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Second locus for late-onset familial Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurologic disorder resulting from the degeneration of upper and lower motor neurons, and is inherited in 10% of cases. About 20% of familial ALS, clinically indistinguishable from sporadic ALS, is caused by mutations of Cu/Zn superoxide dismutase on chromosome 21q22.21 inherited as an autosomal dominant trait. We now report a new locus in the non-SOD1 dominantly inherited ALS.

We screened a large ALS family with 11 affected individuals and one obligate gene carrier with genome-wide ABI polymorphic markers using the ABI 377 automated system. No evidence of linkage was obtained with the autosomal markers. We next screened this family with X chromosome markers as there was no evidence of male-to-male transmission of the disease. Linkage was established with several X chromosome markers with a lod score up to 3.8; almost the maximum possible score in this family.

Our finding imply that a gene for the dominant expression of a neuronal degeneration is coded on X chromosome and raise the question of the role of X-linked genes that escape inactivation in this pathogenesis. More importantly, our finding that a gene causing ALS is localized on X chromosome has direct investigational relevance to sporadic ALS, where epidemiological studies show male gender predominance (1.3:1) and earlier onset in men by 5-10 years.

Key words – Amyotrophic lateral sclerosis, linkage analysis, X chromosome, lod score

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease resulting from degeneration of motor neurons in the brain stem, spinal cord, and cerebral motor cortex, leading to progressive muscular atrophy, weakness, paralysis, and death due to respiratory failure[3]. About 10% of ALS cases are familial ALS, clinically indistinguishable from sporadic ALS except for earlier onset of the disease by 10 years. Although three loci were identified for the juvenile autosomal ALS[2, 5, 6], most cases of familial ALS are inherited as a dominant disorder with the mean age of about 46 years[13]. Approximately 20% of familial ALS cases are associated with mutations in Cu, Zn superoxide dismutase (SOD1) gene[12] located on chromosome 21q21[14]. Over 60 different mutations in SOD1 are associated with familial ALS5 and transgenic mice overexpressing mutated human SOD1 are models for familial ALS[4]. Recent efforts have focused on the discovery of other genes responsible for the remaining 80% of familial ALS cases. However, lower penetrance and late-onset in many non-SOD1 ALS cases have slowed the search for additional genetic loci. We now report the second dominant locus for ALS on X chromosome.

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Materials and methods

Clinical evaluation

All family members diagnosed with familial ALS were examined by a neurologist. Those patients with any signs of ALS on general exam underwent extensive evaluation. Five members of Family 186 were autopsied and two studied in detail: both had finding of typical ALS (Charcots disease) with anterior horn cell loss and corticospinal tract involvement.

Genotype analysis

Blood samples were obtained with the consent of each family members of familial ALS and lymphoblastoid cell lines were established. Genomic DNA was prepared from the lymphoblastoid cells as described in a standard method[8]. Primer sequences of microsatallite markers for radioactive genotyping were obtained from the Genome Database. PCR was performed in 96 well microtiter plate (PE Applied Biosystems 9600) or by Catalyst 877. Amplified alleles were separated in gels run on the 377 Automated DNA Sequencer (PE Applied Biosystems) and analyzed by genotyper software (PE Applied Biosystems) and by use of GenescanTM 350-TAMRA or GenescanTM 500 TAMRA as size standards. CEPH DNA standards were used for the determination of allele sizes.

Linkage analysis

Two-point and multipoint lod score calculations were performed using the computer programs, MLINK and LINKMAP. Disease penetrance was set at 90% for all analyses. Heterogeneity tests were carried out using the HOMOG program.

