

## ARTICLE

# Phenotype-specific adverse effects of *XPD* mutations on human prenatal development implicate impairment of TFIIH-mediated functions in placenta

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Mutations in *XPD* (*ERCC2*), *XPB* (*ERCC3*), and *TTD-A* (*GTF2H5*), genes involved in nucleotide excision repair and transcription, can cause several disorders including trichothiodystrophy (TTD) and xeroderma pigmentosum (XP). In this study, we tested the hypothesis that mutations in the *XPD* gene affect placental development in a phenotype-specific manner. To test our hypothesis and decipher potential biologic mechanisms, we compared all *XPD*-associated TTD ( $n=43$ ) and XP ( $n=37$ ) cases reported in the literature with respect to frequencies of gestational complications. Our genetic epidemiologic investigations of TTD and XP revealed that the exact genetic abnormality was relevant to the mechanism leading to gestational complications such as preeclampsia. Through structural mapping, we localized the preeclampsia-associated mutations to a C-terminal motif and the helicase surfaces of *XPD*, most likely affecting *XPD*'s binding to cdk-activating kinase (CAK) and p44 subunits of transcription factor (TF) IIH. Our results suggested a link between TTD- but not XP-associated *XPD* mutations, placental maldevelopment and risk of pregnancy complications, possibly due to impairment of TFIIH-mediated functions in placenta. Our findings highlight the importance of the fetal genotype in development of gestational complications, such as preeclampsia. Therefore, future studies of genetic associations of preeclampsia and other placental vascular complications may benefit from focusing on genetic variants within the fetal DNA.

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## INTRODUCTION

Mutations in *XPD* (*ERCC2*), *XPB* (*ERCC3*), and *TTD-A* (*GTF2H5*), genes involved in the nucleotide excision repair (NER) pathway and in transcription, are associated with trichothiodystrophy (TTD), a rare (affected frequency of 1 in 10<sup>6</sup>) disorder characterized by hair, skin and neurological abnormalities.<sup>1</sup> Besides TTD, mutations in *XPD* (*ERCC2*) can lead to several other DNA repair disorders including xeroderma pigmentosum (XP), Cockayne syndrome (CS), cerebro-ocular facial syndrome (COFS) or a combination of XP/TTD, XP/CS, or COFS/TTD.<sup>1–4</sup> *XPD*, *XPB*, and *TTD-A* code for components of the seven-subunit core complex of transcription factor (TF) IIH. TFIIH also contains a three-subunit Cdk-activating kinase (CAK) complex composed of cdk7, MAT1, and cyclin H.<sup>5</sup> TFIIH has a role in NER and in both basal and activated transcription,<sup>6</sup> as a component of RNA polymerase-II (Pol-II) transcription machinery, TFIIH has an important role in RNA Pol-II elongation.<sup>7</sup> In addition, TFIIH is an important regulator of the cell cycle.<sup>5</sup> The core complex is sufficient to support the NER function of TFIIH, while the CAK complex is required for phosphorylation of RNA Pol-II and numerous nuclear receptors (NRs).<sup>7</sup>

We recently reported<sup>8</sup> that pregnancies in which the fetus had TTD were at significantly increased risk of preeclampsia, HELLP syndrome (a severe form of preeclampsia with hemolysis, elevated liver enzymes, and low platelets), and elevated mid-trimester maternal serum human chorionic gonadotropin levels. The affected fetuses had decreased fetal movement and were significantly more likely to be small for gestational age (SGA) and to deliver pre-term.<sup>8</sup> Gestational abnormalities were only detected in TTD-affected pregnancies (where infant had two mutations) but not in unaffected pregnancies (where the infant was either a heterozygote or noncarrier),<sup>8</sup> highlighting the relevance of the fetal genotype to development of observed complications.

Impairment of placental development could explain the constellation of abnormalities observed in TTD-affected pregnancies;<sup>8</sup> thus, we hypothesize that mutations in TTD NER/transcription genes affect placental development. Given that pregnancies resulting in XP/TTD, also caused by mutations in *XPD*, were not associated with gestational complications,<sup>8</sup> we further propose that both the fetal genotype and the exact genetic abnormality are relevant to the underlying mechanism.

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To test our hypotheses, we compared all *XPD*-associated TTD and XP cases reported in the literature with respect to frequencies of gestational complications. We then conducted an analysis of the correlation between *XPD* mutations and the presence of preeclampsia in all the reported cases in order to create the first structural map of preeclampsia-associated domains on the three-dimensional (3D) protein structure of *XPD*.

