Molecular characterization of beta-ketothiolase deficiency in 10 Indians: Discovery of 4 novel mutations in *ACAT1* gene

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Disclosure Information

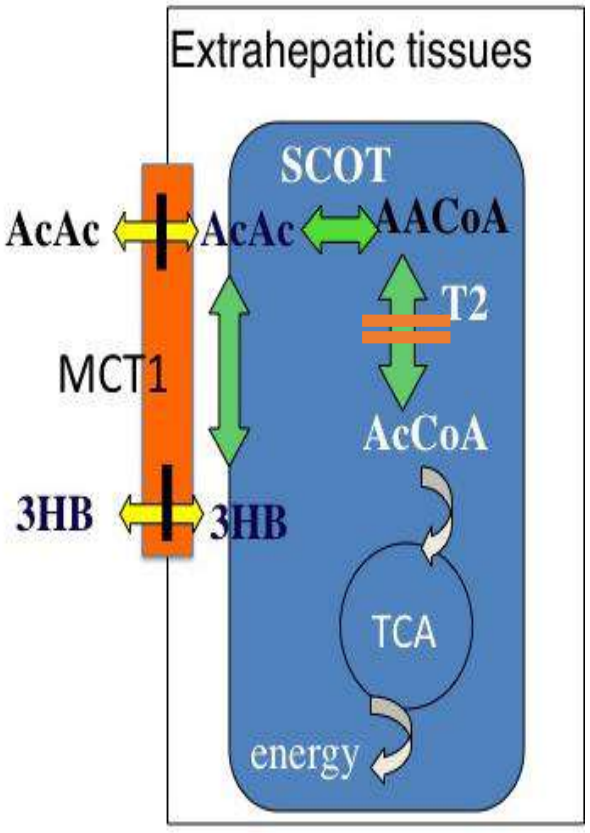
Elsayed Abdelkreem

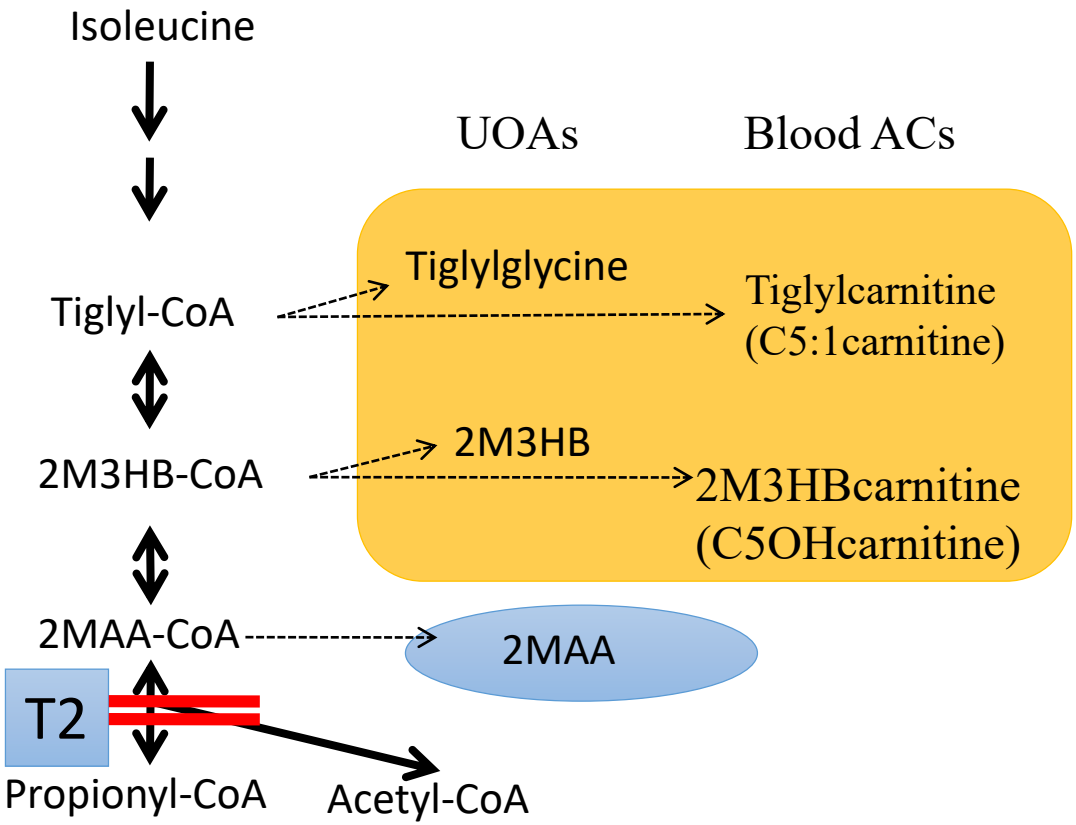
I have no financial relationships to disclose

Beta-ketothiolase deficiency

- Also known as mitochondrial acetoacetyl-CoA thiolase or T2 deficiency.
- Affects ketone body utilization and isoleucine catabolism.

Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency

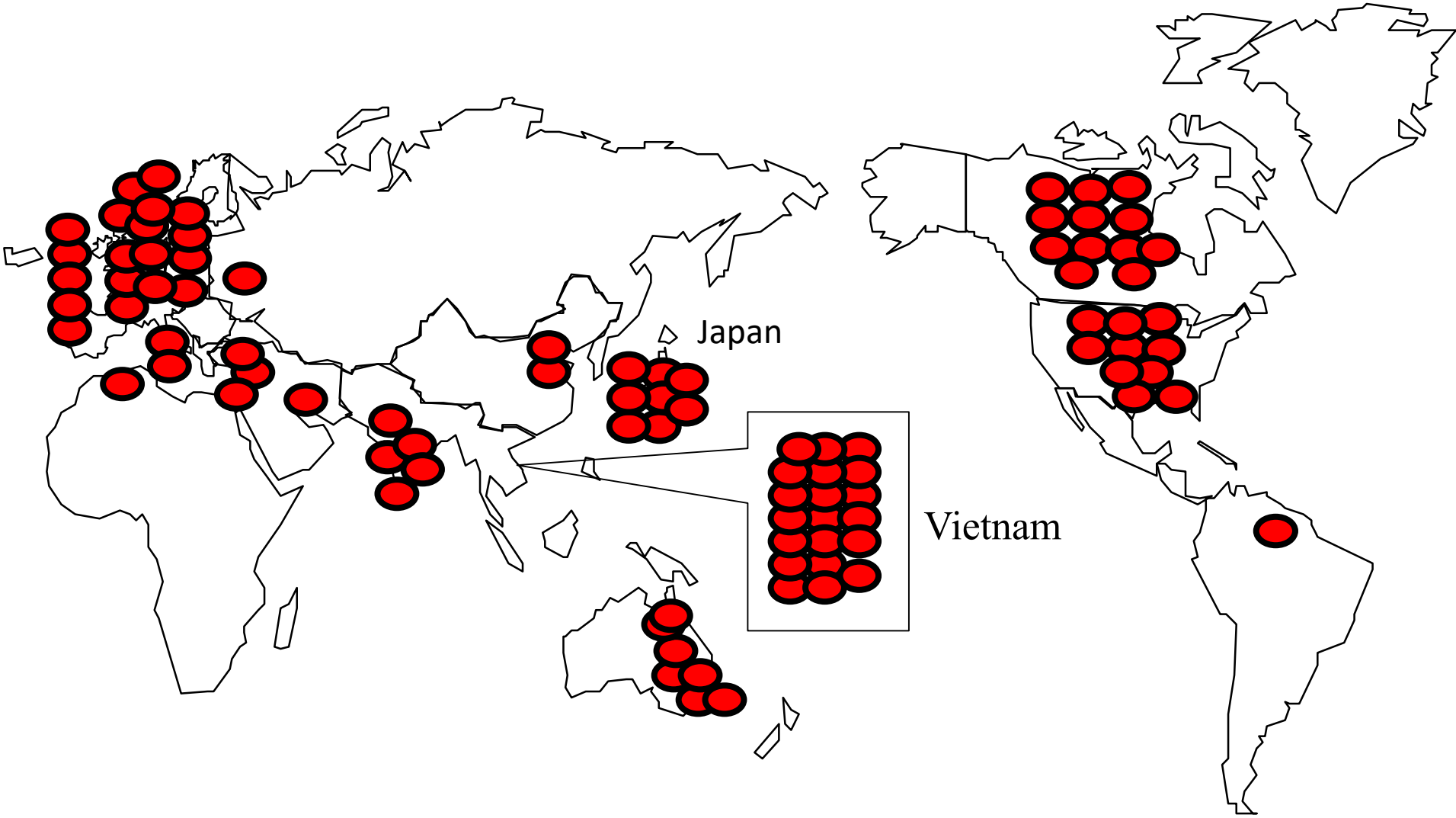




Typical T2 deficiency:

- Presents with ketoacidotic episodes.
- Characteristic biochemical abnormalities.

