Effect of mitochondrial aldehyde dehydrogenase-2 genotype on cardioprotection in patients with congenital heart disease

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Aims
About 40% of East Asians carry an aldehyde dehydrogenase-2*2 (ALDH2*2) allele, and the influence of the ALDH2*2 allele on human cardioprotection has not been studied. This study was designed to evaluate the effect of ALDH2*2 allele on cardioprotection of patients with congenital heart diseases after open-heart surgery.

Methods and results
The right atrial appendage was harvested before performing cardiopulmonary bypass in cyanotic and acyanotic congenital heart disease groups (n = 20 per group). Tissues were assayed to determine the impact of cyanosis on metabolic remodelling. A prospective cohort of Tetralogy of Fallot (TOF) patients (n = 118) was recruited to investigate the influence of the ALDH2*2 allele on cardioprotection after surgical repair. Myocardium samples were dissected after cardioplegia. ALDH2 activity, oxidative stress and glutathione (GSH) levels, and activating transcription factor-4 (ATF4) were analysed. After genotyping and grouping, all of the experimental and clinical results were compared between ALDH2*2 carriers and non-carriers.

Cyanosis inhibited ALDH2 activity and led to aldehyde accumulation in ALDH2*2 carriers. This accumulation in turn increased expression of ATF4 and resulted in larger myocardium GSH pools. The differences in ALDH2 activity and GSH level between carriers and non-carriers disappeared during cardioplegic arrest, and more aldehydes accumulated in the non-carriers. Consequently, ALDH2*2 carriers showed lower postoperative troponin I, inotrope score, and shorter postoperative length of ICU and hospital stay.

Conclusions
ALDH2*2 carriers with cyanotic congenital heart disease were associated with an induced metabolic remodelling phenotype and a compensatory myocardium GSH pool. When ALDH2 activity was impaired during open-heart surgery, this larger GSH pool could lead to unexpectedly better cardioprotection. This may aid in the prediction of cardioprotection outcomes and identification of individualized cardioprotective strategies.

Keywords
Congenital heart disease • Mitochondrial aldehyde dehydrogenase • Cardioprotection • Cardiac surgery

Introduction
Myocardial ischaemia and re-perfusion (I/R) insult remains the most common cause of cardiac morbidity and mortality despite major advances in cardiovascular medicine. Therefore, it is of great importance to identify novel cardioprotective strategies to minimize myocardial I/R injury. Aldehydes have been reported to be highly related to cardiac I/R damage. Activation of aldehyde dehydrogenase-2 (ALDH2), a key enzyme that catalyses the removal of reactive aldehydes, confers profound cardioprotection in rodent models. Furthermore, it was demonstrated that ALDH2-knockout mice had increased infarct size after ischaemia because of the accumulation of reactive aldehydes [4-hydroxynonenal (4-HNE)]. At least 540 million or ~8% of the world population carry a common single nucleotide polymorphism (rs671) in ALDH2, where a glutamate at amino acid 504 is replaced by a glutamine.
lysin (E504K) to form an ALDH2 loss-of-function allele (ALDH2*2 allele). ALDH2*2 carriers display dramatically reduced ALDH2 activities. Since ALDH2*2 carriers exhibit impaired ALDH2 activity in the detoxification of reactive aldehydes, it is warranted that cardioprotective outcomes should be re-evaluated in these individuals.

Although ALDH2 activation leads to obvious cardioprotection, Endo et al. found that carrying an ALDH2 loss-of-function allele (ALDH2*2 allele) also has cardioprotective effects. Using ALDH2*2 transgenic mice, they found that myocardial oxidative stress increased the expression of AFT4 and 3-phosphoglycerate dehydrogenase (PHGDH) in ALDH2*2 transgenic hearts, which further led to metabolic remodelling to produce more glutathione (GSH). Thereby, the increase in the intrinsic myocardial GSH pool made ALDH2*2 transgenic mice have a greater tolerance to I/R damage.

