ORIGINAL ARTICLE

Smith–Lemli–Opitz syndrome carrier frequency and estimates of *in utero* mortality rates†

Gabriel A. Lazarin*  , Imran S. Haque, Eric A. Evans and James D. Goldberg

Counsyl, South San Francisco, CA, USA
*Correspondence to: Gabriel A. Lazarin. E-mail: gabriel@counsyl.com
†Presented at ISPD, ASRM, NSGC all in 2015.

ABSTRACT

Objective To tabulate individual allele frequencies and total carrier frequency for Smith–Lemli–Opitz syndrome (SLOS) and compare expected versus observed birth incidences.

Methods A total of 262 399 individuals with no known indication or increased probability of SLOS carrier status, primarily US based, were screened for SLOS mutations as part of an expanded carrier screening panel. Results were retrospectively analyzed to estimate carrier frequencies in multiple ethnic groups. SLOS birth incidences obtained from existing literature were then compared with these data to estimate the effect of SLOS on fetal survival.

Results Smith–Lemli–Opitz syndrome carrier frequency is highest in Ashkenazi Jews (1 in 43) and Northern Europeans (1 in 54). Comparing predicted birth incidence with that observed in published literature suggests that approximately 42% to 88% of affected conceptuses experience prenatal demise.

Conclusion Smith–Lemli–Opitz syndrome is relatively frequent in certain populations and, because of its impact on prenatal and postnatal morbidity and mortality, merits consideration for routine screening.

Funding sources: None
Conflicts of interest: All authors are employees of Counsyl, a molecular diagnostics laboratory.

BACKGROUND

Smith–Lemli–Opitz syndrome (SLOS, OMIM #270400) is an autosomal recessive disease caused by mutations in the *DHCR7* gene resulting in deficiency of the 7-dehydrocholesterol reductase enzyme and impaired cholesterol metabolism. Individuals with the disease exhibit a wide and variable spectrum of phenotypic abnormalities, including multiple congenital malformations, facial abnormalities, metabolic errors, and intellectual disability. Cholesterol supplementation may improve clinical symptoms, although further studies are needed to develop a dependable management strategy. Demise in the prenatal period may be a relatively common outcome, occurring in up to 80% of affected conceptuses.[1] Variable, and sometimes subtle, presentation can lead to missed or delayed diagnoses.[2,3] Prenatally, nonspecific ultrasound findings may be present, such as cardiac defects or cleft lip/palate. Table 1 lists characteristics that may be observed through a prenatal ultrasound, although such an examination may also be normal. Prenatal biochemical screening approaches are also available.[4]

Carrier frequency estimates have varied because of methods of ascertainment, alleles assessed, and populations studied. In general, existing data suggest a carrier frequency of approximately 1% for common alleles in Caucasians,[5–8] with at least one source extrapolating the total carrier frequency to 3%.[9] The most common allele in North American populations is the null mutation, c.964-1G > C, while other alleles, c.452G > A and c.278C > T, may be more frequent in Central European and Mediterranean ancestry populations, respectively.[10]

Smith–Lemli–Opitz syndrome disease incidence has been studied, primarily in Europe and Canada. Diagnoses have been confirmed by molecular and biochemical methods. Most figures range from 1/60 000[11,12] to 1/20 000.[13,14] A large study of SLOS risk assessed in over a million pregnancies in the United States found a mid-trimester prevalence of 1/101 000 Caucasians, much lower than other estimates.[4] Elevated risk was initially identified by mid-trimester serum analysis. However, because SLOS diagnostic testing was not performed in a number of screen-positive pregnancies (in particular, those with fetal demise), these data underestimate the true incidence when SLOS causes lethality before birth. The authors did not comment on possible reasons for the discrepancy between their findings and those of other population studies.

Table 1 **Reported ultrasound findings in conceptuses with Smith–Lemli–Opitz syndrome**

General
in utero demise
Intrauterine growth retardation
Nervous system
Ventricular dilatation
Abnormal corpus callosum or cerebellum
Dandy–Walker malformation/variant
Holoprosencephaly
Facial
Cleft lip/palate
Bifid uvula
Short nose with anteverted nares
Cardiac
Septal or major vessel defects
Complex malformations
Genital
Ambiguous genitalia
Skeletal
Micromelia
Postaxial polydactyly
2–3 toe syndactyly
Microcephaly
Abdominal
Renal hypoplasia or agenesis
Hydronephrosis

Based on Quelin *et al.*, 2012. Normal ultrasound examination is also reported.

