Meta Gene 15 (2018) 27–30

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Frequency of *SMN1* exon 7 deletion in patients with spinal muscular atrophy in Kashmir

Shafia Syed, Mahrukh H. Zargar*, Arshad Pandith, Nabeela Khan, Rehana Ahmad, Qurteeba Mahajan, Wardha Qazi

Advanced Centre for Human Genetics, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, J & K 190011, India

ARTICLE INFO

Keywords:
Kashmir
Spinal muscular atrophy
Survival motor neuron
Deletion

ABSTRACT

Objective: Spinal muscular atrophy (SMA) is an autosomal recessive neuronal disorder resulting from degeneration of spinal motor neurons. Survival motor neuron (*SMN*) gene is the disease determining gene of SMA. In the present study molecular analysis was performed in 85 SMA patients as compared to 100 healthy controls from Kashmiri population.

Method: The deletion in telomeric exon 7 of *SMN1* gene was analysed in 85 SMA cases and 100 healthy controls by allele specific PCR followed by agarose gel electrophoresis.

Result: The frequency of telomeric exon 7 deletion was 42.35% (36 of 85) in total SMA patients; 32 out of 61 SMA type I cases, 2 out of 13 type II SMA cases, 1 out of 6 type III cases and 1 out of 5 type IV SMA cases. Moreover the consanguinity was found in 57.64% of SMA patients. In healthy controls deletion was not found in any samples.

Conclusion: There is a significant association of *SMN1* homozygous exon 7 deletion and the occurance of SMA as compared to healthy controls. Although the frequency of *SMN1* homozygous deletion in Kashmiri population is lower than the rest of the countries but we conclude the presence of exon 7 deletion in clinically suspected SMA patient should be treated as confirmation of diagnosis and therefore this test can be used as one of the useful tool for SMA diagnosis.

1. Introduction

Spinal muscular atrophy (SMA) is the most common autosomal recessive neurodegenerative disorder and the second most common fatal autosomal recessive disorder after cystic fibrosis. With a worldwide incidence of 1/6000 to 1/10000 births and the carrier frequency of 1/50, it is the leading genetic cause of infant death globally (Roberts et al., 1970; Pearn, 1978; Czeizel and Hamula, 1989). Clinically, SMA is characterised by the progressive loss of alpha motor neurons in spinal cord anterior horn cells, leading to progressive proximal and symmetrical limb and trunk muscle weakness along with muscular atrophy. The routine activities such as walking, sitting up, crawling and controlling head movement are compromised. In more severe cases the muscles involved in breathing and swallowing are also involved. Poor muscle tone in SMA patients has been also associated with contractures and broken bones in children (Dubowitz, 1978).

Depending upon the age of onset of symptoms, motor development milestones, severity of muscle weakness SMA has been categorised into four clinical subtypes by International SMA Consortium classification. The most common and severe type is Type I SMA (Werdnig-Hoffmann I disease). This type has onset within first 6 months of life and the patients are not able to sit. Patients usually do not live past two years of age with severe respiratory distress being the most common cause of death. The type II SMA (also known as Dubowitz disease), is an intermediate form and symptoms usually has an onset from 6 months upto 2 yrs. of life. In this form of SMA children can sit up but still develop progressive muscle weakness and can never stand or walk on their own. The type III SMA (also called Kugelberg-Welander disease) has a chronic evolution and the symptoms appear after 2 yrs. of life. These patients are able to stand and walk unaided at least for some time in infancy but many later loose this ability. The type IV SMA (adult form) has an onset after 30 yrs. of life. The symptoms are mildest of all the forms and it mainly affects the proximal muscles of the extremities. The symptoms start gradually in adulthood with normal longevity (Zerrres and Davies, 1998).

SMA has been frequently associated with a gene known as survival motor neuron 1 (*SMN1*) gene which is responsible for the production of a SMN protein. The SMN protein is essential for the maintenance of

* Corresponding author at: Advanced Centre for Human Genetics, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir 190011, India.
E-mail address: mhameedz@gmail.com (M.H. Zargar).

http://dx.doi.org/10.1016/j.mgene.2017.10.005
Received 14 August 2017; Received in revised form 18 October 2017; Accepted 25 October 2017

Table 1
Frequency of *SMN1* homozygous exon 7 deletion in different populations.

Country	No of Patients	*SMN1* EXON 7 deletion	[Reference]
Korea; 2001	37	32%	Cho et al. (2001)
Vietnam; 2003	17	41%	Duc Bach et al. (2003)
South Africa(Johannesburg); 2007	92	51%	Labrum et al. (2007)
Egypt; 2001	33	55%	Shawky et al. (2001)
Russia; 2001	57	65%	Glotov et al. (2001)
Brasilia; 1999	87	69%	Kim et al. (1999)
Algerian; 2009	92	75%	Sifi et al. (2013)
India; 2005	45	76%	Kesari et al. (2005)
Saudi Arabia; 1997	16	82%	Al Rajeh et al. (1998)
Morocco; 2003	54	83%	Bouhouche et al. (2003)
Turkey; 2000	60	85%	Savas et al. (2000)
Germany; 1995	195	90%	Wirth et al. (1995)
Spain; 1995	54	91%	Bussaglia et al. (1995)
Holland; 1995	103	93%	Cobben et al. (1995)
Japan; 2002	32	94%	Akutsu et al. (2002)
Tunis; 2006	60	95%	M'rad et al. (2006)
Iran; 2004	22	95%	Shafeghati et al. (2004)
UK; 1995	140	97%	Rodrigues et al. (1995)
Kuwait; 2001	46	97%	Haider et al. (2001)
France; 1995	229	98%	Lefebvre et al. (1995)
Taiwan; 1995	42	100%	Tsai et al. (2001)

