Clinical characteristics and risk factors of twin pregnancies with single fetal malformation: a multi-center analysis in Southeastern China

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Received July 30, 2019; Accepted October 9, 2019; Epub December 15, 2019; Published December 30, 2019

Abstract: Objective: Twin pregnancy, which has risen in the incidence across the world, causes an increased risk to both mother and babies. However, there is little knowledge on the clinical characteristics and risk factors of twin pregnancies with single fetal malformation. This multi-center analysis aimed to investigate the clinical characteristics and identify the risk factors of twin pregnancies with single fetal malformation in Fujian Province, Southeastern China. Methods: A total of 216 twin pregnancies with single fetal malformation diagnosed at 6 tertiary hospitals in Fujian Province during the period from October 1, 2013 through June 30, 2016 were sampled as the case group, while 216 normal twin pregnancies were selected as controls. The fetal malformations were identified from the subjects’ medical records, and univariate and multivariate logistic regression analyses were employed to identify the risk factors of twin pregnancies with single fetal malformation. Results: The prevalence of twin pregnancies with single fetal malformation was 15.2% in Fujian Province, Southeastern China from 2013 to 2016. There were 225 types of fetal malformations diagnosed in the 216 twin pregnancies with single fetal malformation, and the most three common organs where the fetal malformation developed were the cardiovascular system, the skeletal system, and the urinary system. Univariate analysis revealed that the development of twin pregnancies with single fetal malformation was positively correlated with natural conception and monochorionic twins ($P = 0$), and multivariate logistic regression analysis identified monochorionic twins as an independent risk factor for twin pregnancies with single fetal malformation ($P = 0$). Conclusions: The results of the present study demonstrate a high rate of twin pregnancies with single fetal malformation mainly occurs in the cardiovascular system, the skeletal system and the urinary system. In addition, having monochorionic twins is an independent risk factor for twin pregnancies with single fetal malformation.

Keywords: Twin pregnancy, single fetal malformation, clinical characteristics, risk factor, multi-center study, southeastern China

Introduction

Twins are defined as two offspring produced by the same pregnancy, which account for 2%-3% of all births [1]. The incidence of twin pregnancy has recently risen across the world due to high maternal age, increasing use of assisted reproductive technology (ART) and other risk factors [2, 3]. Twin pregnancy causes an increased risk to both mothers and babies, including preterm delivery, intrauterine growth restriction, and pre-eclampsia [2]. In addition, monochorionic gestations confer an even higher rate of perinatal morbidity and mortality, which may lead to twin-to-twin transfusion syndrome or twin anaemia-polycythaemia sequence [4-7].

With the increasing incidence of twin pregnancy, the rate of twin pregnancies with single fetal malformation is also on a rise [8]. With the implementation of China’s universal two-child policy [9], the use of ART has sharply increased [10], which may lead to an increase in the incidence of twin pregnancy in China. However, there is little knowledge on the clinical characteristics and risk factors of twin pregnancies with single fetal malformation. This multi-center analysis aimed to investigate the clinical char-
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characteristics and identify the risk factors of twin pregnancies with single fetal malformation in Fujian Province, Southeastern China.

Subjects and methods

Subjects

Twin pregnancies with single fetal malformation diagnosed at 6 tertiary hospitals in Fuzhou City, Fujian Province during the period from October 1, 2013 through June 30, 2016 were sampled as the case group; including Fujian Provincial Hospital, Fujian Provincial Maternity and Children’s Hospital, the First Affiliated Hospital of Fujian Medical University, Fujian Medical University Union Hospital, Fuzhou Municipal First Hospital and Fuzhou Municipal Second Hospital; while pair-matched normal twin pregnancies were selected as controls. All twin pregnancies with single fetal malformation were diagnosed by means of clinical diagnosis or auxiliary tools (ultrasonography, chromosomal examinations or X-ray scan) during the study period were included in this study, while singleton pregnancy, two or more gestations, normal twin pregnancies or twin pregnancies with both fetal malformations were excluded from the study.

Twin pregnancies were classified into monochorionic and dichorionic forms based on the chorionicity and grouped into natural conception and ART conception groups according to the type of conception [1]. All subjects’ demographic and clinical characteristics were captured from the medical records.

