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Highly reduced penetrance in a family with a *THAP1* nonsense mutation: Role of *THAP1* expression?

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A large number of pathogenic mutation carriers eventually do not develop the disease in question, a phenomenon known as 'reduced penetrance'. Understanding the mechanisms underlying reduced penetrance has a particularly high imperative given that it may be viewed as a means of 'endogenous' disease protection [1]. For many genetic dystonias such as DYT-THAP1 (DYT6 dystonia) the penetrance is highly reduced (to ~50%). DYT-THAP1 is caused by heterozygous loss-of-function mutations in *THAP1*. It usually manifests in childhood or adolescence (75% of affected carriers with age at onset < 30 years) and starts with brachial or cervical dystonia. Symptoms often generalize and speech problems are common (www.mdsgene.org). Nonetheless, the THAP1-linked phenotype is highly variable, ranging from unaffected mutation carriers to severe generalized dystonia, even among members of the same family [2]. The genetic factors explaining reduced penetrance of *THAP1* mutations remain elusive. Given the function of THAP1 as a transcription factor that regulates the expression of itself and of *TOR1A* [3,4], another dystonia gene, we hypothesized that the penetrance of *THAP1* mutations may be linked to *THAP1* and *TOR1A* expression. We here report a large Polish family with only one affected and at least 10 unaffected carriers of a THAP1 nonsense mutation. This mutation has previously been reported in three independent cases (www.mdsgene.org) and causes almost completely abolished nuclear import of THAP1 [5] underlining its pathogenicity.

The index patient (IV:1), a 21-year-old man, developed the first signs of dystonia at the age of 9 years in the neck and lower face. At 11 years, he noticed difficulties while writing with his right hand. Three years later, left-hand dystonia and strained voice due to larynx involvement occurred. During adolescence, dystonic movements spread to the trunk and lower limbs. On examination at 18 years, he had mild anterocollis with pronounced platysma involvement, and dystonia in the upper limbs, feet and trunk. Irregular, large amplitude tremor of both arms, and frequent blinking with sporadically appearing blepharospasm were also observed. Family history was negative. The patient received bilateral GPi-DBS and since then, dystonic movements of the lower limbs and the right upper limb resolved and severity of the left upper limb and neck dystonia decreased. His blood tests and brain/cervical spine MRI scans were unremarkable. There was no exposure to neuroleptics. Diagnostic testing was negative for *TOR1A* but revealed a nonsense mutation (c.85C > T; NM_018105; p. Arg29X) in *THAP1*.

The study was approved by the Bioethical Committee at the Medical University of Lodz and written informed consent was obtained from all examined individuals. Mutational analysis in unaffected relatives from four generations identified ten additional mutation carriers (Fig. 1A). All mutation carriers but I:1 (who died before re-examination) were neurologically examined at least twice by movement disorder specialists (A.G., A.B.) and no signs of dystonia were found. Unaffected individuals had a median age of 55 years with a range of 21–84 years.

RNA sampling using PAXgene tubes for expression analysis was possible for seven mutation carriers from the family including the affected carrier. We also included three healthy, mutation negative controls. Total RNA was extracted using the PAXgene Blood RNA Kit (Qiagen) and then reversely transcribed into cDNA with the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific). There was no difference in the ratio of wildtype and mutated peak height in the sequences of THAP1 cDNA between the affected and unaffected mutation carriers. Likewise, quantification of this ratio by pyrosequencing (PyroMarkQ48, Qiagen) revealed the mutant allele in 45–49% of reads (Fig. 1B) in all carriers. The seemingly slightly lower levels of the mutant compared to the wildtype allele (< 50%) in all investigated family members was not statistically significant. Expression analyses of *THAP1, TOR1A,* and of three reference genes (*β-ACTIN, YWHAZ, and HPRT*) were performed on a LightCycler (RocheDiagnostics). Affected and unaffected carriers didn't show any expressional difference neither for *THAP1* (1.53 ± 0.02 vs. 1.50 ± 0.40) nor for *TOR1A*. However, the mutation carriers showed a slight but significant reduction of total *THAP1* expression (Fig. 1C) compared to controls. Further, we demonstrated a trend towards an increased *TOR1A* expression (Fig. 1D), possibly due to the reduced repression [4] caused by lower THAP1 levels.

