Unique genetic features of the naked mole-rat's *THADA* gene

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Abstract

Thyroid Adenoma Associated (THADA) is a protein-coding gene that maps to chromosomal band 2p21 and first has been described as a target of recurrent translocation partner in thyroid tumors. Many genome-wide association studies have revealed an association between *THADA* and two frequent human diseases, i.e. type 2 diabetes and polycystic ovary syndrome. Nevertheless, the function of its protein is not been completely understood. However, recent evidence suggests that in a *Drosophila* model *THADA* can act as a sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA)-interacting protein which uncouples SERCA from this function. Once being uncoupled, SERCA produces an increased amount of heat without transporting calcium thus triggering nonshivering thermogenesis. This data prompted us to compare human *THADA* with that of 65 other eutherian mammals. This includes a comparison of *THADA* of a variety of eutherian mammals with that of the naked-mole rat (*Heterocephalus glaber*) which is known to display unique features of thermoregulation compared to other mammals. Our analysis revealed five positions where only the naked-mole rat presented differences. These latter positions included four single amino acid substitutions and one unique deletion of six or seven amino acids, respectively, between residues 858 and 859. In future studies these changes will be analyzed further in detail for their functional relevance.

Introduction

Human *Thyroid Adenoma Associated* (*THADA*) maps to chromosomal band 2p21. First it has been described as a recurrent translocation partner in thyroid tumors characterized by corresponding reciprocal chromosomal translocations with a predominance of a t(2;7)(p21;p15) (Rippe et al., 2003). Later, *THADA* was found to be expressed at a high level in follicular cells of normal thyroid tissue when compared with other tissues (Kloth et al., 2011) but it was shown that its expression was not altered in thyroid cancers positive or negative for *THADA* fusions. It has been proposed that in these latter rearrangements, *THADA* drives tumorigenesis by juxtaposing its regulatory sequences to a translocation partner thereby activating a gene of this partner. In the majority of cases, the gene encoding insulin-like growth factor 2 mRNA-binding protein 3 (*IGF2BP3*) serves as this activated partner (Panebianco et al., 2017). Accordingly, translocations of *THADA* have been placed among the 20 most-significant significantly recurrent breakpoints (SRBs) observed in non-coding regions in human cancers (Rheinbay et al., 2020). Nevertheless, as a result of the translocations not only *IGF2BP3* becomes upregulated but also a truncated *THADA* is expressed. Thus, it remains to be investigated, if both effects - upregulation of *IGF2BP3* as well as the presence of truncated *THADA*- contribute its potential of driving tumorigenesis. As to the tumor types affected, said *THADA* rearrangements in the majority of cases characterize a particular type of thyroid neoplasms called non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) (Pool et al., 2019) but other histologic types have been reported as well. In a study by Morariu et al. (2021) thirty nodules positive for *THADA-IGF2BP3* fusion on FNA were detected. Of the positive nodules that were managed surgically all were intrathyroidal and all lacked aggressive histology. Surgical pathology revealed seven (32%) malignant nodules (six encapsulated follicular variant papillary thyroid carcinomas (EFVPTC), one minimally infiltrative FVPTC), ten (45%) noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP), and five (23%) nodules that were classified as follicular adenomas (FA).

Despite its important role in the genesis of thyroid neoplasms *THADA*'s normal physiological functions still remained enigmatic. Besides the thyroid *THADA* is abundantly expressed in some other endocrine tissues as pituitary gland and parathyroid gland as well as in the endometrium (<https://www.proteinatlas.org/ENSG00000115970-THADA/tissue>).

Many genome-wide association studies (GWAS) have revealed an association between *THADA* and two frequent human diseases, i.e. type 2 diabetes (T2DM) (e.g. Staiger et al., 2008, Grarup et al., 2008, Kang et al., 2009, Boesgaard et al., 2009, Hu et al., 2009, Simonis-

Bik et al., 2010, Schleinitz et al., 2010, Cheng et al., 2011, Gupta et al., 2012, Hotta et al., 2012, Villegas et al., 2014, DeMenna et al., 2014, Bai et al., 2015, Prasad et al., 2016) and polycystic ovary syndrome (PCOS) (e.g. Zhao et al., 2012, Eriksen et al., 2012, Wang et al., 2012, Cui et al., 2013, Sun et al., 2014, Day et al., 2015, Cui et al., 2015, Xia et al., 2019, Park et al., 2019, Bakhashab et al., 2019, Tian et al., 2020, Vishnubotla et al., 2020). Again, due to a lack of knowledge, for a long time it was impossible to tie these associations with any type of causal relationship. Nevertheless, both associations turned out to be highly reproducible and apparently did not depend on ethnicity (Goodarzi et al., 2012).

