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Reduced atrial connexin43 expression after pediatric heart surgery

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Abstract

Myocardial dysfunction and arrhythmias may be induced by congenital heart defects, but also be the result of heart surgery with cardiopulmonary bypass (CPB), potentially caused by differential expression of connexin40 (Cx40) and connexin43 (Cx43). In 16 pediatric patients undergoing corrective heart surgery, connexin mRNA expression was studied in volume overloaded (VO group, $n = 8$) and not overloaded (NO group, $n = 8$) right atrial myocardium, excised before and after CPB. Additionally, in eight of these patients ventricular specimens were investigated. The atrial Cx43 expression decreased during CPB, which was restricted to the VO group ($p = 0.008$). In contrast, atrial Cx40 mRNA did not change during CPB. In ventricular myocardium compared to atrial mRNA levels, Cx40 was lower ($p = 0.006$) and Cx43 higher ($p = 0.017$) expressed, without significant change during CPB. This study revealed a significant influence of CPB and the underlying heart defect on Cx43 expression.

Keywords: Connexin; Cardiopulmonary bypass; Pediatric; Volume overload; Atrium

Myocardial remodeling may occur after different forms of acquired cardiac disease, particularly in adult patients. In the young this may result from congenital heart defects (CHDs), occuring in about 0.8% of live births [1]. Atrial arrhythmias are important determinants of morbidity and mortality in the natural and post-operative course of patients with significant ASD [2]. Multiple studies have shown the impact of gap-junctional remodeling in different forms of atrial disease [3]. A differential expression of the gap-junctional components connexin40 (Cx40) and connexin43 (Cx43) in adult patients with atrial fibrillation (AF) and in animal models may contribute to changed electrical conduction properties and evolution of arrhythmias [4]. Electrical and mechanical dyssynchrony in cardiomyopathy and other forms of ventricular disease leading to arrhythmia and heart failure may also be attributable to connexin remodeling [5–7].

Moreover, cardiac surgery with cardiopulmonary bypass (CPB) faces the heart with a significant stress, similar to that of ischemia or other conditions with cardiac overload. CPB has been shown to cause differential expression of multiple genes [8,9], and it may complicate the post-operative course leading to contractile dysfunction [10] and arrhythmias [11]. Recently, the influence of CPB on connexin expression has been revealed, particularly patients with coronary artery disease undergoing heart surgery showing a marked downregulation of right atrial Cx43 during CPB [12].

Comparing these studies it has to be kept in mind that the connexin expression is species specific and developmentally regulated [13–16]. Therefore, these results from adults and from animal models cannot be adopted to the situation in childhood. Previously, we have shown that CPB reduced the sodium pump, a key component of the cardiomyocyte

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[17]. We hypothesized that connexin expression is also influenced by the CPB, and that the myocardial susceptibility for this might be influenced by dilatation of the myocardium.

Materials and methods

Samples. In 16 patients undergoing corrective heart surgery before initiation of and immediately after CPB, 20 mg of not injured myocardial tissue from the right atrial free wall was obtained. Moreover, in eight of these patients it was possible to obtain ventricular samples. In all patients the surgical procedure required a right atriotomy. The tissue was snap-frozen in liquid nitrogen and stored at −80 °C until further processing. Written informed consent was obtained from all patients. The study protocol has been approved by the Local Ethics Committee (Request-Nr. 168/2000). Pre-operatively the area of the right and left atrium was calculated in all patients by echocardiography (Acuson Sequoia 512®, Mountain View, CA, USA). According to the atrial dimensions and the ratio between right and left atrial area, the patients were assigned to a group having a right atrial volume overload (VO) and a group without volume overload (NO) of the right atrium. Only in one patient with a complex cardiac anomaly (transposition of the great arteries, ventricular septal defect, dysplastic aortic valve, and hypoplastic aortic arch) an additional right ventriculotomy was performed. After sternotomy and opening the pericardium, a first sample of right atrial myocardium was taken. The ascending aorta and both caval veins were cannulated, patients were cooled to 32 °C, aorta was clamped, and cold blood cardioplegia was given. At the end of the operation, after a warm reperfusion blood cardioplegia, the aortic clamp was opened and reperfusion was started. After weaning from CPB, again about 20 mg of myocardial tissue from the right atrial free wall was obtained. The tissue was snap-frozen in liquid nitrogen and stored at −80 °C until further processing.

