Carrier Screening of SMA in North China

ZhiQiang Wang
Lvliang people's hospital
XiuLi Shen
Department of Obstestric and Gynecolody at Tonghua central hospital
QiRui Qin
Lvliang people's hospital
GuiMei Luo
Lvliang people's hospital
HuanHuan Liu
Lvliang people hospital
QingZhong He
shanghai Findgene clinical laboratory
Phuong Anh Nguyen Thi
Shanghai Findgene clinical laboratory
ZhengYuan Wang (wangzhengyuan@findgene.cn)
Shanghai FindGene Clinical Laboratories https://orcid.org/0000-0003-2724-6396

Research

Keywords: Spinal muscular atrophy, carrier frequency, SMN1

Posted Date: September 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-71381/v1

Abstract

Background: Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by loss of motor neurons and progressive proximal muscular atrophy. Although the SMN1 carrier frequencies were reported between 1:53- 1:83 in the Chinese population, carrier frequencies for many ethnicities, including most ethnic groups in North China, are unknown.

Methods: A total of 3,130 maternal blood samples were collected at the Lvliang People's hospital and Tonghua Central hospital. This research was performed with a three-stage screening procedure. The pregnant women were first examined for exon 7 and exon 8 copy number of *SMN1*. If the woman was determined as a SMA carrier, her partner was also tested, and if both parents were carriers, prenatal diagnosis was recommended. The copy number of both exons 7 and 8 of *SMN1* gene were identified by quantitative real-time polymerase chain reaction according to the manufacture's instructions.

Results: A total of 3,130 pregnant women including 1,405 cases from Lvliang People's Hospital and 1725 cases from Tonghua Central Hospital, were tested for SMA carriers using real-time PCR assay. Seventy six cases were heterozygous deletion of exons 7 and 8 in SMN1 gene [1 + 0 genotype], thus carrier frequency of SMN1 deletion is 1:42 (2.43%). After detailed genetic counseling, 52 related paternal partners were tested. Among those individuals, a couple from Tonghua was found to be high risk for having offspring with SMA and prenatal diagnosis was then implemented, and the fetus was diagnosed with SMA. The carrier of SMA frequency in Lvliang and Tonghua populations were 1: 57 and 1: 34 respectively. Therefore, the carrier frequency in Lvliang (1:56) was significantly lower than that in Tonghua (1:34) (p=0.0330). The prevalence of SMA in Lvliang and Tonghua populations were estimate to be 8.5E-5 and 2.25E-4 respectively.

Conclusion: In conclusion, our research has determined the distribution of SMA carrier frequency in the general pregnancies that are present in the northern of China population. This study also provides an accurate assessment of allele frequencies and estimates of SMA prevalence that were previously unavailable to clinicians and patients considering testing in the north of China.

1. Introduction

Spinal muscular atrophy (SMA) is one of the most common autosomal recessive neuromuscular disorders affecting infants and children, characterized by degeneration of motor neurons and progressive muscular atrophy [1]. The incidence of all types of SMA is estimated to be 1 in every 10,000 live births globally [2]. SMA is caused by mutations in SMN1 gene on chromosome 5q13.2. 95% of SMA patients have homozygous SMN1 deletions, 5% of SMA patients have compound heterozygous SMN1 deletion and point variant. Most patients inherit variants from their parents. Only approximately 2% of SMA patients have de-novo variants [3]. SMA is categorized into five types, which are based on motor function obtained and age of onset. Type 0 and I are the most extreme types with SMA onset beginning at birth. Patients usually achieve minimal motor function and develop respiratory failure, their life expectancy is around six months to two years[4]. Type II and III are the intermediate forms of the disorder where affected children are able to sit or walk for a short distance. Depending on their level of the weakness respiratory muscle, the survival time of patients may be beyond of 10 years of age. Type IV SMA begins during adult where patients have a normal life expectancy [5]. Up today,the US Food and Drug Administration approved NUSINERSEN (in December 2016) and ZOLGENSMA (in May 2019) for treatment SMA patients. NUSINERSEN was the first drug approved to treat pediatric and adult patients with SMA while ZOLGENSMA was the first gene therapy approved to

