The 'Spinal Muscular Atrophy Genotype Probability Calculator' Applied to the ACGS Model Risk Calculations for *SMN1*-Related Spinal Muscular Atrophy

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The Association for Clinical Genomic Science (ACGS) has published examples of how to manually solve a variety of risk calculations relevant to spinal muscular atrophy that include some of the more commonly encountered clinical scenarios (see http://www.acgs.uk.com/quality/model-risk-calculations). In this document, the 'Spinal Muscular Atrophy Genotype Probability Calculator' (SMAGPC) is applied to these same scenarios to demonstrate how it can be used to accurately calculate residual carrier risks. An introduction to SMAGPC and instructions for use are available on the website (http://www.genecalcs.weebly.com/smagpc.html).

Assumptions

The ACGS suggest the following assumptions:

A. *SMN1* mutation allele frequency = 0.01. This accords with a SMA prevalence of 1 in 10,000 and a carrier frequency of 0.0198 (~1 in 50).
B. 4% of normal chromosomes have 2 copies of *SMN1*.
C. 2% of mutations are point mutations (i.e. 98% are *SMN1* deletions or gene conversions).

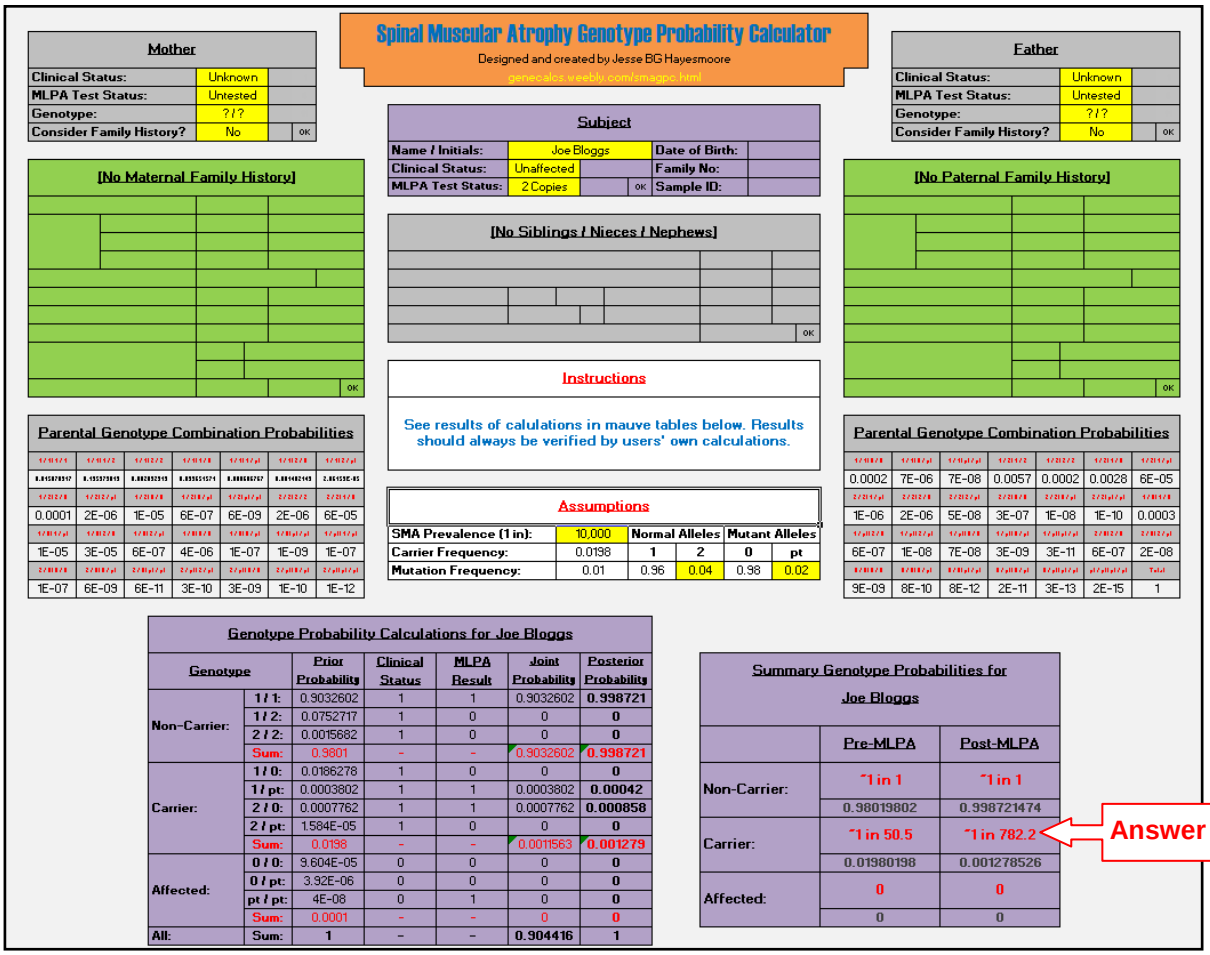
These values can be inputted into SMAGPC in the 'Assumptions' box (yellow cells) as follows; however, the ACGS assumptions are the default values in SMAGPC (i.e. no need to input / alter them).

