J Hum Genet (2006) 51:811–814
DOI 10.1007/s10038-006-0019-z

SHORT COMMUNICATION

Farber lipogranulomatosis: clinical and molecular genetic analysis reveals a novel mutation in an Indian family

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Received: 12 April 2006 / Accepted: 22 May 2006 / Published online: 2 September 2006

Abstract Farber disease is a rare lysosomal storage disorder caused by a deficiency of the acid ceramidase enzyme, leading to the accumulation of ceramide in various tissues. It usually manifests within a few months after birth with a unique triad of symptoms, including painful and progressive deformed joints, progressive hoarseness and subcutaneous nodules. The disease is inherited as an autosomal recessive trait, and mutations in the *N*-acylsphingosine amidohydrolase (*ASAH1*) gene, which codes for the acid ceramidase enzyme, have been shown to cause the disease. In the current study, we report the identification of a novel disease-causing mutation in the *ASAH1* gene that results in Farber disease in an Indian family. The mutation was identified in the eighth exon and is a missense mutation resulting in replacement of Valine by Leucine at codon 182. Two affected siblings harboured the identical mutation. The possible mechanism(s) of disease caused by this mutation are discussed.

Keywords Acid ceramidase · *ASAH1* · DNA sequencing · Farber disease · Mutation

Electronic supplementary material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s10038-006-0019-z and is accessible for authorised users.

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Introduction

Farber Disease, otherwise called the Farber lipogranulomatosis, is a rare sphingolipid disorder inherited as an autosomal recessive genetic trait. Deficiency of acid ceramidase (Sugita et al. 1972) causes abnormal metabolism of neutral lipids with accumulation of ceramide in various tissues, mainly in joints. Clinically, the classic type presents with painful swollen joints of hands and feet, palpable subcutaneous nodules over pressure points and a hoarse voice leading to aphonia due to involvement of vocal cords with the deposits; death usually occurs by 2 years of age. Acid ceramidase is a lysosomal enzyme whose main function is to degrade ceramide to sphingosine and free fatty acid (Gatt 1963). Li et al. (1999) mapped the acid ceramidase gene (ASAH1) to chromosome 8p22-p21.3. Mutations in the *ASAH1* gene have been shown to be the cause of Farber disease (Bar et al. 2001; Koch et al. 1996; Li et al. 1999; Zhang et al. 2000). In the present study, the disease was clinically identified by typical presentation, and by the presence of storage material in the nodules by histopathology, in two siblings from an Indian family presenting with the classical form of Farber disease. Molecular genetic analysis has revealed

a novel mutation to be the cause of Farber disease in this family.

Materials and methods

Patients and samples

Ethical consent was obtained from the Institute ethical committee to carry out genetic testing of the blood samples as per the standard norms of the institute in accordance with the Helsinki declaration of 1975. Informed consent was obtained from the parents of the affected children, as well as from 101 control individuals, before collecting blood samples. The blood samples were obtained and genomic DNA was isolated as previously described (Bashyam et al. 2004).

PCR, DNA sequencing and protein sequence analyses

Sixteen pairs of primers were designed for screening each exon of the human *ASAH1* gene as well as the promoter, 3′UTR and the 5′UTR (including the putative polyadenylation signals) (see Electronic Supplementary Material). Polymerase Chain Reactions (PCR) were carried out on an Applied Biosystems (ABI, Foster City, Calif., USA) Gene Amp 9700 PCR machine, as per standard protocols. PCR products, corresponding to each exon of the *ASAH1* gene, were sequenced using an Applied Biosystems 3100A Genetic Analyzer as per manufacturer's instructions. Homology in the amino acid sequence of the human *ASAH1* protein with that of the predicted acid ceramidase proteins from eight species was determined using the web-based software ClustalW (http://www.ebi.ac.uk/clustalw).

Results

A 1-month-old female child born to first degree consanguineous parents presented with hoarse voice and painful tender joints involving the wrists and ankles. The fingers were swollen with presence of small nodules over the middle finger and wrist. An X-ray showed deformities at the interphalangeal joints with soft tissue swelling. Marginal juxta metaphyseal defect in the anterior-medial aspect of both tibiae was observed. Biopsy of the nodule showed scattered histocytes with peripherally displaced small nuclei and a broad rim of cytoplasm. Proliferating fibroblasts and vascular channels were seen at the periphery. Occasional histocytes had intracytoplasmic diastase resistant PAS positive granules. The child subsequently died of respiratory arrest at the age of 6 months. The clinical triad of painful arthritis, nodules and laryngeal involvement was suggestive of classical Farber disease. The same family returned back after 2 years with another child born subsequently having similar symptoms. The second child died at the age of 2 years.

Routine screening for metabolites in the urine was normal and DNA sequencing revealed a one-base substitution in codon 182 (CUA to GUA) resulting in an amino acid change, from Leucine to Valine, in the eighth exon (Fig. 1); the mutation was confirmed using forward and reverse primers. Both siblings harboured the same mutation and both parents were heterozygous for the same mutation. No other mutation was found in the gene in both siblings. One hundred and one ethnically matched control samples did not harbour the mutation indicating that it was not a single nucleotide polymorphism (SNP).