## MATERIALS AND METHODS

### Comparison of gestational complications in *XPD*-associated TTD versus *XPD*-associated XP

**Study population.** The study population consisted of all TTD, COFS/TTD, XP, XP/CS, and XP/TTD cases with mutations in *XPD* identified through a systematic literature review using Pubmed on MEDLINE. All case reports in English and French were included; reports in other languages were included if the abstract was in either English or French. A total of 43 TTD,<sup>9–22</sup> 37 XP,<sup>23–35</sup> six XP/TTD,<sup>3,8</sup> four XP/CS,<sup>36–38</sup> and 1 COFS/TTD<sup>8</sup> patient with defects in the *XPD* gene were identified and included in our study population.

**Data collection.** Data were obtained by reviewing the case reports using a standard abstraction form, collecting information on reproductive, pregnancy, and delivery histories of each affected pregnancy. Variables for analyses, created from abstraction forms, included presence of preeclampsia, HELLP syndrome, pre-term delivery (<37 weeks gestation), low birth weight (<2500 g), SGA<10th percentile, SGA<3rd percentile, decreased fetal movement, abnormal maternal serum triple screen test results, and history of neonatal intensive care unit admission. The variables were analyzed as present or absent from the record, or do not know if the report did not specifically mention the variable.

**Statistical analysis.** Frequencies of gestational complications were compared between *XPD*-associated TTD ( $n=43$ ) and XP ( $n=37$ ) cases. Analysis was done two ways: first by combining all ‘do not know’ and ‘no’ responses together for comparison with ‘yes’, and second by excluding all ‘do not know’ responses from the analyses. Statistical Analysis System (SAS) software (version 9.1, SAS Institute, Cary, NC, USA) was used for statistical comparisons; all *P*-values were calculated using Fisher’s exact test and all tests were two sided.

### Mapping of preeclampsia-associated domains on *XPD*

In order to limit variability caused by heterogeneity of observed pregnancy complications, structural mapping was restricted to preeclampsia, which was the most common well-documented prenatal complication among our subjects. All XP and TTD cases with specified mutations in the *XPD* gene and information on preeclampsia were included in the analysis. T-Coffee,<sup>39</sup> Promals3D,<sup>40</sup> NCBI BLAST, and TM-Align<sup>41</sup> were used for generating structure-based multiple sequence alignments and mapping of the mutations in the *XPD* gene to the available *XPD* crystal structures (PDB IDs: 3CRV,<sup>42</sup> 2VSE,<sup>43</sup> and 2VL7<sup>44</sup>). PyMOL and Jalview<sup>45</sup> were used for depiction of mapped *XPD* mutations and sequence alignments, respectively.

## RESULTS

### Comparison of gestational complications in *XPD*-associated TTD versus *XPD*-associated XP

Comparison of the frequencies of gestational complications between 43 TTD and 37 XP cases with mutations in *XPD* revealed a significantly higher incidence of preeclampsia ( $P=0.003$ ), abnormal maternal serum triple screen test results ( $P=0.02$ ), pre-term delivery ( $P<0.0001$ ), low birth weight ( $P<0.0001$ ), SGA (<10th percentile) ( $P=0.006$ ), SGA<3rd percentile ( $P=0.003$ ), and NICU admission ( $P<0.0001$ ) among *XPD*-associated TTD pregnancies (Table 1). It is noteworthy that all reported gestational complications were found exclusively among TTD cases (Table 1).

Excluding cases without definitive information on the presence or absence of each complication produced similar results, although

**Table 1 Comparison of pregnancy complications between TTD and XP cases with mutations in *XPD*<sup>a</sup>**

	TTD ( $n=43$ )	XP ( $n=37$ )	<i>P</i> -value
<i>Gender</i>			
Male	20 (46.5)	21 (56.8)	
Female	23 (53.5)	16 (43.2)	
<i>Twin birth</i>			
Yes	1 (2.3)	0 (0.0)	1.00
<i>Preeclampsia</i>			
Yes	9 (20.9)	0 (0.0)	0.003
<i>Decreased fetal movement reported by mother to physician</i>			
Yes	4 (9.3)	0 (0.0)	0.11
<i>Abnormal maternal serum triple screen test results</i>			
Yes	6 (14.0)	0 (0.0)	0.02
<i>Pre-term delivery (&lt;37 weeks gestation)</i>			
Yes	20 (46.5)	0 (0.0)	<0.0001
<i>Low birth weight (&lt;2500g)</i>			
Yes	18 (41.9)	0 (0.0)	<0.0001
<i>Small for gestational age (SGA) (&lt;10th percentile)</i>			
Yes	11 (25.6)	0 (0.0)	0.006
<i>SGA (&lt;3rd percentile)</i>			
Yes	9 (20.9)	0 (0.0)	0.003
<i>Neonatal intensive care unit (NICU) admission</i>			
Yes	11 (25.6)	0 (0.0)	<0.0001