Classification of fetal malformation

All fetal malformation data were captured from the hospital-based birth defects registration cards and perinatal infant reports, and all fetal malformations were classified according to the International Classification of Diseases (ICD)-10 diagnostic categories [11]. Fetal malformations were classified into 11 categories according to the site where the malformation occurred, including the nervous system, cardiovascular system, digestive system, skeletal system, urinary system, acardiac twins sequence, face and neck, respiratory system, abdominal fissure, chromosome abnormality and conjoined twins. A malformation that occurred in two systems or more was defined as multiple malformations.

Ethical statement

This study was approved by the Ethical Review Committee of Fujian Provincial Maternity and Children’s Hospital (date of approval: August 17, 2016; approval no.: 2016-0183). Written informed consent was obtained from all participants following a detailed description of the purpose of the study.

Data analysis

All data were entered into Microsoft Excel 2007 (Microsoft; Redmond, WA, USA), and all statistical analyses were performed using the statistical software SPSS version 13.0 (SPSS, Inc.; Chicago, IL, USA). All measurement data were expressed as mean ± standard deviation (SD). Differences of proportions were compared with chi-square test, and the risk factors of twin pregnancies with single fetal malformation were identified using univariate and multivariate logistic regression analyses.

Results

Subject characteristics

During the period between October 1, 2013 and June 30, 2016, a total of 1421 twin pregnancies were recruited at 6 tertiary hospitals in Fuzhou City, Fujian Province, and there were 216 twin pregnancies with single fetal malformations diagnosed, with a prevalence rate of 15.2%. Among all cases, there were 57.87% (125/216) diagnosed at Fujian Provincial Maternity and Children’s Hospital, 13.42% (29/216) diagnosed at Fujian Provincial Hospital, 8.33% (18/216) diagnosed at the First Affiliated Hospital of Fujian Medical University, 5.56% (12/216) diagnosed at Fujian Medical University Union Hospital, 8.8% (19/216) diagnosed at Fuzhou Municipal First Hospital and 6.02% (13/216) diagnosed at Fuzhou Municipal Second Hospital, respectively.

There were 23.61% twin pregnancies with single fetal malformation (51/216) diagnosed during the antenatal period, and 76.39% (165/216) during the postnatal period, 86.27% (44/51) that received prenatal diagnosis at < 28 weeks of gestational age and 13.73% (7/51) at 28 weeks of gestational age and greater. The malformed fetuses included 115 males (53.24%), 95 females (43.98%) and 6 of unknown gender
Twin pregnancies with single fetal malformation

There were 125 twin pregnancies with single fetal malformation (57.87%) diagnosed using ultrasonography, 75 (34.72%) through clinical examinations, 11 (5.09%) by means of clinical inspections and ultrasonography, 1 (0.46%) using chromosomal test, 1 (0.46%) using ultrasonography combined with chromosomal test and 3 (1.39%) using other tools.

The case and control groups were balanced with the mean maternal age (29 ± 4.6 vs. 27 ± 5.7 years, P > 0.05).

**Fetal malformations**

There were 225 types of fetal malformations diagnosed in the 216 twin pregnancies with single fetal malformation, and the most three common systems where the fetal malformation occurred included the cardiovascular system (40%), the skeletal system (15.1%) and the urinary system (14.2%). Ventricular septal defect was the predominant fetal malformation in the cardiovascular system (72.2%), talipes quinovarus was the predominant fetal malformation in the skeletal system (35.3%), and hypospadias was the predominant fetal malformation in the urinary system (34.4%). In addition, natural conception was the predominant conceptive type (90.2%) and monochorionic twins were the predominant type of twin pregnancies (74.7%) seen in the 216 twin pregnancies with single fetal malformation (Table 1). Table 2 describes the types of fetal malformations diagnosed at the 6 tertiary hospitals included in this study.

**Risk factors of twin pregnancies with single fetal malformation**

Univariate analysis revealed that the development of twin pregnancies with single fetal malformation correlated with natural conception and monochorionic twins (P = 0), and had no associations with maternal age, number of pregnancies, residency or education levels (P > 0.05) (Table 3). Multivariate logistic regression analysis identified monochorionic twins as an independent risk factor for twin pregnancies with single fetal malformation (P = 0) (Table 4).