A genome-wide comparison of the Neandertal genome with recent humans has identified SNPs in *THADA* as the most strongly positively selected SNPs during evolution of modern humans (Green et al., 2010). In 2014, *THADA* was marked as a cold adaptation gene based on population genetic studies (Cardona et al., 2014) but the remarkable and nearly total lack of knowledge about the function of *THADA* remained until 2017 when, using a *Drosophila* model, Moraru et al. (2017) were able to show that *THADA* can act as a sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA)-interacting protein. SERCA is a calcium transporter from the cytosol into the sarcoplasmic reticulum and its action contributes to obligative energy expenditure. Apparently, like sarcolipin, *THADA* is able to uncouple SERCA from this function and, once being uncoupled, SERCA produces an increased amount of heat without transporting calcium thus triggering nonshivering thermogenesis (Chatterjee et al., 2017). Nevertheless, unlike sarcolipin *THADA* does not seem to be inducible by cold, pointing to a more complex network. The *Drosophila* experiments suggest that *THADA* is not only a very „old“ gene in terms of evolution but also has served functions in thermogenesis very early. These functions may explain the association between *THADA* and T2DM as well as with PCOS and suggest a causal link. The anti-obesity effect of *THADA* may be mediated through enhanced thermogenesis or reduced ER (endoplasmic reticulum) calcium. Of note, in mice changes of calcium levels in the endoplasmic reticulum during pancreatic maturation were correlated with the expression of *THADA* (West et al., 2021). Overall, an in depth characterization of the metabolic effects of the *THADA*-driven network will not only aim at a better understanding of its function in general but might also provide new therapeutic targets for the treatment of both conditions associated with *THADA* by GWAS (Chatterjee et al., 2017).

THADA as well as its protein were found to be highly conserved among mammals and even among vertebrates (Drieschner et al., 2007, Soller et al., 2008). To better understand functional relationships between *THADA* and thermogenesis, this study aims at the characterization of Thada of the naked-mole rat (NMR Thada) (*Heterocephalus glaber*) which, when compared to other rodents and mammals in general, is expressing many unusual traits including its limited ability to maintain a stable body temperature and, at least in part together with other

batherygids, appears to have a different thyroid hormone physiology. While some evidence has been presented that NMR are even non-ageing mammals i.e. lack an increase of likelihood of mortality with chronological age (Ruby et al., 2018) this has been a matter of controversial debate (Dammann et al., 2019, Ruby et al., 2019). However, there is still ample data from the current literature allowing to conclude that NMR are at least characterized by a remarkably late onset of senescence (Braude et al., 2021).

Materials and methods

THADA is a well conserved protein among mammalian species. For protein alignment of multiple sequences the NCBI blast tool has been used (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>).

Results

In a first approach, THADA AS-sequences of a small set of eutherian (placental) mammals have been compared with that of the naked-mole rat by multiple sequence alignment. For this first analysis we referred to NMR Thada sequence XP_004839417.1 (NCBI reference sequence) and compared it to a first set of 16 species including:

Bos taurus (European cattle), *Callithrix jacchus* (common marmoset), *Canis lupus familiaris* (dog), *Dasypus novemcinctus*, *Fukomys damarensis* (Damaraland mole-rat), *Gorilla gorilla gorilla* (gorilla), *Homo sapiens*, *Loxodonta africana* (African bush elephant), *Macaca mulatta* (rhesus macaque), *Mus musculus* (house mouse), *Myotis brandtii* (Brandt's bat), *Oryctolagus cuniculus* (European rabbit), *Otolemur garnettii* (northern greater galago), *Pan troglodytes* (chimpanzee), *Papio anubis* (olive baboon), and *Rattus norvegicus* (Norwegian rat).

THADA was unambiguously identified in all these species. Furthermore, this search confirmed the high level of identity between human THADA and that of the other mammalian species investigated ranging between 99.1 % (gorilla and chimpanzee) and 78.3 % (mouse) (Tab.1). Between human and NMR-Thada, an identity of 85.1% was observed.