Isolation of RNA and quantitative transcript analysis. The study protocol has been described previously [7], in essence, 10 mg of right atrial myocardium was used for extraction of total cellular RNA. Frozen samples were homogenized. After DNaseI treatment, RNA extraction was performed with the RNeasy Mini-Kit (Qiagen) using the protocol for heart tissue, including Proteinase K digestion. The amount of isolated RNA was determined using the RiboGreen dye (Molecular Probes, Eugene, OR) according to the manufacturer's instructions. For quantitative one-step RT-PCR a OneStep RT-PCR Kit (Qiagen, Hilden, Germany) was used. Two microliters (approx. 20 ng) of total RNA was reverse transcribed and directly amplified using a LightCycler® system (Roche, Rotkreuz, Switzerland). Online fluorescence monitoring was performed by adding SYBR-Green I. The final reaction volume was 20 μl and final reaction concentrations were as follow: 1× OneStep RT-PCR Buffer, 400 μM of each dNTP, 0.6 μM of each primer, 7.5 units RNase inhibitor, 1:2 105 dilution of freshly prepared SYBR-Green I (Sigma–Aldrich, St. Louis, MO), and 1 μl of the OneStep RT-PCR Enzyme Mix. LightCycler® conditions were as follows: reverse transcription for 30 min at 50 °C, initial RT inactivation and polymerase activation for 15 min at 95 °C, PCR rapid cycling for 40 cycles: denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, elongation 15 s at 72 °C, and final melting curve analysis from 58 to 95 °C with a slope of 0.1 °C/s. For quantification of the initial amount of the specific mRNA a novel mathematical model was used, fitting the kinetics of real-time PCR much better [18,19] than formerly described methods. The amount of the specific mRNA was expressed as gene level per microgram total RNA.

Primer design and PCR product characterization. The primer pairs were designed to discriminate between the different isoforms. Each primer pair contained at least one exon boundary spanning primer, in order to prevent amplification of contaminating genomic DNA. The calculated annealing temperatures were identical for both primer pairs. Building of primer dimers was excluded using software OLIGO 6.1 (MedProbe, Norway). The primer information is summarized in Table 1. In addition to the quantitative analysis of PCR product amplification and the melting curve analysis using the LightCycler® system, RT-PCR products were analyzed by PAGE and silver staining to ensure accurate amplification. Reagent samples without template were included to identify contamination. Additional confirmation of product identity was performed by sequencing of forward and reverse strands of several products with the PRISM Ready Reaction Dye Deoxy Terminator Sequencing Kit (Perkin-Elmer/ABI, Huenenberg, Switzerland), 2500 V at 30 °C for 10 h on an ABI 373 (Applied Biosystems, Rotkreuz, Switzerland).

Data analysis and statistical methods. The statistical analysis of the data was performed by a statistical consultant (R.A.). Because of the small sample size and skewed data distribution, nonparametric exact methods were applied throughout, using commercially available software (StatXact 6 and LogXact 6, both from Cytel Software Corp., Cambridge, USA). The three dichotomous clinical variables gender, VO vs. NO, and infants up to the age of 12 months vs. older children were used to form groups of patients. For univariate analyses, evaluating differences of mRNA levels between these dichotomous groups, Hodges–Lehmann estimates and exact 95% confidence intervals (CI) of the differences were calculated, and exact Wilcoxon–Mann–Whitney tests applied. Exact Jonckheere–Terpstra (JT) [20] tests were used for testing associations between mRNA levels and CPB-related variables. For multivariate analysis of clinical and CPB-related variables that were significantly associated with the respective mRNA levels, exact polytomous regression applying the adjacent category logit model [21] was used. This is a nonparametric exact method suited to the analysis of small datasets, corresponding functionally to the well-known multivariate linear regression method applicable in larger datasets. In order to allow for regression analysis, the CPB-related variables (CPB time, aortic clamp time, and reperfusion time) were dichotomized at their medians, and continuously measured mRNA levels were transformed into ordinal variables with eight levels each. Results of regression analysis are expressed as β regression coefficients with their exact 95% CI. Two-sided tests were used throughout, p levels < 0.050 were considered statistically significant. Because of the exploratory nature of the study, no correction for multiple comparisons was performed.

