Optical Coherence Tomography Angiography in Familial Exudative Vitreoretinopathy: Clinical Features and Phenotype-Genotype Correlation

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Purpose. To evaluate the microstructure of the fovea in patients with familial exudative vitreoretinopathy (FEVR) compared to healthy controls using optical coherence tomography angiography (OCTA).

Methods. In this consecutive, cross-sectional, observational case series, 41 eyes of 41 patients diagnosed as FEVR and 37 eyes in 37 control subjects were studied. OCTA was utilized to automatically measure the foveal avascular zone (FAZ) and the vessel density (VD). Inner retinal thicknesses (IRT) and central retinal thickness (CRT) were measured with the instrument caliper. Targeted next-generation sequencing was performed, and phenotype-genotype association was analyzed.

Results. Small FAZ was found in 31.70% (13/41) FEVR eyes but not in controls. Greater CRT and lower superficial foveal VD were noted in FEVR patients. FAZ is negatively correlated with IRT. Persistence of the inner retinal layer (IRL) in fovea was present in 48.78% (20/41) FEVR eyes but not found in controls. Zero percent (0/10) of patients with the low-density lipoprotein receptor-related protein 5 (LRP5) mutation, 50% (1/2) with the frizzled-4 (FZD4) mutation, and 66.67% (3/4) with the tetraspanin-12 (TSPAN12) mutation had preserved foveal IRL and small FAZ.

Conclusions. Our data indicate FEVR status is associated with a significantly smaller FAZ, decreased vascular density in both the superficial and deep layers of parafoveal area, a thicker fovea, and an abnormally preserved IRL in fovea. In addition, patients with the LRP5 mutation had a milder phenotype than those with the FZD4 or TSPAN12 mutations. These novel findings could provide insight into the understanding of the pathogenesis of FEVR.

Keywords: familial exudative vitreoretinopathy, optical coherence tomography angiography, phenotype-genotype correlation

Familial exudative vitreoretinopathy (FEVR) is a hereditary disorder first described by Criswick and Schepens in 1969.1 Clinically, FEVR exhibits strikingly variable phenotypes, ranging from hardly detectable peripheral retina vascular anomalies to bilateral retinal detachments leading to blindness.2 Classic clinical findings include peripheral avascular retina, retinal neovascularization, exudation, dragging of vasculature, and tractional retinal detachment.3–5 Incomplete vascularization of the retina might lead to various vision-threatening complications, such as vitreous hemorrhage and vitreoretinal traction, primarily on the retina's periphery.6 However, the posterior pole of FEVR patients is believed to be abnormal, even in mild asymptomatic cases. In our previous study,7 we reported abnormal pattern of vessels radiating from the optic disc in mild asymptomatic FEVR individuals. In 2014, Kashani et al.8 had revealed a broad spectrum of features, including diminished foveal contour, persistent fetal foveal architecture, and disruption of the ellipsoid zone in moderate to severe FEVR patients. The anatomical changes also showed a correlation with visual function.9 To date, the macular microvasculature in FEVR remains unknown. In this study, we performed optical coherence tomography angiography (OCTA) in a series of mild to moderate FEVR patients and investigated the correlation between angiographic OCT and structural OCT. It is well documented that LRP5, FZD4, TSPAN12, and NDP are involved in the Wnt/Norrin signaling pathway, which plays a crucial role in normal retinal vasculature development. Mutations in these genes underlie the molecular mechanisms causing FEVR.7–8 Studies also reported recently that mutations in zinc finger protein 408 (ZNF408) and kinesin family member 11 (KF11) related to FEVR pathogenesis.9,10 These six genes were tested in the majority of patients in this study, and the preliminary genotype-phenotype correlation was also analyzed.

Materials and Methods

This study enrolled 41 FEVR patients from 41 families and 37 age-matched healthy controls. Only one eye was designated as the study eye in each FEVR patient or control. One eye that fulfilled all inclusion criteria and none of the exclusion criteria was designated as the study eye; in a case where both eyes from
the same patient fulfilled all inclusion criteria and none of the exclusion criteria, the left eye was chosen as the study eye. The research was conducted in agreement with the Declaration of Helsinki and approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. Informed consent was obtained from the individuals included in our study after explaining the nature and the possible consequences. FEVR was diagnosed in all patients by positive family history, presence of avascular area, and typical peripheral retinal vascular stretching and leakage. The exclusion criteria were: (1) history of preterm birth; (2) any previous ocular surgery; (3) eyes with cataract, radial retinal folds, vitreous hemorrhage, or tractional retinal detachment that might compromise the quality of the OCTA images.

