**REVIEW**

Investigation of 7-dehydrocholesterol reductase pathway to elucidate off-target prenatal effects of pharmaceuticals: a systematic review

MR Boland1,2 and NP Tatonetti1,2,3,4

Mendelian diseases contain important biological information regarding developmental effects of gene mutations that can guide drug discovery and toxicity efforts. In this review, we focus on Smith–Lemli–Opitz syndrome (SLOS), a rare Mendelian disease characterized by compound heterozygous mutations in 7-dehydrocholesterol reductase (DHCR7) resulting in severe fetal deformities. We present a compilation of SLOS-inducing DHCR7 mutations and the geographic distribution of those mutations in healthy and diseased populations. We observed that several mutations thought to be disease causing occur in healthy populations, indicating an incomplete understanding of the condition and highlighting new research opportunities. We describe the functional environment around DHCR7, including pharmacological DHCR7 inhibitors and cholesterol and vitamin D synthesis. Using PubMed, we investigated the fetal outcomes following prenatal exposure to DHCR7 modulators. First-trimester exposure to DHCR7 inhibitors resulted in outcomes similar to those of known teratogens (50 vs 48% born-healthy). DHCR7 activity should be considered during drug development and prenatal toxicity assessment.

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**INTRODUCTION**

Mendelian diseases are genetic conditions that follow a ‘traditional’ pattern of inheritance. Previously, researchers utilized information from Mendelian gene mutations to study shared underlying disease mechanisms that are common to non-Mendelian diseases in complex diseases and cancer. Mendelian diseases are also useful in studying developmental effects of gene mutations and can help researchers understand the effects of a potential pharmaceutical target or off-target effect, increasing the impact of their discoveries. Understanding the underlying mechanisms of Mendelian diseases can enable a priori prediction of fetal outcomes following prenatal pharmaceutical exposure.

In this review, we detail one orphan Mendelian disease—Smith–Lemli–Opitz syndrome (SLOS) resulting from mutations in 7-dehydrocholesterol reductase (DHCR7). These mutations affect a pathway involving vitamin D and cholesterol production. Mutations affecting vitamin metabolism can have an important role in drug response. In-depth study of this biological pathway enables us to explain off-target effects of prenatal drug exposure and highlights DHCR7’s importance in drug development for potential prenatal toxicity assessment.

Clinical characteristics

SLOS was first identified in 1964 when physicians described a similar pattern of congenital anomalies, including mental retardation, incomplete external genitalia and abnormalities of face, hands and feet that followed a familial inheritance pattern. Later, it was discovered that extremely high 7-dehydrocholesterol levels and surprisingly low serum cholesterol levels were common biomarkers of SLOS. This led to the discovery of the exact location in the cholesterol synthesis pathway that was defective in SLOS patients, namely the conversion of 7-dehyrocholesterol into cholesterol (the last step in cholesterol biosynthesis). Subsequently, DHCR7 was identified as the culprit gene. DHCR7 is the only enzyme that converts 7-dehydrocholesterol to cholesterol. Cholesterol cannot be produced without DHCR7.

The physical presentation of SLOS differs widely among individuals, varying by severity, genotype and other environmental factors. The most frequently occurring feature is 2/3 toe syndactyly (that is, ‘webbed toes’) occurring among 97% of patients followed by mental retardation with 95% of patients. Other common signs include microcephaly (84%), postnatal growth retardation (82%), anteverted nares (78%), ptosis (70%), genital anomalies (65%) and congenital heart defects (among 54% of SLOS patients). SLOS severity ranges across a wide spectrum. Some SLOS patients present with a mild form with minimal symptoms and no developmental delay. Others have a severe form that can result in a lack of sexual dimorphism with a functional XY karyotype and female internal and external genitalia.

The importance of cholesterol in prenatal embryonic and fetal development, and its partial to complete absence in SLOS, helps to explain the pleotropic phenotypes within SLOS. In patients possessing homozygous null mutations in DHCR7, cholesterol production is absent and prenatal lethality results. Other mutations reduce DHCR7 expression to < 5%, dramatically reducing cholesterol production in the body.

1Department of Biomedical Informatics, Columbia University, New York, NY, USA; 2Observational Health Data Sciences and Informatics, Columbia University, New York, NY, USA; 3Department of Systems Biology, Columbia University, New York, NY, USA and 4Department of Medicine, Columbia University, New York, NY, USA. Correspondence: Dr NP Tatonetti, Department of Biomedical Informatics, Columbia University, 622 West 168th Street, PH-20, New York, NY 10032, USA.

E-mail: nick.tatonetti@columbia.edu

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SLOS is an inherited autosomal recessive disease with each parent contributing one mutated copy of DHCR7. Inheritance follows a compound heterozygous pattern whereby each parent contributes one copy of different mutations in DHCR7. Therefore, the SLOS patient is heterozygous for two mutations. Being heterozygous for only one mutation generally does not cause the SLOS phenotype, although instances have been reported.\textsuperscript{8,16} Being homozygous for a null mutation in DHCR7 typically results in prenatal death.\textsuperscript{15} This explains why most full-term viable SLOS patients are compound heterozygotes. Figure 1 depicts the autosomal inheritance of SLOS in children and how compound heterozygosity is responsible for the disease phenotype. Many SLOS genetic studies focus on compound heterozygous patients because most homozygous phenotypes result in prenatal fatalities, reducing the detection rate. Both W151X and IVS8-1G>C are null mutations in DHCR7 meaning that they reduce DHCR7 expression to almost 0% in the homozygous state. Therefore, if an individual is homozygous for either of these mutations or heterozygous for the combo then little to no DHCR7 expression would result.\textsuperscript{93} On the other hand, T93M is a non-null mutation in DHCR7 that reduces DHCR7 expression by 5% when compared to normal.\textsuperscript{77} Therefore, a compound heterozygous patient with one IVS8-1G>C null mutation and one T93M mutation would have around 45% functional DHCR7 and SLOS would result, but prenatal fatality would be averted (a). DHCR7, 7-dehydrocholesterol reductase; SLOS, Smith–Lemli–Opitz syndrome.

**Figure 1.** Full-term SLOS patients are typically compound heterozygous for two distinct mutations in DHCR7 (a), whereas homozygous null individuals are detected less frequently due to prenatal lethality (b) depicts the autosomal inheritance of SLOS in children and how compound heterozygosity is responsible for the disease phenotype. Many SLOS genetic studies focus on compound heterozygous patients (a) because most homozygous phenotypes result in prenatal fatalities, reducing the detection rate (b). Both W151X and IVS8-1G>C are null mutations in DHCR7 meaning that they reduce DHCR7 expression to almost 0% in the homozygous state. Therefore, if an individual is homozygous for either of these mutations or heterozygous for the combo then little to no DHCR7 expression would result.93 On the other hand, T93M is a non-null mutation in DHCR7 that reduces DHCR7 expression by 5% when compared to normal.77 Therefore, a compound heterozygous patient with one IVS8-1G>C null mutation and one T93M mutation would have around 45% functional DHCR7 and SLOS would result, but prenatal fatality would be averted (a). DHCR7, 7-dehydrocholesterol reductase; SLOS, Smith–Lemli–Opitz syndrome.