Assumptions					
SMA Prevalence (1 in):	10,000	Normal Alleles		Mutant Alleles	
Carrier Frequency:	0.0198	**1**	**2**	**0**	**pt**
Mutation Frequency:	0.01	0.96	0.04	0.98	0.02

Model Risk Calculation 1: Carrier Test for Individual at Population Risk

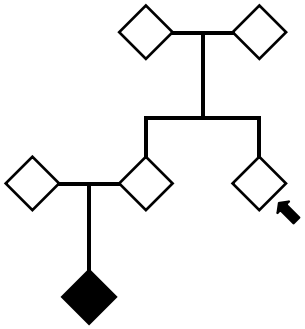
A randomly selected unaffected individual is tested by MLPA and found to have two *SMN1* copies. What is their residual carrier risk?

This scenario is entered into SMAGPC as follows (yellow boxes):



This gives a residual carrier risk of 1 in 782, which accords well with the 1 in 781 stated in the ACGS SMA Model Risk Calculations. The above scenario assumes that the clinical status of the individual's parents is unknown, which leaves open the possibility that they could be affected. It is probably more often the case that the parent's are known to be unaffected. Therefore, to obtain a slightly more accurate figure, the Mother's and Father's clinical status can be altered to 'Unaffected'. This gives a residual carrier risk figure of 1 in 790.