treat SMA children under two years old. These efficacies of novel drugs have recently been demonstrated that improve or ameliorate symptoms in many SMA patients [6]. With the availability of treatment options, the knowledge of carrier frequencies of SMN1 is essential for prevention or early treatment for this disease[7]. Although the SMN1 carrier frequencies were reported between 1:53 – 1:83 in the Chinese population, most of which were studied in south of China [8, 9]. The SMA carrier frequencies may be variable in different geography in China and the information on the prevalence of SMA of the North China is limited, especially in Lvliang (the northwest) and Tonghua (the northeast). Therefore, we conducted a comparative study to estimate the SMN1 allele frequency in People's Hospital at Lvliang city and the Central hospital of Tonghua.

2. Results

2.1 SMN1 carrier frequency

Total of 3,130 cases obtained SMA results, 76 cases 2.43% (95% CI, 1.87%-2.93%) were heterozygous deletion SMN1 carriers and 3054 cases were SMN1 wild type (Fig. 1, Table 1). In 76 healthy carriers, 25 cases (1.779%) were in Lvliang and 51 cases (2.96%) were in Tonghua as summarized in Table 2. Therefore, the carrier frequency in Lvliang and Tonghua were estimated to be 1:56 and 1:34 respectively. All of the SMA carrier pregnancies were given detailed genetic counseling regarding the etiological factors, inheritance pattern, clinical features, reproductive risk and the treatment of SMA. After being made fully aware of the disease, 14/25 (56%) male partners in Lvliang and 38/51 (74.51%) male partners in Tonghua were also recalled and tested for SMN1 copy number by qPCR, of whom 1 partner (1/38, 2.63%) in Tonghua was found to be a SMA carrier (Table 1).

Table 1
SMN1 carrier frequency

The hospital	Lvliang People's hospital	Tonghua Central hospital	Total
Women screened	1405	1725	3130
Carrier	25	51	76
Carrier rate	1.779%	2.957%	2.428%
A priori frequency	1: 56 (95% CI, 1/92 – 1/41)	1: 34 (95%CI, 1:27 – 1:46)	1:41(95%CI, 1:34 – 1:53)
Partners	14	38	52
Recall rate	56%	74.509%	68.421%
Carrier couples	0	1	1
Carrier rate partners	0%	2.631%	1.923%
Prenatal diagnoses	0	1	1
Affected cases	0	1	1
Pregnancies terminated	0	1	1
Termination rate	0	100%	100%

One couple of high risk was offered invasive prenatal diagnosis of SMA. The exon 7 and exon 8 copy number of SMN1 and SMN2 genes of amniotic fluid sample were determined by CNVplex®. The results of amniocentesis indicated that this woman was carrying a fetus with homozygous deletion of SMN1 gene and three copies in exon 7 of SMN2. The detailed copy numbers of SMN1 and SMN2 of patients were shown in Table 2.

Table 2
The copy numbers of SMN1 and SMN2 genes of the amniotic fluid sample were determined by CNVplex® of the amniotic fluid sample.

Amniotic fluid sample	SMN 1 exon 7	SMN 1 exon 8	SMN 2 exon 7	SMN2 exon 8	Results
Patient	0.0	0.0	3	2	Homozygous deletion of SMN1 and 3 copies in exon 7 of SMN2

Previous studies have reported that SMA phenotype is mostly caused by homozygous deletions [11, 12]. After careful prenatal counseling, the pregnancy was terminated, and the fetus tissue then was also confirmed to have SMA with homozygous deletion of the exon 7 and 8 of the SMN1 gene. Results of this family were shown in Table 3 and Fig. 2.

