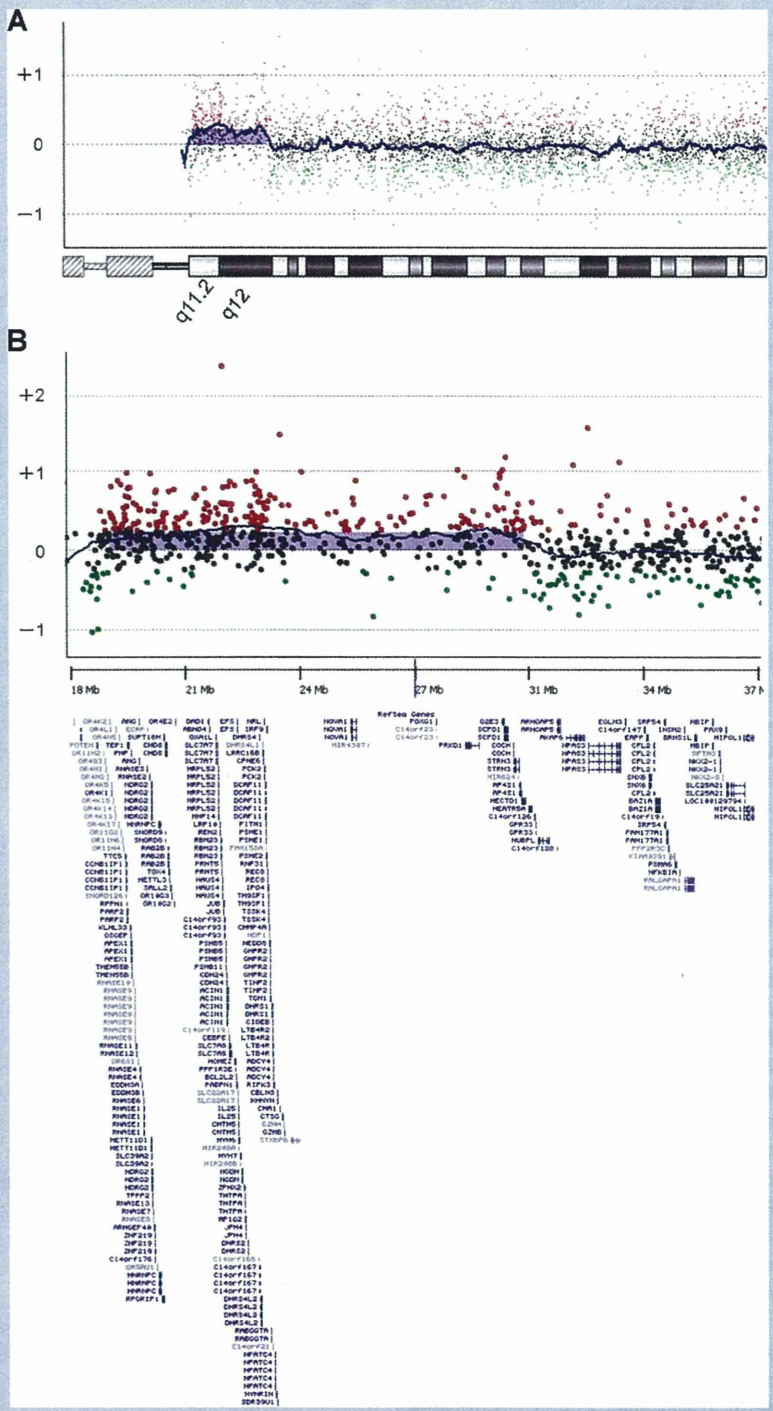

FIG. 2. Result of aCGH analysis for chromosome 14. A: Chromosome view indicating a genomic copy number gain of 14q11.2q12. The mean log2 ratio of this aberration region is 0.24, which indicates mosaicism of this marker chromosome. B: Aberration region expanded in gene view. The locations of the RefSeq Genes from the UCSC genome browser are shown under the gene view. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833].

SKY FISH analysis showed that the marker chromosome was derived from chromosome 14. Suspecting a relationship between the presence of marker chromosome 14 and upd(14)mat, we performed a DNA methylation test at *MEG3* in 14q32.2 [Hosoki et al., 2009], resulting in the abnormal hypomethylation of this gene (Fig. 1B). To confirm the origin of chromosome 14, microsatellite analysis using polymorphic markers on chromosome 14 was performed using ABI PRISM Linkage Mapping Set v2.5 (Applied Biosystems, Foster City, CA). Microsatellite polymorphism analysis indicated that both alleles of chromosome 14 were derived from the patient's mother and marker chromosome 14 was from her father (Fig. 1C). Fragment analysis at D14S275 showed a small peak of paternal inheritance, indicating the mosaic status of the marker of paternal origin (Fig. 1D). To further define the region of marker chromosome 14, microarray-based comparative genomic hybridization (aCGH) analysis performed using a 105K microarray kit (Agilent Technologies, Santa Clara, CA). The gain of genomic copy numbers was detected at 14q11.2-q12 indicating the molecular karyotype as arr14q11.2q12(19,761,035–30,941,609) × 1–1.5 (Fig. 2A,B). *FOXG1* was located in this region. Both parents had normal karyotypes.

DISCUSSION

The present patient showed intrauterine growth retardation, feeding difficulty during the neonatal period, mild hypotonia, and postnatal growth retardation. These findings fit well with those of upd(14)mat. Chromosomal analysis revealed mosaicism of 47,XX, + mar(14)/46,XX. From the association of the clinical findings of this patient and the presence of small SMC 14, we suspected that her clinical symptoms were related to upd(14)mat and performed a DNA methylation test at *MEG3* in 14q32.2 and microsatellite polymorphism analysis. We successfully confirmed that her condition was upd(14)mat. Upd(14)mat is manifested in clinical features overlapping the Prader-Willi phenotype, particularly during infancy. Therefore, this syndrome is considered to be underestimated. Hosoki et al. [2009] recommended performing the *MEG3* methylation test for all undiagnosed infants with hypotonia.

Infantile spasms or seizures are uncommon complications of upd(14)mat. We postulated that an increased dosage of some genes in extra SMC could be responsible for West syndrome. To identify the affecting gene, we performed aCGH analysis. The analysis showed an increased dosage of 14q11.2-q12. These regions contain 124 RefSeq genes including *FOXG1*. Recently, *FOXG1* on 14q12 was reported to be a dose-sensitive gene, and duplication of this gene could cause severe epilepsy and developmental retardation [Yeung et al., 2009; Brunetti-Pierri et al., 2011]. Several patients with *FOXG1* haploinsufficiency have been associated with a Rett-like syndrome and epilepsy [Shoichet et al., 2005; Jacob et al., 2009]. Deletion of this gene could cause seizures, but not infantile spasms. On the other hand, duplication of this gene is reported to cause infantile spasms or seizures during infancy. Yeung et al. [2009] first reported a patient with 4.45 Mb microduplication in 14q12. This patient showed infantile spasms at 6 months. In addition, Brunetti-Pierri et al. [2011] studied six patients with duplication of the 14q12 region. In their series, the size of the duplication varied between ~3 and 14.5 Mb with the patient carrying the largest duplication showing a 14.5 Mb duplication in 14q11.2-q13.1. The shortest region of overlap for the duplicated regions in the six patients contained only three genes, including *FOXG1*. Three of the six patients showed infantile spasms. The authors concluded that *FOXG1* represented the most interesting candidate for explaining the abnormal neurodevelopment phenotypes [Brunetti-Pierri et al., 2011]. The present patient also showed infantile spasms. However, her seizures are not refractory and are well controlled by anti-epileptic drugs and ACTH therapy. Her developmental delay is also not so severe. The 14q11.2-q12 region involved in our patient was almost equal in size to the largest duplication in Brunetti-Pierri's series. *FOXG1* is a dose-sensitive gene, and the results of our patient strongly suggested that an increased dosage of a small amount of this gene might lead to a milder West syndrome and milder intellectual disability.

The West syndrome has a heterogeneous etiology. Recent molecular biological approaches have identified several causative genes. To date, *ARX*, *CDKL5*, *STXBP1*, and *SPTAN1* have been reported as being associated with West syndrome [Kato et al., 2006; Otsuka et al., 2010; Saitsu et al., 2010]. These previous reports state that haploinsufficiency or small mutations of these genes are related to their phenotypes. In addition, duplication of *FOXG1* was recently reported to cause severe epilepsy and developmental delay, including infantile spasms. Epilepsies associated with increasing gene dosage are rare [Ramocki et al., 2010; Brunetti-Pierri et al., 2011]. The results of the study of our patient will provide further evidence that not only duplication but also a small increasing dose of *FOXG1* could cause infantile spasms or seizure during early infancy. Of course, in our patient, the contribution of other genes in 14q11.2-q12 could not be excluded.

The first patient with upd(14)mat had a Robertsonian translocation (13;14) [Temple et al., 1991]. This syndrome was also reported in carriers of Robertsonian translocation involving chromosome 14 and in patients with normal karyotypes [Mitter et al., 2006]. Other chromosomal rearrangements frequently associated with upd are small SMCs [Starke et al., 2003; Liehr et al., 2004]. Mitter et al. [2006] reported 10 patients with upd(14)mat, two of whom had SMC 14. In our patient, we were also able to determine that the marker chromosome was derived from chromosome 14 by SKY FISH, microsatellite polymorphism analysis, and aCGH analysis. The coexisting of small marker chromosome 14 and upd(14)mat is likely to be originated in functional trisomic rescue or gamete complementation in the formation of the chromosome aberration in our patient [Kotzot, 2002].