Multiple alignment of the amino acid sequence of the *ASAH1* gene using ClustalW (http://www.ebi.ac.uk/clustalw) revealed a 38% identity and 59% similarity for the human protein with eight other species. The codon 182 Leucine, completely conserved in all species, is present in close proximity to an N-Glycosylation site (Ferlinz et al. 2001) (codon 173; Asparagine). Alignment of flanking 20 amino acids on either side of the N-glycosylation site revealed a 50% identity and a similarity of 80% of the human protein with the corresponding proteins from eight species (Fig. 2).

Discussion

This is the first clinical and molecular genetic analysis of Farber disease from India. Manifestation of the disease in two siblings in the inbred family, with no affected family members in the last three generations, suggested a recessive genetic disorder. DNA sequencing revealed a novel mutation in the eighth exon of the *ASAH1* gene located in the beta subunit of the acid ceramidase protein. To the best of our knowledge, this is the first report of a disease causing mutation in the eighth exon of the *ASAH1* gene. The p.L182V mutation is the only amino acid change present in both the affected siblings and the parents were heterozygous for the same mutation. Although conversion of Leucine to Valine is unlikely to result in a major change in the protein, it is possible that the p.L182V mutation may affect the glycosylation of the Asparagine residue (amino acid 173 in Fig. 2). It has been reported that, if glycosylation of this Asparagine residue is impaired, there is an 87%

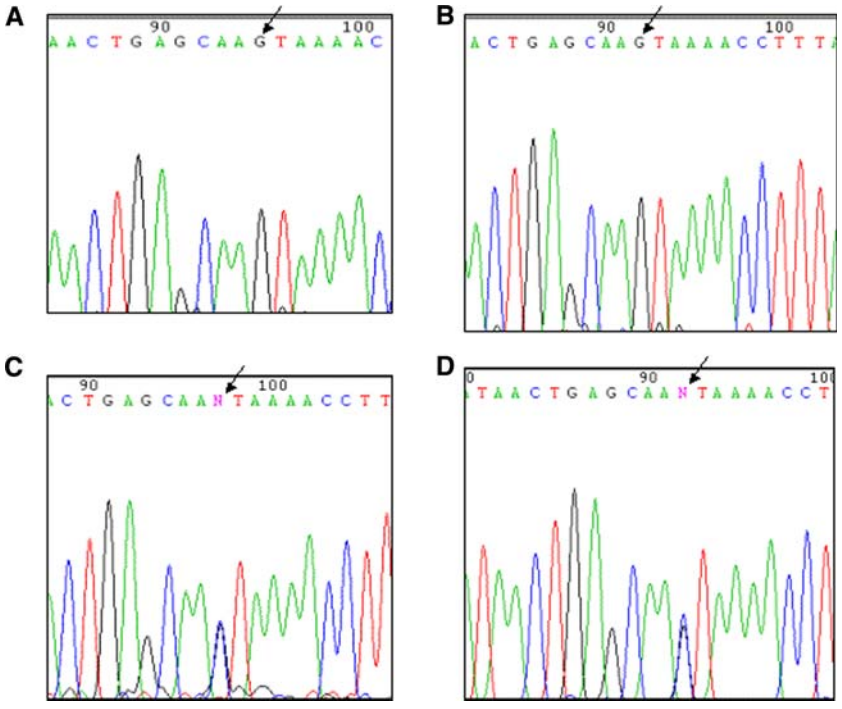

Fig. 1 Eletropherogram of sequencing reactions, carried out for the eighth exon of the *ASAH1* gene using the forward primer, on the two affected siblings and their parents. The mutation is indicated by an *arrow* and was confirmed using the reverse primer. **a** Affected elder sibling; **b** affected younger sibling; **c** father; and **d** mother

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Hum      HGRNMDFGVFLGWNINNDTWVITEQLKPLTVNLDFQRNNKTVFKASSFAGYVGMLTGFKP  216
Chimp    HGRNMDFGVFLGWNINNDTWVITEQLKPLTVNLDFQRNNKTVFKASSFAGYVGMLTGFKP  211
Dog      HGRNMDFGIFLGWNINNNTWVVTEQLKPLSVNLDFQRNNKTVFKASSFAGYVGMLTGFKP  190
Mouse    HGRNMDFGIFLGWNINNNTWVVTEELKPLTVNLDFQRNNKTVFKATSFVGYVGMLTGFKP  215
Rat      HGRNMDFGIFLGWNINNNTWVVTEELKPLTVNLDFQRNNKTVFKATSFAGYVGMLTGFKP  215
Gpuf     HARNLDFGLFMGWDIKNKSWIITEKLKPLVVNLDFRRNNQTVFKSTNFAGYVGMLTGIKP  213
Puf      HARNLDFGLFMGWDVKNKSWIISEKLKPLVVNLDFKRKNQTVFKSTNFAGYVGMLTGIKP  213
Zebra    HGRNLDFGLFMGWDRQNKTWTLTEKLKPLVVNINFERKNQTVFKSTSFAGYVGMLTGIRP  211
Chick    HARNLDFGLFLGWDVKNNSWTVTRELKPTVVNLDFQRNNKTVFRSTNFAGYIGMVSGVKP  216
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Fig. 2 The alignment of amino acids neighbouring the mutated Leucine residue, of the human ASAH1 with its homologues from nine species, performed using the ClustalW alignment software. The amino acid positions for each species are given on the *right side* of the alignment. The Tripeptide LKP (*light shade*) includes the mutated Leucine residue. The essential glycosylation site 'N' is also highlighted (*dark shade*). The amino acid sequences were obtained from the Ensembl database (http://www.Ensembl.org). *Hum* human, *Chimp* chimpanzee, *Gpuf* green puffer fish, *Puf* puffer fish, *Zebra* Zebra fish, *Chick* chicken