Genetic characteristics

SLOS is an inherited autosomal recessive disease with each parent contributing one mutated copy of DHCR7. Inheritance follows a compound heterozygous pattern whereby each parent contributes one copy of different mutations in DHCR7. Therefore, the SLOS patient is heterozygous for two mutations. Being heterozygous for only one mutation generally does not cause the SLOS phenotype, although instances have been reported.\textsuperscript{8,16} Being homozygous for a null mutation in DHCR7 typically results in prenatal death.\textsuperscript{15} This explains why most full-term viable SLOS patients are compound heterozygotes. Figure 1 depicts the autosomal inheritance of SLOS in children and how compound heterozygosity is responsible for the disease phenotype. The discrepancy between the DHCR7 mutation carrier rate and SLOS incidence\textsuperscript{17} is believed to result from prenatal loss of individuals with homozygous null mutations during the first trimester.\textsuperscript{15} As in many inherited genetic conditions, de novo mutations have also been reported.\textsuperscript{18}

Importantly, the relationship between null mutations in DHCR7 and SLOS severity is not one-to-one because variations in the maternal genome can increase the amount of cholesterol passed by the placenta to the fetus\textsuperscript{15} modulating the offspring’s phenotype. Because cholesterol is critical during early development, having increased prenatal cholesterol levels distributed from the mother via the placenta can mitigate many SLOS symptoms.\textsuperscript{15} Diverse factors modulate SLOS severity, therefore it is not possible to completely predict the disease phenotype using genotype information alone or vice-versa.\textsuperscript{19}

**COMPENDIUM CONTAINING SLOS-INDUCING DHCR7 MUTATIONS**

Development of the DHCR7 SLOS mutation compendium

SLOS patients are compound heterozygotes for diverse mutations in DHCR7 or homozygous for non-null mutations. SLOS is a rare disease (~1 in 40,000) and studies typically involve only small cohorts of patients. Even with small cohorts, many DHCR7 mutations have been reported. Therefore, we reviewed the literature and developed a compendium of SLOS-inducing DHCR7 mutations. We collected the reported frequencies for each mutation across all studies, and when available, we extracted the patient genotype (that is, compound heterozygous mutant alleles) and their geographic location or ethnicity. We are interested in the ethnicities of SLOS patients and their corresponding genotypes because certain gene variants affect outcomes only within a given ethnicity (for example, ACBC1 variant in Caucasians).\textsuperscript{20}

To develop our compendium, we analyzed all publicly available DHCR7 mutations contained in the Human Gene Mutation Database (HGMD)\textsuperscript{21,22} and their corresponding publications (HGMD last accessed in May 2015). We kept track of patients’ DHCR7 genotype information and their ethnicity/geographic origin when available (described later). The aggregated allele frequencies for all DHCR7 mutations across 30 studies are given in Supplementary Table S1. Our review focuses only on deleterious mutations to DHCR7, and therefore does not include 13 silent DHCR7 mutations.\textsuperscript{23} Using the HGMD, we found 138 distinct publicly available mutations to DHCR7 and 165 reported (with the additional 27 mutations being proprietary, and only accessible via paid membership).

Our literature review revealed additional DHCR7 mutations (by investigating papers cited in those retrieved papers) and overall our compendium contains 147 DHCR7 mutations, 145 of these being SLOS-inducing mutations. One missense mutation, W158C, was found in an unaffected sibling of a SLOS individual (noted in Supplementary Table S1), and another mutation, G344D, was reported in a patient with holoprosencephaly (noted in Supplementary Table S1) a different mutation at the same position, G344R, was reported in a SLOS patient. Mutations in DHCR7 that result in holoprosencephaly were also included in the compendium and denoted in Supplementary Table S1.

**Common SLOS-inducing DHCR7 mutations**

We found that 12 mutations (of 145 SLOS-inducing mutations) occurred with allele frequency of at least 1% across the 30 studies we reviewed (Table 1). The most common of these were IVS8-1G>C (28.446%), T93M (9.384%), W151X (8.407%) V326L (5.083%), R404C (3.519%) and R352W (3.324%). We compared this result to data obtained from 60,706 assumed healthy individuals (that is, non-SLOS) to determine the frequency of various DHCR7 mutations in that reference population. We used the ExAC database\textsuperscript{24} available at http://exac.broadinstitute.org/ (accessed November 2015). These results are also given in Table 1. We believe the ExAC population are non-SLOS individuals, however it is likely that they have other conditions. Therefore, from henceforth we refer to this population as the ExAC population. The majority of frequent DHCR7 mutations in SLOS patients also occur at higher frequencies in the ExAC population (this is intuitive). There are two exceptions: T93M that was common among SLOS patients (9.4%) but rare in the healthy population (only 3 allele mutations observed out of 60,706 individuals). Another counter-intuitive result was that R242C was found
relatively frequent in the ExAC population (10 allele mutations observed), but was only mutated in 1.2% for SLOS. We present the top 10 DHCR7 SLOS-inducing mutations ranked by their frequency in the ExAC population. Of these, one mutation in V326L and one had a mutation in R352W. Both mutations were shown to reduce expression of DHCR7 upon heterologous expression by > 90%.

### Table 1. DHCR7 mutations implicated in SLOS with allele frequency >1% across 30 studies

| Allele's effect on coding sequence | Genomic chromosome position | Accession number (RS ID) | Intron/exon | Allele freq. in 523 SLOS patients, N = 1037 alleles (%) | Allele freq. in ExAC population, N = 60 706 healthy individuals (%) |
|----------------------------------|----------------------------|--------------------------|-------------|----------------------------------------------------------|------------------------------------------------------------------|
| IVS8-1G > C<sup>a</sup>          | 71146886                   | rs138659167              | Intron 8    | 291 (28.4)                                               | 386 (4.2 × 10<sup>-3</sup>)                                      |
| T93M                             | 71155082                   | rs80338853               | Exon 4      | 96 (9.4)                                                  | 3 (2.7 × 10<sup>-3</sup>)                                        |
| W151X                            | 71152447                   | rs11555217               | Exon 6      | 86 (8.4)                                                  | 82 (6.8 × 10<sup>-3</sup>)                                       |
| Y326L<sup>c</sup>                | 71146873                   | rs80338859               | Exon 9      | 52 (5.1)                                                  | 5 (4.8 × 10<sup>-3</sup>)                                        |
| R404C                            | 71146639                   | rs61757582               | Exon 9      | 36 (3.5)                                                  | 4 (3.5 × 10<sup>-3</sup>)                                        |
| R352W<sup>f</sup>                | 71146795                   | rs80338860               | Exon 9      | 34 (3.3)                                                  | 2 (1.7 × 10<sup>-3</sup>)                                        |
| E448K                            | 71146507                   | rs80338864               | Exon 9      | 23 (2.2)                                                  | 1 (8.5 × 10<sup>-3</sup>)                                        |
| R352Q                            | 71146794                   | rs121909768              | Exon 9      | 22 (2.2)                                                  | 4 (3.4 × 10<sup>-3</sup>)                                        |
| G410S                            | 71146621                   | rs80338862               | Exon 9      | 15 (1.5)                                                  | 5 (4.3 × 10<sup>-3</sup>)                                        |

<sup>a</sup>Obtained from ExAC output, accessed in November 2015 (http://exac.broadinstitute.org/). <sup>b</sup>Annotated as: c.964-1G in some literature articles. <sup>c</sup>SLOS patients with single mutations (true heterozygotes) were found (three patients), two had mutations in V326L and one had a mutation in R352W. Both mutations were shown to reduce expression of DHCR7 upon heterologous expression by > 90%.

### Table 2. Top 10 DHCR7 SLOS-inducing mutations ranked by frequency in ExAC population

| Allele's effect on coding sequence | ExAC allele frequency (%) | Overall frequency (%) among SLOS patients |
|----------------------------------|----------------------------|------------------------------------------|
| Overall                          | African | East Asian | European (Non-Finnish) | Finnish | Latino | Other | South Asian |
| IVS8-1G > C<sup>a</sup>          | 0.420     | 0.295     | 0                        | 0.677    | 0.177  | 0.165 | 0.149      | 0.007    | 28.062 |
| W151X                            | 0.068     | 0.029     | 0                        | 0.116    | 0       | 0.009 | 0.111      | 0        | 8.39   |
| V330M                            | 0.036     | 0.012     | 0.012                    | 0.044    | 0.018  | 0.082 | 0          | 0.007    | 0.096  |
| T154R                            | 0.008     | 0.010     | 0                        | 0.014    | 0       | 0     | 0          | 0        | 0.193  |
| R242C                            | 0.008     | 0.029     | 0.023                    | 0.008    | 0       | 0     | 0          | 0        | 1.157  |
| S169L                            | 0.008     | 0.010     | 0                        | 0.012    | 0       | 0     | 0.110      | 0        | 0.868  |
| G303R                            | 0.007     | 0.010     | 0.046                    | 0.005    | 0       | 0.009 | 0          | 0        | 0.579  |
| R352W<sup>f</sup>                | 0.007     | 0         | 0                        | 0.011    | 0.015  | 0     | 0          | 0        | 0.096  |
| G410S                            | 0.007     | 0         | 0                        | 0.012    | 0       | 0     | 0          | 0        | 0.675  |
| L157P                            | 0.007     | 0         | 0                        | 0.012    | 0       | 0     | 0          | 0        | 0.675  |