A total of six amino acid residues of NMR-Thada turned out to be unique among orthologs present in these 16 other mammalian species with no variation of each of the corresponding amino acid seen in the other mammals analyzed. In addition, NMR-Thada differed from all

other investigated mammalian species by a unique deletion site of six or seven amino acids, respectively, between residues 858 and 859 (Fig. 2).

As a next step, THADA of an extended second set of further 49 non-primate eutherian mammals was compared by multiple alignment with that of *H. glaber*. This second set comprised the following species:

Acinonyx jubatus, *Balaenoptera musculus*, *Camelus dromedarius*, *Camelus ferus*, *Canis lupus dingo*, *Ceratotherium simum simum*, *Choloepus didactylus*, *Chrysochloris asiatica*, *Delphinapterus leucas*, *Eptesicus fuscus*, *Equus asinus*, *Equus caballus*, *Eumetopias jubatus*, *Felis catus*, *Globicephala melas*, *Halichoerus grypus*, *Ictidomys tridecemlineatus*, *Lagenorhynchus obliquidens*, *Lynx canadensis*, *Lynx pardinus*, *Manis javanica*, *Manis pentadactyla*, *Marmota flaviventris*, *Marmota monax*, *Miniopterus natalensis*, *Mirounga leonine*, *Molossus molossus*, *Monodon Monoceros*, *Myotis lucifugus*, *Myotis myotis*, *Neomonachus schauinslandi*, *Neophocaena asiaeorientalis*, *Nyctereutes procyonoides*, *Odobenus rosmarus divergens*, *Orcinus orca*, *Panthera pardus*, *Panthera tigris altaica*, *Phocoena sinus*, *Pteropus vampyrus*, *Puma yagouaroundi*, *Rhinolophus ferrumequinum*, *Rousettus aegyptiacus*, *Talpa occidentalis*, *Trichechus manatus latirostris*, *Tursiops truncatus*, *Ursus arctos horribilis*, *Ursus maritimus*, *Vulpes Vulpes*, *Zalophus californianus*

Four of the previously identified six amino acid residues of NMR-Thada remained to be unique even among the extended set of mammalian species (Tab. 2). Also, the deletion as described above remained unique and represents the most obvious difference between *H. glaber* sequence XP_004839417.1 and all other mammals included in the comparison. Next, all other NMR THADA sequences as available through NCBI were also checked for these differences. In total seven Thada sequences from the naked-mole rat are currently available by NCBI (reference sequence protein isoforms for the Thada gene) and were analyzed (Tab. 3).

Since apparently important insights into the function of THADA have been obtained by in depth analysis of its counterpart in *Drosophila melanogaster* (Moraru et al., 2017), NMR-Thada was also compared with that of the fruit fly. Analysis revealed a 29.8 % identity between THADA of both species which is in the same range as that between the fly and *Homo sapiens* (29.1 % identity).

Discussion and further experimental approach

Human THADA and its gene have been identified nearly twenty years ago by positional cloning of a breakpoint region of chromosome 2 recurrently involved in chromosomal alterations in thyroid tumors (Bol et al., 2001, Rippe et al., 2003). In genome-wide association studies, the SNPs of the *THADA* gene have been found to be associated with T2DM as well as with PCOS and SNPs in *THADA* belong to the most strongly positively selected SNPs during the evolution of the recent human species (Green et al., 2010). Nevertheless, surprisingly little is known about THADA function. Some recent data, however, suggest that, as a SERCA uncoupler, THADA is involved in the control of thermogenesis and energy production.

Compared to other eutherian mammals, naked-mole rats show an extraordinary biology e.g. related to unusual thermogenesis and longevity (Ruby et al., 2018; Dammann et al., 2019). The former prompted us to perform an in-depth analysis of NMRs Thada in comparison with other mammals. This comparison led to the identification of four amino acid exchanges in NMR at positions where all other mammals analyzed share the same amino acids. In addition, NMRs Thada shows one deletion not seen in any of the other mammals investigated.

In *Drosophila*, *THADA* knockout leads to obese hyperphagic flies with reduced energy production and enhanced cold-sensitivity (Muraru et al., 2017). These effects could be reversed by transfer of intact human *THADA*. As a next experimental step an in depth characterization of the NMR-unique changes of THADA as determined herein seems reasonable. It e.g. remains to be determined if transfection of NMR's *Thada* and *Thada* with single changes as present in NMR can also reverse these effects. In order to analyze the possible biological significance of the NMR-unique mutations NMR-derived fibroblasts can also be transfected using human THADA to study the effects on the transcriptome.

