DNA Polymerase Epsilon Deficiency Causes IMAGe Syndrome with Variable Immunodeficiency

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DNA replication is a fundamental cellular process necessary to ensure the faithful transmission of genetic information. In eukaryotes, three highly conserved DNA polymerases, polymerase epsilon, delta, and alpha, act in concert at the replication fork. Polymerase epsilon (Pol ε) is the major enzyme responsible for the synthesis of the leading strand1 and is consequently an essential gene.2 POLΕ encodes the catalytic subunit of Pol ε (POLΕ1), and somatic and germline missense mutations affecting the proofreading domain of POLΕ1 have been associated with colon and endometrial cancer.3–6

Microcephalic primordial dwarfism comprises a group of prenatal-onset extreme growth disorders characterized by intrauterine growth retardation, short stature, and microcephaly. Genes involved in cell cycle progression, including multiple components of the replication licensing machinery, have been identified as monogenic causes of this disorder.7–11 As the molecular basis for many affected individuals remains to be determined, we performed whole-genome sequencing studies to identify further genes and facilitate more comprehensive diagnosis.

During genome replication, polymerase epsilon (Pol ε) acts as the major leading-strand DNA polymerase. Here we report the identification of biallelic mutations in POLΕ, encoding the Pol ε catalytic subunit POLΕ1, in 15 individuals from 12 families. Phenotypically, these individuals had clinical features closely resembling IMAGe syndrome (intrauterine growth restriction [IUGR], metaphyseal dysplasia, adrenal hypoplasia congenita, and genitourinary anomalies in males), a disorder previously associated with gain-of-function mutations in CDKN1C. POLΕ1-deficient individuals also exhibited distinctive facial features and variable immune dysfunction with evidence of lymphocyte deficiency. All subjects shared the same intronic variant (c.1686+32C>G) as part of a common haplotype, in combination with different loss-of-function variants in trans. The intronic variant alters splicing, and together the biallelic mutations lead to cellular deficiency of Pol ε and delayed S-phase progression. In summary, we establish POLΕ as a second gene in which mutations cause IMAGe syndrome. These findings add to a growing list of disorders due to mutations in DNA replication genes that manifest growth restriction alongside adrenal dysfunction and/or immunodeficiency, consolidating these as replisome phenotypes and highlighting a need for future studies to understand the tissue-specific development roles of the encoded proteins.

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Table 1. Biallelic POLE Mutations (GenBank: NM_006231.3)

| ID  | Fam | Sex | Nucleotide Change | Amino Acid Consequence | MAF   | Allele 1 | Nucleotide Change | Amino Acid Consequence | MAF   | Mat Allele | Pat Allele | Country of Origin |
|-----|-----|-----|-------------------|------------------------|-------|----------|-------------------|------------------------|-------|------------|------------|------------------|
| P1  | 1   | M   | c.2091dupC        | p.Phe699Valfs*11       | 0     |          | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 1           | 2           | UK               |
| P2  | 1   | F   | c.2091dupC        | p.Phe699Valfs*11       | 0     |          | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 1           | 2           | UK               |
| P3  | 2   | M   | c.62+1G>A         | Essential Splice Site Introns 1 | 0 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Ireland          |
| P4  | 3   | F   | c.5940G>A         | p.Tyr1980*             | 0.000016 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Australia         |
| P5  | 4   | M   | c.4728+1G>T       | Essential Splice Site Introns 36 | 0 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | USA              |
| P6  | 5   | F   | c.3264_3275+13del | Essential Splice Site Introns 26 | 0.000016 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 1           | 2           | Canada            |
| P7  | 6   | M   | c.1A>T            | p.?                    | 0.000081 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | n/a         | n/a         | USA              |
| P8  | 7   | M   | c.1A>T            | p.?                    | 0.000081 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Ireland          |
| P9  | 7   | F   | c.1A>T            | p.?                    | 0.000081 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Ireland          |
| P10 | 8   | F   | c.3019G>C         | p.Ala1007Pro           | 0.000009 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Ireland          |
| P11 | 9   | F   | c.5265delG        | Ile1756Serfs*5         | 0 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Australia         |
| P12 | 9   | F   | c.5265delG        | Ile1756Serfs*5         | 0 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Australia         |
| P13 | 10  | F   | c.2049C>G         | p.Tyr683*              | 0.000028 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | Australia         |
| P14 | 11  | M   | c.6518_6519delCT  | p.Ser1273Phefs*130     | 0.000089 |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | USA              |
| P15 | 12  | M   | c.801+2T>C        | Essential Splice Site Introns 8 | – |       | c.1686+32C>G      | p.Asn563Valfs*16       | 0.000071 | 2           | 1           | USA              |

