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## Novel mutations in TTC37 associated with Tricho-Hepato-Enteric syndrome

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| Key Words: | Syndromic diarrhea, Tricho-hepato-enteric syndrome, TTC37, intractable diarrhea, Woolly hair, Thespin, Stankler syndrome |
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Novel mutations in *TTC37* associated with Tricho-Hepato-Enteric syndrome

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Key words: Syndromic Diarrhea, Intractable diarrhea, Tricho-Hepato-Enteric syndrome,  
Stankler syndrome, *TTC37*, woolly hair,

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## Abstract

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The Tricho-Hepato-Enteric (THE) syndrome is an autosomal recessive condition marked by early and intractable diarrhea, hair abnormalities and immune defects. Mutations in *TTC37* which encodes the putative protein Thespin, have recently been associated with THE syndrome. In this paper, we extend the pattern of *TTC37* mutations by the description of 11 novel mutations in 9 patients with a typical THE syndrome. The mutations were spread along the gene sequence, none of them being recurrent. Different types of mutation were observed: frameshift mutations, splice site altering mutations or missense mutations, most of them leading to the creation of a premature stop codon. Concurrently, we investigated the pattern of *TTC37* expression in a panel of normal human tissues and showed that this gene is widely expressed, with high levels in vascular tissues, lymph node, pituitary, lung and intestine. In contrast, *TTC37* is not expressed in the liver, an organ which is not consistently affected in THE syndrome. Lastly, we suggested a model for the putative structure of the unknown Thespin protein.

## Introduction

The Tricho-Hepato-Enteric syndrome (THE syndrome, OMIM n°222470) also known as Syndromic Diarrhea, is a rare and severe autosomal recessive condition which associates intractable diarrhea with facial dysmorphism, intrauterine growth retardation, immunodeficiency with low serum concentrations of immunoglobulins, and hair abnormalities characterized by woolly hair (Girault et al., 1994; Verloes et al., 1997).

Parenteral nutrition is usually started in the first weeks of life and maintained all life long in most cases. The liver failure is inconstant and when present, it is observed initially or later on in life. In the past, the presence or absence of liver failure led one to describe 2 different syndromes but it is now admitted that the 2 entities represent 2 sides of the same disease (Fabre et al, 2007, Goulet et al, 2008). Death can occur early in life even though some patients can reach the third decade.

In the past few years, we collected samples for 12 patients from 11 families, all presenting a typical phenotype of THE syndrome. We first excluded several functional candidate genes (Fabre et al, 2009) and then performed a linkage analysis in order to unravel the genetic basis of this syndrome. In 2 of the 3 consanguineous families, homozygosity mapping identified a 5Mb region in 5q as a potential locus for the disease.

Very recently, Hartley et al described molecular defects causative for THE syndrome in *TTC37* (NM\_014639), a gene encoding for a putative protein named Thespin. Their study reported 9 mutations transmitted in a recessive pattern in 12 patients presenting this syndrome.

Since *TTC37* maps in 5q, we assumed that this gene may be responsible for the disease in some of our patients and so performed systematic sequencing of genomic DNA samples. In this paper, we describe 11 novel *TTC37* mutations in 9 patients out of 12 and provide clinical

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3 data for mutated and non mutated patients together with normal patterns of *TTC 37* expression  
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5 in several tissues.  
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## 10 **Material and methods:**

### 11 *Patients*

12  
13 Patients and family members were assessed under approved human subject protocols and all  
14  
15 participants provided informed consent.  
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### 19 *Molecular studies*

20  
21 DNA was isolated from blood via a standard manufacturer's protocol (QIAamp DNA blood  
22  
23 minikit, QIAgen). To analyse the *TTC37* gene, direct sequencing was performed, after PCR  
24  
25 amplification of the 43 exons and intronic flanking sequences, on an ABI 3130XL (Applied  
26  
27 Biosystems).  
28  
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30  
31 The *TTC37* genomic sequence from GenBank accession numbers NM\_014639 was used as  
32  
33 reference sequence. Detailed protocols and primer sequences are available on request. Two  
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35 non-mutated patients and one heterozygous were tested by CGH array using the commercial  
36  
37 Agilent 2x400K SurePrint G3 Human CGH Microarray (Agilent Technologies) array with the  
38  
39 overall median probe spacing of 5,3 kb. Total RNA from 3 patient samples was prepared from  
40  
41 lymphoblastoid cells and reverse transcribed using M-MLV (Sygma). Normal expression  
42  
43 pattern was investigated by qPCR on a panel of normal transcripts from various tissues  
44  
45 (Rapid-Scan cDNA panel, OriGene) using a probe located on exon-intron junction 35-36 of  
46  
47 *TTC37* and compared to *GAPDH* expression in duplex reactions. qPCR were done in  
48  
49 triplicates and 2 sets of experiments were performed.  
50  
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### 52 *Bioinformatics analysis*