T2 deficient patients whose mutations were confirmed in Gifu Univ



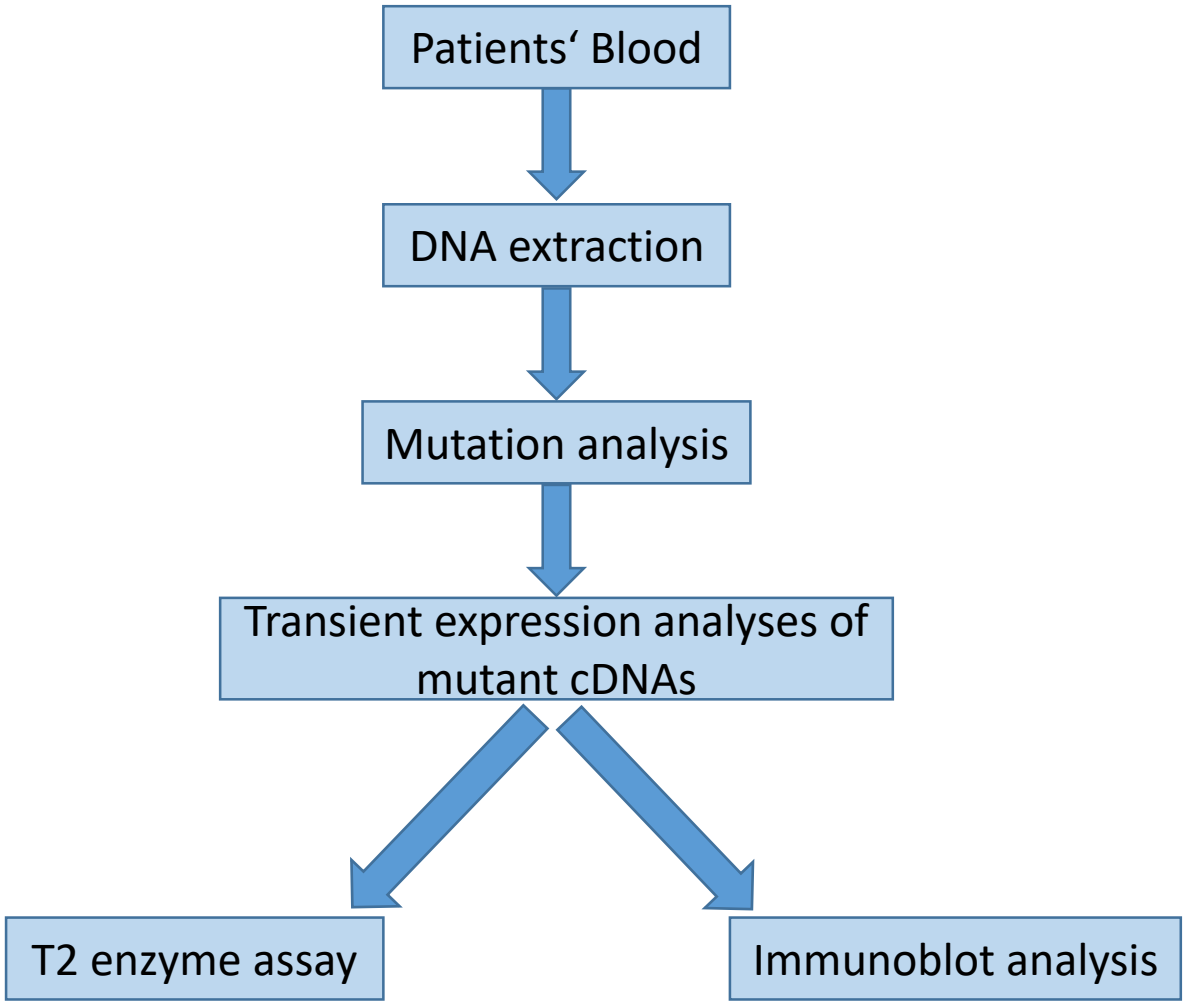
- T2 deficiency is an AR disease encoded by *ACAT1* gene.
- ***ACAT1*** gene is located at chromosome 11q22.3-q23.1.
- Spans about 27kb long, contains 12 exons and 11 introns.
- cDNA is about 1.5kb long and encodes a precursor protein of 427 amino acids.
- To date, more than 70 different mutations have been identified.
- No clear genotype-phenotype correlation.

- Herein, we report 10 Indians with beta-ketothiolase deficiency, describe their molecular characterization, and expression analysis of the identified mutations in *ACAT1* gene.