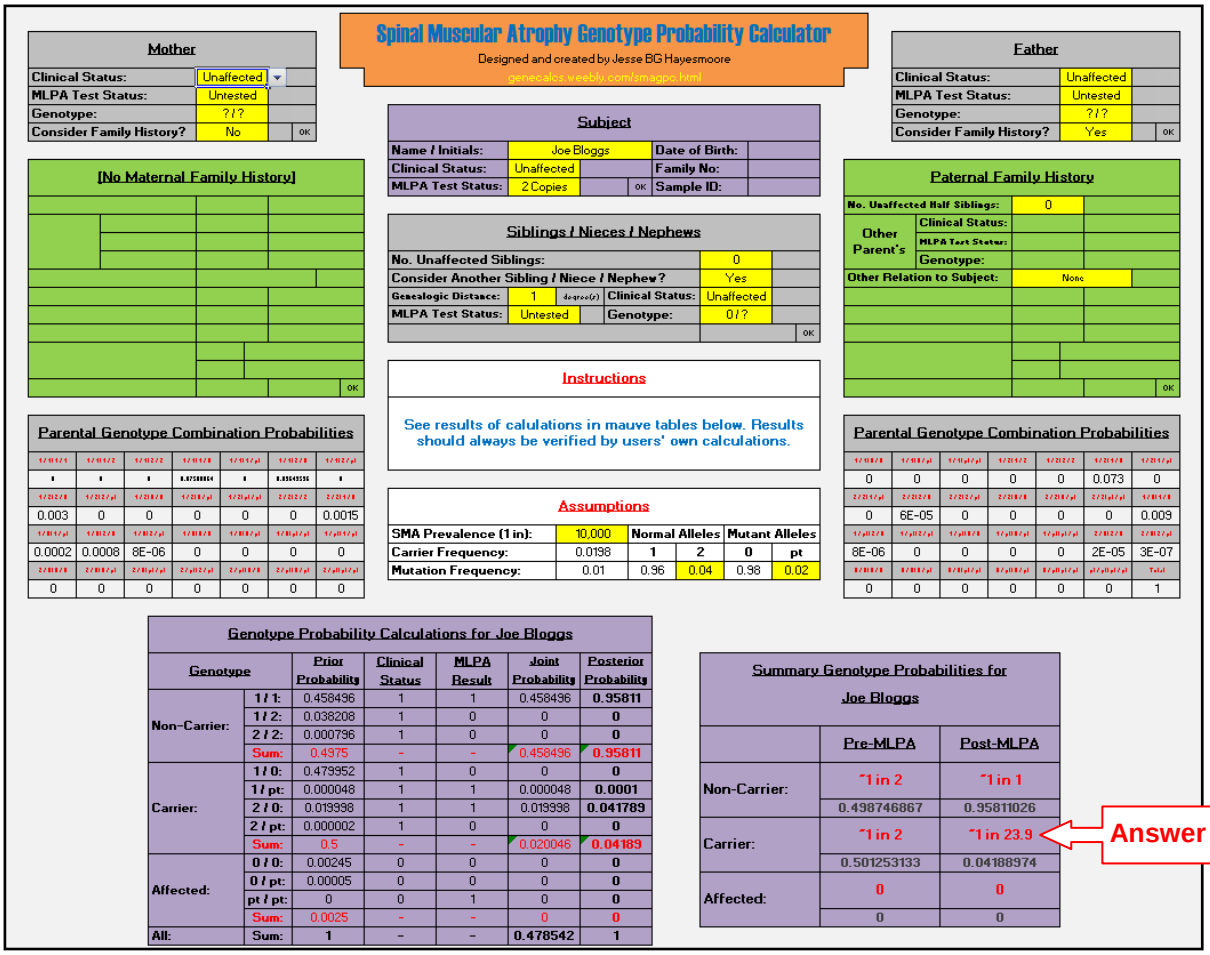
Model Risk Calculation 2: Carrier Test for Unaffected Aunt or Uncle of Affected Individual (Unaffected Carrier Sibling and Parents Not Tested)



0 *SMN1* Copies

In this scenario, the parents of the affected individual are both unaffected, but neither has been tested. However, as the affected individual is homozygous for *SMN1* deletion, each parent can be assumed to have one normal allele (either 1 or 2 *SMN1* copies) and one mutant allele with 0 *SMN1* copies. It should also be noted that in this scenario, both parents of the individual being tested (i.e. the affected individual's grandparents) are assumed to be unaffected (and are not tested). The individual being tested is found to have two *SMN1* copies.

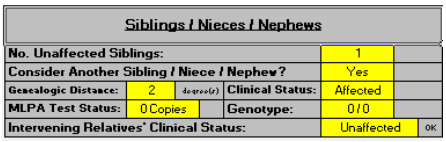
This scenario is entered into SMAGPC as follows:



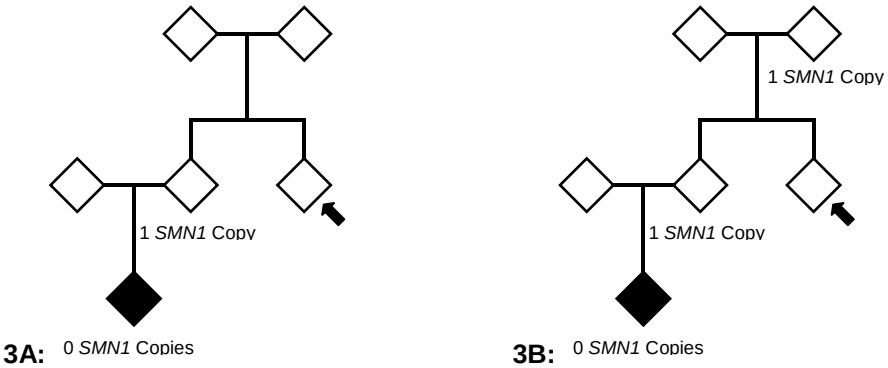
This gives a residual carrier risk of ~1 in 24 (or ~0.0419), as per the figure in the ACGS Model Risk Calculations.