reduction in the activity of the acid ceramidase enzyme (Ferlinz et al. 2001). In a recent study, it was observed that neighbouring residues may be important for proper N-linked glycosylation to occur (Ben-Dor et al. 2004). Another possibility is the generation of a possible new functional splice acceptor site (ACUA to AGUA); this may be less likely since there is an absence of other sequence elements, that are required for efficient splicing to occur, in this region.

Although conversion of Leucine to Valine is a highly conserved change, there have been previous reports of an identical amino acid change resulting in a disease condition, including in Familial Alzheimer Disease (Janssen et al. 2001), in Enhanced S Cone Syndrome (Wright et al. 2004) and also in *bulluos congenital ichthyosiform erythroderma* (Ishiko et al. 2001). Therefore, it appears that the p.L182V mutation, that affects the highly conserved Leucine residue, is the cause of Farber disease in this family.

Acknowledgments We thank all physicians and hospitals of Andhra Pradesh, India, for sending to us patients or their blood samples for this study. We also thank the families of the patients for their co-operation. This work was supported by a Core Grant from the Department of Biotechnology, Government of India, to the CDFD, Hyderabad. RR is thankful for a Junior Research Fellowship from the Council of Scientific and Industrial Research, Govt. of India.

References

Bar J, Linke T, Ferlinz K, Neumann U, Schuchman EH, Sandhoff K (2001) Molecular analysis of acid ceramidase deficiency in patients with Farber disease. Hum Mutat 17:199–209

Bashyam MD, Bashyam L, Savithri GR, Gopikrishna M, Sangal V, Devi AR (2004) Molecular genetic analyses of beta-thalassemia in South India reveals rare mutations in the beta-globin gene. J Hum Genet 49:408–413

Ben-Dor S, Esterman N, Rubin E, Sharon N (2004) Biases and complex patterns in the residues flanking protein N-glycosylation sites. Glycobiology 14:95–101

Ferlinz K, Kopal G, Bernardo K, Linke T, Bar J, Breiden B, Neumann U, Lang F, Schuchman EH, Sandhoff K (2001) Human acid ceramidase: processing, glycosylation, and lysosomal targeting. J Biol Chem 276:35352–35360

Gatt S (1963) Metabolism of (I-14c)Lignoceric acid in the rat. Biochim Biophys Acta 70:370–380

Ishiko A, Akiyama M, Takizawa Y, Nishikawa T, Shimizu Y, Shimizu H (2001) A novel leucine to valine mutation in residue 7 of the helix initiation motif of keratin10 leads to bullous congenital ichthyosiform erythroderma. J Invest Dermatol 116:991–992

Janssen JC, Lantos PL, Fox NC, Harvey RJ, Beck J, Dickinson A, Campbell TA, Collinge J, Hanger DP, Cipolotti L et al (2001) Autopsy-confirmed familial early-onset Alzheimer disease caused by the l153V presenilin 1 mutation. Arch Neurol 58:953–958

Koch J, Gartner S, Li CM, Quintern LE, Bernardo K, Levran O, Schnabel D, Desnick RJ, Schuchman EH, Sandhoff K (1996) Molecular cloning and characterization of a full-length complementary DNA encoding human acid ceramidase identification of the first molecular lesion causing Farber disease. J Biol Chem 271:33110–33115

Li CM, Park JH, He X, Levy B, Chen F, Arai K, Adler DA, Disteche CM, Koch J, Sandhoff K et al (1999) The human acid ceramidase gene (ASAH): structure, chromosomal location, mutation analysis, and expression. Genomics 62:223–231

Sugita M, Dulaney JT, Moser HW (1972) Ceramidase deficiency in Farber's disease (lipogranulomatosis). Science 178:1100–1102

Wright AF, Reddick AC, Schwartz SB, Ferguson JS, Aleman TS, Kellner U, Jurklies B, Schuster A, Zrenner E, Wissinger B et al (2004) Mutation analysis of NR2E3 and NRL genes in enhanced S Cone Syndrome. Hum Mutat 24:439

Zhang Z, Mandal AK, Mital A, Popescu N, Zimonjic D, Moser A, Moser H, Mukherjee AB (2000) Human acid ceramidase gene: novel mutations in Farber disease. Mol Genet Metab 70:301–309