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# Assignment of D-amino-acid oxidase gene to a human and a mouse chromosome

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**Summary.** A part of D-amino-acid oxidase gene was amplified in the human and mouse by polymerase chain reaction. The amplified fragments were ligated to plasmids and then cloned. The plasmids containing the parts of D-amino-acid oxidase gene were biotinylated and hybridized to human and mouse metaphase chromosomes. The chromosomal slides were treated with fluorescein isothiocyanate (FITC)-conjugated avidin. The hybridized signals were amplified with biotinylated anti-avidin antibody and FITC-avidin. The chromosomes were counter-stained with diamidino-phenylindole for assignment of the signal to a specific band. Using this fluorescence *in situ* hybridization (FISH), D-amino-acid oxidase gene was assigned to human chromosome 12q23–24.1 and mouse chromosome 5E3-F. Since these regions are syntenic between human and mouse, the present results indicate that the locus for this enzyme has been conserved through evolution.

**Keywords:** D-Amino-acid oxidase – Chromosomal mapping – FISH – Human – Mouse

## Introduction

D-Amino-acid oxidase (EC 1.4.3.3) catalyzes oxidative deamination of a wide range of D-amino acids, stereoisomers of naturally occurring L-amino acids (Krebs, 1935). This enzyme is present in a variety of organisms (Meister, 1965). In mammals, it is abundantly present in the kidney, liver, and brain (Meister, 1965) and moderately in leukocytes (Cline and Lehrer, 1969; Robinson et al., 1978), the small intestine, epididymis (Gossrau et al., 1991), and preputial and adrenal glands (Goldenberg et al., 1975). It metabolizes D-amino acids of internal and external origin (Konno et al., 1993; D'Aniello et al., 1993). However, its physiological function is unclear (Konno and Yasumura, 1992).

Human D-amino-acid oxidase gene (*DAO*) was mapped on chromosome 12 using a panel of human/Chinese hamster somatic cell hybrids (Fukui and

Miyake, 1992). The spinocerebellar ataxis-2 gene (*SCA2*) was found to have a tight linkage to the *DAO* gene. The *SCA2* gene was assigned to chromosome 12q23–24.1 in a cytogenetic map (Hernández et al., 1995; Gispert et al., 1995). Mouse D-amino-acid oxidase gene (*Dao1*) was mapped at 65 cM on chromosome 5 in a linkage map by a linkage analysis using recombinant inbred strains (Hilgers and Arends, 1985) and cross experiments (Konno et al., 1989). However, neither human nor mouse D-amino-acid oxidase gene has been physically located on a specific chromosome. Therefore, in the present study, the chromosomal positions of these genes were determined using fluorescence *in situ* hybridization (FISH). This is the first report which has mapped the D-amino-acid oxidase gene on human and mouse chromosomes.

## Materials and methods

### *PCR amplification of D-amino-acid oxidase gene*

For an amplification of a part of human D-amino-acid oxidase gene, the following PCR reaction mixture (10 $\mu$l) was used: human genomic DNA (22 ng) purified from the volunteer blood, 2 pmoles each of the sense and antisense primers, 2 nmoles each of dNTP, 0.25 units of Z-Taq DNA polymerase (Takara, Otsu, Shiga, Japan), and the reaction buffer supplied with the polymerase. The mixture was subjected to 40 cycles of 98°C for 1 sec, 55°C for 5 sec, and 72°C for 20 sec in GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA, USA).

For an amplification of a part of mouse D-amino-acid oxidase gene, the following PCR reaction mixture (10 $\mu$l) was used: genomic DNA (83 ng) purified from the liver of a BALB/c mouse, 2 pmoles each of the sense and antisense primers, 2 nmoles each of dNTP, 25 nmoles of $MgCl_2$, 0.5 units of LA Taq DNA polymerase (Takara), and the reaction buffer supplied with the polymerase. The mixture was heated to 94°C for 2 min and then subjected to 30 cycles of 94°C for 45 sec, 62°C for 45 sec, and 72°C for 5 min, followed by a final extension at 72°C for 10 min.

### *Cloning of D-amino-acid oxidase gene*

Cloning of the PCR-amplified DNA fragment was carried out using TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) as specified by the reagent supplier. In brief, the PCR product (1 $\mu$l) above was ligated to a pCR 2.1-TOPO vector (10 ng) for 5 min at room temperature. This solution was mixed with TOP10F′ One Shot competent *Escherichia coli* cells. After being kept for 30 min on ice, the mixture was heat-shocked for 30 sec at 42°C. Then, it was chilled on ice for 2 min and mixed with SOC medium. After being shaken for 30 min at 37°C, the *E. coli* cells were spread onto LB agar plates containing ampicillin, 5-bromo-4-chloro-3-indolyl-$\beta$-D-galactoside, and isopropylthio-$\beta$-D-galactoside. The plates were incubated overnight at 37°C. Resultant white colonies were examined for the presence of the insert.

