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Identification of mutation in *NPC2* by exome sequencing results in diagnosis of Niemann–Pick disease type C

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ABSTRACT

We report identification of a homozygous mutation in *NPC2* in two Iranian siblings with a neurologic dysfunction whose disease had not been diagnosed prior to our genetic analysis. The mutation was identified by exome sequencing. The finding resulted in diagnosis of Niemann–Pick disease type C (NPC) in the siblings, and initiation of treatment with Miglustat. The clinical features of the patients are presented. It has been suggested that NPC is under diagnosed, particularly when presentations are not very severe, as was the situation in the cases studied here. NPC is a fatal autosomal recessive disorder clinically characterized by hepatosplenomegaly and progressive neurological deterioration. At the cellular level, it causes aberrant cholesterol trafficking and accumulation of unesterified cholesterol in lysosomes. Mutations in *NPC1* and *NPC2* are cause of disease in respectively, 95% and 5% of NPC patients. The p.Pro120Ser causing mutation in *NPC2* observed in the Iranian patients was earlier observed in the only other *NPC2* patient reported from the Middle East. The study demonstrates that in addition to greatly facilitating gene discovery, exome sequencing has notable potentials for diagnosis, particularly for diagnosis of atypical cases.

1. Introduction

The name "Niemann–Pick disease" derives from the names of pediatrician Albert Niemann and pathologist Ludwig Pick who first described some of its clinical presentations [1]. The disease is now known to encompass several lysosomal lipid storage diseases. Variability in presentation was recognized as early as 1961, when it was grouped into four subtypes A–D on the basis of rate of disease progression and patterns of organ involvement and lipid storage in only 18 patients [2]. Subtypes A and B proved to be biochemically and genetically related, both being sphingomyelin storage disorders caused by mutations in *SMPD1* that encodes sphingomyelin phosphodiesterase 1 [3,4]. Subtype C was later further subtyped NPC1 and NPC2 based on cell fusion complementation and genetic findings, and subtype D was shown to be allelic to NPC1 [5–7].

Niemann–Pick disease type C (NPC) is a fatal disorder clinically characterized by hepatosplenomegaly and progressive neurological deterioration. Age at onset of symptoms ranges from the perinatal period to adult age, and is most often before the age of 20 years [8]. Severity of disease, rate of progression, and duration till death are additional variant clinical features [8,9]. Its inheritance pattern is autosomal recessive, and its prevalence among living births is estimated at 1/120,000 to 1/150,000 [10]. At the cellular level, NPC is characterized by accumulation of unesterified cholesterol and glycolipids in lysosomes and late endosomes [11]. The precise pattern of stored lipids varies in different tissues [11–14]. Aberrant intracellular translocation of exogenous cholesterol is best evidenced in fibroblasts of NPC patients by a specific pattern of fluorescence in a protocol that includes probing with filipin, a cholesterol binding antibiotic [15]. Exogenous cholesterol in the form of low density lipoprotein (LDL) appears to be normally internalized, transported to endocytic vesicles, and hydrolyzed. However, transport of unesterified cholesterol out of lysosomes to other cellular sites is impaired. Endogenously synthesized cholesterol may contribute to cholesterol accumulation in some cell types [16,17]. Furthermore, intracellular cholesterol homeostasis including cholesterol ester formation is delayed in NPC cells [18].

Two NPC causing genes have been identified. *NPC1* (OMIM # 607623) which encodes an integral 1278 amino acid membrane protein was discovered by linkage analysis [5,19]. The protein is found primarily in late endosomes and interacts transiently with lysosomes and

Abbreviations: NPC, Niemann–Pick type C; *NPC1*, *NPC1* gene; *NPC2*, *NPC2* gene; *SMPD1*, sphingomyelin phosphodiesterase 1 gene; LDL, low density lipoprotein; HGMD, The Human Gene Mutation Database; ALS, amyotrophic lateral sclerosis; DTR, deep tendon reflex; MRI, magnetic resonance imaging; *SOD1*, Superoxide desmutase 1 gene; *C9orf72*, chromosome 9 open reading frame 72 gene; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

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the trans-Golgi network [20,21]. Its precise function with respect to LDL-cholesterol transport and lipid transport in general remains unknown. The vast majority (95%) of NPC patients harbor mutations in *NPC1*. Cell fusion complementation experiments and linkage analysis initially suggested the existence of two NPC causing genes [15,22]. Using ingenious proteomics analysis, *NPC2* (OMIM # 601015) that encodes what was previously known as human epididymis protein HE1 was identified as the second causative gene in 2000 [6]. Mutations in *NPC2* have been identified in almost all NPC patients without mutations in *NPC1*. NPC2 is a ubiquitously expressed cholesterol binding soluble lysosomal protein [23,24]. It binds the mannose-6-phosphate receptor present on lysosomes; the mature protein contains 132 amino acids [6]. Like NPC1, the precise function of NPC2 is not known. Based on evolutionary conservation and mutagenesis analysis, four regions of the protein were predicted to be functionally important [24]. One affects efficient secretion, another affects cholesterol binding, and the roles of the remaining regions are unknown. NPC2 may have other roles in addition to cholesterol trafficking; recently, it was shown to be involved in papillae formation [8,25]. With respect to Niemann–Pick disease, *NPC1* and *NPC2* clearly have related though non-redundant functions [15,26,27]. The clinical and biochemical features of patients with mutations in either of the two genes are very similar, with increased lung involvement during infancy in *NPC2* mutation carriers being the only notable reported difference [9,15,28,29]. Similarly, mice harboring mutations in *NPC1* or *NPC2* orthologous genes and animals with mutations in both genes manifest the same phenotypes [27].

