where $\phi(r, t)$ is the diffuse photon fluence rate at position r and time t, D is the photon diffusion coefficient and expressed in $D = 1/3\mu_s'$, v is the velocity of light within the media and $S(r, t)$ is the light source.

Solutions using this equation are found under different boundary conditions. We used the solution of a semi-infinite homogeneous model (Patterson et al., 1989) for TRS data analysis. In this solution, $R(d, t)$ is expressed by a function of the optode spacing, the reduced scattering coefficient (μ_s') and absorption coefficient (μ_a), as shown in Eq. (2).

$$R(d,t) = (4\pi Dv)^{-\frac{1}{2}} z_0 t^{-\frac{5}{2}} \exp(-\mu_a vt) \exp\left(-\frac{d^2 + z_0^2}{4Dvt}\right) \quad (2)$$

where d is the optode spacing and $z_0 = 1/\mu_s'$.

Using the non-linear least squares method, we fit Eq. (2) into the observed temporal profiles obtained from TRS and determined μ_s' and μ_a at each wavelength (Suzuki et al., 1994). The conversion chi-square (χ_v^2) value was adopted to evaluate fitting accuracy. We confirmed that our observed profiles fitted well with the theoretical curves using this index ($0.8 < \chi_v^2 < 1.2$; Grinvald and Steinberg, 1974).

Appendix B. Calculation of hemoglobin concentration and oxygen saturation by absorption coefficients

The μ_a of the 3 wavelengths (761, 791, 836 nm) that were measured is expressed as shown in simultaneous Eq. (3).

$$\begin{aligned}
\mu_{a761nm} &= \varepsilon_{oxyHb761nm} C_{oxyHb} + \varepsilon_{dcoxyHb761nm} C_{dcoxyHb} \\
&\quad + \varepsilon_{H_2O761nm} C_{H_2O} + \mu_{abkg761nm} \\
\mu_{a791nm} &= \varepsilon_{oxyHb791nm} C_{oxyHb} + \varepsilon_{dcoxyHb791nm} C_{dcoxyHb} \\
&\quad + \varepsilon_{H_2O791nm} C_{H_2O} + \mu_{abkg791nm} \\
\mu_{a836nm} &= \varepsilon_{oxyHb836nm} C_{oxyHb} + \varepsilon_{deoxyHb836nm} C_{deoxyHb} \\
&\quad + \varepsilon_{H_2O836nm} C_{H_2O} + \mu_{abkg836nm} \quad (3)
\end{aligned}$$

where μ_a is the absorption coefficient at the wavelength λ, $\varepsilon_{m\lambda}$ is the molar extinction coefficient of the substance m at the wavelength λ, C_m is the concentration of the substance m and bkg is the chromophores contributing to μ_a in tissue for other than oxygenated hemoglobin (oxyHb), deoxygenated hemoglobin (deoxyHb) and water.

Based on the assumption that light absorption in the living body in this wavelength region occurs from oxyHb, deoxyHb and water, and also that there is no other background absorption in the living body (Tromberg et al., 1997), we determined TRS values for oxygenated hemoglobin (TRS HbO_2) and deoxygenated hemoglobin (TRS Hb) as tissue water concentration is 70%.

TRS total hemoglobin (TRS tHb) and SO_2 were obtained from Eq. (4) as follows.

$$\text{TRS tHb}[\mu M] = \text{TRS } HbO_2 + \text{TRS Hb},$$

$$SO_2[\%] = \frac{\text{TRS } HbO_2}{\text{TRS tHb}} \times 100 \quad (4)$$

Appendix C. Conversion TRS tHb into TRS CBV

We converted the TRS tHb into the CBV by TRS (TRS CBV) using Eq. (5) (Wyatt et al., 1990) for comparison with the CBV by PET (PET CBV).

$$\text{TRS CBV}[\text{cc}/100\text{ g}] = \frac{\text{TRS tHb} \times MW_{Hb}}{Hb \times \eta \times \rho \times 100000} \quad (5)$$

where MW_{Hb} is hemoglobin molecular weight; 64,500, Hb is arterial hemoglobin concentration (g/dl) of subject, η is the cerebral-to-large-vessel hematocrit ratio; 0.85 (Phelps et al., 1979) and ρ is density of cerebral tissue (g/ml); 1.04 (Picozzi et al., 1985).

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ORIGINAL INVESTIGATION

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Molecular characterization of histidinemia: identification of four missense mutations in the histidase gene

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Abstract Histidinemia (MIM235800) is characterized by elevated histidine in body fluids and decreased urocanic acid in blood and skin and results from histidase (histidine ammonia lyase, EC 4.3.1.3) deficiency. It is the most frequent inborn metabolic error in Japan. Although the original description included mental retardation and speech impairment, neonatal screening programs have identified the majority of histidinemic patients with normal intelligence. Molecular characteristics of histidase in histidinemia have not been determined, and cytogenetically visible deletions of 12q22-24.1 in which histidase gene resides have not been identified in histidinemic patients. In order to investigate whether individuals with this disorder have small deletions, additions, or point mutations in the histidase gene, we screened genomic DNA isolated from 50 histidinemic individuals who were discovered by the neonatal screening program. The methods employed included polymerase chain reaction (PCR) amplification of exons 1–21 of the histidase gene, followed by mutation detection enhancement gel electrophoresis and sequencing of the PCR products displaying heteroduplex bands. Four missense mutations (R322P, P259L, R206T, and R208L), two exonic polymorphisms (T141T c.423A → T and P259P c.777A → G), and two intronic polymorphisms (IVS6−5T → C and IVS9+25A → G) were identified. The frequencies of each polymorphism estimated either by dot blot allele-specific oligonucleotide hybridization, restriction enzyme digestion, or direct sequencing of the PCR products amplified from 50 unrelated normal individuals were 0.28, 0.30, 0.40, and less than 0.01, respectively. Mutation analysis of one family demonstrated that the patient inherited R322P from the mother and P259L from the father. This report describes the first mutations occurring in the coding region of the histidase structural gene in patients with histidinemia.

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Introduction

Histidinemia (MIM 235800) is an autosomal recessive disorder resulting from histidase deficiency. Histidase (histidine ammonia lyase, EC 4.3.1.3) catalyses the non-oxidative deamination of histidine to urocanic acid, the first step in the major catabolic pathway of histidine (Taylor et al. 1991b). The metabolic blockade of histidase activity is associated with elevated histidine in body fluids and with decreased urocanic acid in blood and skin (Levy et al. 1995).

Most histidinemic children manifested with clinical abnormalities before the introduction of newborn screening programs in the 1970s. Screening programs

have altered this view substantially by identifying an asymptomatic majority population (Tada et al. 1984). Subsequent analyses, undertaken to evaluate relationships among histidinemia, mental retardation, and speech disturbances, suggest that histidinemia is a generally benign disorder (Scriver and Levy 1983; Tada et al. 1982). This view led to the decision to discontinue newborn screening for this disorder in 1992 in Japan. Few new cases have been diagnosed since the cessation of the screening, although histidinemia is the most frequent (1:8400) of inborn metabolic error in the Japanese population. We have been following 50 patients with abnormal serum histidine levels detected by the neonatal screening program since the initiation of the program in 1977. Our observations, including thorough developmental and pediatric psychiatric analyses, favor the view that histidinemia is a risk factor for the development of behavioral disorders, including learning disabilities, in individuals under specific circumstances such as abnormal perinatal events (Ishikawa 1987).

A molecular approach to this disorder may provide insights into the explanation for the diverse phenotypes of this disease, ranging from the minority of symptomatic patients to the majority who present few or no distinguishing clinical features. The human histidase gene (HAL) has been assigned to chromosome 12q22-12q24.1 (Taylor et al. 1991a). Attempts to identify cytogenetic abnormalities within this region among patients with histidinemia have been unsuccessful, and to this date, no cases with visible deletions of part of the region 12q22-24.1 have been documented with a deletion of the histidase gene locus. In a preliminary study of 17 histidinemia patients described in a review by Levy et al. (2001), a partial deletion or rearrangement of the histidase gene in a patient with normal intelligence was identified using a human histidase cDNA probe. However, there were no gross rearrangements in any other subjects.

In our laboratory, we have isolated and cloned cDNA and genomic DNA encoding human histidase (Suchi et al. 1993, 1995). In order to evaluate whether decreased histidase activity is caused by smaller histidase gene abnormalities, we screened 50 histidinemic patients for the presence of small deletions, additions, splice site mutations, or point mutations by mutation detection enhancement (MDE) gel analysis. We report here the identification of four missense mutations and two exonic and two intronic polymorphisms. These are the first mutations in the coding regions of the histidase gene described in patients with histidinemia.