Plasmids containing the insert were purified using QIAprep Spin Miniprep Kit (QIAGEN, Germany). Nucleotide sequences of the inserts were determined using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and a DNA sequencer (Prism 377, Applied Biosystems).

### *Chromosomal slide preparation*

Chromosomal slides were prepared according to Heng et al. (1992) and Heng and Tsui (1993). Human lymphocytes isolated from the blood of volunteers were cultured in $\alpha$-minimum essential medium ($\alpha$-MEM, Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal calf serum and 0.02% phytohemagglutinin (Gibco BRL) at 37°C for 70 h. Mouse lymphocytes isolated from the spleen were cultured in RPMI 1640 medium (Gibco BRL) supplemented with 15% fetal calf serum, 3 $\mu$g/ml concanavalin A (Sigma, St. Louis, MO, USA), 10 $\mu$g/ml lipopolysaccharides (Sigma), and 50 $\mu$M mercaptoethanol (Sigma) at 37°C for 44 h. The lymphocyte cultures were treated with 5-bromo-2′-deoxyuridine (0.18 mg/ml, Sigma) for 14 h to synchronize the cell population. The synchronized cells were washed three times with serum-free medium to release the block and recultured at 37°C for 4–6 h in $\alpha$-MEM with thymidine (2.5 $\mu$g/ml, Sigma). The cells were harvested and kept in 0.4% KCl at 37°C for 15 min. They were fixed in a fixative (methanol: acetic acid, 3: 1). The solution was spread over slide glasses.

### *Fluorescence in situ hybridization (FISH)*

FISH mapping was performed as described by Heng et al. (1992) and Heng and Tsui (1993). The chromosomal slides were baked at 55°C for 1 h and treated with 100 $\mu$g/ml RNase A in 2 × SSC for 1 h at 37°C. (1 × SSC is a solution of 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) The chromosomes were dehydrated with ethanol and denatured in 70% formamide in 2 × SSC for 2 min at 70°C. The chromosomes were dehydrated with ethanol.

Plamid containing a part of D-amino-acid oxidase gene was biotinylated with biotin-14-dATP for 1 h at 15°C using BioNick Labeling System (Gibco BRL). It was purified by ethanol precipitation. Approximately 20 ng of the biotinylated plasmid DNA, together with 10 $\mu$g of sonicated salmon sperm DNA, were dissolved in 12 $\mu$l of hybridization buffer (50% deionized formamide/1 × SSC/10% dextran sulfate). Two micrograms of sonicated total human DNA (for human chromosomes) or mouse cot-1 DNA (for mouse chromosomes) were included in the solution for suppression of repetitive sequence hybridization. Then, this solution was heated at 75°C for 5 min and chilled on ice. It was loaded on the denatured chromosomal slides. Covered with parafilm, the slides were incubated in a moist chamber for 16–20 h at 37°C.

The slides were washed at 46°C three times in 50% formamide/2 × SSC for 3 min, then three times in 2 × SSC for 3 min. They were immersed in 3% bovine serum albumin (BSA)/4 × SSC for 5 min at room temperature. Fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories, Burlingame, CA, USA) was dissolved in 1%BSA/0.1% Tween 20/4 × SSC at a concentration of 5 $\mu$g/ml and was poured onto the slides. After being incubated for 20 min at 37°C, the slides were immersed three times for 3 min in the same solution without FITC-avidin. Signal amplification was performed using biotinylated goat anti-avidin antibody and a second layer of FITC-avidin according to the direction of the supplier (Vector Laboratories). The slides were then counter-stained with 0.2 $\mu$g/ml 4′,6-diamidino-2-phenylindole (DAPI, Sigma) for 5 min, followed by wash in 2 × SSC for 5 min. The chromosomes were sealed in 90% glycerol in 20 mM Tris-HCl (pH 8.0) containing 2.3% 1,4-diazabicyclooctane (DABCO, Sigma) and 0.05–0.1 $\mu$g/ml propidium iodide (Sigma).

### *Microscopy and assignment of FISH signals to chromosomes*

Micrographs were taken with a Leitz-Aristoplan epifluorescent microscope with a DAPI filter (excitation, BP350-460; reflectior, RKP 510; emission, long path 520) and FITC filter (excitation, BP 450-490; reflector, RKP 510; emission, long path 520). Kodak Ectachrome

P800/1600 "push level 2" E-6P film and Kodak Technical Pan film were used with exposure times of 5–90 sec.