Results and discussion

The main finding of this study in pediatric patients was a differential Cx43 mRNA expression during heart surgery and the contribution of hemodynamic overload for susceptibility to the CPB. In our patients the VO group ($n = 8$) and the NO group ($n = 8$) differed significantly regarding the ratio of right vs. left atrium ($p < 0.0001$, Table 2), whereas, there was no difference regarding age and gender

Table 1
Nucleotide sequence of the primers used in the study

Gene			Product length (bp)
Cx40	fwd	5′ GAGGAGGAAAAGAAGCAGAAGTTT 3′	194
	rev	5′ TCATCCCCCCAGGAAGACT 3′	
Cx43	fwd	5′ CACTACTTTTAAGCAAAAGAGTGGTG 3′	198
	rev	5′ ACAACGAAAGGCAGACTGCT 3′	

Cx40, connexin40; Cx43, connexin43; fwd, forward primer; rev, reverse primer.

Table 2
Patients and cardiopulmonary bypass data

	VO	NO	*p*
n	8	8	
Age (months)	33 (1–133)	6.5 (2–183)	n.s.
Gender (m:f)	5:3	6:2	n.s.
RA:LA ratio	2.1 (1.5–2.9)	0.9 (0.5–1.4)	<0.0001
CPB (min)	60 (17–358)	58 min (50–97)	n.s.
Aortic clamp (min)	36 (7–180)	38 min (9–69)	n.s.
Reperfusion (min)	19 (10–213)	23 min (11–42)	n.s.

CPB, cardiopulmonary bypass; f, female; LA, left atrium; m, male; RA, right atrium; n.s., not significant.

distribution, duration of cardiopulmonary bypass, aortic clamp or reperfusion time. Moreover, the maximal troponin-I, need and length of milrinone therapy, ventilation, and ICU stay were not different between the VO and NO group (data not shown). One patient in the VO group (CAVC) had a post-operative arrhythmia (junctional ectopic tachycardia) and was treated with amiodarone. One other patient with a complex transposition of the great arteries and a large atrial septal defect (VO group) died on the second post-operative day.

Atrial connexin expression

The mRNA expression of atrial Cx40 was not different between specimens with normal hemodynamics (NO group) and those from volume overloaded right atria (VO group), neither pre-operatively, nor after the CPB (data not shown). The Cx40 mRNA expression did not change significantly during the CPB (before CPB, median 9.99×10^{-06}, range 9.16×10^{-08}–3.55×10^{-05}; after CPB, median 7.51×10^{-06}, range 6.55×10^{-08}–1.98×10^{-05}, $p=0.44$). There were no significant associations of the Cx40 mRNA levels and their change during the CPB, neither with the three CPB-related time variables, nor with gender, age group, and volume overload status (Figs. 1A and B). In five patients (two from the VO, three from the NO group) there was an increase of atrial Cx40 mRNA during the CPB. This was not associated with age (range 2–183 months) or gender (two females, three males). In three of them there was also an increase of Cx43 during the CPB.

The Cx43 mRNA expression before CPB (median 1.61×10^{-04}, range 5.39×10^{-06}–3.25×10^{-04}) was not influenced by gender, age group, and volume overload status (data not shown). After the CPB the median Cx43 mRNA expression was 6.17×10^{-05} (range 2.62×10^{-06}–3.32×10^{-04}), hence, there was a decrease during CPB (median decrease, 3.45; 95% CI, 0.05–10.2, $p=0.044$). The decrease was restricted to the VO group (median decrease, 10.8; 95% CI, 2.8–19.9, $p=0.008$, Fig. 2A), while in the NO group there was no significant change of Cx43 mRNA expression during CPB (median change, 0.8; 95% CI, −1.7 to 4.0; $p=0.46$, Fig. 2B). The difference between VO and NO group remained significant in multivariate analysis (β, −1.25; 95% CI, $-\infty$ to −0.23; $p_{\text{multivariate}}=0.006$), hence, it was independent from gender, age group, volume overload status, and the three CPB-related variables. In four patients (all from the NO group) there was an increase of atrial Cx43 mRNA during the CPB. This was not associated with age (range 2–183 months) or gender (two females, two males).

