Screening and Prenatal Diagnosis of Spinal Muscular Atrophy among Reproductive-Age Individuals from the Hubei Region

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ABSTRACT

Objective: To conduct carrier screening for spinal muscular atrophy (SMA) among individuals of childbearing age in the Hubei region, identify the carrier frequency, and provide a relevant basis and reference for prenatal diagnosis.

Study Design: An observational study.

Place and Duration of the Study: Department of Medical Genetics Centre, Maternal and Child Health Hospital of Hubei Province, Hubei, China, from August 2019 to August 2022.

Methodology: Real-time quantitative PCR was performed on 4,816 reproductive individuals from the Hubei region to detect the copy numbers of E7 and E8 in the *SMN1* gene. The screening of SMA carriers and their spouses and prenatal diagnostic analysis of high-risk foetuses were also performed. Statistical analyses were conducted using SPSS version 20.0. Categorical data were compared using Chi-square tests, with statistical significance set at $p < 0.05$.

Results: A total of 105 SMA carriers were identified, with a carrier rate of 2.18%. Among them, 100 carriers had heterozygous deletions of *SMN1* exons 7 and 8, and five carriers had heterozygous deletions of *SMN1* exon 7. The carrier rate was 2.33% in males and 2.15% in females. Four couples were found to be carriers (both with heterozygous deletions of *SMN1* exons 7 and 8). Prenatal diagnosis of their foetuses showed that two were carriers, one foetus was affected with SMA (homozygous deletion of *SMN1* exons 7 and 8), and one had no abnormalities. The result for the foetus with homozygous deletion was verified by multiplex ligation-dependent probe amplification (MLPA).

Conclusion: Screening SMA carriers and population genetic counselling can reduce SMA foetus births, with great significance for eugenics.

Key Words: *Spinal muscular atrophy (SMA), Carrier screening, Prenatal genetic diagnosis, Real-time quantitative PCR, SMN1 gene, Eugenics.*

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INTRODUCTION

Spinal muscular atrophy (SMA) represents a commonly encountered and profoundly severe neuromuscular disorder that is often fatal in children. This hereditary motor neuron disorder manifests as muscle weakness and atrophy, pathologically attributed to the degenerative loss of anterior horn cells in the spinal cord.[1] First documented in 1891, SMA is categorised into five subtypes based on the age of onset and motor functional milestones.[2,3] Type 0 patients are affected at or before birth, with almost no limb movement and requiring ventilator assistance immediately after birth. Type I patients show severe hypotonia within the first six months after birth, accompanied by swallowing and growth difficulties, and generally do not survive beyond two years. Type II patients develop symptoms between 6 and 18 months of age, presenting with gradually worsening generalised muscle weakness and delayed motor development in infancy. Type III patients are affected between 18 months and 10 years of age, gradually developing proximal-dominant muscle weakness during childhood, and later developing scoliosis and respiratory insufficiency. Type IV patients develop the disease during adolescence or adulthood, with slow disease progression.

The population incidence of SMA ranges from approximately 1 in 6,000 to 1 in 10,000, while the carrier rate is estimated to be between 1 in 42 and 1 in 48.[4-6] In China, about 10 million babies are born each year. If one out of every 10,000 newborns has SMA, then there are nearly 1,000 new SMA cases annually. This not only places a heavy economic burden and mental stress on patients' families but also causes a significant consumption of social medical resources.[7]

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SMA is recognised as one of the most prevalent autosomal recessive genetic disorders, a significant contributor to childhood mortality, and a rare disease with high morbidity and mortality rates among infants and young children.[8] Historically, the diagnosis of SMA relied mainly on comprehensive methods, including detailed medical history taking combined with clinical analysis such as electromyography, muscle enzyme tests, and muscle biopsies. However, this diagnostic approach had significant drawbacks, being highly invasive and complex, and could cause adverse effects on children, including pain and an increased risk of infection.[9,10] Currently, given the limited treatment options and high treatment costs for SMA, the American College of Obstetricians and Gynaecologists suggests that women should undergo SMA carrier screening before conception or during early pregnancy.[11] In 2020, China's Expert Consensus on Genetic Diagnosis of Spinal Muscular Atrophy also suggested implanting SMA carrier screening in the general population.[12] However, China still lacks large-scale, systematic data on SMA carrier screening, particularly regarding regional variations in carrier rates remain unclear. This has restricted the precise advancement of SMA prevention efforts.