**Discussion**

Fetal malformation, including specific and non-specific malformation, is a major cause of perinatal mortality, which involves all systems across the body [12]. Previous studies have demonstrated a higher rate of fetal malformation in twin pregnancies than in single pregnancies, with single-twin malformation as the predominant form [13-18]. In this study, the prevalence of twin pregnancies with single fetal malformation was 15.2% in Fujian Province, southeastern China, which was far greater than the national prevalence in China (2.86%) [19], and in 14 European countries from 1984 to 2007 (10.7 per 10,000 births) [20]. The high rate of twin pregnancies with single fetal mal-

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**Table 1. Types of conception and chorionicity of 225 malformations seen in 216 twin pregnancies with single fetal malformation**

<table>
<thead>
<tr>
<th>Site of fetal malformation</th>
<th>Type of conception</th>
<th>Natural conception</th>
<th>Assisted reproductive technology</th>
<th>Monochorionic twin</th>
<th>Dichorionic twin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular system</td>
<td></td>
<td>79</td>
<td>11</td>
<td>66</td>
<td>24</td>
<td>90</td>
</tr>
<tr>
<td>Skeletal system</td>
<td></td>
<td>30</td>
<td>4</td>
<td>23</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>Urinary system</td>
<td></td>
<td>27</td>
<td>5</td>
<td>24</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Face and neck</td>
<td></td>
<td>19</td>
<td>2</td>
<td>14</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Nervous system</td>
<td></td>
<td>15</td>
<td>0</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Digestive system</td>
<td></td>
<td>9</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Conjoined twins</td>
<td></td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Abdominal fissure</td>
<td></td>
<td>8</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Acardiac twins sequence</td>
<td></td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Respiratory system</td>
<td></td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Chromosome abnormality</td>
<td></td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>203</td>
<td>22</td>
<td>168</td>
<td>57</td>
<td>225</td>
</tr>
</tbody>
</table>
### Table 2. Types of fetal malformations seen in 216 twin pregnancies with single fetal malformation in 6 centers included in this study

<table>
<thead>
<tr>
<th>Center at diagnosis</th>
<th>Cardiovascular system</th>
<th>Skeletal system</th>
<th>Urinary system</th>
<th>Face and neck</th>
<th>Nervous system</th>
<th>Digestive system</th>
<th>Conjoined twins</th>
<th>Abdominal fissure</th>
<th>Acardiac twins sequence</th>
<th>Respiratory system</th>
<th>Chromosome abnormality</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fujian Provincial Maternity and Children’s Hospital</td>
<td>55</td>
<td>20</td>
<td>20</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>130</td>
</tr>
<tr>
<td>Fujian Provincial Hospital</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>The First Affiliated Hospital of Fujian Medical University</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Fujian Medical University Union Hospital</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Fuzhou Municipal First Hospital</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Fuzhou Municipal Second Hospital</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>34</td>
<td>32</td>
<td>21</td>
<td>15</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>225</td>
</tr>
</tbody>
</table>
Twin pregnancies with single fetal malformation

Table 3. Univariate analysis of risk factors for 216 twin pregnancies with single fetal malformation

<table>
<thead>
<tr>
<th>Demographic and clinical feature</th>
<th>Case group (n = 216)</th>
<th>Control group (n = 216)</th>
<th>χ² value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35</td>
<td>188</td>
<td>197</td>
<td>1.912</td>
<td>0.167</td>
</tr>
<tr>
<td>≥ 35</td>
<td>28</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>88</td>
<td>95</td>
<td>0.464</td>
<td>0.496</td>
</tr>
<tr>
<td>≥ 2</td>
<td>128</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban areas</td>
<td>116</td>
<td>95</td>
<td>0.337</td>
<td>0.562</td>
</tr>
<tr>
<td>Rural areas</td>
<td>100</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junior high school and lower</td>
<td>54</td>
<td>64</td>
<td>0.948</td>
<td>0.33</td>
</tr>
<tr>
<td>Senior high school and higher</td>
<td>162</td>
<td>152</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of conception</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural conception</td>
<td>194</td>
<td>161</td>
<td>16.213</td>
<td>0</td>
</tr>
<tr>
<td>Assisted reproductive technology</td>
<td>22</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chorionicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monochorionic twin</td>
<td>159</td>
<td>79</td>
<td>56.747</td>
<td>0</td>
</tr>
<tr>
<td>Dichorionic twin</td>
<td>57</td>
<td>137</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Multivariate analysis of risk factors for 216 twin pregnancies with single fetal malformation