Twenty different mutations in *NPC2* have so far been reported (The Human Gene Mutation Database, HGMD V-2012.4, http://www.hgmd.cf.ac.uk/ac/index.php; the NPC database, http://npc.fzk.de/). The distribution of the known mutations in the gene and the encoded protein are shown in Fig. 1. Disease causing mutations are observed through the length of the protein. Here, we report identification of a homozygous mutation in *NPC2* in two siblings with a neurologic dysfunction whose disease had not been diagnosed prior to our analysis. The mutation was identified by exome sequencing. The finding resulted in diagnosis of NPC in the siblings, and initiation of treatment with Miglustat (Zavesca) [30].

2. Subjects and methods

This research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran. Participants or the guardians consented to participate after being informed of the nature of the research.

2.1. Subjects

The Niemann–Pick disease patients studied here were fortuitously identified during our recruitment of Iranian familial amyotrophic lateral sclerosis (ALS) cases [31]. An ALS diagnosed patient (103-26) born to consanguineous parents was referred to us. The diagnosis of definite ALS was based on El Escorial criteria [32]. The parents of the ALS patient reported that their child had cousins who presented with "similar" symptoms (Fig. 2; 103-24 and 103-25). The parents of the cousins were also consanguineous. They informed us that a specific diagnosis had not been made on their children. Upon careful

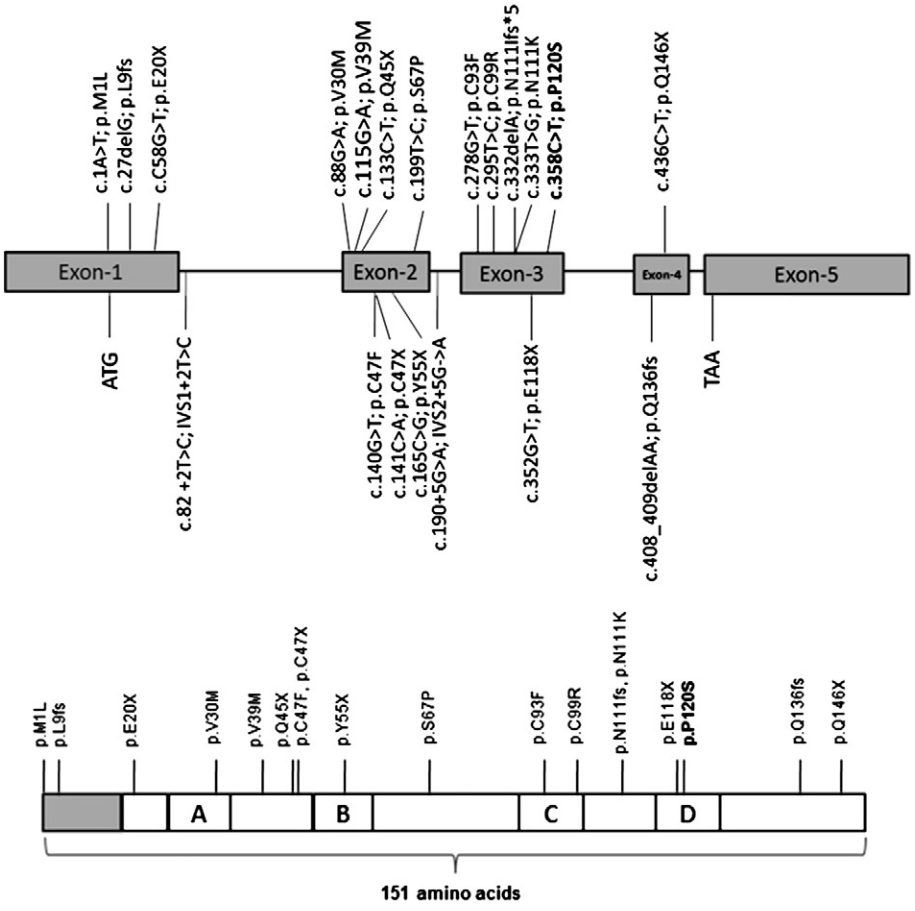

Fig. 1. All reported *NPC2* mutations. (a) Known *NPC2* mutations are shown on schematic view of the gene. A of the initiation codon is designated +1. (b) The coding mutations are shown in schematic view of the encoded protein. The N-terminal fragment (19 amino acids) removed during processing is shown with light shading. The four regions (A–D) of the protein predicted to be functionally important on the basis of evolutionary conservation are shown as boxes [24]. The mutation observed in the Iranian siblings is shown in bold. The schematic drawings are based on reference sequences, NC_000014.8, NM_006432.3, and NP_006423.1.