Received: 3 April 2020 | Revised: 20 May 2020 | Accepted: 22 May 2020

DOI: 10.1002/pd.5762

ORIGINAL ARTICLE

PRENATAL DIAGNOSIS WILEY

Evaluation and classification of severity for 176 genes on an expanded carrier screening panel

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Abstract

Background: Disease severity is important when considering genes for inclusion on reproductive expanded carrier screening (ECS) panels. We applied a validated and previously published algorithm that classifies diseases into four severity categories (mild, moderate, severe, and profound) to 176 genes screened by ECS. Disease traits defining severity categories in the algorithm were then mapped to four severity-related ECS panel design criteria cited by the American College of Obstetricians and Gynecologists (ACOG).

Methods: Eight genetic counselors (GCs) and four medical geneticists (MDs) applied the severity algorithm to subsets of 176 genes. MDs and GCs then determined by group consensus how each of these disease traits mapped to ACOG severity criteria, enabling determination of the number of ACOG severity criteria met by each gene.

Results: Upon consensus GC and MD application of the severity algorithm, 68 (39%) genes were classified as profound, 71 (40%) as severe, 36 (20%) as moderate, and one (1%) as mild. After mapping of disease traits to ACOG severity criteria, 170 out of 176 genes (96.6%) were found to meet at least one of the four criteria, 129 genes (73.3%) met at least two, 73 genes (41.5%) met at least three, and 17 genes (9.7%) met all four.

Conclusion: This study classified the severity of a large set of Mendelian genes by collaborative clinical expert application of a trait-based algorithm. Further, it operationalized difficult to interpret ACOG severity criteria via mapping of disease traits, thereby promoting consistency of ACOG criteria interpretation.

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1 | INTRODUCTION

Carrier screening identifies couples at risk of having offspring affected by a genetic disease, thereby informing reproductive decision-making and pregnancy management. Expanded carrier screening (ECS) accomplishes this for dozens to hundreds of diseases, in comparison to traditional ethnicity-based screening approaches meant to detect a limited number of conditions prevalent within those ethnic groups.[1] The American College of Obstetricians and Gynecologists (ACOG) considers ECS to be an acceptable screening strategy and in a 2017 Committee Opinion proposed that conditions selected for screening panels should meet several of seven criteria for disease inclusion on ECS panels.[2] Four of these criteria address disease severity, stating that conditions should: (a) have a detrimental effect on quality of life, (b) cause cognitive or physical impairment, (c) have an onset early in life, or (d) require surgical or medical intervention.[2] Similarly, the American College of Medical Genetics and Genomics (ACMG) position statement on ECS states that "disorders should be of a nature that most at-risk patients and their partners identified in the screening program would consider having prenatal diagnosis to facilitate making decisions around reproduction."[3] However, individuals have varying perceptions of the concept of severity based on their valuation of phenotypic traits.[4,5]

Disease severity inclusion criteria for ECS panels have not been widely studied. However, multiple studies have shown that disease categorization is helpful for patients in understanding the types of diseases included on ECS panels, and facilitates reproductive decision-making.[5-7] Neither ACOG nor ACMG define or cite processes by which to interpret the severity criteria proposed for gene inclusion on ECS panels. In 2014, Lazarin et al published an algorithm to objectively categorize disease severity.[4] In the study, 192 health care providers rated 13 individual disease traits related to disease severity, independent of any named genetic disease. Disease traits (eg, shortened lifespan, intellectual disability), as opposed to diseases themselves, were evaluated. This is because, despite health care providers' potential lack of familiarity with many rare genetic diseases, the traits associated with them are often encountered by health care providers regardless of the etiology. The analysis established and validated an algorithm (Figure 1) that can be applied to a disease, with its given set of individual phenotypic traits, resulting in a classification of the condition into one of four severity categories: profound, severe, moderate, and mild.[4]

To date, no published studies have rigorously applied the algorithm using health care providers with specialized knowledge of genetic disease, nor has it been applied to the large number of diseases currently included on many ECS panels. In this study, we applied the algorithm to assign objective severity classifications (profound, severe, moderate, mild) to 176 genetic diseases commonly found on ECS panels. Furthermore, in order to bring specificity to ACOG's four severity criteria (have a detrimental effect on quality of life, cause cognitive or physical impairment, have an onset early in life, require surgical or medical intervention), we mapped the disease traits utlilized by the algorithm to the criteria.

What's already known about this topic?

- Disease severity is an important consideration for disease inclusion on expanded carrier screening panels.
- An algorithm that objectively classifies diseases into severity categories has been published and validated.

What does this study add?

- 176 genes were classified into severity categories.
- The algorithm was used to bring clarity to American College of Obstetricians and Gynecologist's (ACOG's) severity criteria that are not easily interpretable.
- 170 of 176 genes met at least one of ACOG's severity criteria.

2 | MATERIALS AND METHODS

2.1 | Institutional review board considerations

This study did not use patient samples or results and therefore was not subject to review by an institutional review board or ethics committee.

2.2 | Gene classifications

Eight board-certified pediatric genetic counselors (GCs) followed by four board-certified medical geneticists (MDs), all of whom are experienced in managing patients with rare genetic disease, applied a

