
Title: Human Genetics
ArticleTitle: Genetic innovations and our understanding of stillbirth
ArticleAuthor: Louise Wilkins-Haug
Date: 2020

Human Genetics (2020) 139:1161–1172
https://doi.org/10.1007/s00439-020-02146-2

REVIEW

Genetic innovations and our understanding of stillbirth

Louise Wilkins-Haug[1]

Received: 26 March 2019 / Accepted: 27 February 2020 / Published online: 21 April 2020

Abstract
Stillbirth after 20 weeks gestation happens in 1 in 200 pregnancies and occurs more commonly than neonatal loss and sudden infant death syndrome (SIDs) combined. The stillbirth rate is several times greater in low as opposed to high-resource countries. However, among high-resource countries, although a lower overall stillbirth rate exists, there has been little change for several decades. Molecular genetic technologies are emerging as important contributors to our understanding of stillbirth. Initially, genetic etiologies included alterations in chromosome number or structure such as aneuploidy and microduplications and deletions. More recently, next-generation sequencing analysis in two genetic conditions, Smith Lemli Optiz Syndrome (SLOs) and the channelopathy disorders (such as long QT syndrome (LQTS)) provide examples into the association of pathogenic gene variants with stillbirth. Although these specific conditions individually account for only a small number of stillbirths, investigating these disorders provides a new and innovative approach for further understanding genetic contributors to adverse pregnancy outcomes. Our knowledge of the role of genetic disease as an etiology for stillbirth is elementary. Genomic interrogation of maternal–fetal genotypes, gene–gene, and genotype–environment interaction is lacking in stillbirth research. At the DNA sequence level, further investigation of variants of unknown significance is an opportunity for exploration of biologic pathways of importance to pregnancy loss. This review concentrates on SLO as an example of a single gene disorder with a high carrier but low affected liveborn proband rate. The channelopathy disorders are included as initial examples of genetic conditions with variable presentation including an association with sudden infant death syndrome. Highlighted are the challenges when numerous genes and variants are involved, and the task of assigning pathogenicity. The advantages and limitations of genetic evaluations are presented and avenues for further research considered.

Introduction

In the United States, stillbirth occurs more commonly than neonatal death and sudden infant death combined. Stillbirth is usually characterized as fetal loss over 20 weeks gestation and occurs in 1 in 160–200 pregnancies (Hoyert and Gregory 2016). Globally, the world health organization (WHO) uses the International Classification of Disease (ICD)-9 definition of greater than 28 weeks, and 500 g (Lawn et al. 2016). Whether weight or gestational age are the best measurements may be country specific (Bose et al. 2015). The *Every Newborn Action Plan* (ENAP) promotes a 2030 goal of worldwide stillbirth reduction to 1 in 80 livebirths. While in high-resource countries, the stillbirth rate is already below this mark, a goal of 1 in 80 worldwide reflects that the majority of stillbirths currently occur in low-resource countries with higher rates and more deliveries (Lawn et al. 2010) (Fig. 1).

Worldwide, one stillbirth occurs every 2 min of every hour of every day. Most significantly, prior to 2006, systematic tracking of stillbirth was not available (Lawn et al. 2016). Understanding the cause of a stillbirth often is handicapped by parental reluctance for investigations, tissue changes with time from stillbirth to delivery and the challenges of varying standardized descriptions of cause. At least 81 classification systems exist containing multiple, even up to 30 etiologies (Korteweg et al. 2006; Leisher et al. 2016). The majority focus on maternal characteristics, placental pathology and when included, fetopsy (Fig. 2). Considerable heterogeneity and overlap in etiologies confound the determination of a primary cause of the stillbirth. For example, even when malformations are present, almost half of stillbirths have a placental finding otherwise associated

✉ Louise Wilkins-Haug
LWilkinshaug@partners.org

[1] Division of Maternal Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 01770, USA

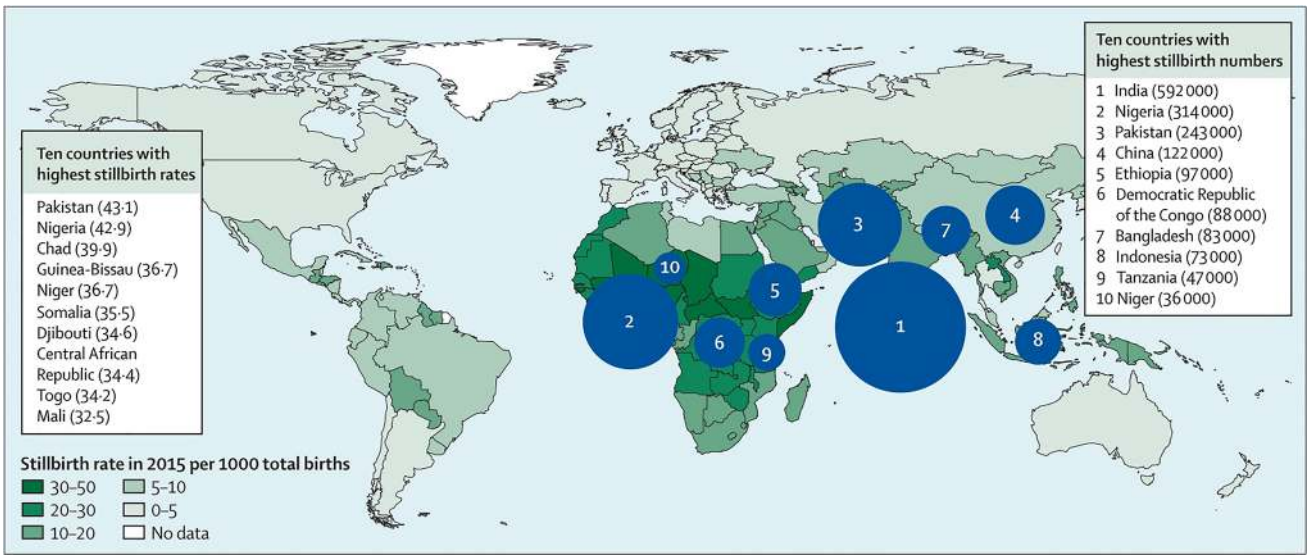

Fig. 1 Distribution of stillbirth worldwide reflecting absolute rates as well as impact of high stillbirth rates in high delivery volume countries (from Lawn et al. 2016)

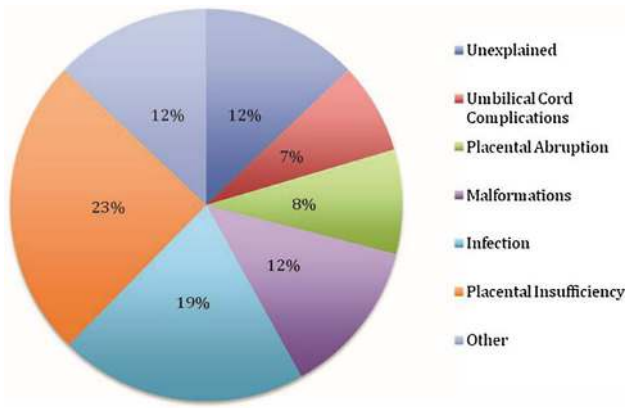

Fig. 2 Distribution of causes of stillbirth (Gravett et al. 2010)

with fetal death (syncytial knots, abruption) (Page et al. 2017). Currently, in the United States, even with dedicated, contemporaneous evaluations, 20–30% of stillbirth remain without a discernible primary etiology (Page et al. 2017).

