Familial hypercholesterolemia - preliminary results of genetic screening by NGS technique

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Familial hypercholesterolemia (FH) is an autosomal dominant monogenic lipid metabolism disorder and is characterized by significantly elevated LDL-cholesterol and premature ischemic heart disease. It is estimated that it occurs in approximately 1/500-1/250 persons in the general population. The detection of a molecular defect confirms the diagnosis of familial hypercholesterolemia. Early diagnosis and initiation of hypolipidemic therapy is important in the prevention of cardiovascular events in this group of patients.

The aim of the study was to assess the frequency of occurrence of genetic variants affecting cholesterol levels in the group of patients from Malopolska region with clinical suspicion of FH using next generation sequencing (NGS).

The material consisted of 99 patients aged 18-70 diagnosed with FH based on the criteria of the Simoon Broome Register.

Methods:

All patients underwent a questionnaire study, including family history, cardiovascular events and hypolipidemic treatment. In addition, a fasting lipidogram was performed using the enzymatic method. The secondary causes of hypercholesterolemia were excluded.

Ilumina MiSeq platform was used to study mutations. A panel including exons of FH-causing genes: LDLR, APOB, PCSK9 and STAP1, as well as genes with lower quantitative impact on cholesterol (apo E, ABCG5, ABCG8, LPL, NPC1, LPL, LDL-RAP1, LIPC, CELSR2) was developed. Genetic variants were classified based on the points determining their pathogenicity and impact: CADD, Fitcons, SIFT2 (Sorting Intolerant From Tolerant), Polyphen2, Mutation Taster, as well as their clinical relevance (ClinVar). Variants described as pathogenic according to at least 3 of the 5 metrics described above were considered pathogenic.

Results:

In the study group 12 patients had pathogenic variants in the LDL receptor gene, previously described as associated with familial hypercholesterolemia. In 6 subjects pathogenic variants in the LDL receptor gene, not described earlier, were detected. 10 patients had mutations in the apo B 100 gene, of which 9 persons had the rs5742904 mutation - previously described as the most frequent mutation in the ApoB100 gene . In 1 person we found an ApoB100 mutation not described earlier. 2 people from one family had a mutation newly described as related to FH of the STAP1 gene. It is interesting to observe the high frequency of pathogenic variants of ApoE in the studied group.

Characteristics of patients

Parameter	N=96		
	n	x/Me	sd/IR
Age (yrs)	93	44	14,09
TC [mmol/l]	87	7,215	2,2072
HDL-C [mmol/l] *	87	1,55	0,55
LDL-C [mmol/l]	87	4,992	2,0692
TG [mmol/l] *	86	1,26	1,1
glucose [mmol/l] *	84	5	0,88
BMI [kg/m2]	95	26,08	4,539
WHR	91	0,866	0,0854
SBP [mmHg]	90	132,4	17,55
DBP [mmHg]	90	83	11,95

Mutations observed in LDL receptor gene in a group of patients with FH from Polish population

Gene	Mutation	Aminoacid change	Exon	Freq	Mutation Taster2	Polyphen-2	SIFT
LDLR	c.58G>A	p.Gly20Arg	1	1	Polymorphism	Benign	Damaging
LDLR	c.100T>G	p.Cys34Gly	2	5	Disease causing	Probably damaging	Damaging
LDLR	c.313+1G>T		3	1	Disease causing		
LDLR	c.442T>C	p.Cys148Arg	4	1	Disease causing	Probably damaging	Damaging
LDLR	c.530C>T	p.Ser177Leu	4	1	Disease causing	Probably damaging	Damaging
LDLR	c.666C>A	p.Cys222*	4	1	Prediction disease causing		
LDLR	c.782G>T	p.Cys261Phe	5	1	Disease causing	Probably damaging	Damaging
LDLR	c.798T>A	p.Asp266Glu	5	1	Disease causing	Probably damaging	Damaging
LDLR	c.986G>T	p.Cys329Phe	7	2	Disease causing	Probably damaging	Damaging
LDLR	c.1061-8T>C		7	1	Polymorphism		
LDLR	c.1222G>A	p.Glu408Lys	9	1	Disease causing	Possibly damaging	Damaging
LDLR	c.1223A>T	p.Glu408Val	9	1	Disease causing	Probably damaging	Damaging
LDLR	c.1328G>A	p.Trp443*	9	1	Disease causing		
LDLR	c.1449G>T	p.Trp483Cys	10	1	Disease causing	Possibly damaging	Damaging
LDLR	c.1705+1G>A		11	1	Disease causing		
LDLR	c.1775G>A	p.Gly592Glu	12	2	Disease causing	Probably damaging	Damaging
LDLR	c.1862C>G	p.Thr621Arg	13	1	Disease causing	Probably damaging	Damaging
LDLR	c.2390-16G>A		16	1	Polymorphism		Damaging

Mutations observed in apo B gene and other genes associated with FH in patients with FH from Polish population

Gen	Mutacja	Zmiana aminokwasowa	Exon	Freq	Mutation Taster2	Polyphen-2	SIFT
LDLRAP1	c.672C>T	p.Ser224Ser	7	1	Disease causing		Tolerated
PCSK9	c.60_65dupGCTGCT	p.Leu21_Leu22dup	1	1	Polymorphism		
STAP1	c.120+6T>C		1	2	Disease causing		
APOB	c.12382G>A	p.Val4128Met	29	1	Polymorphism	Benign	Tolerated
APOB	c.11833A>G	p.Thr3945Ala	27	1	Polymorphism	Benign	Tolerated
APOB	c.10708C>T	p.His3570Tyr	26	1	Polymorphism	Benign	Tolerated
APOB	c.10580G>A	p.Arg3527Gln	26	9	Disease causing	Probably damaging	Damaging
APOB	c.10579C>T	p.Arg3527Trp	26	1	Disease causing	Probably damaging	Damaging
APOB	c.10131G>A	p.Leu3377Leu	26	1	Disease causing		Tolerated
APOB	c.8462C>T	p.Pro2821Leu	26	2	Polymorphism	Benign	Tolerated
APOB	c.8353A>C	p.Asn2785His	26	1	Polymorphism	Benign	Tolerated
APOB	c.7696G>A	p.Glu2566Lys	26	10	Polymorphism	Benign	Tolerated
APOB	c.7615G>A	p.Val2539Ile	26	1	Polymorphism	Benign	Tolerated
APOB	c.6639_6641delTGA	p.Asp2213del	26	1	Disease causing		
APOB	c.3122-6G>A		20	1	Polymorphism		
APOB	c.2068-4T>A		14	1	Disease causing		
APOB	c.1594C>T	p.Arg532Trp	12	1	Polymorphism	Probably damaging	Damaging
APOB	c.538-9C>T		5	1	Polymorphism		

The results suggest that NGS is a useful method in the molecular diagnostics of familial hypercholesterolemia. NGS allows to examine the profile of genes affecting the cholesterol level in a patient, which may be important in determining new pathways of lipid disorders therapy.