Data regarding other ethnic populations are limited, but where available, suggest that SLOS is uncommon or rare in non-Caucasians, particularly among individuals of African or East Asian ancestry.[6,7,14,15]

This study utilizes a large database of individuals tested for SLOS to report observed carrier frequencies and estimate the expected birth incidence resulting from those frequencies. A total of 262 399 individuals with no reported indication of personal or family history of SLOS or infertility were screened for SLOS mutations as part of an expanded carrier screening panel, including samples of more than 10 000 for most major US ethnic groups. Because this population is large and screened without apparent indication or dependency on clinical symptoms, highly accurate allele frequency estimates are possible.

METHODS

This is a retrospective analysis of results from individuals electing expanded carrier screening that included SLOS between January 2012 and December 2015.

The analyses for this study were performed in a Clinical Laboratory Improvement Amendments and College of American Pathologists-certified laboratory using two methods (Family Prep Screen 1.0 and 2.0, Counsyl, South San Francisco, CA). Most ($n = 210\,857$) were screened via targeted genotyping (Family Prep Screen 1.0) for 13 *DHCR7* mutations using TaqMan fluorescent probes on the Fluidigm 96.96 platform.

Another 51 542 were screened via a next-generation sequencing (NGS) test (Family Prep Screen 2.0) using custom hybrid capture followed by sequencing on the Illumina HiSeq 2500 to test for variants in *DHCR7* exons 3-9. This methodology encompasses the 13 mutations identified by genotyping and other mutations previously known or undescribed. Large deletions and insertions, which may account for 4–5% of causative alleles,[16] would typically not be identified by this methodology. Identified variants were classified for pathogenicity based on the American College of Medical Genetics and Genomics' recommendations for interpretation and reporting using the approach described by Karimi *et al.*[17,18] Patients were informed when a known, likely, or predicted deleterious variant was identified. The combination of test methodology, variant classification, and variant reporting will be referred heretofore as NGS. Variants of uncertain significance and known, likely, or predicted benign variants were not routinely reported to the physician or patients, as per the laboratory's routine carrier screening protocol.

This study is exempt from institutional review board oversight, as determined by Western Institutional Review Board. Exemption is applicable because of de-identification of the data presented (45 CFR part 46.101(b)(4)).

Study population

This population totals 262 399 individuals that elected expanded carrier screening that included SLOS between January 2012 and December 2015. Carrier status for up to 109 genes in addition to *DHCR7* could be assessed simultaneously. The laboratory's total tested population within this time range is greater than 262 399, but individuals were excluded from this analysis when any of the following occurred: An indication other than 'no family history (routine carrier screening)' was selected, SLOS was not included in a customized disease panel ordered by the physician, or the patient requested exclusion of his or her results for research purposes.

The ordering physician or the patient directly reported ethnicity. Unknown ethnicity could be selected. These unknown individuals and ones for which no response was selected are reported together.

All tests were ordered by a physician or other healthcare provider. Most were obstetricians, maternal fetal medicine specialists, reproductive endocrinologists, geneticists, and genetic counselors. Follow-up genetic counseling was made available at no cost to all individuals tested. Testing was performed as fee-for-service, typically paid for by a third party and/or the patient.

RESULTS

Data for ethnicities where $n > 9000$ and carrier frequency exceeds 0.5% are detailed in Table 2. Table S1 includes the remaining populations.

