Adverse Effects of Trichothiodystrophy DNA Repair and Transcription Gene Abnormalities on Human Fetal Development

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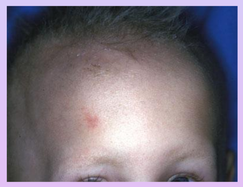
ABSTRACT

The effects of DNA repair and transcription genes in human prenatal life have never been studied. Trichothiodystrophy (TTD) is a rare (affected frequency of 10^{-6}) recessive disorder caused by mutations in genes involved in the nucleotide excision repair (NER) pathway and in transcription. Based on clinical observations, we conducted a genetic epidemiologic study to investigate gestational outcomes associated with TTD. We compared pregnancies resulting in TTD-affected offspring (N=24) with respect to abnormalities in their antenatal and neonatal periods to pregnancies resulting in their unaffected siblings (N=18), accounting for correlation, and to population reference values. Significantly higher incidence of several severe gestational complications was noted in TTD-affected pregnancies. Small for gestational age (SGA<10^{th} percentile) (RR=9.3, P=0.02), SGA<3^{rd} percentile (RR=7.2, P=0.04), and neonatal intensive care unit (NICU) hospitalization (RR=6.4, P=0.02) occurred more frequently among TTD-affected neonates compared to their unaffected siblings. Compared to reference values from general obstetrical population, pregnancies that resulted in TTD-affected infants were significantly more likely to be complicated by hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome (RR=35.7, P=0.0002), elevated mid-trimester maternal serum human chorionic gonadotropin (hCG) levels (RR=14.3, P<0.0001), SGA<3^{rd} percentile (RR=13.9, P<0.0001), preterm delivery (<32 weeks) (RR=12.0, P<0.0001), pre-eclampsia (RR=4.0, P=0.006), and decreased fetal movement (RR=3.3, P=0.0018). Gestational complications were noted in nearly all pregnancies resulting in TTD-affected offspring with *XPD* and *TTDN1*, but not *TTD-A*, gene mutations. Abnormal placental development may explain the constellation of observed complications; therefore, we hypothesize that some TTD genes play an important role in normal placental and fetal development. We investigated this hypothesis by analyzing the expression patterns of the four TTD genes identified to date. Using EST Profile Viewer (NCBI), we determined expression of all four genes in human placenta. We found high expression of all TTD genes in placenta, above the mean of their expression in all organs. All genes appeared to be in the higher range of their expression in placenta than in skin. Expression of *TTDA* was strongly negatively correlated (r=-0.7,P<0.0001) with gestational age, while *XPD*, *XPB* and *TTDN1* were consistently expressed from 14 to 40 weeks gestation. **Conclusion** Our results indicate an important role for *XPD*, *XPB* and *TTDN1* gene products during normal human placental and fetal development.

INTRODUCTION

- TTD is rare (affected frequency of 1 in 10^6 in Europe) autosomal recessive disorder of DNA repair.
- TTD is characterized by:
  - Sulphur deficient brittle hair
  - Small stature
  - Mental retardation
  - Ichthyotic skin
  - Unusual facial features
  - Photosensitivity in some cases

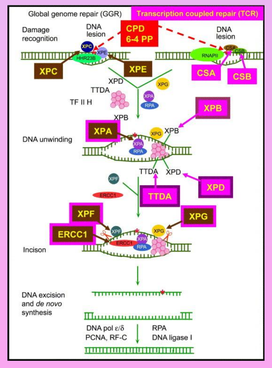


http://www.scalyskin.org/userimages/TTD2.jpg

- Photosensitive TTD caused by mutations in ***XPD*** (***ERCC2***) and ***XPB*** (***ERCC3***) which code for the two helicase subunits of transcription factor **TFIIH** and ***TTD-A*** (***TFB5***) which codes for the 10th subunit of **TFIIH**.