In the above example, details for the testee's sibling were inputted in the 'Siblings / Nieces / Nephews' box. It should be noted that 'No. Unaffected Siblings' is set to 0 in this case, as this box refers only to full unaffected

siblings for which MLPA test status or genotype are not specified. A slightly less efficient, but equally valid, approach that would give an identical residual risk value would be to input details for the affected niece / nephew (instead of the unaffected sibling) as follows:



Model Risk Calculation 3: Carrier Test for Sibling of a Carrier (Parents Not Tested or Only One Tested and Shown to be a Carrier)



3A: 0 *SMN1* Copies **3B:** 0 *SMN1* Copies

This scenario is similar to Model Risk Calculation 2, but the difference is that the sibling of the testee has been tested and found to have one *SMN1* copy (i.e. genotype is '1 / 0'). The testee again is found to have two *SMN1* copies. The ACGS Model Risk Calculations demonstrate two variations for this scenario. In the first (referred hereon as 3A), both parents of the testee are assumed to be unaffected and completely untested. In the second variation (referred hereon as 3B), one of the parents has been tested and is found to have genotype '1 / 0'.

It may be tempting to enter Model Risk Calculation 3A by inputting the mother's genotype as '1 / ?' and the father's as '0 / ?' (or vice versa) without entering the '1 / 0' genotype of the testee's unaffected sibling. However, this would result in an over-estimation of residual carrier risk, as this would not adjust the relative parental genotype combination probabilities with the correct weighting (i.e. having a son with genotype '1 / 0' does not just inform that the parents must each have one of these alleles, but also, for example, makes the parental genotype combination of '1 / 0, 1 / 1' more likely than '1 / 0, 1 / 2'). Therefore, Model Risk Calculation 3A should be inputted as follows:

Spinal Muscular Atrophy Genotype Probability Calculator

Designed and created by Jesse BG Hayesmoore

genecalcs.weebly.com/smagpc.html

Mother	
Clinical Status:	Unaffected
MLPA Test Status:	Untested
Genotype:	? / ?
Consider Family History?	Yes

Father	
Clinical Status:	Unaffected
MLPA Test Status:	Untested
Genotype:	? / ?
Consider Family History?	No

Subject

Name / Initials:	Joe Bloggs	Date of Birth:	
Clinical Status:	Unaffected	Family No:	
MLPA Test Status:	2 Copies	Sample ID:	

Maternal Family History

No. Unaffected Half Siblings:	0	
Other Parent's	Clinical Status:	
	MLPA Test Status:	
	Genotype:	
Other Relation to Subject:	None	

[No Paternal Family History]

Siblings / Nieces / Nephews

No. Unaffected Siblings:		0	
Consider Another Sibling / Niece / Nephew?		Yes	
Genealogic Distance:	1 degree(s)	Clinical Status:	Unaffected
MLPA Test Status:	1 Copy	Genotype:	1 / 0

Instructions

See results of calulations in mauve tables below. Results should always be verified by users' own calculations.

