

# *In silico* Analysis and 3D Modeling of ASAH1 Protein in Farber Lipogranulomatosis

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## Abstract

Farber lipogranulomatosis is an autosomal recessively inherited lysosomal storage disorder caused by acid ceramidase deficiency. The farber disease was caused by the mutation in ASAH1 gene. An *in silico* technique was initiated to characterize the properties and structure of the protein. The ASAH1 protein analyzed in the study showed that this is a stable protein and belong to acid ceramidase like family. The secondary structure prediction of the protein revealed that the presence of maximum number of random coils as its secondary structure elements. The 3D structure was modeled using Swiss model workspace and the structure was validated. The present study gave an outlook on ASAH1 protein and further research was carried out in preventing the disease

**Key words:** Farber lipogranulomatosis, ASAH1, acid ceramidase, lipid storage disease

## Introduction

Farber lipogranulomatosis disease describes a group of inherited metabolic disorder called lipid storage diseases in which excess amount of lipids build up to harmful level in the joints, tissues & central nervous system. The liver, heart & kidney may also affected by this disease. It is an autosomal-recessively inherited lysosomal storage disease caused by acid ceramidase deficiency & associated with distinct clinical phenotypes (Karoline et al., 2007). The symptoms of farber lipogranulomatosis disease seen in the first few weeks of life & impaired motor & mental ability & difficulty with swallowing affected persons may require the insertion of a breathing tube. The liver & spleen are also enlarged in many cases. The disease occurs when both parents carry & pass on the defective gene that regulates the protein sphingomyelin. the disorder affects both males & females.

Children with significant neurological involvement usually die early in infancy. The patients with mild neurological findings suffer from progressive joint deformation, subcutaneous, nodules, a hoarse voice & intestinal pneumonitis leading to death. As the inflammatory component of this disorder caused by some kind of leukocyte dysregulation, allogeneic hemotopoietic, stem cell transplantation can restore a healthy immune system & thus provide a curative option in farber lipogranulomatosis disease patients without neurological involvement (Karoline *et al.*, 2007)

Acid ceramidase is the lipid hydrolase which are mainly responsible for the degradation of ceramide into sphingosine & free fatty acids within

lysosomes. The enzyme activity is deficient in the inherited lipid storage disorder farber lipogranulomatosis. The mutations in the gene encoding Acid ceramidase lead to profound reduction in enzyme activity. Recent studies suggest that Acid ceramidase activity is expressed in several human cancers so that they can also act as a useful cancer drug target (Park and Schuchman, 2006) Julia bar et al., 2001 reported that there are six novel mutations in the acid ceramidase gene causing farber disease. Three point mutations resulting in single amino acid substitutions, one intronic splice site mutation resulting in exon skipping & two point mutations lead to complete exon skipping. The studies revealed that these mutations were the direct cause of acid ceramidase deficiency in the respective patients

The molecular mechanism & pathogenesis of farber disease was studied by isolating and characterizing a full length human acid ceramidase gene. The results showed that the human acid ceramidase gene consist of 14 exons & 13 introns spanning approximately 26.5 kb of genomic DNA. It was reported the physiological importance of Acid ceramidase and establishes a genotype-phenotype correlation in this disease (Zhang *et al.*, 2000)

ASAH1 gene is "N-acylsphingosine amidohydrolase (acid ceramidase 1)1" is the gene responsible for farber disease. This gene instructions for producing ASAH1 protein. These enzymes are found in lysosomes where it break down fats called ceramides into other fats that can be used by the body. More than 15 mutations in ASAH1 gene are found to cause Farber lipogranulomatosis. The lack of acid ceramidase or reduction in the enzyme activity results in the accumulation of ceramide in cells,

tissues of lungs, liver, bone & muscles. This causes signs and symptoms of Farber lipogranulomatosis. This gene was located in the short (p) arm of chromosome 8 at position 22. The base pairs are located from 17,913,924 to 17,942,506

## Materials & methods

To analyze the ASAH1 protein various tools and soft wares were used. The primary sequence of ASAH1 protein Acc.no gi |16877108|gb|AAD11589.1| was obtained from the Genbank at National centre for Biotechnology Information (NCBI). The sequence was analyzed by various tools. For the calculation of the similarity search BLAST tool was used and to find the physicochemical properties tool like Protparam was used. The conserved domain in the sequence was predicted by Conserved Domain search tool in NCBI. Intreproscan and Fingerprint scan were the tools used to predict the signatures and the motif regions in the sequence. GOR IV is used to predict the secondary structure of the protein. Since no structure information was available in the form of X-ray crystallographic data in Protein data bank (PDB). The modeling of the protein was done to deduce the three dimensional structure of the protein. The Homology modeling was done by Swiss model and the structure validation was performed by Protein Structure Validation Suite (PSVS) tool. Then the 3-D coordinate file was visualized and analyzed in Rasmol.