Materials and methods

Subjects

Fifty patients detected as having high histidine levels by the neonatal screening program from 1977 to 1991 have been followed periodically at Nagoya City University Hospital. Serum histidine levels varied among the patients. One patient's highest recorded serum histidine level was 4.9 mg/dl at 1 month of age, although the Guthrie screening value was greater than 6 mg/dl. The highest serum histidine values during the follow-up of the remaining 49 patients were from 6.9 to 17.3 mg/dl (mean 12.1 ± 2.5 mg/dl). Twenty patients were given low histidine formula for variable lengths of time. None are currently receiving low histidine formula or on a restriction diet. Normal control subjects were unrelated and did not have any family history of inborn errors of metabolism.

Mutation detection and sequence analysis

Peripheral leukocyte genomic DNA was isolated as described (Baas et al. 1984) from 50 patients, selected family members of patients nos. 3 and 45, and 50 unrelated healthy Japanese individuals following informed consent.

Exons 1–21 and the flanking sequences of the histidase gene were amplified by polymerase chain reaction (PCR; Saiki et al. 1988). The DNA sequence of the histidase gene was obtained from a nucleotides site (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi/nucleotide; accession nos. AB042205–AB042217). Oligonucleotide sequences and thermocycling conditions used in these amplifications are provided in Table 1. The goal was to include all *cis*-acting elements that participate in pre-mRNA splicing. To this end, the 3′-ends of all primers were designed to be located at least 42 base pairs (bp) away from the splice junctions. For the amplification and screening of exon 9, primers were selected not to include the 4-bp repetitive element in intron 8, 65 bp upstream from the intron–exon junction (Maffei et al. 1997).

MDE gels (AT Biochem, Malvern, Pa., USA) were used to analyze PCR products for the presence of small mutations in the histidase gene. Aliquots of 15 µl PCR product were combined with 3 µl loading dye (50% [w/v] sucrose, 0.6% [w/v] bromophenol blue, and 0.6% [w/v] xylene cyanol). The samples were heated at 95°C for 3 min, slowly cooled to 37°C, and run on 0.5×MDE gels containing 0.6×Tris-borate-EDTA (TBE) buffer (1×TBE = 0.089 M TRIS-borate, 0.002 M EDTA, pH 8.3). Gels were run overnight in 0.6×TBE at 20 V/cm for 12–18 h, depending on the size of the PCR product. MDE gels were stained for 15 min at room temperature in 1 µg/ml ethidium bromide in 0.6×TBE. Bands were visualized on a UV-*trans*-illuminator and photodocumented. In order to detect homozygous mutations, a second MDE gel analysis was performed on the PCRs mixed at a 10:1 ratio with the PCR product of the same region amplified from cloned histidase gene plasmids (Suchi et al. 1995).

Mutations were characterized by nucleotide sequencing whenever a heteroduplex band was detected. PCR-amplified products were subcloned into pCRII

Table 1 Histidase gene (HAL) primers and conditions for PCR amplification

Exons	Primer sequences Forward (5′–3′)Reverse (5′–3′)	Position	Annealing temperature (°C)
1	TCTCTGGCCTTTGCAGTCTTTTATGCAGAAGTG-GCTACC	−223* to c.−50 (exon 2)	54
2	AAGTGGACAGGAGGCT-CACGGGTTCAATGCTGCAAAGAC	c.−157 (exon 1) to IVS2+71	57
3, 4	GA-ACATGGCTGTACAATGTGCCCTGTTCTCCTTGA-CAGTG	IVS2−52 to IVS4+106	55
5, 6	TCAAGCTGTTCAGACTCACTTTATGATGCTATC-TATGACC	IVS4−42 to c.472 (exon 6)	53
6, 7	CCGTGGAAGTCTTTAACGTGAAGCCT-CATCCTCTGATCTG	IVS5−46 to IVS7+114	55
7, 8	AAATCCCTGCATTTAATTGCGGATAACTCA-CACTGTGCTG	IVS6−42 to IVS8+75	54
9	GGAGTTTGCTTTTCTTGGAATT-TCCCACTCATTTCAATT	IVS8−43 to IVS9+126	51
10	ACTCACTGGCAAAGCAAGGATAC-GAAGCATGCAAGCACAG	IVS9−70 to IVS10+73	54
11	GTTCCTGTAGGTCTCATTTCAAGGAATGATTG-AGGCTGAGA	IVS10−131 to IVS11+90	53
12	CGGGTATGTAGGTTCGTTACGTCTAAAGATCT-CAGCCACC	IVS11−59 to IVS12+76	55
13, 14	AAGGTAGGAGGATCGCT-AGATTTCATCCTGAACCTGGGGA	IVS12−124 to IVS14+74	55
15	TATTGGTACTCTTCAGGTAATCGAG-CAATGGAACTGAGAAGC	IVS14−58 to IVS15+103	54
16, 17	TCTCTTGCAGGAAGACTTGTCTGATTGCAGCA-AGTTCATG	IVS15−144 to c.1400 (exon17)	56
17	AACTGCTCTTGACCCAGAAGGAAAGACCCTTA-GATGGACG	IVS16−63 to IVS17+54	55
18	TCTGGAGAGTGTATCC-CATGGTTTCCAGGTGATGCTGATG	IVS17−106 to IVS19+103	56
19, 20	CAT-CTGTTAGTTGGGTGGTGCTGTGGGCTGCCTC-GATGT	IVS18−105 to c.1814 (exon 20)	58
20	ATCGCTTCATGGCCCCG-GACGGTGCACTTAGTGTTTATTA	c.1778 (exon 20) to IVS20+181	51
21	ACA-GCAGCTGTGTCTGGTCATGCCATCAGCCTAAC-CAACG	IVS20−119 to c.2125 (exon 21)	55

vector (TA cloning kit, Invitrogen, Carlsbad, Calif., USA). Nucleotide sequence information was determined by means of a Thermo sequence fluorescent labeled primer cycle sequencing kit (Amersham, Piscataway, N.J., USA) and an automatic A.L.F. red sequencing apparatus (Pharmacia, Peapack, N.J., USA). Base changes detected by sequencing were confirmed by either allele-specific oligonucleotide hybridization or restriction enzyme digestion. At least three clones from at least two separate PCRs were sequenced to verify newly identified base changes.

Allele frequencies

The frequency of two putative missense mutations (R322P and P259L) and a base change associated with a possible polymorphism, P259P (c.777A → G), was assessed by dot blot allele-specific oligonucleotide hybridization. Regions spanning exons 10 and 12 were amplified from genomic DNA samples of 50 normal unrelated individuals. PCR products (10 µl) were mixed with an equal volume of 4×SSC (1×SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) and heat-denatured at 95°C for 5 min. The denatured samples were blotted to Hybond-N+ nucleic acid transfer membrane (Amersham) via a Bio-Dot apparatus (BioRad, Hercules, Calif., USA). Filters were hybridized with allele-specific oligonucleotides labeled with [γ-32P]-ATP (> 5,000 Ci/ml; Amersham) and T4 polynucleotide kinase (Takara Shuzo, Otsu, Shiga, Japan). The allele-specific oligonucleotides were 5′TTGCCCCACTCT-CTCAT3′, 5′TTGCCCTGCTCTCTCAT3′, and 5′TTGCCCCGCTCTCTCAT3′ for 259P, 259L, and 259P (c.777A → G), respectively, and 5′TGTA-GAGCGAGCCAGTG3′ and 5′TGTAGAGCCAG-CCAGTG3′ for 322R and 322P, respectively. Hybridizations and washes were according to the

standard procedure, and the hybridization results were visualized by autoradiographic exposure to New RX Medical X-ray film (Fuji, Tokyo, Japan).

Direct sequencing of the PCR products was employed to calculate the allele frequencies of two other putative missense mutations in exon 9 (R206T and R208L) and another intronic base change in intron 6 (IVS6−5 T → C). The PCR primers used were 5′ CCGTGGAAGTCTTTAACGTG3′ and 5′AAGCCTCATCCTCTGATCTG3′ for intron 6, and 5′GGGCTCAGACAAGTCAGACAG3′ and 5′CTTCTCAGGTGATTCCTCAGC3′ for exon 9 amplification. The latter pair of primers was also used for the direct sequencing of the PCR products from the parents of patient no. 45. The PCRs were purified by the Qiagen PCR purification kit (Qiagen, Valencia, Calif., USA) and sequenced using Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, Calaif., USA).

