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

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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. © 2017 The Authors. Prenatal Diagnosis published by John Wiley & Sons, Ltd.

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 1/60,00011,12 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                  | Cardiac                        | Facial                  | Genital                      | Skeletal                  | Abdominal                  |
|--------------------------|-------------------------------|-------------------------|------------------------------|---------------------------|---------------------------|
| in utero demise           | Septal or major vessel defects| Cleft lip/palate        | Ambiguous genitalia          | Micromelia                | Renal hypoplasia or agenesis|
| Intrauterine growth retardation | Complex malformations       | Bilid uvula             |                              | Postaxial polydactyly     | Hydrenephrosis             |
| Nervous system            |                               | Short nose with anteverted nares |                              | 2–3 toe syndactyly        |                           |
| Ventricular dilatation    |                               |                         |                              | Microcephaly              |                           |
| Abnormal corpus calamus or cerebellum |                    |                         |                              |                           |                           |
| Dandy-Walker malformation/variant |                    |                         |                              |                           |                           |
| Holoprosencephaly         |                               |                         |                              |                           |                           |

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, 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.
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%.

### 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 |                  |                       |          |                  |                  |
| c.1054C>T |        | 13 871 | 19 519 | 66 084 | 20 231 | 58 439 | 9 472 |
| c.1055G>A |        | 2     | 0     | 6     | 1     | 3     | 0    |
| c.1210C>T |        | 5     | 0     | 14    | 2     | 14    | 1    |
| c.1228G>A |        | 0     | 0     | 2     | 2     | 4     | 1    |
| c.1342G>A |        | 0     | 1     | 14    | 0     | 10    | 8    |
| c.278C>T  |        | 2     | 0     | 8     | 7     | 7     | 1    |
| c.452G>A  |        | 5     | 37    | 229   | 11    | 178   | 40   |
| c.506C>T  |        | 0     | 0     | 2     | 1     | 3     | 1    |
| c.724C>T  |        | 2     | 0     | 28    | 0     | 27    | 1    |
| c.725G>A  |        | 1     | 0     | 8     | 2     | 6     | 2    |
| c.906C>G  |        | 0     | 0     | 0     | 3     | 0     | 0    |
| c.964IG>C |        | 59    | 410   | 866   | 90    | 811   | 85   |
| c.976G>T  |        | 0     | 3     | 11    | 0     | 15    | 0    |
|           |        | Tested by NGS, with NGS-specific alleles |                  |                       |          |                  |                  |
| c.964IG>T |        | 3 284 | 4 695 | 13 073| 3 377 | 9 109 | 1 512 |
| c.1057delG|        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.1139G>A |        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.1222T>C |        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.1295A>G |        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.1337G>A |        | 0     | 0     | 3     | 1     | 2     | 1    |
| c.1389insT|        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.1426T>C |        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.1A>G    |        | 0     | 0     | 0     | 0     | 0     | 0    |
| c.292C>T  |        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.355delC |        | 0     | 0     | 0     | 0     | 1     | 0    |
| c.3G>A    |        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.413-2A>G|        | 0     | 0     | 0     | 0     | 1     | 0    |
| c.461C>G  |        | 0     | 0     | 3     | 0     | 3     | 0    |
| c.461C>T  |        | 0     | 0     | 1     | 0     | 0     | 0    |
| c.546G>A  |        | 0     | 0     | 0     | 1     | 0     | 0    |
| c.651C>A  |        | 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.964IG>T |        | 0     | 0     | 2     | 0     | 2     | 0    |
|           | Cumulative frequency (TG and NGS) | 76 (0.55%) | 452 (2.32%) | 1207 (1.83%) | 121 (0.60%) | 1093 (1.87%) | 143 (1.51%) |
|           | 1 in 183 | 1 in 43 | 1 in 55 | 1 in 167 | 1 in 54 | 1 in 66 |

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

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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.429T>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:

\[ q = \sum \text{allele}_1, \text{allele}_2 \ldots \text{allele}_{43} = 0.0093516; \]

\[ 1/q^2 = 11,435 \]

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

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### 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             | 5                               | 1                                 | 67                              |
| Southeast Asian  | 834              | 2                               | 1                                 | 50                              |
| Southern European| 1512             | 31                              | 28                                | 10                              |
| Unknown          | 9518             | 128                             | 115                               | 10                              |

TG, targeted genotyping.

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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. 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 1/20000 to 1/101 000, these data predict an incidence beyond the highest end of that spectrum – at conception, 1/11644 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 1/13924.