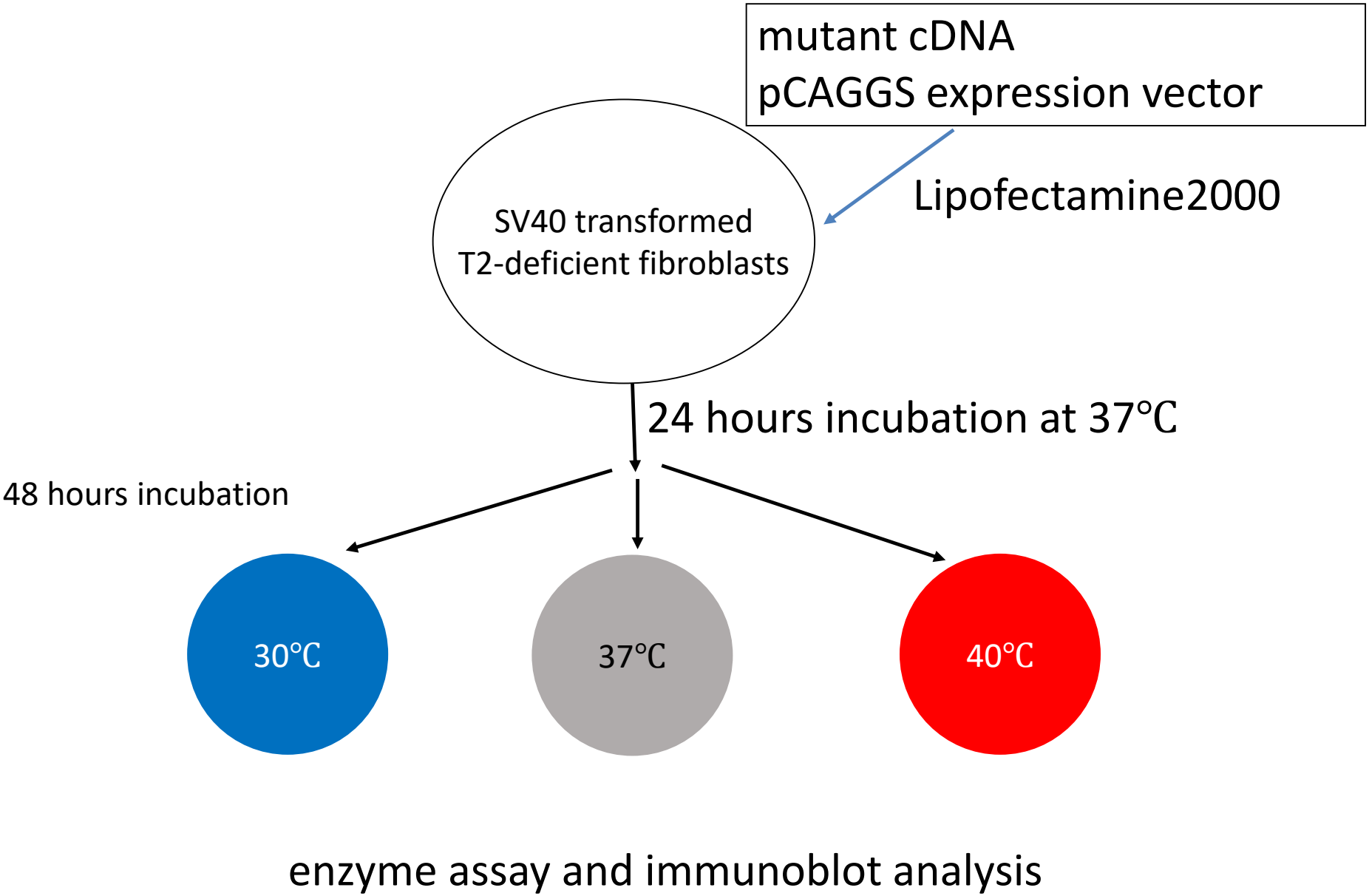
Patients

Identifier	Gender	Predisposing condition	Age at onset (months)	Frequency	pH	Outcome
GK94	M	Fever, cough	7	1	6.5	Normal
GK98	M	Diarrhea	**4**	1	6.8	**GDD, epilepsy**
GK108	F	Poor feeding	11	1	6.9	**Death**
GK109	M	Vomiting	9	1	6.8	Normal
GK110	F	Fever, cough	11	1	7.0	Normal
GK111	M	Poor feeding	6	1	7.1	Normal
GK112	F	Fever, cough	7	3	7.15	**Some hypotonia**
GK113	M	Fever, cough	12	2	7.1	Normal
GK114	M	Fever, cough	**19**	1	6.9	Normal
GK99	Data are currently unavailable					

Methods (summary)



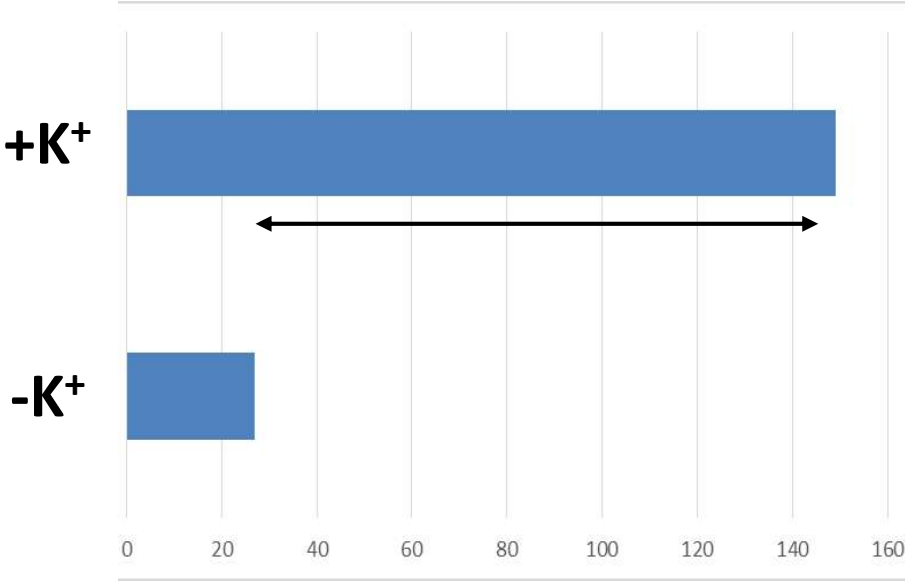
Transient expression analysis of T2 cDNA



enzyme assay and immunoblot analysis

Enzyme assay of Mitochondrial acetoacetyl CoA-thiolase (T2)

- Based on the fact that T2 is the only thiolase activated in presence of K^+.
- Hence, the difference of thiolase activity in presence and absence of K^+ represents the T2 activity.



