diluted 1:200) and PDGFRβ (SantaCruz, sc-6252, diluted 1:200) were used. We also analyzed the expression of KIT and PDGFRβ in 6 ganglioneuroma samples (Table III). GIST specimens were used for the positive controls. The evaluation of immunohistochemistry was performed by two independent observers (AS and JH). We evaluated the complete cytoplasm and membrane staining in more than 30% of cells as positive, and cytoplasm or membrane staining in less than 30% of cells as negative. We considered that the positive specimens showed the expression of the protein.

### Statistical Analysis

Statistical analysis was performed using Statview software (SAS). The $\chi^2$-test was used to correlate the categorical variables. The prognostic significance of the clinical variables was assessed by using Cox proportional hazards model. For all analyses, the *P* values were 2-tailed, and a *P*-value of less than 0.05 was considered statistically significant.

## RESULTS

### Expression and Mutation Analysis of *KIT*

*KIT* mRNA expression was found in 22 (91.7%) of 24 cell lines with RT-PCR (Supplemental Table I). All cell lines predominantly showed a 12 bp (GGTAACAACAAA) deleted product (GNNK$^-$ isoform) at the end of the extra cellular domain (exon 9) compared to the wild-type of *KIT* (Supplemental Fig. 1) [27]. We could not find any activating mutations as previously reported in GIST and AML [10,11]. Two single nucleotide polymorphisms (SNPs) were found [541aa, A > C of 1642 bp in exon 10 (Reference SNP (refSNP) Cluster Report: rs 3822214 by NCBI) in SCMC-N4 and SKNSH, 862aa, G > C of 2,607 bp in exon 18 (rs 3733542) in SJNB-5 and SKNSH]. A silent mutation was also found (I798I, ATC > ATT of 2,414 bp in exon 17 in SJNB-8). All cell lines, except for one, expressed *SCF*. Both soluble and membranous bound forms of *KIT* mRNA were found.

*KIT* expression was detected in 32 (80.0%) of 40 tumor samples by RT-PCR (Table I) and 23 (60.5%) of 38 paraffin sections of tumor samples by immunohistochemistry (Table II). The expression of mRNA and protein was measured in ten patients using both RT-PCR and immunohistochemistry. The expression of *KIT* mRNA and protein was associated with NB patients under 1 year ($P = 0.0016$, 0.0074, respectively) and inversely associated with *MYCN* amplification ($P = 0.0006$, 0.017, respectively) and Stages 3 or 4 NB patients over 1 year old ($P < 0.0001$, 0.014, respectively). *KIT* mRNA expression was significantly associated with *TRKA* mRNA expression ($P < 0.001$). Multivariate analysis showed the coefficient of correlation between *KIT* mRNA and *TRKA* mRNA was 0.627 (0.398–0.785, $P < 0.001$) and between KIT mRNA and survival was 0.665 (0.446–0.809, $P < 0.001$). The KIT protein expression was found in two of four differentiating NB samples and five of six samples of ganglioneuroma (Table III). The difference of expression rate of KIT protein between neuroblastoma (NB) and ganglioneuroma or between differentiating and poorly differentiated NB were not statistically significant.

### Expression and Mutation Analysis of *PDGFRs*

*PDGFRα* mRNA was detected in all cell lines and tumor samples by RT-PCR (Supplemental Table I). As for the mutation of *PDGFRα*, no activating mutations were found. Three SNPs were found (567aa A > G of 1,849 bp in exon12 (rs 1873778) in SJNB4, 603aa G > A of 1957 bp in exon13 (rs 10028020) in SJNB4, NB16, NB69, LAN2, and SKNSH, 824aa C > T of 2,620 bp in exon 18 (rs 2228230) in SJNB-5, SJNB-8, NB-19, LAN-1, LAN-5, and SKNSH). Silent mutation was found in GOTO (V533V, GTG > GTA of 1,747 bp in exon 11). PDGFRα protein was strongly expressed in almost all tumor samples by immunohistochemistry.

*PDGFRβ* mRNA was expressed in 14 (58%) of 24 cell lines and 29 (73%) of 40 NB samples using RT-PCR (Tables I and II). *PDGFRβ* was expressed in 24 (63%) of 38 tumor samples by immunohistochemistry (Table III). The expression of *PDGFRβ* mRNA and protein was associated with NB patients under 1 year ($P = 0.0014$ and 0.019, respectively) and inversely associated with *MYCN* amplification ($P = 0.0198$ and 0.0052, respectively), advanced stage patients one year old and over ($P = 0.0046$ and 0.0011, respectively). The correlation between *PDGFRβ* and *TRKA* mRNA expression was significant ($P = 0.0003$). Multivariate analysis showed the coefficient of correlation between *PDGFRβ* mRNA and *TRKA* mRNA was 0.574 (0.320–0.751, $P < 0.001$) and between *PDGFRβ* mRNA and survival was 0.525 (0.256–0.719, $P = 0.004$). The correlation between PDGFRβ protein expression and a favorable histology was also significant ($P = 0.0021$). The

**TABLE III. Correlation of KIT and PDGFRβ Expression to Histopathology of NB According to INPC System**

| INPC system | Number of patients | KIT (%) | PDGFRβ (%) |
|---|---|---|---|
| Neuroblastoma (Schwannian stroma-poor) | | | |
| Undifferentiated | 1 | 0 | 0 |
| Differentiating | 4 | 2 (50) | 4 (100) |
| Poorly differentiated | 27 | 17 (63) | 16 (59.3) |
| Ganglioneuroblastoma | | | |
| Intermixed (Schwannian stroma-rich) | 4 | 2 (50) | 4 (100) |
| Nodular | 2 | 2 (100) | 1 (50) |
| Total | 38 | 23 (60.5) | 25 (65.8) |
| Ganglioneuroma (Schwannian stroma-dominant) | 6 | 5 (83.3) | 6 (100) |

The difference of expression rate of KIT or PDGFRβ protein between neuroblastoma and ganglioneuroma was not statistically significant. The difference of expression rate of KIT or PDGFRβ protein between differentiating and poorly differentiated neuroblast.

expression of PDGFRβ was found in all four differentiating NB samples and all five ganglineuroblastoma samples (Table III). The difference of expression rate of PDGFRβ protein between NB and ganglioneuroma or between differentiating and poorly differentiated NB were not statistically significant.

### Expression and Mutation Analysis of *FLT3*

*FLT3* mRNA expression was detected in 19 (79.2%) of 24 cell lines and in 32 (80%) of 40 tumor samples by RT-PCR (Table I and Supplemental Table I). No ITDs or kinase domain mutations were observed in any cell lines. *FLT3* expression was associated with NB patients under 1 year ($P = 0.0177$) and *TRKA* expression ($P < 0.0001$; Table I). Inverse correlations were observed for *MYCN* amplification ($P = 0.0019$) and advanced stage patients over one year old ($P = 0.0011$). FLT3 protein expression was not examined.

### Expression and Mutation Analysis of *RET*

*RET* expression was detected in 22 (91.6%) of 24 cell lines and in 17 (42.5%) of 40 tumor samples by RT-PCR (Table I and Supplemental Table I). However, no mutations were found in this study. We identified SNPs (691aa or 769aa) of the *RET* gene. *RET* expression was not associated with any clinical findings (Table I). Furthermore, we examined the expression of both isoforms RET51 and RET9. There were no correlations between the RET isoforms and the clinical findings.

### Expression and Mutation Analysis of *TRKA*

*TRKA* expression was detected in 7 (29.2%) of 24 cell lines and in 28 (70.0%) of 40 tumor samples by RT-PCR (Table I and Supplemental Table I). *TRKA* expression was associated with NB in patients under age 1 year ($P = 0.0006$) and with good prognosis (Table I). We examined the expression of the TRKA isoform, but did not detect isoform III in any cell lines or tumor samples [20]. On the other hand, we found another novel isoform (deletion of exons 7–9) in 6 (25%) in 24 cell lines (SJNB-2, SJNB-6, NB1, TGW, SKNSH, SCMC-N4) with the coexpression of isoforms I or II, which we referred to as isoform IV in this article (Supplemental Fig. 2). However, we could not find this isoform IV in any of 40 tumor samples.