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Figures and tables with legends

Fig. 1:

Legend Fig. 1: Chromosomal assignment of human *THADA* to band 2p21, intron-exon structure of *THADA* (red, exons represented by vertical bars), and localization of SNPs (blue squares) associated with Type 2 Diabetes (T2DM) and Polycystic Ovary Syndrome (PCOS), respectively. 1: rs13429458, PCOS, 2: rs10203174, T2DM, 3: rs12478601, PCOS, 4: rs7578597, T2DM

Figure 2:

NP_001077422.1	803	KILAFDLLMKLSK-TAVHFQDSGKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQDALPSSLSAYL-TQQVACDNGDR	880
XP_004029196.2	803	KILAFDLLMKLSK-TAVHFQDSEKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQDALPSSLSAYL-TQQVACDNGDT	880
XP_001141732.2	803	KILAFDLLMKLSK-TAVHFQDSEKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQDALPSSLSAYL-TQQVACDNGDR	880
XP_002799292.2	803	KMLAFDLLMKLSK-TAVHFQDSEKLQGLFQAAL ~~AL~~ STSTKPYDCVTASYLLNFLIWQDALPSSLSAYL-TQQVACDNGDR	880
XP_017802961.1	803	KMLAFDLLMKLSK-TAVHFQDSEKLQGLFQAAL ~~AL~~ STSTKPYDCVTASYLLNFLIWQDALPSSLSAYL-TQQVACDNGDR	880
XP_035128574.1	803	KILAFDLLMKLSE-TAVHFQDSEKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQDALPSSLSAYV-TQ-LACDNGDR	879
XP_014393943.1	799	KLLAFDLLMKLPR-TVIQFQDSEKLQDLFQAAL ~~AL~~ STSTKPYDCVTASYLLNFLICQNALPSSLSAYVkaQQAACGNGDK	877
XP_004839417.1	794	KTAFDLLMKLSV-TAVQFQDAEKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQDVPSSLHA-----ACSAGDK	865
NP_001103429.1	799	KILAFDLLMKLPK-TVVQFQDSEKLQGLFQAAL ~~EL~~ SSSTKPYDCVTASYLLNFLIWQDVPSSLFDSLkTQQTACEDGDK	877
XP_033619720.1	794	KTAFDLLMKLSV-TAVQFQNAEKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQDAVPSSLSAYL-THGAACSSGDK	871
XP_012379587.1	794	KILAFDLLMKLPK-TVIQFQDPEKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLICQDALSSFLSTYlKtQPVAYGDGDK	872
XP_008252690.2	794	KTAFDLLMKLSK-TAVHFQDSEKLEGLFQAAL ~~EL~~ STSPKPYDCVTASYLLNFLIWQDALPSSLPAYL-TQQTACEGGDQ	871
XP_010808315.1	795	KILAFDLLMKLPK-TFVQFQGSVKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQNALPSSLSAYLkTQRVACDNGDK	873
NP_001178698.1	794	KTAFDLLMNLSSvTAGQIQGSEKLQDLFQAAL ~~EL~~ STSTKPYDCVTASYLLNLLIWQDALPASLSASS-AQQPTCGAGEK	872
NP_898842.2	794	KTAFDLLMKLSSvTAGQFQDSEKLQDLFQAAL ~~EL~~ STSTKPYDCVTASYLLNLLIRQDALPAVLASS-PQQLTRGAGET	872
XP_023367895.1	604	KILAFDLLMKLPK-TAIQFQDSEKLQGLFQAAL ~~EL~~ STSTKPYDCVTASYLLNFLIWQNALPSFLSAHV-NQQVACGHGDK	681
XP_023411086.1	533	KVLAFDLLMKLTK-TVVQFQGSEKLQDLFQAAL ~~EL~~ STSTKPHDCVTASYLLNFLIWQDALPSPLSARLqTQQVAYGDGDK	611