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Original article

A familial case of LEOPARD syndrome associated with a high-functioning autism spectrum disorder

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Abstract

A connection between LEOPARD syndrome (a rare autosomal dominant disorder) and autism spectrum disorders (ASDs) may exist. Of four related individuals (father and three sons) with LEOPARD syndrome, all patients exhibited clinical symptoms consistent with ASDs. Findings included aggressive behavior and impairment of social interaction, communication, and range of interests. The coexistence of LEOPARD syndrome and ASDs in the related individuals may be an incidental familial event or indicative that ASDs is associated with LEOPARD syndrome. There have been no other independent reports of the association of LEOPARD syndrome and ASDs. Molecular and biochemical mechanisms that may suggest a connection between LEOPARD syndrome and ASDs are discussed.

Keywords: LEOPARD syndrome, Noonan syndrome; Autism spectrum disorders (ASDs); RAS/MAPK signal transduction pathway

1. Introduction

LEOPARD syndrome (OMIM#151100) is a rare autosomal dominant disorder characterized by **L**entigines, **E**lectrocardiogram abnormalities, **O**cular hypertelorism, **P**ulmonic valvular stenosis, **A**bnormalities of genitalia, **R**etardation of growth, and **D**eafness. This syndrome is caused by germline missense mutations in the *PTPN11* gene that encodes Src homology 2 domain-containing tyrosine phosphatase 2 (Shp2): non-receptor protein-tyrosine phosphatase comprising two N-terminal SH2 domains, a catalytic domain, and a C terminus with tyrosylphosphorylation sites and a proline-rich stretch. The mutations induce catalytically impaired Shp2 by a "dominant negative effect" [1–2].

In the more common Noonan syndrome, approximately 50% of patients have *PTPN11* mutations scattered over the entire Shp2, including the catalytic domain. The mutations resulting in the Noonan phenotype are the "gain-of-function" mutations, and they exhibit substantially increased catalytic ability. Although LEOPARD syndrome and Noonan syndrome are caused by *PTPN11* mutations resulting in opposite effects, they share many common clinical features, including physical dysmorphic findings and intellectual disability [1].

The term "autism spectrum disorders (ASDs)" was first used by Lorna Wing [3] and then widely used as a category comprised of autistic disorder, Asperger's

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disorder, and other related conditions [4]. These conditions are very common neurobehavioral disorders that are characterized by impairments in three behavioral domains, including social interaction, language/communication/imaginative play, and a range of interests and activities [3–5].

At least ten genes have been reported to be associated with ASDs [6]. Except for Rett syndrome, the other pervasive developmental disorder (PDD) subtypes including autistic disorder, Asperger's disorder, disintegrative disorder, and PDD Not Otherwise Specified (PDDNOS) are not tightly linked to any particular gene mutations. Several common genetic syndromes are known to be associated with ASDs. Autism is frequent in patients with tuberous sclerosis (TSC) [7], with neurofibromatosis type 1 [8,9] and with Fragile X syndrome [10]. Studies of psychological profiles of adults with Noonan syndrome did not suggest a specific behavioral phenotype, but difficulties with social competence and emotional perceptions were noted [11]. A case of Noonan syndrome who was also diagnosed with autism was reported [12]. The present study of neuropsychiatric evaluation in a familial case of LEOPARD syndrome indicates all patients satisfied the criteria of ASDs. An association of LEOPARD syndrome and ASDs has not been reported previously. The familial case presented in this report may suggest such an association.

2. Patients and methods

After obtaining written informed consent, fifteen coding exons in *PTPN11* were sequenced in each patient following the methods described somewhere else [13].

Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR) [5] and The high-functioning Autism Spectrum Screening Questionnaire (ASSQ) [14] were used in neuropsychiatric evaluation of the subjects.

Patient 1 is a 20-year-old male who was born as the second child to a non-consanguineous Japanese couple. His early developmental milestones were reportedly unremarkable. He was clinically diagnosed with LEOPARD syndrome at age 7 years based on findings that included lentigines, multiple café-au-lait spots, electrocardiogram (ECG) abnormalities, ventricular septal defect, ocular hypertelorism, short stature, and unilateral renal hypoplasia. *PTPN11* mutation analysis revealed a heterozygous mutation of 1403C > T (T468 M). The patient was diagnosed as having Asperger's disorder based on ASSQ and DSM-IV-TR, at age 12 years. His intelligence quotient (IQ) by the Wechsler Intelligence Scale for Children-third edition (WISC-III) was 85 (verbal: 77, performance 98). His ASSQ score by mother's rating was 41. He met the DSM-IV-TR diagnostic criteria of Asperger's disorder with all subcategories in the category of Qualitative impairment in social interaction (Category 1), three subcategories (1,2, and 4) in the category of Restricted repetitive and stereotyped patterns of behavior, interests and activities (Category 2), and the rest of the four categories (Table 1).

Patient 2 is a 15-year-old younger brother of Patient 1. His early infantile developmental milestones were unremarkable. He was diagnosed with growth retardation at age 2½ years. At age 12 years his clinical findings of a few café-au-lait spots, ocular hypertelorism, and undescended testes led us to obtain *PTPN11* mutation analysis, which showed the same heterozygous mutation of 1403C > T. At age 9 years, a diagnosis of Asperger's disorder was made based on ASSQ and DSM-IV-TR. His full-scale IQ by WISC-III at age 9 years was 99 (verbal 104, performance 92). His ASSQ score by parental rating was 32 at age 15 years. He also met the Asperger's disorder diagnostic criteria with all subcategories of Category 1, three of Category 2 (1, 2, and 4), and the rest of the categories (Table 1).

Patient 3 is the 22-year-old eldest brother of Patients 1 and 2. His developmental milestones were normal, although his ritualistic behavior and difficulties in relating to peers were noted in his childhood. He had a surgical repair of bilateral undescended testes and inguinal hernia. He was diagnosed with Wolff-Parkinson-White syndrome at age nine years. He has ocular hypertelorism and short stature. The same *PTPN11* heterozygous mutation found in the two younger siblings was identified in this patient. He attends college, and was diagnosed as having PDDNOS, because he also had impaired development of reciprocal social interaction associated with communication skills, repetitive routine, and ritualistic behavior. His ASSQ score was 7 at age 22 years (Table 1).

Patient 4 is a 55-year-old male who is the father of the siblings. He has prominent lentigines, bilateral mild hearing loss, cardiac anomalies, ECG abnormalities, short stature, and apparent ocular hypertelorism. His early developmental milestones are not well known. He has been noted to have obsession with a specific topic, repetitive routine and rituals, and clumsy movements. At age 50 years, his social skills and aggressive behavior were noted to be deteriorating, and consequently he was suspected of having Asperger's disorder based on DSM-IV-TR. He met the diagnostic criteria of Asperger's disorder with Category 1 (1 and 3), Category 2 (1 and 2), and the rest of the four categories. His ASSQ score was 20 at age 55 years by his wife's evaluation. He has the same heterozygous *PTPN11* mutation (Table 1).

3. Discussion

The presented familial case of LEOPARD syndrome included individuals (patients 1, 2, and 4) diagnosed with or suspected of having Asperger's disorder, and

Table 1
Summary of clinical findings and *PTPN11* mutation.

	Pt. 1 Male	Pt. 2 Male	Pt. 3 Male	Pt. 4Male
Age	20 y	15 y	22 y	55 y
Physical findings				
Skin: café-au-lait spots	multiple	a few	a few	a few
Lentigines	+++	+++	–	+++
Cardiac defects	VSD	No	No	No
EKG abnormalities	+	No	WPW	No
Ocular hypertelorism	+	+	+	+
Pulmonary stenosis	No	No	No	No
Abnormal genitalia	No	Und. Testes*	Und. Testes*	No
Renal anomalies	R-hypoplasia	No	No	No
Retardation of growth	Yes	+	+	No
Deafness	No	No	No	Yes
Miscellaneous:				
Rocker bottom feet	Yes	Yes	Yes	No
Macrocephaly	Yes	Yes	Yes	No
PTPN11 mutation	T468 M	T468 M	T468 M	T468 M
Neuropsychological				
Diagnosis	AD**	AD**	PDDNOS***	AD**
ASSQ score[1] (age)	41 (12 y)	32 (15 y)	7 (22 y)	20 (50 y)
WISC-III[2] (age)	85 (12 y)	99 (9 y)	n/a	n/a
-Verbal/performance	77/98	104/92	n/a	n/a

* Und. Testes, undescended testes.
** AD, Asperger's disorder.
*** PDDNOS, Pervasive developmental disorder not otherwise specified.
[1] ASSQ score, Autism Spectrum Screening Questionnaire Score. The cutoff score of 3 predicts 91% of the true positive rate of Autistic spectrum disorders.
[2] WISC-III, Wechsler Intelligence Scale for Children-third edition.

patient 3 was diagnosed as having PDDNOS, which may lead to the diagnosis of ASD. ASDs were first introduced by Lorna Wing, who suggested that Asperger's disorder is a type of ASD and described in detail its various manifestations in speech, nonverbal communication, social interaction, motor coordination, motor clumsiness, and idiosyncratic interests [3]. Patient 3 did not have enough clinical symptoms to meet the diagnostic criteria for Asperger's disorder; however, he had some symptoms suggestive of ASD in his childhood that led to a diagnosis of PDDNOS.