No previous research has been done to investigate the effect of ALDH2 E504K polymorphism on human cardioprotection; therefore, we studied the cardioprotection outcomes of Han Chinese patients, where I/R injury was inevitable because of aortic clamping and unclamping during open-heart surgery. In the present study, young patients with congenital heart diseases were selected as study subjects for the following reasons. First, some interference factors that can affect ALDH2 activity are common in adult patients, including oestrogen, diabetes mellitus, and nitroglycerine application. Secondly, myocardium dissections are usually necessary in many paediatric cardiac surgeries, and therefore we can collect the resected heart tissue to analyse the myocardial metabolic remodelling.

A two-step study was conducted on paediatric patients undergoing elective cardiac surgery. In part I of the study, the effects of cyanosis on myocardium oxidative stress, ALDH2 enzyme activity, and GSH formation were determined. In part II, the influence of ALDH2 genotypes on cardioprotection outcomes after open-heart surgery of cyanotic patients was evaluated and the underlying mechanisms were investigated.

Methods

Patients and study design

All experiments were carried out in accordance with Council for International Organizations of Medical Sciences guidelines. Our local ethics committee (Fuwai Hospital Research Ethics Committee) approved this study, and informed consent was obtained from all patients. The study design is shown in Figure 1. In part I, right atrial appendage tissue of 20 chronic cyanosis patients and 20 acyanotic patients was harvested (Table 1). Transcutaneous oxygen saturation, tissue oxidative stress, GSH levels, and ALDH2 enzyme activity were assayed.

In part II, a prospective cohort of 118 cyanotic Tetralogy of Fallot (TOF) patients undergoing elective surgical correction was recruited. We focused our study on children with TOF because this disease is the most common form of cyanotic congenital heart disease. TOF patients had relatively uniform anatomy and the cardiopulmonary bypass time of TOF repair was comparatively longer to facilitate the evaluation of the cardioprotection. Furthermore, it was convenient for myocardium sample collection because myocardium dissection is necessary when widening the right ventricular outflow tract during surgical repair. All the cyanotic patients included in this study presented with episodes of spells or repeated saturation measurements <90% (details are available in the Supplementary material online). The exclusion criteria were NYHA cardiac function ≥ class III, lung or liver function impairment, associated multiple ventricular septal defects or main aortopulmonary collateral arteries, previous operation history, and recognized genetic or malformation syndrome. Patients who required second aortic cross-clamping and acquired severe postoperative infections were excluded because these factors could significantly influence the clinical outcomes. All patients were in stable condition without pre-operative respiratory or inotropic support. Intra-operative anaesthetic, cardioprotective, and operative strategies were standardized and performed by the same team in our centre.

Histidine–tryptophan–keto glutarate (HTK) cardioplegia (HTK-Custodiol; Koehler Chemi, Alsbach-Haalen, Germany) was used for cardioprotection. In total, four surgeons worked as the first operators and performed all the procedures. All these surgeons were well trained with at least 5 years of experience in TOF repair. Right ventricular approach (through a vertical incision) was chosen in all the patients for ventricular septal defect repair, and decisions for transannular patching were based on the judgement of pulmonary annulus diameters. All patients were admitted to the paediatric intensive care unit after surgery, and were managed according to the same unit protocols. Inotropic support and ventilation decisions were based on haemodynamic status and clinical judgement. The patients were divided into two groups according to their genotypes, the ALDH2*2 carriers and ALDH2*2 non-carriers. The genotypes of these patients were blinded to the researchers before final data analysis. Preoperative characteristics of the two groups are summarized in Table 2.

Heart tissues and blood specimens

In part I, right atrial appendage tissues (40–100 mg net weight) were harvested after sternotomy and before cardiopulmonary bypass. In part II, right ventricular outflow tract myocardium specimens (60–200 mg net weight) were resected during surgical repair. The ischaemic time of the outflow tract myocardium between aortic cross-clamping and tissue storage was recorded. Blood samples (1 mL) were obtained from all subjects before surgery for genotyping. Specimens were collected in sealed vials and stored at −80 °C until analysis.

Tissue assays

Tissue ALDH2 activity was measured using a spectrophotometer. Malondialdehyde (MDA) and 4-HNE adducts were used to measure oxidative stress status, because excessive accumulation of MDA and 4-HNE adducts is accompanied by cardiomyocyte injury. GSH, which can protect the heart from I/R injury, was also tested in our study. ALDH2, PHGDH and ATF4 expression, and 4-HNE adduct amounts were determined by western blotting (detailed methods are available in the Supplementary material online).