<sup>a</sup>Figures represent actual number of patients while numbers in brackets are percentages.

statistical significance was lost for some tests, probably due to smaller numbers. In this restricted analysis, significantly higher risks were still observed for pre-term delivery ( $P=0.002$ ), low birth weight ( $P<0.0001$ ), and NICU admission ( $P<0.0001$ ) in association with TTD pregnancies, despite small sample sizes (Table 2).

Frequencies of gestational complications were also noted among cases with *XPD*-associated combined phenotypes of XP/TTD, XP/CS, and COFS/TTD. Five of six patients with *XPD*-associated XP/TTD were not noted to have any gestational complications;<sup>3,8</sup> one *XPD*-associated XP/TTD case was reported as having low birth weight, SGA<3rd percentile, and NICU admission.<sup>3</sup> Pregnancy with the one patient with COFS/TTD reported in the literature was complicated with preeclampsia, HELLP syndrome, pre-term delivery, low birth weight, SGA<3rd percentile, and NICU admission.<sup>8</sup> Neonatal history was not reported for the one patient with XP/CS identified through the literature.<sup>38</sup>

### Mapping of preeclampsia-associated domains on *XPD*

All TTD ( $n=8$ )<sup>8,20,21</sup> and XP ( $n=3$ )<sup>30,36,37</sup> cases with known *XPD* mutations and explicit information on presence or absence of preeclampsia were included in the analysis to map the preeclampsia-associated alleles onto *XPD*. Of the eight TTD (including one COFS/TTD) pregnancies, four were associated with preeclampsia and all eight were associated with at least one prenatal complication.

**Table 2 Comparison of pregnancy complications between TTD and XP cases with mutations in XPD (restricted analysis with 'do not know' answers excluded)<sup>a</sup>**

	TTD (n=43)	XP (n=37)	P-value
<b>Gender</b>			
Male	20 (46.5)	21 (58.3)	
Female	23 (53.5)	15 (41.7)	
<b>Twin birth</b>			
Yes	1 (2.5)	0 (0.0)	1.00
No	39 (97.5)	7 (100.0)	
<b>Preeclampsia</b>			
Yes	9 (31.0)	0 (0.0)	0.30
No	20 (69.0)	6 (100.0)	
<b>Hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome</b>			
No	23 (100.0)	6 (100.0)	
<b>Decreased fetal movement reported by mother to physician</b>			
Yes	4 (44.4)		
No	5 (55.6)		
<b>Abnormal levels of maternal serum screening markers</b>			
Yes	6 (60.0)	0 (0.0)	0.45
No	4 (40.0)	1 (100.0)	
<b>Pre-term delivery (&lt; 37 weeks gestation)</b>			
Yes	20 (71.4)	0 (0.0)	0.002
No	8 (28.6)	6 (100.0)	
<b>Low birth weight (&lt; 2500g)</b>			
Yes	18 (90.0)	0 (0.0)	<0.0001
No	2 (10.0)	6 (100.0)	
<b>Small for gestational age (SGA) (&lt; 10th percentile)</b>			
Yes	11 (57.9)	0 (0.0)	0.09
No	8 (42.1)	4 (100.0)	
<b>SGA (&lt; 3rd percentile)</b>			
Yes	9 (50.0)	0 (0.0)	0.11
No	9 (50.0)	4 (100.0)	
<b>Neonatal intensive care unit (NICU) hospitalization</b>			
Yes	11 (100.0)	0 (0.0)	<0.0001
No	0 (0.0)	4 (100.0)	

<sup>a</sup>Figures represent actual number of patients while numbers in brackets are percentages.

In contrast, none of the three XP pregnancies were associated with preeclampsia or any other gestational complications.

