Further Genetic and Clinical Insights of Posterior Polymorphous Corneal Dystrophy 3

Petra Liskova, MD, PhD; Michalis Palos, MD; Alison J. Hardcastle, PhD; Andrea L. Vincent, MBChB

IMPORTANCE Posterior polymorphous corneal dystrophy (PPCD) is a very rare disorder characterized by primary changes of the posterior corneal layers. Sequence variants in 3 genes are associated with the development of PPCD, including ZEB1 that is responsible for PPCD3. Evidence suggests at least 1 more gene remains to be identified.

OBJECTIVE To determine the molecular genetic cause of PPCD3.

DESIGN We performed extensive ophthalmological examination, including rotating Scheimpflug imaging technology and specular microscopy, and direct sequencing of the ZEB1 coding region. Comprehensive review of published PPCD3-causing variants was undertaken.

SETTING Ophthalmology department of a university hospital.

PARTICIPANTS Four Czech probands.

MAIN OUTCOMES AND MEASURES Results of ophthalmological examination and direct sequencing of the ZEB1 coding region.

RESULTS The following 2 novel frameshift mutations within ZEB1 were identified: c.2617dup in exon 8 in a 22-year-old woman, considered to be most likely de novo in origin, and c.698dup in exon 6 in a 20-year-old man. The first patient had mild changes consistent with PPCD and bilateral best-corrected visual acuity of 1.00. The corneal phenotype of the patient in the second case was more severe, with best-corrected visual acuity of 0.40 OD and 0.05 OS. Corneas of both probands were abnormally steep (keratometry readings, flat ≥ 47.4 diopters [D] and steep ≥ 49.2 D) with increased pachymetry values but no pattern indicative of keratoconus. Specular microscopy in both patients revealed reduced endothelial cell density (range, 1055/mm² to 1655/mm²). Both probands had a history of surgery for inguinal hernia; the male patient also reported hydrocele.

CONCLUSIONS AND RELEVANCE Nucleotide changes within the coding region of ZEB1 underlie the pathogenesis of PPCD in 4 of 23 Czech probands (17%). The cumulative de novo ZEB1 mutation rate is at least 14%. Possible involvement of ZEB1 sequence variants not readily identified by direct sequencing of coding regions needs to be further investigated. Our findings also have implications for patient counseling.

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Posterior polymorphous corneal dystrophy (PPCD) is a very rare disorder overrepresented in the Czech population compared with other countries, with an estimated prevalence of 1:100,000. The disease is characterized by primary changes of the posterior corneal layers, with a clinical spectrum of presentation including geographical and vesicular-like lesions, bands, opacities, and resulting irregularities of the otherwise smooth posterior corneal surface. Most studies have observed histopathological findings of abnormal endothelium with epithelial-like features. The abnormal endothelium has the ability to proliferate, leading to focal formation of multilayered areas. Descemet membrane is irregularly thickened and multilaminar with a pathological posterior collagenous layer.

Corneal edema may develop in some patients as a result of endothelial dysfunction; occasionally, secondary glaucoma is caused by overgrowth of the pathological endothelial cells over the trabecular meshwork. Anterior corneal surface abnormalities, such as irregular astigmatism and high steepening, have also been observed in PPCD.

Sequence variants in 3 genes are so far associated with the development of PPCD, and evidence suggests that at least 1 more gene remains to be identified. For a few cases, VSX1 is described as the causative gene responsible for PPCD1 (OMIM 122000), and COL8A2-associated PPCD2 (OMIM 609140) is also rare. Pathogenic changes in ZEB1 (GenBank accession number NG_017048.1) underlying PPCD3 (OMIM 609141) are estimated to be responsible for approximately 30% of all probands.

Herein, we report 2 novel ZEB1 mutations identified in 2 Czech probands with PPCD3 and provide a detailed phenotypic description. We show that the prevalence of pathogenic ZEB1 sequence variants responsible for PPCD3 may vary considerably depending on the population studied, summarize published disease-causing changes to date within this gene, and add to the estimation of the de novo mutation rate of ZEB1.

