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Short report

# SLOS carrier frequency in Poland as determined by screening for Trp151X and Val326Leu *DHCR7* mutations

E. Ciara [*], E. Popowska [a], D. Piekutowska-Abramczuk [a], D. Jurkiewicz [a], M. Borucka-Mankiewicz [a], Paweł Kowalski [a], B. Goryluk-Kozakiewicz [a], M.J.M. Nowaczyk [b], M. Krajewska-Walasek [a]

[a] *Department of Medical Genetics, Children's Memorial Health Institute, Al. Dzieci Polskich 20, 04-730 Warsaw, Poland*
[b] *Department of Pathology and Molecular Medicine and Department of Pediatrics, McMaster University, Hamilton, Canada*

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**Abstract**

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis caused by mutations in the *DHCR7* gene. Previous studies estimated the prevalence of SLOS between 1 in 10,000 to 1 in 70,358 based on case frequency surveys. Although panethnic, SLOS appears to be most frequent in Central European populations (Czech Republic 1 in 10,000, Slovakia 1 in 15,000 – 1 in 20,000). In Polish individuals with SLOS two *DHCR7* mutations, c.452G > A (p.Trp151X) and c.976G > T (p.Val326Leu), account for 65.2% of all observed *DHCR7* mutations. We analyzed 2169 samples for the p.Trp151X mutation and 2087 for the p.Val326Leu mutation. The combined carrier frequency of these two mutations of was 2.40 ± 0.32%, yielding a calculated incidence of SLOS in Poland of 2.5 $4 \times 10^{-4}$–4.3 $5 \times 10^{-4}$ (1 in 2,300 to 1 in 3,937) placing SLOS among the most common recessive genetic disorders in Poland.


*Keywords:* SLOS; Carrier frequency; *DHCR7* mutations; Polish population

[*] Corresponding author. Tel.: +48 22 8151075, fax:+48 22 8157457.
*E-mail address:* e.ciara@czd.pl (E. Ciara).

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## 1. Introduction

Smith-Lemli-Opitz syndrome (SLOS, MIM 270400) is an autosomal recessive disorder of cholesterol biosynthesis caused by mutations in the *DHCR7*. In 1998, the human *DHCR7* was mapped to the chromosome region 11q12-13 and the first pathogenic mutations were identified in SLOS patients [6,12,23,24]. To date, 105 different *DHCR7* mutations have been described [4].

SLOS occurs most frequently among populations of Central Europe (Czech Republic: 1 in 10,000, Slovakia: 1 in 15,000–1 in 20,000), while in the United Kingdom and the United States of America the prevalence is estimated as 1 in 60,000 [2,7,20,22]. Based on ascertainment of prenatal and postnatal cases diagnosed during one year, the observed incidence in Ontario, Canada was 1 in 20,358 [14], subsequently, however, the observed incidence during a prospective national 3-year surveillance was 1 in 70,358 live births [18].

The observations of several groups indicate that the disease carrier rate is approximately 1 in 100 for Caucasian population in North America (1%) and possibly as high as 1 in 50 to 1 in 30 in some European populations (2 -3.3%) resulting in the expected incidence of SLOS as high as 1 in 1,590 to 1 in 13,400 in North America [1,15], and 1 in 1,666 to 1 in 22,201 in Central Europe [11]. Although the overall carrier rates are similar in various populations, the frequencies of individual mutations differ significantly. In Central Europe the most frequent mutation is p.Trp151X, while in Western Europe populations and their descendants the most frequent mutation is IVS8-1G > C [10,28]. Among 48 Polish SLOS individuals two mutations, c.452G > A (p.Trp151X) and c.976G > T (p.Val326Leu), were found to account for 65.2% of all observed *DHCR7* changes [3, unpublished data].

## 2. Material and methods

### 2.1. Study subjects

In order to determine the carrier frequency of SLOS in Poland, DNA samples from 4,256 anonymous Polish neonates were obtained from Guthrie cards (previously collected for newborn screening of phenylketonuria). All samples were from individuals of Polish ethnicity. The study was approved by local ethics committees and informed consent was obtained from each of the participants. Genomic DNA was eluted from dried blood spots using 5% Chelex-100 resin [1]. For the purposes of this study, 2169 samples were analyzed for the p.Trp151X mutation and 2087 for the p.Val326Leu mutation.

### 2.2. Allele specific mutation assay

To screen the p.Trp151X and p.Val326Leu mutations we used allele specific assay based on the amplification refractory mutation system (ARMS) as previously described for *DHCR7* assays [13,15]. Our sensitivity studies revealed that each ARMS assay could detect a single carrier in 20 subject pools. In each ARMS analysis four primers, two control primers amplifying intragenic *DHCR7* region (~590 bp) and two allele specific primers amplifying *DHCR7* gene fragment (~220 bp) with two screened mutations, were used. The individual constituents of each positive pool were subsequently analysed and the carrier of the p.Trp151X or p.Val326Leu mutation was identified. For each ARMS assay, PCR was carried out in a reaction volume of 50 μl containing 1.5 μl of the eluted genomic DNA, 1x PCR buffer (DyNAzyme),