## DISCUSSION

The aberrant expression of KIT and SCF has been reported in several solid tumors, such as small cell lung cancer [28], gynecological tumors [29], and breast cancer [30]. However, *KIT* mutations are rarely reported in other cancers [31–33] except for GIST [10] and the core-binding factor AML [11]. An autocrine or paracrine loop of KIT and SCF has been hypothesized in NB cell proliferation [34]. Moreover, the $GNNK^-$ isoform of *KIT* has been shown to be predominantly expressed in varieties of tumors, such as AML and germ cell tumor [35,36], and the $GNNK^-$ isoform has a growth advantage compared with the $GNNK^+$ isoform and phosphorylates downstream signals, such as MAP and STAT kinases [27]. In this study, KIT expression was associated with NB patients under 1 year of age and good prognosis as previously reported [7]. The $GNNK^-$ isoform was predominantly expressed in NB patients. An inverse correlation between KIT expression and *MYCN* amplification was observed and it supported the observation of Krams et al. [7]. On the other hand, KIT expression has been reported to be associated with a poor prognosis and with *MYCN* amplification in NB [4,9]. These different results may due to the differences of experimental method, race or the number of patients analyzed. Moreover, the loss of KIT expression has also been reported in advanced cancer, including breast cancer [32], melanoma [37], thyroid cancer [38], and ovarian cancer [39]. The loss of KIT expression may be associated with NB tumor progression.

PDGFRs and their ligands, PDGFA and PDGFB, have an important role not only in embryogenesis, but also in the progression of some tumors, suggesting the presence of an autocrine or paracrine mechanism [40,41]. PDGFRs can become potent oncoproteins when they are overexpressed or mutated [40–42]. The intensive expression of PDGFRα protein was detected in this study, suggesting that expressed PDGFRα may be the therapeutic target for the kinase inhibitor, imatinib. On the other hand, the expression pattern of PDGFRβ was associated with good clinical outcome in NB similar to KIT. PDGFRβ has been considered to have oncogenic potential compared to PDGFRα [14].

*FLT3* expression was associated with a good clinical outcome of NB in our study. Our results may provide the evidence that neuroectodermal and hematopoietic cells share common regulatory pathways, as previously reported [15]. It was reported that the RET and TRKA pathways collaborate to regulate NB differentiation [16], but RET expression was not associated with TRKA expression or any clinical parameters in present study. We could not find the alternative spliced variant form of TRKA, TRKAIII, which was reported to have the oncogenic potential [20]. We found another new isoform (deletion of exons 7–9) in 6 (25%) of 24 cell lines. Further study is needed to clarify the function of this new isoform.

In conclusion, our data suggest that the loss of expression of several RTKs may be related to disease progression and poor clinical outcome in NB.

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CANCER GENETICS AND CYTOGENETICS

Short communication

# Mutations of *GATA1*, *FLT3*, *MLL*-partial tandem duplication, *NRAS*, and *RUNX1* genes are not found in a 7-year-old Down syndrome patient with acute myeloid leukemia (FAB-M2) having a good prognosis

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**Abstract** The prognosis of leukemia developed in Down syndrome (DS) patients has improved markedly. Most DS leukemia occurs before 3 years of age and is classified as acute megakaryocytic leukemia (AMKL). Mutations in the *GATA1* gene have been found in almost all DS patients with AMKL. In contrast, it has been shown that occurrence of DS acute myeloid leukemia (DS-AML) after 3 years of age may indicate a higher risk for a poor prognosis, but its frequency is very low. Age is one of the significant prognostic indicators in DS-AML. The prognostic factor of gene alterations has not been reported in older DS-AML patients. We here describe the case of a 7-year-old DS boy with AML-M2, who had no history of transient abnormal myelopoiesis or any clinical poor prognostic factors, such as high white blood cell counts or extramedullary infiltration. We molecularly analyzed the *GATA1*, *FLT3*, *MLL*-partial tandem duplication, *NRAS*, and *RUNX1* (previously *AML1*) genes and did not detect any alterations. The patient has lived for more than 5 years after treatment on the AML99-Down protocol in Japan. This suggests that a patient lacking these genes alterations might belong to a subgroup of older DS-AML patients with good prognosis. Accumulation of more data on older pediatric DS-AML patients is needed. 

## 1. Introduction

Children with Down syndrome (DS) have a ~20-fold higher incidence of leukemia than do unaffected children. Most DS leukemia is diagnosed as acute megakaryocytic leukemia (AMKL), which occurs before 3 years of age, and the prognosis has markedly improved [1–3]. Infants with DS and transient abnormal myelopoiesis are at high risk for later development of AMKL, usually by 3 years of age. Recently, it has been reported that mutations of *GATA1* are present in virtually all cases of DS acute myeloid leukemia (DS-AML) [4,5]. The same mutations are seen in transient abnormal myelopoiesis cases as well [5]. Furthermore, in paired samples from transient abnormal myelopoiesis and AMKL in the same children, identical *GATA1* mutations were found [4–6], suggesting that DS with transient abnormal myelopoiesis and AMKL are within a biologically homogeneous group. *GATA1* mutation is a very early event in the development of DS-AMKL and in the process of multistep leukemogenesis [4,7].

On the other hand, DS-AML occurring after the age of 3 years may be completely different from that occurring before the age of 3 years, and may instead be biologically similar to de novo AML in non-DS patients. Multivariate analysis of data showed that children with DS aged ≥2 years at diagnosis had an increased risk of relapse after treatment [2]. There has been no good classification of DS-AML patients between the age of 2 and 4 years. Classification of the biological differences would probably be more useful than a better age cut.

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Here we describe the case of a 7-year-old boy with DS-AML who lacked mutations of *GATA1*, *FLT3*, *MLL*-partial tandem duplication (PTD), *NRAS*, and *RUNX1* (previously *AML1*) genes. The prognostic factors for DS-AML, particularly in older children, are still unknown. The present case supports the hypothesis that DS-AML patients who do not have alteration of these genes have a good prognosis.

## 2. Case report

A 7-year-old boy with DS presenting with a persistent fever was admitted to our hospital because of anemia and thrombocytopenia. On admission, he had a pale face and systemic petechiae and purpuras. No cervical lymphadenopathy or hepatomegaly was noted. Blood testing revealed a white blood cell count of 7,500/μL with 9% myeloblasts, 8% segmented neutrophils, 15% monocytes, 49% lymphocytes, and 6% blasts, a hemoglobin concentration of 6.1 g/dL, and a platelet count of $41.2 \times 10^4$/μL. Bone marrow examination revealed 66% blasts (Fig. 1a) with 39.2% monocytoid blasts and 18.8% myeloblastic cells with Auer bodies (Fig. 1b) and azurophilic granules. The diagnosis of AML-M2 was made according to the morphological and immunophenotypic criteria of the French–American–British (FAB) classification in combination with other laboratory data.

Even though the differential count showed a predominance of monocytic cells, myeloblasts (15.2%) and myeloblastic cells (18.8%) were 34% of total. These cells were positive for peroxidase staining (73.5%), and both nonspecific (5.8%) and specific (55%) esterase staining. Nonspecific esterase-positive cells were <20% among blasts, which matches the criteria of FAB-M2. Immunophenotypic analysis of CD45+ cells showed the presence of CD13 (56.8%), CD33 (86%), CD38 (95.2%), and HLA-DR (26.7%) antigens and the absence of CD34 (2.7%), CD11b (11.7%), and CD14 (0.6%). CD11b and CD14 presented on monocytes were negative in this patient. Cytogenetic analysis demonstrated the 47,XY,+21c karyotype in 20 bone marrow cells.

The serum and urine lysozyme level has been used as an aid in distinguishing AML with maturation (FAB-M2) from acute myelomonocytic leukemia (M4). In this patient, the count of monocytes in peripheral blood was 1,125/μL, which is less than the 5,000/μL of the FAB-M2 criteria. The serum lysozyme level was 25 μg/mL (normal range, 5–10 μg/mL) and the urine lysozyme level was 0 μg/mL. The level of lysozyme of this patient in peripheral blood was less than threefold of the normal range. Collectively, these data led us to diagnose this patient with AML-M2.

The patient was treated on the Japanese Childhood AML Cooperative Study Group Protocol for DS patients (AML99-Down protocol), which consists of pirarubicin (THP-ADR) (25 mg/m$^2$ on days 1 and 2), etoposide (150 mg/m$^2$ on days 3–5), and cytosine arabinoside (Ara-C) (100 mg/m$^2$ on days 1–7) at five cycles every month [8,9]. No prophylaxis for the central nervous system was performed.

On the first cycle of chemotherapy, he had severe mucositis and high fever for 5 weeks. On the second cycle, he had high fever during therapy. We considered this fever a side effect of Ara-C, and therefore methylprednisolone was given for 30 minutes prior to drip infusion of Ara-C. The patient obtained complete remission after the first cycle of chemotherapy and has continued in complete remission for 5 years without any reoccurrence.

