ARTICLE

Genomics & Informatics
Vol. 9(2) 64-68, June 2011

DOI: 10.5808/GI.2011.9.2.64
pISSN 1598-866X eISSN 2234-0742

Association of the X-linked Androgen Receptor Leu57Gln Polymorphism with Monomelic Amyotrophy

Young-Mi Park[1¶], Young-Min Lim[2¶], Dae-Seong Kim[3], Jong-Keuk Lee[1]* and Kwang-Kuk Kim[2]*

[1]Asan Institute for Life Sciences, [2]Department of Neurology, University of Ulsan College of Medicine, Asan Medical Center, Seoul 138-736, Korea, [3]Department of Neurology, Pusan National University Hospital, Busan 626-770, Korea

Abstract

Monomelic amyotrophy (MA), also known as Hirayama disease, occurs mainly in young men and manifests as weakness and wasting of the muscles of the distal upper limbs. Here, we sought to identify a genetic basis for MA. Given the predominance of MA in males, we focused on candidate neurological disease genes located on the X chromosome, selecting two X-linked candidate genes, androgen receptor (*AR*) and ubiquitin-like modifier activating enzyme 1 (*UBA1*). Screening for genetic variants using patients' genomic DNA revealed three known genetic variants in the coding region of the *AR* gene: one nonsynonymous single-nucleotide polymorphism (SNP; rs78686797) encoding Leu57Gln, and two variants of polymorphic trinucleotide repeat segments that encode polyglutamine (CAG repeat; rs5902610) and polyglycine (GGC repeat; rs3138869) tracts. Notably, the Leu57Gln polymorphism was found in two patients with MA from 24 MA patients, whereas no variants were found in 142 healthy male controls. However, the numbers of CAG and GGC repeats in the *AR* gene were within the normal range. These data suggest that the Leu57Gln polymorphism encoded by the X-linked *AR* gene may contribute to the development of MA.

Keywords: X-linked gene, androgen receptor (*AR*) gene, monomelic amyotrophy (MA), case-control study

Introduction

Monomelic amyotrophy (MA; MIM 602440), also known as Hirayama disease, is a rare motor neuron disease mainly afflicting young males that is characterized by weakness and wasting of the muscles confined to the hand and forearm, without sensory or pyramidal tract involvement (Hirayama *et al.*, 1987). It follows a non-progressive course after a few years of unilateral or bilateral progression (Misra *et al.*, 2006). MA was first reported as juvenile muscular atrophy of an upper extremity in Japanese patients (Hirayama *et al.*, 1959). To date, most studies have involved a very limited numbers of patients and have focused on case reports, mainly from Asian countries such as Japan and India. These studies have included 38 cases and 73 cases with MA in a Japanese population (Hirayama, 1972; Hirayama and Tokumaru, 2000), a sibling case in Turkey (Gucuyener *et al.*, 1991), 44 patients and a case from India (Gourie-Devi and Nalini, 2003; Nalini *et al.*, 2004), a sporadic case in an Italian man (Rigamonti *et al.*, 2004), a young Swiss female patient with MA (Jeannet *et al.*, 2005), and 15 male cases from 14 Indian families (Misra *et al.*, 2005). Currently, the pathophysiology of MA is not well understood but various possibilities have been considered, including autoimmune and genetic factors, and ischemic changes of the spinal cord induced by neck flexion (Nalini *et al.*, 2004). Rare familial cases have suggested the possibility of either autosomal-recessive or autosomal-dominant inheritance patterns in different families (Nalini *et al.*, 2004; Schlegel *et al.*, 1987; Sobue *et al.*, 1978). In the few previous genetic association studies, the role of deletions in *SMN1* and SMN2 genes was excluded (Di Guglielmo *et al.*, 1996; Gamez *et al.*, 2007; Misra *et al.*, 2005). In addition, abnormal expansion of CAG repeats of the androgen receptor gene has not been found in patients with MA (Katila *et al.*, 2007). Thus, the genetic cause of MA is still unknown. However, the predominant occurrence of MA in males suggests that gene(s) in the X chromosome may play a role in MA (Misra *et al.*, 2005). Thus, in this study, we tested whether genetic variants found in two X-linked neurological candidate genes-androgen receptor (*AR*) and ubiquitin-like modifier activating enzyme 1 (*UBA1*) -are involved in the development of MA in Korean patients.