Each enrolled patient underwent a comprehensive ophthalmologic examination, including best-corrected visual acuity (BCVA), axial length (AL), funduscopy, fluorescein fundus angiogram (FFA), OCT, and OCTA. FEVR staging was determined according to FFA as previously described: stage 1, avascular periphery; stage 2, avascular periphery with neovascularization; stage 3, macula-sparing retinal detachment; stage 4, macula-involving retinal detachment; stage 5, complete retinal detachment. Structural OCT and OCT angiography were performed with an Avanti RTVue XR system 2.0 (Optovue, Inc., Fremont, CA, USA). The proprietary three-dimensional PAR algorithm developed by Optovue removed projection artifacts from the OCTA volume on a per voxel basis, using information from the OCT and OCTA volume to differentiate an in situ OCTA signal from projection artifacts. With three-dimensional PAR-enabled software, projection artifact was removed from en-face OCTA images and B-scan OCTA images. OCTA images were corrected for magnification using Bennett's formula. The inner capillary layers were imaged from the internal limiting membrane to the ganglion cell layer. The en-face image was segmented with the inner boundary at the outer inner plexiform layer, and the outer boundary was set at the midpoint of the outer plexiform layer to obtain images of the outer layers of capillaries. The foveal avascular zone (FAZ), the vessel density of foveal zone (within the 1-mm central circle on ETDRS grid), and the parafoveal zone (the annual area with a 3-mm diameter circle, eliminating the foveal zone) were automatically measured. The appearance of FAZ was described as abnormal if the FAZ was not recognized or if any vessel crossed within the FAZ area. The FAZ area was categorized as small (area $< 0.18 \text{ mm}^2$; we defined the mean FAZ area $\pm 2$ SD of controls as the normal range, and anyone less than mean $-2$ SD as small, which would be $0.18 \text{ mm}^2$) and non-small (area $\geq 0.18 \text{ mm}^2$; Fig. 1). Foveal contour and thickness was assessed by spectral domain optical coherence tomography B scans performed by the same instrument. Using the built-in software Avanti RTVue XR system 2.0 (Optovue), the inner and outer retinal thickness in the fovea center was measured manually. Inner retinal thickness (IRT) was defined as the distance between the internal limiting membrane (ILM) and outer border of the inner nuclear layer. Outer retinal thickness (ORT) was defined as the
distance between the outer border of the inner nuclear layer and the inner border of the retinal pigment epithelium (RPE). Central retinal thickness (CRT) was identified as the distance between the ILM and RPE. 18

Gene tests on six known genes were performed in the majority (27/41) of patients in the FEVR group. All DNA samples were extracted from peripheral whole blood samples. Using methods previous described,2 targeted next-generation sequencing was performed using a custom genetic pediatric retinal disease panel. Identified mutation was validated by Sanger sequencing through family members. The Human Gene Mutation Database (http://www.biobase-international.com/product/hgmd, in the public domain) was consulted to identify reported pathogenic variants. We then predicted the penetrance and genotype-phenotype correlation of each variant using a family segregation study.2

**Statistical Analysis**

All statistical analyses were performed using SPSS software 23 (SPSS, Inc., Chicago, IL, USA). All quantitative data were expressed as mean ± standard deviation. Variable normality was inspected using the Shapiro-Wilk test. Comparisons of normal distributional data between FEVR patients and control subjects were performed using the Student’s t-test. The Mann-Whitney U test was used to compare data with non-normal distributions. Non-parameters were compared with chi-squared test. Correlations between continuous variables were analyzed using Pearson’s correlation analyses. P values <0.05 were considered statistically significant.

### RESULTS

Forty-one FEVR patients and 37 healthy controls were evaluated. Table 1 summarizes the demographics and clinical features. The average age was 25.61 ± 6.93 years old (range, 8–44) in FEVR group and 25.46 ± 6.45 years old (range, 10–42) in the control group (P = 0.936). In the FEVR group, 63.41% were male, and in the control group, 67.57% were male (P = 0.700). No statistically significant difference was found in refractive status, with an average of −2.68 ± 2.05D and −3.10 ± 1.85D in FEVR and controls, respectively (P = 0.345). Thirty-six eyes with FEVR were graded as stage 1, and five eyes were graded as stage 2.