In the four TTD cases with confirmed history of preeclampsia, one of whom also had COFS, the same spatial region of the folded protein was affected; this region is made up of the faces of the helicase domains distal to the DNA-binding sites and includes the amino- and carboxy-terminal portions of the protein (Figure 1a). Biochemical<sup>46</sup> and biophysical<sup>47</sup> data have implicated these regions in binding to the CAK complex and the p44 subunit of the core complex of TFIH.

In two of these TTD cases with preeclampsia, the defect in the protein could be mapped to a region of 20 amino acids in the XPD C-terminus domain (Figure 1a); the genotypes of each of these cases (case 1: L461V, 716–730Δ:A725P and case 2: E731R,Δ), contained one

null allele<sup>48</sup> and one point mutation each in the C-terminal motif. We searched various databases for homologues of this XPD C-terminus domain, which was associated with preeclampsia. This motif showed strong conservation across species among eukaryotes but was found to be absent from all completed eubacterial genomes in the BLAST database<sup>49</sup> and in Archaea with the exception of a divergent version of this motif identified in three Methanococcales genomes (Figure 1b). A homologous complex to TFIH has not been identified in Archaea.<sup>50</sup>

In the two other TTD cases with preeclampsia, the mutations were not adjacent in the primary sequence; however, they mapped along with the C-terminal mutations to the helicase surfaces distal to the DNA-binding sites (Figure 1a). In one case, the genotype consisted of an allele with the R112H mutation, not associated with preeclampsia (see below), and a T482Δ, a mutation presumed to be null, on the other allele (Figure 1a). In the COFS/TTD case, a D681W allele was coupled with R616W, another null allele (Figure 1a).

Three of the four TTD cases in our study, which were not associated with preeclampsia were R112H homozygotes.<sup>21</sup> In the fourth TTD case, the patient had an allele with severely compromised XPD gene (34–54fs, 55–760Δ) coupled to an allele with an insertion (E81V, S<sub>ins</sub>, K82E) in helix 3 of the second helicase domain. Similar to this latter TTD case, each of the three XP patients had an allele lacking the preeclampsia-associated motif (case 1: G47R:L461V, 716–730Δ; case 2: 189–760Δ:S541R; case 3: G675R:669–707fs, 708–760Δ). In these three XP cases, the other alleles all contained point mutations classically associated with XP (case 1: G47R, case 2: S541R, case 3: G675R) (data not shown).