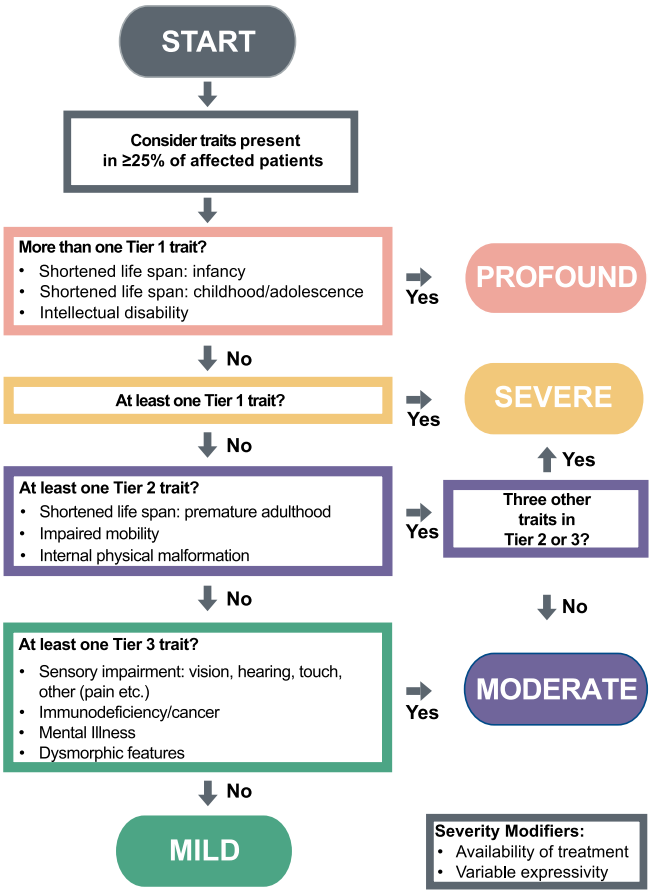

FIGURE 1 Severity classification algorithm evaluating specified disease traits (published by Lazarin et al.[4]). Each genetic counselor (GC) and medical geneticist (MD) used the algorithm to independently review and classify genes and their associated phenotype arising in at least 25% of individuals in the untreated state, as profound, severe, moderate, or mild [Colour figure can be viewed at wileyonlinelibrary.com]

validated severity algorithm[4] to randomly assigned subsets of 176 genes offered on a commercially available ECS panel (Foresight, Myriad Women's Health). The algorithm was applied to *genes*, rather than *diseases*, because some conditions are caused by multiple genes (eg, Usher Syndrome, Fanconi Anemia, Niemann Pick Disease) and some genes are associated with multiple conditions (eg, *HBB*, *FKTN*, *MYO7A*). The algorithm (described in Figure 1) was applied in a two-step review and consensus process as described in Figure 2 and Text S1 in Data S1. Honoraria were provided to each GC and MD participant. Permutation testing was conducted to determine whether particular individuals within each GC (1-4) or MD (1, 2) pair consistently classified disease genes as higher or lower in severity than their counterpart (see Text S2 in Data S1).

2.3 | Mapping disease traits to ACOG criteria

Following GC and MD gene classifications, each disease trait assessed in the algorithm (see Figure 1) was mapped collaboratively by the MDs and GCs to the four ACOG severity criteria: (a) a detrimental effect on quality of life, (b) cognitive or physical impairment, (c) onset early in life, and (d) requires surgical or medical intervention. Disease traits were mapped to ACOG severity criteria only if all GCs and MDs assessing each gene agreed on the traits. To assess ACOG's "detrimental effect on quality of life" criterion, we considered "quality of life" to be defined by the domains included in the Pediatric Quality of Life Inventory (PedsQL) 4.0 Generic Core Scales (physical functioning, emotional functioning, social functioning, and school functioning)[8] and PedsQL Infant Scales (physical functioning, physical symptoms, emotional functioning, social functioning, and cognitive functioning).[9] Disease traits that negatively impact these PedsQL domains were then mapped to the "detrimental effect on quality of life" criterion. We considered ACOG's "cause cognitive or physical impairment" criterion to be captured by the PedsQL Generic Core and Infant Scales domains of physical functioning and physical symptoms (physical impairment) and school functioning and cognitive functioning (cognitive impairment).[8,9] To interpret ACOG's "onset early in life" criterion, we used the American Academy of Pediatrics' (AAP) definitions of infancy (birth to age 2 years of age), childhood (2-12 years of age), and adolescence (12-21 years of age).[10] Though the severity algorithm uses shortened lifespan as a disease trait rather than age of onset, we reasoned that a condition that results in death before the end of adolescence (age 21 years) would be considered "early onset." Thus, to simplify the mapping, tier 1 traits of "shortened life span: infancy" and "shortened life span: childhood/adolescence" were combined into one trait. We interpreted "require surgical or medical intervention" as only interventions that are delivered within the context of the health care setting and that are required to treat, delay, halt the onset of, or lessen the severity of disease symptoms.

2.4 | Data analysis

Descriptive statistics were used to characterize general data trends. Statistical significance between proportions was determined using chi-squared analysis; a result was considered significant when $P < .05$.

3 | RESULTS

3.1 | Genetic counselor review and classification

After initial review across all GC pairs, 107 of the 176 disease-associated genes (60.8%) had concordant severity classifications (Figure 3A, Table 1). Within the four GC pairs, concordances were 68.2% (30/44 genes), 47.7% (21/44 genes), 65.9% (29/44 genes), and 61.4% (27/44 genes), respectively (Figure 3A, Table 1). With the exception of four genes (*NR0B1*, *ABCC8*, *KCNJ11*, *CYP21A2*), all discordant classifications were within one level of classification (Figure 3A, Table 1). A permutation test was conducted to determine whether any individual within each GC pair tended to classify genes as more or less severe than their counterpart and found no significant difference (all $P > .05$; Figure S1 in