There are conflicting studies on gap-junctional remodeling in atrial disease comparing patients with atrial fibrillation (AF) and subjects in sinus rhythm. In adult patients AF was associated with a reduction of right atrial Cx40 [22] and a downregulation of Cx43 [23]. In contrast, Polontchouk et al. [24] found no change in Cx43 protein content, but an increase in Cx40 protein expression in patients with AF. In patients with AF due to mitral valve disease left atrial Cx40 and Cx43 protein content was increased compared to patients in sinus rhythm [3]. The occurrence of AF after heart surgery was associated with an elevated atrial Cx40 expression [25]. However, whether the change of connexin expression is causal or reactive in these studies remains unclear. The CPB itself has been shown to cause a reduction of Cx43 in patients with coronary artery disease [12]. We could show that this

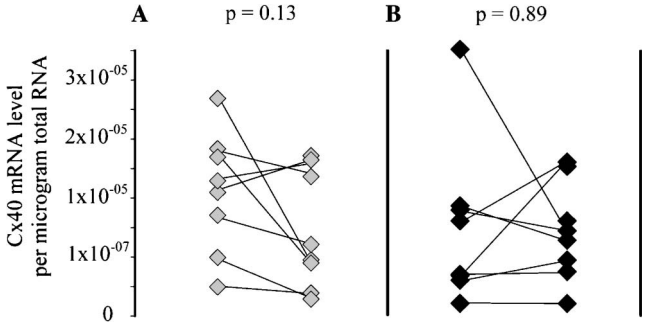

Fig. 1. Atrial Cx40 mRNA expression before and after cardiopulmonary bypass. Cx40 mRNA expression did not change significantly during the CPB (before CPB, median 9.99×10^{-06}, range $9.16\times^{-08}$–3.55×10^{-05}; after CPB, median 7.51×10^{-06}, range 6.55×10^{-08}–1.98×10^{-05}, $p=0.44$), (A) neither in the VO (◇), (B) nor in the NO (♦) group.

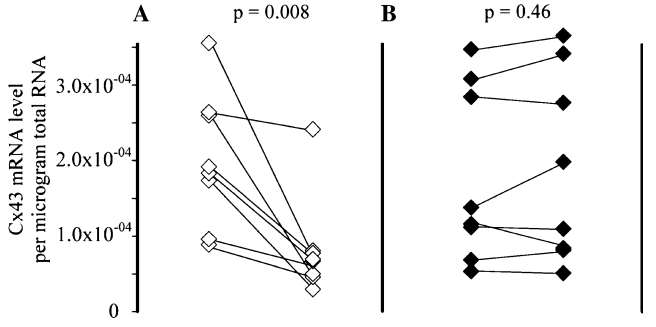

Fig. 2. Atrial Cx43 mRNA expression before and after cardiopulmonary bypass. The median Cx43 mRNA expression before CPB was 1.61×10^{-04} (range, 5.39×10^{-06}–3.25×10^{-04}), and after the CPB 6.17×10^{-05} (range, 2.62×10^{-06}–3.32×10^{-04}). (A) The decrease was restricted to the VO group (◇, median decrease, 10.8; range 2.8–19.9, $p=0.008$). (B) Cx43 mRNA expression during CPB was unchanged in the NO group (♦, median change, 0.8; range −1.7 to 4.0; $p=0.46$).

phenomenon is found not only in the adult human with heart disease but also in the pediatric population with only modest forms of hemodynamic overload. In our patients the CPB had a significant influence on Cx43 mRNA expression, however, this was restricted to patients with dilated myocardium, due to a heart defect with a left-to-right shunt between the atria.

The ratio of atrial Cx43/Cx40 mRNA did not change during the CPB (before CPB, median 18.5, range 4.9–56; after CPB, median 11.1, range 3.9–40, $p = 0.20$). There were no significant associations of the Cx43/Cx40 ratio with gender, age group, volume overload status, CPB time, aortic clamp time, or reperfusion time (data not shown). The importance of the ratio of the both gap-junctional components was suggested in a paper comparing conduction velocity and immunodetectable gap-junctional protein expression [26], showing atrial conduction to decrease with increasing Cx40, however, there was even a stronger correlation between conduction velocity and the ratio of Cx43/Cx40.