## 3. Analysis of *GATA1*, *FLT3*, *MLL*, *NRAS*, and *RUNX1* genes

Written informed consent was obtained from the parents of the patient. RNA extracted from his bone marrow cells at

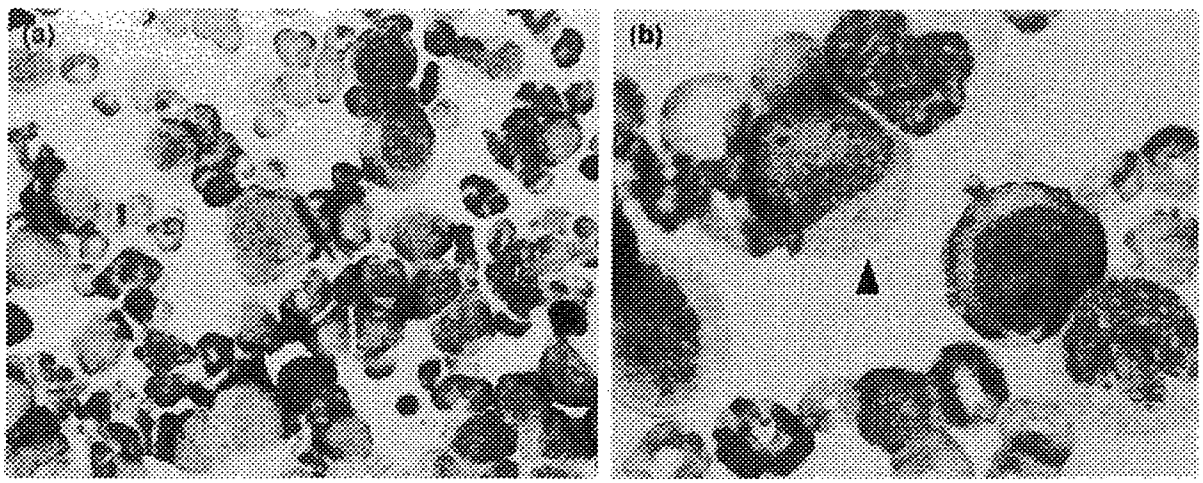


Fig. 1. Initial bone marrow smear at diagnosis. (a) Bone marrow aspirate showing hypercellularity (Giemsa staining). (b) Leukemic cells with Auer bodies (arrowhead).

diagnosis was reverse transcribed to cDNA and alterations of *GATA1*, *FLT3*, *MLL*-PTD, *NRAS*, and *RUNX1* genes were examined as previously described [10–13]. Briefly, mutational analysis of *GATA1* within exon 2, where there are hot spots, was performed with reverse transcription-polymerase chain reaction (RT-PCR) followed by direct sequencing [11]. Point mutations of *FLT3*-D835/I836 were examined with restriction fragment length polymorphism (RFLP)-PCR [12] and *FLT3*-internal tandem duplication (ITD) was analyzed with RT-PCR [11,13]. *MLL*-PTD was examined with simple first-round RT-PCR using the primer pair located between exon 9 and exon 4 [14]. Mutation of *NRAS* and *RUNX1* genes was examined with PCR-single strand conformation polymorphism analysis (SSCP) and direct sequencing [15].

## 4. Discussion

Lange et al. [16] studied 1,206 children with AML, including 118 (9.8%) DS patients. Among these, >95% of AML patients with DS were <5 years old. FAB-M7 (AMKL) was found in 62%, and FAB-M1or M2 in 10%. Children under 2 years ($n$ = 94) treated on Children's Cancer Group (CCG) studies 2861 and 2891 had a 6-year EFS of 86%; those aged 2–4 years ($n$ = 58), 70%; and those older than 4 years ($n$ = 9), 28%. Outcome of children with DS-AML is excellent with standard induction therapy, but declines with increasing age; this report, however, gives no information about patients >4 years old [16].

Although white blood cell count at diagnosis is a significant predictor of outcome in non-DS AML, this is not the case for either DS or antecedent myelodysplastic syndrome patients. Extramedullary infiltration, which includes tumor nodules, skin infiltration, meningeal infiltration, gingival infiltration, or hepatosplenomegaly, has been discussed as a prognostic factor and is generally thought to indicate poor outcome in non-DS AML [8].

Monosomy 7 (−7) or deletion of the long arm of chromosome 7 [del (7q)] is found in only 4–5% of pediatric patients with AML. Although, cytogenetically, −7 is generally associated with a dismal prognosis in AML, even this may not be as unfavorable in those with DS [17]. Our patient did not have an acquired chromosomal abnormality in addition to trisomy 21 at diagnosis. Having no additional chromosomal abnormalities, including absence of −7, might be one of the good prognostic factors.

Our patient had no prior history of transient abnormal myelopoiesis or of the *GATA1* mutation in leukemic cells. In this respect, the leukemogenesis of this patient may differ from that typical of DS-AMKL patients <3 years old. DS-AMKL patients >3 years old at diagnosis often show the absence of a prior history of transient abnormal myelopoiesis. An age of >3 years at diagnosis may indicate only a different biological origin from those with a prior history of transient abnormal myelopoiesis and the *GATA1* mutation. In other words, there may be age-related biologic differences in the nature of AML in DS patients. We suggest that a better way to predict their prognosis would be by analyzing for the presence or absence of *GATA1* mutations and screening for the groups with good prognosis, rather than by the age at diagnosis, because the *GATA1* mutations are tightly associated with AMKL in DS patients, who are mostly younger children and have a good prognosis [1].

There is little clinical and genetic information on older pediatric patients with DS-AML with a poor prognosis. AML-M7 with *GATA1* mutations has a good prognosis among DS patients. This patient was 7 years old and his prognosis was good, suggesting that leukemogenesis in this case was not due to *GATA1* mutation.

DS-AML in older pediatric patients is considered to be similar to de novo non-DS AML. We therefore analyzed the same genetic prognostic factors in this patient as have been reported in de novo pediatric AML. There are no large studies of the genetic prognostic factors associated with older pediatric DS-AML, however, which made it difficult to compare the incidence of those mutations between non-DS AML and DS-AML among children. Particularly for older children with DS-AML, more accumulation of data is needed.

We examined ITD and D835/I836 mutations of *FLT3*. The prevalence and prognostic significance of these features are unknown in DS-AML. *FLT3*-ITD occurs in ~30% of adult AML patients and ~20% of pediatric AML patients [18–21]. *FLT3*-ITD is considered to predict poor prognosis in adult and pediatric AML patients [19,22–24]. On the other hand, ~10% of adult and pediatric AML patients have *FLT3*-D835/I836 mutations. AML patients with *FLT3*-D835/I836 mutations tend to have a poor prognosis as adults, but not as children [25,26]. Alterations of *FLT3* were not detected in the present patient. Given that this case was considered to be the same as de novo AML in a non-DS patient, the absence of *FLT3* alterations suggests a good prognosis.

We analyzed other possible prognostic factors, such as *MLL*-PTD, *NRAS*, and *RUNX1* mutations. *MLL*-PTD was detected in ~10% of AML patients with normal karyotype and in 90% of AML patients exhibiting trisomy 11 as the sole chromosome abnormality. The *MLL*-PTD was reported to be a subgroup of patients with an unfavorable prognosis in adult AML [14]. In a study of the Japanese Childhood AML Cooperative study group, AML patients with *MLL*-PTD comprised 13.3% and correlated with poor prognosis [21]. The prognostic impact of *NRAS* mutations, reported in 11–30% of AML patients, is still under discussion [27,28]. As for *RUNX1* mutation, we have reported that the mutations in pediatric hematologic malignancies are infrequent, but may be related to AML-M0, acquired trisomy 21, and leukemic transformation [10]. Furthermore, non-constitutional chromosome 21 in the leukemic clone may also lead to an unfavorable prognosis. No mutations of these genes were found in our patient, suggesting a good prognosis.

Table 1
Frequency of Down syndrome acute myeloid leukemia and myelodysplastic syndrome patients in published studies, including pediatric patients older than 4 years

| Study group | Accrual period, mo/yr | DS-AML/AML patients, no./no. (%) | DS-AML patients >4 yr old, no. | References |
|---|---|---|---|---|
| POG8498 | July 1984–July 1989 | 12/285 (4.2) | 0 | Ravindranath et al., 1992 [29] |
| Nagoya | Sept. 1986–Aug. 1992 | 9/NI | 0 | Kojima et al., 2000 [1] |
| NOPHO84/NOPHO88 | July 1984–Dec. 1992 | 23/223 (10.3) | 2 | Lie et al., 1996 [30] |
| BFM 87/BFM 93 | July 1987–Dec. 1994 | 40/633 (6.3) | 3 | Creutzig et al., 1996 [31] |
| CCG 2861/2891 | Mar. 1988–Oct. 1995 | 118/1206 (9.8) | 3 | Lange et al., 1998 [16] |
| Japan AT group/Down | Sept. 1987–Aug. 1997 | 33/NI | 0 | Kojima et al., 2000 [1] |
| CCG 2891 | Oct. 1989–Oct. 1999 | 161/1108 (14.5) | 9[a] | Gamis et al., 2003 [2] |
| AML99 | Jan. 2000–Dec. /2003 | 66/418 (15.8) | 2 | Kobayashi et al., 2006 [8] |

*Abbreviations:* DS-AML/MDS, Down syndrome acute myeloid leukemia and myelodysplastic syndrome; NI, no information.
[a] Nine patients are older than 5 years; data are shown separately for patients aged 2–5 years and older than 5 years.