Two other putative polymorphisms, T141T (c.423A → T) and IVS9+25T → C, involve restriction enzyme sites *Pvu*II and *Ava*II, respectively. T141T (c.423A → T) abolishes the *Pvu*II site, and IVS9+23T → C creates a new *Ava*II site. The frequency of these sequence variations was estimated utilizing these restriction sites. PCR-amplified DNA fragments from 50 normal unrelated individuals were purified using the PCR Purification kit, digested by 10 units of *Pvu*II or *Ava*II (New England Biolabs, Beverly, Mass., USA), and separated by electrophoresis in 1.2% agarose gels. The PCR primers were the same sets used to amplify intron 6 and exon 9 (see above) for T141T (c.423A → T) and IVS9+25T → C, respectively.

Results

MDE gel electrophoresis of PCR products spanning exons 6, 9, 10, and 12 revealed two to three bands in five, six, one, and one patient(s), respectively. Sequence analysis of these PCR products identified four missense mutations, two exonic base changes without amino acid changes, and two intronic base changes. These were: P259L (c.776CA → TG), P259P (c.777A → G), R322P (c.965G → C) in patient no. 3, R206T (c.617G → C) in patient nos. 12, 25, and 45, R208L (c.623G → T) in patient no. 44, T141T (c.423A → T) in patients nos. 6, 9, and 11, IVS6−5T → C in patient nos. 3, 6, 9,11, and 44, and IVS9+25A → G in patient nos. 17 and 28. Characteristics of these base changes are summarized in Table 2.

Patients 6, 9, and 11 harbored a silent base change T141T (c.423A → T) and an intronic sequence variation IVS6−5T → C. From each individual, at least five independent clones from at least two independent PCRs had this allele, whereas at least two clones had the same sequence as the reported histidase sequence. The latter intronic variation was also found in two other unrelated histidinemia patients (nos. 3 and 44) and was considered to be a polymorphism. Direct sequencing of PCR products from 50 unrelated normal individual demonstrated the frequency of IVS6−5T → C to be 0.40. As patients 6 and 9 are sister and brother, and as patient 11 is their cousin, T141T could have been a family-specific sequence. However, when *Pvu*II restriction enzyme digestion was applied to the PCR products from 50 normal individuals, it revealed that the frequency of T141T (c.423A → T) was 0.28 in this population.

In patient 3, three base changes were identified in exons 10 and 12. At codon 322 in exon 12, five of eight subclones had the sequence of CCA, which altered the amino acid arginine to proline (R322P), whereas the other three were CGA (Fig. 1). At codon 259 in exon 10, four of seven subclones had CTG, which codes for leucine (P259L), whereas the other three clones were CCG (P259P (c.777A → G)), which did not change the amino acid (proline) but was one base different from the reported CCA (Fig. 2). Sequence analysis and dot blot allele-specific hybridization of PCR products spanning exons 10 and 12 of the histidase genomic DNA of the parents revealed that the mother possessed a normal proline at amino acid 259 (259P/P259P(c.777A → G)) and was heterozygous for R322P, while the father had the normal 322R and was heterozygous for P259L/P259P (c.777A → G) (Fig. 3). The frequency of the polymorphism at codon 259 was estimated to be 0.30 by dot blot allele-specific oligonucleotide hybridization of 50 normal individuals (data not shown). R322P or P259L sequences were not detected in any of the 50 normal individuals by dot blot hybridization.

Table 2 Histidase mutations (*ASO* allele-specific oligonucleotide hybridization, *NA* not applicable)

Nucleotide change	Amino acid change	Position	Methods	Frequency
c.423A → T	T141T	Exon 6	*Pvu*II digestion	0.28
IVS6−5T → C	–	Intron 6	Direct sequencing	0.40
c.617G → C	R206T	Exon 9	Direct sequencing	NA
c.623G → T	R208L	Exon 9	Direct sequencing	NA
IVS9+25A → G	–	Intron 9	*Ava*II digestion	<0.01
c.776CA → TG	P259L	Exon 10	ASO	NA
c.777A → G	P259P	Exon 10	ASO	0.30
c.965G → C	R322P	Exon 12	ASO	NA

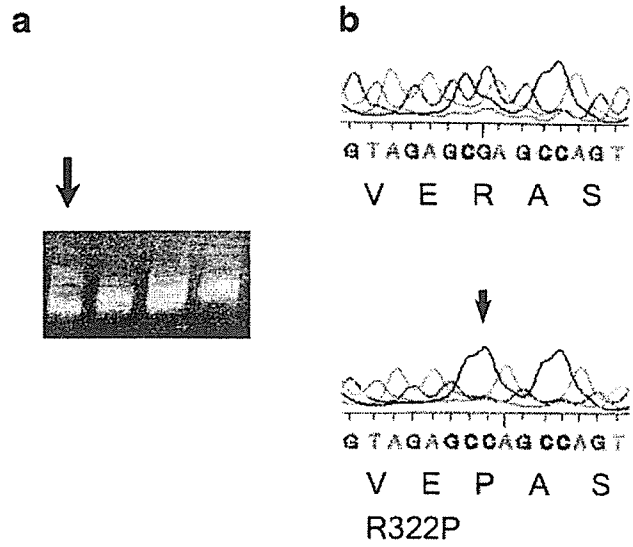

Fig. 1 a Mutation detection enhancement (MDE) gel electrophoresis of histidase exon 12 (*arrow* heteroduplex bands from patient no. 3).b Fluorescent dideoxy sequence analysis of exon 12. *Top* Normal reported sequence. *Bottom* A subcloned PCR product amplified from patient no. 3

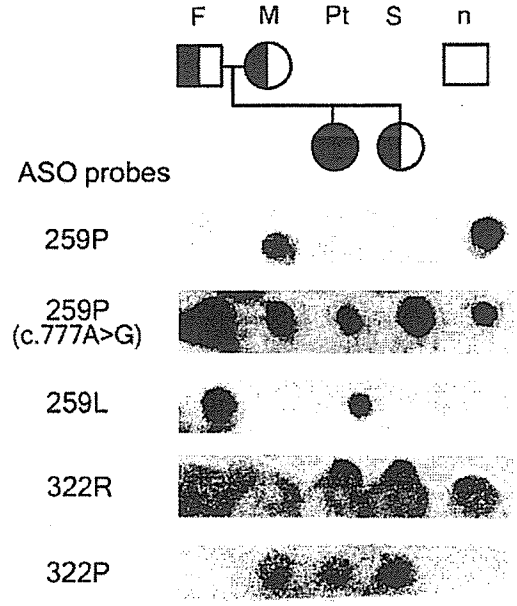

Fig. 3 Allele-specific oligonucleotide (*ASO*) hybridization analysis of PCR-amplified genomic DNAs from family members of patient no. 3 (*F* father, *M* mother, *Pt* patient, *S* sister, *n* unrelated control, *259P, 322R* probes from normal sequences, *259L, 322P* probes containing mutations, *259P (c.777A > G)* polymorphic sequence)

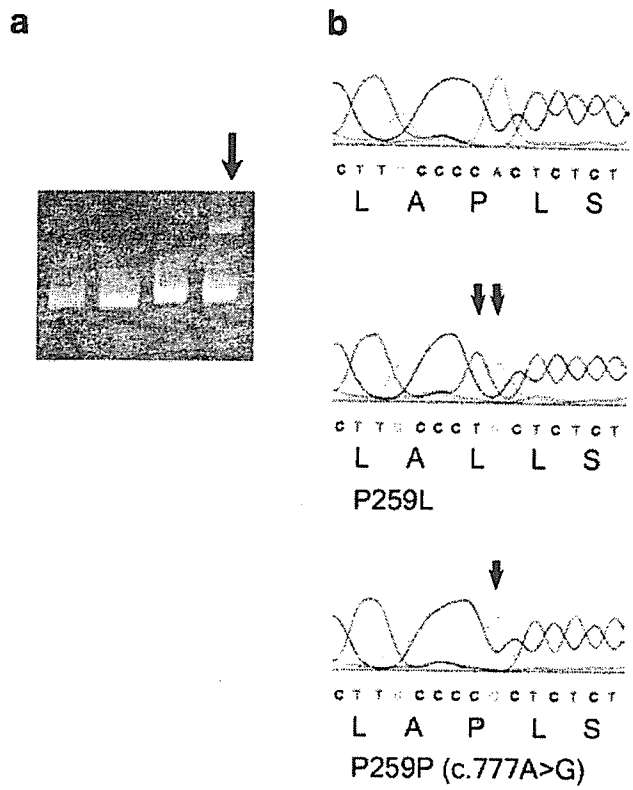

Fig. 2 a MDE gel electrophoresis of histidase exon 10 (*arrow* heteroduplex bands from patient no. 3). b Fluorescent dideoxy sequence analysis of exon 10. *Top* Normal reported sequence. *Middle* One of the subcloned PCR products amplified from patient no. 3. *Bottom* Another subclone

A missense mutation (R206T) was identified in exon 9 of patients 12, 25, and 45 (Fig. 4). At least three clones from each individual showed c.617G → C, while there were at least two clones with the normal sequence. No other mutation was detected in the other exons by MDE analysis in these patients. No R206T base change was detected in 50 normal individuals by direct sequencing of the region. Genomic DNA of the parents of patient no. 45 was obtained. Direct sequencing of the exon 9 region demonstrated the R208T sequence in the father who was heterozygous for this mutation.