53  
54 The topology of normal and mutated Thespin protein was analysed using the algorithms  
55  
56 proposed at TMpred ([http://www.ch.embnet.org/software/TMPRED\\_form.html](http://www.ch.embnet.org/software/TMPRED_form.html)), TOPPRED  
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3 (<http://www.mobyle.pasteur.fr/cgi-bin/portal.py?form=Toppred>), PSORT II  
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5 (<http://psort.ims.u-tokyo.ac.jp>) and SMART ([http://smart.embl.de/smart/show\\_motifs.pl](http://smart.embl.de/smart/show_motifs.pl))  
6  
7 Predicted effects of missense mutations were obtained on Polyphen website  
8  
9 (<http://genetics.bwh.harvard.edu/pph>).  
10  
11

## 12 Results

### 13 *Patients phenotype*

14  
15 The clinical features of the 12 patients are summarised in table 1. Detailed clinical data were  
16  
17 previously published for 3 of them (Fabre et al, 2007, Egritas et al 2010). All the 12 patients  
18  
19 presented the 3 major signs of THE syndrome i.e. dysmorphism, hair abnormalities and  
20  
21 intractable diarrhea. Among the 12 patients, one died of septicemia at age 10, 2 have stopped  
22  
23 parenteral nutrition and 1 never required it.  
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### 28 *Identification and characterization of novel TTC37 mutations in THE patients*

29  
30 Screening for mutations of *TTC37* coding sequence and intron-exon junctions of DNA  
31  
32 samples from THE patients identified 10 novel non ambiguous mutations in homozygous or  
33  
34 compound heterozygous condition, in 8 (out of 12) patients. In addition, one patient presented  
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36 only a heterozygous missense mutation. The mutations were spread along the gene: 3 were  
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38 nonsense or frameshift mutations in the coding sequence leading to premature stop codon, 5  
39  
40 were mutations affecting splice sites either by substitution or deletion and 3 were missense  
41  
42 mutations. All the parents of mutated patients were investigated and carried a mutation in  
43  
44 heterozygous condition. The location and predicted consequences of the mutations on protein  
45  
46 expression or function are reported in table 2.  
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53 Two of the 3 patients without mutation and the one with a single heterozygous mutation were  
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55 investigated by whole genome CGH array with 17 probes mapping in TTC37. No CNV of the  
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57 *TTC37* region or of other regions could be identified.  
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3 The effects of splice sites mutations on sequence transcripts were analysed by direct  
4 sequencing of RNA transcripts from lymphoblastoid cells, for 3 patients. The tested samples  
5 exhibited abnormal sequence due to the modification of splicing: skipping exon 23 in the case  
6 of the c.2515+1 C>G mutation, leading to a frameshift and creation of a premature stop  
7 codon (figure 1a); skipping exon 25 in the case of the c.2577-3\_-7delTTTT, leading to the  
8 deletion of 19 amino-acids in frame (figure 1b); cryptic splice activation in exon 42 in case of  
9 the c.4620+1 G>C mutation promoting an alternative splicing and the replacement of the 41  
10 terminal amino-acids by 61 others.  
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### 22 *Pattern of expression*

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24 Quantitative expression in normal tissues was assessed in a panel of 48 different tissues and  
25 revealed that *TTC37* is widely expressed with the highest levels observed in vascular tissues,  
26 lymph node, pituitary, lung and intestine. Noticeably, we did not find any expression in the  
27 liver (figure 2).  
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### 33 *Bioinformatics*

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35 In silico protein predictions were performed to propose a putative structure for Thespin and to  
36 evaluate the potential effect of *TTC37* mutations identified in this study. The algorithm results  
37 are in accordance to predict that Thespin is mostly cytosolic and may contain 4  
38 transmembrane domains (Figure 3). With all algorithms used in this study, several tetratricopeptide  
39 repeats (TPR) domains are predicted and their number varies from 5 to 22.  
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### 48 **Discussion**