New innovations in genetics can advance our understanding of stillbirth. Relatively noninvasive sample procurement (blood, fetal tissue, placental biopsy) for DNA and RNA affords a "molecular autopsy" for genetic disorders (Quinlan-Jones et al. 2019). Technologies such as whole exome/genome sequencing (WES/WGS), "omics" and functional studies provide avenues to increase our understanding of parturition or conversely, perhaps the lack of an appropriate delivery trigger in an adverse maternal–fetal environment. This review examines the current role of genetics in stillbirth investigation. Established genetic contributions such as from chromosomal imbalance are presented. Additionally, two single-gene disorders are included to highlight possible new associations. Lastly, future opportunities in stillbirth research are proposed to further our understanding at the single gene level and when integrating the complexities of gene–gene and gene–environment interaction.

Recognized genetic contributors to stillbirth

Genetic evaluation of a stillborn infant is classically, and in many countries, remains a karyotype. In 8–13% of stillbirth cases, a chromosomal abnormality is identified (Korteweg et al. 2012). More recently, chromosome microarray (CMA) has become the preferred test for stillbirth (Quinlan-Jones et al. 2019). CMA can be applied to nondividing, nonliving cells and also can detect small chromosome gains and losses not identified by a karyotype. In a large, multicenter United States investigation of over 500 stillbirths, CMA significantly increased the detection of clinically important chromosomal anomalies from 5.8 to 8.3% ($P = 0.007$), a 41.9% increase (detection rate ratio, 1.42; 95% confidence interval [CI] 1.07–1.89) (Reddy et al. 2012). In cases with stillbirth and anomalies, CMA increased detection of pathogenic findings to 29.9% (20/67) as compared to karyotype alone; 19.4% (13/67), $P = 0.008$; a 53.8% increase (detection rate ratio, 1.54; 95% CI 1.03 to 2.26). Furthermore, CMA provides results more often than karyotype analysis (87.4% vs. 70.5%, $P < 0.001$) (Reddy et al. 2012).

Recent advances in genetic technologies include lower cost and faster sequencing. Two current approaches are whole exome sequencing (WES), and whole genome sequencing (WGS). WES focuses on the smaller portion of

the genome (< 2%) which contains the DNA sequences leading to the majority of currently known inherited diseases. In contrast, WGS provides sequence data from across the entire genome. In general, advantages of WES are its speed and relatively lower cost when compared to WGS. Following PCR for capture of the segment, WES detects sequence copy number variants using a high depth of repetitive sequencing (generally about 100×). WGS provides even greater detection of copy number variants across the genome but at a lessor depth of sequencing. Depth of sequencing refers to the number of times a specific nucleotide is identified within short fragments. For WES, increasing the depth helps assure sequencing errors are minimized and uneven coverage resulting from the PCR step is addressed (Sims et al. 2014). WES, however, does not sequence the introns, those adjoining regions which may contain important gene activation and suppression information. WGS, however, sequences the entire genome—the exomes, adjoining regions and intergenic areas. With WGS, PCR is not needed for the initial capture step, so sequencing can be achieved at 30× depth. But as the entire genome is analyzed, the longer sequencing time and pipeline analysis results in a higher cost per sample (Strauss et al. 2018). Sequencing approaches are changing rapidly, however, with high coverage sequencers being developed to handle both the genome and the exome at a reasonable cost and within a practical timeframe.

Clinically, sequencing at the exome level (exome sequencing) is increasingly incorporated into the care of infants in the intensive care unit as well as for specific pediatric populations. The increased detection of genetic conditions varies by the neonatal or pediatric indication. Among pediatric populations with an undiagnosed neurologic condition, 27% had a further diagnosis made by WES (Yang et al. 2014). Diagnostic yield from WES is further increased when both parents as well as the proband (triad or trio testing) undergo testing. Trio testing allows delineation of a "de novo" status of an identified variant as one piece of evidence of potential pathogenicity. Among a pediatric population with unexplained etiology of developmental delay, inclusion of trios increased the discovery rate of possibly pathogenic variants from 9 to 41% of the patients (Lee et al. 2014). In the prenatal setting, WES is just now being introduced among pregnancies in which a fetal malformation is identified. Similar to pediatric studies, the yield of diagnoses increases from 14% (2/14) to 50% (5/10) when trios are included (Drury et al. 2015). A large, multicenter study of over 600 fetuses with ultrasound anomalies found pathogenic variants in 52/610 (8.5%; 95% CI 6.4–11.0). In fetuses with multisystem anomalies, pathogenic variants were present in 22/143 (15.4%). Trios were available in most cases (Lord et al. 2019).

Among stillbirth populations, WES or WGS evaluation is available in only a few, small sample size studies. These studies are difficult to compare as they have different criteria for inclusion of congenital anomalies, consanguinity and unclear gestational age. Also, the sequencing platforms and interpretation guidelines differ. In Saudi Arabia, in 44 families with stillbirths or lethal anomalies, WES revealed pathogenic findings in 50% (22/40). These results may be influenced by a high rate of consanguinity in this study which would potentially bias the results to autosomal recessive disorders. Variants of unknown significance (VOUS) were present in another 15 families (34%) (Shamseldin et al. 2018). Among the 4 cases of IUFD without anomalies, 2/4 (50%) had WES pathogenic variants. Similarly, preliminary WES/WGS findings are available from an Australian population of 43 families with a stillbirth. Again, anomalous stillbirths are included with a rate of 23% with pathogenic variants and an additional 26% with likely pathogenic candidate variants reported. Of note, pursuit of at least one VOUS with functional studies provided evidence of lethality in an animal model. Among all those with a positive WES, anomalies were present (Barnett 2017, personal communication, American Society of Human Genetics annual meeting). Stillbirth remains an area of adverse pregnancy outcome which has undergone only initial evaluation by sequencing.

Single-gene disorders as contributors to stillbirth

Two different approaches could be considered to gauge whether a single gene disorder is associated with stillbirth—(1) Hardy–Weinberg disequilibrium—fewer affected individuals than expected in the pediatric population based on the gene carrier rate and (2) extension of investigations in genetic disorders resulting in infant death syndrome (SIDs). Two genetic disorders were selected for inclusion in this review to exemplify each of these approaches. Smith Lemli Opitz syndrome (SLOs) is due to pathogenic variants in a cholesterol metabolism enzyme with a lower than expected affected proband population. SLO has a high population carrier frequency and is an example of a deviation from Hardy–Weinberg approach. For the second approach, infants with SIDs are associated with channelopathy pathogenic variants which produce altered cardiac rhythms. Among stillbirth cases, a similar increased rate of cardiac-based pathogenic variants is noted. In both instances, the growing specificity, access and decreasing cost of genetic sequencing technologies are informing the recognition of underappreciated genetic disorders which may be associated with stillbirth.

Smith Lemli Opitz and stillbirth

Smith Lemli Opitz syndrome (SLOs) was initially described by David Smith in the early 1960s with the familial recurrence of children with ambiguous genitalia, similar facial

features and intellectual disability (Smith et al. 1964; Opitz 1994). Altered cholesterol levels led to the identification of an enzymatic abnormality within key pathways (Irons et al. 1993). SLO is the result of pathogenic variants in the gene *DHCR7*, a key component of the cholesterol pathway. Most commonly, a G-to-C transversion in the splice acceptor site on exon 9 results in a null, pathogenic variant leading to premature termination and absent enzymatic activity (Yu et al. 2000). This variant, IVS8-1G-C is annotated as c.964-1G4C in some literature articles.