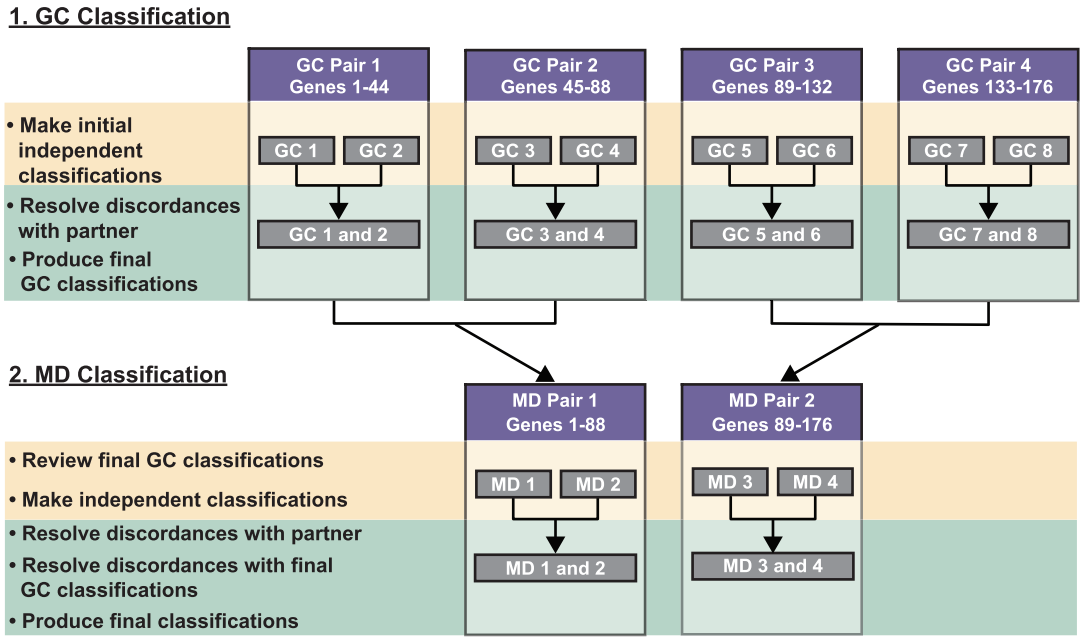

FIGURE 2 Workflow of the severity classification process. Eight GCs were divided into four pairs; each pair was asked to review 44 genes. Once the GC pairs finished their initial review and resolved discordances, the classification process was then passed to the four MDs for review and final classification. Gene numbers in this figure correspond to the gene numbers in Table 1 [Colour figure can be viewed at wileyonlinelibrary.com]

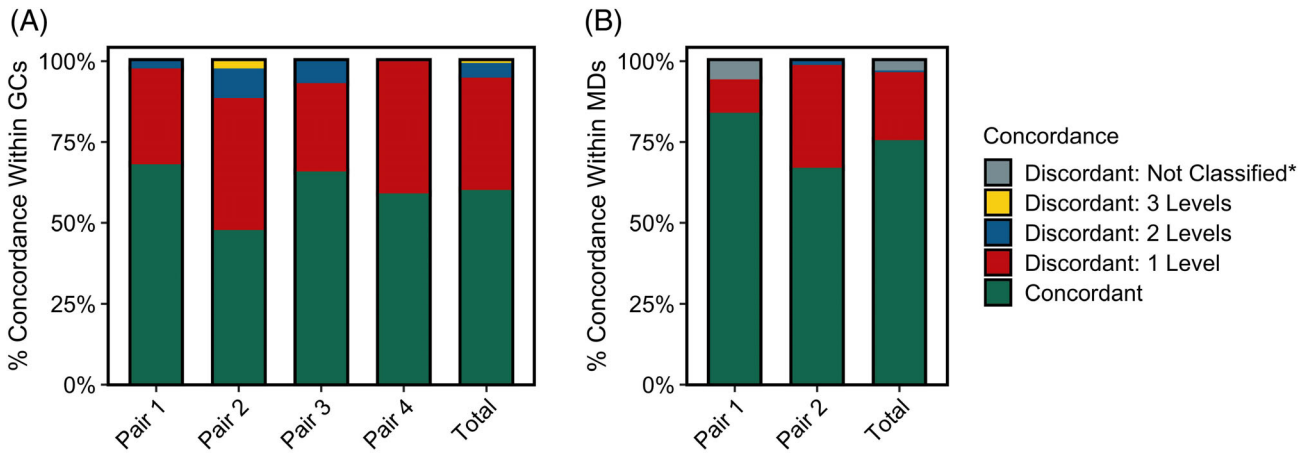

FIGURE 3 Concordance after initial gene severity classification. Concordance of initial severity classifications within each GC pair and in total among all GCs A, and concordance of initial severity classification within each MD pair and in total among all MDs B. Levels of discordance are indicated by color (green: concordant, red: discordant by one level, blue: discordant by two levels, yellow: discordant by three levels, gray: not classified) [Colour figure can be viewed at wileyonlinelibrary.com]

Data S1). Reasons for discordances noted by the GC pairs included difficulties in determining the primary phenotype associated with a specific disease with varying severity, difficulty discerning the level of intellectual disability associated with the disease from the available literature, and inability to locate published data regarding life expectancies and phenotypic differences in various forms of the conditions. After final review of discordances and consensus on final classifications between each GC pair, 65 genes (36.9%) were categorized as profound, 65 (36.9%) as severe, 42 (23.9%) as moderate, and four (2.3%) as mild (Table 1).

3.2 | Medical geneticist review and classification

After initial review across both MD pairs, 133 of the 176 disease-associated genes (76%) had concordant classifications (Figure 3B, Table 1). Within each MD pair, concordances were 84.1% (74/88 genes, MD Pair 1) and 67% (59/88 genes, MD Pair 2) (Figure 3B, Table 1). One member of MD Pair 1 did not definitively classify five genes; these were considered discordances. All but one discordant classification (*SLC22A5*) were within one level of classification (Figure 3B, Table 1). A permutation test was conducted (similar to that for

TABLE 1 Initial and final severity classifications between pairs of genetic counselors (GCs) and medical geneticists (MDs)