Table 3
The copy number of SMN1 genes of the carrier couples and fetus were explored by qPCR

qPCR	SMN1 exon 7	SMN1 exon 8
Pregnancy	1 copy	1 copy
Partner	1 copy	1 copy
Fetus tissue	0 copy	0 copy

2.2 The copy number of the SMN1 gene in 3,130 pregnant women

The observed one-copy genotype (1 + 0) frequency was 1 in 56 (1.78%) in Lvliang, 1 in 34 (2.96%) in Tonghua, and 1 in 41 (2.43%) in total (Table 4). The 1:56 carrier frequency detected in the Lvliang sample group is comparable to the 1:53 and 1:83 that reported in different areas of Southern Chinese populations. However, the carrier frequencies of SMN1 genotypes of Tonghua pregnancies were significant higher than Lvliang specimens (p = 0.0330). Additionally, the two copy and three copy genotype frequencies of SMN1detected in our data are 96.39% and 1.82% respectively. The 96.39% of two copies frequencies is higher than the 92.55% has been reported in the Chinese population (3.84%, p = 0.0001). However, the 1.82% three copy genotype frequencies of SMN1 in this research is lower than the 5.68% that reported in the Chinese population (3.86%, p = 0.0001)[13].

Table 4
Frequency of *SMN1* copy number on two alleles of 3130 healthy pregnant women

Geographic regions	1 copy (1 + 0)		2 copies (1 + 1, 2 + 0)		3 copies (2 + 1)		Total n
	n	Frequency 95%CI	n	Frequency 95%CI	n	Frequency 95%CI	
Lvliang	25	0.0178 (0.0109–0.0247)	1319	0.938(0.926–0.951)	61	0.0433 (0.0328–0.0541)	1405
Tonghua	51	0.0296 (0.0216–0.0376)	1593	0.923(0.911–0.936)	81	0.0469 (0.0369–0.0569)	1725
Total	76	0.0243(0.0189–0.0297)	2911	0.930(0.921–0.939)	142	0.045 (0.038–0.0527)	3130

Table 5
Frequencies of *SMN1* copies per allele and allele pairing for each ethnic group

***SMN1* copies per allele**	**Lvliang**	**Tonghua**
0	0.0092	0.015
1	0.9685	0.9607
2	0.0224	0.0244
Allele pairings		
Non-carrier		
2 + 2	0.0005	0.0006
2 + 1	0.0434	0.0469
1 + 1	0.9380	0.9229
Carrier		
1 + 0	0.01782	0.2882
2 + 0	4.1E-4	7.3E-4
Affected		
0 + 0	8.5E-5	2.25E-4

Hardy–Weinberg equilibrium was used to calculate the frequencies of *SMN1* copies per allele

Table 6
Overview the SMN1 carrier frequencies

City/Ethnicity	Region	Total samples	Subject	Carrier frequency	A priori frequency	Method	References
Shanghai	The southeast of China	4,719	Pregnancies	1.9%	1:53	PCR-DHPLC	[8]
Liuzhou	The south of China	4,931	Pregnancies	1.2%	1:83	PCR-DHPLC	[9]
Lvliang	The northwest of China	1,405	Pregnancies	1.77%	1:56	Realtime PCR	This article
Tonghua	The north east of China	1,725	Pregnancies	2.96%	1:34	Realtime PCR	This article
Hong Kong	The south of China	569	Normal southern Chinese	1.6%	1:63	Realtime PCR	[14]
Chinese	China	13,069	Pregnancies	1.77%	1:56	Realtime PCR	[15]
Taiwanese	Taiwan	107,611	Pregnancies	2.10%	1:48	Realtime PCR	[16]
Korean	Korea	1,581	umbilical cord blood	1.83%	1:55	Realtime PCR	[17]
Thai	Thailand	505	healthy adults	1.78%	1:56	Real time PCR	[18]
Iranian	Iran	200	Healthy couple	5%	1:20	Real time PCR	[19]
Russian	Russia	2,253	Healthy adults	2.78%	1:36	MLPA	*
French	France	229	patients	1.79–2.94%	1:34 – 1:56	Real time PCR	[20, 21]
Australian	Australia/ New Zealand	147	Healthy adults	2.04%	1:49	Real time PCR	[22]
American	North America	5,102	Healthy adults	1.67–2.50%	1:37 – 1:125	Real time PCR	[23]
Moroccan	Morocco	150	New born	4%	1:25	Real time PCR	[24]

*: The paper was presented by professor A.V. Polyakov in the American Society of Human Genetics (ASHG 2016) conference.