Table 1 presents the frequency of DS-AML/MDS in children >4 years old from previous reports [1,2,8,16,29–31]. In BFM 87/BFM93, there were three such patients among 40 patients with DS-AML [31]. These three patients were 12, 15, and 16 years old at diagnosis, their FAB classification was M0, M2, and M4, and their white blood cell count at diagnosis was 2,600/μL, 22,600/μL, and 1,400/μL, respectively. The 12-year-old girl died from sepsis after four weeks of consolidation therapy; the other two patients were not treated [31]. In the CCG-2861 and CCG-2891 studies, three patients were reported to be >5 years old [16], two of whom died of disease and one from toxicity. On the AML99-Down protocol, there were two patients >4 years old (one being the present patient) [8]. A 4-year-old boy with AML FAB-M5a who failed to obtain complete remission after two courses of induction therapy and received cord blood stem cell transplantation was, at writing, still alive [32].

To date, there are only a few individual case reports of children >4 years old [32,33]. For DS patients, immunologic disorders, congenital heart disease, and other factors possibly caused disease-related and treatment-related mortality. Considering the high incidence of therapy-related mortality, overtreatment should be avoided.

No alterations in *GATA1*, *FLT3*, *MLL*-PTD, *NRAS*, or *RUNX1* were found in our patient, suggesting that he belongs to a subgroup, among older DS-AML patients, with good prognosis. Because the prognostic factors for DS-AML are still unknown, particularly in older children, further data accumulation is needed.

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PHN who have insufficient pain relief with standard analgesics and do not tolerate or are contraindicated to tricyclic antidepressants. The model structure allowed for differences in costs, utilities and transition probabilities between the initial 30-day run-in period and maintenance therapy and also took account of other medications that are added in alongside gabapentin/lidocaine plaster and those received by patients discontinuing gabapentin/lidocaine plaster. Most transition probabilities were based on clinical trials identified through a systematic literature review. Missing data, including resource utilization, were obtained from a Delphi panel and cost data from official price lists and tariffs. Utilities derived from a published study using the Health Utilities Index were adjusted for age and were supplemented and validated by the Delphi panel. **RESULTS:** The total cost for lidocaine plaster was £958 per patient, compared with £789 for gabapentin, although lidocaine plaster generated 0.292 quality-adjusted life-years (QALYs), compared with 0.248 for gabapentin. Lidocaine plaster therefore cost £3767 ($7370; 95% confidence interval: dominant, £13,415) per QALY gained relative to gabapentin. Probabilistic sensitivity analysis demonstrated that we can be 98.7% confident that lidocaine plaster is cost-effective relative to gabapentin at a £20,000/QALY threshold and 65% confident at a £5000/QALY threshold. Scenario analyses and extensive one-way sensitivity analyses on all parameters including the time horizon confirmed the robustness of the results. **CONCLUSION:** The lidocaine 5% plaster is a highly cost-effective treatment for PHN in Scotland.

## PODIUM SESSION II: RESPIRATORY DISORDERS

**RS1**

### COST EFFECTIVENESS OF QUANTIFERON IN SCREENING FOR TUBERCULOSIS INFECTION IN CLOSE CONTACTS IN JAPAN

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**OBJECTIVES:** QuantiFERON-TB Gold assay (QFT-G) is a recently approved blood test for detection of tuberculosis (TB) infection. The advantages of tuberculin skin test (TST) are; improved specificity, unaffected by prior BCG vaccination, and single patient visit. The aim of this study was to examine the cost effectiveness of using QFT-G as a screening test followed by the standard treatment of TB infected patients after exposure in close contacts in Japan. **METHODS:** We used a Markov model to compare the cost and effectiveness of QFT-G alone, TST alone, and TST followed by QFT-G using a hypothetical adult cohort (20 years old) of close contact patients with sputum-smear-positive TB patients. Main outcome measure was incremental cost per Quality adjusted life year (QALY) gained during lifetime. In the model, costs and effectiveness of each screening test, further chest X-ray examination, prophylaxis after identifying as infected patients, and treatment for overt tuberculosis patients who is not identified by screening or suffer from the disease even after prophylaxis were considered and estimated with published literatures. Both deterministic one-way and probabilistic sensitivity analysis were performed. **RESULTS:** TST alone was the most expensive and the least effective alternative. The mean QALYs for QFT-G only strategy was slightly higher than that for TST followed by QFT-G strategy (27.0565 vs. 27.0564). Total costs from the societal perspective were higher on average for the TST followed by QFT-G strategy ($120.9) than the QFT-G alone strategy ($112.9). The incremental cost effectiveness ratio for QFT-G alone strategy dominated. The results were robust in deterministic one-way sensitivity analysis while the acceptability curve showed that the QFT-G alone strategy has 99.6% chance of being cost effective at $50,000/QALY gained threshold. **CONCLUSION:** QFT-G alone strategy is the least expensive and the most effective in screening the TB infection after exposure in close contacts in Japan.

**RS2**

### INCREMENTAL COSTS ASSOCIATED WITH ANTIBIOTICS PRESCRIBED FOR COMMUNITY ACQUIRED PNEUMONIA

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**OBJECTIVES:** The purpose of this study was to estimate the total and incremental costs associated with antibiotic treatments for community acquired pneumonia (CAP). **METHODS:** Persons over age 18 were identified in the Marketscan databases July-December 2004. We identified CAP episodes for patients with claims for ICD9 codes 481.XX–486.XX, plus the most frequent antibiotics prescribed as initial treatment for an episode. Logistic regression estimated a propensity score for each patient; which was the predicted probability of using telithromycin. Patients were then matched according to this probability. Then exponential conditional means models (ECM) were specified, controlling for variables that were still significantly different after the propensity score matching. These models allowed the incremental costs to be estimated for treatment of telithromycin relative to other antibiotics. **RESULTS:** The most common initial antibiotic treatments compared to telithromycin for CAP (n = 187 CAP episodes) were amoxicillin, azithromycin, clarithromycin, levofloxacin, moxifloxacin, and a group of all other antibiotics. The mean length of a CAP episode was from 11.5 to 16.5 days. Mean total expenditures among the episodes was $897, with a range of $530 to $1175. Multivariate ECM models were fitted and showed significant incremental cost reductions per episode associated with telithromycin relative to: amoxicillin (–$283, $p = 0.002$), azithromycin (–$686, $p < 0.001$), levofloxacin (–$411, $p < 0.001$), and moxifloxacin (–$484, $p < 0.001$). The difference between telithromycin and clarithromycin (–$52) was not significant. **CONCLUSION:** The results of this study show that the costs differed among episodes of CAP by the initiating antibiotic. Use of propensity score matching and ECM regression controlled for intra-episode differences, so the incremental costs differences may be attributed to factors such as length of episode. The costs of CAP can be substantial and it is important to note that the initiating antibiotic may affect those costs.

**RS3**

### THE HEALTH STATUS BURDEN OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) IN A U.S. MEDICARE POPULATION: FINDINGS FROM THE MEDICARE CURRENT BENEFICIARY SURVEY

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**OBJECTIVES:** With increasing longevity among the elderly, it is important to better understand the impact of chronic disease on general health status. The objective of this study was to quantify the health status burden of COPD for a nationally repre-

Analyses were conducted from both the societal and health care system perspective. Sensitivity analyses were performed. RESULTS: Reduction in disease outcomes, disease sequelae and cost-of-illness by health state was observed in the time period post-Prevnar®, across all age groups. The total cost of the vaccination program to the Canadian health care system (including herd immunity effects), from a payer perspective amounted to $74,682,790; this decreased to $46,197,274 from a societal perspective. The total number of illnesses avoided was 86,164. The incremental cost-effectiveness ratio (ICER) was $28,551 and $17,661 per additional QALY from the health system and societal perspectives, respectively. When herd immunity effects were excluded from the analysis, the ICER increased to $166,560 and $115,995 per QALY, respectively. Sensitivity analysis indicated that total cost and ICER results were most sensitive to changes in the epidemiology and cost of otitis media. However, these changes did not considerably impact the results, indicating a robust model. CONCLUSION: Consistent with previous findings, vaccination with Prevnar® is cost-effective. Administration of Prevnar® results in a substantial reduction in pneumococcal disease in vaccinated children and unvaccinated adults.