Grouping and clinical data collection

Patients were genotyped by direct sequencing, and were grouped according to their genotypes. Inotropic score, which indicates cardiac function, was used to quantify inotropic use, and serum troponin I (TnI) levels at 20 h after aortic cross-clamp removal were determined. Baseline characteristics, variables that can affect cardioprotection and detailed cardioprotection results were prospectively collected (detailed methods are available in the Supplementary material online).

Statistical analysis

Statistical analysis was performed to compare outcomes between ALDH2*2 carriers and non-carriers. Continuous variables are
summarized using means and standard deviations (or medians and inter-quartile range if the distribution was skewed). Numbers and percentages were used to summarize categorical data. In univariate analysis, differences between groups were compared using the Chi-square test for categorical variables and non-parametric tests for continuous variables (due to the small sample size in each group). In multivariate analysis, differences between groups were compared using multiple linear regressions for continuous variables and binary logistic regressions for categorical variables after controlling other co-variates. Exact logistic regression was used in analysing the difference of categorical variables when no event was observed in both univariate and multivariate analysis. All reported P-values are two-sided, and P-values of <0.05 were considered to indicate statistical significance.

**Results**

**Cyanosis and metabolic remodelling**

Eleven ALDH2*2 carriers (all ALDH2*2 heterozygous) were found in part I of the study. ALDH2 genotypes and cyanosis have significant impacts on ALDH2 activities. ALDH2*2 non-carriers had higher enzyme activities in both cyanotic (1.56 ± 0.34 U/mg) and acyanotic patients (2.58 ± 0.71 U/mg). Furthermore, cyanosis inhibited patients’ enzyme activities. Cyanotic ALDH2*2 carriers (0.70 ± 0.15 U/mg) and non-carriers had lower enzyme activities when compared with normoxia ALDH2*2 carriers (1.11 ± 0.25 U/mg) and non-carriers (Figure 2A).
In acyanotic patients, there were no significant differences between non-carriers and carriers in 4-HNE-His adducts (1.14 ± 0.59 vs. 1.54 ± 0.65 μmol/mg), MDA levels (70.17 ± 35.19 vs. 93.90 ± 51.66 nmol/mg), and the tissue GSH pool (1.29 ± 0.45 vs. 1.25 ± 0.23 nmol/mg). Cyanotic ALDH2*2 carriers manifested the highest 4-HNE-His adducts (2.35 ± 0.40 nmol/mg), MDA levels (264.13 ± 116.97 nmol/mg), and tissue GSH pools (2.35 ± 0.40 nmol/mg) (Figure 2B–D).

### Cardioprotection cohort study

#### Genotypes and baseline characteristics

Primarily, 118 TOF patients were included, and 3 patients were excluded for second aortic cross-clamping (n = 2) and severe postoperative infection (n = 1). The remaining 115 patients were successfully genotyped and analysed, and these subjects were found to exist in the Hardy–Weinberg equilibrium (P = 0.63). About 33.91% of the patients carry an ALDH2*2 allele (detailed information is available in the Supplementary material online, Figure S1). There were no significant in-between-group differences in baseline characteristics and intra-operative variables that can affect cardioprotection outcomes (Table 2).

#### Myocardial assay

We randomly selected 20 patients with adequate amounts of myocardium from both groups to measure ALDH2 enzyme activity. For the detection of 4-HNE-His adducts and GSH levels, 20 ALDH2*2 carriers and 40 ALDH2*2 non-carriers were used. The myocardial ischaemic time of ALDH2*2 carriers and ALDH2*2 non-carriers were 34.33 ± 2.33 and 35.42 ± 3.43 min, respectively (P = 0.12). Strong expression of the ALDH2 protein was observed in both groups (Figure 3B and D). Although ALDH2*2 non-carriers exhibited a higher ALDH2 activity before ischaemia, no statistical difference (P = 0.94) of myocardium ALDH2 activity was observed among the two groups following cardioplic arrest (Figure 3A). Lower 4-HNE-His adduct levels were observed in the myocardium dissected during surgery of the ALDH2*2 carriers (Figure 3A and C). Differences in myocardial GSH levels among ALDH2*2 carriers and non-carriers also were lost during surgery (Figure 3A). Increased protein expression of ATF4 and PHGDH were observed in ALDH2*2 carriers (Figure 3B, E, and F).