		Genetic counselors				Medical geneticists			
	Gene	Classification 1	Classification 2	Final classification	Pair	Classification 1	Classification 2	Final classification	Pair
1	*HBB*	Severe	Moderate	Severe	1	Moderate	Moderate	Moderate	1
2	*DHCR7*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
3	*PMM2*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
4	*ACADS*	Mild	Mild	Mild	1	Mild	Mild	Mild	1
5	*LRPPRC*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
6	*SLC26A2*	Severe	Moderate	Moderate	1	Moderate	Moderate	Moderate	1
7	*BTD*	Moderate	Moderate	Moderate	1	Profound	Profound	Profound	1
8	*MMAA*	Profound	Severe	Severe	1	Profound	Profound	Profound	1
9	*VPS13B*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
10	*NPHS2*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
11	*LIPA*	Severe	Moderate	Severe	1	Severe	Severe	Severe	1
12	*SMPD1*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
13	*GLB1*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
14	*FANCC*	Moderate	Moderate	Moderate	1	Severe	Severe	Severe	1
15	*GRHPR*	Moderate	Moderate	Moderate	1	Moderate	Moderate	Moderate	1
16	*IVD*	Severe	Severe	Severe	1	Severe	Profound	Profound	1
17	*MCOLN1*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
18	*PEX12*	Severe	Profound	Profound	1	Profound	Profound	Profound	1
19	*AMT*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
20	*TH*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
21	*AGA*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
22	*SLC12A6*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
23	*SMN1*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
24	*GAA*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
25	*PKHD1*	Profound	Severe	Profound	1	Severe	Severe	Severe	1
26	*NR0B1*	Severe	Mild	Mild	1	Moderate	Severe	Severe	1
27	*BBS1*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
28	*RMRP*	Moderate	Moderate	Moderate	1	Moderate	Severe	Severe	1
29	*PEX1*	Severe	Profound	Profound	1	Profound	Profound	Profound	1
30	*ALMS1*	Severe	Moderate	Severe	1	Severe	Severe	Severe	1
31	*ATM*	Severe	Moderate	Severe	1	Severe	Severe	Severe	1
32	*BLM*	Severe	Moderate	Moderate	1	Severe	Severe	Severe	1
33	*ASPA*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
34	*PEX7*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
35	*MKS1*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
36	*SGCG*	Moderate	Moderate	Moderate	1	Moderate	Moderate	Moderate	1
37	*POMGNT1*	Profound	Profound	Profound	1	Profound	Profound	Profound	1
38	*EVC*	Moderate	Moderate	Moderate	1	Moderate	Moderate	Moderate	1
39	*PTS*	Moderate	Severe	Severe	1	Severe	Severe	Severe	1
40	*ZFYVE26*	Severe	Severe	Severe	1	Severe	Severe	Severe	1
41	*GNPTG*	Moderate	Moderate	Moderate	1	Severe	Severe	Severe	1
42	*GALK1*	Mild	Moderate	Moderate	1	Moderate	Moderate	Moderate	1
43	*CPT1A*	Severe	Moderate	Severe	1	Moderate	Severe	Severe	1
44	*IL2RG*	Profound	Profound	Profound	1	Severe	Severe	Severe	1

TABLE 1 (Continued)

		Genetic counselors				Medical geneticists			
	Gene	Classification 1	Classification 2	Final classification	Pair	Classification 1	Classification 2	Final classification	Pair
45	*CFTR*	Moderate	Severe	Severe	2	Moderate	Severe	Severe	1
46	*GLA*	Moderate	Severe	Severe	2	Moderate	Severe	Severe	1
47	*RS1*	Moderate	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
48	*ASL*	Profound	Profound	Profound	2	Profound	Profound	Profound	1
49	*ATP7A*	Profound	Profound	Profound	2	Profound	Profound	Profound	1
50	*MYO7A*	Moderate	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
51	*ALPL*	Moderate	Moderate	Moderate	2	Not Classified	Moderate	Moderate	1
52	*AGXT*	Mild	Moderate	Moderate	2	Severe	Severe	Severe	1
53	*GCDH*	Severe	Moderate	Moderate	2	Severe	Moderate	Moderate	1
54	*AGL*	Mild	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
55	*LAMB3*	Severe	Moderate	Severe	2	Severe	Severe	Severe	1
56	*ALG6*	Severe	Severe	Severe	2	Severe	Severe	Severe	1
57	*ABCC8*	Mild	Profound	Profound	2	Severe	Severe	Severe	1
58	*MAN2B1*	Severe	Severe	Severe	2	Severe	Severe	Severe	1
59	*GNE*	Moderate	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
60	*RTEL1*	Moderate	Profound	Profound	2	Profound	Profound	Profound	1
61	*HSD17B4*	Moderate	Profound	Profound	2	Profound	Profound	Profound	1
62	*SGCB*	Moderate	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
63	*PEX6*	Profound	Profound	Profound	2	Profound	Profound	Profound	1
64	*HLCS*	Severe	Profound	Profound	2	Profound	Profound	Profound	1
65	*CLN5*	Profound	Profound	Profound	2	Profound	Profound	Profound	1
66	*KCNJ11*	Mild	Severe	Severe	2	Not Classified	Profound	Profound	1
67	*CYP21A2*	Severe	Mild	Severe	2	Not Classified	Severe	Severe	1
68	*USH2A*	Moderate	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
69	*ALDOB*	Severe	Moderate	Moderate	2	Not Classified	Severe	Severe	1
70	*MTM1*	Severe	Severe	Severe	2	Severe	Severe	Severe	1
71	*OTC*	Profound	Severe	Profound	2	Not Classified	Profound	Profound	1
72	*CLN3*	Profound	Severe	Profound	2	Severe	Severe	Severe	1
73	*NPC1*	Severe	Profound	Profound	2	Profound	Profound	Profound	1
74	*DYSF*	Moderate	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
75	*PEX2*	Profound	Profound	Profound	2	Profound	Profound	Profound	1
76	*TGM1*	Mild	Moderate	Moderate	2	Moderate	Moderate	Moderate	1
77	*FAH*	Severe	Severe	Severe	2	Severe	Profound	Profound	1
78	*SACS*	Moderate	Severe	Severe	2	Severe	Severe	Severe	1
79	*SLC17A5*	Profound	Severe	Severe	2	Severe	Severe	Severe	1
80	*FKTN*	Profound	Profound	Profound	2	Profound	Profound	Profound	1
81	*BCKDHB*	Profound	Profound	Profound	2	Severe	Profound	Profound	1
82	*STAR*	Profound	Severe	Severe	2	Severe	Severe	Severe	1
83	*ALDH3A2*	Severe	Severe	Severe	2	Severe	Severe	Severe	1
84	*XPA*	Moderate	Severe	Severe	2	Severe	Severe	Severe	1
85	*MLC1*	Severe	Severe	Severe	2	Severe	Severe	Severe	1
86	*PEX10*	Profound	Profound	Profound	2	Profound	Profound	Profound	1
87	*LAMA3*	Severe	Moderate	Severe	2	Severe	Severe	Severe	1
88	*OPA3*	Moderate	Severe	Moderate	2	Moderate	Moderate	Moderate	1