We here present a large, multigenerational family with highly reduced pentrance (< 10%) despite a loss-of-function *THAP1* mutation. Expressional analyses of *THAP1* and *TOR1A* do not support the hypothesis that penetrance of the mutation is related to the expression of these target genes since there was no difference between affected and

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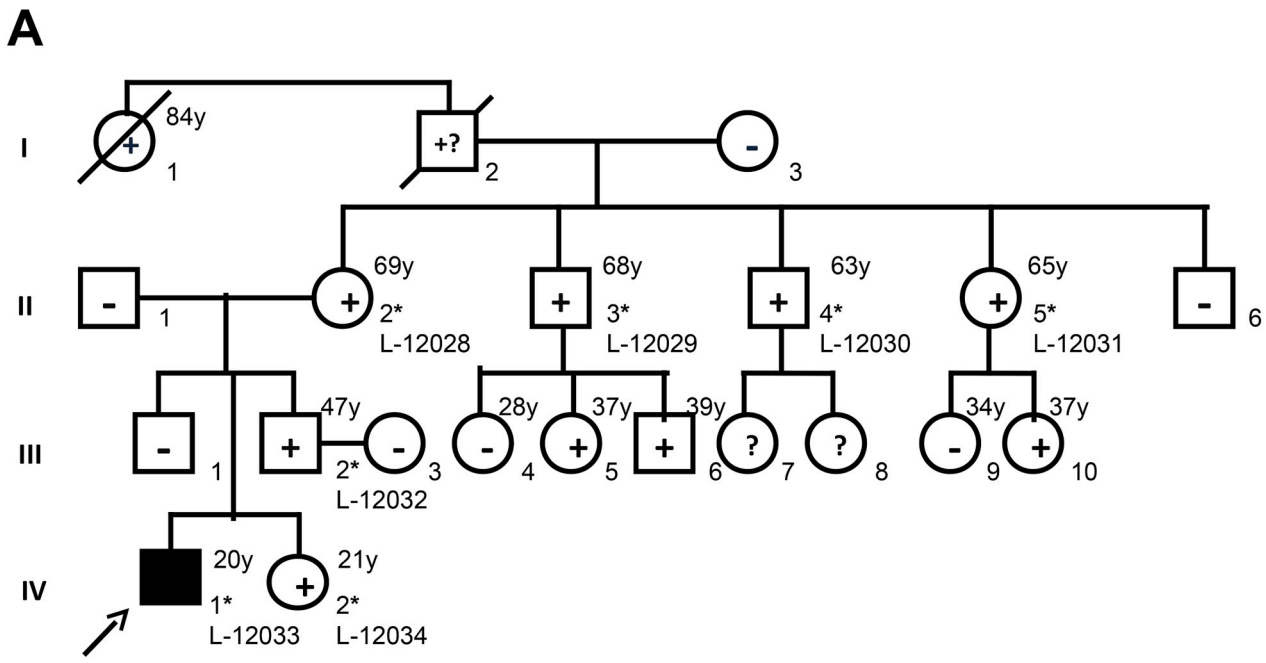
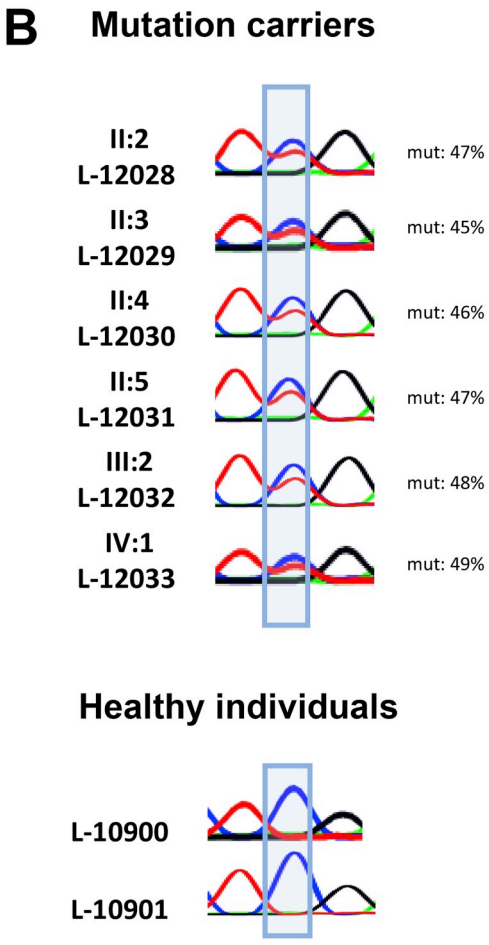
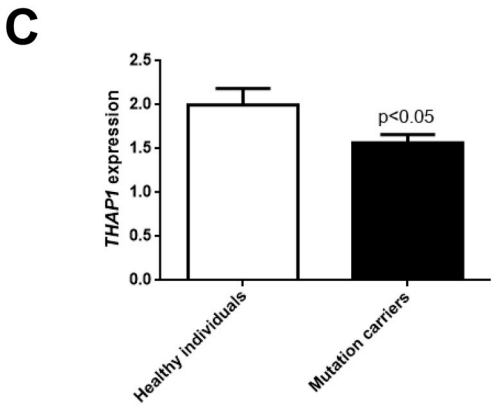
unaffected mutation carriers. Thus, factors of reduced penetrance remain elusive. Since expression is tissue- and time point specific, investigations in other cell types (e.g. neurons) and at different time points (during development) [6] in a multiomic fashion including genome and transcriptome analyses may shed light on causes of reduced penetrance in *THAP1* mutation carriers.