#### E50K variant and cardioprotection outcomes

ALDH2*2 carriers exhibited unexpectedly greater tolerance to I/R injury. Lower postoperative TnI levels and 24 h inotropic scores were observed in ALDH2*2 carriers (Table 3). The ratio of high-dose inotropic application (inotrope score ≥ 10) at 24 h after surgery on ALDH2*2 non-carriers was higher when compared with ALDH2*2 carriers (67.11 vs. 46.15%, P = 0.03) (odds ratio: 2.38; 95% Cl: 1.08–5.25). The duration of mechanical ventilation was similar in both groups (P = 0.33). The ratio of prolonged ICU stay (≥7 days) was lower in ALDH2*2 carriers (ALDH2*2 carriers, 0; ALDH2*2 non-carriers, 11.84%; P = 0.041), as well as the ratio of prolonged postoperative hospital stay (≥14 days) (ALDH2*2 carriers, 0; ALDH2*2 non-carriers, 15.79%; P = 0.01) (Table 3).

After being adjusted for gender, age, weight, left ventricular diastolic diameter, preoperative haemoglobin, Nakata index and cardiopulmonary bypass and cross-clamp time, the differences in TnI, 24 h inotropic score, 24 h high-dose inotrope ratio, prolonged ICU stay ratio, and ratio of prolonged postoperative length of stay were still significant (Table 3).

To account for the impact of surgeon’s skill on the clinical outcomes, every surgeon’s total number of cases and the number of patients with prolonged ICU and hospital stay are listed in the Supplementary material online (detailed information is available in the Supplementary material online, Tables S3 and S4). There were no
statistical differences when comparing the ratios of patients with prolonged ICU and hospital stay among the four surgeons.

Discussion

ALDH2 is the key enzyme in the metabolism of reactive aldehydes and is expressed abundantly in the heart. Transgenic expression of the ALDH2 gene in mice does not only ameliorate the acute cardiac toxicity effects of ethanol, but can also rescue chronic alcoholic myocardial hypertrophy and contractile dysfunction through removal of toxic aldehydes. Furthermore, activation of ALDH2 can significantly reduce ischaemic damage to the rat hearts. The ALDH2 loss-of-function allele (ALDH2*2 allele) has the highest prevalence ratio (~40%) in the East Asians, including the Han Chinese population, and it has been shown that the ALDH2*2 genotype is associated with cardiovascular abnormalities, such as hypertension and coronary heart diseases.

To the best of our knowledge, this is the first study to investigate the influence of the ALDH2*2 allele on human cardioprotection after open-heart surgery. The atrial appendage tissue results demonstrate that cyanosis can significantly inhibit ALDH2 activity, and this inhibition in turn was associated with the metabolic remodelling in ALDH2*2 carriers and produced more GSH. The data from part II of our study further revealed that cyanotic ALDH2*2 carriers obtained better cardioprotection results after cardioplegic arrest possibly due to this elevated GSH pool. Prolonged ICU and postoperative hospital stays are main indicators for cardioprotection. Surprisingly, all the patients with prolonged ICU and postoperative hospital stay were found in the ALDH2*2 non-carriers group. As we retrospectively analysed these patients with prolonged stay, we found that all these patients developed low cardiac output syndrome after surgery. The postoperative inotrope support duration and inotrope scores of these patients were significantly higher than the others. All these results strongly indicate that ALDH2*2 carriers got less I/R injury and better cardiac function after surgery.