An R208L missense mutation was identified in six subclones from two independent PCRs from one patient (no. 44), only six base pairs 3′ to the R206T mutation (Fig. 4). Two clones from the same PCR showed the normal sequence. Except for the presence of the aforementioned IVS6−5T → C, no other heteroduplex bands were detected in the histidase gene from this patient. Direct sequencing demonstrated that this base change was not present in 50 normal individuals.

Two other unrelated patients (nos. 17 and 28) who displayed heteroduplex bands in the exon 9 region had an intronic sequence variation, IVS9+25A → G. One of 50 normal individuals was revealed to harbor this base change by PCR amplification and *Ava*II restriction digestion. This normal individual was also heterozygous for IVS9+25A → G. Three additional normal individuals were assayed for this base change; no PCR segments were digested by *Ava*II.

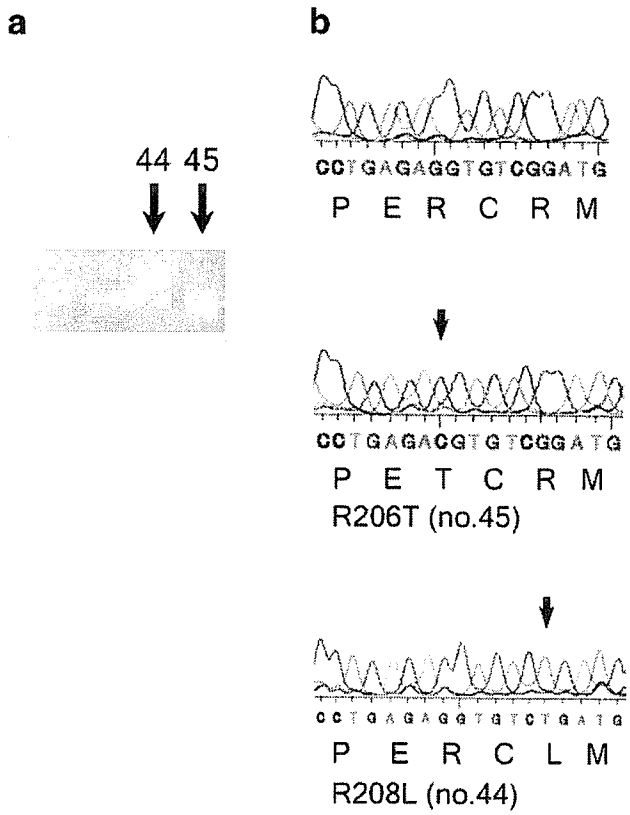

Fig. 4 a MDE gel electrophoresis of histidase exon 9 (*arrows* heteroduplex bands from patient nos. 44 and 45). b Fluorescent dideoxy sequence analysis of exon 9. *Top* Normal reported sequence. *Middle* A subcloned PCR product amplified from patient no. 45. *Bottom* A subcloned PCR product amplified from patient no. 44

Discussion

Histidinemia is an autosomal recessive amino acid metabolism disorder in which the patients have decreased histidase activity in the liver and epidermal stratum corneum (Levy 1988). Mammalian histidase has a molecular mass of about 200 kDa and is described to consist of three identical subunits of 75 kDa each (Okamura et al. 1974; Taylor et al. 1990). Several genetic mechanisms can be considered to cause the deficiency in an enzyme composed of a homotriplex: (1) a large deletion of the gene, either cytogenetically visible and spanning several other genes or detectable by Southern blot analysis, (2) an aberration in the *cis*-element sequence, such as promoter and enhancer regions, (3) a coding sequence abnormality or intronic sequence abnormality that results in qualitatively or quantitatively abnormal mRNA, (4) abnormalities in post-translational modification, and (5) abnormalities in regulatory proteins. Some cases of histidinemia involving mental retardation or other abnormalities might result from a contiguous gene syndrome in which the HAL locus and genes adjacent to it are deleted. However, no cases with a HAL locus deletion have been demonstrated among patients with histidinemia or those with translocations of 12q or deletions in 12q (Levy et al. 2001).

In this report, we have focused on the hypothesis that histidase activity is reduced in patients with histidinemia because of mRNA abnormality. There are suggestions, in at least some patients, that mutations have occurred in the coding region of the histidase structural gene. Kuroda et al. (1982) have demonstrated an altered sensitivity of skin histidase to denaturation in two patients. To investigate this possibility by molecular techniques, we screened histidase genomic DNA for mutations.

Two missense mutations R322P and P259L were identified in exons 12 and 10, respectively, in one patient, no. 3. Dot blot allele-specific oligonucleotide hybridization and sequence analysis of the respective exons revealed that R322P was inherited from the mother and P259L from the father. Both codons are conserved among mouse, rat, and human. R322P changes the basic amino acid to neutral proline. Taylor et al. (1993) have reported an R322Q mutation in a histidinemic mice population. The R322Q mutation in the mouse is expressed in Cos1 cells and has been demonstrated to have decreased histidase activity compared with the enzyme protein with the wild-type arginine at codon 322. P259L also involves the amino acid proline and possibly affects the secondary structure of the polypeptide. The mutation R206T was found in three unrelated histidinemia patients (nos. 12, 25, and 45). Only one patient (no. 44) possessed the R208L mutation, which lies 6 bp 3′ to the R206T mutation. The two arginines, one amino acid apart, are expected to create a strong negative charge in this portion of the polypeptide, and a change to a neutral amino acid in one of these arginines could cause a major conformational alteration in the enzyme protein. Although the effect of these missense mutations on the activity of histidase has not been demonstrated in vitro, all of the base changes lead to nonconservative amino acid substitutions. In addition, these four changes have not been identified in 100 alleles of 50 normal individuals by dot blot hybridization or by direct sequencing, providing additional support that they are disease-causing mutations.

Two intronic sequence variations were also identified within the histidase gene during this study. Both occurred in more than one patient. One of them, IVS9+25A → G, was present in one of 106 alleles from normal individuals. With the incidence of histidinemia among Japanese being 1:8400, one in 46.3 people is expected to be a heterozygous carrier. It is still possible that IVS9+25A → G is a disease-causing base change, however, it is less likely to cause abnormal splicing in the transcribed RNA, because the base change is more than 20 bp away from the intron–exon junctions. The second intronic base change, IVS6−5T → C, is at a −5 position in the respective intron and may affect splicing efficiency. However this base change is seen in normal individuals

with a frequency of 0.40 by PCR amplification and subsequent direct sequencing.

One of the two exonic polymorphisms P259P (c.777A → G) occurs in the normal population with the frequency of 0.30. The polymorphic P259P (c.777A → G) at codon 259 was identified in five of six alleles in the family members of patient no. 3 excluding the alleles with P259L. Although the reported "normal" codon 259 is CCA, the mutated sequence CTG is likely to have arisen from CCG with the second C changing to T, rather than via two base changes from CCA.