Ventricular connexin expression

Alterations in gap-junctional expression and organization, particularly the reduced Cx43 expression, have been implicated in the evolution of arrhythmias and heart failure in different forms of ventricular disease [27–29]. Moreover, the connexin expression differed significantly between the various heart chambers and the conduction tissue. Cx43 is the principal connexin in atrial and ventricular myocytes, Cx40 expression is restricted mainly to atrial and usually not expressed in ventricular cardiomyocytes [30,31]. Therefore, we aimed to study ventricular connexin expression in addition to right atrial samples. Ventricular myocardium was available in eight of the patients. Our study confirmed the reported differential expression of both connexins, showing significantly different mRNA levels in ventricle compared to the atrial expression (Table 3). The mRNA expression of Cx40 in ventricle was significantly lower than in right atrial myocardium. Probably due to tendency of ventricular Cx40 expression to increase during CPB ($p = 0.19$, Fig. 3) this was true before the CPB (ventricle: median 8.53×10^{-07}, range 1.87×10^{-08}–3.82×10^{-06}, vs. right atrium: median 9.99×10^{-06}, range 9.16×10^{-08}–3.55×10^{-05}, $p = 0.006$), but not post-CPB (data not shown).

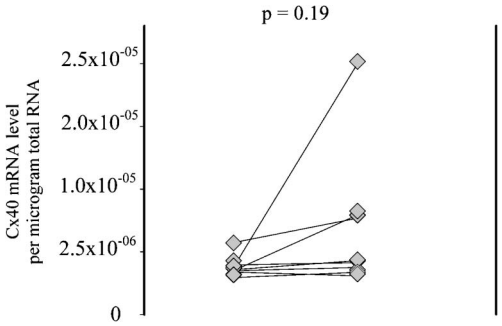

Fig. 3. Expression of ventricular Cx40 mRNA (◇) during the CPB. The pre-operative Cx40 mRNA (median 8.51×10^{-07}, range 1.87×10^{-08}–3.82×10^{-06}) tended to increase during the CPB (median 1.81×10^{-06}, range 1.15×10^{-07}–2.55×10^{-05}), however, statistically this was not significant ($p = 0.19$). Patient with severe increase during CPB : right ventricle, 3 months, VSD-closure via atriotomy.

The same was true for the Cx43 mRNA expression, which was significantly different between ventricular and right atrial myocardium only before the CPB (ventricle: median 3.53×10^{-04}, range 2.97×10^{-05}–3.19×10^{-03}, vs. right atrium: median 1.61×10^{-04}, range 5.39×10^{-06}–3.25×10^{-04}, $p = 0.017$). This seemed to be caused by the reduction of ventricular Cx43 mRNA during CPB, although this did not reach statistical significance ($p = 0.29$, Fig. 4). In contrast to the other patients, the youngest patient of the population (1-week-old newborn) having a complex heart defect (CAVC and coarctation) showed a 2-fold increase of ventricular Cx43 mRNA during CPB (Fig. 4, indicated with an asterisk). His ventricular Cx40 expression (increased) and atrial Cx40 and Cx43 (decreased) during CPB fitted the pattern of most of the other patients. This was the only patient with post-operative arrhythmia (junctional ectopic tachycardia).

Compared to Cx40 we found significantly higher levels of Cx43 in both, atrial and in ventricular myocardium. In ventricular myocardium, the Cx43 mRNA expression was up to 170,000-fold higher than that of Cx40. Interestingly, if the Cx43/Cx40 ratio was calculated only in "normal" LV myocardium the ratio was much lower (23- to 35-fold), probably displaying the increase of Cx43 in diseased ventricular myocardium, compared to that in normal. However, due to the low number of normal LV samples, this did not reach statistical significance.