Patient demographics

Of 210 857 that had the genotyping assay, mixed/other Caucasians represented the largest reported ethnic group (25.14%) followed by Northern Europeans (23.40%). Finnish represented the smallest ethnic group (0.07%), and Native Americans were the smallest of the major US ethnic groups

Table 2 *DHCR7* carrier frequencies in selected populations

Mutation	Effect	African American	Ashkenazi Jewish	Mixed/other Caucasian	Hispanic	Northern European	Southern European
Tested by TG and NGS, with TG-specific alleles		13 871	19 519	66 084	20 231	58 439	9 472
c.1054C > T	R352W	0	1	1	0	2	0
c.1055G > A	R352Q	2	0	6	1	3	0
c.1210C > T	R404C	5	0	14	2	14	1
c.1228G > A	G410S	0	0	2	2	4	1
c.1342G > A	E448K	0	1	14	0	10	8
c.278C > T	T93M	2	0	8	7	7	1
c.452G > A	W151*	5	37	229	11	178	40
c.506C > T	S169L	0	0	2	1	3	1
c.724C > T	R242C	2	0	28	0	27	1
c.725G > A	R242H	1	0	8	2	6	2
c.906C > G	F302L	0	0	0	3	0	0
c.964-1G > C	IVS8-1G > C	59	410	866	90	811	85
c.976G > T	V326L	0	3	11	0	15	0
Tested by NGS, with NGS-specific alleles		3 284	4 695	13 073	3 377	9 109	1 512
c.964-1G > T		0	0	1	0	0	0
c.1057delG	aka V353Wfs*60	0	0	0	0	1	0
c.1139G > A	C380Y	0	0	1	0	0	0
c.1222T > C	Y408H	0	0	1	0	0	0
c.1295A > G	Y432C	0	0	1	0	0	0
c.1337G > A	R446Q	0	0	3	1	2	1
c.1389insT		0	0	1	0	0	0
c.1426T > C	aka p. *476Qext*51	0	0	1	0	0	0
c.1A > G	M1V	0	0	0	0	1	1
c.292C > T	Q98*	0	0	1	0	0	0
c.355delC	aka p.H119Ifs*8	0	0	0	0	1	0
c.3G > A	M1I	0	0	1	0	0	0
c.413-2A > G		0	0	0	0	1	0
c.461C > G	T154R	0	0	3	0	3	0
c.461C > T	T154M	0	0	1	0	0	0
c.546G > A	W182*	0	0	0	0	1	1
c.651C > A	Y217*	0	0	0	1	0	0
c.952delT		0	0	0	0	1	0
c.963 + 1G > A		0	0	1	0	0	0
c.964-1G > T		0	0	2	0	2	0
Cumulative frequency (TG and NGS)		76 (0.55%) 1 in 183	452 (2.32%) 1 in 43	1207 (1.83%) 1 in 55	121 (0.60%) 1 in 167	1093 (1.87%) 1 in 54	143 (1.51%) 1 in 66

NGS, next-generation sequencing; TG, targeted genotyping.

(0.18%). Nearly 14% of the tested population had unknown or unreported ethnicity.

Targeted mutation data

Of ten ethnic groups with $n > 3000$, the highest carrier frequency was found among Ashkenazi Jews (2.35% or 1/42) and the lowest among South Asians (0.07% or 1/1477). In general, the frequency was low among Asian populations. On the other hand, all populations of European origin showed carrier frequencies exceeding 1%.

Of the 13 targeted mutations assayed, all were detected six times at minimum, and 11 of the mutations were detected at

least ten times. Nonetheless, two were predominantly frequent. The null c.964-1G > C mutation was most frequent, accounting for 75.0% of carriers identified. It was the most frequent, or tied for most frequent, mutation identified in non-Asian ethnic groups. But, these latter populations had few carriers identified. Where c.964-1G > C was the most frequent mutation, we observed varying carrier frequency, ranging from 2.14% in Ashkenazi Jewish to 0.10% in Middle Easterners.

The second most frequent allele was c.452G > A, accounting for 16.5% of all carriers' mutations. It was most common in the Cajun/French-Canadian population, with a carrier frequency of 0.52%.

Next-generation sequencing data

Included in the targeted mutation dataset earlier, 51 542 individuals underwent comprehensive mutation analysis through NGS. The same eligibility criteria apply to these data as described in the Methods section.

The patient demographic pattern approximates that of the larger genotyped population. Mixed/other Caucasians (25.4%) and Northern Europeans (17.7%) were the largest populations. Greater than 800 individuals were tested in ten ethnic groups, ranging from 834 (Southeast Asian) to 13 073 (mixed/other Caucasian).