Immunoblot analysis

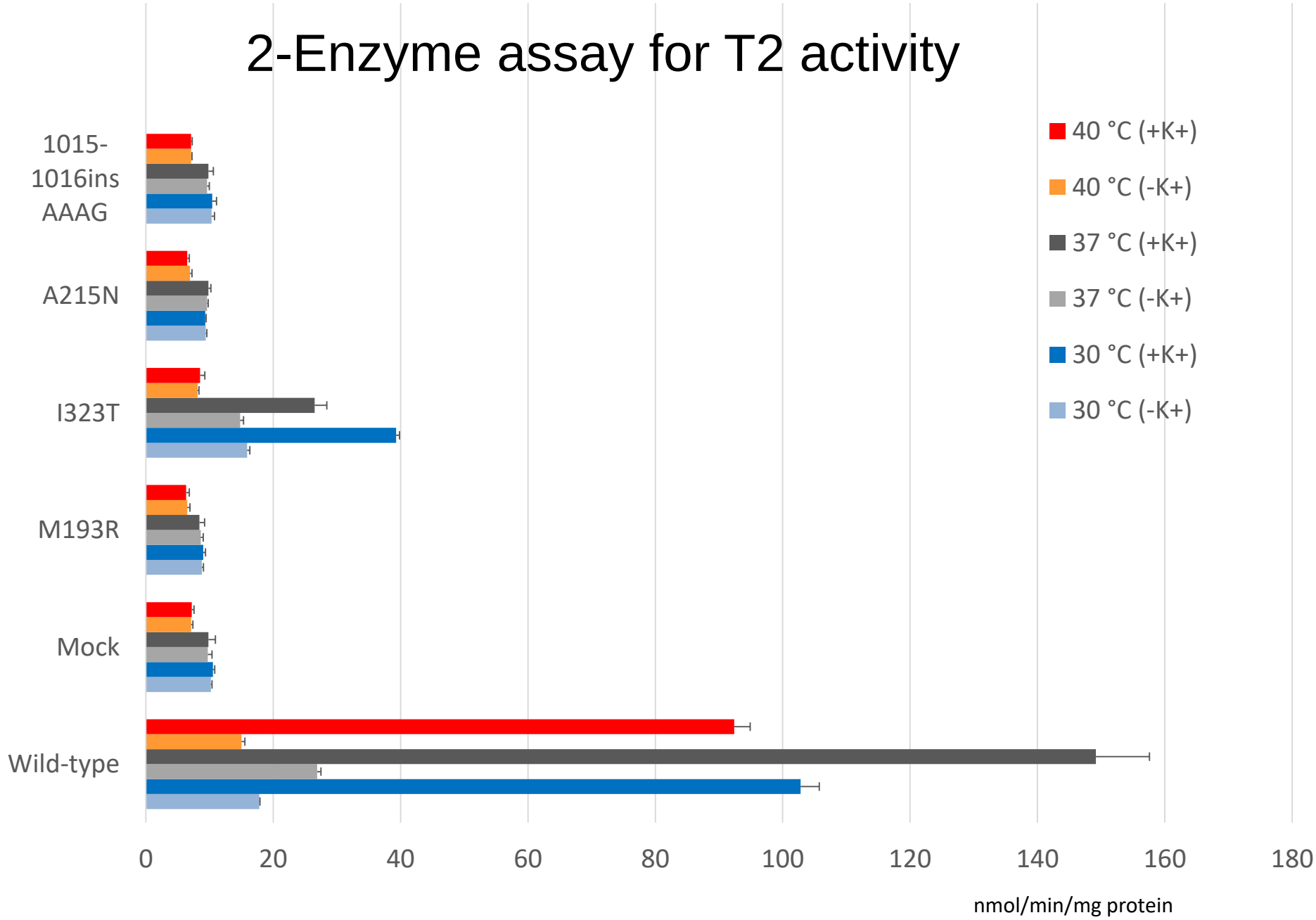
- The 1st antibody was a mixture of an anti-T2 antibody and anti-succinyl-CoA: 3-oxoacid CoA transferase (SCOT) antibody.
- To assess the effects of mutations on protein solubility, we analysed not only the **supernatants** that were used for the enzyme assay but also the **pellets** of the cell extracts.