MDE analysis yielded no heteroduplex bands in a number of amplified exonic regions. Several reasons for the low sensitivity of heteroduplex occurrence can be proposed. One is that a true false-negative might occur in which the MDE gel does not produce heteroduplex bands even when there is a mismatch in the strands of the amplified DNA. False-negative rates are reported to vary depending on the length of the PCR products. Another reason is that, if there is a deletion of the gene or part of the gene in one allele, PCR will only amplify the remaining normal allele, resulting in one band. In addition, when a mutation resides at a point at which PCR primers anneal, the allele containing the mutation will not be amplified. The strategy that we have adapted will also miss 5′ promoter mutations and large intronic deletions that may alter transcription efficiency, RNA stability, or splicing. Moreover, histidinemia, despite a consistent biochemical phenotype, may include more than one form of histidase deficiency, namely a more frequent benign type and a less frequent maladaptive variant. One form could arise because of an abnormality in a protein other than histidase protein, such as one of the regulatory proteins. Lastly, one case in this series demonstrated serum histidine levels at a high normal range despite Guthrie's test results showing a value greater than 6 mg/dl. Determination of histidase activity levels will thus be necessary to confirm the diagnosis of histidinemia.

In summary, four missense mutations have been identified within the histidase gene among 50 patients with abnormal histidine levels detected through the newborn screening program. This is the first report in which mutations have been documented within the coding region of this gene.

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ORIGINAL ARTICLE

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Sibling risk of pervasive developmental disorder estimated by means of an epidemiologic survey in Nagoya, Japan

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Abstract Broad-spectrum autism, referred to as pervasive developmental disorder (PDD), may be associated with genetic factors. We examined 241 siblings in 269 Japanese families with affected children. The sibling incidence of PDD was 10.0% whereas the prevalence of PDD in the general population in the same geographic region was 2.1%. Both of these rates are higher than those reported previously, probably because of the expanded clinical criteria applied. The prevalence in males of the general population was 3.3% and that in females was 0.82%. The sibling incidences were 7.7 and 20.0% for families in which the probands were male and female, respectively. Because the reversed sex ratios correspond to the general rule for a multifactorial threshold model, we suggest that most PDD cases result from the cumulative effects of multiple factors (mostly genetic). The sibling incidences were 0 and 10.9% for families in which the proband had low and normal birth-weight, respectively, suggesting the risk is lower in families with low-birth-weight probands.

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Introduction

Autism is a behaviorally defined syndrome, characterized by pervasive impairment of social interaction and communication and the presence of stereotypical characteristics. Although these clinical symptoms may arise from brain dysfunction, clinical severity is modified by environmental factors. In the past decade, criteria for diagnosis of autism have been expanded, from a strict category (Kanner type) to a broad spectrum, owing to progress in neuropsychological understanding (Wing 1996). The broad spectrum of autism is defined as pervasive developmental disorder (PDD) in the DSM-IV criteria (American Psychiatric Association 1994) and the number of cases has recently increased rapidly, in line with the expanding criteria. A recent study described the prevalence in the Japanese general population to be more than 1% (Honda et al. 2005).

Previous studies of twins have suggested that autism is strongly affected by genetic factors. Ritvo et al. (1985) found that the concordance for autism by pairs was 96% in 23 monozygotic twins and 24% in 17 dizygotic twins; Steffenburg et al. (1989) reported respective values of 91 and 0%. Bailey et al. (1995) reported concordance for classical autism to be 60% in monozygotic and 0% in dizygotic twins, but for the broad spectrum disease it was 92% and 10%, respectively. These results show that autism is strongly affected by genetic factors but is also affected by the environment. Previous sibling studies have also suggested genetic effect on autism, with August et al. (1981), Baird et al. (1985), Chakrabarti et al. (2001), and Icasiano et al. (2004) reporting sibling risks of 2.8, 5.9, 3.9, and 6.3%, respectively. These frequencies are much higher than that in the general population but

much lower than that in single-gene diseases. As far as we are aware, all previous studies involving both twins and siblings were performed on Caucasian populations only. Several genome-wide investigations have proposed susceptibility loci associated with autism (PDD), but neither a single candidate gene nor an inheritance mode for autism have been determined (Shastry 2003). It has also been suggested that PDD may be caused by non-genetic factors, for example neonatal factors.

In this study we estimated the prevalence of PDD in the general population of the western region of Nagoya, Japan, and examined the overall sibling incidence and the incidence after families were classified by the sex and birth-weight of the probands. We suggest a model for the etiology of PDD based on our results.

Subjects and methods

Screening and diagnosis

This study was conducted using a regional support system for children. The western region of Nagoya is a residential area with high population density—half a million people within 97 km^2. Infants with any developmental problems are detected by a screening system which is well organized by local government. The first stage of the system is a routine health check at general health centers; the average percentage attendance in 2001–2003 was 95.3 and 86.5% for 18-month-old and 3-year-old children. Pediatricians and public health nurses examine development and all infants with developmental problems (including mild symptoms) are referred to the West District Care Center for Disabled Children (WDC center). The second stage is based on observation at kindergartens and day nurseries. Psychologists from the WDC center make regular visits and refer cases to the WDC center when necessary. Because the number of infants attending kindergartens or nurseries is 99.7% in this area, most infants with problems should be noticed. The WDC center also cooperates with general hospitals in the area. Because there are no departments of pediatric psychiatry, children are always referred to the WDC center. Medical examinations such as chromosome analyses are performed at hospitals and the results are sent to the WDC center. On initial examination at the WDC center, psychologists obtain detailed histories from parents and also conducted an intelligence quotient test (Tanaka-Binet) on all children. In addition, pediatric psychiatrists observe children's behavior carefully in a play space with the help of public health nurses. If children have developmental problems, they start educational programs (group style or individual therapy) at the center. After repeated observation both by psychologists and pediatric psychiatrists, children are finally diagnosed, at the age of four or above, using the DSM-IV (American Psychiatric Association 1994) criteria.

In this study we excluded autistic children with certain disorders (two cases with Duchenne muscular dystrophy, two with Down syndrome, one with 18p partial monosomy, and one with tuberous sclerosis).

Prevalence and siblings

In this study we first estimated the prevalence of PDD in the general population. At the center we diagnosed 281 infants who were born between 1995 and 1999 as having PDD. The number of affected children (281) was divided by the total number of age-matched children residing in the area (13,568 children, consisting of 6,949 boys and 6,619 girls). The parents were all Japanese.

For the sibling study we excluded families with siblings younger than 4 years. This resulted in a cohort of 269 families whose characteristics are listed in Table 1. The affected siblings had already been diagnosed in our regional system before this study started. To avoid

Table 1 Characteristics of the families, probands, and siblings that formed the cohort of this study

Family factors	Number of families
All families	269
Children per family	
1 child	85
2 children	136
≥3 children	41
Average	1.9
Affected children per family	
1 child	247
2 children	22
≥3 children	1
Average	1.09
Proband factors	**Number of probands**
All probands	269
Male	215
Female	54
Sex ratio	3.98
Birth weight	
≥2,500	240
<2,500	29
Detailed clinical criteria	
Autistic	77
PDD-NOS	119
Asperger	73
Sibling factors	**Number of siblings**
All siblings excluding probands	241
Unaffected siblings	217
Male	105
Female	112
Sex ratio	0.94
Affected siblings	24
Male	13
Female	11
Sex ratio	1.18

Autistic, autistic disorder; PDD-NOS, pervasive developmental disorder not otherwise specified; Asperger, Asperger's disorder

non-detection of autistic siblings, we interviewed parents again about the behavior of their children and made further examinations at the center if they showed even minor problems. This procedure did not identify any new affected siblings. We indicate the firstborn PDD as the proband in multiple incidence families. The sibling risk (concordance rate) was determined as the number of PDD siblings divided by the total number of siblings.

Results

In the general population the prevalence of PDD was 2.1% (281/13,558), 3.3% (227/6,949) for boys and 0.82% (54/6,619) for girls. In the sibling study 23 multiple-incidence families were found as shown in Fig. 1. There were only two families (families 1 and 2) with further births after two affected children, but in family 1 the third child was also affected. Table 2 lists the incidence (concordance rate) among siblings. The incidence of PDD in all siblings was 10.0%, with values of 7.7 and 20.0% for families in which the probands were male and female, respectively. The incidences were 0 and 10.9% for families in which the probands had low and normal birth-weight, respectively.

Discussion

Pervasive developmental disorder, including classical autism, has received much attention in recent decades, especially because the criteria for clinical diagnosis of autism have been expanded from strict categorization to a broad spectrum. PDD is manifested by a difficulty in social communication, but the detailed etiology remains unclear. Although PDD (autism) seems to be strongly affected by genetic factors, several genome-wide investigations have failed to determine a single candidate gene, suggesting that several genes may be associated with this disorder.