### Abnormal FAZ Appearance and Smaller FAZ Area in FEVR Patients

As shown in Figure 1 and Table 2, the most distinct difference between the FEVR group and controls is the abnormal central foveal capillary network. The complete absence of FAZ was noted in four eyes (9.76%) in the FEVR group. However, FAZ is present in all eyes (100%) in the control group. Crossing vessel in the avascular area was found in 2 (4.88%) FEVR eyes. In addition, the average FAZ area was 0.27 ± 0.17 mm² in FEVR groups and 0.36 ± 0.09 mm² in controls (P = 0.003). The average FAZ perimeter was 2.04 ± 0.67 mm in FEVR groups and 2.41 ± 0.32 mm in controls (P = 0.004). Acircularity index (AI; measured as perimeter/standard circle perimeter with equal area) was 1.15 ± 0.08 and 1.11 ± 0.03 in FEVR and controls, respectively (P = 0.02; Table 2, Fig. 2). Small FAZ (area <0.18 mm²) was found in 13 (31.70%) of FEVR eyes but not in controls, resulting in a P value of <0.001.

**Table 1. Demographics and Clinical Features of FEVR and Controls**

<table>
<thead>
<tr>
<th>Variables</th>
<th>FEVR, n = 41 (95%CI)</th>
<th>Control, n = 37 (95%CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eyes (subjects)</td>
<td>41</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>M/F (M%)</td>
<td>26/15 (63.41%)</td>
<td>25/12 (67.57%)</td>
<td>0.700*</td>
</tr>
<tr>
<td>Age (y, range)</td>
<td>25.61 ± 9.63 (8–44)</td>
<td>25.46 ± 6.45 (10–42)</td>
<td>0.936</td>
</tr>
<tr>
<td>RE (Diopeter)</td>
<td>−2.68 ± 2.05</td>
<td>−3.10 ± 1.85</td>
<td>0.345</td>
</tr>
<tr>
<td>AL (mm)</td>
<td>24.97 ± 0.97</td>
<td>25.15 ± 0.96</td>
<td>0.417</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test was used.

### Table 2. Area of Foveal Avascular Zone and Vessel Density in FEVR and Control

<table>
<thead>
<tr>
<th>Variables</th>
<th>FEVR, n = 41 (95%CI)</th>
<th>Control, n = 37 (95%CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAZ Area (mm²)</td>
<td>0.27 ± 0.17 (0.22–0.32)</td>
<td>0.36 ± 0.09 (0.33–0.38)</td>
<td>0.003</td>
</tr>
<tr>
<td>Perimeter (mm)</td>
<td>2.04 ± 0.67 (1.82–2.24)</td>
<td>2.41 ± 0.32 (2.30–2.52)</td>
<td>0.004*</td>
</tr>
<tr>
<td>AI</td>
<td>1.15 ± 0.08 (1.13–1.17)</td>
<td>1.11 ± 0.03 (1.11–1.13)</td>
<td>0.02*</td>
</tr>
<tr>
<td>FD-300 (%)</td>
<td>44.41 ± 5.51 (42.74–46.02)</td>
<td>49.56 ± 3.96 (48.25–50.78)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Foveal VD (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>20.25 ± 7.81 (17.96–22.71)</td>
<td>17.03 ± 6.00 (15.14–19.00)</td>
<td>0.046</td>
</tr>
<tr>
<td>Deep</td>
<td>33.15 ± 8.49 (30.32–35.78)</td>
<td>30.45 ± 5.85 (28.62–32.38)</td>
<td>0.11</td>
</tr>
<tr>
<td>Parafoveal VD (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>43.67 ± 5.35 (41.80–45.03)</td>
<td>46.77 ± 3.82 (45.48–47.95)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Deep</td>
<td>48.62 ± 6.70 (46.58–50.77)</td>
<td>50.61 ± 5.43 (48.99–52.42)</td>
<td>0.103*</td>
</tr>
</tbody>
</table>

FD-300 = vessel density around the 300-μm width of the FAZ region.
Decreased Parafoveal Vessel Density in FEVR Patients

The FEVR eyes had a statistically significant lower density in parafoveal superficial retina vessel density (SRVD; 43.67% ± 5.35% vs. 46.77% ± 3.82%; \(P = 0.001\)) and had a trend of lower density in deep retina vessel density (DRVD; 48.62% ± 6.70% vs. 50.61% ± 5.43%; \(P = 0.103\); Table 2, Fig. 3). Furthermore, the vessel density FD-300 (300 \(\mu\)m around FAZ region) was 44.41% ± 5.51% in FEVR group, which is lower than in controls (49.56% ± 3.96%, \(P < 0.001\); Table 2, Fig. 2).