Results

1- Mutation Analysis

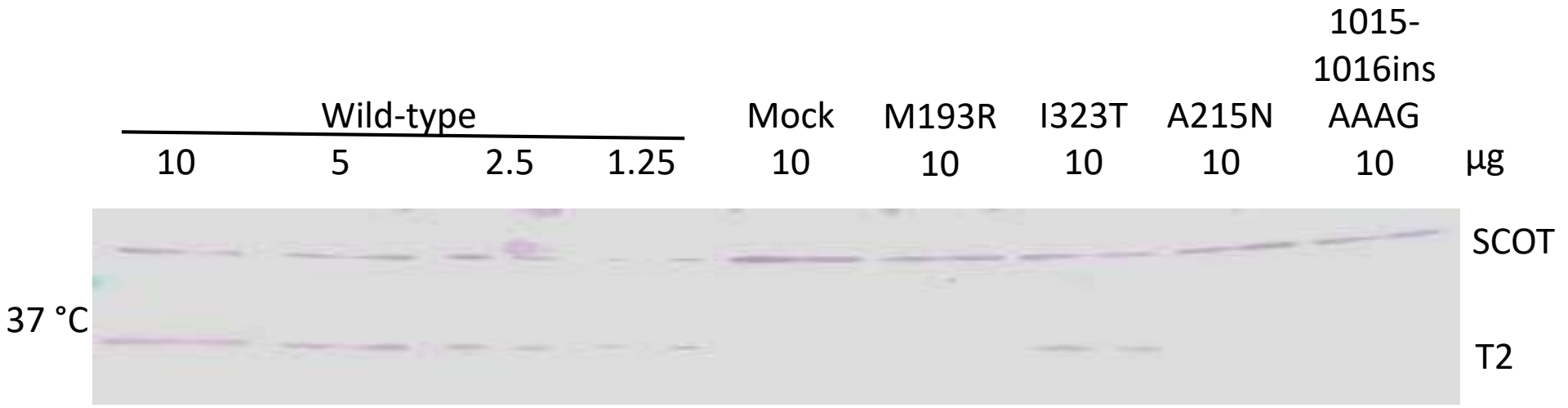
Patients	Mutations in *ACAT1* gene	
GK94	253_255delGAA (E85del)	253_255delGAA (E85del)
GK98	**578T>G (M193R)**	**578T>G (M193R)**
GK99	**578T>G (M193R)**	**578T>G (M193R)**
GK108	**578T>G (M193R)**	**578T>G (M193R)**
GK109	**1015-1016insAAAG**	1015-1016insAAAG
GK110	**IVS7+1g>a**	IVS7+1g>a
GK111	**578T>G (M193R)**	**968T>C (I323T)**
GK112	c.1124A>G	c.1124A>G
GK113	**c.643_644delGCinsAA (A215N)**	c.643_644delGCinsAA (A215N)
GK114	**578T>G (M193R)**	**578T>G (M193R)**

Results


2-Enzyme assay for T2 activity
1015-1016ins AAAG
A215N
I323T
M193R
Mock
Wild-type
40 °C (+K+)
40 °C (-K+)
37 °C (+K+)
37 °C (-K+)
30 °C (+K+)
30 °C (-K+)
0
20
40
60
80
100
120
140
160
180
nmol/min/mg protein

Results

3-Immunoblot analysis (*supernatant*)