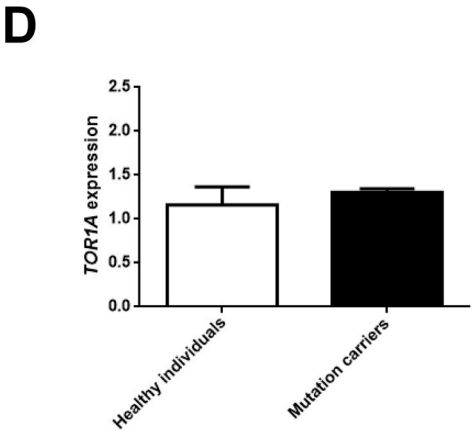


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Fig. 1. Multigenerational family with a nonsense mutation (p.Arg29X) in *THAP1* and expressional analysis. (A) Pedigree of the family. The filled symbol indicates the index patient with the mutation (IV:1). Empty symbols mark asymptomatic family members. L codes denote indivudials for whom RNA was available for expressional studies. The plus sign highlights individuals carrying the mutation while a minus sign marks individuals who were tested but do not carry the mutation. The age at last examination is also provided. I:2 is an obligate carrier but mutational status could not be evaluated since he died before the study. For two additional individuals for whom no sample could be obtained, mutational status is unknown and indicated by a question mark. (B) Representative electropherograms of cDNA sequencing are provided for II:2 (L-12028), II:3 (L-12029), II:4 (L-12030), II:5 (L-12031), III:2 (L-12032), IV:1 (L-12033)) (upper panel) and two unrelated healthy controls (L-10900, L-10901) (lower panel). Pyrosequencing results given as the percentage of mutated (mut) allele as indicated next to the sequences. (C) *THAP1* and (D) *TOR1A* expression levels of the mutation carriers (n = 7) and healthy controls (n = 3) measured by qPCR. Means are shown and standard error of the mean is indicated. The results are based on the mean ratios of *THAP1 and TOR1A* expression compared to three reference genes *β-ACTIN, YWHAZ, and HPRT*.

Declarations of interest

None.

Patient consent

Obtained.

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Ethics approval

This study was conducted with the approval by the Bioethical Committee at the Medical University of Lodz.

Contributors

All authors have approved the final article for submission.

1) Research project: A. Conception, B. Organization, C. Execution;

2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique;

3) Manuscript: A. Writing of the first draft, B. Review and Critique.

M.D.: 1B, C, 2B, 3A.
A.G.: 1B, C, 2C, 3A.
H·B.: 1C, 2A,B, 3B.
J.P.: 1B, 1C, 2B, 3B.
F.J.K.: 1B, 2C, 3B.
A.B.: 1A, 2C, 3B.
K.L.: 1A, 2C, 3B.

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