(Continues)

TABLE 1 (Continued)

		Genetic counselors				Medical geneticists			
	Gene	Classification 1	Classification 2	Final classification	Pair	Classification 1	Classification 2	Final classification	Pair
89	*FMR1*	Severe	Severe	Severe	3	Severe	Severe	Severe	2
90	*MEFV*	Moderate	Moderate	Moderate	3	Moderate	Moderate	Moderate	2
91	*ATP7B*	Moderate	Moderate	Moderate	3	Moderate	Moderate	Moderate	2
92	*GALT*	Profound	Profound	Profound	3	Profound	Severe	Profound	2
93	*CPT2*	Profound	Profound	Profound	3	Profound	Severe	Profound	2
94	*HEXA*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
95	*ARSA*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
96	*BCS1L*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
97	*HEXB*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
98	*AIRE*	Moderate	Moderate	Moderate	3	Moderate	Moderate	Moderate	2
99	*PCCB*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
100	*TPP1*	Profound	Profound	Profound	3	Profound	Severe	Profound	2
101	*NBN*	Profound	Severe	Severe	3	Severe	Severe	Severe	2
102	*NAGLU*	Profound	Severe	Profound	3	Profound	Profound	Profound	2
103	*DLD*	Profound	Profound	Profound	3	Profound	Severe	Profound	2
104	*SLC37A4*	Moderate	Severe	Severe	3	Severe	Severe	Severe	2
105	*COL4A4*	Moderate	Severe	Severe	3	Severe	Severe	Severe	2
106	*ADA*	Profound	Severe	Profound	3	Profound	Severe	Profound	2
107	*BCKDHA*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
108	*MPI*	Profound	Moderate	Severe	3	Severe	Severe	Severe	2
109	*NPC2*	Profound	Severe	Profound	3	Profound	Profound	Profound	2
110	*TAT*	Severe	Severe	Severe	3	Severe	Severe	Severe	2
111	*PAH*	Severe	Severe	Severe	3	Severe	Moderate	Severe	2
112	*ACADM*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
113	*GBA*	Profound	Moderate	Severe	3	Severe	Severe	Severe	2
114	*CAPN3*	Moderate	Moderate	Moderate	3	Moderate	Moderate	Moderate	2
115	*HOGA1*	Mild	Moderate	Mild	3	Mild	Moderate	Moderate	2
116	*NEB*	Severe	Moderate	Severe	3	Severe	Severe	Severe	2
117	*LAMA2*	Severe	Moderate	Moderate	3	Moderate	Moderate	Moderate	2
118	*IKBKAP*	Severe	Severe	Severe	3	Severe	Moderate	Severe	2
119	*HYLS1*	Severe	Profound	Profound	3	Profound	Profound	Profound	2
120	*FKRP*	Profound	Moderate	Profound	3	Profound	Profound	Profound	2
121	*G6PC*	Moderate	Severe	Severe	3	Severe	Severe	Severe	2
122	*SGCA*	Moderate	Moderate	Moderate	3	Moderate	Moderate	Moderate	2
123	*ASS1*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
124	*PCDH15*	Moderate	Moderate	Moderate	3	Moderate	Mild	Moderate	2
125	*CYP11B1*	Moderate	Moderate	Moderate	3	Moderate	Moderate	Moderate	2
126	*BBS2*	Severe	Severe	Severe	3	Severe	Severe	Severe	2
127	*MMAB*	Profound	Profound	Profound	3	Profound	Profound	Profound	2
128	*XPC*	Severe	Severe	Severe	3	Severe	Severe	Severe	2
129	*TMEM216*	Profound	Severe	Profound	3	Profound	Profound	Profound	2
130	*ARG1*	Severe	Severe	Severe	3	Severe	Severe	Severe	2
131	*CLN8*	Profound	Profound	Profound	3	Profound	Severe	Profound	2
132	*SGCD*	Moderate	Moderate	Moderate	3	Moderate	Moderate	Moderate	2

TABLE 1 (Continued)