### Methods

All participants signed informed consent approved by the ethics committee of the General University Hospital in Prague before participation in the study. We recruited 4 Czech probands diagnosed as having PPCD who had not been described previously. Ophthalmological assessment included distance Snellen visual acuity, slitlamp biomicroscopy, corneal topography and pachymetry using a rotating Scheimpflug imaging system (Pentacam; Oculus Optikgeräte GmbH), specular microscopy (Noncon ROBO Pachy SP-9000; Konan Medical Inc), spectral-domain optical coherence tomography (Ophthalmic Technologies Inc), and keratometry (IOL-Master V.5; Carl Zeiss Meditec AG).

We extracted DNA from venous blood samples using a commercially available kit (Genta Puregene blood kit; Qiagen) according to the manufacturer's protocol. Bidirectional direct sequencing of the ZEB1 coding regions was performed as previously described. We used ZEB1 transcript variant 2 (NCBI NM_030751.5) as the reference sequence.

Disease-causing sequence variants in ZEB1 observed in this study and those previously reported in other patients were reviewed and summarized. All published sequence chromatograms were aligned to NM_030751.5. We used a program suite (Mutalyzer 2.0.beta-26) to confirm the manually described sequence variants at DNA and protein levels as per currently recommended nomenclature.

### Results

#### Molecular Genetic Analysis

Two sequence variants, c.2617dup in exon 8 leading to frameshift p.(Thr873Asnfs*2) and c.698dup in exon 6 of ZEB1 leading to frameshift p.(Ser234Valfs*4), were found in 2 probands (eFigure in the Supplement). Case 1 was a single offspring of white Czech origin. Subsequent sequence analysis of her parents revealed that neither carried the ZEB1 mutations. Case 2, also of white Czech origin, had 1 sister. Unfortunately, the sister and parents were not available for our investigation. No other potential disease-causing variants were found in the 2 other probands.

**Patient 1**

The first proband was referred to our academic center at the age of 14 years. At that time, her best-corrected visual acuity (BCVA) was 1.00. Very discrete changes of the posterior corneal layers consistent with the diagnosis of PPCD were observed.

At her last visit at the age of 22 years, BCVA and refraction remained stable (Table 1). Vesicular-like lesions with surrounding halos and patchy opacification with thickening of the posterior corneal layers predominantly located in the corneal periphery were observed bilaterally (Figure 1A). A few geographical lesions and guttae were also observed, but no bands. The right cornea was more severely affected than the left. No corneal edema, anterior synechiae, or corectopia were observed. Specular microscopy detected decreased endothelial cell density ranging from 1253 to 1644 cells/mm² (mean [SD] values in 30 young healthy subjects, 2940 [345] cells/mm²), slight pleomorphism and polymegathism, and a few small black spots most likely representing guttae (Figure 2A).

Rotating Scheimpflug imaging revealed bilateral regular astigmatism with typical symmetric bow-tie patterns and marked corneal steepening indicated by darker shades of red and purple (right eye shown in Figure 3A; left eye shown in Figure 3C). The measurement of the thinnest point in the right eye (Table 1) was greater than the mean (mean [SD] values in a sample of 364 normal eyes, 537 [36.7] μm; 95% CI, 513-562 μm). Bilateral corneal thickening in the corneal periphery was also observed as shown by the pachymetric map (Figure 3B and D) and the corneal thickness spatial profile (Figure 3E and F). Single rotating Scheimpflug images further documented opacification of the posterior corneal surface (Figure 3G and H).
described in association with PPCD3.15,17 Her body mass index (calculated as weight in kilograms divided by height in meters squared) was 24.4. No changes indicative of PPCD were detected on slitlamp examination in the proband’s parents, whose status was negative for the \textit{ZEB1} mutation.

\textbf{Patient 2}

Patient 2 was referred to our academic center at the age of 20 years, having been followed up elsewhere for visual impairment since infancy. Family history was negative for a corneal dystrophy.

At the initial visit, the proband had a left esotropia of less than 5° and was fixating with his right eye only. His BCVA was bilaterally decreased (Table 1). On slitlamp examination, numerous scattered vesicular-like lesions surrounded by a grayish halo were observed bilaterally, with opacification of irregular density of the posterior corneal layers centrally and in the periphery (right eye is shown in Figure 1B and C; left eye is shown in Figure 1D and E). These findings seemed to be slightly more pronounced in the left cornea. No other anterior segment abnormalities were observed. Results of dilated fundus examination and spectral-domain optical coherence tomography of the macular area were unremarkable in both eyes.