As a densely populated central region of China, Hubei has not yet been the focus of any targeted studies investigating SMA carrier status among its reproductive-aged population. Therefore, this study aimed to adopt real-time fluorescence quantitative PCR (real-time PCR) technology to conduct SMA carrier screening among reproductive-aged individuals in Hubei. It aimed to clarify the SMA carrier rate and genotype distribution patterns in this region and, simultaneously, to perform prenatal diagnosis for high-risk foetuses. This research will provide data to support the primary and secondary prevention of SMA in Hubei, thereby contributing to a reduction in the birth rate of SMA-affected children and improving birth quality in the region.

METHODOLOGY

Clinical data of 4,816 reproductive-aged individuals who visited the Maternal and Child Health Hospital of Hubei Province and voluntarily underwent SMA carrier screening from August 2019 to August 2022 were collected and analysed. The inclusion criteria comprised participants undergoing pre-pregnancy examination who had normal male or female phenotypes, were aged 17 years or older (including 859 males and 3,957 females), and voluntarily consented to SMA carrier screening after signing the informed consent document. The exclusion criterion included individuals with a family history or childbearing history of SMA. The Medical Ethics Committee of Hubei Maternal and Child Health Hospital, Hubei, China, granted approval for this study [2021 IEC (LW039)].

Two millilitres (mL) of peripheral venous blood were collected from the reproductive-age individuals participating in the screening. Genomic DNA was extracted from peripheral blood specimens *via* a commercially available DNA isolation kit (Guangzhou Daan Gene Co., Ltd. Product Model: No. 20170666). Pregnant women requiring prenatal diagnosis underwent amniocentesis at 18 weeks of gestation, and 10 mL of amniotic fluid was collected to extract the genomic DNA of the amniotic fluid. The DNA concentration was measured and adjusted to 10-20 ng/μL, and the ratio of OD260nm/OD280nm was between 1.8 and 2.0.

Real-time PCR was performed using a detection kit for exon deletion of the *survival motor neuron 1 (SMN1) gene* (Shanghai Wuse Shi Medical Technology Co., Ltd) to detect the *SMN1* gene copy number. Multiplex real-time PCR employing MGB probes was implemented, with *human RPP40* gene serving as the internal reference (Shanghai Wuse Shi Medical Technology Co., Ltd). The amplification of exons 7 and 8 of the *SMN1* gene was performed, followed by a relative quantitative analysis of the copy number. At the same time, a chemical blocking approach was employed to mitigate the impact of *SMN2* on the detection outcomes. Each sample was divided into two reaction tubes: E7 and E8. Three concentration gradients of normal control products, deletion control products, and blank control products were set, respectively.

The copy number variations of exons 7 and 8 in the *SMN1* and *SMN2* genes were verified using the multiplex ligation-dependent probe amplification (MLPA) P060-B2 SMA kit (MRC-Holland, Netherlands). After DNA denaturation, probe hybridisation with the sample DNA, ligation, and resulting PCR amplification of the ligated probes, the PCR amplicons were analysed using capillary electrophoresis. Data analysis and processing were performed with Coffalyser V8.0 software (https://www.mlpa.com/coffalyser).

Prenatal diagnosis was performed on foetuses whose both parents were carriers. The pregnant women underwent amniocentesis after 18 weeks of gestation. They were placed in the supine position, and their skin was routinely disinfected. Under the guidance of a specialist, the puncture site was located, and amniotic fluid was extracted transabdominally for detection.

Statistical analyses were conducted using SPSS version 20.0. Categorical data were presented as percentages and case counts, and intergroup comparisons were performed *via* the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

The *SMN1* exon copy number deletions were identified in 4,816 samples, including 859 males and 3,957 females. A total of 105 SMA carriers were detected, with an overall carrier rate of 2.18% (105/4,816), comprising 20 males (2.33%, 20/859), and 85 females (2.15%, 85/3,957). No significant difference in carrier frequencies was observed between males and females ($\chi^2 = 0.107$, $p = 0.743$; Table I).