Pathogenic variants in pediatric probands In early studies, 63% (21/33) of children with clinical and metabolic SLO were found to possess the IVS8-1G-C null variant. Interestingly, this pathogenic allele was identified only in a heterozygotic state; one copy of the IVS8-1G-C pathogenic variant with a presumed second pathogenic variant as yet unidentified. The homozygotic combination (IVS8-1G-C/IVS8-1G-C) was not noted suggesting lethality (Yu et al. 2000). Genotyping of larger cohorts revealed a wide range of pathogenic variants (Witsch-Baumgartner et al. 2001). Among SLOs' probands in the literature, a compendium of at least 145 pathogenic alleles reveals several important characteristics (Waterham and Hennekam 2012; Boland and Tatonetti 2016). First, the most common pathogenic allele is IVS8-1G4C (28.5%). The distribution and locations of the next most common pathogenic variants are noted in Table 1. Among this cohort are 130 missense, 8 nonsense, 8 deletions, 2 insertions, 1 indel, and 5 splice site pathogenic variants. Second, most probands possess a combination of one missense and one nonsense pathogenic variant. And lastly, homozygosity for the null allele, IVS8-1G4C, the most common variant allele, is rare among SLO liveborn (Boland and Tatonetti 2016). Most SLOs patients are compound heterozygotes possessing the IVS8-1G-C variant and a range of other pathogenic variants within the gene. The absence of IVS8-1G-C homozygosity, a common feature in other autosomal recessive conditions, is striking.

Phenotypic variation in SLOs individuals is partially a reflection of this heterogeneity of pathogenic variants. Children with SLOs can have minimal dysmorphia with normal development to more commonly, profound developmental delay, multisystem structural anomalies and sexual dimorphism (Nowaczyk and Irons 2012). Homozygous DHCR7 null pathogenic variants result in absent cholesterol production with marked impact on embryonic and fetal development (Lanthaler et al. 2013). The other pathogenic variants affect cholesterol production to variable degrees with lower levels of cholesterol correlated with the most severe phenotypes. However, 70% of the phenotype variation remains unexplained (Yu et al. 2000).

Cholesterol availability from the mother to the fetus also contributes to the variation of SLO phenotype (Lanthaler et al. 2013). For example, increased cholesterol transfer from the mother to the fetus blunts the severity of more severe pathogenic variants. Conversely, the phenotype changes adversely with diminished maternal–placental cholesterol transport. This latter situation can exist with maternal

Table 1 DHCR7 alleles identified in probands with SLO from 30 studies (Boland and Tatonetti 2016)

Allele's effect on coding sequence	Genomic chromosome position[a]	Accession number (RS ID)[a]	Intron/exon	Allele freq. in 523 SLOS patients, $N = 1037$ alleles (%)	Allele freq. in ExAC population, $N = 60{,}706$ healthy individuals (%)
IVS8-1G>C[b]	71146886	rs138659167	Intron 8	291 (28.4)	386 (4.2×10^{-1})
T93M	71155082	rs80338853	Exon 4	96 (9.4)	3 (2.7×10^{-3})
W151X	71152447	rs11555217	Exon 6	86 (8.4)	82 (6.8×10^{-2})
V326L[c]	71146873	rs80338859	Exon 9	52 (5.1)	5 (4.8×10^{-3})
R404C	71146639	rs61757582	Exon 9	36 (3.5)	4 (3.5×10^{-3})
R352W[c]	71146795	rs80338860	Exon 9	34 (3.3)	2 (1.7×10^{-3})
E448K	71146507	rs80338864	Exon 9	23 (2.2)	1 (8.5×10^{-4})
R352Q	71146794	rs121909768	Exon 9	22 (2.2)	4 (3.4×10^{-3})
G410S	71146621	rs80338862	Exon 9	15 (1.5)	5 (4.3×10^{-3})
Unidentified suspected variant	–	–	–	13 (1.3)	–
PS1S	–	–	Exon 4	12 (1.2)	–
R242C	71150032	rs80338856	Exon 7	12 (1.2)	1 (8.3×10^{-3})
F302L	71148915	rs80338858	Exon 8	12 (1.2)	1 (8.3×10^{-4})

[a]Obtained from ExAC output, accessed in November 2015 (http://exac.broadinstitute.org/)

[b]Annotated as: c.964-lG>C in some literature articles

[c]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%

pathogenic variants of APOE and ATP-binding cassette transporter A1 (ABCA1) (Witsch-Baumgartner et al. 2004; Lanthaler et al. 2013) A maternal APOE2 variant profoundly decreases the cholesterol transporter protein and lowers cholesterol availability to the fetus. Lower fetal cholesterol in most studies is associated with a more severe SLO phenotype (Abuelo et al. 1995). Animal knock-out models for SLOs correlate APOE deficiency with more severe phenotype and increased fetal lethality (Solca et al. 2007; Lanthaler et al. 2013).

Pathogenic variants in SLO carriers and Hardy–Weinberg expectations

The lack of individuals among the SLOs pediatric population with homozygosity (two copies of the same variant) for the most common pathogenic variant in carriers does not conform to the HW equilibrium $(p2 + 2pq + q2) = 1$; the mathematical representation of the balance of wildtype and pathogenic variants within a population. First, as noted above, the most common pathogenic variant of DHCR7 is not seen in the homozygotic (q2) state among liveborns. Second, carriers (2pq) of DHCR7 pathogenic variants occur at a greater frequency then would be expected based on the prevalence of the disorder. Carrier frequencies range from 1/20 among persons in Utah to 1/50–1/100 among Caucasians in the United States (Nowaczyk et al. 2006). Based on a carrier average of 1/30, a disease prevalence of 1/3600 would be expected. However, the documented proband prevalence is 10–15 times less common (1 in 20,000–60,000) (Battaile et al. 2001) (Table 1). With these carrier rates, an estimated 42–88% of fetuses with SLO are lost as perinatal demise (Lazarin et al. 2017).

Few studies include analysis of SLOs in stillborn infants. Among ten reported cases of fetal SLOs based on metabolic studies, three fetuses were indeed homozygotic for the null pathogenic variants IVS8-1G-C. While a sample size of ten is small, 30% with homozygotic null pathogenic variants is at marked difference from the liveborn SLO populations in which heterozygosity but not homozygosity for IVS8-1G-C is noted. All three fetuses had multisystem involvement and were terminated preventing assessment of their viability (Quelin et al. 2012). Alternatively, the SLO fetal proband frequency is also indirectly implied by a second trimester population study of women with low unconjugated estriol on aneuploidy screening. Using this marker, the frequency of SLO is similar to that in live probands (1/60,000) suggesting the losses may occur in the first trimester (Schoen et al. 2003).

A recent analysis of a large population of stillbirths did not support a predominance of homozygotic pathogenic variants for SLOs. DNA was extracted on 144 cases and sequencing of DHCR7 revealed no cases with homozygosity of pathogenic variants. A single copy of a pathogenic variant (a heterozygote) was noted in nine stillbirth samples (9/139) (6.5%). An additional case was identified as a compound heterozygote with two different pathogenic variants of DHCR7 (Gibbins et al. 2018). Upon further review of this study, however, other interpretations of the data are worth presenting. While the lack of homozygosity was taken as not supportive of DHCR7 involvement in stillbirth, a rate of 6.5% heterozygosity for pathogenic variants ("carriers") is approximately two times the carrier rate in the general population (1–3%) (Lazarin et al. 2017). One could speculate that the clinical pathogenicity of these heterozygotic alleles may be further influenced by undetected indels, gene–gene or gene–environment interaction. Interestingly, the pathogenic variants described were not those commonly identified among liveborn probands or carriers (Gibbins et al. 2018).