Results and Discussion

A large North American Caucasian family (Family 186) was initially studied, because the family had a potential to give high lod score enough to establish linkage (Fig 1). Since adult-onset familial ALS was thought to

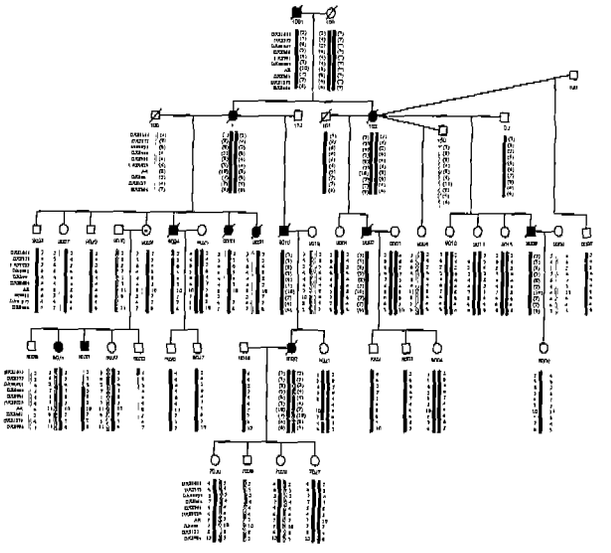

Fig 1. Pedigree and haplotypes of Family 186.

be inherited as an autosomal dominant trait, we screened this ALS family with the affected individuals and one obligate gene carrier with the genome-wide panel of polymorphic markers using the ABI 377 automated system. In the course of screening all the autosomal chromosomes with 350 ABI polymorphic markers spaced ~10 cM apart, we found several potential loci on chromosome 5, 6, 17, and 19. Further investigation of these candidate loci by saturation genotyping of all the available members of the family ruled out linkage to these loci.

As Family 186 had no male-to-male transmission of the disease, we next screened this family with X chromosome markers. The marker DXS991 co-segregated with ALS. Two recombination events were evident with DXS1055 and one recombination with DXS981 on chromosome Xp11-Xq11 in the pedigree. Two-point lod score between DXS991 and ALS resulted in statistically significant linkage (Z=3.8, q=0). We further investigated this chromosomal interval with additional 7 markers known to be mapped in this region. All of these markers gave high lod scores as summarized in Table 1. Haplotype analysis of the genotyped individuals in the Family 186 (Fig 1.) revealed a recombination between DXS1275 and

Table 1. Two-point lod scores between ALS2 locus and Xp11-Xq11 markers.

Locus	Lod scores at recombination fraction(θ)						
	0.00	0.05	0.10	0.15	0.20	0.30	0.40
DXA1055	-∞	0.62	0.97	1.05	1.02	0.75	0.31
DXA573	3.33	3.04	2.73	2.41	2.08	1.36	0.58
DXA8023	2.80	2.55	2.30	2.03	1.74	1.12	0.44
DXA988	3.66	3.35	3.03	2.69	2.33	1.55	0.68
DXA991	3.80	3.47	3.13	2.78	2.40	1.59	0.69
DXA8029	3.72	3.40	3.06	2.71	2.34	1.53	0.63
AR	3.84	3.51	3.17	2.82	2.44	1.64	0.73
DXA981	3.69	3.38	3.05	2.71	2.34	1.56	0.69
DXA1275	-∞	1.90	1.92	1.80	1.61	1.10	0.46
DXA986	0.75	0.68	0.61	0.54	0.47	0.31	0.16

The numbers in parentheses are total combined lod scores for Family 186 and 20 additional two generation families without male-to-male transmission of ALS. The two-point lod scores of Family 186 are shown without parentheses in the table.

DXS1055. The candidate region is 16.0 cM interval including the centromere of X chromosome, and the actual physical distance of this area is 29.6 Mb.

Our results demonstrate that a gene other than SOD1 causes adult-onset dominant ALS. This second ALS gene, ALS2, is linked to an 8.9 cM region between DXS1055 and DXS1275 (Fig 2). This region includes the pericentromeric region of the X chromosome where recombination is less frequent. Therefore, the actual physical distance in this area could be larger than that predicted by the genetic map. Comparison of the genetic map of this region with 75 Kbp resolution physical map[10] shows the physical distance between DXS1055 and DXS1275 is indeed larger at 29.6 megabasepairs (Mbp) and contains about 100 transcripts.

