A new mechanism in THRA resistance: the first disease-associated variant leading to an increased inhibitory function of THRA2

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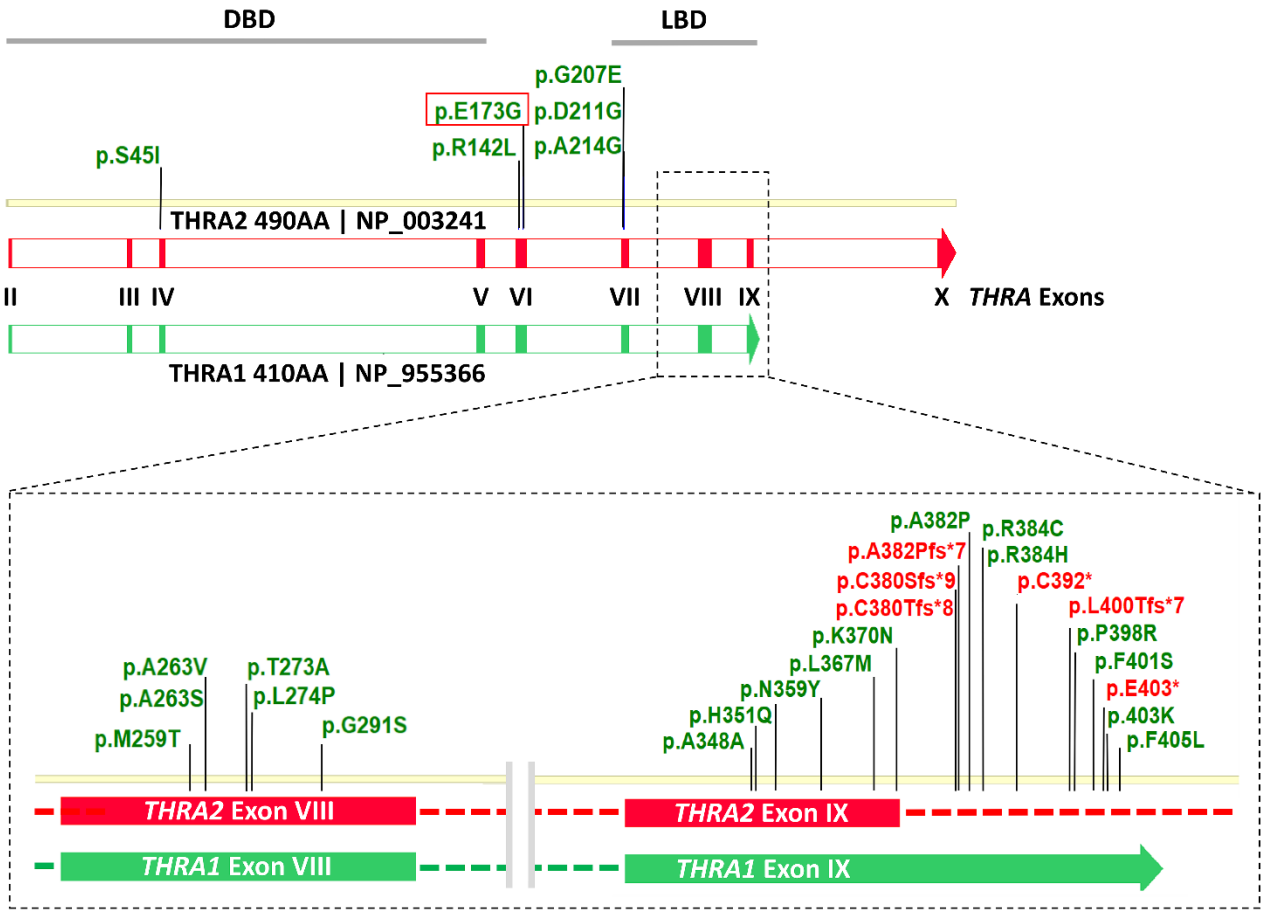


Figure S1: The two main alternative splicing isoforms of the *THRA* gene. The protein coding exons II-X are depicted between the splicing isoforms. Exon I is non-coding. The T3 binding pockets are encoded mainly by exon 9 of the THRA1 isoform (green). Most of the reported disease-associated genetic variants are located in the ligand (T3) binding domain (LBD). The DNA-binding domain (DBD) of the receptor is located at the N-terminus of the protein. The here described disease-associated variant p.E173G is located between DBD and LBD (red square). AA, amino acid. Missense variants are highlighted in green, frameshift and nonsense variants are highlighted in red.

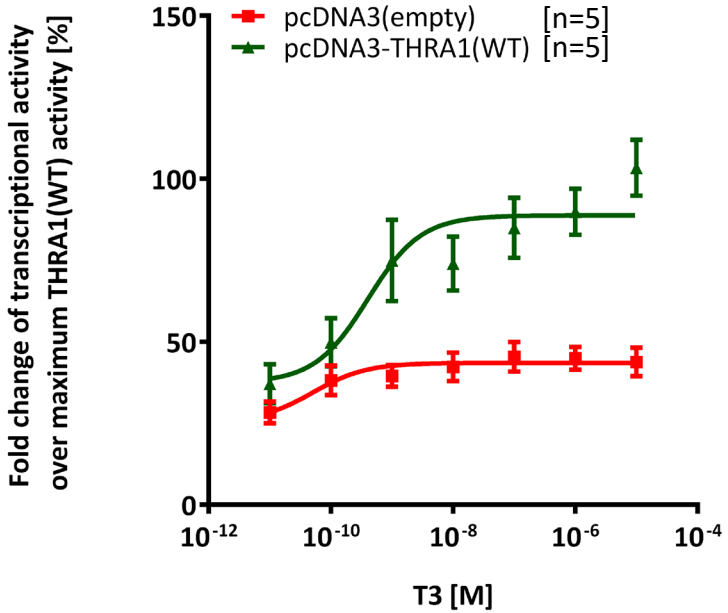


Figure S2: Results from concentration-response experiments of transiently transfected JEG3 cells with either pcDNA3-THRA1(WT) or empty pcDNA3 vector as mock control. Even mock transfected cells did respond to T3, although effects are small in comparison to the transfection with the THRA1(WT) construct. Values from the luciferase assay were normalized to the maximum transcriptional activation of THRA1(WT) [=100%]. The dots and the error bar depict the mean and SEM of five independent experiments performed in triplicates