Lastly, when the pathogenic variants responsible for SLOs are compared between the stillbirth, heterozygotic carriers and pediatric proband populations, differences exist. Except for IVS8-1G>C allele, there is little similarity between the other identified pathogenic alleles in these three populations. These findings highlight the Hardy Weinberg disequilibrium, the range of genetic variation in disease-associated genes, and the potential for influence by ascertainment. Also at question is whether other modifiers are undiscovered such as gene–gene or gene–environment as exemplified by maternal cholesterol availability (Table 2).

The source of this Hardy–Weinberg disequilibrium remains unanswered. Ascertainment may also play a role with milder disease not appreciated, although this would have to involve many affected individuals based on a carrier rate of 1/30. Classification of benign variants as pathogenic also would incorrectly alter the Hardy–Weinberg calculations (Kelley and Herman 2001). Potentially, some variants are relatively "intolerant" leading to early losses and not stillbirth. As the cholesterol pathway is so critical for early development, early pregnancy loss in addition to stillbirth should be considered. Additionally, review of the reproductive histories of SLO carrier couples for miscarriage or stillbirth may be informative, although these rates may be impacted by various factors including limitations to family size, and bias in recall especially for early miscarriage.

Channelopathies

Pathogenic variants of the channelopathy genes are associated with sudden death across the age spectrum from infants (sudden infant death syndrome (SIDs)) to adults. Channelopathy genes are directly responsible for controlling the rhythm of the heart. Maintenance of calcium and potassium transport across membranes in the heart is essential for normal electronic conduction. Pathogenic variants result in prolonged (long QT syndrome) or shortened

Table 2 Population carrier frequencies of *DNCR7* mutation, predicted carrier rate and Hardy–Weinberg expectation of Smith Lemli Opitz syndrome frequency (Nowaczyk et al. 2006)

Author(s) (year)	Region	Ethnicity (number of samples)	Mutation screened	Heterozygotes identified	IVS8-1G>C carrier rate	IVS8-1G>C as a percentage of all mutations observed in patients (%)	Predicate disease carrier rate (%)	Calculated incidence
Yu et al. (2000)	Various	American Caucasians (90) Finns (120) Africans (Sierra Leone) (121) Chinese (95) Japanese (103)	IVS8-1G>C	1	1.11	29	3.8	1 in 2770
Battaile et al. (2001)	Oregon	Anonymous (1503)	IVS8-1G>C	16	1.06%	29	3.7	1 in 2921
Metherall[a]	Utah	Not reported	IVS8-1G>C		1.13%	29	3.9	1 in 3673
Wright et a1. (2003)	Various	African Americans (1378)	IVS8-1G>C	10	0.73%		4.5	1 in 1975
Waye et al. (2002)	Ontario	Caucasians (1557)	IVS8-1G>C	17	1.09%	24	5.4	1 in 1371
Loeffler et al. (2001)	Austria	Austrians (Western Tirol) (640)	IVS8-1G>C W151X	Not reported Not reported	2.7%	50		
Ciara et al. (2006)	Poland	Polish (4256)	W151X V326L	39 12	2.4%	60	4.0	1 in 2500

No carriers of the IVS8-1G>C mutations were found in Southeast Asians (Chinese, Vietnamese, Laotian, Thai, Filipino), East Indians (Indian, Pakistani, Sri Lankan), Middle Eastern and Canadian Aboriginal populations, and Japanese populations

[a]As reported by Opitz et al. (2002)

(short QT syndrome) rhythms, an increased rate (catecholaminergic polymorphic ventricular tachycardia (CPVT)), or a sudden disruption of a normal rhythm (Brugada). The channelopathy genes are dominant variants and contribute to fainting, cardiac arrest and sudden death associated with exertion, stress, catecholamine release or at rest. They are also associated with otherwise unexplained infant demise—sudden infant death syndrome (SIDs) and most recently, stillbirth (Crotti et al. 2013). As a group, these conditions are uncommon, with long QT syndrome (LQTS) the most prevalent at 1 in 2000 persons (Modell and Lehmann 2006). However, in the general population, the presence of a variant and even a pathogenic variant in a channelopathy gene is more common than perhaps previously appreciated. Using sequencing data from the NHLBI GO Exome Sequencing project, 1/31 individuals carry a previously published LTQS gene variant. In this unaffected population, 33 variants were identified, just over half of these variants had previous functional data supportive of pathogenicity published. ECG data indicate these identified variants do not prolong the QT interval in these individuals suggesting they are not monogenic variants responsible for LQTS, or act in conjunction with other genes or environmental exposures (Refsgaard et al. 2012).

Among the general population, up to 1/20 may possess a channelopathy variant not previously associated with symtomatic cardiac arrythmia (Ackerman and Creery 2003; Ackerman 2004; Tester and Ackerman 2005). Given the number of genes and variants, associating pathogenicity to a channelopathy gene variant is challenging (Campuzano et al. 2015; Baruteau et al. 2017). As with all genetic sequencing variations, pathogenicity of a variant should rely on several criteria beyond association with a disease state. The American College of Medical Genetic provides helpful guidance in this area. Comparison of the variant to established databases, segregation within the family, in silico mapping of anticipated changes in the protein produced, and in vitro analysis of protein function all contribute to the determination (Richards et al. 2015). This also requires reanalysis over time (Duzkale et al. 2013; Campuzano et al. 2015).

The genetics of the channelopathy proteins are complex with numerous genes each having multiple variants identified. For the most common disorder, LQTS, variants of three genes, *KCNQ1 (LQT1, ≈ 35%), KCNH2 (LQT2, ≈ 30%) and SCN5A (LQT3, ≈ 10%)* account for 75% of cases. Two additional genes (KCNE1, KCNE2) make up the top five contributors to LQTS variation. (Splawski et al. 2000; Priori et al. 2003; Tester and Ackerman 2005). Eight further genes and

literally hundreds of published variants further complicates the genetics of LQTS (Refsgaard et al. 2012). Variants of the channelopathy genes seem to be relatively well tolerated and continue to be identified although many are of unknown significance. For some variants, their original association with cardiac conditions is now being reassessed when investigated with in vitro and in vivo functional studies. This is particularly true for the numerous limited/disputed evidence genes and their variants (Giudicessi et al. 2018a, b).

Further confounding the analysis of channelopathies is penetrance and, especially for LQTS, the sex of the individual. Females with LQTS1 and LQTS2 are at greater risk for QT prolongation and more severe outcomes (Locati 1998). At baseline, women have longer QT intervals than men with LQTS definition varied by sex (men $QT_c > 440$ ms; women $QT_c > 460$ ms) (Buxton et al. 2006). In males, testosterone at puberty provides a protective effect with shorter QT intervals (Zareba et al. 2003). In women, variation of QT duration is noted with hormonal fluctuations of the menstrual cycle and associated with prolongation with the use of unopposed estrogen in postmenstrual women (Kadish et al. 2004). Similarly, during pregnancy, initial increases in progesterone are protective but as progesterone decreases prior to labor, arrhythmia events become more common, especially so in the postpartum period (Seth et al. 2007). In vitro, progesterone is protective based on cardiac repolarization experiments (Odening et al. 2016). Whether these effects are further modulated by the sex of a fetus with a pathogenic variant remains unexplored.

Among cases of SIDs, cardiac arrhythmia as a causation has long been considered. In a landmark study over 4 decades ago, longer QT intervals were associated with infants who later underwent SIDs (Schwartz 1976). Later, sequencing of the genes associated with LQTS supported these initial cohort findings (Schwartz et al. 2000). Currently, 1–5% of SIDs cases have a of classic LQTS pathogenic variant with estimates of any channelopathy pathogenic variant in 5–10% of SIDs cases. These rates are higher than expected and may be double the general populaiton rate of expected LTQS variants (3–5%). Guidelines exist for molecular testing of channelopathy variants in SIDs cases (molecular autopsy), including clinical cardiology evaluation of parents and further parental genetic testing if indicated (Baruteau et al. 2015, 2017). However, the studies of association of channelopathies with SIDs remain challenging to compare to one another as they have utilized different approaches (Sanger vs. NGS), parental studies are often absent and attempts at validation either in vitro or "in silico" are limited (Baruteau et al. 2017).