A rotating Scheimpflug image of the right cornea showed marked corneal steepening with an oval pattern (Figure 4A).22 Corneal thickness, including its spatial profile, revealed diffuse thickening greater than 2 SDs above the values obtained in a control population (Figure 4B and C and Table 1).23,24 The rotating Scheimpflug images at horizontal and vertical axes demonstrated marked corneal steepening and irregularities, including patchy dense opacification of the posterior corneal layers (Figure 4D and E). Owing to a fixation error, the Scheimpflug evaluation in the left eye could not be performed.

Specular microscopy showed a very disordered endothelial structure with unclear cell borders and an irregular posterior cornea surface with ridges and dark irregular geographical areas in most of the captured locations. Some cells were enlarged and peculiarly extended with interdigitations and pits (Figure 2B–G). An area of confluent endothelial cells allowing for density counting could be generated in a very few images. Normal endothelium of an age-matched control for comparison is shown in Figure 2H.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>BCVA OD</th>
<th>BCVA OS</th>
<th>Refraction, D</th>
<th>IOP, mm Hg</th>
<th>ECD, /mm²</th>
<th>CCT, μm</th>
<th>Thinnest Pachymetry, μm</th>
<th>K1/K2, D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>1.00</td>
<td>−0.75/−3.00 × 17°</td>
<td>16</td>
<td>16</td>
<td>1253-1290</td>
<td>1577-1644</td>
<td>599</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>0.05</td>
<td>−10.00/−0.75 × 165°</td>
<td>17</td>
<td>16</td>
<td>1059-1153</td>
<td>1055-1655</td>
<td>623</td>
</tr>
</tbody>
</table>

Abbreviations: BCVA, best-corrected visual acuity; CCT, central corneal thickness; D, diopter; ECD, endothelial cell density; ellipses, not measured; IOP, intraocular pressure; K1/K2, flat/steep keratometry readings; OD, right eye; OS, left eye; PPCD3, posterior polymorphous corneal dystrophy 3.

\footnote{Standard K1 and K2 readings (the corneal curvature in the central 3.0-mm zone), CCT, and thinnest pachymetry were generated by rotating Scheimpflug imaging (Pentacam; Oculus Optikgeräte GmbH), except for the left cornea of case 2, for which an alternative instrument (IOL-Master V.5; Carl Zeiss Meditec AG) was used.}

\footnote{The CCT and thinnest pachymetry measurements for the left eye of case 2 could not be taken by rotating Scheimpflug imaging owing to a fixation error.}
The patient underwent surgery for bilateral inguinal hernia in childhood. He had a persistent hydrocele (diagnostic confirmation from a specialist was not sought) but denied any other extraocular findings associated with PPCD. His body mass index was 19.9.

**Discussion**

Herein we report 2 novel disease-causing sequence variants, c.698dup in exon 6 and c.2617dup in exon 8 of the ZEB1 gene, in 2 probands with extensively clinically characterized PPCD phenotypes. This study is the first, to our knowledge, to use rotating Scheimpflug imaging of the PPCD phenotype demonstrating corneal steepening and monitoring pachymetry in all parts of the cornea. Diffuse corneal thickening in one patient and increased peripheral thickness in the other, both without visible edema, were observed. Although molecular characterization of most cases is not routine, increased corneal steepening seems to be a common feature of PPCD, likely regardless of the underlying molecular genetic cause.

Patient 2 demonstrates the association of PPCD and astigmatism in children that may be amblyogenic. Although the right cornea was steeper with a larger spherical refractive error, the greater astigmatism in the left eye together with posterior corneal changes were likely more amblyogenic and also contributed to reduced BCVA. Therefore, we recommend regular ophthalmological assessment to all children of parents with PPCD for appropriate refractive correction.