As expected, in most ethnic groups, the carrier frequency by comprehensive analysis was higher compared with that by targeted analysis. The relative increase varied. A greater increase was observed among non-Caucasian groups, which also had the lowest initial frequency. This is logical; the targeted panel was based on studies primarily conducted in European populations, and even the most common alleles were infrequent among non-European groups. Therefore, discovery of additional infrequent alleles would have greater impact on overall carrier tabulations.

Finally, in order to elucidate the benefit conferred by the NGS approach, the percentage of carriers identified by NGS and not identified by targeted analysis was calculated. This ranged from 0% (four ethnic groups) to 80% (East Asians), and overall, the targeted approach detected 92.4% of all of the mutations detected in this predominantly European population (59% of individuals). Table 3 details, among only the population tested by NGS, the numbers of mutations that were included on the 13 mutation panel or the NGS panel.

In total, the NGS approach identified 58 occurrences of 30 unique mutations that were not on the targeted mutation panel. Three mutations were identified in more than three individuals; c.1337G > A was identified nine times in five patient populations.

One potentially 'affected' individual was identified in the NGS dataset: A person that was compound heterozygous for two *DHCR7* mutations: c.111G > A and c.429 T > G. The individual underwent genetic counseling, and no related symptoms were apparently reported. Further investigation was not initiated at that time. Possible explanations include unreported or unknown clinical symptoms or diagnosis, *cis* configuration of alleles, genetic 'diagnosis' with other modifying/alleviating factor, or laboratory error.

Impact on conceptus survival rates

Published disease incidence estimates at birth range from 1/20 000 to 1/101 000. The largest non-mixed population, Northern Europeans ($n = 58\,439$), were commonly studied in those literature sources as well. SLOS birth incidence based on Hardy–Weinberg principles is predicted to be 1/11 435 based on the following calculation:

$$q = \sum \text{allele1, allele2 } \ldots \text{ allele43} = 0.0093516;$$
$$1/q^2 = 11\ 435$$

Using the highest and lowest birth incidence estimates earlier, these data suggest an *in utero* demise rate of 42% to 88%.

Table 3 **Comparison of NGS and TG methodologies for Smith–Lemli–Opitz syndrome carrier detection in selected populations ($n > 800$)**

Ethnicity	Tested by NGS, *n*	All carriers detected by NGS, *n*	Carriers detectable by TG panel, *n*	Carriers missed by TG panel (%)
African	3284	14	14	0
Ashkenazi Jewish	4695	103	103	0
Mixed/other Caucasian	13 073	258	240	7
East Asian	3102	5	1	80
Hispanic	3377	29	27	7
Middle Eastern	861	4	2	50
Northern European	9109	178	165	7
South Asian	1872	3	1	67
Southeast Asian	834	2	1	50
Southern European	1512	31	28	10
Unknown	9518	128	115	10

TG, targeted genotyping.

DISCUSSION

Accurate carrier frequencies for SLOS are reported here, based on screening of a large general population cohort. Frequencies are approximately 2% (1/50) in Caucasians and Ashkenazi Jews and exceed 0.5% (1/200) in Hispanics and African Americans. These are meaningful because current carrier screening guidelines include diseases of similar frequency and specifically identify that as one factor in favor of population screening.[19] Comparisons of the disease's predicted birth incidence from the data presented here and observed birth incidences from the literature suggest a substantial proportion of affected conceptuses do not survive.

The overall carrier frequency for this population is 1.4%, although this has limited application to an individual clinical setting, given substantial ethnic variability. SLOS carriers are most frequent among individuals of European ancestry, in particular, Northern Europeans and Ashkenazi Jews. While previous disease incidence estimates have ranged from 1/20 000 to 1/101 000, these data predict an incidence beyond the highest end of that spectrum – at conception, 1/11 664 in Northern Europeans and 1/7396 in Ashkenazi Jews. Combining all Caucasian populations yields a carrier frequency of 1.7% and a predicted disease incidence at conception of 1/13 924.

In Hispanics and African Americans, carrier frequencies are 1/167 and 1/183, respectively. In these populations, predicted disease incidences are approximately 1/111 556 to 1 in 133 956. Carrier status for SLOS is very rare among all Asian populations we studied.