Table 3
Comparison of atrial and ventricular connexin expression

	Atrium	Ventricle	*p* value
Cx40			
Pre-CPB	9.99×10^{-06} (9.16×10^{-08}–3.55×10^{-05})	8.53×10^{-07} (1.87×10^{-08}–3.82×10^{-06})	0.006
Post-CPB	7.51×10^{-06} (6.55×10^{-08}–1.98×10^{-05})	1.76×10^{-06} (1.15×10^{-07}–2.55×10^{-05})	n.s.
Cx43			
Pre-CPB	1.61×10^{-04} (5.39×10^{-06}–3.25×10^{-04})	3.53×10^{-04} (2.97×10^{-05}–3.19×10^{-03})	0.017
Post-CPB	6.17×10^{-05} (2.62×10^{-06}–3.32×10^{-04})	2.90×10^{-05} (6.03×10^{-06}–2.59×10^{-03})	n.s.

CPB, cardiopulmonary bypass; Cx40, connexin40; Cx43, connexin43; n.s., not significant.

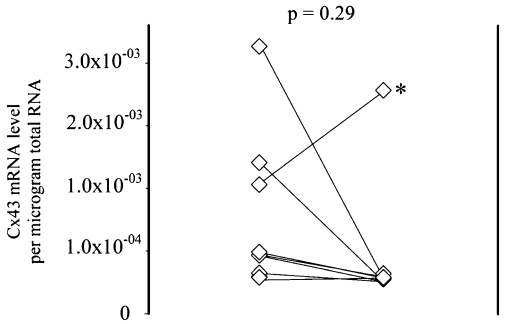

Fig. 4. Expression of ventricular Cx43 mRNA (◇) during the CPB. Apart from one patient the ventricular Cx43 mRNA decreased during CPB (pre-CPB, median 3.74×10^{-04}, 2.97×10^{-05}–3.19×10^{-03}; post-CPB, median 2.93×10^{-05}, range 6.03×10^{-06}–2.59×10^{-03}). In one patient ventricular Cx43 mRNA increased significantly (∗, 200%) during CPB. This was a right ventricular specimen from the youngest patient of the population (1-week-old newborn) having a complex heart defect (AVSD and coarctation). This was the only patient with post-operative arrhythmia (junctional ectopic tachycardia).

Conclusion

This study revealed a significant influence of the CPB on Cx43 expression. Whereas, the volume overload itself had no effect on connexin expression, it changed the susceptibility to the CPB. Cx43 mRNA expression was significantly reduced during CPB in volume overloaded right atria but entirely unchanged by CPB in normal atria. During cardiopulmonary bypass the mRNA expression of ventricular Cx40 increased, whereas that of Cx43 decreased. Due to this, for both connexins the pre-operatively significant difference between atrial and ventricular expression was blunted after the CPB. Based on our results, we believe that the additional influence of the CPB itself has to be taken into account if connexin remodeling is studied in atrial and ventricular disease.

Limitations of the study

In this study, we investigated only mRNA expression. Due to the limited amount of myocardial tissue available during routine corrective surgery in pediatric patients with congenital heart defects, Western blotting for protein expression or immunohistochemistry was not performed as particularly for investigating the low abundant Cx40 large amounts of tissue are needed. Several groups found similar mRNA and protein expression levels, indicating that the expression is controlled primarily at the level of transcription or of messenger stability, this was true not only for Cx43 but also for atrial Cx40 expression [28,31]. The atrial samples could be separated into volume overloaded and not overloaded. Unfortunately, due to the low number of ventricular specimens and the heterogeneity of hemodynamic overload in diseased right and left ventricular myocardium, this was not further separated into different groups. More normal LV specimens should be investigated in the future, to prove this result, to date still lacking statistical significance.

As reports on connexin expression during the cardiopulmonary bypass are sparse, particularly in pediatric patients, we believe that despite these limitations this study adds important new data in the field of myocardial connexin remodeling.