This study further confirms that the endothelial cell density is decreased in patients with PPCD. The visualized endothelium varied in size, ranging from slightly to greatly enlarged cells. The endothelium shape also varied, ranging from a normal appearance to cells with unusual shapes likely to correlate with the microscopy findings that describe cells with collapsed cytoplasm, are peculiarly extended, and are connected with neighboring cells by numerous interdigitations, with clusters of multilayered fusiform cells focally replacing the endothelium.

Of the 7 Czech patients with disease-causing changes in ZEB1 identified to date, 3 unrelated subjects (2 female and 1 male) had a history of inguinal hernia surgery, which supports...
Figure 3. Rotating Scheimpflug Imaging Data of the Corneas in Case 1

A and C, Front sagittal curvature maps of the right and left eyes, respectively. B and D, Pachymetric maps (Pachy) of the right and left eyes, respectively. E and F, Corneal thickness spatial profiles of the right and left eyes, respectively, represented by a red line with triangles and compared with dotted black lines indicating the mean and 2 SD values obtained in a healthy population. G and H, Single image of the right and left eyes on the vertical meridian, respectively. Arrows indicate areas of irregularity and opacification. Abs indicates absolute scale with 61 colors for each refractive step shown in diopter (D) or 10 μm in pachymetry maps.

Figure 4. Rotating Scheimpflug Imaging Data of the Right Cornea in Case 2

A, Front sagittal curvature map. B, Pachymetric map (Pachy). C, Corneal thickness spatial profile, represented by a red line with triangles and compared with dotted black lines indicating the mean and 2 SD values of a healthy population. D and E, Single images on the horizontal and vertical meridians, respectively. Arrows indicate areas of irregularity and opacification. Abs indicates absolute scale with 61 colors for each refractive step shown in diopter (D) or 10 μm in pachymetry maps.
### Table 2. Summary of ZEB1 Pathogenic Changes Identified in Patients With PPCD3