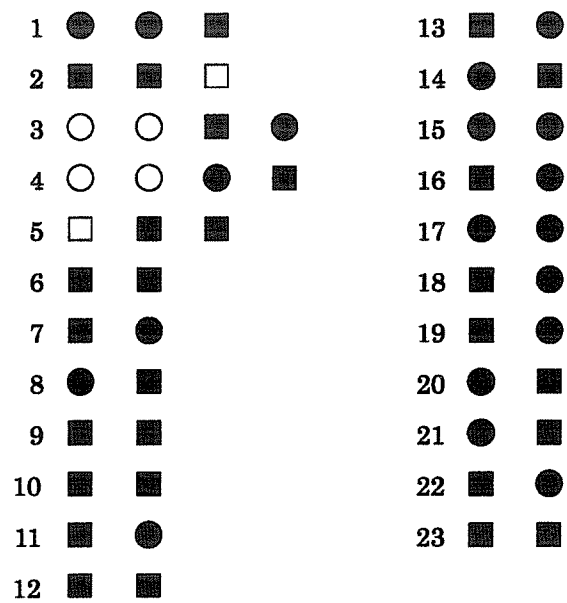

Fig. 1 Birth order of PDD probands and their siblings in 23 families with multiple affected children: *filled square*, male with PDD; *filled circle*, female with PDD; *open square*, normal male; *open circle*, normal female

In this study sibling incidence was 10.0%, in contrast with 2.1% in the general population. Both values were higher than in a previous report using DSM-III criteria (American Psychiatric Association 1980). The differences presumably reflect our application of the DSM-IV criteria. We believe our results may be used in genetic counseling for Japanese families, but further data are necessary to establish better guidelines.

The sibling incidence may also provide evidence to judge the mode of inheritance. It is unlikely that PDD has an autosomal dominant or an autosomal recessive mode of inheritance, because, theoretically, sibling incidence would then be 50 and 25%. In multifactorial inheritance, sibling incidence (Q) is estimated from the prevalence in general population (p): $Q=\sqrt{p}$ (Edwards' method; Emery 1986). Theoretical sibling incidence based on the prevalence in the population is 14.4%. Sibling incidence in our survey (10.0%) is therefore much closer to that for multifactorial inheritance mode than that for autosomal inheritance.

We examined the effect of sex on the incidence risk. The number of male probands was approximately four times that of female probands (Table 1). Sibling incidence for families with a male proband was less than half that for families with a female proband, however. The reversed sex ratios correspond to the general rule for a multifactorial threshold model (Carter 1969; Harper 2004), i.e. where there is unequal sex incidence, the risk is higher for relatives of a proband of the sex in which the condition is less common. A proband of the more rarely affected sex requires a greater genetic factor to develop the disorder. Such reversed sex ratios in autistic families were also observed by Ritvo et al. (1989). Other phenomena which corresponded to a multifactorial threshold model were also found in previous studies—a high sibling risk (35.3%) was noted in families with multiple affected children (Ritvo et al. 1989), with a wide discrepancy of risk between monozygotic and dizygotic twins (Ritvo et al. 1985; Steffenburg et al. 1989; Bailey et al. 1995).

X-linked recessive inheritance can be excluded using our data for prevalence in the general population. Because the prevalence in males was 3.3%, the expected frequency of affected females (assuming Hardy–Weinberg equilibrium) is the square of this number, 0.11%. The actual observed prevalence in females was 0.82%, however. If PDD had an X-linked dominant inheritance mode, the prevalence in females should be higher than in males. An imperfect X-linked dominant cannot, however, be excluded using the data for prevalence. It is, moreover, difficult to prove the mode of inheritance if several inheritance types are mixed or if non-genetic

Table 2 Incidence rates in siblings

Family category (Number of families)	Number of siblings and incidence rate			
	All siblings	Affected siblings	Incidence (%)	95% cl
All families [269]	241 (male 118, female123)	24 (male 13, female 11)	10.0 (male 11.0, female 8.9)	6.5–14.5 (6.0–18.1, 4.5–15.4)
Sex				
Families with male probands [219]	196	15	7.7	4.3–12.3
Families with female probands [50]	45	9	20.0	9.6–34.6
Birth-weight				
Families with NBW probands [240]	220	24	10.9	7.1–15.8
Families with LBW probands [29]	21	0	0.0	0.0

cl, confidence limits; NBW, normal birth weight; LBW, low birth weight

factors are mixed. A larger cohort is thus necessary for statistical examination and for drawing conclusions about the mode of inheritance.

Non-genetic factors have also come under consideration. A case control study showed that obstetric complications did not increase the risk of autism (Cryan et al.1996), but Indredavik et al. (2004) demonstrated that the rate of incidence with the Asperger syndrome was higher (4/56) than in controls (0/81). In this study sibling incidences were 0 and 10.9% for families in which the proband had low or normal birth-weight, respectively. Although there were few low-birth-weight siblings, this suggests the risk in families with low-birth-weight probands is lower.

Several models have been proposed for the etiology of PDD. Gillberg et al. (2000) described PDD (autism) to be a syndrome resulting from many individual diseases (factors), but this has been criticized by Jones et al. (2002), who proposed a risk factor model, supposing cumulative effects of multiple factors (mostly genes), in general agreement with a multifactorial threshold model.

Because our results for sibling incidence also provide support for such a model, we suggest that most PDD cases result from the cumulative effects of multiple factors (mostly genetic). Although there remains a possibility that one (or a few) major factor(s) could also cause PDD, this would not affect the results of our survey if the proportion were very small. Many factors, including single gene disorders and infection and neonatal factors, have been reported to be associated with PDD. For example, Jamain et al. (2003) suggested that defects in a single X-linked gene encoding neuroligin (cell-adhesion molecules at synapses) might cause autism. This needs to be taken into consideration in further investigations.

Finally we propose a model for the etiology for PDD encompassing two groups:

1 a large group in which the syndrome results from the cumulative effects of multiple factors (mostly genetic), namely a multifactorial disorder; and
2 a small group in which it results from separate individual factors.

This is in line with the classical model of Penrose (1963) who divided mental retardation into two groups:

1 a physiological group which is biased in the normal distribution for intelligence; and
2 a pathological group resulting from various kinds of neurological disease.

Further investigations of larger numbers of families, genes, and non-genetic factors are necessary for clarification.

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第93回日本小児精神神経学会
シンポジウム「子どものこころの臨床における発達について再考する」

自閉症臨床から

杉　山　登志郎*

Ⅰ．発達精神病理学とは何か

今回のシンポジウムのテーマである，自閉症のこころの臨床における発達というテーマを考察するうえで，精神病理学の重要性を最初に取り上げておきたい．

精神病理学(psychopathology)という学問は，今日ほとんど顧みられなくなった感があるが，本来は身体医学における病理学と同様，精神医学における臨床の基礎をなすものである．精神病理学には実は二つの意味がある．一つは，ほとんど症状学と同義語として用いられていて，精神科疾患のさまざまな症状に関しその概念を疾患ごとにまとめたものである．しかし真の意味は別にあり，一般的な心理学では届きにくい病的心理を扱う医学的心理学である(笠原，1987)．この領域の存在こそが，他の身体医学から精神医学を分ける独自の部分であり，いわゆるこころの臨床を特徴づけるものである．また逆に，こころの臨床に立ち入ることが可能か否かは，精神病理学の研鑽を積んでいるか否かによって分けられるといって過言ではない．身体科のみを基盤とする，こころの臨床における限界の大部分がこの点にあり，筆者としては発達障害における臨床も同一であると考える．

精神病理学の持つ意味を具体的な例で示してみよう．例えば幻覚，妄想が明らかな青年を前にすれば，医学教育課程を学んだすべての医師にとって統合失調症の診断を下すことは可能であろう．また精神科薬理学の知識を持つ医師であれば，適切な薬物療法の処方を行うことも可能である．しかしながら，統合失調症の患者に対し治療者として寄り添うためには，その患者がどのような内的世界にあるのかを知ることが必要不可欠となる．それは健常者にとって容易に理解ができる世界ではない．精神科疾患の一部の体験世界は，通常の常識的心理学では届かないからこそ，独自の医学的心理学を必要とするのである．統合失調症の患者が先へ先へと自分の存在を投企させようとしているとき，その焦りに共感しつつ治療者が掛けることばによってのみ，その激しい生き焦りを少しでも引き留めることが可能となるのであって，その内的な世界を無視した対応は極めて敏感な患者に対して侵襲的に作用こそすれ，治療的にはならないであろう(中井，1974)．このような体験世界を学んではじめて統合失調症者の持つ脆弱性の理解も可能となる．