Abbreviations: ID, individual number; Fam, family number; Mat, maternal; Pat, paternal; n/a, not available. All subjects harbored a loss-of-function mutation in combination with an intronic variant on the alternate allele identified as part of a shared haplotype and found to alter splicing in RNA studies. MAF indicates minor allele frequency in European (non-Finnish) population observed in gnomAD. None of the variants were present in any Non-European population in gnomAD.

Whole-genome sequencing (WGS) of 48 individuals with microcephalic primordial dwarfism identified heterozygous POLE (GenBank: NM_006231.3) loss-of-function (LoF) variants in three subjects (P1, P3, P4; Table 1). These LoF variants were significantly enriched in our cohort compared to a control WGS dataset (GnomAD,12 p = 5.1 × 10−5, Fisher's exact test, Table S1). As these variants were present in the unaffected parents, the WGS data were further evaluated and a second rare intronic variant in POLE identified, c.1686+32C>G (dbSNP: rs762985435). This was present in trans with the LoF mutation in all three probands (Table 1). Targeted sequencing of POLE and interrogation of existing whole-exome sequencing (WES) data in additional cases of primordial dwarfism identified five additional subjects compound heterozygous for LoF alleles and the c.1686+32C>G variant (P5–P9, Table 1). Notably, a clinical diagnosis of IMAGe syndrome (GeneReviews in Web Resources) (MIM: 614732) had been considered in individuals P1 and P3, with adrenal failure also reported in P5, P6, and P7. We therefore investigated cases of IMAGe syndrome drawn from other cohorts without an existing molecular diagnosis (i.e., CDKN1C mutation negative). These included three previously published IMAGe-affected case subjects.13,14 Analysis of their WGS data identified additional POLE LoF variants inherited in trans with the intronic variant in individuals P11–P15 (Table 1). The c.1686+32C>G variant was part of a common haplotype in all individuals where WES/WGS performed, extending over 921 kbp (Figure S2, chr12:132341818–133263107, GRCh38). In P10 a missense variant (c.3019G>C) encoding a p.Ala1007Pro substitution was found, at a residue conserved to yeast (Figure S1) within the polymerase domain of the protein (Figure 1). All variants identified were sufficiently rare (MAF < 0.000112) and, where DNA available, segregation in families was consistent with an autosomal recessively inherited disorder (Table 1).

Phenotypically, affected individuals had severe growth failure of prenatal onset (Figure 2, Table S2). IUGR was present in all case subjects (birth weight was −3.0 ± 0.8 SD) with significant short stature evident postnatally (height −8.1 ± 2.4 SD). While head circumference was also significantly reduced (OFC −5.4 ± 1.5 SD), this was less severe, resulting in a relative macrocephaly. Those affected had a common facial appearance with micrognathia, crowded dentition, long thin nose, short wide neck, and small, low-set, posteriorly rotated ears (Figure 2). 12 individuals had adrenal insufficiency and all affected males had genitourinary abnormalities including bilateral cryptorchidism and/or hypospadias, with the majority of case subjects fulfilling clinical criteria for IMAGe syndrome (GeneReviews in Web Resources; Table 2, Table S3, Supplemental Note).
Osteopenia and developmental dysplasia of the hip (DDH) were frequently observed and café-au-lait patches were notably present in a third of individuals.

