Supplemental Table 1. Variant filtering criteria[a]

Primary criteria for autosomal dominant inheritance models	● Not in GATK tranche, AD >10, GQ >50 ● AF ≤0.0001 in public databases ● Genes with ≥2 loss-of-function variants or ≥3 loss-of-function or missense variants ● GDI <50th percentile
Secondary criteria for autosomal dominant inheritance models	● Not in GATK tranche, AD >10, GQ >50 ● AF ≤0.001 in public databases ● Genes with ≥2 loss-of-function variants or ≥3 loss-of-function or missense variants ● GDI <75th percentile
Criteria for compound heterozygotes	● Not in GATK tranche, AD >10, GQ >50 ● AF ≤0.01 in public databases ● Genes with ≥2 loss-of-function or missense variants in the same sample that were inherited from different parents. ● GDI <75th percentile
Criteria for previously reported genes	● ≥1 loss-of-function or missense variants in gene with prior evidence for association with sacral agenesis ● AF ≤0.001 in public databases ● No quality filters
Criteria for *de novo* screen	● Each member of trio with variant GQ >40 and AD >8; Alternate AD ≥5 in child ● AF ≤0.001 in public databases ● Focused on loss-of-function or missense variants
Criteria for autosomal recessive inheritance models	● Not in GATK tranche, AD >10, GQ >50 ● AF ≤0.01 in public databases ● Homozygous for loss-of-function or missense variants ● GDI <75th percentile
Criteria for X-linked recessive inheritance models	● Not in GATK tranche, AD >10, GQ >50 ● AF ≤0.0001 in public databases ● Homozygous (female) or hemizygous (male) loss-of-function or missense variants on chromosome X ● GDI <75th percentile

AD allelic depth, *AF* allele frequency, *GDI* gene damage index, *GQ* genotype quality, *WGS* whole genome sequencing, *ESP* Exome Sequencing Project.

[a]The quality of all variants was reviewed using the Integrated Genomics Viewer.

When filtering by AF, the maximum AF was used from the following populations/public databases, as annotated by ANNOVAR v2018Apr16: October 2014 and August 2015 releases of 1000 genomes - (all + AFR/EUR/EAS), ESP (all + AFR/EUR, ExAC-non-TGCA (all + AFR/AMR/EAS/FIN/NFE/OTHER/SAS), gnomAD Whole genome sequencing (all + AFR/AMR/ASJ/EAS/FIN/NFE/OTHER), gnomAD Whole exome sequencing (all + AFR/AMR/ASJ/EAS/FIN/NFE/OTHER/SAS)), plus AF from approximately 50,000 internal WGS controls (all + AFR/AMR/EUR/EAS/SAS).

Supplemental Table 2. Known candidate genes meeting filtering criteria[a], ordered by gene damage index. National Birth Defects Prevention Study, 1997-2011.

Child	Gene	Variant	Locus	[Allele1, Allele2]	GQ	AD	AF[b]	CADD	GDI
19	*CYP26A1*	p.R65S	10:93074313	[G, C]	99	[17, 16]	0	12.6	44.9%
20	*BOC*	p.H151P[c]	3:113250909	[A, C]	99	[57, 56]	0.0010	0.8	68.8%
21	*SIDT1*	p.H24D[c]	3:113533091	[C, G]	99	[24, 19]	0	9.0	83.2%
22	*PDZD2*	p.P1033R	5:32074204	[C, G]	99	[24, 28]	0.00010	23.8	84.9%
19	*USF3*	p.N607S[c]	3:113659862	[T, C]	99	[68, 61]	0.00020	0.8	85.0%
23	*SPTBN5*	p.R2992Q	15:41856432	[C, T]	99	[7, 9]	0.00040	24.0	99.7%
2	*SPTBN5*	p.S583L	15:41883140	[G, A]	99	[26, 14]	0.00040	8.7	99.7%

AD allelic depth for the [Allele1, Allele2], *AF* allele frequency, *CADD* combined annotation dependent depletion score, *GDI* gene damage index, *GQ* genotype quality.

[a]AF ≤0.001, No quality filters.

[b]Maximum allele frequency observed in any public database for any subpopulation.

[c]Variants not found in ClinVar.