このような問題はもっと一般的な，健常心理の近くにある現象についても事情は同じであ

Toshiro SUGIYAMA : The Case of Antism
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る．例えば不登校青年の他者への不信感情(森下，2000)，強迫性障害患者における周囲を巻き込んだ不安と表裏一体の万能感の存在など(成田，1994)，われわれがこころの臨床において日常的に接する患者に関しても，通常の心理学では届きにくい世界が存在するからこそ，医療あるいは臨床心理における治療の意味が生まれるのである．一般的な小児科医のみならず，今日の身体科医学を基盤とする医師がこころの臨床を学ぶうえで，基本的な欠落がこの点にある．診断は可能であっても，それから先の「今，何が必要なのか」を提示できなくてはこころの臨床を専門とする臨床医とは認め難い．

そのうえでさらに，自閉症をはじめとする発達障害臨床においては，発達に伴う独自性をふまえた医学的心理学を構築することが必要となる．この問題を論じるために，最近になって新たに見直されるようになった，発達における氏と育ちの問題に先に触れておきたい．

発達を支えるものは子どもが持つ遺伝子と環境である．発達障害臨床に限定すれば生物学的負因と環境因ということになるが，これまでの研究では圧倒的に負因の持つ重みが環境因よりも大きいことが示されてきた．それでは負因によってすべてが決まるのであろうか．もしそうであるなら，近年の自閉症圏患者の世界レベルでの著しい増加というトピックスは説明が不可能である．近年の分子レベルでの遺伝子研究によって，遺伝子は環境によって発現の仕方が異なることが明らかとなってきた．多くの状況依存的なスイッチが存在し，環境との相互作用の中で合成されるタンパク質や酵素レベルの差異が生じるのである(Marcus, 2004)．高名な例を一つあげれば，MAO-Aと呼ばれる酵素がある(Caspi et al, 2002)．この酵素を生じる遺伝子を持つ児童は，攻撃的な傾向を発現する傾向があることが知られている．しかしながらそれはすべての児童においてそうなるのではない．虐待環境下においてのみ，攻撃的な傾向が現れるのである．この例にも示されるように，遺伝子の持つ情報は，学習，記憶，脳の発達，感情コントロールのレベルで環境との相互作用が生じることが明らかになった．この環境因の持つ重みは，被虐待児の臨床に携わっていれば容易に実感される．被虐待児において，脳梁の体積減少をはじめとする脳の器質的な変化が現れることは周知のとおりであり，しかも明らかに後天的な外傷である性的虐待において，最も明瞭に脳の器質的な変化が認められるのである(Putnum, 2003)．虐待の世代間連鎖など世代を超えて継続する虐待環境のような問題は，遺伝子の最も基本的な発現のレベルにおいて変化が生じる可能性を考慮することが今や必要となった．

発達障害臨床，特に自閉症臨床においては，その独自の体験世界を無視しては治療的な対応は不可能である．だが体験世界のみを重視しその場にとどまることは必ずしも治療的には働かない．子どもは発達する存在だからである．われわれに求められているのは，生物学的負因という強い制約が存在したとしても，その体験世界をふまえたうえで，より治療的な環境を用意することであり，発達障害治療の基盤となる治療的教育を円滑に進めることである．このような前提をふまえたうえで，この小論では自閉症の精神病理学をまとめ，ついでわれわれが現在取り組んでいる自閉症臨床におけるトピックスを取り上げる．この作業をとおして，発達障害におけるこころの臨床の特徴の検討を行う．

Ⅱ．自閉症の発達精神病理

筆者はこれまで，自閉症の精神病理学の確立を己のライフワークと考えてきた．寡症状統合失調症よりも自己表出を行わない自閉症の体験世界は，生涯を掛けて取り組む意義があるもの

と考えたのであるが，この領域に踏み込み始めた20年以上前は，今日のように饒舌な自閉症者がこれだけ世界的に多数出現するとは，またその方々に自ら会うとは想像もつかなかった．これは後に考えれば簡単なパズルである．彼らは聞かれたときには著しく内面を語ることは苦手であるが，書かせればより容易に自分の体験を表すことが可能となる．そして大きな問題は，途中から顕わになった問題(例えば統合失調症の病的体験など)と違って，彼らにとってみれば，生まれてからずっとその中に生きている体験世界は意外でも何でもない普通の世界であり，その独自性は外から指摘をされて初めて気づくことができるのである．Dona Williamsの自伝(1992)が彼らの世界を開くきっかけとなったのは，この彼らにとって当たり前の世界が実は普遍的ではなかったことに，多くの自閉症者が初めて気づいた点にある．

筆者自身は自閉症の精神病理学的な中心について，体験世界との心理的な距離の欠如と述べたことがある(杉山，2000)．自閉症者は認知対象に対し，自己意識があたかも認知対象に部分的に飛び込んでしまうような認知の仕方をしている．狭く，深く，細かく認知しており，その結果，大枠や曖昧な把握が非常に不得手である．一方，心理的な現象そのものは，健常者に比してそれほど大きな変化や逸脱はない．自閉症者の自伝を読めば，彼らの感情の持ち方はわれわれと同一であり，さらにそこにごく普通の防衛メカニズムが認められることにも気づく．深い愛着の形成は，健常児よりも遅れるが形成される．認知の特異さのために，自己意識のあり方は健常者とは幾らか異なっており，最近筆者は自閉症者とはある種の異文化に生きる者と考えるようになった(杉山，2002)．

自閉症の幼児は，対人的な情報への選択的注意という機能が十全に働いていない．その結果，お母さんの出す情報も，機械からでる雑音も等価的に流れ込んでしまうという情報の洪水状態で立ち往生している状態となる．Grandinは，自分の幼児期の耳は調整の効かないマイクロフォンのようだったと述べている(Sacks, 1995)．この状態から徐々に彼らは認知の焦点を合わせることが可能になってくる．しかし健常児の認知が広く開かれたものであるのとは異なって，恐らくは意識的な焦点の絞り込みによって初めて成り立つがために，自閉症の注意はあるものに注意が向いているときには，他の情報が無視をされるという単焦点(Williams, 1996)，単車線(Grandin, 2000)と呼ばれる強い過剰選択性を抱えるようになる．さらに注意の障害のために知覚の雑音の除去ができない．この結果，大きな声が聞こえずに，小さな機械音(例えばエアコンの音など)が強烈に聞こえるといった現象が生じることもある．

筆者は自閉症者の記憶の特徴を，タイムスリップ現象として取り上げた(杉山，1994)．これは自閉症児・者が過去の記憶のフラッシュバックを頻々と生じる現象であるが，これも記憶表象に対する心理的距離の欠如として考えることが出来る．この現象は，特定の刺激が過去の不快場面の記憶を開けてフラッシュバックを生じるという鍵構造を作ることもある．また自閉症者において，現在と過去とがモザイク状に体験されているということも稀ではない．

さらに自閉症者は感覚過敏に対抗するために，成人になると解離を利用して，自ら感覚モードを意図的に切り替え，不快刺激を遮断することすらある．そこまで意識的でなくとも，不快な状況や退屈な状況において，解離を用いて意識を飛ばすという技術を持っている自閉症児・者は多い(杉山ら，2003)．

むしろ臨床上の大きな支障は二次的な問題である．このような幅の狭い認知の中に生きるた

表1　触法行為を繰り返した青少年の一覧

#	性別	初診年齢	診断	非行・犯罪内容	既往他
1	m	5	PDDNOS	子兎を踏み殺す	虐待
2	f	7	PDDNOS	人のものを持ってくる	
3	m	8	アスペルガー症候群	万引き，衝動的乱暴	虐待
4	m	8	アスペルガー症候群	万引き，お金の持ち出し	虐待
5	m	9	PDDNOS	万引き	
6	m	13	高機能自閉症	お金の恐喝	
7	m	13	アスペルガー症候群	放火，乱暴	虐待
8	m	15	アスペルガー症候群	お金の持ち出し，万引き，放火	不登校
9	m	15	PDDNOS	下着を盗む，強制わいせつ未遂	
10	m	15	PDDNOS	下着の窃盗	虐待
11	m	15	アスペルガー症候群	万引き，乱暴，家出	不登校
12	m	17	アスペルガー症候群	強制わいせつ	
13	m	18	アスペルガー症候群	お金の持ち出し，親戚の家から窃盗	
14	m	18	アスペルガー症候群	下着の窃盗，隣家への忍び込み	緘黙
15	m	18	PDDNOS	幼児への強制わいせつ	虐待
16	m	20	アスペルガー症候群	ストーカー行為にて逮捕	不登校
17	m	21	高機能自閉症	幼児の隠し撮り，下着の隠し撮り	
18	m	24	高機能自閉症	暴力行為	