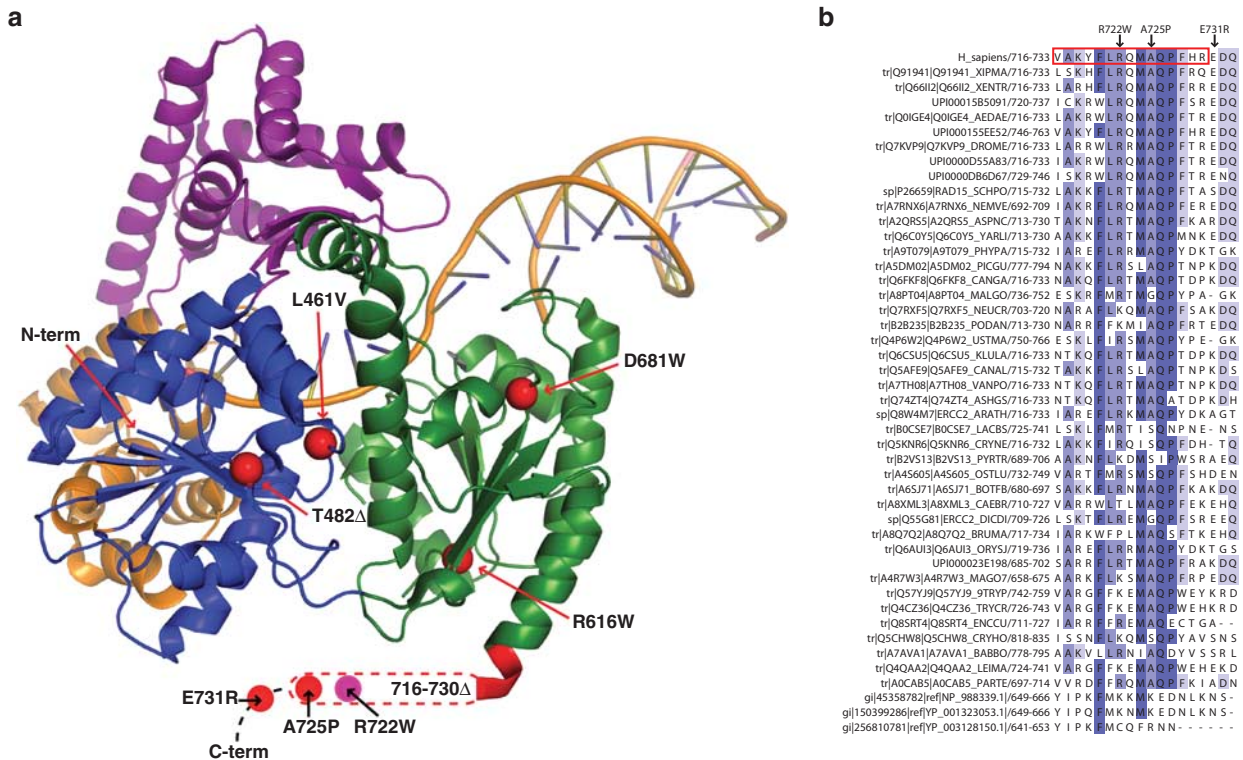
## DISCUSSION

Gestational complications were first reported to be part of the spectrum of abnormalities associated with TTD based on our novel clinical observations and subsequent genetic epidemiologic investigation in TTD families.<sup>8</sup> In the current study, we found elevated risks of preeclampsia and other placental vascular pregnancy complications in XPD-associated TTD- but not XP-affected pregnancies; thus, the exact genetic abnormality is relevant to the underlying mechanism in placental maldevelopment. Our molecular analysis revealed that preeclampsia-associated mutations in XPD affect protein-protein interactions, such as with CAK and p44 subunits of TFIH, leading to impairment of TFIH-mediated functions in placenta.

The majority of TTD cases had at least one of the gestational complications noted in Table 1. None of the XPD-associated XP cases were reported as having any of the noted gestational complications. Reporting bias is an unlikely explanation for the observed differences between XP and TTD cases in our study because both conditions are associated with similarly severe (although different) phenotypes.

Genetic susceptibility factors associated with pregnancy complications such as preeclampsia have long been studied with small success, possibly due to the multifactorial causes of the disease. Role of the maternal and fetal genotype in the development of preeclampsia is a topic of heated scientific debate.<sup>51–53</sup> Here, we demonstrate that at least for TTD-associated mutations in XPD, preeclampsia is unequivocally mediated by the fetal genotype. To the best of our knowledge, the penetrance of XPD mutations with respect to preeclampsia is highest compared to mutations reported in other genes.

In effect, the complete absence of pregnancy complications reported in XP patients stands in clear contrast to TTD patients, all of whom had at least one pregnancy complication. These observations also raise important issues concerning the role of the TFIH complex in normal prenatal development. Different types of mutations in XPD result in the development of either XP or TTD phenotype, with some



**Figure 1** Structural mapping of preeclampsia-associated mutations in *XPD*. (a) Changes in the amino acid sequences resulting from mutations associated with gestational complications mapped as red spheres to the *T. acidophilum* XPD crystal structure.<sup>43</sup> The DNA present in the model was generated by superposition of the *T. acidophilum* XPD helicase domains with the helicase domains of the co-crystal structure of the Hel308 complex<sup>58</sup> and is shown to provide general orientation of functional regions. A gross schematic of the region occupied by the C-terminal motif is indicated by red coloring of the amino acids at the *T. acidophilum* XPD C-terminus and the box outlined in red dashes that follows. The R722W mutation associated with TTD is shown as a pink sphere within the box. The coloring scheme is as follows: helicase 1 domain, blue; 4Fe-4S domain, gold; arch domain, purple; helicase 2 domain, green; DNA, gold backbone and purple bases. (b) Multiple sequence alignment of the C-terminal motif of eukaryotic XPD proteins. The A725P, E731R mutations associated with gestational complications are indicated with arrows above the alignment. The 716–730Δ is outlined in a red box. The R722W mutation associated with TTD maps to this region and is indicated with an arrow above the alignment. The last three sequences in the alignment are putative XPD proteins from Methanococcales species and represent the only prokaryotic XPD-like proteins that could be found with any significant similarity to eukaryotic proteins in this motif. The alignment is colored according to sequence identities and sequences are labeled by their UniRef or GenBank identifiers.

overlapping symptoms. It has been hypothesized that mutations affecting XPD's function in DNA repair cause XP, which is associated with 1000-fold increased risk of skin cancer, while mutations affecting XPD's role in RNA Pol-II-mediated transcription lead to TTD, which is associated with abnormalities of the nervous system, skin and hair. Further experimental studies would be required to address the nature of the cellular dysfunction in placental tissues of TTD patients.