City/Ethnicity	Region	Total samples	Subject	Carrier frequency	A priori frequency	Method	References
Germans	West Thuringen	3/1,778,200	Patients	2.86-4%	1:25 – 1:35	Real time PCR	[25]
*: The paper was presented by professor A.V. Polyakov in the American Society of Human Genetics (ASHG 2016) conference.							

3. Discussion

In present study, the total SMN1 one copy genotype frequency from Lvliang (1:56) and Tonghua (1:34) were similar or higher than reported in the previous Chinese population studies (1: 56)[13]. Reports of carrier frequencies in South of China vary from lower in Liuzhou (1:83), intermediate in Hongkong (1:63) Shanghai (1:53) (Table 6). However, the carrier frequency in Tonghua (1:34) was the highest frequency reported for the Chinese population studies. This result indicated that the distribution gene pools of SMA of the Chinese Han ethnic groups between the North West, the North East and the South regions were different. Reports of carrier frequencies in other countries vary from lower in the United Kingdom (from 1:60 to 1:80), intermediate in Thailand (1:56), Taiwan (1:48), United States (1:41), Korea (1:47), and Australia (1:49), to higher frequencies (1:20) in Iran and European (1:25 – 1:35) populations (Table 6). Compared to other countries, the carrier frequency Tonghua was higher than that reported for the United Kingdom, East Asia, Australia, and United States population. However, it was similar to or lower than that reported for the European and Iranian population (Table 6). According to data from the Lvliang and Tonghua Statistical Information Service (http://www.xiandaiyuwen.com/news/shuju/697913.html; accessed on 13 July 2020), in 2019, the total population in Lvliang and Tonghua were 3,885,600 with 380,000 births and 2,159,400 with 13,711 births respectively. Based on the carrier frequencies in this study, the number of carriers in Lvliang was estimated to be 68,168 in total and 667 in newborns per year and the number of carriers in Tonghua was estimated to be 63,512 in total, and 403 in newborns per year. Using the observed genotype data and assuming Hardy–Weinberg equilibrium, maximum likelihood estimation was employed to determine frequencies for alleles and allele pairings within all sample groups (Table 5). These calculations reveal that the expected the prevalent of *SMA* in the Lvliang and Tonghua group is 8.5E-5 and 2.25E-4 respectively. The prevalent of SMA in Tonghua population is comparable to the Russian populations that reported in previous study (p = 0.9979) (* Table 6). Our study had some limitations. Due to the limitation of qPCR method, 5% of SMA patients with SMN1 missense variant and silent carrier genotype SMN1 '2 + 0' could not be identified by the qPCR. Therefore, the SMA carrier frequency might be underestimated than that could be. By contrast, this study also had several advantages. This is the first large-scale population study on the SMA carrier frequency in Northern of China conducted using samples from pregnant women. Further, this study also provides an accurate assessment of allele frequencies and estimates of SMA prevalence that were previously unavailable to clinicians and patients considering testing in the north of China.

4. Conclusion

In conclusion, our research has indicated SMA carrier frequency in the northern of China. The results also gave a valuable data for a nationwide program of genetic counseling, population screening and clinical/prenatal diagnosis to prevent SMA in China. Such an approach should provide Chinese couples who are undergoing genetic counseling with improved choices for their family planning.

5. Method

5.1 Study design

This study was approved by the institutional review board of the People's Hospital of Lvliang and Central Hospital of Tonghua. This was a retrospective study conducted with data collection from 20 August 2018 to April 28, 2020. The inclusion criteria for participants were: pregnant women with no family history of SMA. A total of 3,130 pregnant women with no family history of SMA were accepted for SMA carrier screening, of whom 1,405 cases came from Lvliang and 1,725 cases came from Tonghua. This research was performed with a three-stage screening procedure. At the first stage, the pregnant women were examined for exon 7 and exon 8 copy number of *SMN1*. If a woman was found to be a SMA carrier, her partner was also recommended for testing, and if both parents were carriers, prenatal diagnosis was recommended.