specialised nerve cells called motor neurons. The SMN gene has been mapped to chromsomome 5q13.2. It contains 9 exons and encodes 294 aa protein. There are two highly identical copies of this gene: telomeric *SMN1* and centromeric *SMN2*. Both share 99.8% sequence homology with only five nucleotide differences, one in intron 6, one in exon 7, two in intron 7, and one in exon 8 (Melki et al., 1990). These subtle differences do not alter the amino acids sequence, but it has an effect on mRNA splicing. The *SMN1* transcript includes the exon 7 and produces a full-length and stable protein while *SMN2* excludes the exon 7 and results in loss of a chunk of protein (Lorson and Androphy, 2000). The majority of SMN protein is produced by *SMN1* gene and a small amount produced by the *SMN2* gene. SMA phenotype is a result of *SMN1* gene dysfunction. The *SMN2* gene copy number affects the disease severity only because of the insufficient amount of protein produced by *SMN2* gene as it excludes the exon 7.

Several studies have shown a vast majority of SMA patients having the homozygous deletion in exon 7 in *SMN1* gene but with varied frequency in different populations (Table 1) (Cho et al., 2001; Duc Bach et al., 2003; Labrum et al., 2007; Shawky et al., 2001; Glotov et al., 2001; Kim et al., 1999; Sifi et al., 2013; Kesari et al., 2005; Al Rajeh et al., 1998; Bouhouche et al., 2003; Savas et al., 2000; Wirth et al., 1995; Bussaglia et al., 1995; Cobben et al., 1995; Akutsu et al., 2002; M'rad et al., 2006; Shafeghati et al., 2004; Rodrigues et al., 1995; Haider et al., 2001; Lefebvre et al., 1995; Tsai et al., 2001). The *SMN1* exon 7 deletion has been reported to be more prevalent among Spain, Holland, Japan, Tunis, Iran, UK, Kuwait, France and Taiwan with frequencies of > 90%, although lower frequencies have been found in Korea, Vietnam, Egypt and Johannesburg. Keeping in view the varied results of *SMN1* exon 7 deletion in SMA patients across different populations, the present study has been undertaken to study the frequency of *SMN1* exon 7 deletion in Kashmiri SMA cases.

2. Materials and methods

2.1. Study population

A total of 85 SMA patients and 100 healthy controls from the Kashmiri population were included in the present study. The patient diagnosis was made in accordance with the guidelines set by the International SMA Consortium. The clinical classification of patients into four groups was done based on criteria given by International *SMA* Consortium (Zerrres and Davies, 1998). The patients were selected from both the inpatient and outpatient services from the Department of Neurology and Department of Paediatrics, Sher-i-Kashmir Institute of Medical Sciences Hospital and GB Panth Paediatrics Hospital in Kashmir. Data from all SMA patients was obtained from clinical examinations of patients and/or interviews with guardians. The study was approved by the ethics committee of the institute and all the patients/guardians (in case of minors) and controls gave informed consent to participate in the study.

2.2. Sample collection and DNA extraction

About 2–3 mL of peripheral blood was collected from SMA patients and healthy controls in tubes containing ethylenediamine tetraacetic acid (EDTA) and DNA was isolated using a Zymogen (Irvine, CA, USA) DNA extraction kit. The quality of the DNA was checked by agarose gel electrophoresis. The extracted DNA was stored at − 20 °C for further use.

2.3. Mutation detection

Allele specific PCR was used to detect exon 7 deletion in *SMN1* gene using the primers described previously (Feldkötter et al., 2002). The primers specifically amplify exon7 of *SMN1* gene and not *SMN2* gene. To monitor the efficiency of PCR reaction, an internal control for the PCR was used. A control primers pair AAT1/2 was used to amplify a fragment of exon V of α1-antitrypsin gene (Newton et al., 1989). The primer sequences are given in Table 2. The PCR reaction was set in a final volume of 25 µl mixture containing 1 × PCR buffer (Biotools), 0.2 mM dNTP mixture (biotools), 150 ng each primer (Sigma), 1 U Taq DNA polymerase (Biotools 5 U/µl), and 200 ng genomic DNA (0.2 µg/µl). Amplification was done at 94 for 7 min, 30 cycles of 94 °C for 30 s, 58.5 °C for 30 s min, 72 °C for 30 s min followed by extension at 72 °C for 7 min. The PCR product was directly visualised under UV light after electrophoresis using 2–3% agarose gel (Genie, Bangalore, India). The PCR product of *SMN1* was visualised at the corresponding size of 307 bp, while that of internal control AAT1/2 at 220 bp (Fig. 1).

3. Result

A total of 85 SMA patients and 100 healthy controls were studied for *SMN1* exon 7 deletion from Kashmiri population. Of the 85 SMA patients, 61 cases were classified as type I SMA, 13 as type II SMA, 6 as