The ASSQ is a 27-item checklist for completion by lay informants when assessing characteristic symptoms of Asperger's disorder and high-functioning autism in children and adolescents with normal intelligence or mild mental retardation. The ASSQ allows for rating on a 3-point scale (0, 1, or 2; 0 indicating normality, 1 some abnormality, and 2 definite abnormality). The range of possible scores is 0–54. The mean ASSQ parent scores in the Asperger's disorder validation sample were 25.1 (SD, 7.3) [14]. The cutoff score of 13 is 91% of the true positive rate of ASDs. The ASSQ score was established as a screening tool primarily for children between 6 and 17 years of age by parents and/or teachers. The delayed evaluation of patient 3 may account for the difference in diagnosis between this patient and his siblings.

ASDs are known to be associated with particular genetic disorders such as fragile X syndrome [10,15,16], tuberous sclerosis (TSC) [7], and neurofibromatosis type 1 [8,9]. Fifty percent of children with TSC have behavioral problems in the form of ASDs. Gene mutations in either *TSC1* or *TSC2* influence neural precursors, resulting in abnormal cell differentiation and dysregulated control of cell size. These cells migrate to the cortex to generate an abnormal collection of inappropriately positioned neurons, causing widespread cortical disorganization and structural abnormalities [7]. Mutations in *PTPN11* causing LEOPARD syndrome induce catalytically impaired Shp2. In situ hybridization detected Shp2 expression in the neural ectoderm and nervous system in mouse embryos, suggesting an involvement of Shp2 in neural development. Shp2 is a critical signaling molecule in the coordinated regulation of progenitor cell proliferation and neuronal/astroglial cell differentiation. The studies with mutant mouse strains with Shp2 selectively deleted in neural precursor cells showed a dramatic phenotype of growth retardation, early postnatal lethality, and multiple defects in proliferation and cell fate specification in neural stem/progenitor cells [17]. The product of the TSC2 gene tuberin is known to up-regulate the B-RAF/MEK/MAPK signal transduction pathway. B-RAF is required for neuronal differentiation, suggesting another possible link between B-RAF signaling and the clinical manifestations of TSC including ASDs [18]. Disturbed neuronal cell differentiation and development due to mutations in

the TSC genes and the *PTPN11* gene are likely to contribute to the development of ASDs in patients with these syndromes.

NF-I is well known to be associated with ASDs. The prevalence of autism in patients with NF-I was reported to be 4% [9]. The well-known function of the NF-I protein is to act as a RAS-GTPase-activating protein known to be involved in the regulation of the RAS-mitogen-activated protein kinase (MAPK) pathway. Mutations in the NF-I gene are thought to result in activation of the RAS/MAPK signal transduction pathway [2]. Clinical overlap between LEOPARD syndrome and NF-I is also well known [19].

Approximately 50% of patients with Noonan syndrome are due to missense *PTPN11* mutations [20]. *PTPN11* encodes SHP2, a protein tyrosine phosphatase, that is involved in the activation of the RAS/MAPK cascade [2]. Noonan syndrome is caused by "gain of function" *PTPN11* mutations [1,2], and the SHP2 mutants due to the *PTPN11* mutations causing Noonan syndrome cause prolonged activation of the RAS/MAPK pathway [2]. Schubbert et al. [21] reported that germline KRAS mutations cause Noonan syndrome through the hyperactive RAS/MAPK pathway.

Herault et al. [22] reported a positive association of the HRAS gene and autism. The psychological profiles of adults and children with Noonan syndrome have been studied, and deficiencies in social and emotional recognition and expression have been identified in adults, while low verbal IQ, clumsiness, and impairment of developmental coordination have been reported in children [23].

To date, there have been no reports to suggest an association of LEOPARD syndrome and ASDs. Our observations in this familial case may suggest at least some degree of association between LEOPARD syndrome and ASD phenotypes possibly through the RAS/MAPK signal transduction pathway. Further studies with more patients with LEOPARD syndrome are needed to establish the association and to investigate the genetic contributing factors causing ASDs, leading to the prevention and earlier detection of ASDs and better management of patients with these disorders.

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Original Article

Attitude to extended use and long-term storage of newborn screening blood spots in Japan

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Abstract ***Background***: Residual dried blood spots (DBS) remaining after routine newborn screening (NBS) tests are candidate specimens for extended uses such as quality assurance and the development of new technology. A trial of NBS using tandem mass-spectrometry was launched in 2004 in Japan. The aim of the present study was to analyze the attitudes of the public, patient families, and medical professionals toward the extended use and long-term storage of residual DBS, and to construct a standardized informational brochure.

Methods: A questionnaire was sent to randomly selected members of the public, members of the Japanese Phenylketonuria (PKU) Association, medical staff of a general hospital, staff of a children's hospital, obstetricians and gynecologists, pediatricians and NBS personnel. Associated responses, which were given in a free comment format, were analyzed by text mining.

Results: The awareness ratio of NBS was low in the public (26.6%), but despite this, when a brief explanatory note on NBS was provided, 71.7% of them recognized the necessity of NBS. They were less positive than medical professionals and PKU patient families regarding the extended use of DBS for forensic investigation, for the study of health problems, or long-term storage of residual DBS, regardless of whether these factors affected them personally or not. Among the medical professionals, obstetricians and pediatricians exhibited a higher ratio of negative responses toward the extended use and long-term storage of DBS than others.

Conclusion: The general public is more conservative than PKU patients and their families or medical professionals about the extended use or long-term storage of residual DBS. Presentation to the public, particularly to couples of childbearing age, of appropriate explanatory information on NBS itself, or the extended use or long-term storage of residual DBS, is recommended.

Key words biobank, dried blood spots, long-term storage, newborn screening, text mining.

The newborn screening (NBS) program commenced in 1977 in Japan.[1] Dried blood spots (DBS) remaining after regular NBS tests are the best candidates for use not only as quality control specimens for NBS and the development of new screening technology, but also for scientific research and forensic investigation. Systematic, large-scale collection and storage of biological specimens in facilities generally termed "Biobanks", is now in operation in some countries, following the first implementation in Iceland and the UK in 1998.[2] Residual DBS are a potential source for biobanking owing to the high coverage rate among populations.[3]

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The rationale for extended use and long-term storage of residual DBS is currently under debate. It is controlled by regulations or statutes specific to the NBS program, or as part of governmental policy, in certain European countries,[4,5] Australia, New Zealand,[6] and in some States of the USA.[7,8] Guidelines for the handling of DBS, including the general rules for extended use, were presented in Japan in 1998.[9] These guidelines require that prior parental consent be given for the extended use of DBS for each specific purpose. A trial of screening on tandem mass-spectrometry was launched in 2004 in Japan. With implementation of this pilot study, substantiation of the requirement outlined in the guidelines has become mandatory. The rationale for long-term storage of residual DBS is also another issue to be discussed. The aim of the present research was to analyze the public's comments on the extended use and long-term storage of residual DBS and to offer proposals for socially acceptable policy thereof in Japan.

Methods

The questionnaire consisted of brief explanatory notes and seven questions (Table 1). This questionnaire was based on a poll questionnaire developed in the UK.[5] The questionnaire was sent to the following: (i) members of the public; (ii) members of the Japanese Phenylketonuria (PKU) Association (JPA); (iii) medical staff (excluding clerical employees) of Kurume University Hospital (KUH); (iv) personnel (including clerical employees) of the National Center for Child Health and Development (NCCHD); (v) members of the Japan Pediatric Society (JPS); (vi) members of the Japanese Society of Obstetrics and Gynecology (JSOG); and (vii) members of the Japanese Society for Mass-screening (JSM). Selection of addressees was randomized using SPSS version 14 (SPSS Japan, Tokyo, Japan). The questionnaire was sent to members of the public and members of the JPS (parents of patients) and JSOG via email, and to the other groups via regular mail. The period of survey was 1 April 2006–30 November 2007.

Analysis of free comments by text mining

Free comments were analyzed by text mining using the True Teller program (Nomura Research Institute, Tokyo, Japan), as follows.

Free comments written in Japanese were first subjected to word segmentation. Nouns were then extracted and ranked in the order of their usage frequency in the texts. Clusters of semantically close words were constructed first from the top 10 nouns, and then groups of words with lower frequency were added to the parent cluster. Semantic similarity was determined at each step by dependency parsing and by directly examining the original text.