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50 Mutations previously identified in THE syndrome are heterogeneous and include frameshift,  
51 nonsense and splice site mutations. Here, we describe further 11 different mutations that add  
52 heterogeneity to the molecular genetics of THE syndrome (figure 3). These 11 novel  
53 mutations, in addition to the 9 mutations previously described, indicate strongly that *TTC37* is  
54 the main gene responsible for THE syndrome. There is no clear hot spot region for mutations  
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3 even though, in 70% of the mutated alleles (Hartley et al 2010 and our study), the mutation is  
4 localised in the 3' half of the gene. Most of the mutations are nonsense, frameshift or splice  
5 mutations that are predicted to produce premature stop codon. At least, 2 of the 3 missense  
6 mutations might affect splicing as well, as they are located in the vicinity of splice sites. For  
7 one of the patients, a single missense mutation in heterozygous condition has been identified  
8 to date. As we exclude the presence of an intragenic deletion or duplication on the other  
9 allele, we assume that a non-identified intronic substitution producing an aberrant splicing is  
10 probably associated with the missense mutation.  
11

12 The phenotypes of the 3 patients who did not carry any mutation in *TTC37* have been  
13 carefully re-evaluated and were confirmed as being typical THE syndrome, suggesting  
14 strongly that at least one other gene may be implicated in this disease.  
15

16 Multi-tissue transcript expression analysis showed wide expression of *TTC37* mRNA with a  
17 high expression level in intestinal tissue but not in the liver, indicating that liver dysfunction  
18 observed in THE syndrome is probably secondary to another genetic cause or to long term  
19 parenteral nutrition. More surprising is the high level of expression observed in vein, arteria  
20 and lung as no abnormalities have been noticed in these tissues.  
21

22 Up to date, the function of Thespin is unknown. We identified orthologs of human *TTC37*  
23 using HomoloGene ([www.ncbi.nlm.nih.gov/homologene](http://www.ncbi.nlm.nih.gov/homologene)) and found a nucleotide sequence  
24 conservation in canis (92%), bos (92%), rattus (83%), mus (82%), gallus (66%), danio (55%)  
25 and drosophila (27%) in favour of an essential role for Thespin.  
26

27 Protein motifs and domains are predicted in Thespin by several algorithms such as TMpred,  
28 PSORT and TOPPRED. Four transmembrane domains located in the C-terminal half of the  
29 protein are predicted, the last one in the C-terminal part being predicted with a stronger score.  
30 The putative protein also contains several tetratricopeptide repeats (TPR) domains which are  
31 structural motifs consisting of 34 Amino acid residues and found in over 300 human proteins  
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3 (D'andrea et al, 2003). Despite the fact that the amino-acids are poorly conserved, these  
4 motifs assemble into a characteristic Helix-Turn-Helix structure. The basic function of these  
5 TPR motifs is to mediate protein-protein interactions and therefore, can be involved in a  
6 variety of biological process such as cell-cycle regulation, transcriptional control, protein  
7 transport or folding. Consequently, no hint is given by the presence of TPR domains  
8 regarding a specific function. Interestingly, one of the splice site mutations of this series leads  
9 to a deletion of 19 residues in frame, following the skipping of exon 25. This deletion does  
10 not modify the predicted transmembrane domains but deleted several putative TPR domains  
11 of the C-terminal part, suggesting a major functional role for these structures.  
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24 Beside the TPR domains, neither the gene nor its derived protein show any significant  
25 sequence similarity to other known human DNA or protein sequences. In the literature,  
26 mutations in other TPRs containing proteins have been described as causative in several  
27 neurological human diseases such as Leber congenital amaurosis (Sohocki et al, 2000) or  
28 CMT type 4C (Senderek et al, 2003) but no clear link can be made between these proteins and  
29 their putative function.  
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38 Collectively, our results confirmed the role of *TTC37* in THE syndrome, extended the pattern  
39 of mutations associated with this syndrome and provided new data on the normal expression  
40 pattern of *TTC37* and the putative structure of Thespin. Assigning an unknown protein to a  
41 specific disease as done for THE syndrome, may enable one to describe new protein functions  
42 and will lead surely to characterize the localisation and functional properties of Thespin.  
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8 Figure 1:  
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10 Sequencing analysis of transcripts in 2 patients with splice mutations:  
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12 a: scheme representation of the splice site mutation c.2515+1 C>G, cDNA PCR amplification  
13 with primers located in exon 22 and 24 for the homozygous patient, his heterozygous mother  
14 and a normal control and sequence obtained for the patient showing exon 23 skipping.  
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18 b: scheme representation of the splice site mutation c.2577-3\_-7delTTTTT, cDNA PCR  
19 amplification with primers located in exon 23 and 26 for the homozygous patient and a  
20 normal control and sequence (forward and reverse) obtained for the patient showing exon 25  
21 skipping.  
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32 Figure 2:  
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34 qPCR analysis on normal human tissues: 48 tissues have been tested, 34 are shown on the  
35 graph. Experiments have been done twice with probe located on exons 35-36 junction. RQ:  
36 Relative Quantification to GAPDH expression.  
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43 Figure 3:  
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45 a: Scheme representation of *TTC37* with mutations described previously (underneath) and in  
46 our study (above); red: non sense or frameshift, blue missense, green: splicing mutations.  
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50 b: Model of human Thespin as predicted by TMPred algorithm.  
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|                               | Patients with mutations in TTC37 | Patients without mutations in TTC37 | Hartley et al. 2010 |
|-------------------------------|----------------------------------|-------------------------------------|---------------------|
| Number of patients (families) | 9 (8)                            | 3 (3)                               | 12 (11)             |
| Consanguinity                 | 4/8                              | 1/3                                 | 7/11                |
| Median age                    | 10 y                             | 11 y                                | 3 y                 |
| Male/female                   | 4/5                              | 0/3                                 | 6/6                 |
| Hair abnormalities            | 9/9                              | 3/3                                 | 12/12               |
| Dysmorphism                   | 9/9                              | 3/3                                 | 12/12               |
| Intractable diarrhea          | 9/9                              | 3/3                                 | 12/12               |
| Onset in the first month      | 7/9                              | 2/3                                 | NR                  |
| Parenteral nutrition          | 9/9                              | 2/3                                 | 12/12               |
| Immunodeficiency              | 9/9                              | 2/3                                 | 12/12               |
| IUGR                          | 7/9                              | 2/3                                 | 10/11               |
| Weight<3percentile            | 6/9                              | 2/3                                 | NR                  |
| Liver involvement             | 4/9 (initial 1/4)                | 2/3 (initial 2/3)                   | 5/10                |
| Mental retardation            | 5/8                              | 2/3                                 | 7/9                 |