## Results and Discussion

The similarity search for the sequence was carried out with the help of BLAST tool. The results indicated 100% similarity to acid ceramidase isoform c [Homo sapiens] (Table .1).

Accession	Description	Score	E value
AAH16828.1	ASAH1 protein [Homo sapiens]	808	0.0
NP_001120977.1	acid ceramidase isoform c [Homo sapiens]	806	0.0
EAW63794.1	N-acylsphingosine amidohydrolase (acid ceramidase) 1, isoform CRA_d [Homo sapiens]	798	0.0
NP_004306.3	acid ceramidase isoform b [Homo sapiens]	772	0.0

Table 1. BLAST result of the ASAH1 protein

The domain search was done by Conserved domain search on the Blast site and it showed two domains N-acylethanolamine hydrolyzing acid ceramidase (NAAA) and Choloylglycine hydrolase family. (Figure1). The physicochemical properties of the protein revealed the number of amino acids to be 389, Molecular weight: 44003.5 and theoretical isoelectric point as 7.53. The maximum number of amino acid present in the sequence was found to be Leucine (10%) and the least was that of Histidine (1.3%) the total number of positively charged residues (Arg+ Lys) was 42 and total number of negatively charged residues (Asp+ Glu) was 41. The instability index of the protein was computed to be 37.51. This classifies the protein as stable protein. The grand hydropathicity was calculated to be -0.171. The amino acid composition was given in the (Table.2).

Amino acid	No. of residues	Percentage of residues
Ala (A)	20	5.1%
Arg (R)	18	4.6%
Asn (N)	20	5.1%
Asp (D)	19	4.9%
Cys (C)	8	2.1%
Gln (Q)	8	2.1%
Glu (E)	22	5.7%
Gly (G)	32	8.2%
His (H)	5	1.3%
Ile (I)	23	5.9%
Leu (L)	39	10.0%
Lys (K)	24	6.2%
Met (M)	8	2.1%
Phe (F)	23	5.9%
Pro (P)	21	5.4%
Ser (S)	21	5.4%
Thr (T)	30	7.7%
Trp (W)	10	2.6%
Tyr (Y)	16	4.1%
Val (V)	22	5.7%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

Table 2. Amino acid composition

Fingerprint	No.of motifs
CDATPASE	2
ACONITASE	2
OTCASE	2
Y414FAMILY	2
ACRIFLAVINRP	2
EMR1HORMONER	2

Table 3. Fingerprint scan result of ASAH1 protein

Secondary element	Percentage
Alpha helix (Hh)	15.68%
310 helix (Gg)	0.0%
Pi helix (Ii)	0.0%
Beta bridge (Bb)	0.0%
Extended strand (Ee)	29.05%
Beta turn (Ti)	0.0%
Bend region (Ss)	0.0%
Random coil (Cc)	55.27%

Table 4. Secondary elements of ASAH1 protein

The fingerprint scan of the sequence showed six fingerprints having two motifs for each fingerprint in the sequence (Table.3) The secondary structure prediction was done & random coils was found to be frequent (55.27%), followed by Extended strand (29.05%) and alpha helix was found to be least frequent (15.68%) (Table.4).

This was graphically represented in Figure 2. The tertiary structure was modeled by Swiss model workspace by using the templates from PDBSum (Figure.3) the modeled structure showed 80-Hbonds, 3 helices, 13 strands & 26 turns. The modeled structure was validated by PSVS and Ramachandran plot was plotted (Figure.4)

The analysis of ASAH1 protein showed sequence similarity mostly to the acid ceramidase c (Homo sapiens). The two domains was identified by Conserved domain search. Acid ceramidase is a lipid hydrolase responsible for farber disease. The mutation in the gene encoding acid ceramidase lead to