Results

Question 1

The demographic background of the participants is summarized in Table 2. The recovery rate ranged from 26.9% (JSOG members) to 60.4% (JSM members). There was a predominance of female responders from the JPA, and a predominance of male

Table 1 Content of questionnaire

QUESTIONNAIRE ON THE USE OF BLOOD SPOTS REMAINING AFTER NEWBORN SCREENING TESTS

Question 1. Please provide your gender, birth year and month, and occupation (please specify).
Question 2a. Did you know that newborn screening tests are conducted in our country?
1. Yes, I knew.
2. No, I have never heard of it /I only came to know of it through this questionnaire.
Question 2b. If you answered "1. Yes, I knew." to question 2a:
How did you know of newborn screening tests? Please mark every option that applies.
1. Because I have undergone the test
2. Because my child(ren) has (have) undergone the test
3. Because I am engaged in medical service
4. Because I am a member of a newborn screening test-associated organization.
5. Because I am a member of a society of patients' or their families.
6. Other (please specify)
Question 3. Do you think newborn screening tests is necessary?
1. Yes 2. No 3. Unsure
Question 4. How do you feel about the use of residual blood spots by screening laboratories or research laboratories in order to maintain and improve newborn screening technology?
1. Agree 2. Neither agree nor disagree 3. Disagree 4. Unsure
Please give your free comments.
Question 5. Stored residual blood spots can be used to identify victims of a fire or natural disaster with the aid of DNA analysis. How do you feel about residual blood spots being used for such purposes?
1. Agree 2. Neither agree nor disagree 3. Disagree 4. Unsure
Please give your free comments.
Question 6a. How do you feel about your own or your family's residual blood spots being used to study health problems or medical research that directly involve you or your family?
1. Agree 2. Neither agree nor disagree 3. Disagree 4. Unsure
Please give your free comments.
Question 6b. How do you feel about your own or your family's residual blood spots being used to study health problems or medical research that do not directly involve you or your family?
1. Agree 2. Neither agree nor disagree 3. Disagree 4. Unsure
Please give your free comments.
Question 7a. How do you feel about the storage of residual blood spots for a long period (several decades), if they may be used in the future for the benefit of you or your family?
1. Agree 2. Neither agree nor disagree 3. Disagree 4. Unsure
Please give your free comments.
Question 7b. How do you feel about the storage of residual blood spots for a long period (several decades), if they may be used in the future for the benefit of society in general?
1. Agree 2. Neither agree nor disagree 3. Disagree 4. Unsure
Please give your free comments.
End of Questionnaire

Occupation was excluded from these questions when they were addressed by the members of the public and Japanese Phenylketonuria Association.

Table 2 Participant demographic data

	Members of the public	JPA members	KUH medical staff	NCCHD personnel	JPS members	JSOG members	JSM members
No. addressees	2127	351	1177	751	1012	992	396
No. respondents	1030	182	651	355	390	267	263
Recovery rate (%)	48.8	36.2	55.3	47.3	38.5	26.9	66.4
Median age (years) (range)	41.5 (21–61)	43.0 (19–80)	36.0 (19–62)	35.0 (22–76)	49.0 (25–81)	49.0 (23–80)	48.5 (26–93)
Male/Female ratio (%)	50.7/49.2	20.9/79.1	37.0/62.7	33.0/67.0	63.0/37.0	87.1/12.9	58.2/41.8

JPA, Japan Phenylketonuria Association; JPS, Japan Pediatric Society; JSM, Japanese Society for Mass-screening; JSOG, Japan Society of Obstetrics and Gynecology; KUH, Kurume University Hospital; NCCHD, National Center for Child Health and Development.

responders from the JSOG. Medical personnel respondents consisted of physicians (n = 1106), nurses (n = 324), laboratory technicians (n = 36), pharmacists (n = 15), radiologists (n = 12), midwives (n = 3), dieticians (n = 3) and others (n = 24). Occupation was not requested for members of the public or the JPA (Table 1).

The responses to questions in each group are given in Table 3. Data on differences between male and female responses are not presented unless described specifically. In Table 3, data are presented for "yes" or "no," and "agree" or "disagree" responses alone. The remaining answers were classified as "ambiguous" collectively, and are not given in the table.

Question 2

The ratio of members of the public who answered that they knew that NBS was conducted (26.6%) was lower than that of any of the other groups of subjects (question 2a). This ratio was lower in male (13.2%) than in female (40.4%) members of the public.

The leading reasons whereby members of the public had become aware that NBS was conducted (question 2b) were as follows: "because I am engaged in medical service" (56.2%), followed by "because my child(ren) underwent the test" (28.8%). Among 507 female members of the public who responded, 324 (63.9%) answered that they had ever given birth. Of the 324, 183 (56.5%) replied that they had known of NBS. Among these 183, 164 (89.6%) knew of NBS because their babies had undergone the test.

Question 3

The ratio of those who answered that NBS was necessary was 71.7% in members of the public, while it was ≥85.7% in the other groups. The ratio of respondents who were opposed to NBS was <0.8% in every group.

Question 4

The ratio of respondents who responded positively towards the use of residual DBS to maintain and improve NBS technology was lower in members of the public and medical staff of a general hospital (KUH) than in the groups that were involved in conducting NBS or had benefited from it (patients and their families).

Question 5

The ratio of positive responses toward the use of residual DBS for forensic purpose in members of the public was closer to the ratios in the other groups, when compared to other questions.

Question 6

The ratio of positive responses toward the use of residual DBS to study health problems or medical problems that directly involved themselves or their family (Question 6a) varied from 63.1% (members of the public) to 81.4% (JSM members). In the event that the purpose of DBS use was unrelated to themselves or their family, the ratio was lower in every group, except JSOG members, who gave identical ratios of affirmative answers for both questions (Question 6b).

Question 7

The ratio of responses that were positive towards storage of residual DBS in the event that they might be used for themselves or their family was relatively uniform among all the groups, except members of the public, who had a lower ratio (Question 7a). This tendency was reproduced in responses regarding the event that residual DBS might be used for the benefit of society in general, although the positive response ratio was generally lower in every group, with the exception of JMS members.

In every group the ratio of negative responses was generally higher in questions 6b and 7b than in all the other questions.

Text mining analysis

Three concepts were generated from the clusters of words that were constructed as described in the previous section. The clustered words (shown in parentheses) and concepts deduced from them (shown in brackets following an arrowhead) were as follows: (i) (privacy, administration, information, identity, etc.) > [personal data]; (ii) (agreement, approval, written consent etc.) > [availability of consent]; and (iii) (progress, research, medical science, therapy etc.) > [progress in medicine]. The frequency of usage of words that are relevant to each concept is illustrated in Figure 1.

In brief, words related to the "personal data" concept appeared at approximately the same frequency in each group (Fig. 1a). The usage frequency of words related to the "availability of consent" concept, however, was lower in members of the public and JPA members than in the other groups (Fig. 1b). In contrast, words related to the "progress in medicine" concept were more frequently used in members of the public and JPA members than in the other groups (Fig. 1c).

Discussion

The ratio of members of the public who were aware that NBS was being conducted in Japan was <27% (Question 2a). Nearly half

Table 3 Responses

Groups		Members of the public	JPA members	KUH medical staff	NCCHD personnel	JPS members	JSOG members	JSM members	Total
No. respondents		1030	182	651	355	390	267	263	3138
Questions[†]	Answers (%)[‡]								
Question 2a (Awareness of NBS)	Yes	26.6	93.4	84.3	82.0	99.0	99.3	99.2	69.9
	No	73.4	6.0	14.7	17.7	0.8	0.4	0.8	29.7
Question 3 (Necessity of NBS)	Yes	71.7	99.5	85.7	88.4	97.9	97.4	98.9	85.8
	No	0.8	0	0.8	0.6	0.3	0.4	0.4	0.6
Question 4 (Extended use for NBS itself)	Agree	54.4	81.3	66.8	73.2	86.2	87.4	90.1	69.8
	Disagree	2.8	0.5	1.5	2.0	2.6	5.6	3.8	2.6
Question 5 (Forensic use)	Agree	74.0	78.6	82.3	80.2	85.9	87.9	83.3	79.5
	Disagree	1.8	1.1	1.8	2.8	2.3	5.2	2.3	2.3
Question 6a (Use for own health problems)	Agree	63.1	78.6	76.5	76.3	80.8	72.3	81.4	73.0
	Disagree	3.6	2.2	2.9	2.5	4.1	7.9	0.4	3.8
Question 6b (Use for health problems of others)	Agree	53.4	69.8	70.5	73.2	74.6	72.3	79.1	66.5
	Disagree	8.2	4.4	3.8	3.9	7.4	10.5	0.4	6.5
Question 7a (Long-term storage for own benefit)	Agree	67.8	79.7	79.1	81.7	80.8	80.1	79.8	76.1
	Disagree	3.6	2.7	2.9	1.4	4.4	6.4	6.1	3.7
Question 7b (Long-term storage for benefit of others)	Agree	59.9	70.9	76.0	75.5	77.5	77.2	80.6	71
	Disagree	4.5	2.7	3.2	2.5	5.0	8.2	5.3	4.4

[†]Synopses of questions are shown in brackets. Please refer to Table 1 for full text of questions. [‡]Answers "yes" or "no" and "agree" or "disagree" alone are indicated. The remaining answers were classified as "ambiguous" collectively, and are not given in the Table.

JPA, Japan Phenylketonuria Association; JPS, Japan Pediatric Society; JSM, Japanese Society for Mass-screening; JSOG, Japan Society of Obstetrics and Gynecology; KUH, Kurume University Hospital; NBS, newborn screening; NCCHD, National Center for Child Health and Development.

of the female members of the public who had ever given birth replied that they did not know that NBS was being implemented. This result would imply that briefing on NBS was not provided in enough detail to be remembered by mothers, or was not given at all. This low awareness ratio appears to be closely linked with the lower usage frequency of words related to the "availability of consent" concept in members of the public than in other groups (Fig. 1b).

Approximately 70% of members of the public agreed with the significance and necessity of NBS, when some information on NBS was provided (Question 3). In addition, members of the public used words related to the "progress in medicine" concept most frequently among all the seven groups, when their questionnaire responses were analyzed by text mining (Fig. 1c). All these results indicate that the public is potentially interested in NBS and anticipates benefit from it. Therefore, intensive publicity regarding the purpose and benefit of NBS among the general public, and in particular couples who intend to have children, is expected to be useful.