The following 34 genes previously reported to be associated with sacral agenesis were evaluated: *ACD, ATP6V1A, BOC, CCDC191, CDX2, CDX4, CFAP44, CLTCL1, CYP26A1, DRD3, GRAMD1C, HOXA13, HOXB13, HOXC13, HOXD13, MNX1, MORN1, NAA50, NOP53, PCSK5, PDZD2, PTEN, PTF1A, QTRT2, SIDT1, SPTBN5, TBX4, TBXT, USF3, VANGL1, WNT3A, ZDHHC23, ZNF330, ZNF80.*

Supplemental Table 3. De novo missense and loss-of-function variants meeting filtering criteria[a], ordered by gene damage index. National Birth Defects Prevention Study, 1997-2011.

Child	Gene	Variant	Locus	[Allele1, Allele2]	GQ	AD	AF[b]	CADD	GDI
4	*METTL1*	p.R137X	12:57769384	[G, A]	99	[30, 47]	0.00080	9.7	7.4%
5	*EIF3E*	p.I196V	8:108229081	[T, C]	99	[39, 30]	0.000020	21.0	10.6%
6	*EXOSC1*	p.N141S	10:97437250	[T, C]	99	[19, 32]	0.0000090	12.3	10.9%
30	*KHDRBS1*	p.Y397H	1:32039528	[T, C]	99	[46, 29]	0	31.0	11.4%
7	*BRI3BP*	p.S169A	12:125025179	[T, G]	99	[41, 37]	0	20.8	16.5%
8	*RBPJ*	p.I419L	4:26430804	[A, C]	99	[31, 37]	0	12.8	20.8%
9	*FAM175B*	p.F132L	10:124826723	[C, A]	99	[34, 28]	0	24.0	25.8%
3	*OTUD6B*	p.R50H	8:91070443	[G, A]	99	[22, 19]	0	33.0	48.7%
10	*PRKG1*	p.P100L	10:51074889	[C, T]	99	[178, 39]	0	23.5	58.1%
11	*REST*	p.I644M	4:56930790	[A, G]	99	[39, 40]	0	0.0	69.4%
10	*DYNC1H1*	p.L525V[c]	14:101985798	[C, G]	99	[57, 42]	0	16.1	74.5%
12	*ADRB2*	p.M171V	5:148827342	[A, G]	99	[35, 41]	0	21.0	88.4%

AD allelic depth for the [Allele1, Allele2], *AF* allele frequency, *CADD* combined annotation dependent depletion score, *GDI* gene damage index, *GQ* genotype quality.

[a]AD >8, GQ >40, Max AF ≤0.001 in public databases.

[b]Maximum allele frequency observed in any public database for any subpopulation.

[c]Variant confirmed in proband via Sanger sequencing. Variant not detected in either parent.

None of the variants were found in ClinVar.

Supplemental Table 4. Variants that met secondary inclusion criteria[a], ordered by gene damage index. National Birth Defects Prevention Study, 1997-2011.