[¶]These authors contributed equally to this work.
*Corresponding authors: E-mail kkkim@amc.seoul.kr, cookie_jklee@hotmail.com
Tel +82-2-3010-3444, Fax +82-2-474-4691
Accepted 11 April 2011

Methods

Subjects

For the analysis of clinical characteristics of MA, clinical data were collected from 34 patients who were diagnosed at Asan Medical Center. For the genetic study, genomic DNA samples from a total of 24 patients, including 22 males and two females, were collected from Asan Medical Center and Pusan National University Hospital. All patients were diagnosed by neurologists according to the following diagnostic criteria: (1) insidious onset between age 14 and 25 years; (ii) unilateral or asymmetric muscle weakness and wasting in the hand and forearm; (iii) lack of involvement of cranial nerves, pyramidal tracts, sphincters and sensory systems, and absence of reflex changes in upper extremities; (iv) nonprogressive course and spontaneous arrest of disease within several years after onset; (v) no history of toxin exposure, poliomyelitis, or other causes for clinical presentations; and (vi) normal motor and sensory nerve conductions and neurogenic changes confined to C7, C8, and T1 myotomes in electrodiagnostic tests (Hirayama *et al*., 1987; Singh *et al*., 1980). Control samples with no history of disease were obtained from the Biobank for Health Sciences at the Center for Genome Sciences in Seoul, Korea. The study was approved by the Institutional Review Board of Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea. All patients or their parents provided written informed consent.

Candidate gene sequencing

To identify candidate causal variants, we sequenced two X-linked candidate genes (*AR* and *UAB1*) using genomic DNA from nine patients with MA and one healthy control subject. Genomic sequences of *AR* and *UAB1* for sequencing analysis were obtained from the GenBank (http://www.ncbi/nlm.nih.gov/) database, and polymerase chain reaction (PCR) primers to amplify coding regions and promoter regions (500 bp upstream from exon 10 of the genes) were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/). Each fragment amplified by PCR was sequenced with an ABI Prism 3730 sequencer (Applied Biosystems, Foster City, CA, USA). DNA polymorphisms were identified using the PolyPhred program (http://droog.gs.washington.edu/polyphred/). For the genetic association study, candidate variants found in the *AR* gene were genotyped by resequencing using all 24 patients with MA and 142 healthy control subjects.

Statistical analysis

Statistical analyses for the case-control study were performed using SPSS (version 18) software (SPSS Inc., Chicago, IL, USA). The association with MA was tested using Fisher's exact test.

Results

Clinical characteristics of MA in Koreans

Since 1989, a total of 34 patients, including two females, have been diagnosed with MA at Asan Medical Center. The clinical characteristics of MA in these Korean patients were analyzed based on clinical data. Similar to previously reported by another groups, MA exhibited a predominantly juvenile onset, between age 15 and 25 (mean age at disease onset, 17.65 years), and mainly affected males (Fig. 1). In 27 of 34 patients (79.4%), symptom onset occurred before 20 years of age. There was no family history of MA or other neurologic disorders affecting anterior horn cells. Arm weakness started unilaterally in all patients, but subsequently affected the other side within 1 year in five patients (14.7%). The initial progression was stabilized 1-5 years

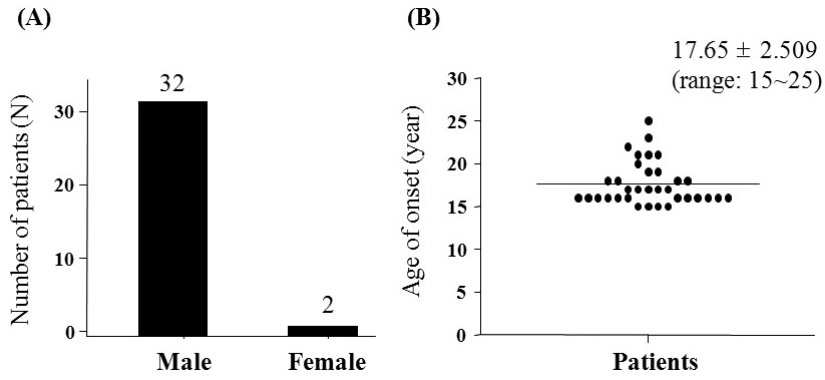

Fig. 1. Clinical characteristics of MA. (A) Male-dominant occurrence of MA. (B) Age of onset in MA. The sex distribution and clinical onset age of MA patients were plotted.