Table 1:

Clinical features of the 12 patients of this study and the 12 previously published by Hartley et al, 2010. Facial dysmorphism is characterized by hypertelorism, broad flat nasal bridge, prominent forehead; Observed hair abnormalities are sparse, fragile and uncombable hair with trichorrhexis nodosa. NR: not reported

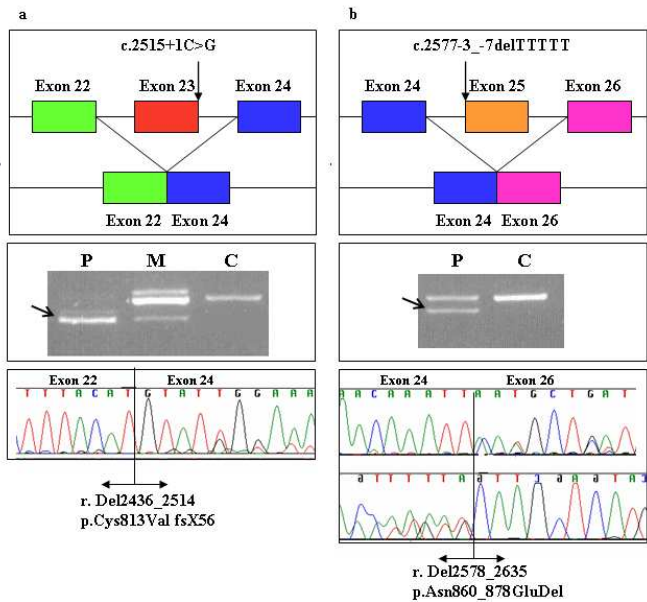
**Table 2. Mutations in *TTC37*, geographical origin and consanguinity in the families of the patients with THE syndrome (NM\_014639; NP\_055454)**