めに，彼らはさまざまな誤解や誤学習を抱えて生活をしており，筆者はこれを「自閉症の認知の穴」と呼んでいる．自閉症臨床における専門家の役割の一つは，彼らの抱えるこの認知の穴の修正にある．

Ⅲ．高機能広汎性発達障害の触法行為に関する研究

われわれは，日本自閉症協会が主任となる厚生科学研究において，連続して高機能広汎性発達障害の社会的不適応の問題に関する臨床研究を継続的に取り組んできた．今日，社会的な不適応の代表といえば，何といっても高機能広汎性発達障害者による犯罪であろう．実に，毎年のように，高機能広汎性発達障害と診断される青少年による重大事件がわが国において生じている事態をどう考えれば良いのであろう．このような社会問題化してしまった不幸な事件だけでなく，強制わいせつなどの事件が多発しており，矯正に関わる者の間で大きな問題となっているのである(杉山，2003)．

筆者が継続的なフォローアップを行っている高機能広汎性発達障害386名(3歳から48歳：平均年齢11.1±7.6歳)の中で，行為障害と診断される少年，あるいは触法行為を繰り返した青年は18名(4.7%)であった．ここでまず強調をしたいのは，専門医療機関を受診するに至った比較的重症の症例においても，95%以上は触法行為とは無関係であるという事実である．この一覧を表1に示した．下位分類としてはアスペルガー症候群が9名と半数を占めていたが，これは言語的な発達が良好で早期診断を受けにくいことが影響をしているものと考えられる．触法に至る要因を見るために，対照群の選別を行った．非触法群と触法群の数に大きな差があるため，フォローアップ症例の中から，同年齢同姓で，できるだけ知能指数が近い者を同数選んだ．候補となる者が複数存在した場合には，ランダム抽出を行った．こうして対照群との比較をしてみると，知能指数に関しては当然ながら有意差

表2　治療とその後の転帰

#	性別	年齢	早期診断	治療手技	再犯	その後の適応
1	m	5	+	精神療法	−	良好
2	f	7	−	薬物療法・精神療法	−	改善
3	m	8	−	入院治療	−	良好
4	m	8	−	入院治療	+	改善
5	m	9	−	薬物療法・精神療法	−	良好
6	m	13	+	精神療法	−	改善
7	m	13	−	薬物療法・精神療法	−	改善
8	m	15	−	薬物療法・精神療法	+	不変
9	m	15	−	薬物療法・精神療法	+	改善
10	m	15	−	薬物療法・精神療法	−	改善
11	m	15	−	薬物療法・精神療法	+	改善
12	m	17	−	薬物療法・精神療法	−	良好
13	m	18	−	薬物療法・精神療法	+	不変
14	m	18	+	精神療法	−	良好
15	m	18	−	精神療法	−	不変
16	m	20	−	薬物療法・精神療法	+	不変
17	m	21	+	薬物療法・精神療法	−	不変
18	m	24	−	薬物療法・精神療法	+	不変

はなく，しかし，DSM-Ⅳの第5軸GAF得点によって現在の適応水準を見ると，触法群51.2±6.0と非常に不良なのに対し対照群74.2±8.0であり，統計学的な高い有意差($t = 7.8$, $p < 0.01$)が認められた．また小学校入学前に診断を受けていた者は触法群4名に対し対照群14名とこれも有意差(χ^2 ($f = 1$) $= 11.1$, $p < 0.1$)が示された．さらに，表1を見ると，6名に虐待といわざるを得ない生育歴の問題があり，3名に不登校，1名に緘黙の既往が認められた．また青年期に至ったほぼ全員が激しいいじめを学校教育の中で受けていた．

これらの要因をまとめると，抽出されるのは次の3点である．第1に，診断の遅れと治療の遅れである．第2に，第1の問題に直結する虐待，いじめなどの迫害体験の存在である．第3に，非常に不良な現在の適応状態である．言い換えれば，早期に診断が可能となるシステムを構築し，虐待やいじめなどの迫害体験から児童を守り，現在の適応を良好に保つことで，このグループの触法行為は予防が可能であることが示唆される．

治療を試みた結果を表2に示した．当然ではあるが，年齢が上がるにつれ全体的な改善も，再犯の予防も困難となる傾向が明確に示されている．一方，青年期に至って再犯がなかった者の半分は早期療育を受けており，触法行為を犯した後の再犯の予防という点からも，どうやら早期療育を受けていることがどうやら有効である．これらの事例は，治療期間は1年から3年程度の者が多く，まだ転帰を確定するには不十分であるが，それにしても，青年期に至って触法行為によって初めて治療を受けた群においては特に治療に難航している．

先にわが国において高機能広汎性発達障害の少年，青年による重大犯罪が続いて生じていることを述べた．国際医学雑誌に掲載されたAsperger障害による殺人の報告は3例に過ぎず(Mawson et al, 1985; Baron-Choen, 1988; Howlin, 1997)，毎年のように生じている現在のわが

国の状況はやはり異常である．この事実は，わが国においてこのグループへの医療的，教育的対応が立ち後れていることを何よりも象徴しているものと筆者には思える．

Ⅳ．母子とも高機能広汎性発達障の症例への親子平行治療

広汎性発達障害において，強い遺伝的負因が認められることは従来から知られていた．しかし男性が女性より圧倒的に多いということもあって，これまで強調をされてきたのは主として父親－息子というパターンであった．あいち小児保健医療総合センターは2002年5月に，心療系の病棟が開設され，さまざまな児童の入院治療が開始された．その中で，特に子ども虐待が絡み入院治療児を必要とするに至った高機能広汎性発達障害の症例において，母親－子どもという組み合わせが少なからず存在し，それらが例外なく難治例であることに気づいた．翻ってみれば，重大事件に至った高機能広汎性発達障害の事例において，しばしばこのような組み合わせが認められていた．

発達障障害の臨床家であれば誰しも，高機能広汎性発達障害児の治療，フォローアップに際して母親への対応に苦慮する事例があることは経験しているであろう．このような事例についてこれまでは，高機能広汎性発達障害を育てるうえでの困難によって二次的に生じた母親の側のストレス性の反応と周囲の無理解によって悪循環が生じたものと見なされてきた．しかしながら母親にも同質の社会性の問題が潜んでいることを想定し，それに沿った対応を組むことで著しい進展がみられることにわれわれは気づき，積極的に親の側の診断を行い，必要に応じて親のカルテを作成し，薬物治療を含む母子の平行治療を行うに至った．そうして平行治療を積極的に行ってみると，高機能広汎性発達障害の母子例は稀ではなく，母親自身の発達障害的な要因を考慮することがむしろ有効な治療に繋がる鍵となっていた．

このようにして平行治療を行った母子は25例に及ぶ．あいち小児センター心療科は高機能広汎性発達障害治療センターとして機能しており，年間新患は約300名に及ぶ．高機能広汎性発達障害の母数は約1,000名であり，その2.5％ということになる．母親の診断は，いずれも当科においてなされたものであり，子どもの問題で受診した際に，母親自身の問題が明らかになったものである．診断の時期は受診当初のものが16例で，そのうち6例は子どもの診断についての説明を聞き，母親自らが自己の発達障害の存在に気づいた例である．残りの10例は当初から独特の認知パターン，対人関係のあり方が顕著で，発達障害という視点でみれば診断は容易な症例であった．また9症例は経過中に診断が可能となったもので，治療経過の中で母親自身がパニックを起こしたものや，母親のこだわりの強さ，独特の認知のあり方から，治療の過程で徐々に問題の存在が明らかになった症例が見られた．母親自身の障害についての告知を行った者は16症例，行わなかった者が9症例であった．障害告知を行った16症例中，6例は子どもの診断の過程で母親が自らの障害に気づいた例であり，自身の対人関係のあり方や社会的な能力に対して不全感を持っていたこともあり，受容は良好であった．治療経過中に診察場面で母親自身がパニックになる，あるいは広汎性発達障害の認知的特徴が明らかになるなどの，契機によってそれをきっかけとして母親に告知をしたもので，最終的には受容は良好であった．

母親に精神科における治療歴がある者は18症例であった．最も多い診断はうつ病の15例で，次いで境界性人格障害(BPD) 5例，パニック障