**PIN19**

**COST-EFFECTIVENESS OF GARGLING FOR PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS**

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OBJECTIVES: To investigate the cost effectiveness of gargling to prevent upper respiratory tract infections (URI) from a societal perspective. METHODS: The effectiveness of gargling for preventing URI has been demonstrated in a randomized controlled trial in which the participants recorded the frequency of gargling, incidence and severity of URI and duration of daily medicine. Costs of gargling, visiting physicians, medicine, and lost productivity were considered. The cost of gargling was estimated as the opportunity cost of the time required. The utility of severe and moderate URI was also considered. Average costs and utility during 60 days of observation in the trial were estimated as the sum of the average daily cost and utility of the participants remaining staying in the trial. The incremental cost effectiveness ratio (ICER) of gargling when compared with the absence of gargling was calculated, and bootstrap sampling generated an acceptability curve. RESULTS: The estimated unit cost of gargling was 49.2 yen. Assigned participants gargled 4.5 times per day on average. The gargling group had higher costs and utility than the group that did not gargle. The incremental cost and effectiveness for 60 days were 4750 yen and 0.43 quality-adjusted life days respectively. The gargling group required 8020 yen more for gargling, but saved 3270 yen by preventing URI for 60 days. This showed that the ICER of gargling was 4.07 million yen/QALYs (34,400 US$/QALYs). The acceptability curve showed 67.1% was less than 6 million yen/QALYs, and 88.7% less than 12 million yen/QALYs. CONCLUSION: Although it can prevent URI, gargling is more costly than not gargling because the cost of gargling exceeded the savings derived from URI prevention. However, the ICER of gargling was comparable with that of other widespread medical technologies.

**PIN20**

**PHARMACOECONOMIC ANALYSIS OF SEVERE COMMUNITY-ACQUIRED PNEUMONIA TREATMENT**

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OBJECTIVES: Selection of the most cost-effective treatment regimen of severe community-acquired pneumonia. METHODS: Direct medical expenditure on courses of treatment with levofloxacin and ceftriaxone were evaluated during this trial. "Cost minimization" analysis was chosen as a pharmacoeconomic method, and a "lost opportunities" index was calculated when using less cost-effective drugs. RESULTS: Direct medical costs in the group of patients who received levofloxacin amounted to 16,097.99 rubles, and in the group of patients who were treated with ceftriaxone they totaled 32,573.47 rubles per patient. They were made up of levofloxacin and ceftriaxone antibacterial drug treatment costs and the costs of patients' hospital stay. The cost of the drug treatment course amounted to 3,997.99 rubles for the first group (levofloxacin) and to 19,073.47 rubles for the second group (ceftriaxone); the cost of hospital stay amounted to 12,100 rubles and 13,500 rubles respectively. In the breakdown of expenditure on treatment of community-acquired pneumonia with levofloxacin, patients' hospital stay accounted for 75% of expenses, whereas drug treatment accounted for only 25% thereof; when treating with ceftriaxone, the expenditure on patients' hospital stay amounted to 40% and that on drug treatment—to 60%. The "lost opportunities" index equaled one and thus indicated that when using a more cost-effective drug (levofloxacin) for the treatment of one patient compared to a less cost-effective drug (ceftriaxone) it is possible to theoretically treat an additional patient, taking into account the difference in the costs of treatment with the drugs compared, provided the profile of antibiotic resistance is congruent with that under the conditions of the clinical study used herein. CONCLUSION: Antibacterial treatment of severe community-acquired pneumonia with levofloxacin is more cost-effective, enabling the reduction of costs by 16,475 rubles per patient compared to treatment with ceftriaxone owing to lower expenditure on drugs.

**PIN21**

**THE CLINICAL AND ECONOMIC BURDEN OF COMPLICATED SKIN AND SKIN STRUCTURE INFECTIONS DUE TO *STAPHYLOCOCCUS AUREUS*: FINDINGS FROM A NATIONAL DATABASE**

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OBJECTIVES: Complicated skin and skin structure infections (cSSSIs) are a common complication among hospitalized patients. There are limited national data on the costs of cSSSIs due to *Staphylococcus aureus*, a common hospital-acquired pathogen. METHODS: This retrospective cohort study used data from the 2004 Health care Cost and Utilization Project Nationwide Inpatient Sample (HCUP-NIS). Patients with *S. aureus* cSSSIs were identified based on ICD-9-CM diagnosis codes and compared to patients without skin infections. Excess mortality, length of stay (LOS), and costs were estimated for both groups. Multivariate models (with log transformation) were used to adjust costs for potential confounding factors, including age, gender, mortality, hospital region, and comorbidity. RESULTS: We identified 55,585 hospitalized patients with cSSSIs due to *S. aureus*. The comparison cohort consisted of 7,618,776 patients without skin infections. The mortality rates were similar for the *S. aureus* cSSSI and comparison cohorts (3.9% and 2.0%, respectively). For com-

# 5）遺伝子検査ネットワーク

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5. 遺伝子診断を取り巻く最近の動向

# 5）遺伝子検査ネットワーク

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〔KEYWORDS〕 遺伝子検査，ネットワーク，遺伝子診断

## 遺伝子診断実施の現状

近年の遺伝医学研究の進歩に伴い，様々な疾患ならびに健康状態への遺伝子の関与が明らかにされ，遺伝子診断が可能な疾患も増加してきている．しかし，その一方で，遺伝子診断を行うに際して，以下のような技術的・経済的な問題点が生じてきている．

（1）個々の遺伝子疾患は頻度が少なく，また，遺伝子変異のパターンも多様性に富むため，診断・検査の1件当たりの解析費用が高価となり，コマーシャル・ベースに乗りにくい．

（2）疾患遺伝子が明らかになった直後は，研究的に遺伝子解析を行う施設も多いが，研究期間の終了や，解析を行う研究者の異動によって，遺伝子解析が中断されることが多い．したがって，現時点でどの遺伝子検査をどの施設が行っているかの情報を得ることが困難．

（3）症例の病態に応じて，類似の他の遺伝子疾患との鑑別が必要となるが，それぞれが稀少な疾患であることが多く，特に診療経験が少ない疾患の場合は，疾患の絞り込みが難しく，結果として必要以上の遺伝子検査が実施されることがある．

（4）類似の病態を示す遺伝子疾患がある場合には，同時に複数の遺伝子解析を行う必要性が出てくるため解析費用が高価となるほか，必ずしも同一施設での解析が可能ではないことから，複数の施設に解析を依頼する必要が出てくる．

（5）疾患遺伝子や遺伝医学に関する知識が十分でない場合には，遺伝子解析結果の解釈が困難となる場合もある．

（6）生殖細胞系列の遺伝子検査で保険診療が認められているのはごく一部にすぎないことから，検査会社に依頼して検査を行うに際しては自費での高額な費用負担が生じる．一方，研究レベルでの検査依頼を行う場合には，解析結果受理までにしばしば長い時間がかかる．

（7）それぞれの遺伝子疾患における遺伝子変異や遺伝子欠失の状況とその表現型をリアルタイムに知ることのできるデータベースがほとんどないため，日本国内における日本人の遺伝子疾患の頻度や診断の現況を知ることが困難である．

これらは遺伝医療にかかわる研究者・医療者の長年にわたる問題であり，状況の改善が望まれてきた．こうしたなかで，最近，いくつかの研究班や研究者・医療者などからなるNPOが，これらの解決をはかるべく活動を開始している．本稿では，それらの動向も含めて，遺伝子検査ネットワークの現況を報告する．

## いでんネット

「いでんネット（臨床遺伝医学情報網）」（http://

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2）MATSUBARA Yoichi　東北大学大学院医学系研究科遺伝病学
3）KOSAKI Kenjiro　慶應義塾大学医学部小児科

www.kuhp.kyoto-u.ac.jp/idennet/)は，京都大学医学部附属病院遺伝子診療部が運用している臨床遺伝医学に関する情報サイトである．内容としては，

（1）遺伝相談施設(カウンセラー)情報(施設新規登録・変更も含む)
（2）ヒトGermline遺伝子・染色体検査オンラインデータベース(付：エビデンスに基づいた日本人のための遺伝子診断ガイド2005)
（3）遺伝子治療施設情報(2001年国内の遺伝子治療・臨床研究開発状況のまとめ)
（4）遺伝医学・遺伝医療に関するガイドライン(付：京都大学遺伝子解析遵守事項・遺伝子解析計画書書式)

などからなる．そのうち，「遺伝相談施設(カウンセラー)情報」は，全国の遺伝子診療施設の情報を掲載したもので，臨床遺伝専門医が遺伝相談に応じている施設の情報が，一般向けならびにあらかじめ登録を行ってIDとパスワードを付与されている医療関係者向けに分けて掲載されている．「いでんネット」の特徴は，これらの情報を登録した施設の担当者自らが編集・更新することができる点にある．遺伝カウンセリング担当者の異動や遺伝カウンセリング実施日の変更が起きた場合には，施設担当者が情報をアップデートすることにより，最新の情報を掲載することができる．