Preserved Foveal Inner Retinal Layer Noted in 48.78% of Eyes With FEVR

Based on the structural OCT, the foveal CRT was significantly higher in the FEVR group (219.56 ± 34.95 \(\mu\)m vs. 189.97 ± 11.52 \(\mu\)m; \(P < 0.001\)). Moreover, the average IRT was 15.59 ±
FIGURE 3. Retinal vascular density in FEVR patients and healthy controls. (A, C) Representative images of the superficial and deep retinal vascular density in a FEVR and (B, D) a healthy control. (A) In a FEVR patient, the SRVD (superficial retina vessel density) is 26.8% in the foveal area, and 44.1% in the parafoveal zone. (C) DRVD (deep retina vessel density) is 33.1% and 39.3%, respectively. (B) However, in a control healthy, the SRVD is only 8.9% in the foveal zone, and 47.0% in the parafoveal zone. (D) The DRVD is 22.5% in the foveal zone, and 46.1% in the parafoveal zone. The foveal SRVD and DRVD in FEVR eyes were significantly higher than control eyes. However, (G) the superficial and (H) deep vessel densities of FEVR eyes in the parafoveal zone were lower than those in control. *P < 0.05, ***P < 0.001.
19.09 in FEVR and 0 ± 0 in controls (P < 0.001). In individuals with FEVR, the preserved foveal IRL was noted in 48.78% (20/41) of eyes using structural OCT, while it was not found in any control eyes (P < 0.001). Interestingly, in the patients with preserved central foveal IRL, the IRT was negatively correlated with the FAZ area (r = -0.53, P = 0.016; Fig. 4).

Phenotype-Genotype Association for Microvascular Structure in FEVR Patients

For genetic diagnosis, 27 families received genetic tests. Causative mutations were identified in 59.26% (16/27) of families, including LRP5 mutations in 10 patients, FDZ4 in 2 patients, and TSPAN12 mutations in 4 patients. No NPD, ZNF408, or KIF11 mutations were found in our series. No mutations were noted in these six genes in the other 11 patients. No patient with LRP5 mutation had preserved foveal IRL or small FAZ area. However, 50% (1/2) of patients with the FZD4 mutation and 75% (3/4) with the TSPAN12 mutation had preserved foveal IRL and a small FAZ area (Fig. 5).

DISCUSSION

In this study, we performed a cross-sectional study of structural OCT and OCTA images obtained from a cohort of individuals with FFA-confirmed FEVR. Compared to controls, FEVR patients had a smaller and irregular FAZ. The FAZ area negatively correlates with the inner retinal thickness at the fovea. In addition, superficial and deep parafoveal capillary density had decreased in FEVR patients. These findings suggest that not only the periphery retinal vessels but the vessels in the macular area are also involved in this disease. Understanding the subtler changes in FEVR patients could allow for improved patient identification, and these patients then could benefit from noninvasive angiography for more definitive diagnosis. To our knowledge, this study is the first to report on OCTA changes in FEVR patients. It is well known that FAZ is critically important in visual development. Traditionally, FAZ could only be investigated by high-quality FFA images. In 1999, Mintz-Hittner et al. reported the absence of FAZ by FFA in preterm individuals. However, FFA requires intravenous dye injection, which is invasive and time-consuming while posing some risks. Recently, OCTA has provided a more accurate way to observe and measure FAZ. OCTA is a novel, noninvasive imaging technology that can acquire volumetric angiographic information without the need for injection of intravenous dye. OCTA has been employed to reveal the microvascular changes of early stage DR, AMD, and some rare diseases, such as Best vitelliform macular dystrophy, Stargardt disease, and small melanocytic choroidal tumors. It has the advantage of obtaining high-resolution images of the vascular tissue at various depths of the retina, therefore providing an additional way to diagnose and monitor patients with posterior pole abnormalities. In our study, we analyzed different OCTA plexuses in FEVR patients qualitatively and quantitatively to investigate vascular alterations while gathering insights into FEVR pathogenesis. The OCTA images showed that the FAZ area was decreased dramatically. In FEVR eyes, 16.92% had a small FAZ area (<0.18 mm²). Our analyses further found a significant negative correlation between central foveal IRL thickness and the FAZ.
area, which might indicate that the preserved IRL was associated with a persistent central foveal capillary network. Our results suggest that FAZ developed abnormally in some FEVR patients. A previous study considered that the development of the fovea involves bidirectional movements of the retinal neuronal cells. The cells of the inner retina are displaced centrifugally to form the foveal pit, and cone photoreceptor cells migrate centripetally to increase the concentration of foveal cones in the foveal pit. Smaller FAZ in some FEVR patients might be related to the incomplete migration of photoreceptor and inner retina and, consequently, lead to incomplete development of fovea. The absence of FAZ was also reported in eyes with threshold/prethreshold ROP and Stickler syndrome.