Figure 1. Graphical Representation of conserved domain in ASAH1 protein

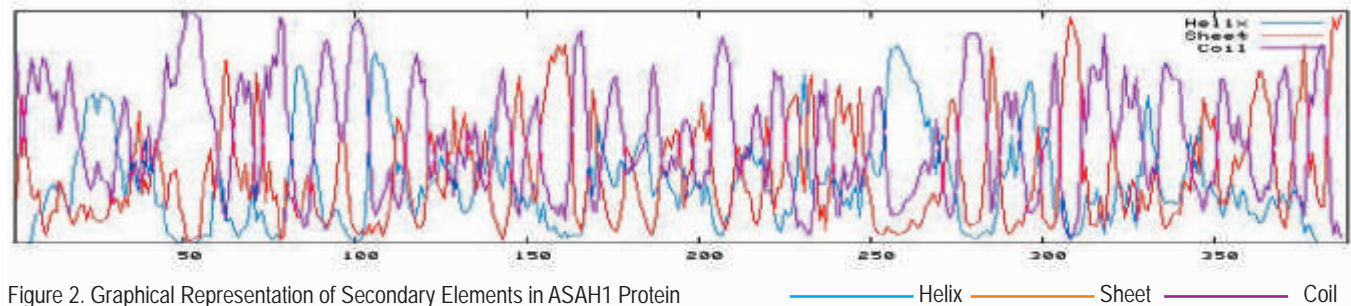


Figure 2. Graphical Representation of Secondary Elements in ASAH1 Protein

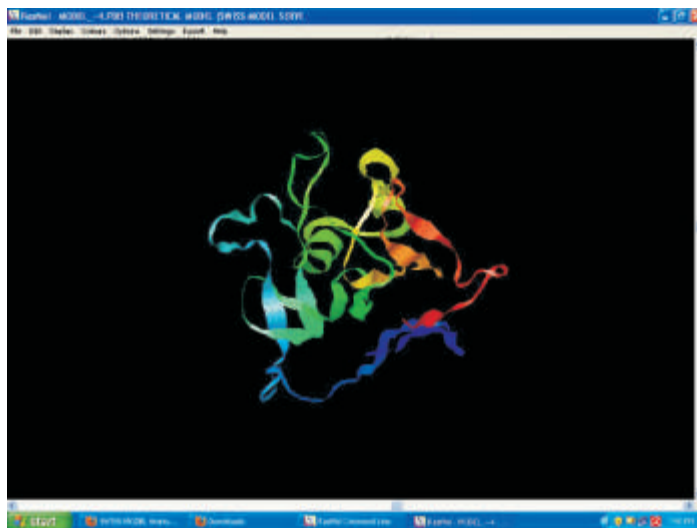


Figure 3. Three dimensional structure of ASAH1 protein

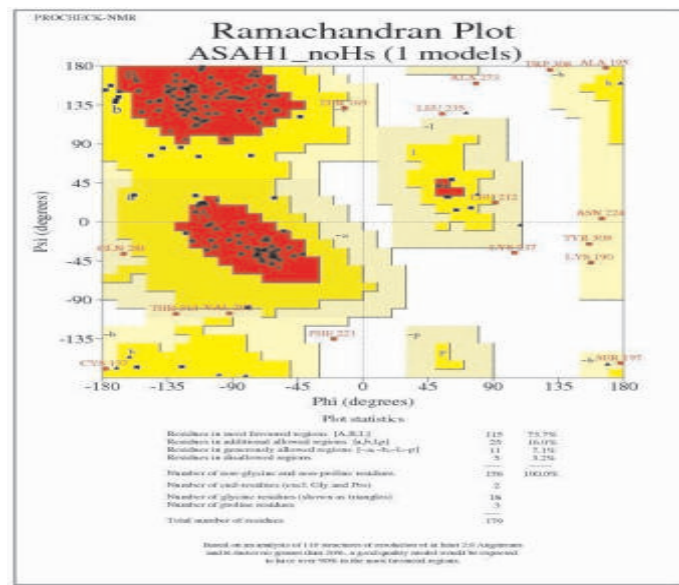


Figure 4. Graphical representation of Ramachandran plot by PSVS

reduction in the enzymatic activity. The modeled structure revealed that 73.7 % are of allowed regions. Further research involving development of appropriate strategies for studying this protein could be of significance in preventing the farber lipogranulomatosis.

### Conclusion

In the present study the sequence and structure analysis of ASAH1 protein was done by various tools and softwares. Based on the findings it could be concluded that further research involving the development of appropriate strategies for studying this protein could be of significance in preventing the farber lipogranulomatosis.

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