ALDH2*2 non-carriers displayed higher ALDH2 activities in both cyanotic and acyanotic groups, in accordance with Seitz’s results. ALDH2 enzyme activity can be significantly inhibited by oxidative stress such as excessive 4-HNE directly or by adduct formation on ALDH2. Therefore, the cyanotic ALDH2*2 carriers possessed the lowest ALDH2 activity and accumulated the highest

Figure 2 Metabolic remodelling in Aldh2*2 carriers. Comparison of right atrial appendage specimens from cyanotic and acyanotic patients with different ALDH2 genotypes. Blue bars represent cyanotic patients, and red bars represent acyanotic patients. Aldh2*2 carriers possessed lower ALDH2 activities in both cyanotic and acyanotic groups (A). Cyanosis inhibited ALDH2 activity in Aldh2*2 carriers and Aldh2*2 non-carriers (A). Cyanotic Aldh2*2 carriers got the highest glutathione pools (B) and accumulated the greatest 4-hydroxynonenal-His adducts and malondialdehyde levels (C and D). MDA, malondialdehyde; other abbreviations as in Figure 1. The data were presented as median ± inter-quartile range (Box).
Figure 3  Myocardium assays after ischaemia. Comparisons of myocardial aldehyde dehydrogenase-2 activity, GSH, 4-hydroxynonenal-His adducts, and expressions of aldehyde dehydrogenase-2, activating transcription factor-4, and 3-phosphoglycerate dehydrogenase among ALDH2*2 carriers and non-carriers. Aldehyde dehydrogenase-2 activity differences between ALDH2*2 carriers and non-carriers disappeared during open-heart surgery (A). Higher 4-hydroxynonenal-His adducts levels were observed in ALDH2*2 non-carriers (A and C). Representative immunoblots found no difference of aldehyde dehydrogenase-2 expression among ALDH2*2 carriers and non-carriers (B and D), but ALDH2*2 carriers showed greater protein expression of activating transcription factor-4 and 3-phosphoglycerate dehydrogenase (B, E, and F). ATF4, activating transcription factor-4; PHGDH, 3-phosphoglycerate dehydrogenase; other abbreviations as in Figure 1. #P, 0.05, € and *P, 0.05.

Table 3  Clinical outcomes of the patients after surgical repair in part II of the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALDH2*2 non-carriers (n = 76)</th>
<th>ALDH2*2 carriers (n = 39)</th>
<th>P-value</th>
<th>Adjusted P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median TnI, ng/mL</td>
<td>18.19 (13.87–25.99)</td>
<td>15.43 (11.72–20.21)</td>
<td>0.014</td>
<td>0.026</td>
</tr>
<tr>
<td>Median 24 h inotropic scoreb</td>
<td>12 (8–17)</td>
<td>9.5 (0–14)</td>
<td>0.014</td>
<td>0.001</td>
</tr>
<tr>
<td>24 h high-dose inotrope, n (%)c</td>
<td>51 (67.11)</td>
<td>18 (46.15)</td>
<td>0.030</td>
<td>0.008</td>
</tr>
<tr>
<td>Median mechanical ventilation (h)</td>
<td>19.5 (11–36.75)</td>
<td>19 (8–25)</td>
<td>0.325</td>
<td>0.073</td>
</tr>
<tr>
<td>Median ICU stay (h)</td>
<td>46.5 (26.25–93.50)</td>
<td>46 (22–96)</td>
<td>0.533</td>
<td>0.121</td>
</tr>
<tr>
<td>Prolonged ICU stay, n (%)d</td>
<td>9 (11.84)</td>
<td>0 (0)</td>
<td>0.041</td>
<td>0.023</td>
</tr>
<tr>
<td>Median postoperative LOS (days)</td>
<td>9 (7–11)</td>
<td>8 (7–10)</td>
<td>0.338</td>
<td>0.085</td>
</tr>
<tr>
<td>Prolonged postoperative LOS, n (%)e</td>
<td>12 (15.79)</td>
<td>0 (0)</td>
<td>0.010</td>
<td>0.009</td>
</tr>
</tbody>
</table>

TnI, troponin I; ICU, intensive care unit; LOS, length of stay.

bAdjusted for gender, age, weight, left ventricular diastolic diameter, preoperative haemoglobin, Nakata index, cardiopulmonary bypass time, and cross-clamp time.

cInotropic score is calculated by obtaining the total amount of inotropic support the patients received at each sampling point, and a higher inotrope score indicates poorer cardiac function.

dPatients with high-dose inotrope application at 24 h after surgery referred to those who had an inotrope score  10.

eProlonged ICU stay means an ICU stay  7 days.

fProlonged postoperative LOS means postoperative LOS  14 days.
oxidative stress levels before surgery. It is known that increased oxidative stress can activate ATF4, and translational activation of ATF4 can induce members of the ATFCREB family of transcription factors to up-regulate genes involved in amino acid biosynthesis.\(^9\)\(^{28}\) Consistent with this, more ATF4 and PHGDH were expressed in the myocardium of cyanotic ALDH2*2 carriers, and higher GSH pools were found in them.