Table 1. Genetic variants identified in the X-linked candidate genes, *AR* and *UBA1*, by direct sequencing of genomic DNA from patients with MA

Gene	SNP (rs# or new)	Allele (1 : 2)	Amino acid change	PolyPhen prediction	Flanking sequences (SNP±10 bp)
AR	rs78686797	T : A	p.Leu57Gln	Probably damaging	TTGCTGCTGC[T:A]GCAGCAGCAG
	rs5902610	$[CAG]_n$	$[Gln58]_{19-28}$		GCTGCTGCTG[(CAG)n]CAAGAGACTA
	rs3138869	$[GGC]_n$	$[Gly457]_{12-18}$		TGGGGGTGGT[(GGC)$_n$]GAGGCGGGAG
	new	C : A			CCTCTCTCCC[C:A]CTTCTCCCTC
UBA1	rs4239963	C : G			GTACCCTGGG[C:G]CTGTTTCTGA
	new	C : T	p.His240His		AGGCCCGACA[C:T]GGGTTTGAGA
	rs5906356	C : T			CAGGCTGCTG[C:T]CTCTCCGCCC

Allele 1 refers to a reference allele.

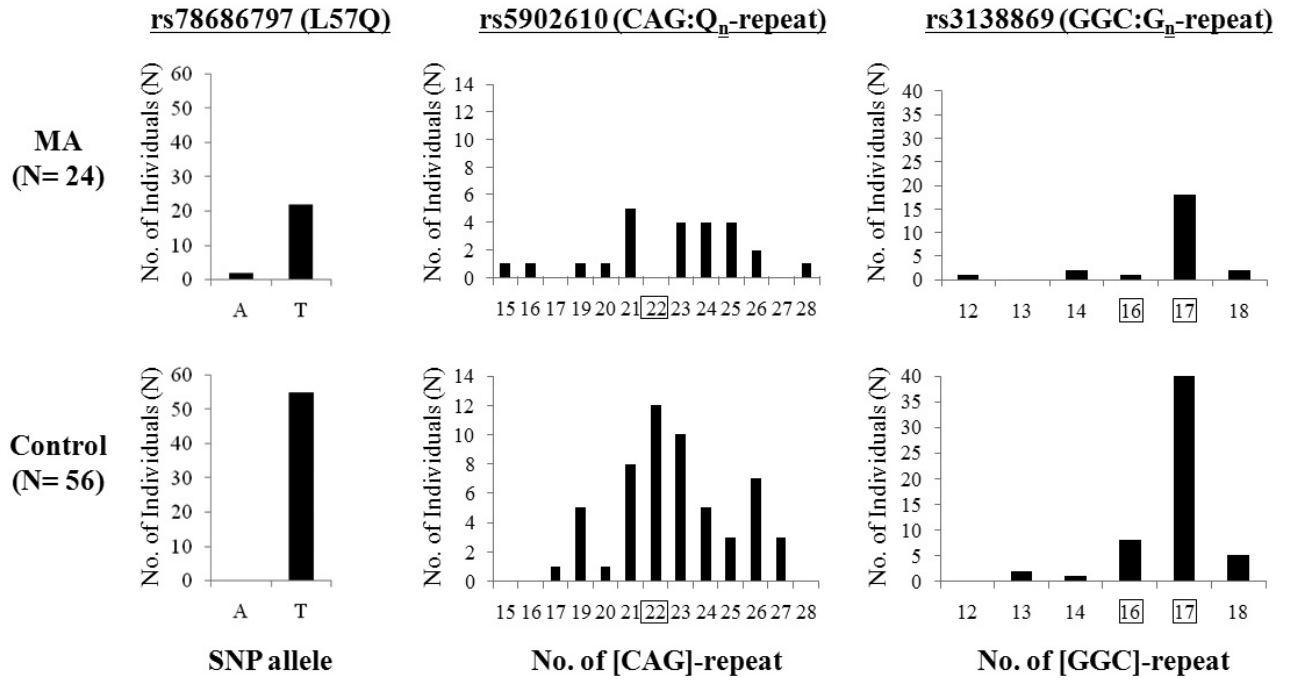

Fig. 2. Genetic Variants of the *AR* gene identified in MA patients. Three genetic variants (rs78686797, rs5902610, rs3138869) were detected in the *AR* gene. Compared with normal controls, MA patients had a rare L57Q variant and various ranges of CAG and GGC repeats. The number in the box is the repeat number of the reference sequence.

after onset.

Identification of genetic variants in X-linked *AR* and *UBA1* genes

The clinical features of MA bear some resemblance to spinal muscular atrophy (SMA), Kennedy disease, and familial amyotrophic lateral sclerosis (ALS). We hypothesized that a different mutation in that same gene that causes these neurological diseases (i.e., allelic heterogeneity) could result in a slightly different phenotype in MA. In order to test our hypothesis, we initially selected 20 candidate genes (*ALS2, ANG, AR, BSCL2, DCTN1, FIG4, FUS, GARS, HMN7B, HSPB8, IGHMBP2, NEFH, PRPH, PLEKHG5, SETX, SMN1, SMN2, SOD1, TARFBP, UBA1, VAPB*) that are involved in either SMA, Kennedy disease or familial ALS. In addition, 94% of our Korean MA patients were males, suggesting that genetic defects on the X chromosome might play a crucial role in MA. Thus, among the initially selected 20 neurological candidate genes, we focused on the X-linked genes, *AR* and *UBA1*. After sequencing of promoter regions and coding regions in the *AR* and *UBA1* genes using genomic DNA from nine MA patients and one normal control, we