All of the screening participants were given standard genetic counseling. Information on the participants was listed in Fig. 1 and Table 2.

5.2 SMA screening test

A total of 5 mL of peripheral blood was collected from each participant. Genomic DNA was extracted using GO-BTCD-400 Kit (Gene on Biotech, China) according to the manufacturer's instructions. According to the Chromysky Medical Research protocol, the copy numbers of exon 7 and exon 8 of SMN1 gene were performed by quantitative real-time polymerase chain reaction (qPCR) (Chromysky Medical Research, Shanghai, China). The amplification of SMN1 and *RPP40* genes (internal standard) were done by multiplex PCR. Each assay included five control samples, which were a no-DNA control, one SMN1 exon 7 or exon 8 deletion control, and three gradients control of two-copy of *SMN1*. All samples were performed by an ABI StepOne plus real-time PCR system. The data from FAM and VIC channels were used to calculate the florescence and evaluate the cycle threshold (Ct). ΔCt and ΔΔCt were then calculated to define the *SMN1* copy number by using the $2^{-\Delta\Delta Ct}$ method (Table 7).

Table 7
The calculation SMN1 copy number of exon 7 and exon 8 were based on ΔΔCt value.

ΔΔCt value for exon 7	
Wild type	ΔΔCt ≤ -0.55
Heterozygous deletion	-0.45 ≤ ΔΔCt ≤ 0.45
Homozygous deletion	ΔΔCt > 0.8
Retest	-0.55 < ΔΔCt< -0.45
Retest	0.45 < ΔΔCt ≤ 0.8
ΔΔCt value for exon 8	
Wild type	ΔΔCt ≤ -0.55
Heterozygous deletion	-0.45 ≤ ΔΔCt ≤ 0.45
Homozygous deletion	ΔΔCt > 1.5
Retest	-0.55 < ΔΔCt< -0.45
Retest	0.45 < ΔΔCt ≤ 1.5

The copy number of exon 7 and exon 8 of SMN1 gene were calculated according to scheme shown in the Table 1. Re-testing would be performed when ΔΔCt values ranged in -0.55 < ΔΔCt< -0.45 or 0.45 < ΔΔCt ≤ 1.5.

5.3 Clinical follow- up

Pregnancies with wild type SMA screening results were advised for regular prenatal care; genetic counseling was provided if routine ultrasound examination showed abnormalities. Pregnant women with heterozygous deletion SMN1 results were given detailed genetic counseling regarding the etiological factors, inheritance pattern, clinical features, reproductive risk and the treatment of SMA and her partners were offered SMA screening test. If both partners were defined as SMA carriers, the genetic counseling was provided for invasive prenatal genetic diagnostic testing of the fetus. The copy number of SMN1 and SMN2 genes of amniotic fluid sample were explored by CNVplex® (a technique for high-throughput detection of sub-chromosomal copy number aberrations) as described before[10]. The homozygous deletion SMN1 of fetus result was defined as true positive upon postnatal genetic diagnostic confirmation or clinical follow-up results. Patients without confirmatory diagnostic results were excluded from this research.

5.4 Statistical analysis

Statistical analysis between the different groups was performed using a chi-square test or Fisher's exact test, and *P* values of < 0.05 were considered statistically significant.

Abbreviations

SMA: Spinal muscular atrophy

qPCR: quantitative real-time polymerase chain reaction

Declarations

Funding Statement

This research received no external funding.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

The authors are very grateful to the pregnant women and their spouses or partners who participated in this research and to the whole team in the laboratory of Findgene.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Medical Ethics Committee of Lvliang People's hospital and Tonghua Central hospital. Informed consent was waved because of the retrospective design. The authors had no access to information that could identify individual participants during and after data collection.