The number of available mutant *XPD* alleles with confirmed association to preeclampsia was small, but sufficient to allow us to create the first structural map of preeclampsia-associated regions of XPD. Our structural analysis demonstrated a strong association between preeclampsia and a short motif in the C-terminal region of XPD as well as spatially proximal regions of the helicase domains distal to the DNA-binding sites. These regions are implicated in binding of XPD to the CAK complex and the p44 subunit of the core complex of TFIH.<sup>46,47</sup> Individual analysis of each TTD and XP case harboring two mutations in *XPD* provided further insight into the compensation mechanisms of different *XPD* alleles. In two TTD cases with preeclampsia, a null allele was coupled with a point mutation in the C-terminal motif. There is some evidence that proteins produced from null mutations retain some function and can complement orthogonal mutations on the sister allele<sup>54</sup>; however, in both of these cases the null alleles lacked a functional complement to the region mutated in the sister alleles. This strongly suggested that

the observed preeclampsia in these cases was due to the disruption of a specific function mediated by this C-terminal motif. The absence of this motif in prokaryotes coupled with the experimental data suggests that this motif has a function in eukaryotic transcription.

Disruption of the C-terminal motif alone could not fully account for association with preeclampsia because this motif was not affected in either allele of two of our TTD cases with preeclampsia. However, the regions disrupted in these cases were spatially proximal to the C-terminal motif and were also implicated in transcription by binding of XPD to the CAK complex. Thus, we propose that the alleles associated with preeclampsia in these two cases may be disrupting functions similar to those mediated by the C-terminal motif. In one of those cases, the genotype consisted of a T482Δ on one allele and an R112H mutation on the sister allele. R112H maps to a residue in contact with the 4Fe-4S cluster and disrupts the helicase activity of XPD.<sup>42</sup> Three of the four TTD cases in our study, which were not associated with preeclampsia were R112H homozygotes. These observations suggested that gestational complications in the R112H:T482Δ heterozygote TTD case were driven by the T482Δ allele. In the COFS/TTD case, R616W, a null allele, was coupled to D681W on the sister allele; another mutation, D681N, in the same location as D681W has been shown to interfere with p44 binding.<sup>55</sup> Therefore, the overall result of the mutations in these two cases may be a change in the nature of interaction between XPD and p44; there is evidence that

failure on the part of XPD to interact with p44 can lead to abolition of transcription without any significant effects on the helicase activity of this protein.<sup>56</sup>

XPD is an evolutionarily conserved ATP-dependent helicase, which is only fully active when assembled in the multi-subunit TFIH. XPD is specifically stimulated by its interaction with p44<sup>44,55</sup> and its binding to the CAK complex is required for the function of TFIH in RNA Pol-II-mediated transcription, NER, and phosphorylation of NRS.<sup>13,57</sup> Future functional studies involving larger sample sizes will determine which particular activities of TFIH are most severely impacted because of specific mutations in XPD.

Limitations of our study include lack of explicit information on presence or absence of some complications in a subset of subjects; this limitation is unavoidable because gestational complications were not routinely noted during investigation of TTD and XP cases until we reported prenatal complications as part of the spectrum of abnormalities associated with TTD. It would be premature to draw conclusions based on the observation that one of six XP/TTD and the one COFS/TTD case in our study had gestational complications, given the lack of standard diagnostic criteria for and the rarity of these combined phenotypes. Other limitations include a small sample size for structural mapping of preeclampsia-associated domains and the absence of experimental structures for human XPD and XPD/TFIH/CAK complexes. Despite these limitations, we obtained consistent and biologically plausible results from our analyses with respect to the involvement of the CAK domain of TFIH in the development of observed gestational complications.

In summary, our analyses revealed that a specific subset of XPD mutations, which lead to TTD, but are unrelated to XP, result in higher risk of development of preeclampsia and other gestational complications possibly due to their adverse effects on binding of XPD with CAK and p44 subunits of TFIH, leading to impairment of TFIH-mediated functions in placenta. Our findings should help drive future research focusing on the role of abnormalities in the various domains of XPD in the development of human prenatal complications.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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