Another novel finding in the current study is that both the superficial and deep parafoveal retinal vascular density (VD) was lower in the FEVR group than in the normal controls. There are no reports on the change of VD in ROP or Stickler syndrome, so the underlying mechanism remains unclear. However, we suggest that this phenomenon is at least one possible reason for lower visual density in the parafoveal area of FEVR patients.

According to the structural OCT images, on average, the fovea is thicker in FEVR patients, mainly due to the presence of retentive IRL and the absence of the foveal pit. Nearly half of FEVR patients (48.78%) had a persistence of IRL with a reduced or absent foveal pit. A study by Yonekawa included more variable phototypes, from asymptomatic to retinal dragging, observing the persistence of the IRL in only 20% of FEVR eyes. We believe that this discrepancy might result from different study populations. OCTA is a measurement that depends on strong fixation ability while OCT does not. The phenotype in the current study should be milder than in Yonekawa’s.

There are several other retinal diseases known to have preserved foveal IRL, such as retinopathy of prematurity (ROP), albinism, and Stickler syndrome. Previous studies have shown an absence of FAZ in eyes with threshold/prethreshold ROP and an absence of the foveal depression in half the eyes in preterm birth children, even without ROP. Although the underlying mechanisms of these diseases differ, this similar finding in different disease entities suggests that the persistence of foveal IRL might be a common result of abnormal macular development in early infancy. Further studies are needed to investigate the possible links between these diseases.

Rao et al. have reported that mutations in the six genes account for 38.7% of FEVR patients in Chinese patients and Salvo’s report has shown 48.9% of disease-causing variants. Previous studies showed that the LRP5 mutations showed broader phenotypic spectra, while NDP mutations were correlated with severe phenotypes. We also investigated the potential association between clinical features and gene results, with positive genetic findings in 59.26% of our tested FEVR patients. This finding aligns with our prior studies and those of others. In our previous studies, we showed that Han Chinese may have a unique mutation spectrum, primarily with FZD4 involvement instead of LRP5. However, in the current study’s series, LRP5 was identified as the causative mutation in the majority of patients. Interestingly, our results suggest that the microstructural abnormalities—including the FAZ disappearance/decrease and persistent foveal IRL—are very common in patients with FZD4 or TSPAN12 mutations but not in those with LRP5 mutations.

There are some limitations to our study. First, despite the relatively large sample size, FEVR is a rare disease, and the number of patients is limited. Further studies should be performed to determine the repeatability of our findings. Second, our center is a tertiary referral center for pediatric vitreoretinal diseases, including pediatric retinal detachment. Referral bias could very well be present. Third, 27 out of 41 families agreed to undergo genetic testing, but the other 14
families did not. There could be cultural or social factors dictating family decision-making, which may introduce unanticipated bias.

In summary, our study produced significant and novel findings regarding the microvascular structure of FEVR patients. Some patients with FEVR tended to have a significantly thicker fovea, an abnormally preserved IRL at the fovea, a smaller FAZ, and decreased vascular density in both the superficial and deep layers of the parafoveal area. These changes were noted in the majority of FEVR patients secondary to \textit{TPSN12/FZD4} mutation but were absent in patients secondary to \textit{LRP5} mutation. Further studies are necessary to further explore the genotype-phenotype association.

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