A single homozygous intronic variant (c.4444+3A>G) in POLE has previously been reported to be associated with immunodeficiency, lymphopenia, and short stature (facial dysmorphism, immunodeficiency, livedo, and short stature, aka FILS syndrome [MIM: 615139]).\(^{15,16}\) Five affected individuals identified in this study also had increased susceptibility to respiratory tract infections, with lymphocyte subset deficiencies and/or IgM hypogammaglobulinemia identified in P1, P3, P4, P8, P9, P14, and P15 (Table 2, Table S4). Deficiency of natural killer cells was present in P1, P3, and P8. P1 had the most profound immunodeficiency, developing CMV pneumonitis and then subsequently developed EBV haemophagocytic lymphohistiocytosis, requiring an allogeneic bone marrow transplant. Notably, this subject’s sister (P2), who had the same compound heterozygous POLE mutations, died at 22 months from HSV infection. Therefore, our findings establish that the phenotype spectrum of biallelic POLE mutations extends from IMAGe syndrome to include immunodeficiency, in line with the phenotype and pathogenicity of the previously reported c.4444+3A>G mutation.\(^{15,16}\)

To establish whether the c.1686+32C>T variant affected the POLE transcript, RNA studies were performed on primary fibroblast lines derived from two subjects (P1, P3). RT-PCR using primers spanning POLE intron 15 demonstrated the presence of a larger PCR product (Figure 3), which capillary sequencing established to be due to retention of part of intron 15 within POLE transcripts (Figure S3). A minigene assay was then performed to assess splicing of this genomic segment and to directly confirm the contribution of the c.1686+32C>T variant. This demonstrated that the c.1686+32C>T variant markedly impaired splicing of the usual exon 15 splice donor site, although some canonical splicing also occurred (Figure 3). The inclusion of 47 bp of intronic

Figure 1. Mutations Causing POLE-Associated IMAGe Syndrome Are Distinct from Mutations Conferring a Non-syndromic Susceptibility to Cancer
Schematic of the POLE gene, which encodes POLE1, the catalytic subunit of DNA polymerase epsilon. Domains: Pol, polymerase; Exo, exonuclease. Mutations identified in POLE subjects indicated above gene and protein (green). Recurrent intronic mutation underlined. For comparison, heterozygous germline missense mutations located in the exonuclease domain predisposing to colorectal cancer and other malignancies highlighted below (red).

Figure 2. Individuals with Biallelic POLE Mutations Have Severely Impaired Pre- and Post-natal Growth and a Recognizable Facial Gestalt
(A) Photographs of POLE-deficient subjects demonstrating facial similarities. Written consent obtained from all families for photography.
(B and C) Severe pre-natal onset growth restriction occurs in POLE-deficient individuals.
(B) Adult POLE-deficient subject next to a control individual of average stature.
(C) Growth is severely impaired pre- and postnataally. Z-scores (standard deviations from population mean for age and sex) for birth weight and postnatal height and head circumference (OFC). Dashed lines 95% confidence interval for general population. Circles, individual subject data points; red bars, mean values.
DNA in the variant transcript results in a frameshift, which would lead to premature termination (p.Asn563Valfs*16). While this transcript might be targeted for nonsense-mediated decay, any translated protein would also be non-functional given that this frameshift occurs at the start of the polymerase catalytic domain. Combined with a LoF mutation on the second allele, substantial reduction in POLE1 was therefore anticipated. Subsequent immunoblotting of total protein extracts from of primary fibroblasts from affected subjects confirmed that POLE1 levels were indeed markedly depleted (Figure 3; 5%–3% for P1 and 11% ± 4% P3, relative to the mean of both control subjects and normalized to vinculin loading control; mean ± SD for n = 2 independent experiments), with chromatin fractionation experiments demonstrating reduction of POLE1 in both soluble and chromatin-bound fractions (Figure S4). Taken together with the consistent clinical phenotype across case subjects, we concluded that the identified POLE variants were pathogenic, resulting in a phenotype spectrum substantially overlapping IMAGe syndrome.