Child	Gene	Variant	Locus	[Allele1, Allele2]	GQ	AD	AF[b]	CADD	GDI
3	*SLC35F3*	p.K260R	1:234318782	[A, G]	99	[31, 31]	0.000045	24.1	33.7%
27	*SLC35F3*	p.N355S	1:234323041	[A, G]	99	[23, 29]	0.0010	22.7	33.7%
20	*SLC35F3*	p.R413K	1:234323215	[G, A]	99	[50, 48]	0.00011	18.5	33.7%
11	*SLIT2*	p.K561E	4:20533588	[A, G]	99	[15, 30]	0.000030	23.1	45.0%
29	*SLIT2*	p.D1042N	4:20589703	[G, A]	99	[25, 23]	0.00070	25.8	45.0%
9	*SLIT2*	p.P1316L	4:20617033	[C, T]	99	[24, 29]	0.0010	18.0	45.0%
17	*COLGALT1*	p.R102W	19:17559354	[C, T]	99	[24, 21]	0.00041	34.0	52.9%
30	*COLGALT1*	p.R221H	19:17568546	[G, A]	99	[28, 27]	0.00080	28.9	52.9%
26	*COLGALT1*	p.T237I	19:17568594	[C, T]	99	[27, 16]	0.0000097	24.7	52.9%
7	*SH3BP4*	p.R234W	2:235041469	[C, T]	99	[43, 38]	0.0010	24.7	56.4%
20	*SH3BP4*	p.P612L	2:235042604	[C, T]	99	[86, 60]	0	17.2	56.4%
12	*SH3BP4*	p.V801I	2:235043170	[G, A]	99	[24, 20]	0.0010	22.1	56.4%
20	*ENTPD8*	p.R249W	9:137436562	[G, A]	99	[23, 16]	0.00010	28.0	61.2%
17	*ENTPD8*	p.A124fs	9:137437183	[GC, G]	99	[11, 8]	0.00020	.	61.2%
13	*ENTPD8*	p. M1	9:137438284	[A, C]	99	[16, 10]	0.00080	23.6	61.2%
1	*SLC12A4*	p.L842V	16:67946254	[G, C]	99	[33, 30]	0.00010	14.0	61.9%
2	*SLC12A4*	p.L594F	16:67948128	[G, A]	99	[22, 8]	0.00030	25.7	61.9%
10	*SLC12A4*	p.L594F	16:67948128	[G, A]	99	[17, 17]	0.00030	25.7	61.9%
1	*SLC12A4*	p.M587T	16:67948148	[A, G]	99	[14, 11]	0.000018	26.7	61.9%
29	*MYO6*	p.V781D	6:75881744	[T, A]	99	[21, 16]	0.0010	25.2	62.7%
23	*MYO6*	p.Q999R	6:75892579	[A, G]	99	[22, 20]	0	7.3	62.7%
6	*MYO6*	p.N1144D[c]	6:75911689	[A, G]	99	[20, 37]	0	17.8	62.7%
2	*BPTF*	p.S1005C	17:67910898	[C, G]	99	[25, 24]	0.000036	10.5	64.5%
21	*BPTF*	p.P1925L	17:67928377	[C, T]	99	[47, 37]	0.00020	24.8	64.5%
4	*BPTF*	p.Q2402E	17:67945912	[C, G]	99	[83, 77]	0	12.0	64.5%
24	*MEGF8*	p.R26W	19:42326319	[C, T]	99	[19, 15]	0.000018	20.2	65.5%
29	*MEGF8*	p.R808C	19:42350271	[C, T]	99	[5, 14]	0.00010	35.0	65.5%
12	*MEGF8*	p.R1511H	19:42356884	[G, A]	99	[10, 12]	0.00050	23.2	65.5%
5	*MEGF8*	p.E2662K	19:42376422	[G, A]	99	[14, 6]	0	24.0	65.5%
6	*CHD7*	p.A1953V[c]	8:60852211	[C, T]	99	[46, 41]	0.00020	23.3	66.6%
4	*CHD7*	p.D2038E	8:60852839	[C, A]	99	[35, 37]	0.000009	24.1	66.6%
7	*CHD7*	p.M2482V	8:60856724	[A, G]	99	[45, 39]	0.00030	14.3	66.6%
10	*CHD7*	p.A684T	8:60865136	[G, A]	99	[30, 38]	0.00030	26.5	66.6%
4	*NCAN*	p.T468R	19:19226816	[C, G]	99	[27, 22]	0.000019	4.1	69.2%
7	*NCAN*	p.A703S	19:19227727	[G, T]	99	[41, 26]	0	0.0	69.2%

19	*NCAN*	p.E855K	19:19228183	[G, A]	99	[51, 43]	0.0010	0.8	69.2%
13	*NCAN*	p.V1007G	19:19233789	[T, G]	99	[28, 17]	0	0.0	69.2%
13	*PCK2*	p.T109M	14:24098253	[C, T]	99	[37, 23]	0.00040	33.0	72.6%
8	*PCK2*	p.T483A[c]	14:24103234	[A, G]	99	[23, 21]	0.00010	28.0	72.6%
19	*PCK2*	p.R537H	14:24103651	[G, A]	99	[43, 34]	0.00050	33.0	72.6%
26	*SGSM1*	p.V53I	22:24847651	[G, A]	99	[5, 6]	0.0010	20.7	73.2%
19	*SGSM1*	p.R58Q	22:24847667	[G, A]	99	[11, 7]	0.000045	24.6	73.2%
10	*SGSM1*	p.I224V	22:24855549	[A, G]	99	[46, 40]	0.0010	13.8	73.2%

AD allelic depth for the [Allele1, Allele2], *AF* allele frequency, *CADD* combined annotation dependent depletion score, *GDI* gene damaging index, *GQ* genotype quality.

[a]AD>10, GQ>50, AF≤0.001, GDI<75th percentile, ≥2 loss-of-function variants or ≥3 loss-of-function or missense variants.

[b]Maximum allele frequency observed in any public database for any subpopulation.

[c]Variants are present in ClinVar but predicted to be benign or likely benign or of uncertain significance; sacral agenesis was not one of the phenotypes reported.