Among stillbirth populations, investigators explored the channelopathy genes based on the premise of an age continuum from sudden infant death syndrome to fetal death (Schwartz 2004). Isolated cases of fetal loss and LTQS were reported and followed by larger cohort studies (Ishikawa et al. 2013). Three studies contribute intriguing findings to this area (Table 3). Two of the three studies found higher rates of channelopathy variants while the smallest sample size study ($N = 70$) did not (Crotti et al. 2013; Munroe et al. 2018; Sahlin et al. 2019). In the Shalin study, pathogenic variants associated with Brugada syndrome occurred 3× more commonly but did not reach statistical significance. All three studies were limited by their retrospective nature with lack of access to parental samples and familial segregation information. Additionally, in vitro analysis was applied to only a small subset of the newly identified possibly pathogenic variants.

Such data are an entry point to the study of channelopathies as an etiology for stillbirth. A comparison between the frequency of LQTS pathogenic variants in adults, in cases of SIDs and in stillborn infants is preliminary due to small sample size. Among infants who are stillborn, SCN5A pathogenic variants play a larger role than in other populations

Table 3 Occurrence of pathogenic variants of channelopathy and cardiomyopathy genes in cases of unexplained stillbirth

Author	Stillbirth population—unexplained, retrospective	Genes assessed	% Positive findings (pathogenic or probably pathogenic sequence variations	Specifics
Crotti et al. (2013)	91	KCNQ1 (LQTS1), KCNH2 (LQTS2) and SCN5A (LQTS3)	8.8% [95% CI 3.9–16.6%][a]	3 pathogenic, 5 rare and with functional effect
Munroe et al. (2018)	70	35 channelopathy gene panel	1.4% (N.S.)	Probably pathogenic on functional in vitro studies
Sahlin et al. (2019)	174	70 channelopathy and cardiomyopathy genes	12.1%**	Only channelopathy pathogenic variants identified

** Significantly greater than controls

[a]Pathogenic variants not present in 1300 sequenced healthy controls or in the Helmholtz Zentrum exome database or publicly available: 1000 Genome Project 19 (http://www.1000genomes.org/ensembl-browser); NHLBI GO Exome Sequencing Project20 (http://evs.gs.washington.edu/EVS), Exome Chip Design 21 (http://genome.sph.umich.edu/wiki/Exome_Chip_Design), uncertain pathogenicity defined as present in above populations at 0.05–0.3%

(Sahlin et al. 2019). Such a finding may suggest the SCN5A pathogenic variants are less tolerated by a fetus. However, as noted previously, the numerous genes and hundreds of identified variants of these channelopathy disorders continue to make the assessment of the channelopathies challenging. By extension from studies of SIDs, further investigation of gene–gene and gene–environment may be informative. Additionally, given the unique setting of the fetus, the combination of variants in the mother–fetus dyad may provide information.

Future opportunities

Genetics is the study of DNA sequence variations while genomics is a broader consideration of the entire extent of an individual's gene sequences, gene–gene interactions and gene function. Genomic technologies support investigations into system biology and functionals pathways. Related areas of research, "omics", has become a wide-ranging area incorporating pathways from gene variants to the protein produced, metabolites and even the innate microbiology of an individual. All these forms of investigation into the etiology of stillbirth are in their preliminary stages. Prospective stillbirth studies are needed that incorporate standardized phenotyping of both the infant and placenta, trio sampling (DNA from the stillborn infant and both parents), biobanking from the placenta and maternal circulation and systematic collection of antepartum and parental variables. As genomic technologies are moving at a rapid pace, a contemporaneous, prospective collection should include sample procurement and storage conditions which will permit not only the technologies of today but also those emerging such as single-cell RNA sequencing.

Genetics as represented by single gene changes may eventually represent only a fraction of stillbirth etiology. Among other pregnancy complications such as preterm labor, genewide association studies have not fulfilled the promises of earlier candidate genes (Strauss et al. 2018). However, singe-gene changes such as with SLO or a channelopathy are of importance for understanding stillbirth etiology, recurrence risk counseling and phenotype characterization. Trio investigation at the exome and whole genome levels will provide the opportunity to further characterize candidate regions as "de novo" and assess maternal–fetal dyads. As included in this review, association of a variant with a disease state is not enough for a delineation of pathogenicity. In further characterization of SLO, the channelopathies and newly recognized candidate genes, identified gene variants should undergo multistep assessment before pathogenicity is assigned. Detailed investigation and consistent reporting of in silico modeling of the effect of an amino acid change, frequency in population databases, familial segregation and in vitro or in vivo analyses are essential (Richards et al. 2015). Gene variant association studies alone should be considered as preliminary with further work to be done to investigate pathogenicity.

Stillbirth, similar to preterm labor and other pregnancy complications, is likely to be a multiple pathway event. Opportunities exist to learn from the current approach to the genetics of preterm labor. Sequencing (WES and WGS) studies and genome wide association studies (GWAS) are narrowing the focus to genes in the maternal inflammatory pathways. The genes identified to date lead to alerted responses to inflammation whether from an infectious cause or tissue stress. As sequencing develops these connections, WES or WGS is proposed as the current research modality to advance the understanding and potential treatment of preterm labor (Strauss et al. 2018). A combination of WES and WGS entails a comprehensive approach to assure a more complete investigation limited not only to candidate regions but also able to detect multiple gene changes in intergenic regions and the noncoding regions. With such approaches, new variant discovery, pathway interrogation with "omics" analysis and gene–gene interaction can be approached. Bringing this broad multifactorial approach using new and evolving genomic technologies to the assessment of inflammation or other key pathways in the stillbirth population is needed.

Lessons also can be learned from the investigations of SIDs. A "triple risk model" proposed by Filiano includes a vulnerable infant (perhaps with a genetic variation), a critical period in development and an exogenous stressor (such as smoking in SIDs) (Filiano and Kinney 1994). Genetic vulnerability is being approached by sequencing studies in both the SIDs and stillbirth populations. Trio studies are critical in the assessment of a genetic variation as parental studies can inform whether a "de novo" change exists or whether it is present in a parent with a different penetrance or vulnerability. Vulnerability also may be attributable not to a single gene but to gene–gene or even maternal–fetal dyad gene combinations. The idea of a critical period in pregnancy for stillbirth currently remains undefined and should be considered in light of the hormonal changes during pregnancy. In a subset of potentially vulnerable fetuses with a channelopathy variant, the fetal sex and timing of stillbirth in relationship to the lowered progesterone levels in later gestation should be explored more fully. Lastly, the concept of exogenous stressors brings forward a range of investigations—a role for inflammation, whether or not infection based, has emerged in preterm labor and SIDs research but evaluation is needed in assessment of stillbirth (Strauss et al. 2018; Ferrante et al. 2010, 2016). In particular, the influence of bacterial or viral presence, the innate and genetically controlled fetal and maternal responses, and the tolerance of inflammation are prime candidates for exploration in a stillbirth population.

Like the SIDs population, a reasonable assumption to pursue is that stillbirth will be due to at least two factors—(1) single-gene pathogenic variants and (2) variants which may predispose to death when a critical period of development or an exogenous stressor is present (Opdal and Rognum 2011).