References

[1] J.H. Moller, J.I.E. Hoffman, Pediatric Cardiovascular Medicine, first ed., Churchill Livingstone, Philadelphia, 2000.
[2] H.A. Huysmans, M. Vrakking, W.J. van Boven, Late follow-up after surgical correction of atrial septal defect of the secundum type, Z. Kardiol. 78 (1989) 43–45.
[3] U. Wetzel, A. Boldt, J. Lauschke, J. Weigl, P. Schirdewahn, A. Dorszewski, N. Doll, G. Hindricks, S. Dhein, H. Kottkamp, Expression of connexins 40 and 43 in human left atrium in atrial fibrillation of different aetiologies, Heart 91 (2005) 166–170.
[4] D.E. Gutstein, G.E. Morley, D. Vaidya, F. Liu, F.L. Chen, H. Stuhlmann, G.I. Fishman, Heterogeneous expression of gap junction channels in the heart leads to conduction defects and ventricular dysfunction, Circulation 104 (2001) 1194–1199.
[5] H. Kitamura, Y. Ohnishi, A. Yoshida, K. Okajima, H. Azumi, A. Ishida, E.J. Galeano, S. Kubo, Y. Hayashi, H. Itoh, M. Yokoyama, Heterogeneous loss of connexin43 protein in nonischemic dilated cardiomyopathy with ventricular tachycardia, J. Cardiovasc. Electrophysiol. 13 (2002) 865–870.
[6] N.J. Severs, S. Rothery, E. Dupont, S.R. Coppen, H.I. Yeh, Y.S. Ko, T. Matsushita, R. Kaba, D. Halliday, Immunocytochemical analysis of connexin expression in the healthy and diseased cardiovascular system, Microsc. Res. Tech. 52 (2001) 301–322.
[7] D.D. Spragg, C. Leclercq, M. Loghmani, O.P. Faris, R.S. Tunin, D. DiSilvestre, E.R. McVeigh, G.F. Tomaselli, D.A. Kass, Regional alterations in protein expression in the dyssynchronous failing heart, Circulation 108 (2003) 929–932.
[8] M.V. Podgoreanu, G.A. Michelotti, Y. Sato, M.P. Smith, S. Lin, R.W. Morris, H.P. Grocott, J.P. Mathew, D.A. Schwinn, Differential cardiac gene expression during cardiopulmonary bypass: ischemia-independent upregulation of proinflammatory genes, J. Thorac. Cardiovasc. Surg. 130 (2005) 330–339.
[9] M. Ruel, C. Bianchi, T.A. Khan, S. Xu, J.R. Liddicoat, P. Voisine, E. Araujo, H. Lyon, I.S. Kohane, T.A. Libermann, F.W. Sellke, Gene expression profile after cardiopulmonary bypass and cardioplegic arrest, J. Thorac. Cardiovasc. Surg. 126 (2003) 1521–1530.
[10] W.M. Breisblatt, K.L. Stein, C.J. Wolfe, W.P. Follansbee, J. Capozzi, J.M. Armitage, R.L. Hardesty, Acute myocardial dysfunction and recovery: a common occurrence after coronary bypass surgery, J. Am. Coll. Cardiol. 15 (1990) 1261–1269.
[11] J.E. Tamis, J.S. Steinberg, Atrial fibrillation independently prolongs hospital stay after coronary artery bypass surgery, Clin. Cardiol. 23 (2000) 155–159.
[12] H.I. Yeh, S.H. Hou, H.R. Hu, Y.N. Lee, J.Y. Li, E. Dupont, S.R. Coppen, Y.S. Ko, N.J. Severs, C.H. Tsai, Alteration of gap junctions and connexins in the right atrial appendage during cardiopulmonary bypass, J. Thorac. Cardiovasc. Surg. 124 (2002) 1106–1112.
[13] N.S. Peters, N.J. Severs, S.M. Rothery, C. Lincoln, M.H. Yacoub, C.R. Green, Spatiotemporal relation between gap junctions and fascia adherens junctions during postnatal development of human ventricular myocardium, Circulation 90 (1994) 713–725.
[14] M.J.A. Van Kempen, J.L.M. Vermeulen, A.F.M. Moorman, D. Gros, D.L. Paul, W.H. Lamers, Developmental changes of connexin40 and connexin43 mRNA distribution patterns in the rat heart, Cardiovasc. Res. 32 (1996) 886–900.
[15] B. Delorme, E. Dahl, T. Jarry-Guichard, J.P. Briand, K. Willecke, D. Gros, M. Theveniau-Ruissy, Expression pattern of connexin gene