FISH signals and the DAPI banding pattern were recorded separately by taking photographs. The assignment of the FISH signals to DAPI-banded chromosomes was achieved by superimposing the photographic images.

## Results and discussion

In order to obtain a part of D-amino-acid oxidase gene of the human and mouse, several PCR primers were designed from the nucleotide sequences of cDNA encoding human and mouse D-amino-acid oxidases (Momoi et al., 1988; Tada et al., 1990). Using these primers, PCR was performed with human and mouse genomic DNA as a template. A pair of primers (a sense primer, 5′-GGACACAGTTCTGGGATTTCG-3′ and an antisense primer, 5′-CCTC CTCAAAAGACTCCACTT-3′ which correspond to the nucleotides 524–544 and 688–708 of human D-amino-acid oxidase cDNA, respectively) successfully amplified a single DNA fragment of about 3.5 kb in the human. Similarly, a pair of primers (a sense primer, 5′-GCTGCCTCCATTCTCCA AACG-3′ and an antisense primer, 5′-GATAAGCTTCACTCCCCTCTC-3′ which correspond to the nucleotides 295–315 and 525–545 of mouse D-amino-acid oxidase cDNA, respectively) amplified a single 5-kb fragment in the mouse. These fragments were ligated to a plasmid vector and cloned in *E. coli*. Sequencing of these inserts indicated the presence of the exact D-amino-acid oxidase cDNA sequences at both ends flanking the intron sequence, confirming that parts of the human and mouse D-amino-acid oxidase genes were cloned.

The plasmids were biotinylated and hybridized to human and mouse metaphase chromosomes spread on slide glasses. The slides were overlaid with FITC-conjugated avidin and further treated with biotinylated anti-avidin antibody and FITC-avidin for signal amplification. Chromosomes were counter-stained with DAPI. The FISH signals were localized to the DAPI-banded chromosomes.

Figure 1 shows human chromosomes hybridized with the D-amino-acid oxidase gene probe. Among 100 checked mitotic figures, 52 of them showed FISH signals on a pair of chromosomes (Fig. 1, left panel). DAPI banding showed that these signals were on the distal part of the long arm of human chromosome 12 (Fig. 1, right panel). Superimposing the photographic image of the FISH signals on the DAPI-banded chromosome allowed the localization of the D-amino-acid oxidase gene to q23–24.1 (Fig. 2).

This position of human D-amino-acid oxidase gene agrees with the results of linkage analysis by Gispert et al. (1995). They have shown that the *SCA2* gene, mapped on 12q23–24.1, has a tight linkage with the *DAO* gene.

Mouse D-amino-acid oxidase gene (*Dao1*) was similarly mapped (Fig. 3). Among 100 mitotic figures examined, 78 of them showed hybridization signals (Fig. 3, left panel). They were on the distal part of chromosome 5 (Fig. 3, right panel). Superimposing the photographic image of the FISH signals on the

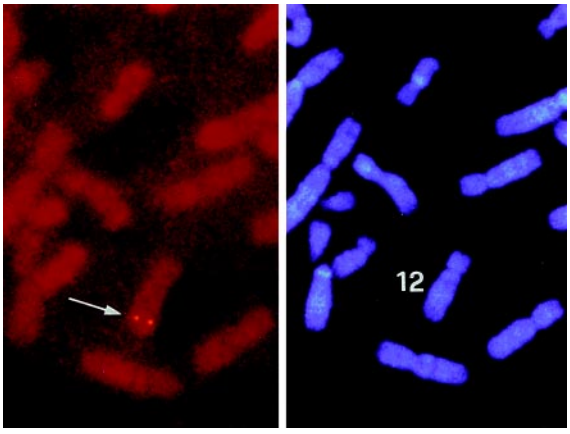


**Fig. 1.** FISH mapping of human *DAO* gene. Left panel shows the FISH signals on human chromosome. Right panel shows the same mitotic figures counterstained with DAPI to identify human chromosome 12

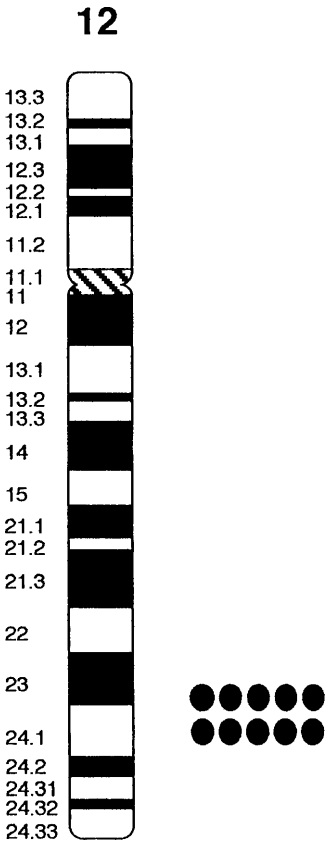


**Fig. 2.** Diagram of FISH mapping result of human *DAO* gene. Each dot represents double FISH signals detected on human chromosome 12

