Is Trisomy 21 a Risk Factor for Rapid Progression of Pulmonary Arteriopathy? — Revisiting Histopathological Characteristics Using 282 Lung Biopsy Specimens —

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Background: Pulmonary hypertension (PH) is more progressive in trisomy 21 patients. However, pulmonary arteriopathic lesions in these patients have not been fully characterized histopathologically.

Methods and Results: A retrospective review of a lung biopsy registry identified 282 patients: 188 patients with trisomy 21 (Group D) and 94 without (Group N). The mean age at lung biopsy was 3 and 7 months (P<0.0001). Pulmonary arterial pressure (PAP) and pulmonary vascular resistance were similar between the 2 groups. There were no significant differences in the proportion of patients with irreversible intimal lesions or the index of pulmonary vascular disease (IPVD; a measure of the degree of pulmonary arteriopathy progression) between the 2 groups. In addition, after propensity score matching for patient background (n=43 in each group), there were no significant differences in IPVD (P=0.29) or the ratio of irreversible intimal changes between the D and N groups (P=0.39). Multivariate analysis identified age (P<0.0001) and PAP (P=0.03) as the only risk factors for progression of pulmonary arteriopathy.

Conclusions: Histopathologically, early progression of pulmonary arteriopathy in patients with trisomy 21 was not proved compared with patients without trisomy 21. Although we cannot exclude the possibility of bias in the Group D and N patients who were slated for lung biopsy, factors other than pulmonary arteriopathy may affect the marked progression of clinical PH in trisomy 21 patients.

Key Words: Atrioventricular septal defect; Pulmonary arteriopathy; Trisomy 21, Ventricular septal defect
identified 591 (45.7%) patients who were diagnosed with VSD or AVSD (Figure 1). Among these patients, those with other genetic diseases (e.g., trisomy 18), complex lesions (e.g., hypoplastic left ventricle and total anomalous pulmonary venous connection) were excluded, as were patients who had undergone pulmonary artery banding. The remaining simple VSD or AVSD patients were selected as the cohort for the present study. These patients were divided into 2 groups, namely patients with (Group D; n=188) and those without (Group N; n=94) trisomy 21. Hemodynamic and histomorphometric data were collected retrospectively from the institution’s database and compared between the 2 groups. The protocol of the present study was approved by the Institutional Review Board of Tohoku University Graduate School of Medicine, and the requirement for informed consent was waived.

Tissue Preparation
Lung tissue was obtained from a lobe of the lung and was fixed in 10% formalin; paraffin-embedded sections were then prepared. In each case, 30 semi serial histological sections at 50-µm intervals (each 3-µm thick) were prepared, as described previously, and elastic-Masson staining was performed.

Measurements
To evaluate the severity of the intimal lesions, the diagnostic criteria of the Heath-Edwards (HE) classification and the index of pulmonary vascular disease (IPVD) score were used. The IPVD was determined on the basis of findings relating to the intima and media as follows: 1, no intimal lesions; 2, cellular proliferation of the intima; 3, fibrous thickening of the intima; and 4, destruction of the media. More than 50 small pulmonary arteries between 30 and 500 µm in diameter were evaluated, and the mean score was calculated as the IPVD score for each patient.

Propensity Score Matching
Because the present retrospective observational study carries multiple confounding factors for direct comparison of each parameter between the 2 groups, we elected to perform propensity score matching to select 2 comparable subgroups of patients with and without trisomy 21. Pulmonary arteriopathy was generally progressing until approximately 3 years of age, and so patients <3 years of age were selected. The following variables were included as covariates: age, sex, diagnosis, systolic pulmonary arterial blood pressure, and the ratio of systolic pulmonary arterial blood pressure to systolic systemic blood pressure.

Statistical Analysis
Continuous variables are expressed as the mean±SD or median (range) according to parametric or non-parametric data distribution, and were compared using Student’s t-test or the Wilcoxon rank sum test. For categorical variables, which are expressed as a frequency or percentage, the Chi-squared test was used for comparisons between groups. In addition, multiple linear regression analysis was performed to determine independent risk factors for the progression of the intimal lesion in the small pulmonary arteries. Statistical significance was set at P<0.05. Data were analyzed using JMP software (SAS Institute, Cary, NC, USA).