Approximately 66.8–90.1% of respondents in each group, excluding members of the public, had positive responses toward the extended use of residual DBS to evaluate and improve current NBS technology and to develop new technologies (Question 4). The extended use of DBS, and the duration of storage of residual DBS and associated personal data, are governed by legislation or by recommendations proposed by academic societies in Denmark,[4] UK,[5] Australia, New Zealand,[6] France,[10] The Netherlands[10] and 16 States in the USA.[8] In Japan, the general guidelines that control the extended use of DBS were set out in 1998.[9] For routine screening tests, oral or written information is provided to parents in various ways in accordance with this guideline in individual areas. Notification on the storage policy, however, for residual DBS is generally not included in such information. Explicit documentation and publicity regarding storage policy is essential to ensure transparency about the outcomes of DBS and to avoid public confusion about their storage, as was encountered in the Enschede disaster.[10] The articulation of policies regarding storage of human materials and data is strongly recommended in the operation of human biobanks and genetic databases in general, according to a recent OECD recommendation draft.[11] Residual DBS are systematically registered and stored nationwide and used for research in Denmark[4] and the UK.[5] Purposes of storage are specified in these countries. They are, in brief, later retesting, quality assurance, improvement and development of new NBS programs, and research. Residual DBS are used for research purposes and the system is termed "biobank" in these countries.[4,5] In laboratories in Japan, the purposes, duration and conditions of storage of residual DBS are diverse.[12] A common purpose of storage is to provide for possible retesting. It is crucial to make the purpose of storage of residual DBS clear in order to first determine the duration and conditions of storage, because the stability of analytes on DBS is largely unknown.[8] All of these results indicate that presentation to the public, particularly couples of child-bearing age, of appropriate explanatory information on the extended use and long-term storage of residual DBS is necessary. Presentation of information on the NBS

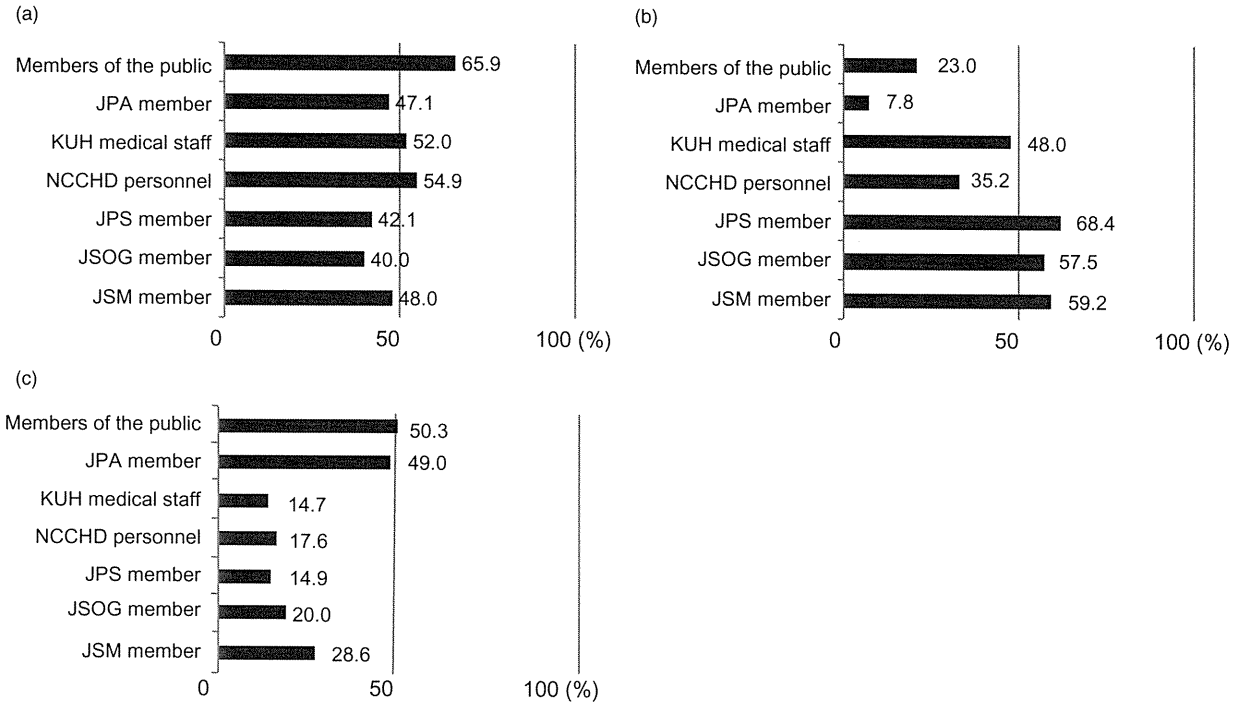

Fig. 1 Usage frequency of words (%) related to the concepts (a) personal data; (b) availability of consent; and (c) progress in medicine. The usage frequency number is defined as the sum of every word that is related to each concept divided by number of comments made for every question. JPA, Japanese Phenylketonuria Association; JPS, Japan Pediatric Society; JSM, Japanese Society for Mass-screening; JSOG, Japanese Society of Obstetrics and Gynecology; KUH, Kurume University Hospital; NCCHD, National Center for Child Health and Development.

program to couples during pregnancy may allow better understanding of the program.[13]

Attitudes toward the use of DBS for forensic purposes were similar among all the groups (Question 5). It remains open whether or not consent for extended use for such a purpose should be included in routine explanatory brochures. Legislation or regulation regarding whether or not a screening laboratory can release stored DBS upon judicial order should also be discussed.

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Original article

Epilepsy in autism: A pathophysiological consideration

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Abstract

Eighty cases of idiopathic autism with epilepsy and 97 cases without epilepsy were studied to evaluate the pathophysiology of epilepsy in autism. The initial visit to this clinic ranged 8 months–30 years 3 months of age, and the current ages are 5 years 8 months–42 years 3 months, 60% reaching to over 30 years of age. The average follow up duration is 22.2 years ± 9.4 years. The ages of onset of epilepsy were from 7 months to 30 years of age, with the two peaks at 3.2 years and 16.7 years. EEG central focus appeared earlier than frontal focus. Abnormality of locomotion and atonic NREM were observed more frequently in epileptic group. These suggest the neuronal system related to abnormality of locomotion and atonic NREM, which are the hypofunction of the brainstem monoaminergic system, is the pathomechanism underling the epilepsy in autism. By showing the abnormal sleep–wake rhythm and locomotion being the very initial symptoms in autism, we had shown the hypofunction of the brainstem monoaminergic system is the initial pathomechanism of autism. Thus, epilepsy in autism is not the secondary manifestation, but one of the pathognomonic symptoms of autism. The brainstem monoaminergic system project to the wider cortical area, and the initial monoaminergic hypofunction may lead to the central focus which appears earlier. The failure of the monoaminergic (serotonergic) system causes dysfunction of the pedunculo-pontine nucleus (PPN) and induces dysfunction of the dopamine (DA) system, and with development of the DA receptor supersensitivity consequently disinhibits the thalamo-frontal pathway, which after maturation of this pathway in teens cause the epileptogenesis in the frontal cortex.

Keywords: Autism; Epilepsy; Brainstem monoamine; Serotonergic neuron; Noradrenergic neuron; Dopaminergic neuron; Frontal and central focus; Locomotion; Atonic non-REM

1. Introduction

Autism is defined as qualitative impairment in reciprocal social interaction, in verbal and nonverbal communication, and in imaginative activity, markedly restricted repertoire of activities and interests, and onset during in infancy and childhood [1].

However, autism starts in early infancy, and the early features include the abnormalities of sleep–wake (S–W) rhythm, poor in activation of the antigravity muscles, and failure in locomotion [2]. The characteristic symptoms and signs of autism appear age dependently through the developmental course and all the characteristic symptoms appear by 3 years [2].

Based on these clinical characteristics of autism, particularly of the early stage, e.g. abnormal S–W rhythm and locomotion, we hypothesized the pathophysiology of autism as an early disorder of brainstem monoaminergic system [2].

The frequency of epilepsy is high in idiopathic autism but the prevalence is unclear. It increases with age [3,4] and the cumulative risk of epilepsy in adults with autism is estimated at 20–35% [5]. The two peaks of occurrence of epilepsy in autism in early childhood and adolescence are known [6–8]. Frontal foci in EEG and frontal lobe

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epilepsy were shown to appear later from teens [9]. However, the underlying pathophysiologies of these characteristics of epilepsy in autism remain to be clarified.

In this paper, we show our clinical studies on epilepsy in autism, particularly in relation to the age factors, and discuss the pathophysiology of epilepsy in autism.

1.1. Subject and method

Subjects are the patients with autism spectrum disorders followed at Segawa Neurological Clinic for Children for 5–33 years, and had several precise neurological evaluations, and one or more EEG studies from April 1, 2008 to March 31, 2009. There were 485 patients in total; the cases with epilepsy were 244 and without epilepsy were 241. Among these autistic spectrum disorder cases, idiopathic autism who have been followed for 5–33 years were 177 cases in total. They consisted with 80 patients with epilepsy (male 64 and female 16) and 97 patients without epilepsy. The cases with epilepsy were subjected to the present study with non-epileptic cases as pathological controls.