<table>
<thead>
<tr>
<th>Family No.</th>
<th>Exon</th>
<th>DNA</th>
<th>Protein</th>
<th>Race/Origin</th>
<th>No. of Affected Members Undergoing Testing by Sexa</th>
<th>Nonpenetrant Cases by Sex</th>
<th>No. With Inguinal Hernia by Sex</th>
<th>Sourceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>c.1A&gt;G</td>
<td>p.(Met1?)</td>
<td>White New Zealander</td>
<td>1 F/1 M</td>
<td>0</td>
<td>0</td>
<td>Vincent et al, 18 2009</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>c.2T&gt;G</td>
<td>p.(Met1?)</td>
<td>American (race not specified)</td>
<td>1 M</td>
<td>0</td>
<td>NA</td>
<td>Aldave et al, 17 2007</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>c.34C&gt;T</td>
<td>p.(Gln12*)</td>
<td>American (race not specified)</td>
<td>1 F/2 M</td>
<td>0</td>
<td>NA</td>
<td>Aldave et al, 17 2007</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>c.640C&gt;T</td>
<td>p.(Gln214*)</td>
<td>American (race not specified)</td>
<td>1 M</td>
<td>0</td>
<td>NA</td>
<td>Aldave et al, 17 2007</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>c.672del</td>
<td>p.(Gly225Gufs+7)</td>
<td>British (race not specified)</td>
<td>1 F/2 M</td>
<td>0</td>
<td>1 M</td>
<td>Nguyen et al, 26 2010</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>c.698dup</td>
<td>p.(Ser234Valfs*4)</td>
<td>White Czech</td>
<td>1 M</td>
<td>0</td>
<td>1 M</td>
<td>Present study</td>
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<tr>
<td>7</td>
<td>7</td>
<td>c.929dup</td>
<td>p.(Cys311Valfs*25)</td>
<td>American (race not specified)</td>
<td>1 F/1 M</td>
<td>0</td>
<td>NA</td>
<td>Aldave et al, 17 2007</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>c.973C&gt;T</td>
<td>p.(Arg325*)</td>
<td>American (race not specified)</td>
<td>2 F</td>
<td>0</td>
<td>NA</td>
<td>Aldave et al, 17 2007</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>c.1124del</td>
<td>p.(Phe375Serfs*31)</td>
<td>White British</td>
<td>1 F</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>10</td>
<td>7</td>
<td>c.1332, 1335del</td>
<td>p.(Ile444Metfs*48)</td>
<td>White American</td>
<td>1 M (sporadic)</td>
<td>0</td>
<td>1 M</td>
<td>Krafchak et al, 15 2005</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>c.1348C&gt;T</td>
<td>p.(Gln450*)</td>
<td>White American</td>
<td>1 M (sporadic)</td>
<td>0</td>
<td>1 M</td>
<td>Krafchak et al, 15 2005</td>
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<tr>
<td>12</td>
<td>7</td>
<td>c.1387, 1390del</td>
<td>p.(Pro463Trpfs*29)</td>
<td>White British</td>
<td>2 M</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>13</td>
<td>7</td>
<td>c.1482dup</td>
<td>p.(Glu495Argfs*10)</td>
<td>American (race not specified)</td>
<td>3 F</td>
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</tr>
<tr>
<td>14</td>
<td>7</td>
<td>c.1569del</td>
<td>p.(Val526*)</td>
<td>American (race not specified)</td>
<td>3 F/1 M</td>
<td>0</td>
<td>NA</td>
<td>Aldave et al, 17 2007</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>c.1576dup</td>
<td>p.(Val526Glyfs*3)</td>
<td>White American</td>
<td>1 F/2 M</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>16</td>
<td>7</td>
<td>c.2157C&gt;G</td>
<td>p.(Tyr719*)</td>
<td>White Czech</td>
<td>1 F</td>
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<td>1 F</td>
<td>Liskova et al, 16 2007</td>
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<tr>
<td>17</td>
<td>7</td>
<td>c.2182G&gt;T</td>
<td>p.(Glu728*)</td>
<td>White American</td>
<td>2 F</td>
<td>0</td>
<td>0</td>
<td>Krafchak et al, 15 2005</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>c.2324dup</td>
<td>p.(Glu776Glyfs*44)</td>
<td>White Czech</td>
<td>3 M</td>
<td>1 F</td>
<td>0</td>
<td>Liskova et al, 16 2007</td>
</tr>
<tr>
<td>19</td>
<td>8</td>
<td>c.2617dup</td>
<td>p.(Thr873Asnfs*2)</td>
<td>White Czech</td>
<td>1 F (sporadic)</td>
<td>0</td>
<td>1 F</td>
<td>Present study</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>c.2916,2917del</td>
<td>p.(Gly973Valfs*14)</td>
<td>White American</td>
<td>6 F/7 M</td>
<td>1 F/1 M</td>
<td>8 M</td>
<td>Krafchak et al, 15 2005</td>
</tr>
<tr>
<td>21</td>
<td>9</td>
<td>c.2990,2991del</td>
<td>p.(Glu977Alafs*7)</td>
<td>American (race not specified)</td>
<td>1</td>
<td>0</td>
<td>NA</td>
<td>Aldave et al, 17 2007</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male; NA, not available; PPCD3, posterior polymorphous corneal dystrophy 3.

*Each mutation refers to a separate family. ZEB1 transcript variant 2 (NM_030751.5) was used as the reference sequence. Nonpenetrance and inguinal hernia status are shown only for family members confirmed as positive for a mutation. Sporadic status indicative of a likely de novo origin is shown for cases in which both parents were negative for the identified pathogenic change. Aldave et al17 reported the presence of inguinal hernias in 2 men and 1 woman. No further genotype correlation was provided; therefore,

the exact number of members with inguinal hernia is not shown for individuals from families 2, 3, 4, 7, 8, 13, 14, and 21. In family 21, sex was not reported for the affected subject.

†The summary of patients does not include 1 male with confirmed ZEB1 mutation but unknown phenotype.

‡Because of inconsistencies in mutation descriptions and alignment to different reference sequence versions, only those reporting changes permitting manual curation and verification (ie, not published abstracts) are listed.

Mutations responsible for PPCD3 within ZEB1 have been described only in 4 patient populations from the United Kingdom, United States, Czech Republic, and New Zealand.15-18,26 Including the 2 novel changes reported herein, the number of ZEB1 pathogenic sequence variants has reached 21 (Table 2).