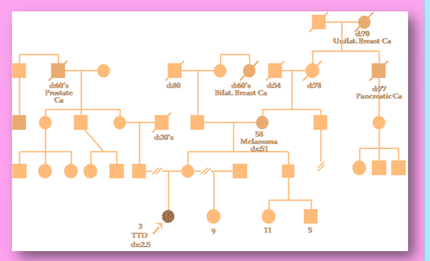
NER and TFIIH

- **TFIIH** has various roles in several pathways including nucleotide excision repair (**NER**), basal transcription and activated transcription.
- Non-photosensitive TTD has been associated with mutations in ***TTDN1*** **(*C7ORF11*)**, a gene of unknown function
- Mutations in genes coding for other **NER** proteins may Lead to other **NER** disorders besides TTD:
  - Xeroderma Pigmentosum [***XPA – XPG***, ***XPV***]
  - Cockayne Syndrome [***CS-A***, ***CS-B***]
  - Cerebro-oculo-facial Syndrome (COFS) [***XPD***, ***CS-B***]
  - XP/TTD [***XPD***]
  - COFS/TTD [***XPD***]



STUDY CONCEPTION and HYPOTHESIS

Based on clinical observations by Dr. Moslehi, we designed a genetic epidemiologic study to investigate pregnancy and gestational outcomes associated with TTD in order to test the hypothesis of involvement of TTD genes in human fetal development.



OBJECTIVE

- **Compare pregnancies resulting in TTD-affected offspring with respect to abnormalities noted during their antenatal and neonatal periods to:**
  - **Pregnancies resulting in unaffected siblings of TTD offspring**
  - **Population (general obstetrical) reference values**
- **Analyze expression Patterns of TTD genes in various tissues including placenta.**

STUDY DESIGN

Study Population. Obligate heterozygote mothers of all TTD patients studied at the NIH between 2001-2006 (N=15)

- Detailed Reproductive Epidemiologic Questionnaire
- Medical and Birth Records
- Multi-generation Pedigrees
- Biological Samples

RESULTS

Characteristics of Families, Pregnancies, and Outcomes

Family ID	Age of mother at birth of first affected child (yr)	Phenotype of Affected Children	Number of Pregnancies Resulting in an Affected	Number of Affected Live births	Number of Unaffected Live births	Number of Miscarriages	Stillbirth
R01	26	TTD	1	1	0	2	0
R02	28	TTD	1	1	1	0	0
R03	28	TTD	1	1	1	0	0
R04	31	TTD	3	3	0	0	0
R05	19	TTD	1	1	1	0	0
R06	23	TTD	1	1	1	1	0
R07	23	TTD	1	1	1	0	0
R08	31	TTD	2[a]	2[a]	1[a]	0	0
R09	30	TTD	2[b]	3[b]	2	0	0
R10	25	TTD	1	1	1	1	0
R11	23	TTD	2	2	0	0	0
R12	21	XP/TTD	4	4	4	3	1[c]
R13	25	TTD	1	1	3	0	0
R14	36	TTD	1	1	2	1	0
R15	25	COFS/TTD	1	1	0	0	0
Total			23	24	18	8	1

[a] Twins (1 affected, 1 unaffected)
[b] Twins (both affected) died shortly after birth
[c] Decreased fetal activity at 15-16 weeks and induced delivery at 21 weeks due to intrauterine fetal demise

Features of Pregnancies Resulting in Live Births (N=42) of Carrier Mothers (N=15)

		Affected (n=24) N (%)	Unaffected (n=18) N (%)	RR (95% CI)	*P*-Value
Gender					
	Male	13(54.2)	10 (55.6)	1.0 (0.6-1.7)	1.00
	Female	11 (45.8)	8 (44.4)		
Twin Births					
Yes		3 (12.5)	1 (5.6)	2.0 (0.2-20.7)	0.57
No		21 (87.5)	17 (94.4)		
Pre-eclampsia					
	Yes	7 (25.0)	1 (5.6)	5.2 (0.4-61.3)	0.19
	No	17 (70.8)	17 (94.4)		
Hemolysis, Elevated Liver Enzymes and Low Platelets (HELLP) Syndrome					
Yes		3 (12.5)	0		
No		21 (87.5)	18 (100.0)		
Decreased Fetal Movement Reported by Mother to Physician					
Yes		9 (37.5)	1 (5.6)	6.3 (0.9-45.0)	0.07
No		15 (62.5)	17 (94.4)		
Abnormal Levels of Maternal Serum Screening Markers (n=11)					
Yes		6 (85.7)[a]	0		
No		1	4 (100.0)		
Pre-term Delivery (<37 weeks gestation)					
Yes		11 (45.8)	2 (11.1)	2.9 (1.0-8.8)	0.06
No		13 (54.2)	16 (88.9)		
Low Birth Weight (<2500 grams)					
Yes		15 (62.5)	2 (11.1)	3.5 (1.1-11.0)	0.03
No		9 (37.5)	16 (88.9)		
Small for Gestational Age (SGA) (<10^{th} percentile)					
	Yes	13 (54.2)	1 (5.6)	9.3 (1.4-60.5)	0.02
	No	11 (45.8)	17 (94.4)		
SGA (<3^{rd} percentile)					
	Yes	10 (41.7)	1 (5.6)	7.2 (1.1-48.1)	0.04
	No	14(58.3)	17(94.4)		
Neonatal Intensive Care Unit (NICU) Hospitalization					
	Yes	15 (62.5)	1 (5.6)	6.4 (1.4-29.5)	0.02
	No	9 (37.5)	17 (94.4)		