Because the abnormality in crawling is the initial sign of locomotion and implicates a failure in antigravity muscle activity in autism [2], the patterns of crawling were assessed. We have also evaluated the activity of mentalis muscle by surface EMG during EEG examination to see if the atonia in non-REM (NREM) stage being abnormally present. This leak out of REM sleep component into NREM stage (atonic NREM) is the sign of failure in antigravity muscle activity revealing one of the cause of abnormality of locomotion [10,11]. Atonic NREM was compared between the cases with or without epilepsy.

All clinical charts and EEG reports were reviewed.

IQ/DQ examinations were performed by Revised Kyoto Development test, Tanaka–Binet test, WISC-III or WAIS-R during the course.

2. Results

2.1. Ages of first visit to this clinic and follow up period

The ages of the initial visit to this clinic ranged from 8 months to 30 years 3 months of age, mostly before 10 years of age (46.3% before 5 years of age, and 30% between 5 and 10 years of age) (Fig. 1 Upper Plate).

The current age of these patients as of February 28, 2009 were 5 years 8 months to 42 years 3 months of age (Fig. 1 Lower Plate). Sixty percent of the patients are reaching to over 30 years of age.

The average years of follow up in this clinic is 22.2 years ± 9.4 years.

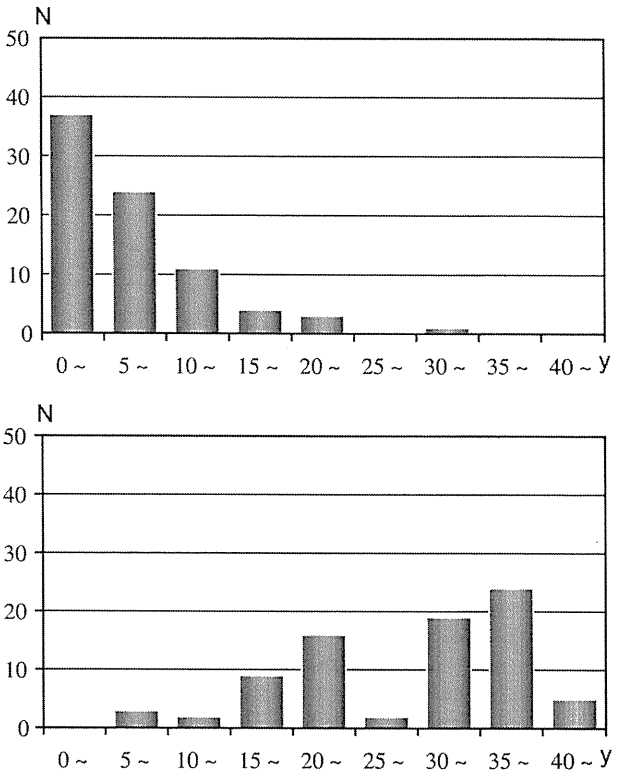

Fig. 1. Upper Plate: Ages of first visit to this clinic, Lower Plate: Age by year as of. Feb. 28, 2009, abscissa; age by year, ordinate; number of cases, Duration of follow up at Segawa Neurological Clinic for Children: 22.2 ± 9.4 years.

2.2. Ages of onset of epilepsy

The ages of onset of epilepsy ranged from 7 months to 30 years of age. There were two peaks of onset, the highest was before 5 years of age (34 cases; 42.5%), and followed by 10–15 years of age (17 cases; 21.3%) (Fig. 2). We assessed these patients by dividing into two groups with the ages of onset, that is, before 10 years of age

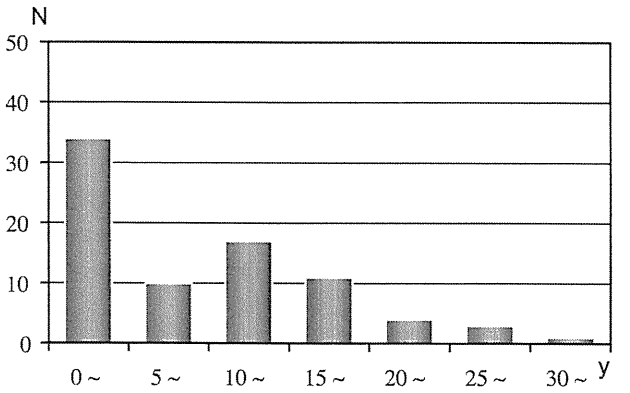

Fig. 2. Age of onset of epilepsy, abscissa; age by year, ordinate; number of cases, Average age (years) ± SD; Group with the onset younger than 10 years of age; 3.2 ± 2.7 years, Group with the onset older than 10 years of age; 16.7 ± 4.8 years.

(44 cases, 55%) (younger group) and after 10 years of age (36 cases, 45%) (older group). The average age of onset of the former was 3.2 years ± 2.7 years, and of the latter was 16.7 years ± 4.8 years.

The clinical types of epilepsy seen in autism were various and showed changes through the course.

2.3. EEG characteristics

The central spike tended to occur earlier than the frontal spike in the both groups (Fig. 3). The onset of epilepsy and appearance of central ($r = 0.461$, $p = 0.0002$) and frontal ($r = 0.542$, $p < 0.0001$) spikes showed moderate correlation.

The relation of the age of onset of central and frontal spikes was assessed. The time lag between central and frontal spike tended to be longer in the older group than younger group (Fig. 4).

The average age of appearance of central focus was 6.0 ± 3.3 years of age in the younger group, and 10.3 ± 6.1 years in the older group. Frontal focus appeared at 8.7 ± 4.1 years of age in the younger group, and at 15.1 ± 7.3 years in the older group.

2.4. The characteristics of the locomotion

Crawling was evaluated in the younger and older groups with epilepsy, and the group without epilepsy.

The information of crawling and walking in infancy and early childhood was available in 37 among 44 cases in younger group, and 26 of 36 cases in older group.

The most cases crawled and walked at appropriate age except a few cases whose epilepsy started before 5 years (Fig. 5).

The characteristics of the crawling were divided into the four groups; the group with normal crawling, with minimum crawling (crawled but short period less than one month), crawled but with abnormal pattern, such as shuffling, and did not crawl. The cases who did not crawl showed the differences among the epilepsy groups with younger and older onsets, and non-epileptic groups. The rate of no crawling was the highest in epilepsy group with older onset, and the lowest in the no epilepsy group (Table 1).

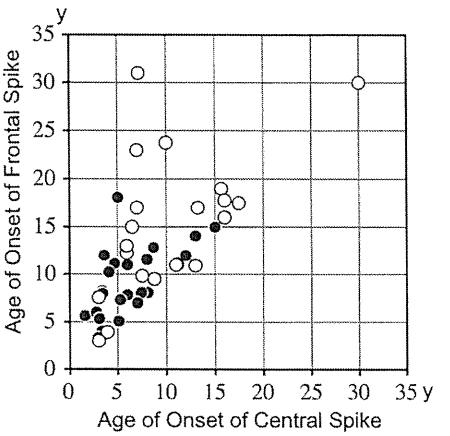

Fig. 4. Ages of onset of central (abscissa) and frontal (ordinate) spike ● < 10 years, ○ ⩾ 10 years.

2.5. Atonia in NREM sleep

Atonic NREM was evaluated in both epilepsy and non-epilepsy cases on the initial records, and was plotted against the age of the examination. The cases with epilepsy showed higher frequency of atonic NREM than non-epilepsy cases (Fig. 6). The rate of atonic NREM was compared among the younger and older epilepsy group, and non-epilepsy group in the latest records. The rate of the atonic NREM was the highest in the older group and the lowest in the non-epilepsy group (Fig. 7).

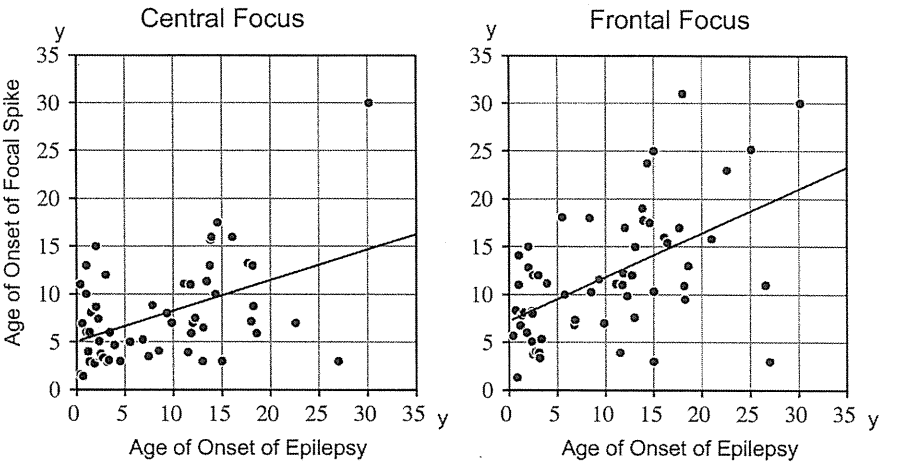

Fig. 3. Ages of appearance of central and frontal spike.