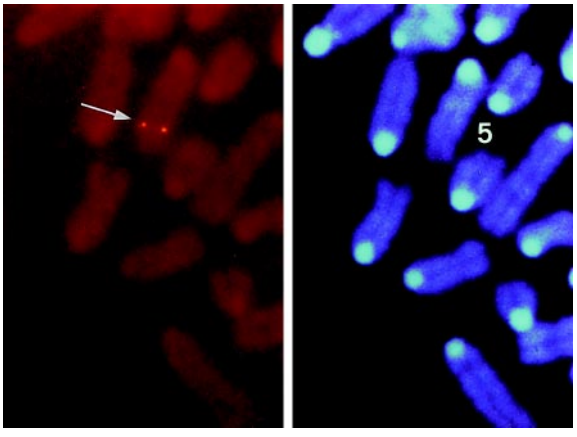


**Fig. 3.** FISH mapping of mouse *Dao1* gene. Left panel shows the FISH signals on mouse chromosome. Right panel shows the same mitotic figures counterstained with DAPI to identify mouse chromosome 5

DAPI-banded chromosome allowed the localization of the D-amino-acid oxidase gene to E3-F (Fig. 4).

In the mouse, a precise linkage map has been constructed but a cytogenetic map is still under development. The correspondence between these two maps has not been well established. The positions of the *Dao1* gene at 65 cM on chromosome 5 in the linkage map and at E3-F on the same

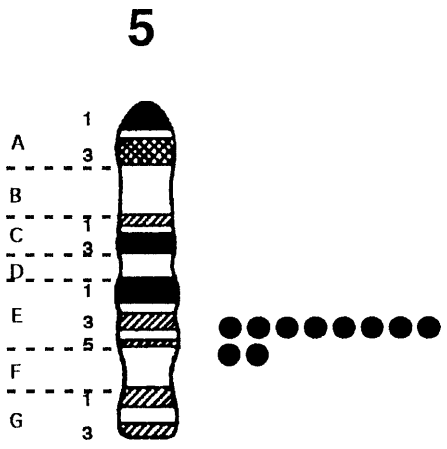


**Fig. 4.** Diagram of FISH mapping result of mouse *Dao 1* gene. Each dot represents double FISH signals detected on mouse chromosome 5

chromosome in the cytogenetic map may be used as a landmark for assignment of other genes to the cytogenetic map.

The present experiments have visualized the D-amino-acid oxidase locus on human and mouse chromosome for the first time. The human *DAO* gene is on chromosome 12q23–24.1 and the mouse homologue *Dao1* gene is on chromosome 5E3-F. These chromosomal regions are shown to be syntenic between human and mouse. Several homologous genes have been mapped in these regions (Mouse Genome Informatics, The Jackson Laboratory, http//www.informatics.jax.org). The gene order on the long arm of human chromosome 12 is

*TBX5* (q21) – *ACADS* (q22) – *TBX3* (q23–24.1) – *NOS1* (q24.2–24.31) – *TCF1* (q24.3)

and the gene order at 65.0 cM on mouse chromosome 5 is

*Acads – Dao1 – Nos1 – Tbx3 – Tbx5 – Tcf1*

From the present data, the human *DAO* gene would be mapped between the loci for ACRDS (acetyl coenzyme A dehydrogenase, short chain) and NOS1 (nitric oxide synthase 1, neuronal). The loci for human *TBX3* and *TBX5* may need to be re-examined because their positions are different from those of the mouse.

It is shown that amino acid sequence of D-amino-acid oxidase is highly homologous between human and mouse (Tada et al., 1990; Konno et al., 1999). In addition to this homology, the presence of the D-amino-acid oxidase genes in the homologous chromosomal regions suggests that D-amino-acid oxidase has a functional significance and has been conserved through evolution.

Since the evolutionary divergence between humans and mice, chromosomal changes, such as inversion, translocation, duplication, and

deletion, have occurred on their chromosomes. However, the present results indicate that the human and mouse chromosomes still have a very similar gene order in part. Comparative mapping of genes to human and mouse chromosomes would facilitate our understanding of the genome structure and its evolution.

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