<table>
<thead>
<tr>
<th>Variable</th>
<th>b</th>
<th>SE</th>
<th>Wald</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural conception</td>
<td>0.452</td>
<td>0.301</td>
<td>2.257</td>
<td>0.133</td>
<td>1.304</td>
<td>0.871, 2.837</td>
</tr>
<tr>
<td>Monochorionic twin</td>
<td>1.459</td>
<td>0.222</td>
<td>43.334</td>
<td>0</td>
<td>3.507</td>
<td>2.785, 6.639</td>
</tr>
</tbody>
</table>

formation may be attributed to the advanced maternal age [3]; however, the exact causes require further investigations.

In this multi-center study, 57.87% twin pregnancies with single fetal malformation were diagnosed at Fujian Provincial Maternity and Children’s Hospital. As the only province-level maternity and children’s healthcare center, more than 18 thousand pregnancies were delivered at the hospital, and most of the twin pregnancies received prenatal diagnosis and were delivered at this hospital. In addition, Fujian Provincial Center for Prenatal Diagnosis is built in this hospital, which undertakes the diagnosis of birth defects, notably for the malformations on the fetal body surface, such as hypospadias, accessory ear, hemangioma and talipes equinovarus, and a careful physical examination may reduce the missing diagnosis of these malformations. In this study, the circulatory system was found to rank first as the site of fetal malformation development, which may be explained by the fact that most of the malformations in the circulatory system are diagnosed using ultrasound. These data indicate that improving the detection of malformations during pregnancy and the subsequent termination of pregnancy is of great importance to reduce fetal death and the birth of unstable and malformed infants. In addition, the 51 twin pregnancies with a single fetal malformation receiving prenatal diagnosis all had severe malformations, which mainly occurred at the nervous system (19.6%) and the circulatory system (19.6%). Therefore, selective fetal reduction may be done to reduce the delivery of the fetus with severe malformations in the context of the other normal fetus in twin pregnancies.

In the current study, the most three common organs where fetal malformation developed were the cardiovascular system, the skeletal system, and the urinary system, which was similar to previous reports [22, 23]. We found that congenital heart disease was predominant in cardiovascular disorders, and ventricular septal defect was the predominant fetal malformation in the cardiovascular system, which was in
Twin pregnancies with single fetal malformation

agreement with previous findings that congenital heart defects are the most common type of birth defects in both single and twin pregnancies [24-26]. Malformations of the skeletal system include talipes equinovarus, polydactyly, limb shortening and deformity, spinal dysplasia, strephexopodism, ankyloactylia, rib deformity and muscular spasm, and most of these malformations may be identified by prenatal diagnosis during the pregnancy and have a satisfactory prognosis [20]. However, severe limb shortening and deformity and muscular spasm may cause neonatal disabilities, leading to psychological and physiological burdens. Malformations of the urinary system mainly included hypospadias, enorchia, hydrocele of tunica vaginalis and concealed penis, and most of these malformations are diagnosed after birth. Therefore, active postpartum follow-up and treatment should be given to these malformations and prevention is required at all three levels.

With great advances in maternal-fetal medicine [27], complex twin pregnancies are paid increasingly attention [1], and remarkable improvements have been achieved in intrateruitive therapy [28-31]. Most complex twin pregnancies are considered as specific complications of enzygotic twins [1]. In this study, we detected a low proportion of acardiac twins and conjoined twins in the study subjects; however, these highly fatal and unstable malformations require a lot of attention. Acardiac twins sequence mainly includes acephalocardia, acardius anencephalus, acardius amorphus and acardius acormus [32]. During the embryonic period, the twins may develop obvious placental vascular anastomosis, and the fetus with normal structure (blood pumper) pumps and transfers blood to the fetus with abnormal structure (blood receptor), which aggravates the cardiac burden in the blood pumper and leads to a high likelihood of developing heart failure, resulting in pericardial effusion and polyhydramnios [33, 34].