Ethnicity with highest allele frequency for each DHCR7 SLOS-inducing mutation is bolded. <sup>a</sup>c.964-1G>C in some literature articles.

patients, we would expect the common allele frequencies to change somewhat and become less biased towards Northern Europeans. A couple of DHCR7 mutations were reported to cause SLOS in the heterozygous form, however, it is likely that an unidentified suspected variant was present in these instances, as SLOS results from two defective copies of DHCR7, either in a compound heterozygous state (2 different DHCR7 mutations) or a homozygous mutated state. Importantly some asymptomatic siblings of SLOS patients were heterozygous carriers of the null W151X mutation (they should have around 50% expression of DHCR7) and they exhibited no symptoms of SLOS suggesting that the disease causing state requires DHCR7 expression to be < 50%. This also confirms that one copy of a DHCR7 mutation does not result in SLOS.

### DHCR7 MUTATIONS VARY BY GEOGRAPHICAL LOCATION

Specific DHCR7 mutations exhibit geographic dependency. SLOS incidence ranges by geographical location and ethnicity because carrier frequencies vary by region. The incidence in both the United States of America (USA) and Europeans is 1 in 30 000 live births. Variations in reported incidence rates are thought to be due to sampling bias differences. The carrier rate, or the
| Genotype | Prenatal lethal | Reference(s) | No. of patients (N = 229) | Incidence (%) |
|----------|----------------|--------------|----------------------------|---------------|
| **Homozygous** | | | | |
| IVS8-1G > C | IVS8-1G > C | Yes/or shortly after birth | 15, 95–99 | 18 | 7.860 |
| W151X | W151X | Yes | 15,100 | 8 | 3.493 |
| R352Q | R352Q | No | 26,44,101 | 6 | 2.620 |
| T93M | T93M | No | 16,37,41 | 4 | 1.747 |
| R352W | R352W | No | 26 | 2 | 0.873 |
| P467L | P467L | No | 43 | 2 | 0.873 |
| E448K | E448K | No | 16,102 | 2 | 0.873 |
| IVS8-1G > T | IVS8-1G > T | Yes | 15 | 1 | 0.437 |
| G963 134 bp frameshift | G963 134 bp frameshift | Died shortly after birth | 103 | 1 | 0.437 |
| L109P | L109P | No | 100 | 1 | 0.437 |
| N287K | N287K | No | 44 | 1 | 0.437 |
| R446Q | R446Q | NA | 97 | 1 | 0.437 |
| R352L | R352L | No | 44 | 1 | 0.437 |
| **Total homozygous** | | | | 48 | 20.961 |
| **Compound heterozygous** | | | | |
| IVS8-1G > C | T93M | No | 30,37,41,98,99,102 | 21 | 9.170 |
| IVS8-1G > C | W151X | No | 15 | 7 | 3.057 |
| IVS8-1G > C | G410S | No | 97 | 6 | 2.620 |
| IVS8-1G > C | T154M | Died shortly after birth/no | 95,99 | 3 | 1.310 |
| G147D | F302L | Yes for ref. 1, NA for ref. 2 | 97 | 3 | 1.310 |
| R352Q | G303R | No/died shortly after birth | 26,101 | 3 | 1.310 |
| IVS8-1G > C | C183Y | Died shortly after birth in some cases | 95 | 2 | 0.873 |
| T93M | Q98X | No | 38,41 | 2 | 0.873 |
| W151X | V326L | No | 8 | 2 | 0.873 |
| W151X | C380S | No | 8 | 2 | 0.873 |
| V326R | L68P | No | 38 | 2 | 0.873 |
| IVS8-1G > C | M1V | No | 38,98 | 2 | 0.873 |
| IVS8-1G > C | P51S | No | 8,97 | 2 | 0.873 |
| IVS8-1G > C | L99P | No | 8,102 | 2 | 0.873 |
| IVS8-1G > C | S169L | Died shortly after birth/no | 97,99 | 2 | 0.873 |
| IVS8-1G > C | A247V | No | 99 | 2 | 0.873 |
| IVS8-1G > C | F302L | Possibly | 97 | 2 | 0.873 |
| IVS8-1G > C | V326L | No | 96,97 | 2 | 0.873 |
| W151X | I145L | No | 100 | 2 | 0.873 |
| W151X | L157P | No | 8,100 | 2 | 0.873 |
| W151X | V326L | No | 100 | 2 | 0.873 |
| H119L | G244R | Died shortly after birth in some cases | 95,103 | 2 | 0.873 |
| H301Q | R242H | No | 97 | 2 | 0.873 |
| F302L | L470Q | No | 99 | 2 | 0.873 |
| G303R | R352W | No | 26 | 2 | 0.873 |
| H405Y | G138V | No | 29 | 2 | 0.873 |
| T93M | W151X | No | 16,30 | 2 | 0.873 |
| W151X | R352W | No | 100 | 2 | 0.873 |
| T93M | G147D | No | 97 | 1 | 0.437 |
| T93M | T154R | No | 98 | 1 | 0.437 |
| T93M | S192F | No | 38 | 1 | 0.437 |
| T93M | R228W | No | 38 | 1 | 0.437 |
| T93M | D234Y | No | 37 | 1 | 0.437 |
| T93M | R242H | Died shortly after birth | 95 | 1 | 0.437 |
| T93M | G244R | No | 30 | 1 | 0.437 |
| T93M | N274K | No | 41 | 1 | 0.437 |
| T93M | V281M | No | 37 | 1 | 0.437 |
| T93M | F302L | No | 37 | 1 | 0.437 |
| T93M | G410S | No | 98 | 1 | 0.437 |
| T93M | 720-735del frameshift | No | 8 | 1 | 0.437 |
| T93M | IVS5+1 del | No | 16 | 1 | 0.437 |
| IVS8-1G > C | A50N | No | 38 | 1 | 0.437 |
| IVS8-1G > C | P51H | No | 102 | 1 | 0.437 |
| IVS8-1G > C | Q98X | No | 15 | 1 | 0.437 |
| IVS8-1G > C | L109P | Died shortly after birth | 95 | 1 | 0.437 |
| IVS8-1G > C | H119L | No | 97 | 1 | 0.437 |
| IVS8-1G > C | H119fsX8 | No | 38 | 1 | 0.437 |
Table 3. (Continued)