Assumptions

SMA Prevalence (1 in):	10,000	Normal Alleles		Mutant Alleles	
Carrier Frequency:	0.0198	1	2	0	pt
Mutation Frequency:	0.01	0.96	0.04	0.98	0.02

Parental Genotype Combination Probabilities

1	1	1	0.51294	1	0.88016	1
0.0016	0	0	0	0	0	0
0.0002	0.0004	0	0	0	0	0
0	0	0	0	0	0	0

Parental Genotype Combination Probabilities

0	0	0	0	0	0.038	0
0	0	0	0	0	0	0.0094
8E-06	0	0	0	0	0	0
0	0	0	0	0	0	1

Genotype Probability Calculations for Joe Bloggs

Genotype		Prior Probability	Clinical Status	MLPA Result	Joint Probability	Posterior Probability
Non-Carrier:	1 / 1:	0.468096	1	1	0.468096	0.97899
	1 / 2:	0.029008	1	0	0	0
	2 / 2:	0.000396	1	0	0	0
	Sum:	0.4375	-	-	0.468096	0.97899
Carrier:	1 / 0:	0.489952	1	0	0	0
	1 / pt:	0.000048	1	1	0.000048	0.0001
	2 / 0:	0.009998	1	1	0.009998	0.02091
	2 / pt:	0.000002	1	0	0	0
	Sum:	0.5	-	-	0.010046	0.02101
Affected:	0 / 0:	0.00245	0	0	0	0
	0 / pt:	0.00005	0	0	0	0
	pt / pt:	0	0	1	0	0
	Sum:	0.0025	-	-	0	0
All:	Sum:	1	-	-	0.478142	1

Summary Genotype Probabilities for Joe Bloggs

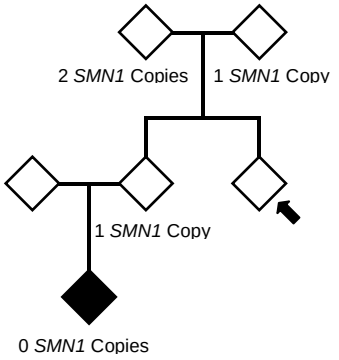
	Pre-MLPA	Post-MLPA
Non-Carrier:	~1 in 2	~1 in 1
	0.498746867	0.978989505
Carrier:	~1 in 2	~1 in 47.6
	0.501253133	0.021010495
Affected:	0	0
	0	0

Answer

This gives a residual carrier risk of ~1 in 48 (or ~0.0210), as per the figure in the ACGS Model Risk Calculations.