Fluorescence quantitative PCR detection results for exons 7 and 8 of the *SMN1* gene, including their amplification curves, are illustrated in Figure 1. Five carriers showed heterozygous deletions in the *SMN1* gene exon 7 (0.10%, 5/4,816); 100 carriers had heterozygous deletions involving exons 7 and 8 of the *SMN1* gene (2.08%, 100/4,816).

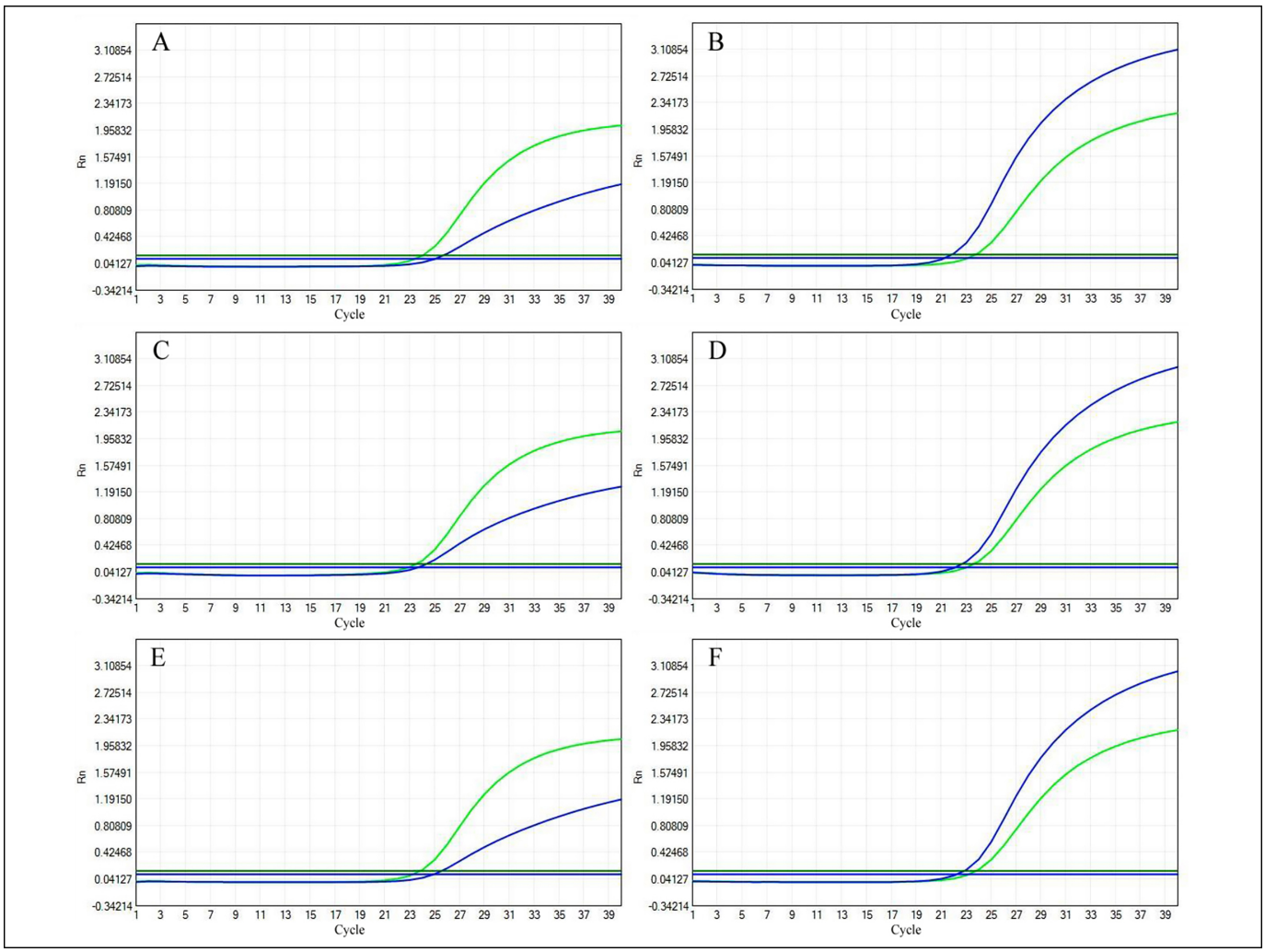

Figure 1: Fluorescence quantitative PCR amplification curves for exons 7 and 8 of the *SMN1* gene. (A) Amplification curve showing a heterozygous deletion of *SMN1* E7; (B) Amplification curve showing no deletion of *SMN1* E8; (C) Amplification curve showing no deletion of *SMN1* E7; (D) Amplification curve showing a heterozygous deletion of *SMN1* E8; (E) Amplification curve showing a heterozygous deletion of *SMN1* E7; (F) Amplification curve showing a heterozygous deletion of *SMN1* E8. Colour-coded by fluorescence channel: FAM channel (Blue); VIC channel (Green).

Table I: Statistics of SMA carrier screening [n (%)].

Variables	Male	Female	Total
SMA Carrier	20 (2.33)	85 (2.15)	105 (2.18)
Negative	839 (97.67)	3,872 (97.85)	4,711 (97.82)
Total	859	3,957	4,816

Table II: Statistics of SMA carrier screening [n (%)].

Variables	Male	Female	Total
SMN1 E7 heterozygous deletion	0	5	5
SMN1 E8 heterozygous deletion	8	27	35
SMN1 E7 and E8 heterozygous deletion	20	80	100
Total	28	112	140

Table III: Prenatal diagnosis of 14 foetuses in the families with SMA carriers.