| Genotype | Prenatal lethal | Reference(s) | No. of patients | Incidence (%) |
|----------|-----------------|--------------|-----------------|---------------|
| IVS8-1G > C | F174S | No | 41 | 1 | 0.437 |
| IVS8-1G > C | W182C | No | 97 | 1 | 0.437 |
| IVS8-1G > C | W182L | Died shortly after birth | 95 | 1 | 0.437 |
| IVS8-1G > C | K198E | Died shortly after birth | 95 | 1 | 0.437 |
| IVS8-1G > C | F235S | No | 29 | 1 | 0.437 |
| IVS8-1G > C | R242H | NA<sup>b</sup> | 97 | 1 | 0.437 |
| IVS8-1G > C | W182C | Died shortly after birth | 95 | 1 | 0.437 |
| IVS8-1G > C | S254A | No | 97 | 1 | 0.437 |
| IVS8-1G > C | F255L | Died shortly after birth | 95 | 1 | 0.437 |
| IVS8-1G > C | I297T | No | 29 | 1 | 0.437 |
| IVS8-1G > C | H301R | No | 41 | 1 | 0.437 |
| IVS8-1G > C | P329L | No | 30 | 1 | 0.437 |
| IVS8-1G > C | 356delH | No | 96 | 1 | 0.437 |
| IVS8-1G > C | G366V | No | 104 | 1 | 0.437 |
| IVS8-1G > C | C380Y | No | 99 | 1 | 0.437 |
| IVS8-1G > C | R242H | NA | 97 | 1 | 0.437 |
| IVS8-1G > C | I297T | No | 29 | 1 | 0.437 |
| IVS8-1G > C | S254A | No | 97 | 1 | 0.437 |
| IVS8-1G > C | I297L | Died shortly after birth | 95 | 1 | 0.437 |
| IVS8-1G > C | H301R | No | 41 | 1 | 0.437 |
| IVS8-1G > C | F235S | No | 38 | 1 | 0.437 |
| IVS8-1G > C | R242H | No | 30 | 1 | 0.437 |
| IVS8-1G > C | E448K | No | 96 | 1 | 0.437 |
| IVS8-1G > C | R450L | No | 102 | 1 | 0.437 |
| IVS8-1G > C | F475S | No | 38 | 1 | 0.437 |
| IVS8-1G > C | Unidentified suspected variant | No | 41 | 1 | 0.437 |
| W151X | L109P | Died shortly after birth | 100 | 1 | 0.437 |
| W151X | N146K | No | 100 | 1 | 0.437 |
| W151X | I178F | No | 38 | 1 | 0.437 |
| W151X | W248C | No | 100 | 1 | 0.437 |
| W151X | G347S | No | 38 | 1 | 0.437 |
| W151X | R352Q | No | 100 | 1 | 0.437 |
| W151X | L360P | No | 38 | 1 | 0.437 |
| W151X | R446Q | Died shortly after birth | 100 | 1 | 0.437 |
| W151X | Unidentified suspected variant | Yes | 100 | 1 | 0.437 |
| W151X | G322 frameshift | No | 100 | 1 | 0.437 |
| P51S | N274K | Died shortly after birth | 97 | 1 | 0.437 |
| G322 frameshift | Unidentified suspected variant | No | 100 | 1 | 0.437 |
| V326R | L360P | No | 38 | 1 | 0.437 |
| Q98X | Unidentified suspected variant | No | 38 | 1 | 0.437 |
| L99P | G410S | No | 8 | 1 | 0.437 |
| T154M | Y219D | No | 100 | 1 | 0.437 |
| I178F | R242H | No | 38 | 1 | 0.437 |
| W182L | E224K | No | 38 | 1 | 0.437 |
| P243R | Y408H | Died shortly after birth | 97 | 1 | 0.437 |
| A247V | R404C | No | 8 | 1 | 0.437 |
| V273G | Y432C | No | 38 | 1 | 0.437 |
| H301R | W182L | No | 41 | 1 | 0.437 |
| V326L | G244R | No | 97 | 1 | 0.437 |
| V326L | R352W | No | 8 | 1 | 0.437 |
| V326L | E448K | No | 100 | 1 | 0.437 |
| Intron: G963 insertion of 134 bp frameshift | W248C | No | 103 | 1 | 0.437 |
| T93M | N407Y | No | 16 | 1 | 0.437 |
| R352W | R407Y | Died shortly after birth | 97 | 1 | 0.437 |
| R352W | K376R fs*37 | No | 26 | 1 | 0.437 |
| R352W | L317R | No | 98 | 1 | 0.437 |
| G322 frameshift | L109P | Yes | 100 | 1 | 0.437 |
| V330M | R363C | No | 30 | 1 | 0.437 |
| R352Q | R242H | No | 101 | 1 | 0.437 |
| R352Q | X476Q | No | 101 | 1 | 0.437 |
| R352Q | S192F | No | 101 | 1 | 0.437 |
| P227S | G303R | No | 26 | 1 | 0.437 |
| L68P | R404C | No | 29 | 1 | 0.437 |
| Q107H | C444Y | No | 99 | 1 | 0.437 |
| I145L | Y408H | No | 29 | 1 | 0.437 |
| N274K | V466A | No | 98 | 1 | 0.437 |
| N274K | G410S | No | 98 | 1 | 0.437 |
| R242C | G344R | No | 29 | 1 | 0.437 |
proportion of individuals with one copy of a known SLOS-inducing DHCR7 mutation, is estimated to be 3% among persons of European ancestry, whereas another study found 1–2% among Caucasians. In the USA the carrier frequency is 1%, although in the state of Utah the carrier rate is 4%.  

Mutation frequencies are known to vary by geographic location/ethnicity. Likewise, the spectrum of DHCR7 mutations varies by geographical location. There is evidence that T93M is the founder mutation with origins in the Mediterranean basin and is common in Italy, Spain and Portugal. On the other hand, Northern Europeans (Austrians, Germans, Dutch) present with the W151X mutation more frequently. In Korea, three mutations account for most of the observed variants. Persons of African ancestry rarely develop SLOS, however some carriers were observed among persons of African ancestry. One SLOS patient with Spanish-African mixed ancestry was identified, this patient had T93M (the common Spanish SLOS allele) and V281M. The carrier frequency of DHCR7 mutations among African Canadians was low at 0.79%. The relationship between the specific combinations of DHCR7 mutations and ethnicity/geographic location of SLOS patients warranted further exploration. Therefore, we extracted all genotype data for SLOS patients that also contained ethnicity and geographic information for DHCR7 mutations. The combination of exons 4 and 9, and also 7 and 12 occurred more frequently among Southern Europeans, whereas the null mutation W151X occurs more frequently in Northern Europeans. We also found exon ‘hotspots’ for the pairs of SLOS-inducing DHCR7 mutations. The combination of exons 4 and 9 or the exon 4–9 ‘hotspot’ were more frequent among Southern Europeans, whereas the exon 6–9 hotspot occurred frequently in Northern Europeans. We observed marked differences for the Asian population with the exon 8–9 hotspot occurring more frequently. Individuals from South America (South America: Brazil, United States of America-Hispanic) had three patterns that were equally frequent, the exon 4–9 hotspot (also common among S. Europeans), the exon 7–9 hotspot (unique to S. Americans), and the exon 8–9 hotspot (common among Asians). Importantly, the frequent N. European hotspot (exon 6–9 hotspot) was absent from the South American result.

A clear relationship exists between geography/ethnicity and specific DHCR7 mutations as shown in Figure 2d and has been described previously. In addition, we demonstrate that the specific exon ‘hotspots’ also vary by geographic location/ethnicity (Figure 2d).
selective pressure and a plausible heterozygote advantage for those variants. Researchers found certain DHCR7 variants were associated with lower vitamin D levels in the general population and among individuals with polycystic ovary syndrome. This further links vitamin D levels with DHCR7 variants.

Historically, there would have been an evolutionary pressure to maximize the small amount of sunlight available for vitamin D production in Northern climates due to lower sunlight exposure. In addition, benefits from prenatal vitamin D supplementation are also modulated by geographic location and other factors. Therefore biological mechanisms that maximize vitamin D production and absorption would be selected for in the North. A heterozygote advantage for individuals with one DHCR7 mutation would therefore exist to allow most of the body's 7-dehydrocholesterol to be converted into vitamin D (and not cholesterol). This has been used to explain why Northern populations have a higher prevalence of SLOS. Carriers also are hypothesized to have a reproductive advantage because of the reduced fetal death due to rachitic cephalopelvic disproportion. This is again related to increased vitamin D production.
production causing improved bone formation. Improved hip formation is thought to enable females to produce more offspring and thereby provide another carrier advantage. In addition, vitamin D has been shown to increase chances of pregnancy for infertile women receiving in vitro fertilization. Vitamin D has many pleiotropic roles in reproductive outcomes and can affect both male and female fertility. Therefore, there could be multiple mechanisms that could explain why evolution favors vitamin D production in Northern climates through mutations in DHCR7 (and other genes).