Results

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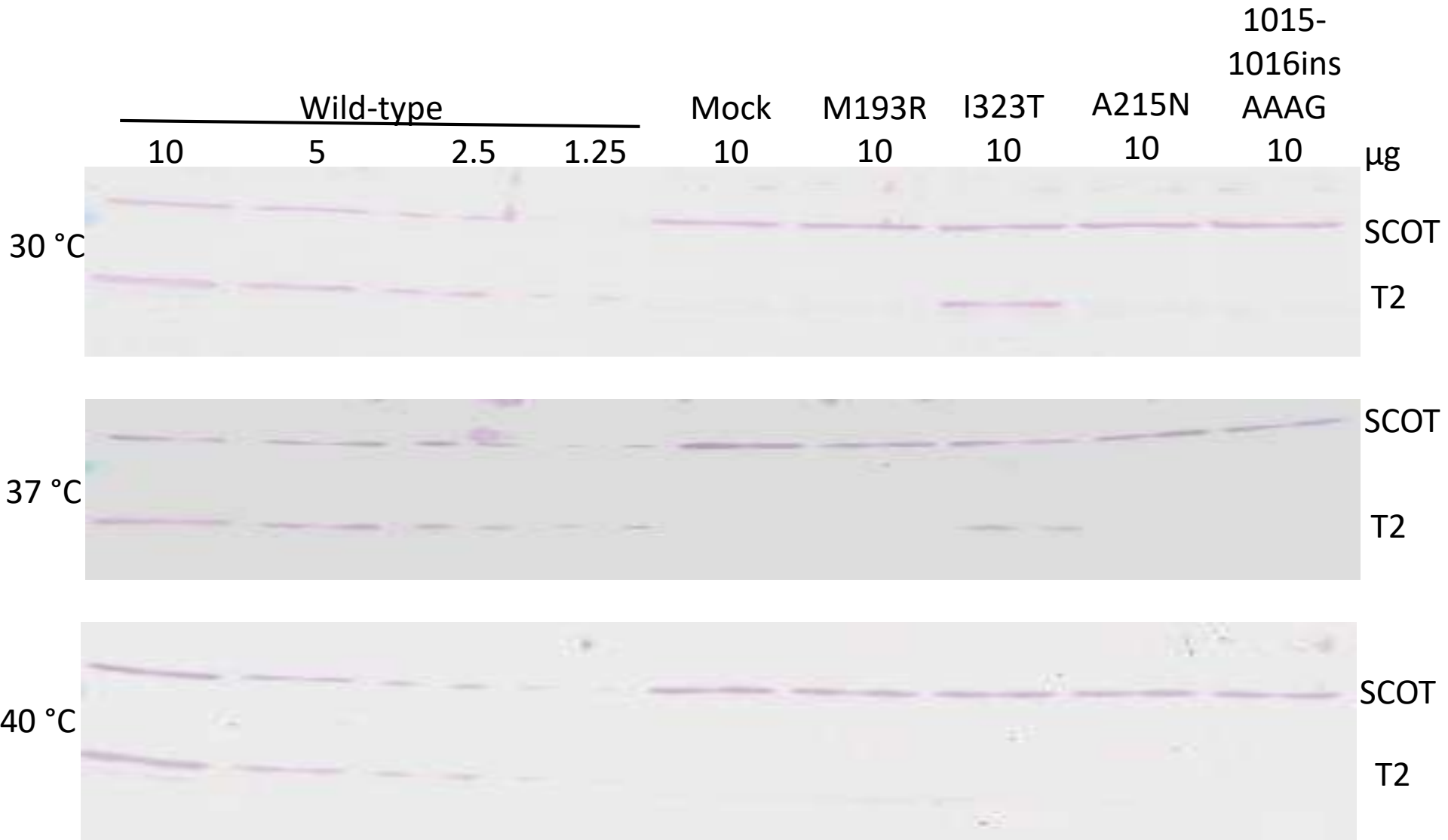
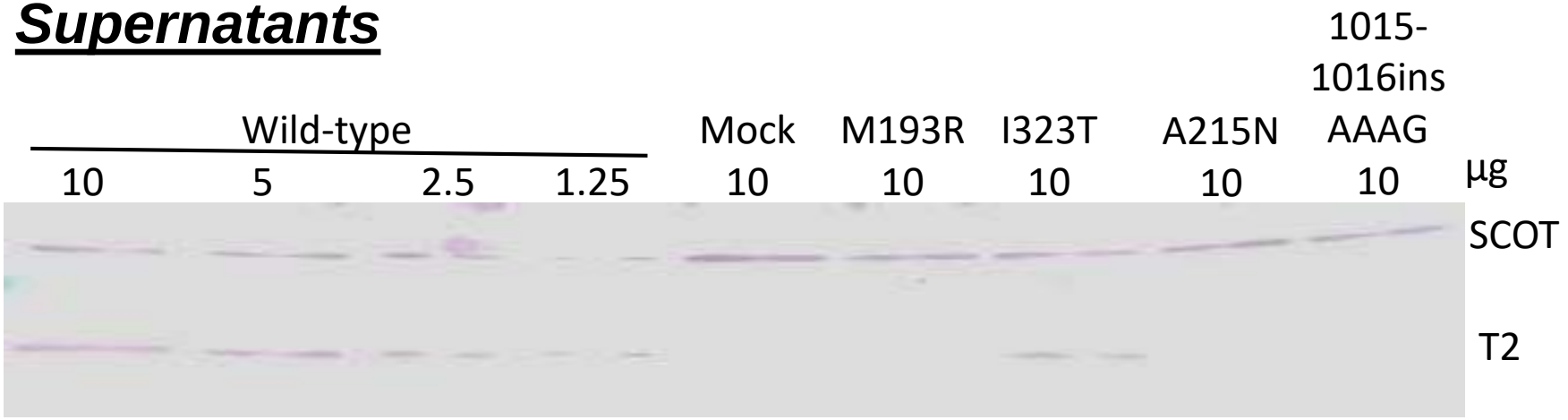