Although ALDH2*2 non-carriers have greater ALDH2 activities, no difference in myocardium ALDH2 activity was observed after cardioplegic arrest. HTK is a widely used cardioprotective solution in open-heart surgery to alleviate ischaemic damage.\(^29\) Although it contains anti-oxidants, HTK cannot block aldehyde formation completely, and no specific agents are included in HTK solution to remove toxic aldehydes. Therefore, aldehyde accumulation is inevitable during cardiopulmonary bypass surgery. Hypothermia is indispensable for open-heart surgery, and ALDH2 is a bio-enzyme whose activity can be easily inhibited by hypothermia. The inhibition of ALDH2 activity would significantly impair the clearance of active aldehydes and result in excessive aldehyde accumulation. Excessive aldehydes in turn inhibit ALDH2 activity to form a vicious cycle in the hypothermic ischaemic heart and can make the ALDH2 activity differences between ALDH2*2 carriers and non-carriers disappear. ALDH2*2 carriers had higher preoperative GSH levels, and more GSH would be consumed to antagonize ischaemic injury. Hence, the myocardium GSH level was declined and less 4-HNE His adducts were formed in ALDH2*2 carriers after cardioplegic arrest. 4-Hydroxynonenal is a toxic aldehyde highly related to ischaemic damage.\(^5\) Therefore, we presumed that the higher intrinsic GSH pool in ALDH2*2 carriers allowed them to achieve a greater tolerance to ischaemic and re-perfusion damage.

Sulphydryl donors are powerful anti-oxidants, and mounting evidence has proved that they are capable of protecting the heart from ischaemic attack.\(^30\)\(^{31}\) Both exogenous glutathione supplementation\(^32\) and intrinsic glutathione elevation\(^9\) can increase the cardiac tolerance to ischaemic damage. And furthermore, exogenous glutathione supplementation has been a therapeutic option for liver and kidney diseases in clinical practice.\(^33\) In agreement with these findings, we found that ALDH2*2 carriers with higher intrinsic glutathione levels fared better with respect to cardioprotection results.

In previous studies, it was reported that a greater tolerance to I/R injuries is associated with higher ALDH2 activities and greater ALDH2 expression,\(^4\)\(^5\)\(^{21}\) which contradicted our results. The clinical conditions of our study were quite different from the animal models of Chen and Ma's experiments. Chen et al. used an isolated rat ischaemic and re-perfusion model to investigate the relationship between ALDH2 activity and cardioprotective results, and Ma et al. used an ALDH2 transgenic mice model to study the influence of ALDH2 activity or expression on cardioprotection outcomes. In contrast, the subjects included in our study were patients who endured the ischaemic and re-perfusion process with cardiopulmonary bypass and hypothermia. When patients received cardiopulmonary bypass and hypothermia, the activity of ALDH2 was inhibited, and the enzymatic difference was equalized. The cardioprotection result then mainly relied on the cardioprotective solution and the intrinsic cardiac compensatory ability. Furthermore, our patients got ischaemic preconditioning due to chronic cyanosis. Chronic ischaemia can cause strong oxidative stress in the heart to result in metabolic remodelling when ALDH2 activity is impaired. Increased oxidative stress is also manifested in other ischaemic diseases. Nagasawa et al. found that although no difference in single lacunar infarct morbidity was detected among patients with different genotypes, ALDH2*2 non-carriers tended to develop more severe ischaemic events (multiple lacunar infarcts).\(^34\) No data of brain metabolism were presented in Nagasawa’s study. However, according to our findings, the metabolic remodelling could occur in ALDH2*2 carriers with lower ALDH2 activity and result in the lower occurrence of multiple lacunar infarcts.