| Family identifier (number of affected individuals) | Mutation 1                                      | Mutation 2                                      | Consanguinity | Geographical origin |
|--|---|---|---------------|---------------------|
| 1 (2)  | c.326_330delTGCCT<br>p.Leu96TrpfsX10            | c.326_330delTGCCT<br>p.Leu96TrpfsX10            | Yes           | Middle-East         |
| 2 (1)  | c.1168delA<br>p.Val390PheFsX419                 | c.3564-2A>G<br>(nd)                             | No            | France              |
| 3 (1)  | c.2515+1G>C<br>p.Cys813ValfsX56                 | c.2515+1G>C<br>p.Cys813ValfsX56                 | Yes           | North Africa        |
| 4 (1)  | c.2577-3_-<br>7DelTTTTT<br>p.Asn860_878GluDel   | c.4620+1G>C<br>p.Trp1524_1564<br>DelIns61       | No            | France              |
| 5 (1)  | c.3015-1C>T<br>nd                               | c.4454T>G<br>p.Leu1485Arg,<br>possibly damaging | No            | France              |
| 6 (1)  | c.3808C>G<br>p.Pro1270Ala,<br>probably damaging | c.3808C>G<br>p.Pro1270Ala,<br>probably damaging | Yes           | North Africa        |
| 7 (1)  | c.3960C>A<br>p.Tyr1320X                         | c.3960C>A<br>p.Tyr1320X                         | Yes           | North Africa        |
| 8 (1)  | c.3230C>A<br>p.Ala1077Asp,<br>probably damaging |   | No            | France              |

Regarding missense mutations, predictions at the protein level, were done using Polyphen algorithm. For frameshift mutations, predictions are theoretical and for splice site mutations, experimental at the transcript level. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.



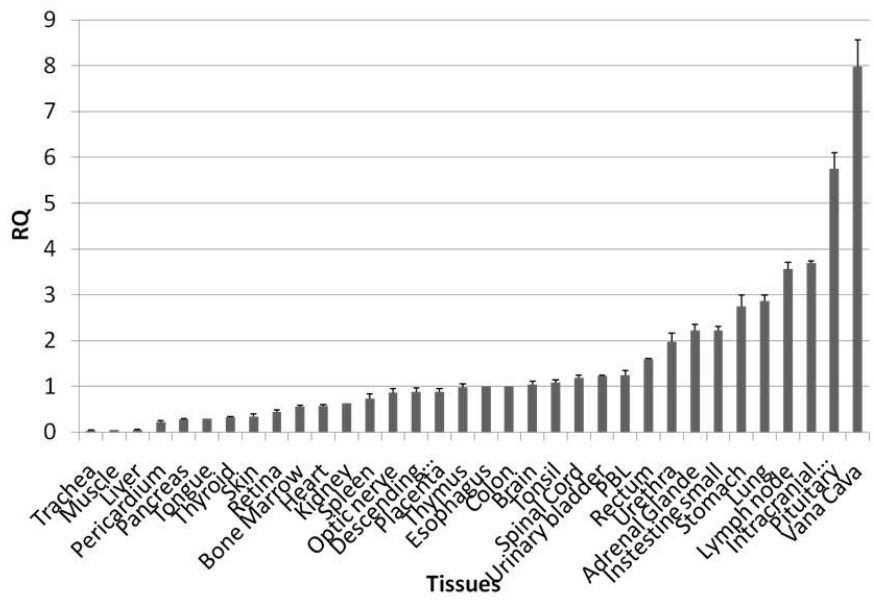
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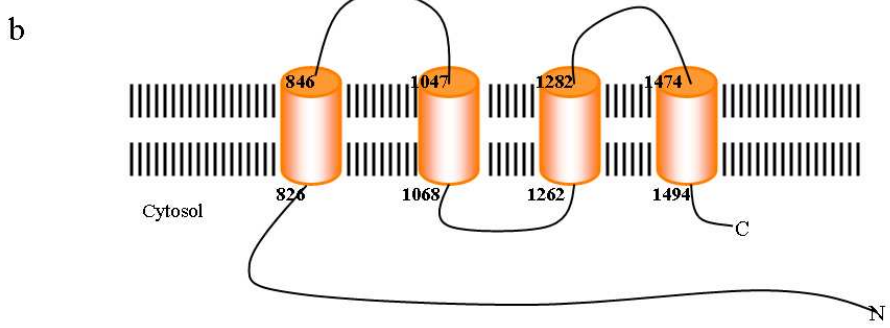
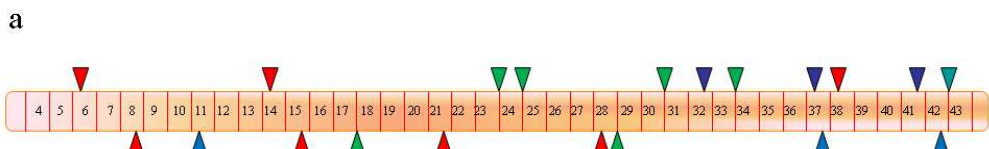
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