Biological mechanism: DHCR7’s effect on vitamin D and cholesterol synthesis

The biological mechanism that connects DHCR7 mutations (SLOS carriers) and Northern climate involves regulation of 7-dehydrocholesterol, vitamin D and cholesterol. Typically, 7-dehydrocholesterol can be converted into either cholesterol or vitamin D (cholecalciferol). The conversion of 7-dehydrocholesterol to vitamin D occurs in the skin upon exposure to ultraviolet B light (290–320 nm). Under normal sunlight conditions, only around 15% of available 7-dehydrocholesterol will be converted to vitamin D, whereas excess 7-dehydrocholesterol in the skin will be converted to inert compounds for degradation. DHCR7 is the sole enzyme used to convert 7-dehydrocholesterol to cholesterol. A shortage of DHCR7 would decrease the body’s ability to convert 7-dehydrocholesterol into cholesterol thereby causing a build-up of 7-dehydrocholesterol, which could be converted into vitamin D in the skin. Each piece of this mechanistic pathway is depicted in Figure 3 along with the corresponding literature references.

An indirect negative feedback loop, established in laboratory studies, allows vitamin D levels to regulate DHCR7 activity and prevent hypervitaminosis D (that is, toxically high vitamin D levels). SLOS patients have high 7-dehydrocholesterol levels. Therefore, from Figure 3 and the literature, we would expect them to have high vitamin D levels. However, vitamin D levels in SLOS patients are reported to be low. This could be due to many lifestyle factors that occur when a patient is seriously ill with SLOS. One possible biological explanation that fits with the literature-derived mechanism (Figure 3) is that SLOS patients spent less time outdoors thereby reducing their ultraviolet B exposure (another critical requirement for vitamin D synthesis).

GENETIC UNDERSTANDING OF SLOS-INDUCING DHCR7 MUTATIONS: IMPLICATIONS FOR FUTURE WORK

Changing our understanding of SLOS genetics using large-scale genomics studies

Using ExAC, we were able to compare the frequencies of DHCR7 SLOS-inducing mutations in the SLOS population (extracted from the literature) and the assumed-healthy ExAC population. We found that there were many differences. To illustrate some of these differences, we plotted the overall incidence of DHCR7 mutations in SLOS against the incidence in the healthy population (ordered by healthy population). Several DHCR7 mutations are much more frequent among SLOS individuals then expected given the background population rate (Figure 4). For example, T93M is the second most frequent DHCR7 mutation in SLOS patients, but it is comparatively rare among the ExAC population (Figure 4). This could be due to sampling bias differences between the SLOS cohort and the ExAC population. For example, T93M is thought to be the founder SLOS mutation and is common...
In Italy, Spain and Portugal, the ExAC population may be under-represented for Southern Europeans. In ExAC they distinguish a ‘Finnish’ European cohort from a non-Finnish European cohort (representing Northern American peoples and other Europeans), but it is unclear how many Southern Europeans are represented in that cohort. Therefore, some of these differences are likely due to sampling bias.

In addition, we were able to identify DHCR7 mutations that were predicted to be damaging using ExAC. This revealed 24 mutations (Table 4). Eight of those mutations were already known to be implicated in SLOS and were contained in our compendium. Six more mutations occurred at the same amino acid position of another mutation that has been implicated in SLOS (demonstrating the importance of that amino acid position in SLOS). However, 12 mutations were never reported as being found in SLOS patients. There are two potential reasons for this: (1) the mutation is not disease causing (this is difficult to ascertain without the protein structure); and (2) the mutation only exists in an under-studied research population. For example, one mutation (R207X) was found to have 2.2% frequency among the healthy African population from ExAC. This means that R207X is polymorphic among Africans. However, this mutation has never been implicated in SLOS. It is possible that Africans are not often diagnosed with SLOS and then sequenced because they often come from resource-poor environments. The 12 mutations in Table 4 that have never been implicated in SLOS are worthy of further investigation by researchers to ascertain whether they are deleterious. If these mutations are deleterious, the next research question would be why African populations are under-diagnosed for SLOS.

Implications for causality

Hill established a set of nine criteria for determining whether an association was causal or not. We will focus our discussion on three of these criteria: strength, plausibility and coherence.

Table 4. DHCR7 mutations predicted to be damaging from ExAC cohort (60,706 individuals) includes both known SLOS-inducing mutations and unknown mutations

| Implicated in SLOS | Position | RSID | Protein consequence | Transcript consequence | Annotation | Overall allele count (freq. %) |
|-------------------|----------|------|---------------------|-----------------------|------------|--------------------------------|
| Yes               | 71146886 | rs138659167 | p.Trp5151Ter        | c.964G>T               | Stop gained | 386 (4.2 × 10⁴)               |
| No                | 71146229 | rs115538563 | p.Arg207Ter         | c.611G>T               | Stop gained | 6 (5.4 × 10⁻¹)                |
| Yes               | 71146886 | rs138659167 | —                   | c.964G>T               | Splice donor| 8 (6.8 × 10⁻¹)                |
| Yes               | 71146679 | rs138659167 | p.Leu138CysfsTer10  | c.412deIC              | Stop gained | 3 (5.7 × 10⁻¹)                |
| No                | 71146790 | rs138659167 | p.Trp5151Ter        | c.964G>T               | Stop gained | 5 (4.4 × 10⁻¹)                |
| AA                | 71146782 | rs138659167 | p.Tyr555Ter         | c.165C>G               | Stop gained | 5 (1.1 × 10⁻¹)                |
| No                | 71153313 | rs138659167 | p.Val134CysfsTer90  | c.400_408delGTGACTCTTinsT | Frameshift | 2 (2.7 × 10⁻¹)               |
| Yes               | 71146791 | rs138659167 | p.Leu138CysfsTer10  | c.412deIC              | Stop gained | 5 (1.1 × 10⁻¹)                |
| No                | 71146659 | rs140791666 | p.Tyr430Ter         | c.1290C>G              | Stop gained | 1 (8.5 × 10⁻¹)                |
| AA                | 71146709 | rs138659167 | p.Cys380Ter         | c.1140C>A              | Stop gained | 1 (8.5 × 10⁻¹)                |
| Yes               | 71155015 | rs138659167 | p.Tyr217Ter         | c.615C>A               | Stop gained | 1 (8.3 × 10⁻¹)                |
| No                | 71148951 | rs138659167 | p.Tyr217Ter         | c.615C>A               | Stop gained | 1 (8.3 × 10⁻¹)                |
| No                | 71155917 | rs138659167 | p.Gln28Ter          | c.82C>T                | Stop gained | 1 (8.3 × 10⁻¹)                |
| No                | 71155938 | rs138659167 | p.Asp214ArgfsTer42  | c.60_61insA             | Frameshift | 1 (8.3 × 10⁻¹)                |

*AA: indicates that another mutation at that same amino acid (AA) position number has been implicated in SLOS. However, the mutation is different from the one found by ExAC. Dots in the RSID column indicate that the RSID was not provided by ExAC for that mutation.
Prenatal effects of 7-dehydrocholesterol reductase modulators
MR Boland and NP Tatonetti

Strength is difficult to assess in SLOS because the DHCR7 mutations are often only found among SLOS patients (and researchers are biased to look specifically for mutations in DHCR7). As we can see from Table 2 the incidence of these mutations in the ExAC population is very low. This helps to strengthen our belief in the relationship between DHCR7 mutations and SLOS, especially for mutations that are disproportionately high among SLOS patients (for example, T93M in Figure 4). However, the fact that some mutations are very high among SLOS patients and very low among the ExAC population could be due to sampling bias between the two populations. Some DHCR7 mutations were found in the ExAC population that is predicted to be functionally deleterious. However, not all of these have been reported to be SLOS-Inducing. This could be due to ethnic differences between the two datasets (that is, certain groups are under-studied and therefore some of the predicted damaging mutations are actually un-reported SLOS-Inducing mutations) or it could be because some predicted damaging mutations are not functionally damaging (this would be easier to determine if the structure of DHCR7 were known). Because the protein structure of DHCR7 remains unknown it is difficult to predict for certain whether a mutation is deleterious or not.

A paper by Lanthaler et al.15 found that certain maternal genetic signatures could help rescue the SLOS phenotype by increasing the amount of cholesterol passed to the offspring via the placenta. This variation in the maternal genome could perhaps explain some of the discrepancies between our literature-derived SLOS compendium and the ExAC population. For example, in addition to the requirement that two DHCR7 mutations be inherited, it may also be necessary to have a certain placenta state (or placental gene mutation) to acquire the disease. This could perhaps explain why some common DHCR7 mutations from ExAC are not found to be SLOS-Inducing (even though they are predicted to be damaging). However, it is too early to determine yet because of sampling bias issues and under-reporting among certain ethnicities. Further investigation could help provide additional coherence to reported DHCR7 mutations in the literature. Therefore, this remains an open area of research.