products at the early developmental stages of the mouse cardiovascular system, Circ. Res. 81 (1997) 423–437.
[16] S.R. Coppen, R.A. Kaba, D. Halliday, E. Dupont, J.N. Skepper, S. Elneil, N.J. Severs, Comparison of connexin expression patterns in the developing mouse heart and human foetal heart, Mol. Cell. Biochem. 242 (2003) 121–127.
[17] M. Pavlovic, A. Schaller, J.P. Pfammatter, T. Carrel, P. Berdat, S. Gallati, Age-dependent suppression of SERCA2a mRNA in pediatric atrial myocardium, Biochem. Biophys. Res. Commun. 326 (2005) 344–348.
[18] W. Liu, D.A. Saint, Validation of a quantitative method for real time PCR kinetics, Biochem. Biophys. Res. Commun. 294 (2002) 347–353.
[19] S.N. Peirson, J.N. Butler, R.G. Foster, Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis, Nucleic Acids Res. 31 (2003) 14–73.
[20] D.G. Altman, Practical Statistics for Medical Research, Chapman & Hall, Boca Raton, FL, 1991.
[21] A. Agresti, Categorical Data Analysis, Wiley, Chichester, 2002.
[22] T. Nao, T. Ohkusa, Y. Hisamatsu, N. Inoue, T. Matsumoto, J. Yamada, A. Shimizu, Y. Yoshiga, T. Yamagata, S. Kobayashi, M. Yano, K. Hamano, M. Matsuzaki, Comparison of expression of connexin in right atrial myocardium in patients with chronic atrial fibrillation versus those in sinus rhythm, Am. J. Cardiol. 91 (2003) 678–683.
[23] S. Kostin, G. Klein, Z. Szalay, S. Hein, E.P. Bauer, J. Schaper, Structural correlate of atrial fibrillation in human patients, Cardiovasc. Res. 54 (2002) 361–379.
[24] L. Polontchouk, J.A. Haefliger, B. Ebelt, T. Schaefer, D. Stuhlmann, U. Mehlhorn, F. Kuhn-Regnier, E.R. De Vivie, S. Dhein, Effects of chronic atrial fibrillation on gap junction distribution in human and rat atria, J. Am. Coll. Cardiol. 38 (2001) 883–891.
[25] E. Dupont, Y. Ko, S. Rothery, S.R. Coppen, M. Baghai, M. Haw, N.J. Severs, The gap-junctional protein, connexin40, is elevated in patients susceptible to post-operative atrial fibrillation, Circulation 103 (2001) 842–849.
[26] P. Kanagaratnam, S. Rothery, P. Patel, N.J. Severs, N.S. Peters, Relative expression of immunolocalized connexins 40 and 43 correlates with human atrial conduction properties, J. Am. Coll. Cardiol. 39 (2002) 116–123.
[27] N.S. Peters, C.R. Green, P.A. Poole-Wilson, N.J. Severs, Reduced content of connexin43 gap junctions in ventricular myocardium from hypertrophied and ischemic human hearts, Circulation 88 (1993) 864–875.
[28] E. Dupont, T. Matsushita, R.A. Kaba, C. Vozzi, S.R. Coppen, N. Khan, R. Kaprielian, M.H. Yacoub, N.J. Severs, Altered connexin expression in human congestive heart failure, J. Mol. Cell. Cardiol. 33 (2001) 359–371.
[29] R.R. Kaprielian, M. Gunning, E. Dupont, M.N. Sheppard, S.M. Rothery, R. Underwood, D.J. Pennell, K. Fox, J. Pepper, P.A. Poole-Wilson, N.J. Severs, Downregulation of immunodetectable connexin43 and decreased gap junction size in the pathogenesis of chronic hibernation in the human left ventricle, Circulation 97 (1998) 651–660.
[30] L.M. Davis, H.L. Kanter, E.C. Beyer, J.E. Saffitz, Distinct gap junction protein phenotypes in cardiac tissues with disparate conduction properties, J. Am. Coll. Cardiol. 24 (1994) 1124–1132.
[31] C. Vozzi, E. Dupont, S.R. Coppen, H.I. Yeh, N.J. Severs, Chamber-related differences in connexin expression in the human heart, J. Mol. Cell. Cardiol. 31 (1999) 991–1003.