In the current study, we found that the development of twin pregnancies with single fetal malformation was associated with the chorionicity and type of conception (P < 0.05). Univariate analysis identified natural conception and monochorionic twins as risk factors for twin pregnancies with single fetal malformation (P < 0.05), and further multivariate logistic regression analysis revealed that monochorionic twins as an independent risk factor for twin pregnancies with single fetal malformation (P < 0.05).

The chorionicity of twin pregnancies includes dichorionic and monochorionic twins; dichorionic twins mainly develop from dizygotic twins, while monochorionic twins, which only develop in monozygotic twins, arise from the division of a single fertilized egg [35]. Most of dichorionic twins occur in natural conception; however, they may also develop from unnatural conception. Following single fetal growth arrest in twin pregnancies, the surviving fertilized eggs develop cleavage, and then develop into monozygotic twins [36]. It is theoretically speculated that monochorionic twins share almost consistent genetic materials, and have an equal possibility of malformations; therefore, monochorionic twins are more likely to develop malformations, or exhibit a lower possibility to have a single fetal malformation than dichorionic twins [37]. Currently available data show a stronger correlation between the chorionicity and fetal malformation in monochorionic twins than in dichorionic twins [24, 33], which is in agreement with the findings from the present study. It is hypothesized that because of uneven cell distribution during the division of monozygotic twins, there is arteriovenous and placental sharing discordance in the placenta, which leads to unequal fetal nutritional distribution or impact of teratogens on a single fetus, thereby resulting in inconsistent growth trends of twins [38, 39]. Nevertheless, dichorionic twins have their respective independent placentas, and there is no vascular communication between the twins, thereby resulting in a low possibility of fetal malformations [40].

Currently, whether the conception by ART increases the incidence of fetal malformations remain in dispute, and the underlying mechanisms have not been fully demonstrated [41-44]. Twins conceived by natural ways had a greater possibility of anencephalus than those conceived by ART, which was considered to be attributed to ovarian hyperstimulation [23]. However, ART was also reported to have no associations with the development of fetal malformations [45-47]. In this study, our data showed that natural conception was the predominant type of conception in twin pregnancies with single fetal malformation, and natural conception was identified as a risk factor for
Twin pregnancies with single fetal malformation. This may be explained by the fact that twins conceived by natural ways are mostly monochorionic, while the pregnancies conceived by ART are mostly dichorionic [48]. In addition, some diseases may be screened and diagnosed prior to implantation with the advances in ART, which reduces the development of malformations [49, 50].

Fetal malformation is a major cause of intrauterine fetal death in dichorionic twins [51]. In monochorionic twins, however, the single fetal malformation may cause poor pregnancy outcomes in the second healthy fetus due to vascular communication, including abortion, premature birth, stillbirth and cerebral injuries [1]. Therefore, the decision on the malformed fetus should be made based on a systematic assessment of the maternal and fetal conditions. Reduction of a malformed fetus is recommended for fetuses with deadly or severe disabling malformations following definitive diagnosis, notably exclusion of chromosome abnormality in the other fetus. Currently, intrauterine therapy has shown effective and safe for the treatment of severe fetal malformations, including acardiac twins sequence, and improvements in the outcomes of the normal fetus [25-27].

In summary, the results of the present study demonstrate a high rate of twin pregnancies with single fetal malformation in Fujian Province, southeastern China, and the fetal malformation mainly occurs in the cardiovascular system, the skeletal system and the urinary system. In addition, monochorionic twin is an independent risk factor for twin pregnancies with single fetal malformation. More health education and primary prevention is strongly recommended to reduce the development of malformations in twin pregnancies. Furthermore, precision diagnosis and management of twin pregnancies with single fetal malformation is of great significance to improve the outcomes [52].

Acknowledgements

The authors would like to thank the staff from the Department of Gynecology and Obstetrics, Fujian Provincial Maternity and Children’s Hospital during the data collection. JYL was funded by a grant from Natural Science Foundation of Fujian Province (grant no. 2018J0-1234). JYY was funded by the Key Clinical Specialty Discipline Construction of Fujian Province (grant no. Min[2015]-593).

Disclosure of conflict of interest

None.

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References

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