Consent for publication

Not applicable

Authors' contributions

ZY W and Z-Q W designed the study. All the authors contributed to the generation, collection, assembly, analysis and/or interpretation of data. PA NT wrote the manuscript, QZ H and ZY W revised the manuscript. All the authors have read manuscript and approved the final manuscript.

Corresponding author

Correspondence to Dr. ZhengYuan Wang

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. D'Amico, A., et al., *Spinal muscular atrophy.* Orphanet Journal of Rare Diseases, 2011. **6**(1): p. 71.
2. Verhaart, I.E.C., et al., *Prevalence, incidence and carrier frequency of 5q–linked spinal muscular atrophy – a literature review.* Orphanet Journal of Rare Diseases, 2017. **12**(1): p. 124.
3. Arnold, W.D., D. Kassar, and J.T. Kissel, *Spinal muscular atrophy: diagnosis and management in a new therapeutic era.* Muscle Nerve, 2015. **51**(2): p. 157-67.
4. Kolb, S.J. and J.T. Kissel, *Spinal Muscular Atrophy.* Neurol Clin, 2015. **33**(4): p. 831-46.
5. Swoboda, K.J., et al., *Perspectives on clinical trials in spinal muscular atrophy.* J Child Neurol, 2007. **22**(8): p. 957-66.
6. Dangouloff, T. and L. Servais, *Clinical Evidence Supporting Early Treatment Of Patients With Spinal Muscular Atrophy: Current Perspectives.* Ther Clin Risk Manag, 2019. **15**: p. 1153-1161.
7. Lin, Y., et al., *Newborn Screening for Spinal Muscular Atrophy in China Using DNA Mass Spectrometry.* Frontiers in Genetics, 2019. **10**(1255).
8. Gong, B., et al., *[Carrier screening for spinal muscular atrophy in 4719 pregnant women in Shanghai region].* Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 2013. **30**(6): p. 670-2.
9. Tan, J., et al., *[Screening for spinal muscular atrophy mutation carriers among 4931 pregnant women from Liuzhou region of Guangxi].* Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 2018. **35**(4): p. 467-470.
10. Liang, Q., et al., *Molecular and clinical profile of VWD in a large cohort of Chinese population: application of next generation sequencing and CNVplex(®) technique.* Thromb Haemost, 2017. **117**(8): p. 1534-1548.
11. Ahn, E.J., et al., *Genotype-Phenotype Correlation of SMN1 and NAIP Deletions in Korean Patients with Spinal Muscular Atrophy.* J Clin Neurol, 2017. **13**(1): p. 27-31.
12. Kesari, A., et al., *Genotype-phenotype correlation of SMN locus genes in spinal muscular atrophy patients from India.* Exp Mol Med, 2005. **37**(3): p. 147-54.
13. Zhang, J., et al., *Carrier Screening and Prenatal Diagnosis for Spinal Muscular Atrophy in 13,069 Chinese Pregnant Women.* The Journal of Molecular Diagnostics, 2020. **22**(6): p. 817-822.
14. Chan, V., et al., *Carrier incidence for spinal muscular atrophy in southern Chinese.* J Neurol, 2004. **251**(9): p. 1089-93.
15. Zhang, J., et al., *Carrier Screening and Prenatal Diagnosis for Spinal Muscular Atrophy in 13,069 Chinese Pregnant Women.* J Mol Diagn, 2020. **22**(6): p. 817-822.
16. Su, Y.N., et al., *Carrier screening for spinal muscular atrophy (SMA) in 107,611 pregnant women during the period 2005-2009: a prospective population-based cohort study.* PLoS One, 2011. **6**(2): p. e17067.
17. Park, J.E. and S.A. Yun, *Carrier Frequency of Spinal Muscular Atrophy in a Large-scale Korean Population.* 2020. **40**(4): p. 326-330.
18. Dejsuphong, D., et al., *Carrier frequency of spinal muscular atrophy in Thailand.* Neurol Sci, 2019. **40**(8): p. 1729-1732.
19. Hasanzad, M., et al., *Carrier frequency of SMA by quantitative analysis of the SMN1 deletion in the Iranian population.* Eur J Neurol, 2010. **17**(1): p. 160-2.
20. *Abstracts from the 51(st) European Society of Human Genetics Conference: Posters*. Eur J Hum Genet. 2019 Jul;27(Suppl 1):1-688. doi: 10.1038/s41431-019-0404-7. Epub 2019 Jul 3.
21. Bürglen, L., et al., *Structure and organization of the human survival motor neurone (SMN) gene.* Genomics, 1996. **32**(3): p. 479-82.