The ALDH2 genotype was related to higher prevalence of hypertension. This association is likely due to alteration of alcohol consumption.\(^23\) In a recent study, it was found that ALDH2*2 carriers exhibited an increased risk for acute coronary syndromes. The authors suggested that reduced alcohol intake, decreased endothelial progenitor cell number, and high-sensitivity C-reactive protein may affect plaque vulnerability thereby prompting a higher occurrence of acute coronary syndromes in ALDH2*2 carriers.\(^35\) Distinct from these studies, our study was focused on the clinical outcomes following an acute ischaemic and re-perfusion attack. Furthermore, only paediatric patients were included where certain environmental intervention factors, such as alcohol consumption, were absent.

Our study reported that the ALDH2 E504K polymorphism significantly affected the cardioprotection results after cardioplegic arrest. Until now, no commercially available cardioprotection solutions depend on the activation of ALDH2 under consideration. A small molecule, Alda-1, has profound ability in activating ALDH2,\(^36\) and Chen et al.\(^6\) demonstrated that Alda-1 administered to rats before ischaemia reduced the infarct size by 60% through clearance of toxic aldehydes. Modification of cardioplegia solutions by adding an ALDH2 agonist suggests an alternative individualized cardioprotection strategy for ALDH2*2 non-carriers.

There were some limitations in this study. Firstly, the sample size of our cohort was relatively small. To avoid the impact of surgical skill on the outcomes of part II of our study, we only enrolled young patients with congenital heart diseases. Whether our conclusions can be applied to the adult patients with acquired heart diseases is not known currently.

**Conclusions**

Cyanosis inhibited ALDH2 activity and led to accumulation of aldehyde in ALDH2*2 carriers. This accumulation was associated with greater expression of ATF4 and larger myocardium GSH pools. Inhibited ALDH2 activity during surgery and lower intrinsic myocardium GSH levels could result in more aldehydes accumulated in the heart of ALDH2*2 non-carriers, and these excessive toxic aldehydes in turn caused deleterious cascades through...
adducts formation on a variety of macromolecules to impair heart function. Consequently, ALDH2*2 non-carriers exhibited poor tolerance to IR injury caused by cardiopulmonary bypass and manifested worse clinical outcomes. Our results have implications for evaluating the risks associated with ischaemic and re-perfusion injury and for developing individualized cardioprotection strategies.

Supplementary material
Supplementary material is available at European Heart Journal online.

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Conflict of interest: none declared.

References
Left atrial appendage invagination during MitraClip implantation

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MitraClip appears to be a promising new approach to mitral regurgitation treatment in select high-surgical-risk patients. A 72-year-old female patient affected by dilated cardiomyopathy and severe symptomatic restrictive mitral regurgitation was remitted by MitraClip implantation. Poor results were observed after the first valve grasp, so a new orientation in the left atrium was tried to improve the left ventricular axial alignment. After a device manoeuvre in the left atrium (apparently arms clip locked position), transoesophageal echocardiogram showed a new thumb-like mass image near the MitraClip device (Figure 1). After a differential diagnostic approach, invagination of the left atrial appendage (LAA) was suggested. Careful push–pull manoeuvres did not resolve the problem. Finally, the partial arms clip opening released the LAA tissue, and the normal anatomy was recovered (see Supplementary material online, Video S1); probably not fully closed the clip had been hooked with LAA tissue during device manoeuvres. No pericardial effusion was observed, and the implantation was performed successfully.

The inversion of LAA is a rare complication after open-heart surgery (not previously reported in percutaneous interventions). It could be corrected with digital manipulation during the surgery. Lack of awareness of this entity can result in a misdiagnosis (thrombus, vegetation?) and diagnosis of unnecessary procedures, even reoperation.

Supplementary material is available at European Heart Journal online.

Figure. A two-chamber view: (A) normal anatomy, MitraClip device (arrow); (B) left atrial appendage invagination noted close to the MitraClip device (hooked tissue); (C–F) evolutive images: the partial arms clip opening released the left atrial appendage tissue, and the normal anatomy was recovered. LAA, left atrial appendage; LA, left atrium; LV, left ventricle; MV, mitral valve.