また，「ヒトGermline遺伝子・染色体検査オンラインデータベース」は，ヒトの生殖細胞系列の遺伝子検査を行っている研究施設の情報が，解析可能な遺伝子，遺伝子解析の方法，解析の費用，資料の送付先など細部にわたって細かく登録されている．このサイトも，遺伝子解析施設の担当者自らがアップデートすることが可能である．

しかし，定期的にアップデートのお願いを送付しているにもかかわらず，上記のいずれの情報もアップデートされていない施設が少なくないのも現状である．また，現時点では，臨床検査会社が受託している遺伝子解析情報も掲載されていない．

## GeneTest

「GeneTest」(http://www.genetests.org/)はNIH(National Institutes of Health)の予算を得てWashington大学により運営されている遺伝子検査に関する情報サイトである．内容としては各種遺伝子検査を行っている検査施設に関する情報のほか，GeneReviewsとして遺伝子疾患に関する最新の総説や自助団体に関する情報なども得ることができる．日本からも8大学・研究施設がエントリーしている．

それぞれの総説を執筆する責任者が定期的に内容をアップデートしているため，内容が新鮮で，遺伝子診断に関する情報も信頼性の高いものとなっている．

なお，上記GeneReviewsは，GeneReviews Japan (http://grj.umin.jp/)として一部が日本語訳されて公開されている．

## オーファンネット・ジャパン

「オーファンネット・ジャパン」は全国の稀少遺伝性疾患に対する遺伝子検査を提供する研究室をネットワーク化し，検査を依頼する医療機関との間のコーディネートを行うNPO法人である．遺伝子検査を提供する研究室の精度管理や，遺伝子検査の結果報告に関する標準化の作業，遺伝子検査にかかわる倫理・遺伝カウンセリングについて医療機関への教育・啓発も行う役割を担っている．依頼元医療機関からの検体の集荷や検査先研究室への検体の送付は，医療機関と契約している検査会社が請け負う形を取っている(図)．

現在，東北大学，信州大学，慶應義塾大学，東北文化学園大学，東邦大学，東京女子医科大学，島根大学，京都大学，国立成育医療センター，エスアールエルなどのメンバーにより設立準備中であり，設立後は全国の医療施設から多くの遺伝子検査が本NPOを通じて行われることになるであろう．

## 遺伝子診断臨床応用支援機構

2006年10月24日に設立されたNPO法人で，慶應義塾大学の小児科専門医のグループを中心に，熱変性高速液体クロマトグラフィー法に基づく効率的な遺伝子診断法の開発，国内外の臨床遺伝専門医からの要望に応じた各種遺伝子診断の実施，効率的な遺伝子診断に必要な技術・情報の公開 http://www/dhplc.jp，遺伝子診断技術者の教育・育成支援，遺伝子診断の意義についての社会啓発，国内外で遺伝子診断を行う施設の連携などを行っている．

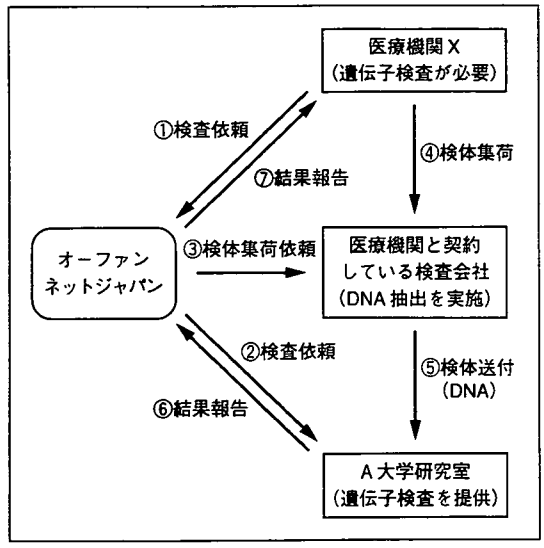



図　オーファンネット・ジャパンにおける検体処理(案)

## 研究班の活動

厚生労働科学研究費補助金(子ども家庭総合研究事業)による小児先天性疾患および難治性疾患における遺伝子診断法の標準化と国内実施施設の整備に関する研究班(班長：国立成育医療センター研究所小児思春期発育研究部長 緒方勤)では，全国の希少疾患症例のコンサルテーションシステムの構築を行っており，小児内分泌疾患や小児遺伝子疾患の診療に携わる医師からの相談に専門医が応じ，必要となる遺伝子診断などのコンサルテーションを行う体制作りが進められている．

厚生労働省精神・神経疾患研究委託費による精神遅滞リサーチ・リソースの拡充と病因・病態解明を目ざした遺伝学的研究班(班長：国立精神・神経センター神経研究所疾病第二部部長 後藤雄一)では，原則として家系内に2名以上の精神遅滞(知能指数もしくは発達指数が70未満)のいる家系の解析を行っており，全国レベルでの遺伝学的診断サービスが開始されている．

文部科学省特定領域研究「応用ゲノム」ゲノム医学研究支援委員会の支援を受け検体を収集し，「応用ゲノム」ヒトゲノム構造解析ツールとしての高密度ゲノムDNAマイクロアレイの開発と応用(東京医科歯科大学難治疾患研究所 稲澤譲治教授，井本逸勢准教授)研究班により作成されたgenome disorder arrayを用いた解析が，原因不明の多発奇形精神遅滞患児を対象に行われている．本CGHアレイ実用化プロジェクトには，先天奇形の診断に習熟した臨床遺伝医が属する医療機関で，倫理委員会での研究承諾が得られた14施設が現在のところ参加している．高密度ゲノムDNAマイクロアレイの概要は，東京医科歯科大学難治疾患研究所遺伝疾患研究部門・分子細胞遺伝のCGH data baseホームページ(http://www.cghtmd.jp/)に公開されている．

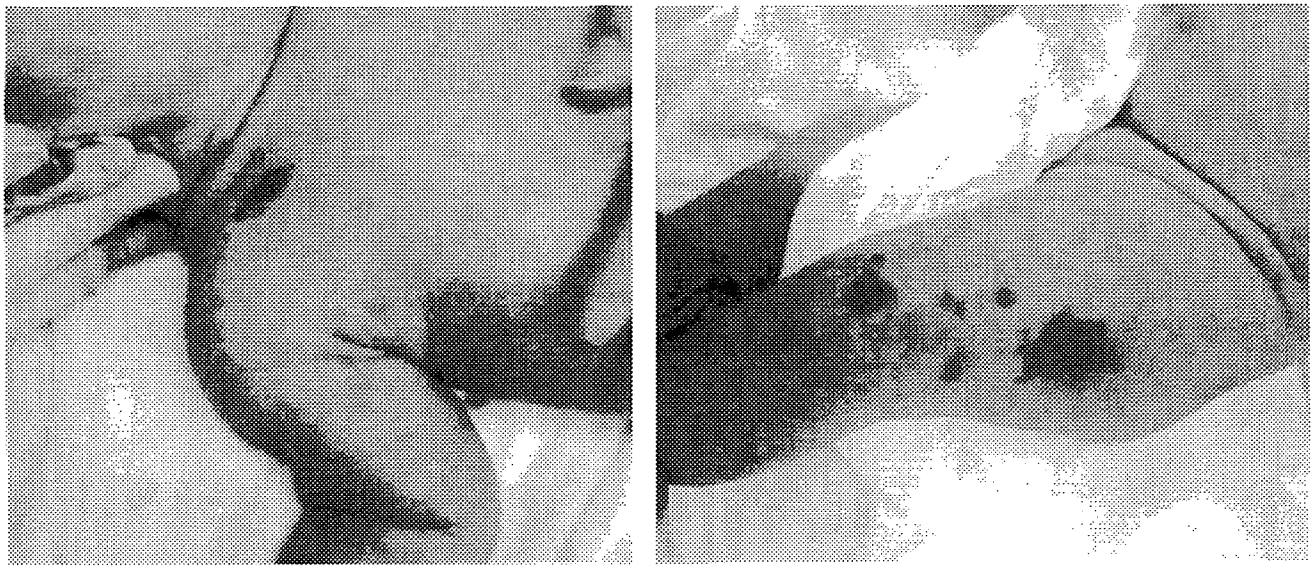
上記に紹介したのは，公的研究費により行われている全国ネットの遺伝子解析研究の一部であり，ほかにも様々な疾患・病態を対象にした研究が多施設共同研究の形態で行われている．