Table S1: **Disease associated variants in *THRA* that have been published to date**. In case *in vitro* functional tests have been performed using the recombinant protein with the respective variant inserted (e.g. *via* T3 stimulated transactivation by THRA1 in a reporter gene assay), we mention, whether the authors had found a transactivation defect that could be (partially) overcome by rising T3 concentrations. *, variant was only published in the ClinVar database (URL https://www.ncbi.nlm.nih.gov/clinvar/?term=THRA[gene]) and no functional tests have been performed. The ClinVar accession number is provided. n.i., no information available. Missense variants are highlighted in green, frameshift and nonsense variants are highlighted in red.

THRA1 isoform		THRA2 isoform		T3 stimulated transactivation by THRA1	Genomic location	Publication
NM_199334	NP_955366	NM_003250	NP_003241		GRCh37.p13 (hg19)	
c.134G>T	p.(S45I)	c.134G>T	p.(S45I)	n.i.	17:38233773G>T	CinVar VCV000029913*
c.425G>T	p.(R142L)	c.425G>T	p.(R142L)	n.i.	17:38240917G>T	CinVar VCV000803389*
c.518A>G,	p.(E173G)	c.518A>G,	p.(E173G)	**increased**	17:38241010A>G	**this report**
c.620G>A	p.(G207E)	c.620G>A	p.(G207E)	n.i.	17:38243003G>A	(van Gucht et al. 2017)
c.632A>G	p.(D211G)	c.632A>G	p.(D211G)	decreased	17:38243015A>G	(van Gucht et al. 2016)
c.641C>G	p.(A214G)	c.641C>G	p.(A214G)	n.i.	17:38243024C>G	CinVar VCV000847657*
c.776T>C	p.(M259T)	c.776T>C	p.(M259T)	decreased	17:38244547T>C	(le Maire et al. 2020)
c.787G>T	p.(A263S)	c.787G>T	p.(A263S)	weak decrease	17:38244558G>T	(Demir et al. 2016)
c.788C>T	p.(A263V)	c.788C>T	p.(A263V)	decreased	17:38244559C>T	(Moran et al. 2017)
c.817A>G	p.(T273A)	c.817A>G	p.(T273A)	decreased	17:38244588A>G	(le Maire et al. 2020)
c.821T>C	p.(L274P)	c.821T>C	p.(L274P)	decreased	17:38244592T>C	(Moran et al. 2017)
c.871G>A	p.(G291S)	c.871G>A	p.(G291S)	n.i.	17:38244642G>A	(Korkmaz et al. 2019)
c.1053C>G	p.(H351Q)	c.1053C>G	p.(H351Q)	decreased	17:38245529C>G	(Kalikiri et al. 2017)
c.1075A>T	p.(N359Y)	c.1075A>T	p.(N359Y)	decreased	17:38245551A>T	(Espiard et al. 2015)
c.1099C>A	p.(L367M)	c.1099C>A	p.(L367M)	n.i.	17:38245575C>A	(Kalikiri et al. 2017)
c.1110G>C	p.(K370N)	c.1110G>C	p.(L367M)	n.i.	17:38245586G>C	CinVar VCV000029914*
c.1138_1141delTGCC	p.(C380Tfs*8)	intron 9		decreased	17:38245614_38245617delTGCC	(Demir et al. 2016)
c.1139_1139delG	p.(C380Sfs9*)	intron 9		n.i.	17:38245615_38245615delG	(Furman et al. 2020)
c.1144_1144delG	p.(A382Pfs*7)	intron 9		decreased	17:38245620_38245620delG	(Moran et al. 2013)
c.1144G>C	p.(A382P)	intron 9		n.i.	17:38245620G>C	(Kalikiri et al. 2017)
c.1150C>T	p.(R384C)	intron 9		n.i.	17:38245626C>T	(Yuen et al. 2015)

c.1151G>A	p.(R384H)	intron 9		decreased	17:38245627G>A	(Demir et al. 2016)
c.1176C>A	p.(C392*)	intron 9		n.i.	17:38245652C>A	(Tylki-Szymańska et al. 2015)
c.1183G>T	p.(E395*)	intron 9		n.i.	17:38245659G>T	(Sun et al. 2019)
c.1193C>G	p.(P398R	intron 9		n.i.	17:38245669C>G	(Tylki-Szymańska et al. 2015)
c.1190_1191insT	p.(L400Tfs*7)	intron 9		decreased	17:38245666_38245667insT	(van Mullem et al. 2012, 2013)
c.1202T>C	p.(F401S)	Intron 9		weakly decreased	17:38245678T>C	(Kalikiri et al. 2017)
c.1207G>T	p.(E403*)	intron 9		decreased	17:38245683G>T	(Bochukova et al. 2012)
c.1207G>A	p.(E403K)	intron 9		n.i.	17:38245683G>A	(Tylki-Szymańska et al. 2015)
c.1213T>C	p.(F405L)	intron 9		n.i.	17:38245689T>C	(Kalikiri et al. 2017)

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