[a] Human chorionic gonadotropin (hCG) levels were elevated in all six. Alpha-fetoprotein levels were elevated in one and reduced in three of the affected pregnancies.

Comparison of TTD-Affected Pregnancy Characteristics to Reference Values

		Affected (n=24) N (%)	Reference Values[a] (%)	RR (95% CI)	*P*-Value
Gender					
	Male	13 (54.2)	(51.1)	1.1 (0.7-1.4)	0.84
	Female	11 (45.8)	(48.9)		
Twin Gestation					
		3 (12.5)	(2.6)	5.5 (1.7-12.0)	0.023
Delivery					
	Vaginal	15 (62.5)	(78.7)		
	Cesarean	9 (37.5)	(20.5)	1.9 (1.0-2.8)	0.072
Hemolysis, Elevated Liver Enzymes and Low Platelets (HELLP) Syndrome					
		3 (12.5)	(0.35)	35.7 (7.6-92.5)	0.0002
Elevated Human Chorionic Gonadotropin (hCG) on Maternal Serum Screening (n=7)					
		6 (85.7)	(6)	14.3 (7.0-16.6)	<0.0001
Birth Weight[b]					
		2215 ± 990.1 g	3353 ± 581 g		<0.0001
Low Birth Weight (<2500 grams)					
		15 (62.5)	(6.9)	9.2 (6.2-11.5)	<0.0001
Very Low Birth Weight (<1500 grams)					
		5 (20.8)	(1.4)	16.3 (6.7-29.5)	<0.0001
Small for Gestational Age (SGA)					
	<10th percentile	13 (54.2)	(10)	5.4 (3.3-7.4)	<0.0001
	<3rd percentile	10 (41.7)	(3)	13.9 (7.4-21.1)	<0.0001
Preterm Delivery					
	<37 weeks	11 (45.8)	(10.9)	4.3 (2.6-6.0)	<0.0001
	<32 weeks	5 (20.8)	(1.9)	12.0 (4.9-21.6)	<0.0001
Pre-eclampsia (n=23)					
		6 (26.1)	(6.5)	4.0 (1.6-7.4)	0.006
Decreased Fetal Movement					
		9 (37.5)	(11.5)	3.3 (1.6-5.2)	0.0018

[a] Elevated hCG; Pre-eclampsia; HELLP; Decreased fetal movement; All others based on 1996 US population rates
[b] Values for Birth Weight are Mean SD

SUMMARY OF FINDINGS

- Highly significant increased risk of the following gestational complications associated with pregnancies resulting in nearly all TTD and COFS/TTD offspring:
  - Pre-term birth
  - Low birth weight
  - Small for gestational age
  - Pre-eclampsia
  - HELLP syndrome
  - Elevated hCG levels
  - Decreased fetal movement

Proposed Mechanism: Abnormal Placental Development?

- High expression of *XPD*, *XPB*, and *TTDN1* in placenta from 14 to 40 weeks of gestation, above the mean of their expression in all other organs including skin.

CONCLUSION

- **Our systematic genetic epidemiologic investigation indicated a greatly increased risk of many adverse pregnancy outcomes such as small for gestational age, HELLP syndrome, pre-eclampsia, preterm delivery, and decreased fetal movement associated with mutations in TTD genes.**
- **Abnormal placental development can explain the constellation of complications noted in our study.**
- **We hypothesize that TTD genes, in particular *XPD*, *XPB* and *TTDN1* are important for normal placental development.**

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