Results

Patients’ Characteristics
Patient characteristics, hemodynamic data, and histomorphometric data are given in Table 1. Patients in Group D were significantly younger than those in Group N (P<0.0001). In both groups, approximately 40% of patients were male. Regarding clinical diagnoses, there were 109 VSD patients (58%) and 79 AVSD patients (42%) in Group D, compared with 83 VSD patients (88%) and 11 AVSD patients (12%) in Group N (P<0.0001). Hemodynamic data obtained at preoperative catheterization indicated similar pulmonary arterial pressure (PAP) in both groups; however, the systolic and mean systemic arterial blood pressures were significantly lower in Group D than in Group N (P=0.0001 and 0.005, respectively). The ratio of systolic pulmonary pressure to systemic pressure was higher in Group D. Pulmonary vascular resistance was not significantly different between the 2 groups. In addition, the data for the 79 patients (Group D: n=40; Group N: n=39) for whom pulmonary vascular resistance data were available before and after oxygen loading during the catheter examination indicated that the vascular response to oxygen was not significantly different between the 2 groups (Figure S1).

Histologic and Histomorphometric Examinations
Histopathological examination revealed that pulmonary arteriopathic lesions, which were graded from 1 to 6 according to the HE classification, were observed in both groups, and the severity of these lesions did not differ significantly between the 2 groups (P=0.51; Figure 2A). IPVD scores for the severity of pulmonary arteriopathy tended to be lower in Group D than in Group N (median [range] 1.05 [1.00–2.26] vs. 1.10 [1.00–2.84], respectively; P=0.06; Figure 2B). The severity of pulmonary arteriopathy stratified by age at biopsy in each group is shown in Figure 3. The intimal lesions progressed with age in both groups. Irreversible pulmonary vascular diseases (PVDs), such as intimal fibrosis and complex lesions, which were graded...
The proportion of patients with an extremely thickened media of small pulmonary arteries did not differ significantly between Group D and Group N either before propensity score matching (9.0% vs. 5.3%, respectively; P=0.35) or after propensity score matching (9.3% vs. 2.3%, respectively; P=0.36). These results also indicated that the early progression of pulmonary arteriopathy was not proved histologically in patients with trisomy 21.

Propensity Score Matching

Patient characteristics after propensity score matching are given in Table 1 and Table S1. There were no significant differences in any of the variables, including age, diagnosis, and hemodynamic parameters, between the 2 groups. In addition, the intimal lesions in the 2 groups after propensity score matching from 4 to 6 according to the HE classification, were detected more frequently and at an earlier age in Group D than in Group N (Figure 3A). Irreversible PVD was occasionally detected even in patients aged 2 months, and the proportion of patients with these lesions increased up to approximately 40% at 1 year of age in Group D. In Group N, these lesions were initially detected at 3–4 months of age; the proportion of patients with these lesions did not increase by 1 year of age (approximately 10%), but it did increase after 1 year of age. Conversely, even before 1 year of age, IPVD scores remained low in both groups. There was little difference between the 2 groups (Figure 3B). In addition, we examined the prevalence of an extremely thickened media of small pulmonary arteries, which is referred to as abnormal thickening of media of small pulmonary arteries and is a finding of pulmonary vascular obstructive disease. The proportion of patients with an extremely thickened media of small pulmonary arteries did not differ significantly between Group D and Group N either before propensity score matching (9.0% vs. 5.3%, respectively; P=0.35) or after propensity score matching (9.3% vs. 2.3%, respectively; P=0.36). These results also indicated that the early progression of pulmonary arteriopathy was not proved histologically in patients with trisomy 21.

### Table 1. Characteristics of Patients With (Group D) and Without (Group N) Trisomy 21

<table>
<thead>
<tr>
<th></th>
<th>Overall cohort</th>
<th>Propensity score-matched cohort</th>
<th>P-value</th>
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<tr>
<td></td>
<td>Group D (n=188)</td>
<td>Group N (n=94)</td>
<td>P-value</td>
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<td>Age (months)</td>
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<tr>
<td>3 (0.5–408)</td>
<td>7 (0.9–696)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Male</td>
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<tr>
<td>78 (41.5)</td>
<td>35 (37.3)</td>
<td>0.52</td>
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<tr>
<td>Diagnosis</td>
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<tr>
<td>VSD</td>
<td>109 (58.0)</td>
<td>83 (88.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AVSD</td>
<td>79 (42.0)</td>
<td>11 (11.7)</td>
<td>&lt;0.0001</td>
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<tr>
<td>PDA</td>
<td>62 (33.2)</td>
<td>21 (22.3)</td>
<td>0.07</td>
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</table>

Data are expressed as the median (range), mean ± SD or n (%). AVSD, atrioventricular septal defect; mABP, mean aortic blood pressure; mPAP, mean pulmonary arterial blood pressure; PDA, patent ductus arteriosus; Qp/Qs, ratio of pulmonary blood flow to systemic blood flow; Rp, pulmonary vascular resistance; sABP, systolic aortic blood pressure; sPAP, systolic pulmonary arterial blood pressure; sPp/Ps, ratio of systolic pulmonary arterial pressure to systemic systemic blood pressure; VSD, ventricular septal defect.
According to the findings of the present study, the chromosomal anomaly of trisomy 21 may not be directly associated with the progression of pulmonary arteriopathy. This finding is in contrast with the common understanding and is a new insight.