## おわりに

本稿では，遺伝子検査のネットワークを中心に，日本での現状ならびに今後の動向について概説した．その一方で，遺伝子解析結果のデータベースの構築も進んでおり，遺伝子検査の精度管理の提案もなされ，その具体的な方法を巡って意見が交わされている．また，遺伝子検査のコストダウンや保険収載の問題など，未解決の課題は少なくないが，よりよい遺伝子医療の実現を目指して関係者の努力が続けられている．

case 15 PART.4 皮膚以外の症状が主体の症候群（皮膚症状が高率に出るもの）

# Maffucci 症候群



生後１カ月，女児．2002 年９月初診
右上前胸部に９×14 mm の，また左大腿部にも多発する血管腫を認めた．

生後１カ月の女児．１カ月健診時に右上前胸部に単発の，左大腿部に多発する血管腫を認めた．手背部ならびに足背部はやや肥厚しているが，１カ月児としては正常範囲であった．妊娠・分娩歴に異常なく，身体発育ならびに精神神経発達にも問題はない．ほかに小児科学的診察でも異常を認めない．家系内に血管腫や内軟骨腫を有する者はおらず，若年発症の腫瘍性病変に罹患している者もいない．

## 臨床診断と経過

本症例は，月齢３カ月ならびに８カ月時にも乳児健診を行っているが，血管腫を除き，小児科学的診察では特記すべき異常を認めなかった．血管腫は消褪傾向に乏しく，海綿状血管腫の形態を呈していた．

多発する海綿状血管腫では，Maffucci 症候群ならびに blue rubber bleb nevus（青色ゴム乳首様母斑）症候群などが疑われるが，形状より blue rubber bleb nevus 症候群は否定的であったため，まだ内軟骨腫は認められなかったが，将来的に悪性腫瘍が発生する可能性のあることが知られている[1～3] Maffucci 症候群を念頭に置いてフォローを開始した．

また，本症候群では，第１番染色体逆位を伴った症例の報告もされていることから，本症例にも両親の同意を得て染色体検査を施行したが，46,XX の正常女性核型であった．

本症例は生後８カ月の受診を最後に，以後のフォローは残念ながら行われていない．

| 同義語 |
| --- |
| Maffucci 症候群 |
| dyschondroplasia with hemangiomata |

## 本症例のポイント

Maffucci 症候群は，1881 年に Maffucci により最初に報告された，多発性血管腫を伴った多発性内軟骨腫（multiple enchondromatosis）を主徴とする症候群である[2]．発生頻度はきわめて稀であり，性差もみられない．ほとんどが遺伝性を認めない散発例である．

多発性内軟骨腫症のみを呈するものは，Ollier 病として知られ，いずれの疾患も同様の分子生物学的な発症機

転に基づくものと推定されている[4].

2002年にHopyanらがPTH/PTHRP I型受容体遺伝子(*PTHR1*)の変異が多発性内軟骨腫患者6名中2名に認められたと報告したが[5], Rozemanらは2004年にヨーロッパ3カ国のOllier病28例, Maffucci症候群3例のPTHR1蛋白発現やシーケンス解析を行ったが異常は認められなかったと報告しており[6], いまだに病因は明らかではない.

出生時から1歳の間に多発性内軟骨腫あるいは多発性血管腫を呈する症例も約25%認められるが, 平均発症年齢は5歳で, 大部分が思春期までに発病する. また, 通常は血管腫の発生が内軟骨腫の発生に先行する. さらに, 新生児期に手背部や足背部の軟部組織の腫脹が認められ, 診断に有用とされる. 精神神経発達は正常で, ほかに先天的な異常は伴わない.

骨格症状は, 多発性内軟骨腫が主たるもので, 片側性のものと両側性のものとがほぼ同頻度である. 両側性のものでも, 個々の病変は通常は非対称性で不規則である. 内軟骨腫の発現部位は手89%, 足61%, 脛骨・腓骨52%, 大腿骨36%, 上腕骨34%, 橈骨・尺骨29%, 肋骨27%, 骨盤25%, 肩甲骨20%, 頭骨18%の頻度と報告されている[7]. ほかに, 病的骨折や長管骨の変形, 脚長差, 脊柱側彎, 低身長などを認めることもある. 内軟骨腫は外科的手術の適応となるが, 多発性で再発もあることから根治は困難である.

皮膚症状は, 多発性血管腫が主たるもので, 海綿状血管腫が多いが毛細血管腫を認めることもある. 自然消褪傾向には乏しい. 血管腫の発現部位は手57%, 足41%, 腕39%, 下肢38%, 体幹29%, 頭頸部25%の頻度と報告されている[7]. とくに指, 拇指球部, 足底, 踵部などに生じることが多い. 口腔粘膜や消化管, 神経系組織, 骨格筋, 骨などにも発現することもある. 海綿状血管腫に連なる静脈では連珠状の膨隆を認めるほか, 血栓形成や石灰化を認めることが多い.

ほかにリンパ管腫, リンパ管拡張症, 静脈瘤, 静脈拡張症などを合併することもある.

厳重な注意が必要な合併症は悪性腫瘍の発生である. 内軟骨腫の軟骨肉腫化の確率がきわめて高いので, 注意を要する. ほかに星状細胞腫, 神経膠腫, 神経鞘腫, 下垂体腺腫, 甲状腺癌, 消化器腺癌, 膵癌, 胆管癌, 卵巣腫瘍, 血管芽細胞腫, 血管肉腫, 悪性リンパ管腫, 扁平上皮癌, 多発性内分泌腫瘍症1型(MEN1), 急性リンパ性白血病などの合併が知られている. したがって, 頭部・腹部を中心とした定期的検査が必須となる.

Maffucci症候群は腫瘍好発先天異常症候群のひとつであることから, 乳幼児期で内軟骨腫を認めない症例でも, 多発性血管腫がみられた場合には, 本症候群を念頭に置いて定期的な検査・診断フォローアップを行うことが必要であると考える. 本症例でも, Maffucci症候群である可能性を両親に告げたうえで, 定期的な受診を勧めたが, 生後8カ月の乳児健診受診を最後に来院なく, フォローが中断されている. いたずらに両親を心配させることなく, 定期的かつ長期の経過観察が必要であることを十分な時間をかけて説明できていたかどうか, 反省させられた症例である.

## 文献

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## Key words

Maffucci症候群, 多発性内軟骨腫, 多発性血管腫, multiple enchondromatosis, Ollier病

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5. 遺伝子診断を取り巻く最近の動向

# 1）遺伝学的検査に関連する指針

## ガイドライン，インフォームド・コンセント

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## ガイドライン，インフォームド・コンセント

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〔KEYWORDS〕 ガイドライン，倫理，インフォームド・コンセント

## はじめに

近年，ゲノム解析の進歩や遺伝学的検査技術の向上に伴い，診療の場における遺伝学的検査の需要は高まってきている．遺伝学的検査には遺伝性疾患を診断する目的で行われる遺伝子検査，染色体検査，蛋白質や代謝産物の測定などが含まれる．

一般に臨床で実施されている広義の遺伝子検査は大きく以下の3つに分けられるが，本稿の対象である「遺伝学的検査」に含まれるのは，③のみである．[1)2)]①の核酸検査と②の遺伝子検査は次世代へ受け継がれるという性質もなく，診療上も有効な情報となるため，通常行われている臨床検査の体制で対応できる．また，これらを対象とする研究においても本人へのインフォームド・コンセントが十分に行われることで混乱はないものと考えられる．

①細菌・ウイルスなどの外来性遺伝子の検出（核酸検査：nucleic acid-based testing）．

②腫瘍組織など後天的に一部の体細胞に起こった遺伝子変異の検出（遺伝子検査：gene-based testing）．

③体内のすべての細胞に共通で次世代へ受け継がれる生殖細胞系列に起こった遺伝子変異の同定（遺伝学的検査：genetic testing）．

③を含め，遺伝学的検査においては，血縁者間で一部共有されており，次世代にも受け継がれる可能性があることから，本人だけでなく家族全体の問題になることが考えられる．疾患によっては発症前診断，出生前診断やそれを目的とした保因者診断ができるが，なかには治療法や予防法のない疾患の発症前診断，重篤という判断が難しい疾患の出生前診断，知る権利や知らない権利，遺伝学的検査実施の時期，代諾の問題など倫理的・法的・社会的諸問題（ethical, legal and social issues；ELSI）への対応に迫られる場面も多い．

詳細は個々のケースによるにせよ，多くの配慮が必要な生殖細胞系列の遺伝学的検査について，遺伝医療に携わる医療者・研究者が共通認識を持ち，検査が適切に行われるために，日本でもいくつかのガイドラインが作成された．遺伝学的検査に関連する内容を含む主要な4つについて紹介する．

また，遺伝学的検査の目的や被検者の状況によってインフォームド・コンセントの内容も異なり，対応が複雑になっている．ガイドラインにおけるインフォームド・コンセントと遺伝カウンセリングについての記述を最後にまとめたいと思う．

### 1. 診療におけるガイドライン：「遺伝学的検査に関するガイドライン」遺伝医学関連10学会2003年策定

（URL：http://www.mext.go.jp/a_menu/shinkou/seimei/genomeshishin/05062701/003/001.