22. Smith, M., et al., *Population screening and cascade testing for carriers of SMA.* European Journal of Human Genetics, 2007. **15**(7): p. 759-766.
23. Hendrickson, B.C., et al., *Differences in SMN1 allele frequencies among ethnic groups within North America.* J Med Genet, 2009. **46**(9): p. 641-4.
24. Lyahyai, J., et al., *Spinal muscular atrophy carrier frequency and estimated prevalence of the disease in Moroccan newborns.* Genet Test Mol Biomarkers, 2012. **16**(3): p. 215-8.
25. Verhaart, I.E.C., et al., *Prevalence, incidence and carrier frequency of 5q-linked spinal muscular atrophy - a literature review.* 2017. **12**(1): p. 124.
26. Vijzelaar, R., et al., *The frequency of SMN gene variants lacking exon 7 and 8 is highly population dependent.* PLoS One, 2019. **14**(7): p. e0220211.

Figures

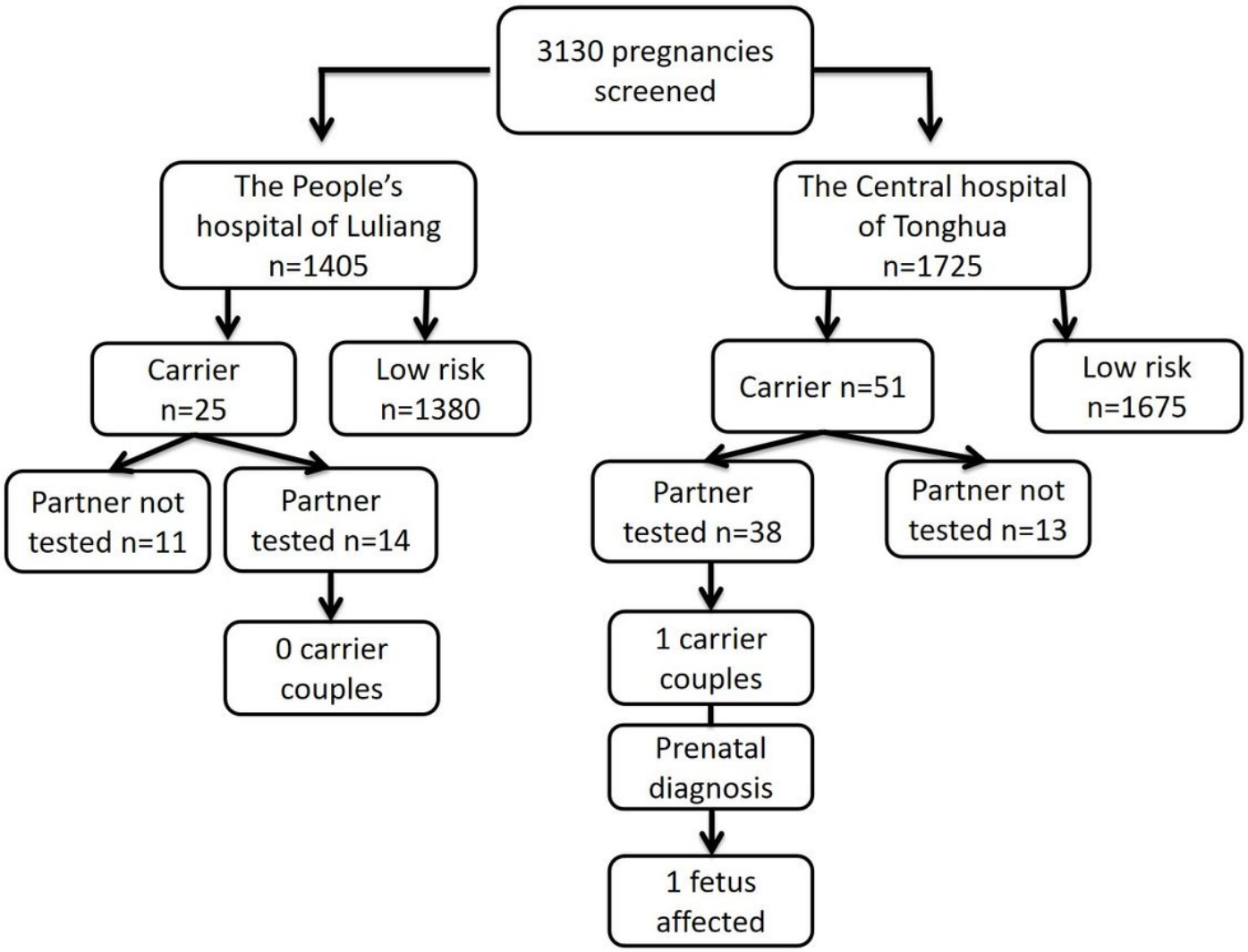

Figure 1

Flowchart of SMA screening results of pregnant women in the People's hospital of Lvliang and the Central hospital of Tonghua

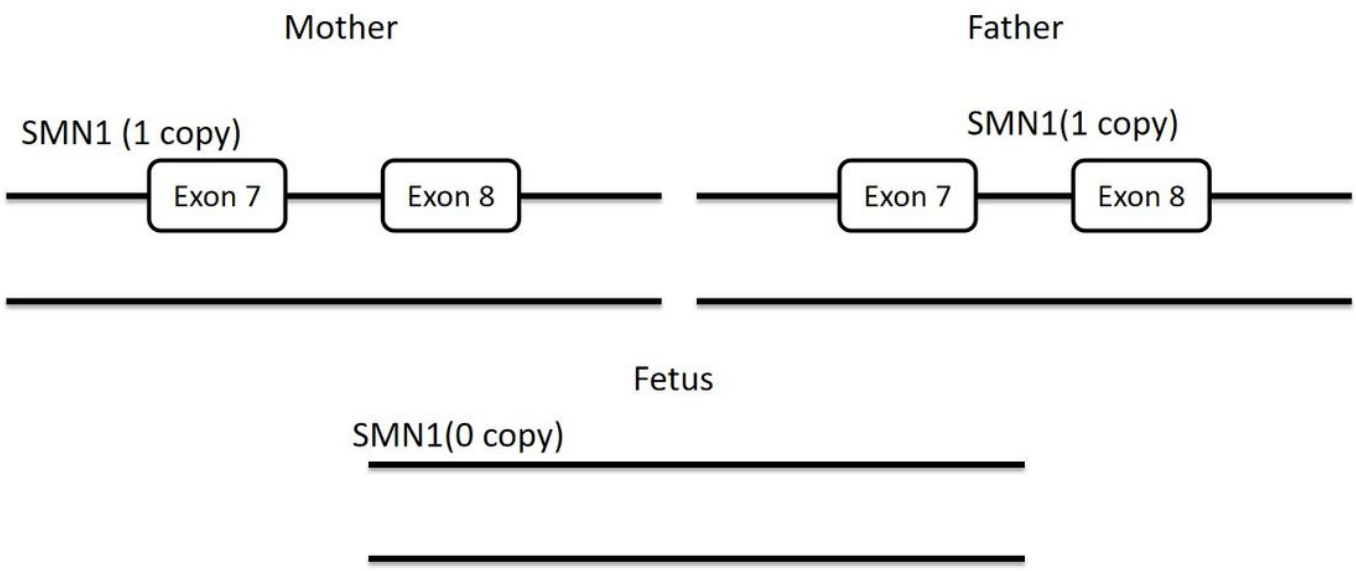

Figure 2

The copy numbers of SMN1 of the family members had SMA patient