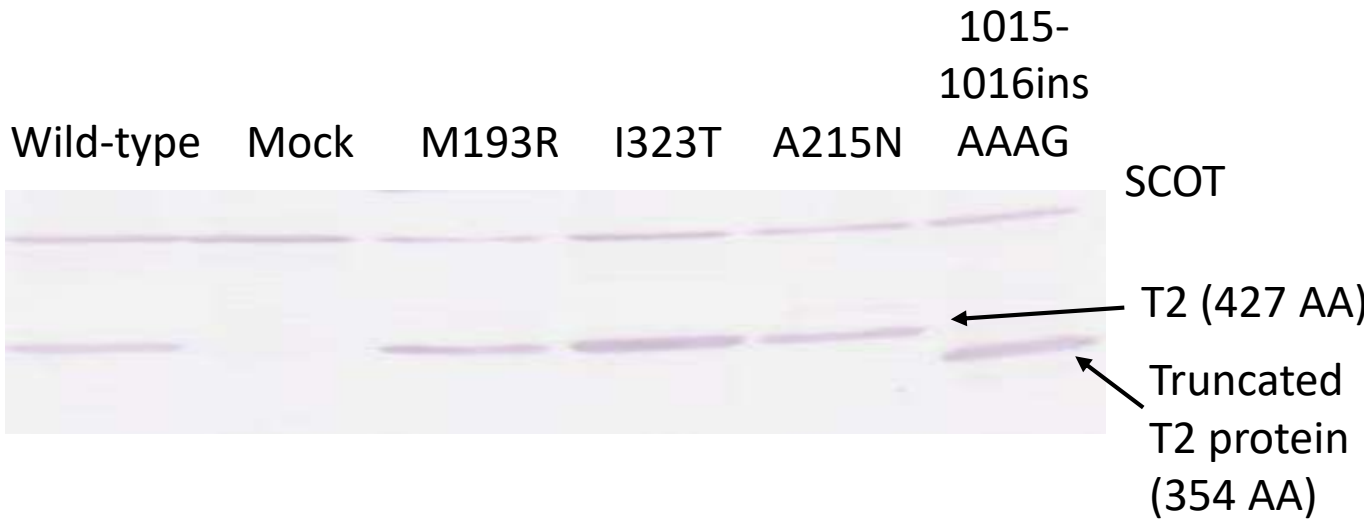
Results

3-Immunoblot analysis (*Supernatant vs Pellet*)

Supernatants



Pellets



Discussion

- Mutations in *ACAT1* gene are highly heterogeneous;
  Only a few common mutations were identified.

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Vietnamese

Case	Mutations		Case	Mutations	
GK70	R208X	c.163_167del5ins2	GK89	IVS10-1g>c	IVS10-1g>c
GK72	R208X	R208X	GK90	R208X	A410V
GK73	R208X	R208X	GK91	R208X	R208X
GK74	IVS10-1g>c	R208X	GK93	R208X	R208X
GK75	R208X	R208X	GK100	IVS10-1g>c	Ex6-11del
GK76	R208X	R208X	GK101	R208X	R208X
GK79	R208X	R208X	GK102	R208X	S284N
GK80	R208X	R208X	GK103	R208X	IVS10-1g>c
GK86	IVS10-1g>c	IVS10-1g>c	GK104	R208X	R208X
GK87	R208X	R208X	GK105	R208X	c.1032_1033insA

Mutant allele frequency	
R208X	70%
IVS10-1g>c	17.50%

Discussion

Indians

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M193R 45%

Vietnames

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GK72	R208X	R208X	GK90	R208X	A410V
GK73	R208X	R208X	GK91	R208X	R208X
GK74	IVS10-1g>c	R208X	GK93	R208X	R208X
GK75	R208X	R208X	GK100	IVS10-1g>c	Ex6-11del
GK76	R208X	R208X	GK101	R208X	R208X
GK79	R208X	R208X	GK102	R208X	S284N
GK80	R208X	R208X	GK103	R208X	IVS10-1g>c
GK86	IVS10-1g>c	IVS10-1g>c	GK104	R208X	R208X
GK87	R208X	R208X	GK105	R208X	c.1032_1033insA

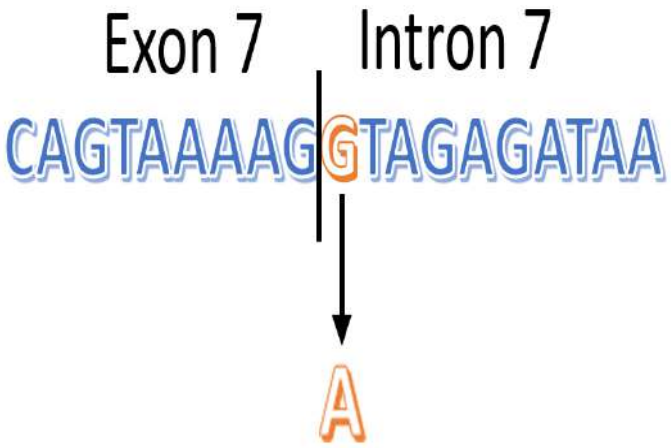
Mutant allele frequency

R208X 70%

IVS10-1g>c 17.50%

Discussion

- Genetic analysis of the 10 Indians revealed 4 novel mutations in *ACAT1 gene:*
- **IVS7+1g>a** mutation : affects a highly conserved point at the splice donor site of intron 7; hence, aberrant splicing (exon 7 skipping) is the usual result.
- Transient expression analysis of mutant T2 cDNA showed that M193R, **A215N**, and **1015-1016insAAAG** are null mutations (no T2 enzymatic activity) and the corresponding mutant T2 proteins are insoluble.



- In contrast, **I323T** mutation resulted in an unstable mutant T2 protein that retained some activity (20%) at 37°C; Such activity increased to 40% at 30°C but ablated at 40 °C .

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GK99						

Summary and conclusions

- We confirmed the diagnosis of beta-ketothiolase deficiency in 10 new patients from India.
- We identified 4 novel mutations in *ACAT1* gene.
- M193R appears to be a common mutation in T2-deficient Indians.

Thank you