1）ONO Akiko　京都大学大学院医学研究科社会健康医学系専攻 遺伝カウンセラー・コーディネーターユニット 遺伝カウンセラーコース
2）KOSUGI Shinji　同・コースディレクタ


0485-1420/07/¥250/論文/JCLS

pdf)

このガイドラインでは「生涯変化しない個人の重要な遺伝学的情報が扱われるため，検査実施時のインフォームド・コンセント，個人の遺伝学的情報の保護，検査に用いた生体試料の取り扱い，検査残後の遺伝カウンセリングなど慎重に検討すべき問題が存在している」とし，それぞれの項目について詳細に記載している．各項目とその概略を挙げる．

**1）対象**

このガイドラインは生殖細胞系列における遺伝子変異もしくは染色体異常に関する検査，あるいはそれらに関連する検査を対象とし，体細胞変異や細菌・ウイルスなどの病原体の核酸検査，親子鑑定などの法医学的なDNA検査は対象外である．

**2）遺伝学的検査の実施**

遺伝学的検査は臨床的・遺伝医学的に有用と考えられる場合に検査を受ける人(被検者)を十分に支援できる体制のもとで実施しなければならないこと，被検者やその血縁者・家族の人権を尊重し，結果から生じる遺伝的差別がないように努め，必要に応じて臨床心理的，社会的支援を受けられるようにすることなどが記載されている．さらに試料は当該検査の目的以外に使用してはならないこと(のちに新たな検査に用いる場合は改めてインフォームド・コンセントが必要/研究に用いる場合は後述するヒトゲノム・遺伝子解析研究に関する倫理指針の遵守)や試料の保管，個人識別情報および検査結果としての個人遺伝学的情報の保護について述べられている．

検査を実施しない可能性がある場合として，被検者が望んでもその検査が倫理的・社会的規範から妥当でないと判断されるときや，治療法・予防法が確立されていない成人期以後に発症する疾患の小児期の遺伝学的検査などが挙げられている．検査実施時のインフォームド・コンセントについても詳細な記載があるが，これに関しては後の項で取り上げる．

**3）結果の開示**

結果開示に際しては被検者の「知る権利」および「知らない権利」を尊重すること，被検者の承諾なしに第三者へ結果開示することは血縁者であっても許されないこと，本人の同意があっても雇用者や保険会社，学校が結果にアクセスすることがあってはならないなどの記載がある．本人の同意がある場合，血縁者への結果開示を行うことができるが，同意が得られない場合でも，開示ができる条件が6項目示されている(血縁者における重大な疾患の発症予防や治療に役立つ情報として利用できるなど)．ガイドラインではこれらの項目がすべて満たされるとき，倫理委員会等の判断のもとに開示ができるとしている．

**4）遺伝学的検査と遺伝カウンセリング**

遺伝学的検査は十分な遺伝カウンセリングを行った後に実施するとしたうえで，遺伝カウンセリングは十分な遺伝医学的知識・経験をもち，遺伝カウンセリングに習熟した臨床遺伝専門医などにより被検者の心理状態をつねに把握しながら行われるべきであり，必要に応じて精神科医，臨床心理専門職，遺伝看護師，ソーシャルワーカーなどの協力のもとチームで行うことが望ましいとしている．また，検査実施後も必要に応じて行われるべきとされている．

**5）目的に応じた遺伝学的検査の留意点**

具体的に，①発症者を対象とする遺伝学的検査，②保因者の判定を目的とする遺伝学的検査，③発症予測を目的とする遺伝学的検査(発症前検査，易罹患性検査，家族性腫瘍に関する検査)，④薬物に対する反応性の個体差を判定することを目的とする遺伝学的検査，⑤出生前検査と出生前診断，⑥新生児マススクリーニング検査の6つの項目について検査実施の条件や配慮すべき点が挙げられている．

**2. 検査受託に関する指針：「ヒト遺伝子検査受託に関する倫理指針」社団法人日本衛生検査所協会2007年改正**

(URL：http://www.jrcla.or.jp/info/info/dna190401.pdf)

現在，検査会社に外部委託される遺伝子検査が増加している．検査会社においても遺伝子検査の意義・影響を認識し，検査受託から結果報告までの体制を整える必要がある．日本衛生検査所協会が制定したこの指針では「ヒト遺伝子検査は，被検者及びその血縁者の遺伝情報等を含む検査であることから，その取扱いによっては様々な倫理

的・法的・社会的問題が生ずる可能性があり，その実施に当たっては十分な配慮が求められる」としたうえで，①被検者やその家族および血縁者の人権の保障，②ヒト遺伝子検査の一次委託元を医療機関に限定，③医療機関における事前の十分な説明と被検者の自由意思による同意(インフォームド・コンセント)の確認，④個人情報の保護の徹底，⑤一般市民への宣伝広告の禁止，⑥適正な検査実施に向けた衛生検査所内の体制整備の6つを基本方針としている．ヒト遺伝子検査受託における遵守事項では，「衛生検査所が，医療機関からヒト遺伝子検査を受託するに当たっては，その特性に鑑み，倫理的・法的・社会的問題に対する十分な配慮が必要である．すなわち，ヒト遺伝子検査の中には治療に直結しない疾患の診断を目的としたものが含まれること，その検査結果が被検者個人にのみならず家族及び遺伝学的情報を共有する血縁者にも影響を与える可能性があること，検査の実施前にはインフォームド・コンセントが必要であること，検査によっては実施前後に遺伝カウンセリングが必要であること等を十分に認識することが必要である」とし，基本方針にのっとった項目が詳細に説明されている．

**3. 研究に関する指針：「ヒトゲノム・遺伝子解析研究に関する倫理指針」文部科学省・厚生労働省・経済産業省 2005 年改正**

(URL：http://www.mext.go.jp/a_menu/shinkou/seimei/genomeshishin/05062701.htm)

この指針はヒトゲノム・遺伝子解析研究において人間の尊厳および人権が尊重され，社会の理解と協力を得て研究の適正な促進が図られることを目的とし，保護すべき個人情報，研究者等の責務，提供者に対する基本姿勢や試料等の取扱いなどについて記載されている．インフォームド・コンセントの項目では，口頭説明とともに説明文書の交付が求められており，必要に応じて遺伝カウンセリングの機会を提供するとしている．

この指針では，診療において実施され，解析結果が提供者およびその血縁者の診療に直接生かされることが医学的に確立されている臨床検査およびそれに準ずるヒトゲノム・遺伝子解析は，医療に関する事項として対象としていない．診療で行われる遺伝学的検査については前述した「遺伝学的検査におけるガイドライン」などの遵守が求められる．しかし，実際には臨床的側面と研究的側面の両方を持つものもある．このような場合は両方のガイドラインに則して実施することが求められる．

**4. 個人情報保護に関する指針：「医療・介護関係事業者における個人情報の適切な取扱いのためのガイドライン」厚生労働省 2006 年改正**

(URL：http://www.mhlw.go.jp/topics/bukyoku/seisaku/kojin/dl/170805-11a.pdf)

2005 年，個人情報保護法が完全施行され，それに伴いこのガイドラインが告示された．ガイドラインの中の項目の1つに「遺伝情報を診療に活用する場合の取扱い」が挙げられている．遺伝情報は，本人の遺伝子・染色体の変化にも基づく体質，疾病の発症等に関する情報が含まれるほか，その血縁者にもかかわる情報でもあり，さらに生涯変化しないことから，これが漏洩した場合，大きな被害・苦痛となるおそれがある．そのため検査結果の取り扱いについては関連ガイドラインを参考とし，特に留意する必要があるとしている．また，検査に同意していても，検査結果が示す意味を正確に理解することが困難であったり，不安を抱く場合も多いとし，医療機関等が遺伝学的検査を行う場合には臨床遺伝学の専門的知識を持つ者による遺伝カウンセリングの実施など，本人および家族の心理社会的支援を行う必要があるとされている．

## 遺伝学的検査のインフォームド・コンセントと遺伝カウンセリング

「遺伝学的検査に関するガイドライン」において，インフォームド・コンセント時に求められている情報提供などの詳細を**表1**に示す．また，遺伝カウンセリングとは，遺伝性疾患の患者・家族またはその可能性のある人(クライエント)が生活設計上の選択を自律的に決定し行動できるよう，クライエントと遺伝カウンセリング担当者の信頼関係に基づいたコミュニケーションの中で遺伝医学に関する情報提供や心理社会的支援を行う医療