Table 2. Genotype distribution of the rs78686797 SNP (Leu57Gln polymorphism) in the X-linked *AR* gene in case and control groups

Group	Genotype			MAF	Fisher's exact test
	TT	TA	AA		p-value
Case	22	0	2	0.08	0.02
Control	142	0	0	0.00	

MA, monomelic amyotrophy; MAF, minor allele frequency.

found a total of seven genetic variants, four in the *AR* gene and three in the *UBA1* gene (Table 1). Notably, three *AR* gene variants contained substitutions located in the coding region that altered the amino acid sequence: one was a nonsynonymous single-nucleotide polymorphism (SNP; rs78686797) encoding Leu57Gln, and two were polymorphic trinucleotide repeat segments that encoded polyglutamine (rs5902610; CAG-repeat) and polyglycine (rs3138869; GGC-repeat) tracts. We mainly focused on these coding polymorphisms in *AR* gene for genetic association study with MA.

Association of the Leu57Gln polymorphism encoded by the X-linked *AR* gene with MA

Among 24 patients with MA, two male patients possessed the Leu57Gln polymorphism, whereas none of the normal controls (n=56) used in an initial case-control study carried this variant (Fig. 2). Furthermore, this variant was not detected even in a larger set of control samples (n=142), demonstrating a significant association with MA (p=0.02; Fisher's exact test; Table 2). In addition, an *in silico* prediction of the functionality of the leu57Gln polymorphism using the program PolyPhen showed the polymorphism site to be "probably damaging" (Table 1). Therefore, it is likely that the Leu57Gln polymorphism is biologically functional, suggesting that it may contribute to the development of MA. On the other hand, however, the numbers of variable CAG repeats (rs5902610) and GGC repeats (rs3138869) in the *AR* gene were within the normal range, and their frequencies were very similar to those of healthy controls (Fig. 2). This result indicates that variation in CAG and GGC repeats does not likely contribute to the development of MA.