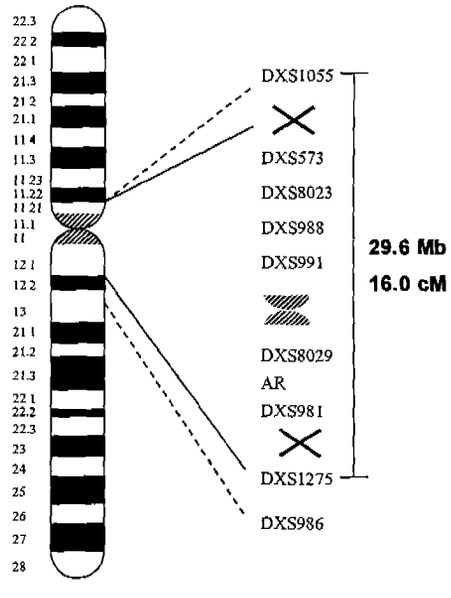

Fig 2. The X chromosomal region locating the ALS2 gene and the actual map distance of the area.

One of the two X chromosomes of normal females becomes inactivated in each somatic cell during early development. This compensates for the dosage difference of X-linked genes between males and females (Lyons hypothesis). For females having the mutated ALS2 gene, it would be expected that the mutant allele would be expressed in only 50% of motor neurons, while the wild type allele would be expressed in the other 50% of motor neurons. Because motor neurons are mononucleated cells, the product of the mutant allele could not have a direct dominant negative effect in the 50% of motor neurons where only the wild type product is expressed. Thus, 50% of motor neurons should remain healthy and reinervate. Studies of post-polio spinal cords indicate that a 50% loss of motor neurons is not reflected in the clinical measurement of strength[1, 15]. Therefore it is unlikely that loss of 50% of motor neurons would cause an ALS phenotype. In another X-linked familial motor neuron disease, Kennedy disease[7], females are not usually affected. A different scenario would prevail if the mutated

gene escapes X-chromosome inactivation or is activated in an age-dependent manner. The gene could then be expressed in all motor neurons and lead to motor neuron degeneration. Eight expressed sequences (representing at least six different genes) clustered in the Xp11.21-p11.22 region are known to escape X-chromosome inactivation [9]. These genes are excellent candidate genes for ALS2.

The finding of SOD1 mutations in familial ALS cases has led to hypotheses of mechanism of pathogenesis, such as loss of dismutase activity, generation of free radicals, and acquired new toxic enzyme activities (peroxidase and peroxynitrate, etc). But it is still unclear how SOD1 mutations lead to motor neuron degeneration. The identification of the ALS2 gene may identify a cellular pathway that intersects with SOD1 and provide an important piece of the puzzle of ALS. Finally, our proof of principle that an X-linked gene can cause dominant ALS has direct investigational relevance to sporadic ALS, where epidemiological studies have shown male gender predominance (1.3:1) and earlier onset in men by 5-10 years[11].