**PHARMACOLOGICAL EFFECTS OF DHCR7 MODULATORS**

Importantly, pharmaceutical drugs have been shown to modulate DHCR7 activity in various ways. Several anti-psychotic drugs were found to enhance DHCR7 activity.57–59 Other drugs, not otherwise known to modulate DHCR7, can cause high 7-dehydrocholesterol in the absence of SLOS.60 Currently, pharmaceuticals are being designed to inhibit DHCR7 as a suggested treatment for hepatitis C.51 However, diverse therapeutic uses exist for targeting DHCR7. In this section, we review all known pharmaceuticals, compounds, chemicals, and toxins known to modulate DHCR7.

DHCR7 modulators

Expression. We used the Comparative Toxicogenomics Database (CTD)62–63 to retrieve articles describing compounds that modulate DHCR7 expression (data retrieved February 2015). We then manually reviewed the resulting literature references and only retained compounds with direct evidence of modulating DHCR7 expression in studies using human tissue. Table 5 contains the list of modulators with corresponding references. Several other compounds were found in CTD to modulate DHCR7, but failed our requirements listed above these are presented as potential DHCR7 modulators in Supplementary Table S3. Overall, we found five pharmaceuticals and two inorganic compounds increased DHCR7 expression and three toxins decreased DHCR7 expression. Antipsychotic drugs, such as haloperidol, clozapine and chlorpromazine, are known to alter expression in cardiovascular genes64 in addition to DHCR7 (increased DHCR7 activity would increase the rate of cholesterol synthesis, Figure 3). Details on the three toxins are described in the Supplementary Material.

Inhibition. We used ChEMBL, a freely-available semi-curated database of bioactive molecules,65 to find all pharmacological compounds that directly inhibit DHCR7 (Table 5). This includes approved chemotherapeutics, and investigational chemotherapeutics either in clinical or preclinical trials. When available we report IC50 values (Table 5). Drugs with IC50 values < 10 000 nm or 10 μM are considered potentially pharmacologically relevant inhibitors.66–67 We included tamoxifen (IC50 = 12 nm) and doxorubicin (IC50 = 150 nm) as FDA-approved DHCR7 inhibitors. DHCR7 inhibitors are used to treat cancer, typically breast cancer. For example, nafoxidine inhibits DHCR7 (ref. 68) and is a known estrogen antagonist. Several clinical trials were performed in the 1970s for nafoxidine.69–72 The major reaction to the drug consisted of dermatitis (55% of patients) exacerbated by sunlight.72 This indicates that the drug affected patients’ dermal response to sunlight exposure (which could be related to its inhibition of DHCR7 and dysregulation of vitamin D synthesis pathways).

Summary modulators. Overall, we found that five approved pharmaceuticals increase DHCR7 expression, whereas two inhibit DHCR7. Two inorganic compounds increase DHCR7 expression, whereas three toxins (including a metabolite of arsenic) decrease DHCR7 expression and one toxin decreases protein activity. Ten pharmaceutical inhibitors of DHCR7 are in various stages of clinical development. This includes seven inhibitors in the preclinical stage and three inhibitors in the clinical trials stage (Supplementary Table S4). DHCR7 Inhibitors are used or will be used (if approved) for cancer treatment.

Investigating fetal outcomes following prenatal exposure to DHCR7 modulators

Overview. The biological mechanisms underlying pharmacological teratogenicity and adverse fetal outcomes are complex involving many genetic and environmental factors. In the United States of America, developmental defects occur in ~3–5% of liveborn children.73 Among these defects between 2 and 3% are classifiable as teratogen-induced meaning that the defect occurred due to a known or suspected prenatal environmental exposure.74 It is further estimated that <1% of teratogenic effects have a pharmacological origin.74 Interactions between pharmaceutical drugs and genetics could result in increased teratogenicity among certain individuals. For example, holoprosencephaly clusters in families.75 Among these defects between 2 and 3% are classifiable as teratogen-induced meaning that the defect occurred due to a known or suspected prenatal environmental exposure.74 It is further estimated that <1% of teratogenic effects have a pharmacological origin.74 Because of the importance of functioning DHCR7 during fetal development, we decided to investigate the outcomes of prenatal exposure to two approved pharmaceutical DHCR7 inhibitors (Supplementary Table S4) and compare them against five approved pharmaceutical drugs that increase the DHCR7 expression. We were interested in the fetal outcomes of both DHCR7 inhibitors and those that increase expression to determine if DHCR7 inhibitors resulted in increased detrimental fetal outcomes. We made this hypothesis because DHCR7 inhibition results in a pharmacologically induced SLOS-like fetal development environment and therefore prenatal exposure to these inhibitors should result in SLOS like adverse outcomes.
| Chemical type* | Chemical/drug | Fetal risk category | Reference(s) | Source | Structure |
|---------------|---------------|---------------------|--------------|--------|-----------|
| **Increases expression (up-regulates)** | | | | | |
| Pharmaceutical drugs | | | | | |
| Antipsychotic (dibenzazepine) | Clozapine | B | 57,59,106 | Literature review/CTD | ![Clozapine structure](image1) |
| Antipsychotic (phenothiazine) | Chlorpromazine | C | 57-59 | Literature review | ![Chlorpromazine structure](image2) |
| Antipsychotic (butyrophenone) | Haloperidol | C | 57-59 | Literature review | ![Haloperidol structure](image3) |
| Statin (naphthalene) | Simvastatin | X | 107,108 | Literature review/CTD | ![Simvastatin structure](image4) |
| Corpus luteum hormone | Progesterone | B | 9 | Literature review/CTD | ![Progesterone structure](image5) |
| **Decreases expression (down-regulates)** | | | | | |
| Inorganic compounds | | | | | |
| Inorganic element (fullerene) | Nanotubes, carbon | — | 109 | Literature review/CTD | NA |
| Hormone antagonist (inorganic) | Potassium dichromate | — | 110 | Literature review/CTD | ![Potassium dichromate structure](image6) |
| **Decreases protein activity** | | | | | |
| Poisonous steroidal jerveratrum alkaloid | Cyclopamine (11-deoxojervine) | Poison | 54 | Literature review | ![Cyclopamine structure](image7) |
| **Direct inhibitors** | | | | | |
| Approved chemotherapeutics | | | | | |
| Chemotherapeutic, antagonist of the estrogen receptor (benzylidene) | Tamoxifen (IC_{50} = 12 nM; K_{d} = 1 nM) | D | 68,114-116 | Chembl | ![Tamoxifen structure](image8) |
| Chemotherapeutic (daunorubicin) | Doxorubicin (IC_{50} = 150–10 000 nM) | D | 117 | Chembl | ![Doxorubicin structure](image9) |
| Investigational chemotherapeutics | | | | | |
| Clinical trials | SERM (active metabolite of tamoxifen) | Afimoxifene | Clinical trials | 116 | Chembl | ![Afimoxifene structure](image10) |
PubMed literature review on fetal toxicity. Using PubMed, we retrieved all relevant observational studies on fetal outcomes due to prenatal exposure of each drug. The full details regarding the semi-automated query and retrieval process for included articles are described in the Supplementary Material. We reviewed each observational study carefully recording the number of pregnancy outcomes per drug treatment. Seven main pregnancy outcomes were used: born-healthy, spontaneous abortion

| Chemical type   | Chemical/drug    | Fetal risk category | Reference(s) | Source | Structure |
|-----------------|------------------|---------------------|--------------|--------|-----------|
| Antagonist of intracellular histamine | Tesmilifene | Clinical trials | 68 | Chembl | ![Chembl structure](image) |
| Non-steroidal anti-estrogen (pyrrolidine) | Nafoxidine | Clinical trials (in 1978) | 68 | Chembl | ![Chembl structure](image) |
| Pre-clinical Tamoxifen-induced antiestrogen activity | &pm;Pachysamine B (IC_{50} = 600 nM) | — | 118 | Chembl | ![Chembl structure](image) |
| Pre-clinical Tamoxifen-induced antiestrogen activity | Epipachysamine D (IC_{50} = 20 000 nM) | — | 118 | Chembl | ![Chembl structure](image) |
| Antifertility agent, ormeloxifene is a SERM | Rel-ormeloxifene | — | 68 | Chembl | ![Chembl structure](image) |
| Inhibitor of Cytochrome P450 (fatty acid, valerate) | Proadifen | — | 68 | Chembl | ![Chembl structure](image) |
| Metabolite of Clomiphene (Benzylidene, Stilbene) | 3′-Methoxy-4′hydroxy clomiphene (IC_{50} = 22 nM) | — | 114 | Chembl | ![Chembl structure](image) |
| SERM | Trioxifene | — | 119 | Chembl | ![Chembl structure](image) |
| Anti-estrogen (raloxifene analog) | LY-117018 | — | 68,119 | Chembl | ![Chembl structure](image) |