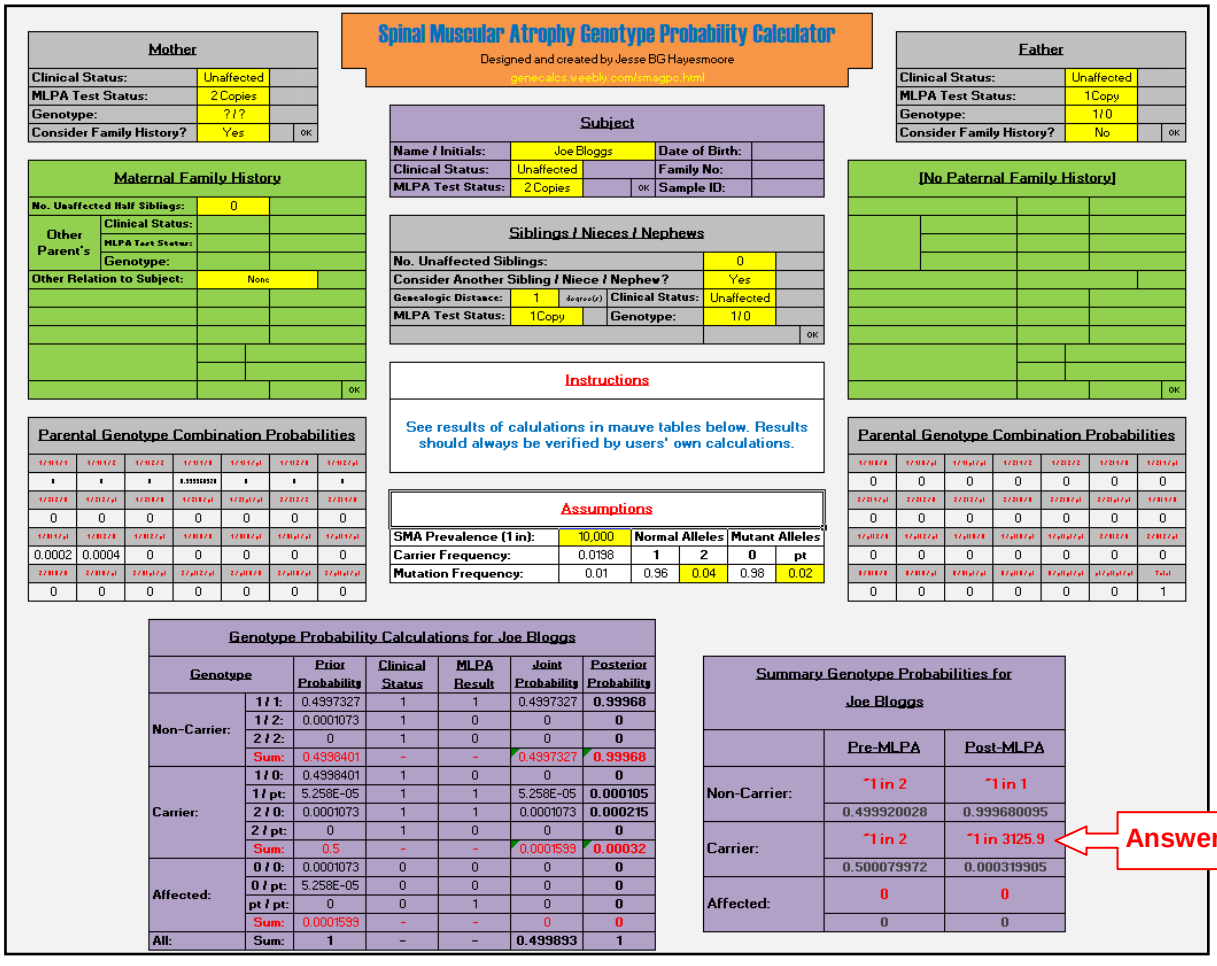
The above input for Model Risk Calculation 3A can be amended for Model Risk Calculation 3B by simply changing the ‘MLPA Test Status’ and ‘Genotype’ input for the mother or the father from ‘Untested’ and ‘? / ?’ to ‘1 Copy’ and ‘1 / 0’. This very slightly lowers the residual carrier risk of the testee to ~1 in 50 (or ~0.0201).

Model Risk Calculation 4: Carrier Test for Sibling of a Carrier (Both Parents Tested and Non-Carrier Parent Has Two Copies)



This scenario is similar to Model Risk Calculation 3B above; however, in this scenario, the other parent has also been tested and found to have two *SMN1* copies. The testee is again found to also have two *SMN1* copies.

Model Risk Calculation 4 should be inputted into SMAGPC as follows:



This gives a residual carrier risk of ~1 in 3,126, which accords well with the ~1 in 3,100 figure that was calculated in the ACGS Model Risk Calculations.

Concluding Remarks

This document demonstrates how SMAGPC can be used to accurately calculate residual carrier risks in what are likely to be commonly encountered clinical scenarios. These calculations are relatively simple, and, as demonstrated in the ACGS Model Risk Calculations document, do not necessarily require use of a calculator. However, clinical scenarios are not always as simple as those described. For example, the individual being tested may have one or more unaffected siblings (or half siblings), or the affected family member may be a more distant relation with several unaffected intervening relatives. To undertake manual calculations on what can be quite complex pedigrees, although possible, may be very time-consuming, impractical, and prone to error, especially if correcting for intervening relative's unaffected status. For these more complex scenarios, a program, such as SMAGPC, could be of great assistance. SMAGPC is designed to handle complex pedigrees, as it allows users to input information (clinical status, MLPA test status, and genotype) on multiple family members of all categories (i.e. siblings, nieces, nephews, aunts, uncles, cousins, and half-relatives) up to 20 genealogic degrees from the testee. For more information about SMAGPC, which is free to use, please visit http://www.genecalcs.weebly.com/smagpc.html.

Please note: Whilst every effort has been made to ensure that SMAGPC is error-free, this cannot be 100% guaranteed. Therefore, results should always be verified by users' own calculations. This is particularly important where results are to be used in a clinical setting.