Discussion

Emergence and access to innovative genomic technologies will drive the next step in understanding stillbirth. Since the first stillbirth awareness series in Lancet in 2011, and re-enforced in 2016, numerous fields of investigators have improved care with projects aimed to increase knowledge, identify stillbirth as an event included with all pregnancy events, provide psychosocial support, examine the impact on the next pregnancy, and bring attention to characterization standards (de Bernis et al. 2016). A gap remains, though, in the pace of basic science and now genomic research on stillbirth as compared to other pregnancy events. These gaps are highlighted in both Lancet series with calls for improved phenotyping, standardized clinical data collection and an approach to stillbirth as a system-based phenomenon (Froen et al. 2016). While such approaches are not initially cost effective, refinements and the knowledge gained in systems biology has the potential for eventual transference to low resource countries where the greater number of stillbirths occur.

Evidence presented here suggests at least two relatively common genetic disorders, Smith Lemli Opitz and channelopathies, may be contributors to stillbirth. Of interest from these initial studies is that the distribution of pathogenic variants identified among stillbirth varies from that noted among affected individuals and carriers. This raises the possibility of pathogenic variants which have such an impact on fetal development that they are incompatible with a livebirth. Trio analysis, proband and each parent, will be essential to investigating the distributions of these pathogenic variants. Expansion of a Hardy–Weinberg disequilibrium analysis to other relatively common single gene disorders should be considered.

Additionally, the identification of new pathogenic variants will provide a starting point for further functional analysis and a systems biology approach. As stillbirth is an infrequent occurrence, the now robust nature of sequencing tools allows analysis of archived samples. However, usually parental samples are not stored and parents not available for contact. In a prospective fashion, the genetic advances in assessing cell-free DNA of placental origin in the maternal circulation may prove to be fruitful. In all settings, the use of trios and standardized criteria for variation classification will refine our knowledge surrounding the role of genetic sequencing in stillbirth. While the focus of this review was primarily single nucleotide alterations, such work is just the beginning of a genomic analysis of stillbirth. Further molecular technologies are likely to provide insight into functional pathways as well as gene–gene and gene–environment interactions. A broad genomic approach to stillbirth will require large datasets and sophisticated data analysis but will contribute important information at both the clinical and basic biology levels.

References

Abuelo DN, Tint GS, Kelley R, Batta AK, Shefer S, Salen G (1995) Prenatal detection of the cholesterol biosynthetic defect in the Smith-Lemli-Opitz syndrome by the analysis of amniotic fluid sterols. Am J Med Genet 56(3):281–285

Ackerman MJ (2004) Cardiac channelopathies: it's in the genes. Nat Med 10(5):463–464

Ackerman K, Creery D (2003) Cardiorespiratory arrest in children. Clin Evid (9):341–349

Barnett (2007) – personal communication

Baruteau AE, Probst V, Abriel H (2015) Inherited progressive cardiac conduction disorders. Curr Opin Cardiol 30(1):33–39

Baruteau AE, Tester DJ, Kapplinger JD, Ackerman MJ, Behr ER (2017) Sudden infant death syndrome and inherited cardiac conditions. Nat Rev Cardiol 14(12):715–726

Battaile KP, Battaile BC, Merkens LS, Maslen CL, Steiner RD (2001) 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 72(1):67–71

Boland MR, Tatonetti NP (2016) Investigation of 7-dehydrocholesterol reductase pathway to elucidate off-target prenatal effects of pharmaceuticals: a systematic review. Pharmacogenomics J 16(5):411–429

Bose CL, Bauserman M, Goldenberg RL, Goudar SS, McClure EM, Pasha O, Carlo WA, Garces A, Moore JL, Miodovnik M, Koso-Thomas M (2015) The Global Network Maternal Newborn Health Registry: a multi-national, community-based registry of pregnancy outcomes. Reprod Health 12(Suppl 2):S1

Buxton AE, Calkins H, Callans DJ, DiMarco JP, Fisher JD, Greene HL, Haines DE, Hayes DL, Heidenreich PA, Miller JM, Poppas A, Prystowsky EN, Schoenfeld MH, Zimetbaum PJ, Heidenreich PA, Goff DC, Grover FL, Malenka DJ, Peterson ED, Radford MJ, Redberg RF, C. American College of and S. American Heart Association Task Force on Clinical Data (2006) ACC/AHA/HRS 2006 key data elements and definitions for electrophysiological studies and procedures: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards (ACC/AHA/HRS Writing Committee to Develop Data Standards on Electrophysiology). J Am Coll Cardiol 48(11):2360–2396

Campuzano O, Allegue C, Fernandez A, Iglesias A, Brugada R (2015) Determining the pathogenicity of genetic variants associated with cardiac channelopathies. Sci Rep 5:7953

Crotti L, Tester DJ, White WM, Bartos DC, Insolia R, Besana A, Kunic JD, Will ML, Velasco EJ, Bair JJ, Ghidoni A, Cetin I, Van Dyke DL, Wick MJ, Brost B, Delisle BP, Facchinetti F, George AL, Schwartz PJ, Ackerman MJ (2013) Long QT syndrome-associated mutations in intrauterine fetal death. JAMA 309(14):1473–1482

Ciara E, Popowska E, Piekutowska-Abramczuk D, Jurkiewicz D, Borucka-Mankiewicz M, Kowalski P, Goryluk-Kozakiewicz B, Nowaczyk MJ, Krajewska-Walasek M (2006) SLOS carrier frequency in Poland as determined by screening for Trp151X and Val326Leu DHCR7 mutations. Eur J Med Genet 49(6):499–504

de Bernis L, Kinney MV, Stones W, Ten Hoope-Bender P, Vivio D, Leisher SH, Bhutta ZA, Gulmezoglu M, Mathai M, Belizan JM, Franco L, McDougall L, Zeitlin J, Malata A, Dickson KE, Lawn JE, g. Lancet Ending Preventable Stillbirths Series study, G. Lancet Ending Preventable Stillbirths Series Advisory (2016) Stillbirths: ending preventable deaths by 2030. Lancet 387(10019):703–716

Drury S, Williams H, Trump N, Boustred C, Gosgene, Lench N, Scott RH, Chitty LS (2015) Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities. Prenat Diagn 35(10):1010–1017

Duzkale H, Shen J, McLaughlin H, Alfares A, Kelly MA, Pugh TJ, Funke BH, Rehm HL, Lebo MS (2013) A systematic approach to assessing the clinical significance of genetic variants. Clin Genet 84(5):453–463

Ferrante L, Opdal SH, Vege A, Rognum T (2010) Cytokine gene polymorphisms and sudden infant death syndrome. Acta Paediatr 99(3):384–388

Ferrante L, Rognum TO, Vege A, Nygard S, Opdal SH (2016) Altered gene expression and possible immunodeficiency in cases of sudden infant death syndrome. Pediatr Res 80(1):77–84

Filiano JJ, Kinney HC (1994) A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. Biol Neonate 65(3–4):194–197

Froen JF, Friberg IK, Lawn JE, Bhutta ZA, Pattinson RC, Allanson ER, Flenady V, McClure EM, Franco L, Goldenberg RL, Kinney MV, Leisher SH, Pitt C, Islam M, Khera A, Dhaliwal L, Aggarwal N, Raina N, Temmerman M, Lancet Ending Preventable Stillbirths Series study (2016) Stillbirths: progress and unfinished business. Lancet 387(10018):574–586

Gibbins KJ, Reddy UM, Saade GR, Goldenberg RL, Dudley DJ, Parker CB, Thorsten V, Pinar H, Bukowski R, Hogue CJ, Silver RM (2018) Smith-Lemli-Opitz mutations in unexplained stillbirths. Am J Perinatol 35(10):936–939