		Genetic counselors				Medical geneticists			
	Gene	Classification 1	Classification 2	Final classification	Pair	Classification 1	Classification 2	Final classification	Pair
133	*DMD*	Severe	Moderate	Severe	4	Severe	Severe	Severe	2
134	*COL4A5*	Moderate	Moderate	Moderate	4	Moderate	Severe	Moderate	2
135	*IDS*	Severe	Moderate	Severe	4	Profound	Severe	Profound	2
136	*ABCD1*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
137	*MMACHC*	Profound	Profound	Profound	4	Profound	Severe	Profound	2
138	*ACADVL*	Moderate	Severe	Moderate	4	Severe	Moderate	Severe	2
139	*GALC*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
140	*CTNS*	Moderate	Severe	Severe	4	Severe	Severe	Severe	2
141	*NPHS1*	Moderate	Severe	Severe	4	Profound	Severe	Profound	2
142	*CYP27A1*	Severe	Severe	Severe	4	Severe	Moderate	Severe	2
143	*GNPTAB*	Profound	Severe	Profound	4	Profound	Profound	Profound	2
144	*MUT*	Profound	Severe	Profound	4	Profound	Severe	Profound	2
145	*TCIRG1*	Profound	Severe	Profound	4	Profound	Profound	Profound	2
146	*PROP1*	Moderate	Moderate	Moderate	4	Moderate	Moderate	Moderate	2
147	*EVC2*	Severe	Moderate	Severe	4	Moderate	Severe	Severe	2
148	*CLRN1*	Mild	Mild	Mild	4	Moderate	Moderate	Moderate	2
149	*PCCA*	Severe	Profound	Profound	4	Profound	Profound	Profound	2
150	*HGSNAT*	Severe	Severe	Severe	4	Profound	Severe	Profound	2
151	*MESP2*	Severe	Moderate	Severe	4	Moderate	Severe	Severe	2
152	*LAMC2*	Severe	Severe	Severe	4	Severe	Moderate	Severe	2
153	*CPS1*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
154	*PC*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
155	*GJB2*	Moderate	Moderate	Moderate	4	Moderate	Moderate	Moderate	2
156	*HBA1/ HBA2*	Severe	Moderate	Moderate	4	Severe	Moderate	Moderate	2
157	*SLC26A4*	Moderate	Moderate	Moderate	4	Moderate	Moderate	Moderate	2
158	*CBS*	Severe	Severe	Severe	4	Severe	Severe	Severe	2
159	*IDUA*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
160	*FANCA*	Moderate	Severe	Severe	4	Severe	Severe	Severe	2
161	*SLC22A5*	Moderate	Severe	Moderate	4	Profound	Moderate	Profound	2
162	*SGSH*	Severe	Profound	Profound	4	Profound	Profound	Profound	2
163	*COL4A3*	Severe	Moderate	Moderate	4	Moderate	Mild	Moderate	2
164	*BBS10*	Severe	Severe	Severe	4	Severe	Severe	Severe	2
165	*HADHA*	Severe	Severe	Severe	4	Severe	Severe	Severe	2
166	*ERCC6*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
167	*PPT1*	Profound	Profound	Profound	4	Profound	Severe	Profound	2
168	*DBT*	Profound	Profound	Profound	4	Profound	Severe	Profound	2
169	*GLDC*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
170	*BBS12*	Severe	Severe	Severe	4	Severe	Severe	Severe	2
171	*USH1C*	Moderate	Moderate	Moderate	4	Moderate	Moderate	Moderate	2
172	*TTPA*	Moderate	Severe	Severe	4	Moderate	Severe	Severe	2
173	*ERCC8*	Profound	Profound	Profound	4	Profound	Profound	Profound	2
174	*HMGCL*	Severe	Profound	Severe	4	Profound	Severe	Profound	2
175	*CTSK*	Moderate	Moderate	Moderate	4	Moderate	Moderate	Moderate	2
176	*CLN6*	Profound	Profound	Profound	4	Profound	Severe	Profound	2

GCs) to determine whether any individual within each MD pair tended to classify genes as more or less severe than their counterpart and found no significant difference (all $P > 0.05$; Figure S1 in Data S1). Reasons for discordances included no definitive classification, multiple phenotypes associated with the gene, unknown percentages of individuals with intellectual disability or shortened lifespan, and difficulties in determining life expectancy in the untreated state for conditions for which treatment is available. After final MD review of discordances and consensus on final severity classifications as defined by the algorithm, 68 (38.6%) genes were categorized as profound, 71 (40.3%) as severe, 36 (20.5%) as moderate, and one (0.6%) as mild (Table 1). Comparison of GC and MD classifications revealed no significant differences in the number of genes ultimately classified within each severity category (36.9% vs 38.6% profound, $P = .74$; 39.6% vs 40.3% severe, $P = .51$; 23.9% vs 20.5% moderate, $P = .44$; 2.3% vs 0.6% mild, $P = .18$, chi-squared test).

3.3 | Disease traits and their relationships to ACOG severity criteria

Disease traits most frequently cited by GCs and MDs across all genes as they applied the severity algorithm were sensory impairment (85 genes), intellectual disability (70 genes), and impaired mobility (67 genes) (Figure 4A). The disease traits that were least frequently cited were immunodeficiency/cancer (12 genes) and mental illness (9 genes) (Figure 4A). The list of genes and their assigned disease traits are provided in Table S1 in Data S1.

To determine which of the diseases evaluated in this study met ACOG's four severity-related criteria (have a detrimental effect on quality of life, cause cognitive or physical impairment, have an onset early in life, or require surgical or medical intervention), MDs and GCs mapped the disease traits used in the severity algorithm (Figure 1) to the ACOG criteria using validated scales and definitions (See Methods). The disease traits and their mapping to each ACOG severity criterion are described in Table 2. The list of genes and their ACOG severity criteria classification are provided in Table S1 in Data S1.

The "detrimental effect on quality of life" criterion was the most frequently met ACOG criterion (155 [88.1%] genes; 65 profound, 57 severe, and 33 moderate, Figure S2 in Data S1).This was followed by the "cause cognitive or physical impairment" criterion (97 [55.1%] genes; 54 profound, 31 severe, and 12 moderate, Figure S2 in Data S1). Seventy-five (42.6%) genes met the "onset early in life" criterion (56 profound, 17 severe, and 2 moderate, Figure S2 in Data S1). The least frequently met ACOG criterion was "require surgical or medical intervention" (62 [35.2%] genes; 26 profound, 27 severe, and 9