Posterior polymorphous corneal dystrophy 3 is caused by frameshift or stop codon mutations most likely leading to transcript degradation by nonsense-mediated decay, resulting in haploinsufficiency.15-17,26 Only 2 pathogenic changes not creating an early stop codon, both located within the methionine used to initiate translation, were found. Sequence variants in the first codon are known to have an uncertain effect on the protein. Most variants are expected to prevent translation; however, translation initiation may also occur upstream or downstream.34-37 Therefore, unless experimentally verified, the possibility remains that haploinsufficiency underlies all PPCD3 cases.
The 2 sequence variants found in this study and the changes previously reported to cause PPCD3 are not listed in the Exome Variant Server (NHGRI Exome Sequencing Project; http://evs.gs.washington.edu/EVS/), which includes data for more than 6000 genomes, or in the 1000 Genomes Project (http://browser.1000genomes.org/index.html [both accessed October 4, 2012]), which supports their pathogenic nature.

The parents of our first proband did not have the pathogenic sequence variant, indicating that the change is most likely a de novo mutation. This feature has also been documented in 2 other families with PPCD3, allowing us to estimate a de novo mutation rate in ZEB1 leading to PPCD3 of at least 14%, not including pedigrees with reportedly unaffected parents but no molecular genetic investigation undertaken.

The pathological mechanism leading to PPCD3 is still elusive. ZEB1 binds to a promoter of the COL4A3 gene, and the temporal expression or the amount of expression of the COL4A3 protein has been suggested to be altered when ZEB1 is mutated, which may influence the endothelial cell to manifest a different phenotype. Alterations in expression of other collagen subtypes during development may also be involved in the pathogenesis. However, the ZEB1 protein has been implicated in many other metabolic pathways, including epithelial-mesenchymal transition, a highly conserved biological process resulting in the change of polarized, immotile epithelial cells into mesenchymal cells with a motile phenotype involved in critical phases of embryonic development and promotion of cancer cell invasion and metastasis. Because the PPCD endothelium is known to gain characteristics such as abnormal proliferation and migration, changes in ZEB1 expression in the corneal endothelium may lead to modulation of cellular phenotype independent of effects on collagens.

A recent study has shown that, in the Czech Republic, PPCD affects at least 1 in 100 000 inhabitants. The finding of pathogenic mutations in 2 of 4 newly detected probands has established that, at present, 112 affected living Czech individuals have a PPCD phenotype. These individuals are from 23 apparently unrelated families, of which 6 patients from 4 families and 1 additional case of nonpenetrance exhibit ZEB1 disease-causing variants. Therefore the proportion of Czech PPCD families is approximately 17%, and the current proportion of PPCD3 patients within the Czech cohort is only 5%. This figure is in contrast to previous studies reporting the contribution of ZEB1 in PPCD pathogenesis, approximately 45% in one US cohort of families, 25% in another study of US families, and 60% of UK families. On the other hand, only 1 proband of 11 (9%) had a ZEB1 pathogenic change in a cohort of patients collected in New Zealand. The contribution of ZEB1 to PPCD disease appears, therefore, to vary extensively depending on the population studied.

Some patients with PPCD but without a molecular diagnosis may have pathogenic ZEB1 sequence variants located in noncoding regions or larger deletions or duplications that are not readily detected by the standard technique of direct sequencing of the coding regions. Future studies may show whether the currently reported rates of ZEB1 involvement are underestimated in some populations. To further elucidate the pathological mechanism of PPCD3 caused by haploinsufficiency of ZEB1, a greater knowledge and understanding of the targets of this protein is required.

**ARTICLE INFORMATION**

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Author Contributions: Dr Liskova had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Liskova and Vincent. Acquisition of data: Liskova, Palos, and Vincent. Analysis and interpretation of data: Liskova, Hardcastle, and Vincent. Drafting of the manuscript: Liskova, Palos, Hardcastle, and Vincent. Critical revision of the manuscript for important intellectual content: Liskova and Vincent. Statistical analysis: Liskova. Obtained funding: Liskova and Vincent. Administrative, technical, and material support: Liskova, Palos, and Vincent. Study supervision: Hardcastle.

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