Discussion

MA is a very rare neurological disease, making genetic studies very difficult. Most studies conducted to date have included a very limited numbers of patients and have focused on case reports. Rare familial MA cases have suggested that MA can be inherited in a dominant or recessive manner in different families (Nalini *et al*., 2004). Moreover, MA occurs almost exclusively in male patients (Misra *et al*., 2005), thus, we hypothesized that gene(s) on the X chromosome may play a crucial role in MA. In this study, we selected the two X-linked genes, *AR* and *UBA1*, from among the candidate genes of the similar neurological diseases, SMA, Kennedy disease and familial ALS. Interestingly, a polymorphism of the *AR* gene encoding Leu57Gln was found in two of 24 MA patients, whereas no variation at the same SNP site was found in 142 healthy male controls (Table 2). Furthermore, the resulting substitution was probably damaging based on an *in silico* prediction of the functional effect of the nonsynonymous SNP. The biological importance of the Leu57Gln polymorphism is not yet clear. However, the Leu57Gln substitution, located immediately adjacent to the CAG-repeat, might be important in the biological functions of the *AR* protein, since the glutamine tracts encoded by the CAG-repeats region in exon 1 of the *AR* gene are involved in receptor function and are associated with human neurodegenerative disease (Lieberman, 2008; Rusmini, 2010). However, variations in two polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts of the *AR* protein do not seem to directly contribute to the MA phenotype, because their expansions are in the normal range (~15-28 repeats for CAG and ~12-18 repeats for GGC) and their frequencies are similar to those of control groups. Notably, whereas expansion ($\geq$40) of the CAG repeat encoding polyglutamine tracts causes spinal bulbar muscular atrophy (Kennedy disease) (La Spada *et al*., 1991), it does not seem to influence the development of MA. Our study and those of others have reported a sex-dependent prevalence of MA, predominantly in males (Misra *et al*., 2005). Thus, it is reasonable to speculate that the high prevalence of MA in males may be mediated by defects in the androgen response attributable to the Leu57Gln substitution in the *AR* protein, because mutations in the *AR* gene are associated with androgen insensitivity (McPhaul *et al*., 1992). Furthermore, it has been reported that expansion of the number of glutamine repeats increases neurotoxicity in motor neurons of the spinal cord (Lee *et al*., 2003). Therefore, the change of leucine to glutamine at residue 57 of the *AR* protein could cause neurotoxicity in spinal motor neurons-a characteristic feature of MA. We also cannot exclude the possible joint involvement of environmental factors, such as testosterone or androgen effects, in the development of MA. Although we found the Leu57Gln polymorphism in MA patients only, it is premature to

conclude that the *AR* gene is a causal or susceptibility gene for MA. One major limitation of our study is its small sample size, a limitation inherent in studies of rare diseases and one that is shared by previous genetic studies of MA (Misra *et al*., 2005; Di Guglielmo *et al*., 1996). Therefore, further studies using other independent sample sets are necessary to validate our results.

In summary, we found a polymorphism in the *AR* gene encoding a Leu57Gln variant in MA patients only. However, no significant changes in polymorphic trinucleotide repeat segments were observed in MA patients. Our results suggest that disruption of AR protein function caused by the Leu57Gln substitution may play a role in the development of MA.

Acknowledgements

We thank all patients with monomelic amyotrophy and their families for participating in this study. This work was supported by a grant (No. 2010-196) from the Asan Institute for Life Sciences, Seoul, Korea, and a grant from the Ministry of Health & Welfare of the Republic of Korea (A010384).

References

Di Guglielmo, G., Brahe, C., Di Muzio, and A., and Uncini, A. (1996). Benign monomelic amyotrophies of upper and lower limb are not associated to deletions of survival motor neuron gene. *J. Neurol. Sci.* 141, 111-113.

Gamez, J., Also, E., Alias, L., Corbera-Bellalta, M., Barcelo, M.J., Centeno, M., Raquer, N., Gratacos, M., Baiget, M., and Tizzano, E.F. (2007). Investigation of the role of SMN1 and SMN2 haploinsufficiency as a risk factor for Hirayama's disease: clinical, neurophysiological and genetic characteristics in a Spanish series of 13 patients. *Clin. Neurol. Neurosurg.* 109, 844-848.

Gourie-Devi, M., and Nalini, A. (2003). Long-term follow-up of 44 patients with brachial monomelic amyotrophy. *Acta. Neurol. Scand.* 107, 215-220.

Gucuyener, K., Aysun, S., Topaloglu, H., Inan, L., and Varli, K. (1991). Monomelic amyotrophy in siblings. *Pediat. Neurol.* 7, 220-222.

