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

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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.
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 re-sequencing 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.](image-url)
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, TARBP2, 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 identified genetic variants in the X-linked candidate genes, AR and UBA1, by direct sequencing of genomic DNA from patients with MA.

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]GCAAGCCAGCAG  |
|      | rs5902610       | [CAG]n      | [Gln58]16-28      |                     | GCTGCTGCTG([CAG]n)[CAAGAGACTA] |
|      | rs3138869       | [GGC]n      | [Gly457]12-19     |                     | TGGGCTGTTT[G(GGC)]GAGGAGGAGGAG |
|      | new             | C:A         |                   |                     | CTCCTTCCCG(C:A)CTCTCTCCCTAA  |
| UBA1 | rs4239963       | C:G         |                   |                     | GTACCCTGGG[C:G]CTGTTTCGTA    |
|      | new             | C:T         | p.His240His       |                     | AGGCCCCACAC[C:T]GGTTTTGAGA   |
|      | rs5906356       | C:T         |                   |                     | CAGGCTGCTG[C:T]CTCTCCGCCCC   |

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, TARBP2, 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
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 (≥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

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 |
|-------|----------|-----|---------------------|
| Case  | TT       | 0.08| 0.02                |
|       | TA       | 0   |                      |
|       | AA       | 2   |                      |
| Control | TT | 142 | 0.00 |                      |
|        | TA | 0   | 2                   |
|        | AA | 0   | 0                   |

MA, monomelic amyotrophy; MAF, minor allele frequency.

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 |
|-------|----------|-----|---------------------|
| Case  | TT       | 0.08| 0.02                |
|       | TA       | 0   |                      |
|       | AA       | 2   |                      |
| Control | TT | 142 | 0.00 |                      |
|        | TA | 0   | 2                   |
|        | AA | 0   | 0                   |
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).

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