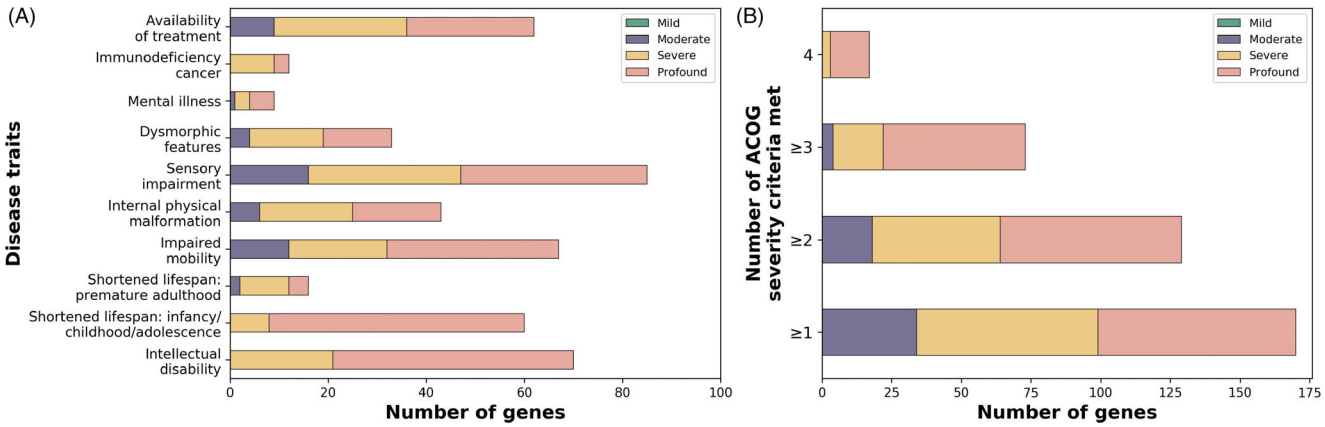

FIGURE 4 Genes classified by disease trait and American College of Obstetricians and Gynecologists (ACOG) severity criteria. A, Genes classified by disease trait. B, Genes classified by number of ACOG severity criteria met [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Mapping of the algorithm's disease traits to American College of Obstetricians and Gynecologists (ACOG) severity criteria

ACOG severity criteria	Have a detrimental effect on quality of life	Cause cognitive or physical impairment	Have an onset early in life	Require surgical or medical intervention
Algorithm disease traits	Intellectual disability Impaired mobility Internal physical malformation Sensory impairment Dysmorphic features Mental illness Immunodeficiency/ cancer	Intellectual disability Impaired mobility	Shortened lifespan: infancy/ childhood/adolescence	Availability of treatment

moderate, Figure S2 in Data S1). The number of ACOG criteria met across all conditions was proportional to the categorized severity of the gene: on average, profound genes met 2.8 ACOG severity criteria, severe genes met 1.9 criteria, moderate conditions met 1.6 traits, and the one mild condition met 0 criteria.

In total, 170 out of 176 genes (96.6%) met at least one of the four ACOG severity criteria, 129 genes (73.3%) met at least two ACOG severity criteria, 73 genes (41.5%) met at least three ACOG severity criteria, and 17 genes (9.7%) met all four criteria (Figure 4B). Cystic fibrosis and spinal muscular atrophy, two conditions recommended for panethnic screening by ACOG,[11] met one and four criteria, respectively (Table S1 in Data S1). Of the remaining six genes that did not meet at least one of the four ACOG severity criteria, one was classified as mild, two were classified as moderate, and three were classified as severe (Figure 4B, Table S1 in Data S1). The results of this disease trait-ACOG criteria mapping show that 170 of the 175 (97.1%) genes that were classified as profound, severe, and moderate met at least one of the ACOG severity criteria (Figure 4B, Table S1 in Data S1).

4 | DISCUSSION

Here, we have demonstrated the application of a severity classification algorithm applied by health care providers with expertise in pediatric and genetic disease to systematically classify the severity of 176 genes on a commercially available ECS panel. In addition, heath care providers operationalized the ACOG severity criteria via mapping of disease traits to the criteria, thereby determining which of the genes analyzed in this study met the criteria.

4.1 | Bringing clarity to severity criteria in guidelines

Guidelines stipulate disease severity as an important factor in the selection of conditions for ECS panel inclusion yet describe severity in terms that are not easily interpreted. Our study brings more clarity to ACOG's severity criteria; MDs and GCs who are experts in rare disease unanimously agreed on the mapping of disease traits to the criteria. When evaluating 176 genes disease traits, 170 met at least one of ACOG's severity criteria (Figure 4B), suggesting that they are appropriate to consider for inclusion on ECS panels.

MDs and GCs mapping disease traits to ACOG's four severity criteria interpreted the criteria based on definitions available in the literature, whenever possible, to avoid assumptions about ACOG's intent. For example, ACOG's "detrimental effect on quality of life" criterion was assessed using a definition of "quality of life" developed based on domains in the PedsQL, a validated instrument for assessing quality of life in infants and children with chronic and acute disease.[8,9] Social and family support, environment, and socioeconomic status may modify detriments to the quality of life experienced by those with genetic disease, but were not included in the evaluation of the ACOG criteria because they are not clearly accounted for by the PedsQL instrument. Interpretation of the "onset early in life" criterion was based on the age limit of adolescence as defined by the AAP.[10] To unambiguously map "shortened lifespan" (the trait assessed in the algorithm) to age of onset, only conditions that result in death before the end of adolescence were considered to meet the criterion. Therefore, genetic conditions that result in death during adulthood but that have an onset during or before adolescence, such as familial dysautonomia and glycogen storage disease type 1a, did not meet the "onset early in life" criterion, even though ACOG implicitly acknowledges the severity of such conditions by suggesting both familial dysautonomia and glycogen storage disease type 1a as examples of conditions to include on ECS panels.[11] "Require surgical or medical intervention" was defined as interventions delivered in the formal health care setting that are required to treat, delay, halt the onset of, or lessen the severity of disease symptoms. For example, surgery to correct a cardiac defect and treatment with chelating medications were considered to meet the ACOG criterion. Palliative or supportive care, as well as interventions delivered outside the context of the medical setting such as specialized early education for those with learning disabilities were not considered to meet the criterion, though their benefits have been demonstrated.[12]

While the majority of genes met at least one ACOG criterion, conservative interpretation of ACOG criteria resulted in 8.5% of profound conditions and 28.0% of severe conditions, including cystic fibrosis and Bloom syndrome, meeting only one ACOG criterion. For example, cystic fibrosis met the "require surgical or medical intervention" criterion because treatments are available to lessen the severity of disease symptoms. However, it does not usually cause death before the end of adolescence (the trait corresponding to ACOG's "early onset in life" criterion), nor is it characterized by disease traits that map to the other ACOG severity criteria. Similarly, Bloom syndrome met the "detrimental effect on quality of life" criterion because it is associated with high rates of cancer and immune deficiency leading to recurrent infections, but not with any of the other traits defining ACOG's severity criteria. Given ACOG's recommendation for panethnic cystic fibrosis carrier screening[11] and for consideration of Bloom syndrome carrier screening in Ashkenazi Jewish individuals,[13] it appears that ACOG supports carrier screening for conditions that meet at least one of its four severity criteria.