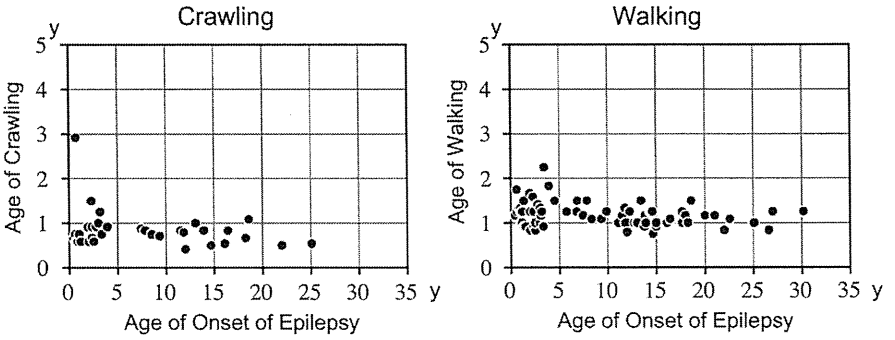

Fig. 5. Age of crawling and walking against the age of onset of epilepsy.

Table 1
Crawling in Autism (%).

	Onset of Epilepsy <10 years	Onset of Epilepsy ⩾10 years	No epilepsy
Normal crawling	59.5	53.8	66.0
Minimum crawling	8.1	3.8	5.1
Abnormal crawling pattern	18.9	15.4	20.6
No crawl	13.5	26.9	8.2

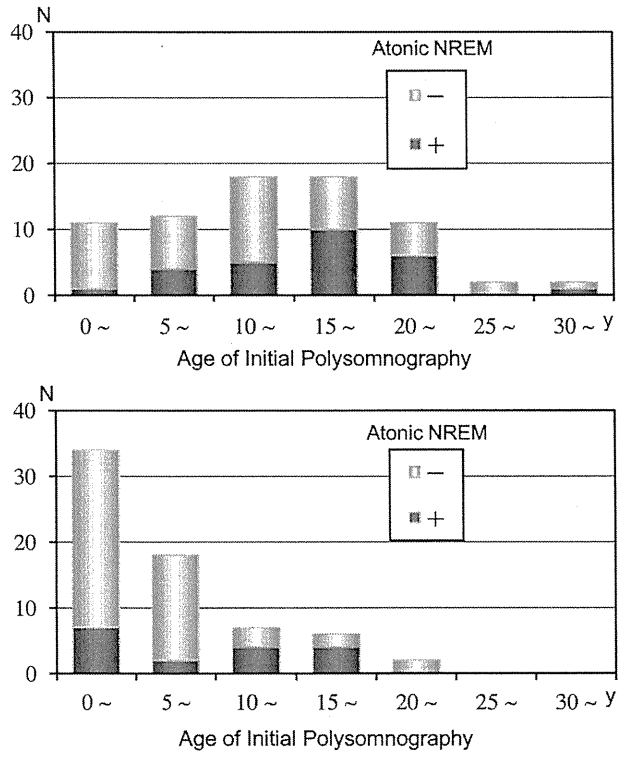

Fig. 6. Number of the cases who showed atonic NREM against the ages of polysomnography recording; Upper plate: cases with epilepsy, Lower plate: cases without epilepsy.

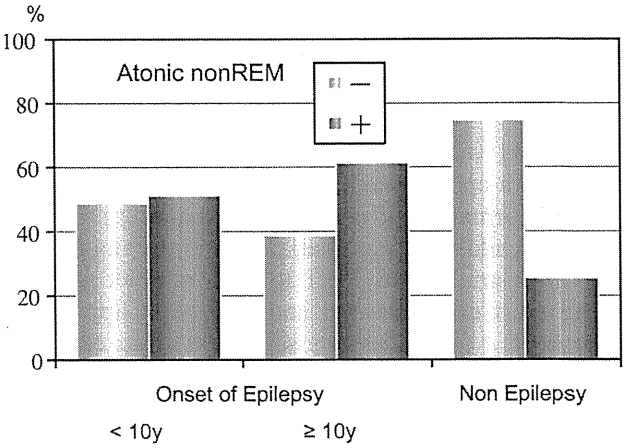

Fig. 7. Rate of atonic NREM in the latest paolysomnography in epilepsy (younger and older) groups, and non-epilepsy group.

2.6. IQ/DQ

IQ/DQ showed deterioration with age in some cases, but most of the patients remained to be essentially unchanged.

The most recently evaluated IQ/DQ was compared in the younger (37 patients) and older group (35 patients) with epilepsy. The average ages of examination in the younger and older groups were 22.9 ± 8.1 years and 24.5 ± 7.5 years, respectively. The average of IQ/DQ of both groups were 33.3 ± 22.8 and 26.6 ± 17.1, respectively, i.e. higher in the younger group.

2.7. IQ/DQ and locomotion

IQ/DQ and the features of locomotion were correlated. Crawling feature was divided to two groups, cases who crawled normally and cases who did not crawl or crawled with abnormal pattern.

The younger group with epilepsy showed similar average IQ/DQ levels in both normal and abnormal crawl groups, 33.9 ± 23.5 and 32.2 ± 21.4, respectively.

The older group with epilepsy also showed similar average IQ/DQ levels in both normal and abnormal crawl groups, 27.6 ± 16.9 and 30.8 ± 15.6, respectively.

However, when we checked the age of walking, in younger group with epilepsy the cases who walked at normal age (younger than 18 months of age) showed higher average IQ/DQ than the cases walked later (later than 19 months of age), 38.1 ± 23.3 and 16.0 ± 7.0, respectively.

3. Discussion

Abnormal sleep–wake rhythm and locomotion are the very initial symptoms in autism, and reflect the initial pathomechanism of autism [2]. These abnormalities suggest the hypofunction of the brainstem monoaminergic systems, serotonergic and noradrenergic neuronal systems [2,10]. Age dependent appearance of the characteristic symptoms of autism suggests the sequential changes of the involved neuron or neuronal system through the development [2].

The epilepsy in autism is one of the major symptoms of autism, and is shown to occur at particular ages with two peaks [6–8]. Our data shown in this study revealed the similar two peaks in young childhood and adolescent. The first peak of the age of the onset in autism as shown in this study is similar to the age of onset of the childhood epilepsy in general, but the second peak in adolescent is unique to autism.

In reference to the EEG epilepsy foci, central spikes appeared earlier than frontal spikes with the specific relation to the onset of clinical seizure in both younger and older groups. These results also confirmed the previous study [9]. In our study it was shown that the both central and frontal EEG foci appeared before the onset of the clinical seizure. The duration between the appearance of central focus and frontal focus showed the longer duration in the older onset group. These processes may speculate the underlying pathomechanism of later onset of epilepsy in autism.

The higher rate of no crawling in the epilepsy group in contrast to the non-epilepsy group suggests the neuronal system related to crawling is involved in pathophysiology of the epilepsy in autism. When compared between younger and older groups, older group showed higher rate of no crawling.

Atonic NREM was higher in epilepsy group in contrast to non-epilepsy group, and it was higher in older group than younger group.

From these features of crawling and atonic NREM, hypofunction of the brainstem monoaminergic neuron modulating antigravity muscle activity and locomotion was thought to be the earliest pathomechanism underling the epilepsy in autism. Thus epilepsy in autism is not the secondary manifestation, but one of the pathognomonic symptoms of autism.

The IQ/DQ of younger and older groups did not show the significant differences, and suggest the locomotion did not show the differences except the features of walking in younger group, showing the lower IQ/DQ in delayed walking group.

In our clinical studies [2], we revealed that failure in two pedals walking in autism causes orofacial and limb kinetic apraxia in early childhood, that is, failure in functional specialization of the cortex. Mori et al. [12] reported that the fastigial nucleus is one of the generators of locomotion. By training Japanese monkey for two pedal walking with upright posture, activation of the cortex through the spinal stepping generator, the fastigial nucleus–thalamus–cortex pathway was shown by functional MRI (Mori S, personal communication). The fastigial nucleus is shown to be affected in autism in neuropathological study [13]. This could be one of the causes of failure in locomotion and failure in mental activity in autism. So the features observed in relation to two pedal walking is due to different pathophysiology from the failure in crawling.

The hypofunction of the brainstem monoaminergic system is suggested to be the initial occurrence in autism. The monoaminergic system project to the wider cortical area, and initial monoaminergic hypofunction may lead to the central focus which appears earlier. The higher rate of no crawling in older group suggests the severer involvement of the monoaminergic deficiency. Atonic NREM caused by the hypofunction of the brainstem monoaminergic system induces the hypofunction of the pedunculo-pontine nucleus (PPN) which lead to the hypofunction of the ventrotegmental and the nigrostriatal dopamine (DA) systems. The hypofunction of the DA system during the development cause the compensatory DA receptor upregulation. This disinhibits the thalamocortical pathway and causes the frontal epileptic focus. Earlier onset of central focus and later onset of frontal focus suggest the progression of this pathomechanism in autism and development of the cortical dysfunction.

Thus failure in locomotion is the most susceptible factor for epileptogenesis in autism and for which atonic NREM might be involved.

In our clinic we have unique opportunity to follow the developmental disorders till adulthood. This is very useful to evaluate how the clinical features change into the adulthood, and to discuss what are the underlying mechanisms of the age dependent changes. Our present data on the epilepsy in autism give the valuable data of the characteristics of longitudinal courses of epilepsy in autism.

4. Conclusion

From these observations, we implicate that the pathophysiology of Epilepsy of idiopathic Autism differ in younger and older onset groups.

Younger onset group may be similar to the usual childhood onset epilepsy. Failure in development of the function and morphogenesis of the cortex may cause the earlier development of spike focus in EEG before clinical seizure.