Abbreviations: NA, not available; SERM, selective estrogen receptor modulator. *Chemical type determined using the Anatomical Therapeutic Chemical Classification Browser: http://mor.nlm.nih.gov/RxClass/; bFetal risk categories are based on FDA's Criteria for the United States of America. Note: Structure diagrams are from Chembl and/or Wikipedia.
| Drug              | Effect on DHCR7 | Fetal risk category | Fetal pregnancy outcome (N = no. of patients)                                                                 | Reference(s) on Fetal Outcome |
|------------------|----------------|---------------------|-------------------------------------------------------------------------------------------------------------|-------------------------------|
| Levothyroxine    | None           | A                   | Born healthy (612)  
Spontaneous abortion (151)  
Elective termination (79)  
Fetal malformation/congenital anomaly (0)  
Stillbirth/intrauterine death (0)  
Ectopic pregnancy (0)  
Neonatal deathb (0)  
Unspecified outcome (249) | 120–124                                                    |
| Clozapine        | Increases expression | B                 | Born healthy (113)  
Spontaneous abortion (15)  
Elective termination (27)  
Fetal malformation/congenital anomaly (20)  
Stillbirth/intrauterine death (0)  
Ectopic pregnancy (0)  
Neonatal deathb (0)  
Unspecified outcome (28) | 125–129                                                    |
| Progesterone     | Increases expression | B                 | Born healthy (531)  
Spontaneous abortion (8)  
Elective termination (13)  
Fetal malformation/congenital anomaly (11)  
Stillbirth/intrauterine death (0)  
Ectopic pregnancy (0)  
Neonatal deathb (0)  
Unspecified outcome (0) | 130–132                                                    |
| Haloperidol      | Increases expression | C                 | Born healthy (268)  
Spontaneous abortion (19)  
Elective termination (19)  
Fetal malformation/congenital anomaly (12)  
Stillbirth/intrauterine death (0)  
Ectopic pregnancy (1)  
Neonatal deathb (0)  
Unspecified outcome (0) | 129,133–136                                                   |
| Chlorpromazine   | Increases expression | C                 | Born healthy (65)  
Spontaneous abortion (0)  
Elective termination (0)  
Fetal malformation/congenital anomaly (0)  
Stillbirth/intrauterine death (42)  
Ectopic pregnancy (0)  
Neonatal deathb (1)  
Unspecified outcome (0) | 129,137,138                                                  |
| Simvastatin      | Increases expression | X                 | Born healthy (184)  
Spontaneous abortion (30)  
Elective termination (87)  
Fetal malformation/congenital anomaly (24)  
Stillbirth/intrauterine death (7)  
Ectopic pregnancy (0)  
Neonatal deathb (1)  
Unspecified outcome (0) | 139–142                                                    |
| Tamoxifen        | Direct inhibition | D                  | Born healthy (146)  
Spontaneous abortion (12)  
Elective termination (31)  
Fetal malformation/congenital anomaly (26)  
Stillbirth/intrauterine death (3)  
Ectopic pregnancy (1)  
Neonatal deathb (0)  
Unspecified outcome (70) | 82–86,143–147                                               |
| Doxorubicin      | Direct inhibition | D                  | Born healthy (140)  
Spontaneous abortion (20)  
Elective termination (0)  
Fetal malformation/congenital anomaly (9)  
Stillbirth/intrauterine death (1)  
Ectopic pregnancy (0)  
Neonatal deathb (1)  
Unspecified outcome (70) | 87,88,148–160                                               |
| Isotretinoin     | None            | X                  | Born healthy (91)  
Spontaneous abortion (38)  
Elective termination (296)  
Fetal malformation/congenital anomaly (83)  
Stillbirth/intrauterine death (0)  
Ectopic pregnancy (0)  
Neonatal deathb (0)  
Unspecified outcome (0) | 161–163                                                    |

*Fetal risk categories are based on FDA’s Criteria for the United States of America. bDied within 1st week of life.
(or miscarriage), elective termination (or induced abortion), neonatal death (died within 1st week of birth), still birth (or intrauterine death), ectopic pregnancy and major/minor fetal malformations or defects or congenital anomalies. Whenever possible we included information regarding the trimester of drug exposure, as this is critical information for understanding teratogenic effects, realizing that SLOS-like symptoms would result mainly from first-trimester exposure (when cholesterol

Figure 5. Fetal outcomes of prenatal exposure to DHCR7 modulators compared to CDC prevalence and a known teratogenic drug (i.e., isotretinoin or accutane) and a known pregnancy safe drug (that is, levothyroxine). (a) contains aggregated results across all trimesters for regarding pregnancy outcomes. (b) Only born-healthy and born with fetal malformations, defects or congenital anomalies. Therefore, we excluded spontaneous abortions, elective terminations, stillbirths, ectopic pregnancies and neonatal deaths. The CDC provides data on live-born babies, elective terminations and fetal losses. We report fetal losses as spontaneous abortions in (a). We also split the number of live-born babies into born with defects and born-healthy using the statistic that 3% of live-born babies has a congenital anomaly. Notice that in (a) the number of fetal malformations/anomalies is higher among DHCR7 modulators including those that increase expression of DHCR7 (5.5%). However, drugs that inhibit DHCR7 have an increase in fetal malformations (10.9%). None of these levels comes close to the known teratogen, isotretinoin or accutane, with 47.7% born with malformations (out of total born). Many patients that are pregnant elect to terminate their pregnancy (a), which may be due to detect anomalies, however data on malformations among aborted fetuses is typically not available. We took all reported results where first-trimester exposure occurred (even if exposure persisted throughout the pregnancy) or where the exposure was listed as ‘early pregnancy’ as this appeared to indicate first-trimester exposure and these are shown in (c). Many known first-trimester accutane or isotretinoin resulted in elective terminations or induced abortions. Therefore, we included elective terminations and spontaneous abortions in (d) along with live births (healthy or malformed).
production is critical for proper structural formation). As controls, we included one known teratogen in our search and one known pregnancy-safe drug. We selected isotretinoin (tradename: accutane) as our known teratogen and levothyroxine as our known pregnancy-safe drug (that is, FDA category A).

Our method uses data gleaned from literature reports on fetal outcomes following prenatal drug exposure. Therefore, this data suffers from reporting bias. This occurs because reports on a drug’s effects may be more likely if the effect is deleterious or severe. To address this issue, we included two ‘control’ drugs and followed the same procedure (that is, literature review and extraction of outcomes). One ‘control’ drug was a known pregnancy safe drug—levothyroxine and the other ‘control’ drug was a known teratogen drug—isotretinoin. We can compare our findings of drugs that modulate DHCR7 to these two ‘controls’ to determine if any effect we observe is significant and worthy of further investigation.

A certain number of adverse fetal outcomes occur in the general population. For additional comparison purposes, we also included data from the Centers of Disease Prevention and Control (CDC) on pregnancy outcomes (that is, live born, spontaneous abortion, elective termination) collected across all races from both 1990 and 2008 for reference.\(^7^9\) We coupled this with data stating that 3% of live-born babies (1 in 33) have a birth defect.\(^8^0\) Table 6 displays our findings along with their corresponding references.