Initial GC and MD discordance when categorizing genes into severity categories using the algorithm's disease traits explains why five conditions did not meet any ACOG criteria despite being categorized as severe or moderate. In order for a disease trait to be mapped to ACOG criteria, all GCs and MDs assessing each gene had to initially agree to the presence of that trait (eg, both GCs or MDs had to agree that intellectual disability was present in ≥25% of affected individuals). However, the phenotypic spectrum caused by variable expressivity was a common cause of initial discordant classification. For example, 21-hydroxylase deficient congenital adrenal hyperplasia (21-OH CAH), caused by mutations in the *CYP21A2* gene, can present in a severe form characterized by life threatening salt-wasting crises, or a less severe but more prevalent form characterized by physical

malformation (ambiguous external genitalia). This variability in presentation led some GCs to initially assign disease traits differently for *CYP21A2*. For example, one GC stated that *CYP21A2* had shortened life expectancy in infancy while the other GC did not state this trait. Although a consensus severity categorization was reached, discordances in initial trait analysis precluded mapping to ACOG criteria and resulted in *CYP21A2* not meeting any ACOG criteria. The severity algorithm applied here takes variable expressivity into consideration and focuses on traits present in at least 25% of individuals with mutations in a given gene, but the complexity of phenotypic presentation in many rare diseases exemplifies one difficulty in interpreting ACOG criteria.

4.2 | Utility of screening for moderate severity conditions by ECS

The term "moderate" as a descriptor of severity may connote to some that the disease does not rise to a level of significance for a couple wanting to know their risk for having a pregnancy affected by genetic disease. However, we found that 94.4% of moderate severity conditions met at least one of ACOG's four severity criteria. As an example, *GJB2*, variants in which cause non-syndromic hearing loss and deafness, was categorized as having moderate severity. Early identification and interventions for individuals with hearing loss are critical; hearing loss complicates childhood development in language, socialization, academic performance, and most importantly human development and self-actualization.[14-17] Interventions such as cochlear implants can effectively avert these developmental delays.[17] Previous studies have also shown that couples find value in screening for moderate severity conditions. A majority of couples identified by ECS to be at risk for pregnancies affected by moderate severity conditions made reproductive and pregnancy management decisions for future family planning based on knowledge of such risk.[6,7] In addition, patients preferred to receive ECS results on conditions in all severity categories, including moderate, reporting that severity classifications informed their choice.[5]

4.3 | Complexity of ECS panel design

ECS panel design is a complicated endeavor that must take into account a number of factors.[18-21] ACOG suggests several criteria other than those related to severity that should guide ECS panel design, including a carrier frequency of one in 100 or greater, a well-defined phenotype, and the ability to diagnose the disease prenatally.[2] Clear definitions of ACOG criteria are needed to ensure consistency of ACOG criteria interpretations across different laboratories. In addition to the in-depth analysis of the severity criteria provided by this study, other criteria have been systematically evaluated. Ben-Shachar et al and Guo and Gregg concluded that a panel meeting the "carrier frequency of 1 in 100 or greater" criterion would include approximately 40 conditions when that threshold is applied to any ethnicity.[19,22] Balzotti et al examined the "well-defined phenotype" criterion by applying the ClinGen framework for gene-disease association evidence[23] to more than 200 genes commonly included on commercial ECS panels, and found that the vast majority had the highest level gene-disease association evidence.[24] These studies, taken together with ours, provide evidence-based interpretations of ACOG panel design criteria and act as guidance for laboratories in the design of ECS offerings.

4.4 | Limitations

This study had limitations that should be noted. First, the GC and MD participants were selected to review the conditions because of their expertise in pediatrics and medical genetics, but their application of the severity algorithm may not replicate application by other clinicians with similar or different expertise. Second, the MDs were not blinded to the final GC classifications, so it is possible that their classification was influenced by that of the GC pair. However, this paradigm imitates clinical practice, in which GCs (or other providers) routinely present initial findings to their clinical colleagues as the entire care team manages the patient. Third, genes were assessed based on the available literature, which may skew toward more severe disease presentations, particularly in the case of rare diseases. As understanding of phenotypes evolves, severity classifications may change. And fourth, the mapping of disease traits to ACOG criteria was not exhaustive, rather, it was limited to the traits in the algorithm. However, these traits were themselves determined by genetics professionals with experience in rare disease.[4]

5 | CONCLUSIONS

This study applied a systematic and transparent process to evaluate the severity of genes on a commercially-available ECS panel by engaging with genetics providers with expertise in rare disease. The severity algorithm applied in this study brings clarity to ACOG's severity criteria and can be used consistently across laboratories when considering genes for inclusion on ECS panels.

ACKNOWLEDGEMENTS

The authors thank Danielle Fanslow and Anna Gardiner for design and editorial assistance. This study was funded by Myriad Women's Health. J.T. was an employee of Children's Hospital of Philadelphia at the time of study and has since become an employee of Myriad Women's Health. G.A.L. was an employee of Myriad Women's Health at the time of study.

CONFLICT OF INTEREST

Aishwarya Arjunan, Holly Bellerose, Raul Torres, Rotem Ben-Shachar, Jennifer Tarpinian, Gabriel A. Lazarin, and Katherine Johansen Taber are all current or former employees of Myriad Women's Health, which markets an expanded carrier screening panel.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study have been completely reported in this manuscript and shared in the Figures and Supplementary Material.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Arjunan A, Bellerose H, Torres R, et al. Evaluation and classification of severity for 176 genes on an expanded carrier screening panel. *Prenatal Diagnosis*. 2020;40:1246–1257. https://doi.org/10.1002/pd.5762