Older onset group with frontal focus suggests the involvement of the specific system, that is failure in the brainstem monoaminergic (serotonergic) system causes dysfunction of the PPN and induces dysfunction of the DA system, and with development of the DA receptor supersensitivity consequently disinhibits the thalamo-frontal pathway, which after maturation of this pathway in teens cause the epileptogenesis in the frontal cortex.

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Hideki Fukuda, Segawa Neurological Clinic for Children, helped the statistical and data analysis. This article was presented at the 12th Annual Meeting of Infantile Seizure Society held at Kurume, Japan, in 2009.

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Clinical and biochemical features of aromatic L-amino acid decarboxylase deficiency

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Editorial, page XXX

Supplemental data at www.neurology.org

ABSTRACT

Objective: To describe the current treatment; clinical, biochemical, and molecular findings; and clinical follow-up of patients with aromatic L-amino acid decarboxylase (AADC) deficiency.

Method: Clinical and biochemical data of 78 patients with AADC deficiency were tabulated in a database of pediatric neurotransmitter disorders (JAKE). A total of 46 patients have been previously reported; 32 patients are described for the first time.

Results: In 96% of AADC-deficient patients, symptoms (hypotonia 95%, oculogyric crises 86%, and developmental retardation 63%) became clinically evident during infancy or childhood. Laboratory diagnosis is based on typical CSF markers (low homovanillic acid, 5-hydroxyindoleacidic acid, and 3-methoxy-4-hydroxyphenolglycole, and elevated 3-O-methyl-L-dopa, L-dopa, and 5-hydroxytryptophan), absent plasma AADC activity, or elevated urinary vanillactic acid. A total of 24 mutations in the *DDC* gene were detected in 49 patients (8 reported for the first time: p.L38P, p.Y79C, p.A110Q, p.G123R, p.I42fs, c.876G>A, p.R412W, p.I433fs) with IVS6+4A>T being the most common one (allele frequency 45%).

Conclusion: Based on clinical symptoms, CSF neurotransmitters profile is highly indicative for the diagnosis of aromatic L-amino acid decarboxylase deficiency. Treatment options are limited, in many cases not beneficial, and prognosis is uncertain. Only 15 patients with a relatively mild form clearly improved on a combined therapy with pyridoxine (B6)/pyridoxal phosphate, dopamine agonists, and monoamine oxidase B inhibitors. *Neurology*® 2010;75:1–1

GLOSSARY

5HIAA = 5-hydroxyindoleacetic acid; **AADC** = aromatic L-amino acid decarboxylase; **HVA** = homovanillic acid; **MAO-A** = monoamine oxidase A; **MET** = metanephrine; **MHPG** = 3-methoxy-4-hydroxyphenylglycol; **PLP** = pyridoxal phosphate; **VLA** = vanillactic acid.

Aromatic L-amino acid decarboxylase (AADC) deficiency (OMIM 107930) is an inborn error of neurotransmitter biosynthesis with an autosomal recessive inheritance.[1,2] Mutations in the gene encoding for the enzyme AADC (*DDC*) lead to a severe combined deficiency of serotonin and catecholamines,[3] clinically characterized by vegetative symptoms, oculogyric crises, dystonia, and severe neurologic dysfunction in infancy.[4-6] Serotonin and dopamine are formed following the hydroxylation of tryptophan and tyrosine by tryptophan and tyrosine hydroxylases and by a subsequent decarboxylation of the corresponding intermediates 5-hydroxytryptophan and L-dopa by a pyridoxal phosphate (PLP)–dependent AADC (figure 1).[7]

The exact diagnosis of AADC deficiency relies on the analysis of neurotransmitters and their metabolites in CSF; however, urinary vanillactic acid (VLA) is also highly diagnostic.[8-11] Measurement of AADC activity in plasma and *DDC* gene sequencing are essential for the final diagnosis.[3,8,12]

The therapy is aimed at correcting the neurotransmitter abnormalities, offering the following medications: dopamine receptor agonists, anticholinergics, monoaminoxidase inhibitors, α-adrenergic agonists, selective serotonin reuptake inhibitors, therapeutic doses of the cofactor

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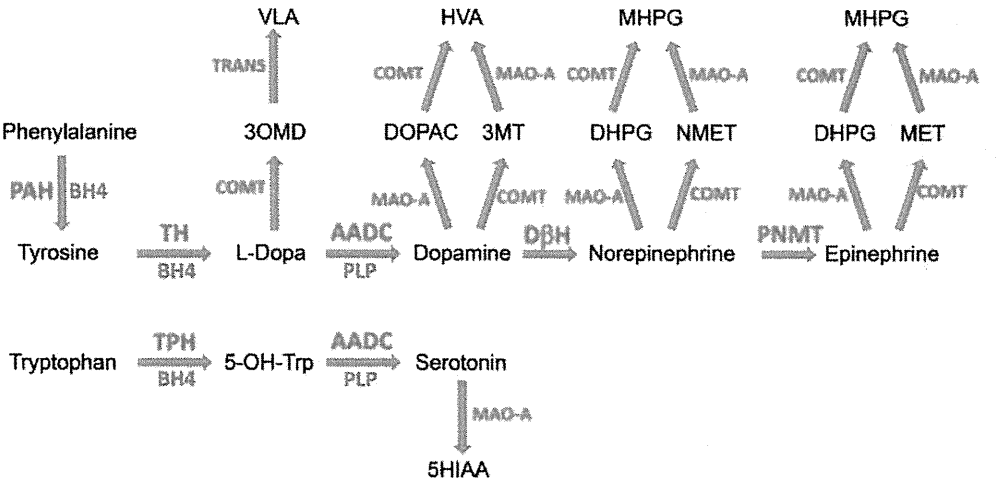
Figure 1 Biosynthesis and metabolism of catecholamines and serotonin



Enzymes and abbreviations: AADC = aromatic L-amino acid decarboxylase; COMT = catechol-O-methyltransferase; DβH = dopamine-β-hydroxylase; MAO-A = monoamine oxidase A; PAH = phenylalanine-4-hydroxylase; PNMT = phenylethanolamine *N*-methyltransferase; TH = tyrosine-3-hydroxylase; TPH = tryptophan-5-hydroxylase; TRANS = transaminase. Reactions catalyzed by MAO-A are coupled with an additional step catalyzed by either aldehyde or aldose dehydrogenase (catabolism of dopamine and serotonin; not shown) or aldehyde or aldose reductase (catabolism of norepinephrine and epinephrine; not shown). Metabolites and abbreviations: 3MT = 3-methoxytryptamine; 3OMD = 3-O-methyldopa; 5-OH-Trp = 5-hydroxytryptophan; 5HIAA = 5-hydroxyindoleacetic acid; DHPG = 3,4-dihydroxyphenylglycol; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; L-dopa = 3,4-dihydroxyphenylalanine; MET = metanephrine; MHPG = 3-methoxy-4-hydroxyphenylglycol; NMET = normetanephrine; VLA = vanillactic acid. Cofactors and abbreviations: BH4 = tetrahydrobiopterin; PLP = pyridoxal phosphate.

of AADC (pyridoxine or PLP), catechol-*O*-methyltransferase inhibitor, precursors of dopamine and serotonin (L-dopa, 5-OH-Trp), folinic acid, and melatonin. Response to treatment varies, but in many cases the therapy shows no or little benefit.[13-22]

In this article, we summarize the biochemical and molecular findings and the course of the disease in 78 patients with AADC deficiency tabulated in the international JAKE database (http://www.biopku.org/BioPKU_Databases JAKE.asp).

METHODS Biochemical investigations. Neurotransmitter metabolites in CSF were investigated by high-performance liquid chromatography with electrochemical detection, with slight modifications in different laboratories, but essentially as described elsewhere.[23] VLA was investigated by a standard method for organic acids profile in urine.[23]

Standard protocol approvals, registrations, and patient consents. Written informed consent was obtained from all patients or their physicians who participated in this study. No approval was required from the regional ethical committees. All biochemical and clinical data were collected within the routine diagnostic procedures.

Case reports. The age at diagnosis ranged from 4 months to 24 years (median 3.9 years) and was available from 60 of 78 patients. Twenty patients were diagnosed at the Children's Hospital in Zürich.

A questionnaire with the following sections was distributed to physicians managing AADC-deficient patients: 1) general patient information, 2) birth information and laboratory tests, 3) clinical information with signs and symptoms and treatment protocols, 4) EEG/CT/MRI data, 5) DNA analysis, and 6) follow-up information. A written consensus was provided for all submitted data by physicians.

A literature search was conducted using MEDLINE (1990–August 2009) for the following key words: aromatic L-amino acid decarboxylase, monoamine decarboxylase, dopa decarboxylase, AADC, and DDC.

Detailed information on AADC-deficient patients is tabulated in the JAKE database (http://www.biopku.org). Clinical information is summarized in table 1, biochemical and molecular data in table e-2 on the *Neurology*® Web site at www.neurology.org, and therapy in table 2. Detailed information on DNA variations is available from the BIOMDB database (http://www.biopku.org). Most important information is included in the case reports (table e-1).

RESULTS Signs and symptoms. All patients showed symptoms typical for deficiency of catecholamines and serotonin. In 96% of them, symptoms became clinically evident during infancy (≤18 months) or during childhood (≤10 years). Only 6 patients were clinically conspicuous at adolescence or adulthood.

Almost all patients (95%) presented with muscular hypotonia. Episodes of oculogyric crises were doc-