Legend Fig. 2: Part of human THADA (first line) aligned that of a variety of eutherian mammals including *H. glaber* (light orange) showing a unique deletion present in Thada of *H. glaber*. Accession numbers refer to the following species (see Tab. 1): *Homo sapiens*: NP_001077422.1, *Gorilla gorilla gorilla*: XP_004029196.2, *Pan troglodytes*: XP_001141732.2, *Macaca mulatta*: XP_002799292.2, *Papio anubis*: XP_017802961.1, *Callithrix jacchus*: XP_035128574.1, *Myotis brandtii*: XP_014393943.1, *Canis lupus familiaris*: NP_001103429.1, *Fukomys damarensis*: XP_033619720.1, *Dasybus novemcinctus*: XP_012379587.1, *Oryctolagus cuniculus*: XP_008252690.2, *Bos taurus*: XP_010808315.1, *Rattus norvegicus*: NP_001178698.1, *Mus musculus*: NP_898842.2, *Otolemur garnettii*: XP_023367895.1, *Loxodonta africana*: XP_023411086.1

Tab. 1:

☒ select all 17 sequences selected		GenPept	Graphics	Distance tree of results	Multiple alignment	New MSA Viewer			
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	thyroid adenoma-associated protein isoform a [Homo sapiens]	Homo sapiens	4026	4026	100%	0.0	100.00%	1953	NP_001077422.1
☒	thyroid adenoma-associated protein [Gorilla gorilla gorilla]	Gorilla gorilla gorilla	3992	3992	100%	0.0	99.13%	1953	XP_004029196.2
☒	thyroid adenoma-associated protein isoform X1 [Pan troglodytes]	Pan troglodytes	3992	3992	100%	0.0	99.13%	1953	XP_001141732.2
☒	thyroid adenoma-associated protein isoform X6 [Macaca mulatta]	Macaca mulatta	3860	3860	99%	0.0	96.24%	1942	XP_002799292.2
☒	thyroid adenoma-associated protein isoform X1 [Papio anubis]	Papio anubis	3747	3747	99%	0.0	96.23%	1934	XP_017802961.1
☒	thyroid adenoma-associated protein isoform X1 [Callithrix jacchus]	Callithrix jacchus	3672	3672	100%	0.0	92.83%	1952	XP_035128574.1
☒	PREDICTED: thyroid adenoma-associated protein isoform X1 [Myotis brandtii]	Myotis brandtii	3336	3336	100%	0.0	84.23%	1950	XP_014393943.1
☒	thyroid adenoma-associated protein isoform X3 [Heterocephalus glaber]	Heterocephalus glaber	3303	3303	98%	0.0	85.09%	1928	XP_004839417.1
☒	thyroid adenoma-associated protein homolog [Canis lupus familiaris]	Canis lupus familiaris	3301	3301	99%	0.0	83.35%	1948	NP_001103429.1
☒	thyroid adenoma-associated protein isoform X1 [Fukomys damarensis]	Fukomys damarensis	3299	3299	98%	0.0	84.21%	1934	XP_033619720.1
☒	thyroid adenoma-associated protein isoform X2 [Dasypus novemcinctus]	Dasypus novemcinctus	3291	3291	100%	0.0	84.54%	1945	XP_012379587.1
☒	PREDICTED: thyroid adenoma-associated protein [Oryctolagus cuniculus]	Oryctolagus cuniculus	3276	3276	97%	0.0	85.14%	1942	XP_008252690.2
☒	thyroid adenoma-associated protein isoform X1 [Bos taurus]	Bos taurus	3253	3253	100%	0.0	83.80%	1946	XP_010808315.1
☒	thyroid adenoma-associated protein [Rattus norvegicus]	Rattus norvegicus	3111	3111	100%	0.0	79.13%	1937	NP_001178698.1
☒	thyroid adenoma-associated protein homolog [Mus musculus]	Mus musculus	3068	3068	100%	0.0	78.36%	1938	NP_898842.2
☒	thyroid adenoma-associated protein isoform X1 [Otolemur garnettii]	Otolemur garnettii	3053	3053	90%	0.0	87.12%	1753	XP_023367895.1
☒	thyroid adenoma-associated protein isoform X1 [Loxodonta africana]	Loxodonta africana	2847	2847	86%	0.0	84.47%	1683	XP_023411086.1

Tab. 2:

Aa position refers to *H. glaber* sequence: XP_004839417.1

NMR Thada, Aa position	NMR	all other mammals analyzed
696	histidine	glutamine
1118	arginine	lysine
1314	lysine	arginine
1358	leucine	phenylalanine

Tab. 3: *H. glaber* Thada sequences as available through (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>).

*: several differences to the other clones

sequence ID
XP_004839417.1
EHA97799.1*
XP_021109460.1
XP_021109461.1
XP_021109459.1
XP_021109457.1
XP_021109458.1