

Molecular characteristics of the Niemann-Pick type C Polish patients

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Objectives:

The Niemann-Pick type C is a rare autosomal recessive neurodegenerative disease resulting from a failure of cholesterol trafficking within and out of the endosomal-lysosomal compartment, due to mutations in the *NPC1* (95% of cases) or the *NPC2* (5% of cases) gene. Both genes encode cholesterol-binding proteins. *NPC1* is a large 1278-amino acid polytopic membrane protein that is localized to the membranes of endosomes and lysosomes (Figure 1), while *NPC2* is a small soluble protein of 132 amino acids that resides in these compartments and can also be secreted. To date, more than 260 different loss of function mutations of *NPC1* and *NPC2* genes have been reported. Only one mutation - I1061T in exon 21 - is frequent and present in approximately 20% of all known mutations for *NPC1* among individuals of Caucasian origin (mainly of Western European descent). Other mutations are rare and often have familial character.

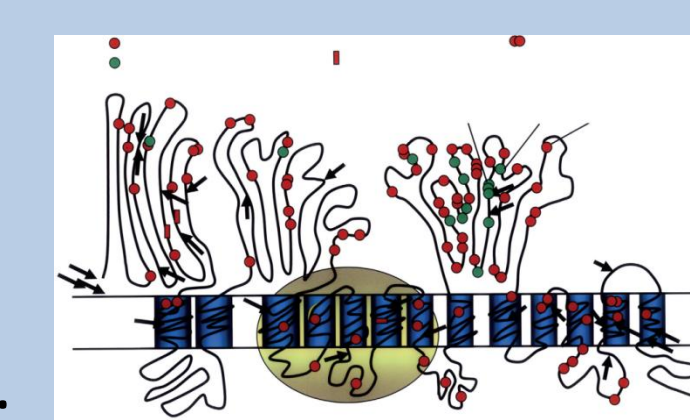


Figure 1. Topology of *NPC1* mutations, compiled from published data [1].

Patients:

A group of 10 unrelated Polish origin patients, either with a diagnosis or a suspicion of diagnosis after biochemical examinations, was for the first time enrolled for molecular diagnostics. Biochemical examinations included measurement of LDL-stimulated cholesterol esterification and filipin fluorescence test (Figure 2).

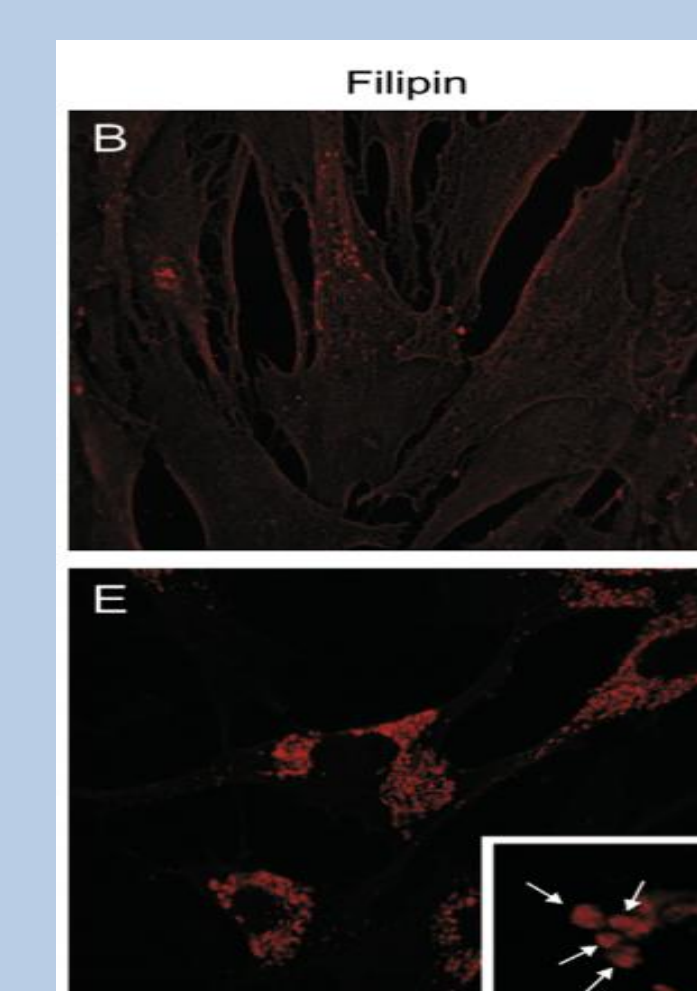


Figure 2. Distribution of cholesterol in control (B) and NPC fibroblasts (E) analysed by fluorescence microscopy. Cholesterol-rich structures (white arrows) are indicated [2].

Methods:

We analysed all exons (25 for *NPC1* and 5 for *NPC2*) with flanking intronic sequences using direct sequencing. Primers were designed with Primer 3 and Mutation Discovery software. For analysis of the sequencing results the Mutation Surveyor by Softgenetics was employed.