**Fetal outcomes from prenatal exposure to DHCR7 modulators.** The overall results for each of the seven FDA approved DHCR7-modulating pharmaceuticals are given in Figure 5. For comparison purposes, known pregnancy-safe levothyroxine and known teratogen isotretinoin (or accutane) are also included. The breakdown of these drugs across the seven pregnancy outcomes is given in Figure 5a. The number of deformations (possible teratogenic effects) vs number of healthy babies is shown in Figure 5b. Drugs that inhibit DHCR7 resulted in deformities among 10.9% of babies born, whereas drugs that increase DHCR7 expression resulted in deformities among 5.5%. This can be compared to the CDC background of 3.0% and levothyroxine’s rate of 0.0% (the known pregnancy-safe drug). Although DHCR7 inhibitors result in increased risk of deformities, the rate was still much lower than the well-known teratogen—accutane or isotretinoin—at 47.7%. We also statistically compared how DHCR7 activity affected fetal outcomes. Using the CDC background rate from 2008 (http://www.cdc.gov/nchs/data/nvsr/nvsr66/nvsr66_07.pdf)\(^7^9\) (accessed in January 2016), we performed a Fisher’s exact test for healthy vs non-healthy (this includes born with birth defect/deformity, spontaneous abortion, elective terminations). As expected, levothyroxine (pregnancy safe drug) was not statistically different from the background rate (\(P\)-value = 0.1974). We found that DHCR7 inhibitors were highly enriched for adverse fetal outcomes (odd ratio = 6.0, \(P\)-value < 0.001) with DHCR7 promoters showing less enrichment (odd ratio = 3.3, \(P\)-value < 0.001). Neither came close to the known teratogen isotretinoin (odd ratio = 34.8, \(P\)-value < 0.001).

We also investigated the relationship between first-trimester exposure to DHCR7 modulators and increased risk of teratogenic effects (Figures 5c and d). We were especially interested in first-trimester effects because reduction in cholesterol has been shown to cause severe teratogenic effects when the exposure occurs during the first-trimester.\(^7^8\) By inhibiting DHCR7 during the first-trimester, cholesterol production is also inhibited, therefore, we would expect similarly severe teratogenic effects.\(^7^8\) In addition, studies have shown that exposure to chemotherapeutic drugs, for example, doxorubicin and tamoxifen, during the first-trimester is associated with increased risk of complications to the fetus\(^8^1\) then exposure during other trimesters. First-trimester results for all pregnancy outcomes are shown in Figure 5c. We grouped drugs by their DHCR7 effect given in Figure 5d. Counts and percentages of pregnancy outcomes for first-trimester exposure to DHCR7 modulating drugs are provided in Table 7.

We found that first-trimester exposure to drugs that increase DHCR7 expression was comparable to first-trimester exposure of a known pregnancy-safe drug with 73.5% born-healthy among drugs increasing DHCR7 expression vs 72.7% for levothyroxine. Even more importantly, we found that first-trimester exposure to DHCR7 inhibitors was comparable to a known teratogen (that is, isotretinoin) with 50% born-healthy among drugs that inhibit DHCR7 vs 48.3% for isotretinoin. However, the sample size for first-trimester exposures was small (\(N = 40\) for known DHCR7 inhibitors) indicating that caution must be taken when interpreting these results.

**Interpretation of fetal results.** We found that tamoxifen has an \(IC_{50}\) of 12 \(\mu\)mol indicating that it inhibits DHCR7 at a pharmacologically potent level.\(^6^6,6^7\) We also found that tamoxifen exposure (especially during 1st trimester) resulted in increased fetal malformation risk. When compared to tamoxifen, doxorubicin had a lower ability to inhibit DHCR7 (\(IC_{50} = 150–10 000 \mu\)mol) and likewise a lower risk of adverse fetal outcomes.

In total, five pregnancies resulted in fetal malformations/defects or congenital anomalies from first-trimester tamoxifen exposure while two pregnancies resulted from first-trimester doxorubicin exposure. The anomalies resulting from first-trimester tamoxifen exposure appeared to have phenotypes similar to SLOS. These included, two cases of genitalia deformities including ambiguous genitalia,\(^8^2\)\(^–^8^4\) cleft palate (a form of holoprosencephaly),\(^8^4,8^5\) and Goldenhar’s syndrome, which is characterized by facial deformities\(^8^3,8^6\) and one hand deformation.\(^8^4\) Note that the facial deformities and cleft palate (a form of holoprosencephaly) can occur in SLOS patients. Genital anomalies occur frequently among SLOS patients.\(^1^0,1^1,1^4\) Hand deformations are rare among SLOS patients, however toe anomalies (for example, toe syndactyly) are common.\(^1^0,1^1\)

Two deformed babies reported in the literature were born after first-trimester doxorubicin exposure. One had genitalia issues,\(^8^7\) whereas the other had hydrocephalus.\(^8^8\) These congenital defects are highly related to the pleiotropic effects of SLOS (and defective functioning of DHCR7) indicating the distinct possibility that pharmacological inhibition of DHCR7 during the first-trimester of
pregnancy results in teratogenic effects similar to the physical manifestations of SLOS.

Comment on vitamin D3’s in-direct inhibition of DHCR7. Of note, vitamin D (cholecalciferol) decreases the DHCR7 activity through an indirect mechanism. There has been some speculation on the possibility of teratogenic effects resulting from high doses of vitamin D when taken as a prenatal supplement. However, a recent review of vitamin D supplementation among pregnant women found that vitamin D supplementation may reduce the risk of pre-eclampsia, low birth weight, preterm birth and adverse kidney outcomes in offspring. Because the mechanism of vitamin D3’s inhibition of DHCR7 is indirect (unlike our studied pharmacological DHCR7 inhibitors) it is likely to be mediated through a complex pathway with many interacting feedback loops. Studies involving moderate prenatal vitamin D supplementation have reported no adverse effects. Therefore, we would not expect vitamin D to result in SLOS-related teratogenic effects, although additional studies would be needed to ascertain the effects of large doses of prenatal vitamin D supplementation.

FUTURE DIRECTIONS AND CONCLUSION
In this review, we demonstrate the utility of an in-depth exploration of one Mendelian orphan disease: SLOS. We contribute a compendium of SLOS-inducing DHCR7 mutations, their incidence and geographic distribution. We also include the incidence of these mutations in a large population from ExAC. This helped us illustrate important differences between mutation frequencies in SLOS vs an assumed healthy population. Comparing our SLOS mutation compendium to ExAC allele frequencies allowed us to raise some important research questions both for studying under-represented ethnic groups, such as Africans (found to exhibit theoretically damaging DHCR7 mutations in ExAC), and for understanding genetic drift of DHCR7 mutations.

We went one step further and explored the prenatal effects of pharmaceuticals that target DHCR7. We posited that pharmacologically induced DHCR7 inhibition would result in fetal outcomes similar to those seen in SLOS (miscarriage, fetal malformations, ambiguous genitalia). We reviewed the literature for observational studies on fetal outcomes due to drug exposure. We found that exposure to DHCR7 inhibitors during the first-trimester of pregnancy resulted in fetal deformities/malformations or anomalies in 50% of conceptions born-healthy vs 48% for a known teratogen control. Contrastingly, 73% were born-healthy to those on drugs that increased DHCR7 expression compared to 73% for a known pregnancy-safe control. These results indicate that screening for DHCR7 inhibition during the pre-clinical phase of drug toxicity may be helpful in identifying drugs with potential to induce adverse fetal outcomes before the drug is released to the market.

CONFLICT OF INTEREST
The authors have no conflicts of interest.

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AUTHOR CONTRIBUTIONS
Mary Regina Boland performed literature review and analyses, synthesized literature and wrote the manuscript. Nicholas P Tatonetti provided feedback on the review findings, provided insights regarding the cohesiveness of the review, and edited the text of the manuscript in addition to providing funding support.

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