Foetal genotypes	Foetal phenotypes	Number of cases	Pregnancy outcomes
Homozygous deletion of E7 and E8	Patient	1	Termination of pregnancy
Heterozygous deletion of E7 and E8	Carrier	2	Full-term delivery
Normal	Normal	1	Full-term delivery

The proportion of heterozygous deletions of exons 7 and 8 of the *SMN1* gene was relatively high, which was consistent with the trends reported in other studies.[13] Thirty-five carriers had heterozygous deletions of exon 8 of the *SMN1* gene (0.73%, 35/4,816). According to a professional genetic analysis, the heterozygous deletion of exon 8 of the *SMN1* gene had little clinical significance, so it was not further analysed (Table II).

Among the 4,816 samples, four couples, both of whom were carriers, were detected. Prenatal diagnosis by amniocentesis was carried out on high-risk foetuses of the above four couples at 18 weeks of gestation. The results showed that one foetus had homozygous deletions of exons 7 and 8 of the *SMN1* gene, indicating a diagnosis of SMA. Two foetuses had heterozygous deletions of exons 7 and 8 of the *SMN1* gene and were identified as SMA carriers, while one foetus had no detectable abnormalities in the *SMN1* copy number. The copy number of exons 7 and 8 of the *SMN1* gene in the amniotic fluid sample from a foetus prenatally diagnosed with homozygous deletion was verified and diagnosed through MLPA technology.