Giudicessi JR, Roden DM, Wilde AAM, Ackerman MJ (2018a) Classification and reporting of potentially proarrhythmic common genetic variation in long QT syndrome genetic testing. Circulation 137(6):619–630

Giudicessi JR, Wilde AAM, Ackerman MJ (2018b) The genetic architecture of long QT syndrome: a critical reappraisal. Trends Cardiovasc Med 28(7):453–464

Gravett MG, Rubens CE, Nunes TM, G. R. Group (2010) Global report on preterm birth and stillbirth (2 of 7): discovery science. BMC Pregnancy Childbirth 10(Suppl 1):S2

Hoyert DL, Gregory EC (2016) Cause of fetal death: data from the fetal death report, 2014. Natl Vital Stat Rep 65(7):1–25

Irons M, Elias ER, Salen G, Tint GS, Batta AK (1993) Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. Lancet 341(8857):1414

Ishikawa S, Yamada T, Kuwata T, Morikawa M, Yamada T, Matsubara S, Minakami H (2013) Fetal presentation of long QT syndrome–evaluation of prenatal risk factors: a systematic review. Fetal Diagn Ther 33(1):1–7

Kadish AH, Greenland P, Limacher MC, Frishman WH, Daugherty SA, Schwartz JB (2004) Estrogen and progestin use and the QT interval in postmenopausal women. Ann Noninvasive Electrocardiol 9(4):366–374

Kelley RI, Herman GE (2001) Inborn errors of sterol biosynthesis. Annu Rev Genomics Hum Genet 2:299–341

Korteweg FJ, Gordijn SJ, Timmer A, Erwich JJ, Bergman KA, Bouman K, Ravise JM, Heringa MP, Holm JP (2006) The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG 113(4):393–401

Korteweg FJ, Erwich JJ, Timmer A, van der Meer J, Ravise JM, Veeger NJ, Holm JP (2012) Evaluation of 1025 fetal deaths: proposed diagnostic workup. Am J Obstet Gynecol 206(1): 53.e51–53 e12

Lanthaler B, Steichen-Gersdorf E, Kollerits B, Zschocke J, Witsch-Baumgartner M (2013) Maternal ABCA1 genotype is associated with severity of Smith-Lemli-Opitz syndrome and with viability of patients homozygous for null mutations. Eur J Hum Genet 21(3):286–293

Lawn JE, Gravett MG, Nunes TM, Rubens CE, Stanton C, G. R. Group (2010) Global report on preterm birth and stillbirth (1 of 7): definitions, description of the burden and opportunities to improve data. BMC Pregnancy Childbirth 10(Suppl 1):S1

Lawn JE, Blencowe H, Waiswa P, Amouzou A, Mathers C, Hogan D, Flenady V, Froen JF, Qureshi ZU, Calderwood C, Shiekh S, Jassir FB, You D, McClure EM, Mathai M, Cousens S, g. Lancet Ending Preventable Stillbirths Series study, g. Lancet Stillbirth Epidemiology investigator (2016) Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet 387(10018):587–603

Lazarin GA, Haque IS, Evans EA, Goldberg JD (2017) Smith-Lemli-Opitz syndrome carrier frequency and estimates of in utero mortality rates. Prenat Diagn 37(4):350–355

Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, Das K, Toy T, Harry B, Yourshaw M, Fox M, Fogel BL, Martinez-Agosto JA, Wong DA, Chang VY, Shieh PB, Palmer CG, Dipple KM, Grody WW, Vilain E, Nelson SF (2014) Clinical exome sequencing for genetic identification of rare Mendelian disorders. JAMA 312(18):1880–1887

Leisher SH, Teoh Z, Reinebrant H, Allanson E, Blencowe H, Erwich JJ, Froen JF, Gardosi J, Gordijn S, Gulmezoglu AM, Heazell AE, Korteweg F, Lawn J, McClure EM, Pattinson R, Smith GC, Tuncalp, Wojcieszek AM, Flenady V (2016) Seeking order amidst chaos: a systematic review of classification systems for causes of stillbirth and neonatal death, 2009–2014. BMC Pregnancy Childbirth 16(1):295

Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Lehmann MH, Towbin JA, Priori SG, Napolitano C, Robinson JL, Andrews M, Timothy K, Hall WJ (1998) Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the International LQTS Registry. Circulation 97(22):2237–2244

Loeffler J, Witsch-Baumgartner M, Menze HJ, Kraft HG, Utermann G (2001) Carrier frequencies of DHCR7 mutation in WestAustria indicate that Smith-Lemli-Opitz syndrome is among the most common autosomal recessive disorders. Eur J Hum Genet 9:S282

Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, Prigmore E, Keelagher R, Best SK, Carey GK, Mellis R, Robart S, Berry IR, Chandler KE, Cilliers D, Cresswell L, Edwards SL, Gardiner C, Henderson A, Holden ST, Homfray T, Lester T, Lewis RA, Newbury-Ecob R, Prescott K, Quarrell OW, Ramsden SC, Roberts E, Tapon D, Tooley MJ, Vasudevan PC, Weber AP, Wellesley DG, Westwood P, White H, Parker M, Williams D, Jenkins L, Scott RH, Kilby MD, Chitty LS, Hurles ME, Maher ER, G. Prenatal Assessment of and C (2019) Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet 393(10173):747–757

Modell SM, Lehmann MH (2006) The long QT syndrome family of cardiac ion channelopathies: a HuGE review. Genet Med 8(3):143–155

Munroe PB, Addison S, Abrams DJ, Sebire NJ, Cartwright J, Donaldson I, Cohen MM, Mein C, Tinker A, Harmer SC, Aziz Q, Terry A, Struebig M, Warren HR, Vadgama B, Fowler DJ, Peebles D, Taylor AM, Lally PJ, Thayyil S (2018) Postmortem genetic testing for cardiac ion channelopathies in stillbirths. Circ Genom Precis Med 11(1):e001817

Nowaczyk MJ, Irons MB (2012) Smith-Lemli-Opitz syndrome: phenotype, natural history, and epidemiology. Am J Med Genet C Semin Med Genet 160C(4):250–262

Nowaczyk MJ, Waye JS, Douketis JD (2006) DHCR7 mutation carrier rates and prevalence of the RSH/Smith-Lemli-Opitz syndrome: where are the patients? Am J Med Genet A 140(19):2057–2062

Odening KE, Koren G, Kirk M (2016) Normalization of QT interval duration in a long QT syndrome patient during pregnancy and the postpartum period due to sex hormone effects on cardiac repolarization. HeartRhythm Case Rep 2(3):223–227

Opdal SH, Rognum TO (2011) Gene variants predisposing to SIDS: current knowledge. Forensic Sci Med Pathol 7(1):26–36

Opitz JM (1994) RSH/SLO ("Smith-Lemli-Opitz") syndrome: historical, genetic, and developmental considerations. Am J Med Genet 50(4):344–346

Opitz JM, Gilbert-Barness E, Ackerman J, Lowichik A (2002) Cholesterol and development: the RSH ("Smith-Lemli-Opitz") syndrome and related conditions. Pediatr Pathol Mol Med 21(2):153–181

Page JM, Christiansen-Lindquist L, Thorsten V, Parker CB, Reddy UM, Dudley DJ, Saade GR, Coustan D, Rowland Hogue CJ, Conway D, Bukowski R, Pinar H, Heuser CC, Gibbins KJ, Goldenberg RL, Silver RM (2017) Diagnostic tests for evaluation of stillbirth: results from the stillbirth collaborative research network. Obstet Gynecol 129(4):699–706

Priori SG, Napolitano C, Vicentini A (2003) Inherited arrhythmia syndromes: applying the molecular biology and genetic to the clinical management. J Interv Card Electrophysiol 9(2):93–101