Hirayama, K. (1972). Juvenile non-progressive muscular atrophy localized in the hand and forearm: observations in 38 cases. *Rinsho Shinkeigaku* 12, 313-324.

Hirayama, K., and Tokumaru, Y. (2000). Cervical dural sac and spinal cord in juvenile muscular atrophy of distal upper extremity. *Neurology* 54, 1922-1926.

Hirayama, K., Tomonaga, M., Kitano, K., Yamada, T., Kojima, S., and Arai, K. (1987). Focal cervical poliopathy causing juvenile muscular atrophy of distal upper extremity: a pathological study. *J. Neurol. Neurosurg. Psychiat.* 50, 285-290.

Hirayama, K., Toyokura, Y., and Tsubaki, T. (1959). Juvenile muscular atrophy of unilateral upper extremity: a new clinical entity. *Psychiatr. Neurol. Japan.* 61, 2190-2197.

Jeannet, P.Y., Kuntzer, T., Deonna, T., and Roulet-Perez, E. (2005). Hirayama disease associated with a severe rhythmic movement disorder involving neck flexions. *Neurology* 64, 1478-1479.

Kalita, J., Misra, U.K., Mishra, D.K., Thangaraj, K., Mittal, R.D., and Mittal, B.R. (2007). Nonprogressive juvenile-onset spinal muscular atrophy: a clinic-radiological and CAG repeat study of androgen receptor gene. *J. Neurol. Sci.* 252, 24-28.

La Spada, A.R., Wilson, E.M., Lubahn D.B., Harding, A.E., and Fischbeck, K.H. (1991). Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 352, 77-79.

Lee, D.K., and Chang, C. (2003). Endocrine mechanisms of disease: expression and degradation of androgen receptor: mechanism and clinical implication. *J. Clin. Endocrinol. Metab.* 88, 4043-4054.

Lieberman, A.P., and Robins D.M. (2008). The androgen receptor's CAG/glutamine tract in mouse models of neurological disease and cancer. *J. Alzheimers Dis.* 14, 247-255.

McPhaul, M.J., Marcelli, M., Zoppi, S., Wilson, C.M., Griffin, J.E., and Wilson, J.D. (1992). Mutations in the ligand-binding domain of the androgen receptor gene cluster in two regions of the gene. *J. Clin. Invest.* 90, 2097-2101.

Misra, U.K., Kalita, J., Mishra, V.N., Kesari, A., and Mittal, B. (2005). A clinical, magnetic resonance imaging, and survival motor neuron gene deletion study of Hirayama disease. *Arch. Neurol.* 62, 120-123.

Misra, U.K., Kalita, J., Mishra, V.N., Phadke, R.V., and Hadique, A. (2006). Effect of neck flexion on F wave, somatosensory evoked potentials, and magnetic resonance imaging in Hirayama disease, *J. Neurol. Neurosurg. Psychiatry* 77, 695-698.

Nalini, A., Lokesh, L., and Ratnavalli, E. (2004). Familial monomelic amyotrophy: a case report from India. *J. Neurol. Sci.* 220, 95-98.

Rigamonti, A., Usai, S., Curone, M., D'Amico, D., and Bussone, G. (2004). Hirayama disease: description of an Italian case. *Neurol. Sci.* 25, 102-103.

Rusmini, P. Bolzoni, E., Crippa, V., Onesto, E., Sau, D., Galbiati, M., Piccolella, M., Poletti, A. (2010). Proteasomal and autophagic degradative activities in spinal and bulbar muscular atrophy. *Neurobiol. Dis.* 40, 361-369.

Schlegel, U., Jerusalem, F., Tackmann, W., Cordt, A., and Tsuda, Y. (1987). Benign juvenile focal muscular atrophy of upper extremities: a familial case. *J. Neurol. Sci.* 80, 351-353.

Singh, N., Sachdev, K.K., and Susheela, A.K. (1980). Juvenile muscular atrophy localized to arms. *Arch. Neurol.* 37, 297-299.

Sobue, I., Saito, N., Iida, M., and Ando, K. (1978). Juvenile type of distal and segmental muscular atrophy of upper extremities. *Ann. Neurol.* 3, 429-432.