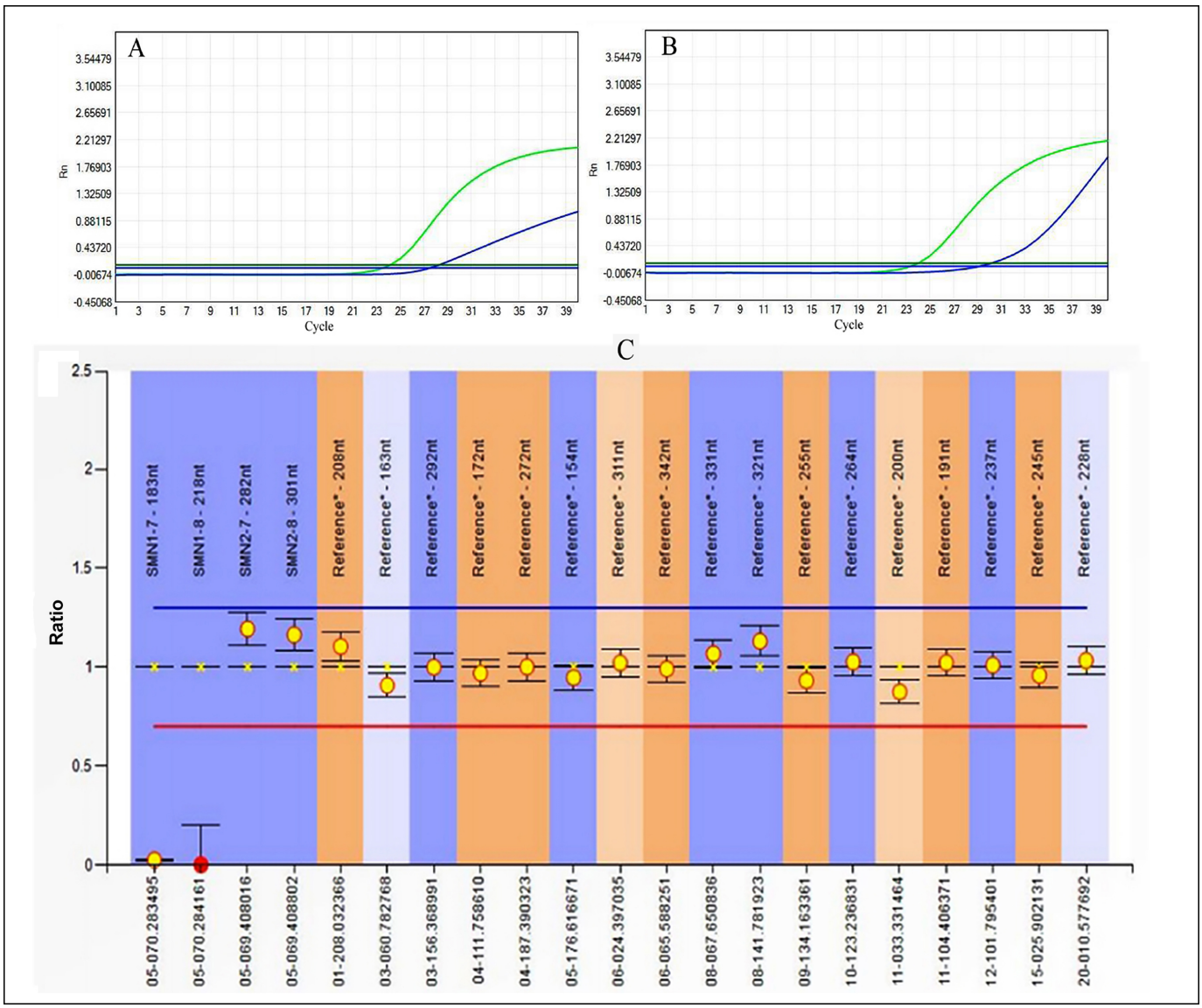

Figure 2: Test results of the amniotic fluid sample from a foetus with homozygous deletion. (A) Amplification curve showing a homozygous deletion of *SMN1* E7 detected by fluorescence quantitative PCR; (B) Amplification curve showing a homozygous deletion of *SMN1* E8 detected by fluorescence quantitative PCR; (C) MLPA detection result: *SMN1* gene exons 7 and 8 showed a copy number of 0, whereas the *SMN2* gene had two copies of exons 7 and 8.

The results showed that the amniotic fluid cell sample of the foetus detected homozygous deletion variations within the *SMN1* gene exons 7 and 8, with zero copy number. However, the *SMN2* gene exhibited two copies of exons 7 and 8. The MLPA analysis results were in agreement with those of real-time PCR, as shown in Figure 2. After genetic counselling, the pregnancy was terminated for the foetus identified with homozygous deletions of exons 7 and 8 through genetic testing, while the two foetuses detected as carriers and the one foetus with a normal gene test result continued their pregnancy (Table III).

DISCUSSION

Various molecular biological methods can be used for the screening and diagnosis of SMA, such as MLPA,[14] denaturing high-performance liquid chromatography (DHPLC),[15] and real-time PCR.[16] The real-time PCR method offers several advantages, including high detection sensitivity, good accuracy, low cost, and simple operation, with good repeatability and universality. Based on these characteristics, fluorescence quantitative PCR has become an ideal method for population carrier screening and clinical the diagnosis of SMA.[17,18]

For the present study, real-time PCR method was opted for SMA carrier screening and prenatal diagnosis analysis. Among the 4,816 samples, a total of 105 carriers were detected, with a carrier frequency of 2.18%. This proportion was slightly higher than the reported SMA carrier rates in the Yunnan region and the Jiangsu region (1/49 and 1/58, respectively)[19,20] and slightly lower than the reported SMA carrier rates in the Shijiazhuang and Guiyang regions (1/36

and 1/44, respectively).[21] Regional variations were observed in the carrier rate of the SMA population due to various factors, such as different migration histories and intermarriage patterns that have shaped genetic distributions across regions. Environmental influences might also be involved.