In keeping with an essential requirement for POLE in eukaryotes, the “leaky” c.1686+32C>G splice mutation permitted residual expression of functional POLE1 in all case subjects. This mutation in trans with truncating mutations would then be expected to lead to marked but partial loss of function. As POLE encodes POLE1, the catalytic subunit of the major leading-strand DNA polymerase Pol ε, reduced chromatin levels of POLE1 would therefore be expected to impact on the availability of Pol ε DNA polymerase activity during its canonical function in DNA replication. Consistent with this, time-course FACS analysis demonstrated delayed cell-cycle progression of BrdU-labeled primary fibroblasts from P1 and P3, indicative of impaired S-phase progression (Figure 3). While no viable model of POLE1 deficiency exists, a Pole4/C0/C0 mouse has been generated, which is similarly deficient for the Pol ε holoenzyme.17 This mouse also has significant prenatal onset growth failure, reduced brain size, and markedly reduced lymphocyte levels. Analysis of embryonic fibroblasts derived from this mouse alongside POLE primary human fibroblasts (derived from P1 and P3 in this study) established that in both cases Pol ε deficiency leads to reduced levels of chromatin-loaded Pol ε complexes, resulting in replication stress arising from reduced numbers of active replication origins.17

Table 2. Individuals with Biallelic Mutations in POLE Were Clinically Diagnosed with Primordial Dwarfism and Features of IMAGe Syndrome

| ID  | Fam | Sex | Age | I  | M_st | A  | Ge | –I | Other Features                                      |
|-----|-----|-----|-----|----|------|----|----|----|-----------------------------------------------------|
| P1  | 1   | M   | 18  | Y  | Y    | Y  | Y  | Y  | scoliosis, osteopenia, small patella, seizures, gastrostomy, eczema |
| P2  | 1   | F   | 1   | Y  | Y    | Y  | –  | Y  | –                                                  |
| P3  | 2   | M   | 7   | Y  | Y    | Y  | Y  | Y  | midline accessory incisor, osteopenia, infant eczema |
| P4  | 3   | F   | 50  | Y  | Y    | N  | –  | Y  | IgM paraproteinaemia                                 |
| P5  | 4   | M   | 12  | Y  | NA   | Y  | Y  | Y  | hypopituitarism, T cell lymphoma, gastrostomy, absent patella |
| P6  | 5   | F   | 10  | Y  | Y    | Y  | –  | Y  | bilat coxa valga, 11 ribs, 6 lumbar vertebrae, scoliosis, gastrostomy, infant eczema |
| P7  | 6   | M   | 13  | Y  | Y    | Y  | Y  | N  | hypopituitarism, atrial septal defect, brachydactyly, gastrostomy |
| P8  | 7   | M   | 3   | Y  | Y    | N  | Y  | Y  | DDH, gastrostomy                                    |
| P9  | 7   | F   | 2   | Y  | Y    | N  | –  | Y  | DDH, gastrostomy                                    |
| P10 | 8   | F   | 39  | Y  | Y    | Y  | –  | N  | DDH, 11 ribs, clinodactyly, osteopenia, cafe au lait patches |
| P11 | 9   | F   | 0.2 | Y  | NA   | Y  | –  | Y  | cafe au lait patch                                  |
| P12 | 9   | F   | 12  | Y  | Y    | Y  | –  | N  | –                                                  |
| P13 | 10  | M   | 22  | Y  | Y    | Y  | Y  | N  | DDH, cafe au lait patch                             |
| P14 | 11  | F   | 18  | Y  | Y    | Y  | –  | Y  | gastrostomy, hypercalcaemia in infancy, cafe au lait patches, DDH, kyphoscoliosis |
| P15 | 12  | M   | 31  | Y  | NA   | Y  | Y  | Y  | cafe au lait patches, seizures, osteopenia, osteoporosis, nodular sclerosis, Hodgkin’s lymphoma |