Differences between birth observation rates and these predictions may be due to the significant *in utero* mortality rate, which has previously been suggested to occur in up to 80% of conceptuses affected with SLOS.[20] Hydrops has been described in several cases of fetuses later diagnosed with SLOS, although it is also clear that this is not an inevitable outcome. It is noteworthy that a study in the Icelandic population predicted finding 19.1 individuals homozygous for c.964-1G > C in a population of 104 220 but actually found none, further suggesting early lethality of this genotype.[21] Craig *et al.* reported a large study of over a million pregnancies biochemically screened for SLOS.[4] They estimated a mid-trimester prevalence of 1/101 000 Caucasians. Two considerations in evaluating the difference between that prevalence and the data herein are that 30% of SLOS screen-positive fetuses were excluded from the Craig *et al.* analysis because of fetal demise and the biochemical screening performed in the second trimester does not detect conditions with first trimester lethality. Continued research may provide explanation, but the data here, in combination with those of Craig *et al.* suggest that first-trimester or second-trimester demise are the most likely outcome of SLOS-affected conceptuses. That likelihood depends on the true live birth incidence, but based on most estimates the prenatal mortality rate is 42–88%.

The data here are unique in that comprehensive exon analysis through NGS was utilized in over 51 000 individuals. In the only other SLOS study located using NGS, Cross *et al.* examined the frequency of *DHCR7* pathogenic variants in the 1000 genomes population.[22] In that, they found a 1.01% carrier frequency and predicted a disease incidence of 1/39 215 conceptions. However, they pool a number of non-Northern European populations (Colombian, Iberian, Puerto Rican, and Toscani) into their Northern European pool. The data here indicate that this pooling undercounts the actual frequency, because Hispanics and Southern Europeans have lower carrier frequencies. Restricting analysis to Northern European populations (British, Utah, and Finnish) shows six of 290 (2.01%) individuals to be carriers for the c.964-1G > C variant alone.

A comparison of detection by targeted genotyping or NGS in this study's population (Table 3) finds that NGS yielded a higher detection rate, particularly in the multiple Asian populations where 50–80% of carriers would not have been detected by the genotyping panel. Another assessment of a larger number of carriers will better define the benefits that NGS may provide.

This study's foremost limitation is that ethnicity reporting is based on the patient or clinic's report and may therefore be erroneously classified. In addition, the laboratory restricts selection to a single ethnic group – an unknown number of individuals have multiple ancestral backgrounds, and these are not accounted for. Ascertainment is also incomplete, because an individual had to elect carrier screening to be included in the dataset. Bias is minimized by limiting the dataset to individuals that reported no indication that increased the probability of positive SLOS carrier status, but this does not account for how the data may differ from an untested cohort, and there may be individuals included with unknown/unreported predisposition (e.g. pregnancy loss of undiagnosed SLOS etiology). Lastly, neither test methodology routinely detected large copy number variants. A similar large-scale study inclusive of these variant types would help further define the full mutation spectrum.

Carrier screening enables couples to plan and optimize reproductive outcomes, through preimplantation or prenatal genetic testing and/or educational and psychosocial preparations.[23] For SLOS specifically, an opportunity exists to eliminate the potential diagnostic odyssey that can arise in a subset of recurrent pregnancy loss scenarios.

These data present SLOS carrier frequencies obtained from large-scale routine carrier screening and suggest a substantial *in utero* mortality rate. These are the largest sample sizes reported to date of every major US-based population. Given the relatively high carrier frequency in a subset of populations, significant postnatal clinical impact, and the risk for pregnancy loss, routine preconception carrier screening is suggested.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Smith–Lemli–Opitz syndrome is an autosomal recessive multiple congenital anomaly syndrome with varying frequency estimates.
- Smith–Lemli–Opitz syndrome is presumed to be associated with an increased risk for pregnancy loss, although this risk has not been quantified.

WHAT DOES THIS STUDY ADD?

- By reporting results from a large, diverse tested population, these data define the carrier frequency in multiple ethnic groups.
- Predicted Smith–Lemli–Opitz syndrome frequency at birth is compared with actual frequencies from previous studies, enabling estimation of the pregnancy loss frequency.

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SUPPORTING INFORMATION

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