SMA is associated with pathogenic genes situated on the *SMN* gene locus on chromosome 5, at position 5q13. Among them, *SMN1* and *SMN2* are highly homologous. *SMN1*, located on the telomere side, is the pathogenic gene, while *SMN2*, located on the centromere side, is a modifier gene that regulates the severity of the disease.[10] Roughly 95% of individuals with SMA exhibit homozygous deletions of exons 7 and 8 in the *SMN1* gene, or a homozygous deletion specifically affecting exon 7. About 5% of SMA cases are caused by heterozygous deletions, point mutations in *SMN1*, or the transformation of *SMN1* into its paralog *SMN2 via* gene conversion.[22] The real-time PCR technique was employed to identify *SMN1* exon copy deletions in 4,816 carrier-screening samples. The results showed that there were four couples in which both spouses were carriers. For these four couples, exons 7 and 8 within the *SMN1* gene coding sequence were heterozygously deleted, making them typical SMA carriers.

As shown in the results, diagnosing and screening families in which both spouses are carriers is conducive to clinically clarifying the gene types of both spouses and providing fertility guidance for couples in high-risk families. Considering the detected results, these four couples were typical SMA carriers, suggesting that the carriage of SMA pathogenic genes in the population has a certain degree of concealment. Although only four couples with both spouses being carriers were found in this study, considering the limitations of the research sample size and the overall carrier frequency of SMA in the population, the actual number of couples with both spouses being carriers may be higher. This highlights the necessity of conducting SMA carrier screening on a wide scale. Comprehensive screening can expand the detection range of SMA carriers, especially among populations that have not received sufficient attention. Detecting potential carrier couples in a timely manner and helping them make appropriate reproductive decisions under scientific guidance can minimise the risk of giving birth to children with SMA, which is of profound significance for improving population quality and reducing the medical burden on families and society.

Since SMA is a single-gene genetic disease, its genetic pattern follows Mendel's laws of inheritance. If both spouses are carriers, the probability of them having a child with SMA is 25%.[5] Therefore, prenatal diagnosis of SMA in high-risk families is of great importance, as shown in this study.

The prenatal diagnosis results of these 4 high-risk families in this study further verify the scientific nature of the genetic pattern of SMA. This not only provides clear foetal health information for these families but also offers a solid basis for subsequent reproductive decisions. The families of the two detected SMA carrier foetuses, although the current foetuses are not patients, still face a relatively high risk of having children with SMA when they form families and give birth to offspring in the future. For these families, the provision of ongoing genetic counselling alongside fertility guidance remains critical.

The study only included 4,816 individuals of childbearing age from Hubei Maternal and Child Health Hospital, which may not fully represent the genetic diversity of the entire regional population. Additionally, only four couples were identified as double carriers, and the small sample size makes it difficult to accurately assess the recurrence risk or outcomes of high-risk pregnancies. Larger-scale, multi-centre studies are needed to improve the carrier rate data and for further validation.

CONCLUSION

This research has tentatively established the carrier frequency of SMA in the Hubei region *via* carrier screening for SMA and identified regional variations in this prevalence. Additionally, through prenatal diagnosis, the birth of one SMA-affected child was successfully prevented. Actively promoting SMA carrier screening and providing reasonable and standardised fertility counselling and guidance for carriers can effectively improve the quality of the newborn population and the living standards of families, and this holds substantial importance for population health and social progress as well as.

ETHICAL APPROVAL:
Ethical approval for this study was granted by the Medical Ethics Committee of Hubei Maternal and Child Health Hospital, Hubei, China, with the approval number [2021 IEC (LW039)].

PATIENTS' CONSENT:
Informed consent was obtained from all the patients included in the study.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
JS: Conception, drafting, and revision of the study.
HL, CZ, YJ, MY: Data acquisition, analysis, processing, and interpretation.
MS: Conception, design, data collation, analysis, and writing of the manuscript.
All authors approved the final version of the manuscript to be published.

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