In Hispanics and African Americans, carrier frequencies are 1/167 and 1/183, respectively. In these populations, predicted disease incidences are approximately 1/111556 to 1 in 133956. 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. 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. Craig et al. reported a large study of over a million pregnancies biochemically screened for SLOS. 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 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. 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.
REFERENCES

1. Kelley RI, Herman GE. Inborn errors of sterol biosynthesis. Annu Rev Genomics Hum Genet 2001;2:299–341.
2. Kelly MN, Tull SY, Tull SS, et al. Brothers with Smith–Lemli–Opitz syndrome. J Pediatr Health Care 2014;29:97–103.
3. Kelley RI, Hennekam RCM. The Smith–Lemli–Opitz syndrome. J Med Genet 2000;37:321–35.
4. Craig WY, Haddow JE, Palomaki GE, et al. Identifying Smith–Lemli–Opitz syndrome in conjunction with prenatal screening for Down syndrome. Prenat Diagn 2006;26:842–89.
5. Nowaczyk MJM, McCaughey D, Whelan DT, Porter FD. Incidence of Smith–Lemli–Opitz syndrome in Ontario, Canada. Am J Med Genet 2001;102:18–20.
6. Wright BS, Nwokoro NA, Wassil CA, et al. Carrier frequency of the RSH/Smith–Lemli–Opitz IVS8-1G > C mutation in African Americans. Am J Med Genet Part A 2003;128A:139–41.
7. Lazarin GA, Haque IS, Nazareth S, et al. Detection of a common mutation in the RSH or Smith–Lemli–Opitz syndrome. Clinical genomics and prevalence of the RSH/Smith–Lemli–Opitz syndrome. Prenat Diagn 2006;26:842–89.
8. Waye JS, Nakamura LM, Eng B, et al. Smith–Lemli–Opitz syndrome: carrier frequency and spectrum of DHCR7 mutations in Canada. J Med Genet 2002;39:e31.
9. Battaile KP, Battaile BC, Merkens LS, et al. Carrier frequency of the common mutation IVS8-1G > C in DHCR7 and estimate of the expected incidence of Smith–Lemli–Opitz syndrome. Mol Genet Metab 2001;72:67–71.
10. Waterham HR, Hennekam RCM. Mutational spectrum of Smith–Lemli–Opitz syndrome. Am J Med Genet C 2012;160C:263–84.
11. Ryan AK, Bartlett K, Clayton P, et al. Smith–Lemli–Opitz syndrome: a variable clinical and biochemical phenotype. J Med Genet 1998;35:558–65.
12. Nowaczyk MJM, Zeesman S, Waye JS, Douketis JD. Incidence of Smith–Lemli–Opitz syndrome in Canada: results of a three-year population surveillance. J Pediatr 2004;145:530–5.
13. Opitz JM, Penchasazadeh VB, Holt MC, Spano LM. Smith–Lemli–Opitz (RSH) syndrome bibliography. Am J Med Genet 1987;28:745–50.
14. Nowaczyk MJM, McCaughey D, Whelan DT, Porter FD. Incidence of Smith–Lemli–Opitz syndrome in Ontario, Canada. Am J Med Genet 2001;102:18–20.
15. Wright BS, Nwokoro NA, Wassil CA, et al. Carrier frequency of the RSH/Smith–Lemli–Opitz IVS8-1G > C mutation in African Americans. Am J Med Genet Part A 2003;128A:139–41.
16. Lanthaer B, Hinderhofer K, Maas B, et al. Characterizations of large deletions in the DHCR7 gene. Clin Genet 2014;88:149–54.
17. Richards CS, Bale S, Bellisimo DB, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2017. Genet Med 2018;10:294–300.
18. Karimi K, Kang P, Haque I, Evans E. Curation and classification of inherited disease variants in a high-throughput clinical-grade genetic screening laboratory environment. 2015. Available at: http://research.counsyl.com/posters/2015/Biocuration/Biocuration-poster-2015_V2_R1.pdf. Accessed Feb 8 2016.
19. Gross SJ, Fletcher RA, Monaghan KG. Carrier screening in individuals of Ashkenazi Jewish descent. Genet Med 2008;10:54–6.
20. Putnam AR, Szakacs JG, Opitz JM, Byrne JLB. Prenatal death in Smith–Lemli–Opitz/RSH syndrome. Am J Med Genet 2005;138A:61–5.
21. Sulem P, Helgason H, Oddson A, et al. Identification of a large set of rare complete human knockouts. Nat Genet 2015;47:448–52.
22. Cross JL, Iben J, Simpson CL, et al. Determination of the allelic frequency in Smith–Lemli–Opitz syndrome by analysis of massively parallel sequencing data sets. Clin Genet 2015;87:570–5.
23. Edwards JG, Feldman G, Goldberg J, et al. Expanded carrier screening in reproductive medicine – points to consider. Obstet Gynecol 2015;125:653–62.

SUPPORTING INFORMATION

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