Results:

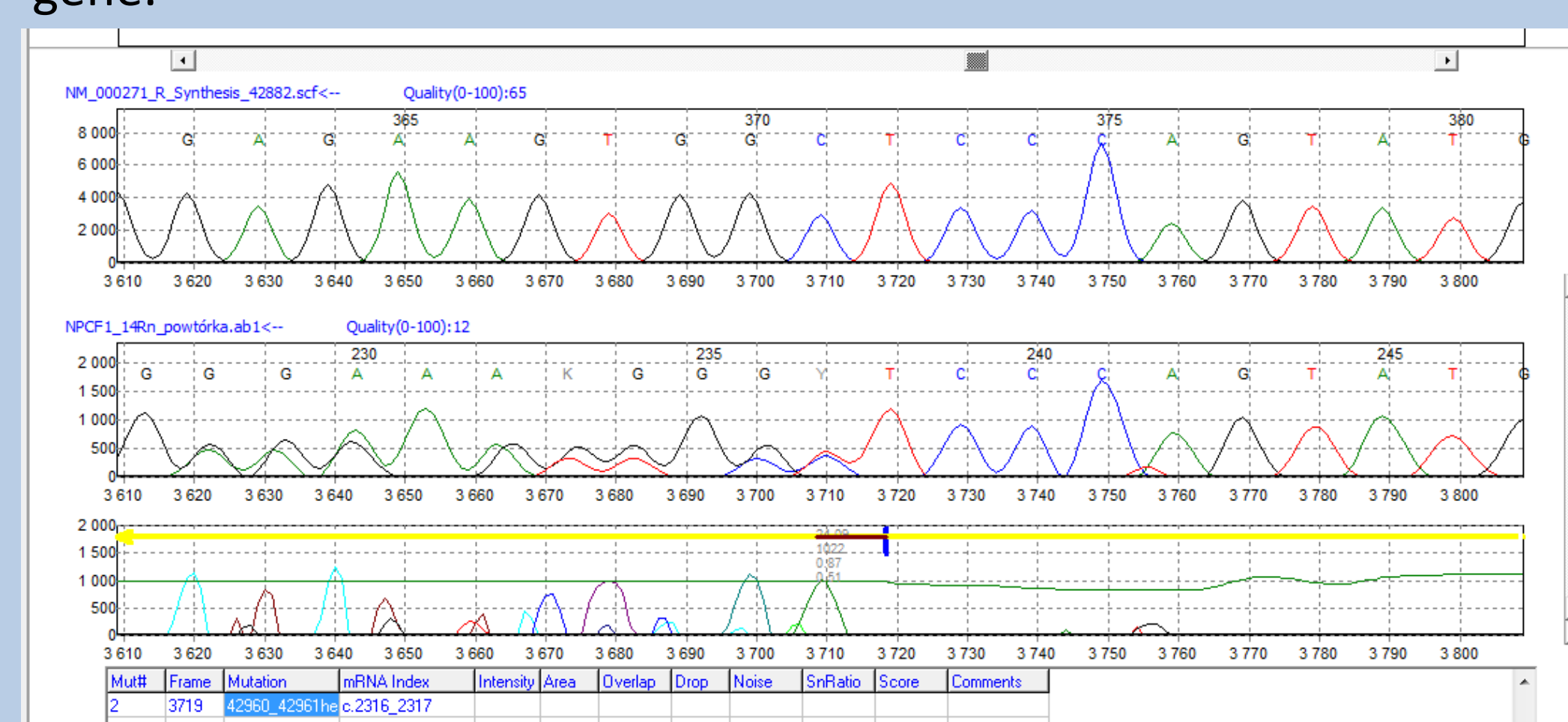
In 7 patients *NPC1* mutations in both alleles were identified, and in one case new intronic *NPC2* mutation in one allele (IV4S+1G>A) was observed (Table 1). Among 11 identified *NPC1* mutations, 6 were unpublished. Mutation 2316_2317 insT in exon 14 [2], so far unsubmitted to the Niemann-Pick Type C Disease Gene Variation Database (<http://npc.fzk.de>), was the most frequent. This mutation in compound heterozygotic genotypes was identified 3 times (3 of the 7 cases of the patients with *NPC1* mutations). The mutation 2316_2317insT (Figure 2) results in introducing premature termination codon (PTC mutation) into *NPC1*. Two patients with the same *NPC1* genotype (S954L/2316_2317insT) have similar clinical outcome: with mild clinical symptoms in childhood with psychotic phenotype in adulthood and late diagnosis. The known (published) S954L pathogenic mutation was identified in 2 cases.

Table 1. Characteristics of results of *NPC1* and *NPC2* gene analysis.

Patient	Sex	Allele1	<i>NPC1</i> exon	Allele2	<i>NPC1</i> exon	Age of first symptoms	Age of diagnosis
1	M	p.S954L	19	2316_2317insT*	14	10 y	16 y
2	M	p.S954L	19	2316_2317insT*	14	childhood	20 y
3	M	p.W1145R	22	c.2913delG*	20	infancy	8 y
4	M	2316_2317insT*	14	c.1841delA*	12	infancy	2.5 y
5	M	p.R404Q	8	p.S734G*	14	infancy	5 y
6	M	unknown	---	unknown	---	2 y	3 y
7	F	p.R348X*	8	p.R1186H	23	infancy	4 y
8	F	1519_1520insC*	9	p.I1061T	21	6 y	6 y [#]
10	M	unknown	---	unknown	----	no data	no data
9	M	IVS4+1G [^]	intron4	unknown	----	no data	no data

*unpublished mutation, [#] older brother with the NPC diagnosis, [^] *NPC2* gene defect

Figure 2. Identification of the 2316_2317insT mutation in the *NPC1* gene.



Summary:

Because of high frequency of the 2316_2317insT among Polish *NPC1* patients, we hypothesised an ancestral effect. In all cases genetic counselling should be proposed. After confirmation of biparental inheritance of identified mutations in the *NPC1* gene, prenatal diagnostics and carrier test for other familial members should be proposed.

[1] Am J Hum Genet. (2001) 68:1373-85.

[2] Arch Biochem Biophys (2010) 493: 221-233.