Abbreviations: ID, individual number; Fam, family number; I, intrauterine growth restriction; M_st, skeletal involvement: metaphyseal dysplasia or other skeletal abnormalities reported in CDKN1C IMAGe-affected individuals (NA, not assessed); A, adrenal insufficiency; Ge, genitourinary abnormalities in males (– female, genitourinary anomalies not applicable); –I, immunodeficiency, either increased susceptibility to infections or documented lymphopenia/hypogammaglobulinemia; DDH, developmental dysplasia of the hip; Y, yes; N, no. See Tables S1–S4 for extended clinical data and morphometrics.
The IMAGe syndrome has previously been found to be caused by dominant gain-of-function mutations in the imprinted gene, CDKN1C. Here, we establish mutations of POLE as an autosomal-recessive cause of the IMAGe phenotype. These mutations contrast with heterozygous germline and somatic cancer-predisposing mutations that affect the exonuclease domain of POLE. IMAGe and cancer mutations are likely to have differing functional outcomes, respectively leading to deficient DNA replication or to impaired proof-reading. Hence, a similar cancer predisposition in POLE-deficient individuals or POLE heterozygous carriers cannot be assumed. However, P5 developed a T cell lymphoma at age 11 and P15 developed Hodgkin’s lymphoma at age 28. Given also the increased lymphoma rates in *Pole*/*C0* mice, POLE deficiency may therefore confer an increased risk of lymphoma.

**Common Intrinsic Variant Identified Causes Aberrant Splicing and POLE-Deficient Cells Show Deficiency of Polymerase Epsilon and Slowed S-phase Progression**

(A) The c.1686+32C>G mutation causes aberrant splicing of intron 15 in subject cells. RT-PCR of POLE transcripts from primary fibroblasts. Primers indicated by arrows in schematic. P1, P3, POLE-deficient subjects; C1, C2, control subjects.

(B) Minigene assay demonstrating that aberrant splicing is a direct consequence of the c.1686+32C>G mutation. +ve control, point mutation in splice donor site, c.1686+1G>A. S’ & 3’ indicate artificial vector-associated exons.

(C) POLE1 levels are markedly reduced in subject fibroblasts. Immunoblot of total cell extracts. POLE1 antibody raised against AA1-176. Vinculin, loading control. * non-specific band.

(D and E) Fibroblast cells from affected individuals exhibit delayed S phase progression. Schematic, experimental set-up.

(E) Quantification of n = 3 affected and n = 3 control cell lines from representative experiment (of n = 3 expts with n ≥ 2 biological replicates per group). Mid-S-phase mean (± SEM) BrdU-labeled cells, normalized to t = 0 time point are plotted for each group. p value, two-way ANOVA.
replication proteins during development, along with the cellular and biochemical basis for the relationship between CDKN1C and Pol ε, will therefore be of interest.

Supplemental Data

Supplemental Data include Supplemental Note, seven figures, five tables, and Supplemental Material and Methods and can be found with this article online at https://doi.org/10.1016/j.ajhg.2018.10.024.

 Consortia

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Declaration of Interests

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from the genetic testing services offered by Baylor Genetics.

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Web Resources

dbSNP, https://www.ncbi.nlm.nih.gov/projects/SNP/
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
GeneReviews, Bennett, J., Schriever Vergano, S.A., and Deardorff, M.A. (1993). IMAGe syndrome, https://www.ncbi.nlm.nih.gov/books/NBK190103/
gnomAD Browser, http://gnomad.broadinstitute.org/
OMIM, http://www.omim.org/

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