Acknowledgement

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References

1. Bradley, W. G., P. Good, C. G. Rasool and L. S. Adelman. 1983. Morphometric and biochemical studies of peripheral nerves in amyotrophic lateral sclerosis. *Ann. Neurol.* **14**, 267-277.
2. Chance, P. F., B. A. Rabin, S. G. Ryan, Y. Ding, M. Scavina, B. Crain, J. W. Griffin and D. R. Cornblath. 1998. Linkage of the gene for an autosomal dominant form of juvenile amyotrophic lateral sclerosis to chromosome 9q34. *Am. J. Hum. Genet.* **62**, 633-640.
3. Griffin, J. W. 1995. Motor neuron disease. *Trends in Neurosci.* **18**, 513-514.
4. Gurney, M. E., H. Pu, A. Y. Chiu, M. C. Dal Canto, C. Y. Polchow, D. D. Alexander, J. Caliendo, A. Hentati, Y. W. Kwon and H. X. Deng. 1994. Motor neuron degeneration in mice that express a human Cu/Zn superoxide dismutase mutation. *Science* **264**, 1772-1775.
5. Hentati, A., K. Bejaoui, M. A. Pericak-Vance, F. Hentati, M. C. Speer, W. Y. Hung, D. A. Figlewicz, J. Haines, J. Rimmler and C. Ben Hamida. 1994. Linkage of recessive familial amyotrophic lateral sclerosis to chromosome 2q33-q35. *Nat. Genet.* **7**, 425-428.
6. Hentati, A., K. Ouahchi, M. A. Pericak-Vance, D. Nijhawan, A. Ahmad, Y. Yang, J. Rimmler, W. Hung, B. Schlotter, A. Ahmed, M. Ben Hamida, F. Hentati and T. Siddique. 1998. Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15-q22 markers. *Neurogenetics* **2**, 55-60.
7. La Spada, A. R., E. M. Wilson, D. B. Lubahn, A. E. Harding and K. H. Fischbeck 1991. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77-79.
8. Maniatis, T., E. F. Fritsh and J. Sambrook. 1989. Molecular cloning: A laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
9. Miller, A. P. and H. F. Willard. 1998. Chromosomal basis of X chromosome inactivation: identification of a multigene domain in Xp11.21-p11.22 that escapes X inactivation. *Proc. Natl. Acad. Sci. USA* **95**, 8709-8714.
10. Nagaraja, R., S. Macmillan, J. Kere, C. Jones, S. Griffin, M. Schmatz, J. Terrell, M. Shomaker, C. Jermak, C. Hott, M. Masisi, S. Mumm, A. Srivastava, G. Pilia, T. Featherstone, R. Mazzarella, S. Kesterson, B. McCauley, B. Railey, F. Burough, V. Nowotny, M. D'Urso, D. States, B. Brownstein and D. Schlessinger. 1997. X chromosome map at 75 kb STS resolution, revealing extremes of recombination and GC content. *Genome Research* **7**, 210-222.
11. Nelson, L. M. 1995-6. Epidemiology of ALS. *Clin. Neurosci.* **3**, 327-331.
12. Rosen, D. R., T. Siddique, D. Patterson, D. A. Figlewicz, P. Sapp, A. Hentati, D. Donaldson, J. Goto, J. P. O'Regan and H. X. Deng. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial Amyotrophic lateral sclerosis. *Nature* **362**, 59-62.
13. Siddique, T., D. Nijhawan and A. Hentati. 1996. Molecular genetic basis of Familial ALS. *Neurology* **47**, 27-35.
14. Siddique, T., D. A. Figlewicz, M. A. Pericak-Vance, J.

L. Haines, G. Rouleau, A. J. Jeffers, P. Sapp, W. Y. Hung, J. Bebout and D. McKenna-Yasek. 1991. Linkage of gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence of genetic-locus heterogeneity. *N. Engl. J. Med.* **324**, 1381-1384.

15. Wolf, H. K., B. J. Crain and T. Siddique. 1991. Degeneration of the substantia nigra in familial amyotrophic lateral sclerosis. *Clin. Neuropathol.* **10**, 291-296.

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초록 : 가족성 근위축성측삭경화증을 유발시키는 두번째 유전자 위치

홍성출
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근위축성측상경화증은 운동신경의 퇴행으로 인한 진행성 신경질환으로 전체의 10%가 유전적 요인에 의해 발생한다. 유전적 요인에 의해 발병되는 근위축성측상경화증의 20%는 염색체 21q22.21에 위치한 Cu/Zn superoxide dismutase (SOD) 유전자에 돌연변이가 생기는 경우다. 본 연구에서는 SOD 유전자 이외에도 근위축성측상경화증을 일으키는 새로운 유전자 위치를 발견하였다.

11명의 근위축성측상경화증 환자와 한 명의 보인자가 있는 근위축성측상경화증 가계를 ABI 377 자동염기서열분석기를 이용하여 염색체표지들을 지놈수준에서 검색하였다. 22쌍의 체염색체에서는 연관을 전혀 찾아볼 수 없었다.

이 가계의 경우 남성에서 남성으로의 유전자 전달이 없었으므로 X 염색체표지들에서의 연관 여부를 조사한 결과 여러 개의 X 염색체표지들에서 로드 스코아 3.8 정도의 높은 수준의 연관이 관찰되었다. 이 결과는 운동신경퇴행에 관여하는 우성유전자가 X 염색체에 위치해 있음을 말해준다. 또한 역학조사결과에 의하면 근위축성측상경화증은 남성에서 1.3배 많이 걸리고 여성에 비해 5-10년 일찍 발병하므로 무작위적 근위축성측상경화증과 본 연구에 의해 찾아진 X 염색체의 유전자와 상관관계가 있음을 암시한다.