associated with CHD are shared among several patients in the database, suggesting that these loci contain genes involved in development of CHD (figure 1).Our strategy for isolation of disease genes using DBCRs is outlined in figure 2.

Results

We have identified three CHD patients with DBCRs of potential interest. Clinical phenotypes and karyotypes are listed in table 1. Two of the breakpoints, 5q13 and 8p23, have been mapped using FISH (figure 3 and 4).

The 5q13 breakpoint is shared among five unrelated CHD cases in MCN*db* (figure 1). Deletions in 8p23 often include CHD in the clinical spectrum [2].

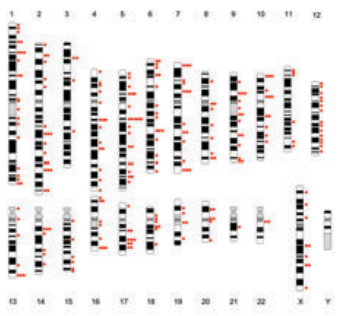

Figure 1. *Location of chromosomal breakpoints reported among patients with congenital heart defects, which have been registered in MCNdb. Each dot represents a patient with a chromosomal breakpoint in the corresponding band.*

Table 1. CHD patients included in this study.

Clinical phenotype	Karyotype
Ebstein´s anomaly Mild tricuspid valve regurgitation Small atrial septal defect	46, XY, inv (5) (q34q13) *de novo*
Ventricular septal defect	46, XY, inv (5) (p13q13) *mat*
Atrial septal defect Cleft palate	46, XY, inv (8) (p23q13) *mat*

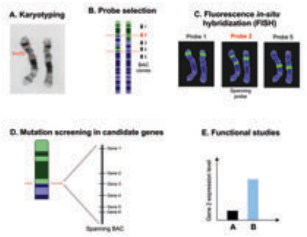

Figure 2. *Strategy for isolation of disease genes using disease associated balanced chromosome rearrangements. A. Karyotyping of a patient with CHD reveals a balanced chromosomal rearrangement (e.g. an inversion). B. Bacterial artificial chromosome (BAC) clones are selected in the breakpoint area and used as template for FISH probes. C. The breakpoint is mapped using FISH. Probes located distal or proximal to the breakpoint results in two signals (e.g. probe 1 and 5) while probes which span the breakpoint results in three signals (probe 2). D. Candidate genes located in the breakpoint area are screened for mutations in a cohort of CHD patients. E. Candidate genes are further characterized by functional studies.*

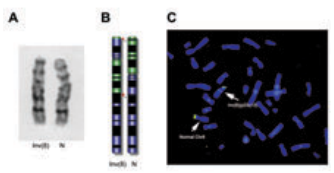

Figure 3. *FISH mapping of a breakpoint in 8p23. The karyotype of the patient is 46, XY, inv (8) (p23q13)* mat. *A. G-banded chromosomes. The pericentric inversion is marked by an arrow. B. Ideogram of a normal chromosome 8 and the chromosome 8 with the inversion. C. Result of FISH analysis. The probe derived from the BAC spanning the 8p23 breakpoint gives two signals on the chromosome with the inversion.*

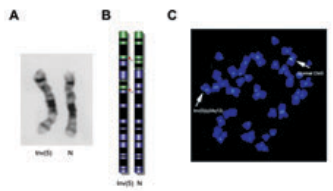

Figure 4. *FISH mapping of a breakpoint in 5q13. The karyotype of the patient is 46, XY, inv (5) (q13q34)* de novo. *A. G-banded chromosomes. The paracentric inversion is marked by an arrow. B. Ideogram of the normal chromosome 5 and the chromosome 5 with the in-version. C. Result of FISH analysis. The probe derived from the BAC spanning the 5q13 breakpoint gives two signals on the chromosome with the inversion.*

Materials and Methods

BAC clones from the RP11 library was obtained from the Max Planck Institute for Molecular Genetics, Berlin. FISH was carried out using standard protocols. 250 ng BAC DNA was labelled using a biotin-14-dATP nick translation kit (Invitrogen) and hybridized to metaphase chromosomes prepared from peripheral blood lymphocytes. Probe signals were visualized using avidin-FITC. Chromosomes were visualized by DAPI staining. A DMRB epifluorescence microscope (Leica) equipped with a sensys 1400 CCD camera (Photometrics) and IPLAP spectrum imaging software (Vysis) was used to inspect the signals.

Acknowledgements

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References

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[2] Giglio *et al.* (2000) *Circulation.* 102:432-7.

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