Several studies have reported that patients with trisomy 21 rapidly develop PAH, with greater damage to the pulmonary vascular bed. In addition, trisomy 21 is considered a risk factor for the progression of pulmonary arteriopathy. Pulmonary hypertension (PH) is more fre-

Figure 3. (A) Correlation between age and the severity of the intimal lesion (Heath-Edwards [HE] classification) between in patients with (Group D) and without (Group N) trisomy 21. Irreversible pulmonary vascular disease (PVD) was observed earlier in Group D than in Group N. (B) Correlations between age and the severity of intimal lesions (index of pulmonary vascular disease [IPVD] scores) in Groups D and N shown in box-and-whisker diagrams. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the 1.5 interquartile ranges and symbols indicate outliers. Even before 1 year of age, IPVD scores remained low in both groups. There was little difference between the 2 groups.

Discussion

According to the findings of the present study, the chromosomal anomaly of trisomy 21 may not be directly associated with the progression of pulmonary arteriopathy. This finding is in contrast with the common understanding and is a new insight.

Several studies have reported that patients with trisomy 21 rapidly develop PAH, with greater damage to the pulmonary vascular bed. In addition, trisomy 21 is considered a risk factor for the progression of pulmonary arteriopathy. Pulmonary hypertension (PH) is more fre-
In the present study, only aging and high PAP were identified as risk factors for the progression of pulmonary arteriopathy. In patients with VSD or AVSD, exposure of the pulmonary artery to severe excessive pressure overload for a prolonged period may exacerbate endothelial dysfunction and induce the development of severe PVD. D’Alto et al.\textsuperscript{14} have reported that the greater frequency of AVSD in trisomy 21 patients was one of the possible causes of the early progression of PH. However, AVSD per se was not identified as a risk factor for the progression of PVD in the present study.

Other than pulmonary vasculopathy, the mechanisms that contribute to the progression of PH in trisomy 21 patients include respiratory abnormalities. Pulmonary hypoplasia, in which the density of the alveoli is decreased and the peripheral airways are enlarged, has been reported in trisomy 21 patients.\textsuperscript{15,16} In addition, upper respiratory tract obstruction due to macroglossia, laryngomalacia, and sleep apnea syndrome are often associated with the chromosomal anomaly of trisomy 21.\textsuperscript{17,18} This respiratory dysfunction leads to hypoxia, hypercapnia, and alveolar hypoventilation, and may promote PH in trisomy 21 patients.
Study Limitations
The present study was retrospective study based on a library of lung biopsy specimens. The lung specimens were collected from many institutions; therefore, details regarding medications and clinical course after biopsy could not be elucidated in the present study. In addition, lung biopsy specimens were not taken from the same portion of the lung in all cases, and we cannot rule out the possibility that the results may be attributable to some degree of sample bias. Moreover, we only evaluated the pulmonary arterial lesions, and not the respiratory system; therefore, the correlation between respiratory dysfunction and clinical PH in trisomy 21 patients could not be elucidated in the present study. Further investigations are needed to clarify the mechanisms of clinical PH in trisomy 21 patients.

Conclusions
The severity of pulmonary arteriopathy in patients with trisomy 21 was not different from that in baseline-matched patients without trisomy 21. Early progression of pulmonary arteriopathy in patients with trisomy 21 was not proved by the histopathological analysis of lung biopsy specimens. One of the possible reasons why the severity of pulmonary arteriopathy was not distinctly different between the groups may be related to biased in the Group D and N patients, who were slated for lung biopsy. Another possibility is that factors other than pulmonary arteriopathy may have an effect on the pronounced progression of clinical PH in trisomy 21 patients.

Acknowledgment
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Conflict of Interest
None declared.

References

Supplementary Files

Figure S1. Changes in pulmonary vascular resistance before and after oxygen loading in the catheter examination of 79 patients with (Group D; n=40) and without (Group N; n=39) trisomy 21.

Figure S2. Comparison of histomorphometric measurements of intimal lesions in patients with (Group D) and without (Group N) trisomy 21 after propensity score matching for patient background in patients ≤12 months of age: (A) Heath-Edwards (HE) classification and (B) box-and-whisker diagram of the distribution of index of pulmonary vascular disease (IPVD) scores.

Table S1. Patient characteristics in cohorts aged ≤12 months

Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-17-0754