Quelin C, Loget P, Verloes A, Bazin A, Bessieres B, Laquerriere A, Patrier S, Grigorescu R, Encha-Razavi F, Delahaye S, Jouannic JM, Carbonne B, D'Herve D, Aubry MC, Mace G, Harvey T, Ville Y, Viot G, Joye N, Odent S, Attie-Bitach T, Wolf C, Chevy F, Benlian P, Gonzales M (2012) Phenotypic spectrum of fetal Smith-Lemli-Opitz syndrome. Eur J Med Genet 55(2):81–90

Quinlan-Jones E, Lord J, Williams D, Hamilton S, Marton T, Eberhardt RY, Rinck G, Prigmore E, Keelagher R, McMullan DJ, Maher ER, Hurles ME, Kilby MD (2019) Molecular autopsy by trio exome sequencing (ES) and postmortem examination in fetuses and neonates with prenatally identified structural anomalies. Genet Med 21(5):1065–1073

Reddy UM, Page GP, Saade GR, Silver RM, Thorsten VR, Parker CB, Pinar H, Willinger M, Stoll BJ, Heim-Hall J, Varner MW, Goldenberg RL, Bukowski R, Wapner RJ, Drews-Botsch CD, O'Brien BM, Dudley DJ, Levy B, Network NSCR (2012) Karyotype versus microarray testing for genetic abnormalities after stillbirth. N Engl J Med 367(23):2185–2193

Refsgaard L, Holst AG, Sadjadieh G, Haunso S, Nielsen JB, Olesen MS (2012) High prevalence of genetic variants previously associated with LQT syndrome in new exome data. Eur J Hum Genet 20(8):905–908

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424

Sahlin E, Green A, Gustavsson P, Lieden A, Nordenskjold M, Papadogiannakis N, Pettersson K, Nilsson D, Jonasson J, Iwarsson E (2019) Identification of putative pathogenic single nucleotide variants (SNVs) in genes associated with heart disease in 290 cases of stillbirth. PLoS ONE 14(1):e0210017

Schoen E, Norem C, O'Keefe J, Krieger R, Walton D, To TT (2003) Maternal serum unconjugated estriol as a predictor for Smith-Lemli-Opitz syndrome and other fetal conditions. Obstet Gynecol 102(1):167–172

Schwartz PJ (1976) Cardiac sympathetic innervation and the sudden infant death syndrome. A possible pathogenetic link. Am J Med 60(2):167–172

Schwartz PJ (2004) Stillbirths, sudden infant deaths, and long-QT syndrome: puzzle or mosaic, the pieces of the Jigsaw are being fitted together. Circulation 109(24):2930–2932

Schwartz PJ, Priori SG, Dumaine R, Napolitano C, Antzelevitch C, Stramba-Badiale M, Richard TA, Berti MR, Bloise R (2000) A molecular link between the sudden infant death syndrome and the long-QT syndrome. N Engl J Med 343(4):262–267

Seth R, Moss AJ, McNitt S, Zareba W, Andrews ML, Qi M, Robinson JL, Goldenberg I, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori SG, Schwartz PJ, Towbin JA, Vincent GM, Zhang L (2007) Long QT syndrome and pregnancy. J Am Coll Cardiol 49(10):1092–1098

Shamseldin HE, Kurdi W, Almusafri F, Alnemer M, Alkaff A, Babay Z, Alhashem A, Tulbah M, Alsahan N, Khan R, Sallout B, Al Mardawi E, Seidahmed MZ, Meriki N, Alsaber Y, Qari A, Khalifa O, Eyaid W, Rahbeeni Z, Kurdi A, Hashem M, Alshidi T, Al-Obeid E, Abdulwahab F, Ibrahim N, Ewida N, El-Akouri K, Al Mulla M, Ben-Omran T, Pergande M, Cirak S, Al Tala S, Shaheen R, Faqeih E, Alkuraya FS (2018) Molecular autopsy in maternal–fetal medicine. Genet Med 20(4):420–427

Sims D, Sudbery I, Ilott NE, Heger A, Ponting CP (2014) Sequencing depth and coverage: key considerations in genomic analyses. Nat Rev Genet 15(2):121–132

Smith DW, Lemli L, Opitz JM (1964) A newly recognized syndrome of multiple congenital anomalies. J Pediatr 64:210–217

Solca C, Pandit B, Yu H, Tint GS, Patel SB (2007) Loss of apolipoprotein E exacerbates the neonatal lethality of the Smith-Lemli-Opitz syndrome mouse. Mol Genet Metab 91(1):7–14

Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, Keating MT (2000) Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation 102(10):1178–1185

Strauss JF 3rd, Romero R, Gomez-Lopez N, Haymond-Thornburg H, Modi BP, Teves ME, Pearson LN, York TP, Schenkein HA (2018) Spontaneous preterm birth: advances toward the discovery of genetic predisposition. Am J Obstet Gynecol 218(3):294–314. e292

Tester DJ, Ackerman MJ (2005) Sudden infant death syndrome: how significant are the cardiac channelopathies? Cardiovasc Res 67(3):388–396

Waterham HR, Hennekam RC (2012) Mutational spectrum of Smith-Lemli-Opitz syndrome. Am J Med Genet C Semin Med Genet 160C(4):263–284

Waye JS, Nakamura LM, Eng B, Hunnisett L, Chitayat D, Costa T, Nowaczyk MJ (2002) Smith-Lemli-Opitz syndrome: carrier frequency and spectrum of DHCR7 mutations in Canada. J Med Genet 39(6):E31

Witsch-Baumgartner M, Loffler J, Utermann G (2001) Mutations in the human DHCR7 gene. Hum Mutat 17(3):172–182

Witsch-Baumgartner M, Gruber M, Kraft HG, Rossi M, Clayton P, Giros M, Haas D, Kelley RI, Krajewska-Walasek M, Utermann G (2004) Maternal apo E genotype is a modifier of the Smith-Lemli-Opitz syndrome. J Med Genet 41(8):577–584

Wright BS, Nwokoro NA, Wassif CA, Porter FD, Waye JS, Eng B, Nowaczyk MJ (2003) Carrier frequency of the RSH/Smith-Lemli-Opitz IVS8-1G>C mutation in African Americans. Am J Med Genet A 120A(1):139–141

Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, Veeraraghavan N, Hawes A, Chiang T, Leduc M, Beuten J, Zhang J, He W, Scull J, Willis A, Landsverk M, Craigen WJ, Bekheirnia MR, Stray-Pedersen A, Liu P, Wen S, Alcaraz W, Cui H, Walkiewicz M, Reid J, Bainbridge M, Patel A, Boerwinkle E, Beaudet AL, Lupski JR, Plon SE, Gibbs RA, Eng CM (2014) Molecular findings among patients referred for clinical whole-exome sequencing. JAMA 312(18):1870–1879

Yu H, Lee MH, Starck L, Elias ER, Irons M, Salen G, Patel SB, Tint GS (2000) Spectrum of Delta(7)-dehydrocholesterol reductase mutations in patients with the Smith-Lemli-Opitz (RSH) syndrome. Hum Mol Genet 9(9):1385–1391

Zareba W, Moss AJ, Locati EH, Lehmann MH, Peterson DR, Hall WJ, Schwartz PJ, Vincent GM, Priori SG, Benhorin J, Towbin JA, Robinson L, Andrews ML, Napolitano C, Timothy K, Zhang L, Medina A, Q. T. S. R. International Long (2003) Modulating effects of age and gender on the clinical course of long QT syndrome by genotype. J Am Coll Cardiol 42(1):103–109