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研究課題名（和文）Genomic Profiling of Childhood Acute Leukemia in North of Iraq

研究課題名（英文）Genomic Profiling of Childhood Acute Leukemia in North of Iraq

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研究成果の概要（和文）：イラクにおける小児急性リンパ性白血病ならびに小児急性骨髄性白血病の計66例について次世代シークエンス(NGS)解析を行った。イラクからサンプルを輸送するために用いた濾紙（FTAカード）から抽出したDNAは、NGSに十分な質を備えていることを確認した。点変異、染色体コピー数変化、ならびに染色体構造変化を解析し、B細胞性ALLの89%において原因となる遺伝子変異を特定した。それらの頻度は、高頻度に認めたTCF3-PBX1融合遺伝子（22%）を除いて、他国における頻度と同等であった。これに加えて、非常に高頻度なRAS経路遺伝子の機能獲得変異（45%）も特定された。

研究成果の学術的意義や社会的意義
Through collaboration with Iraqi doctors, we could perform the molecular diagnosis of Iraqi childhood acute leukemia.
Our results showed the unprecedented high frequency of TCF3-PBX1 in Iraqi childhood ALL, and confirmed our previous finding of high RAS mutations in Iraqi childhood acute leukemia.

研究成果の概要（英文）：Diagnostic/remission (paired) samples from 66 Iraqi children with acute leukemia including, 55 acute lymphoblastic leukemia (ALL) cases, and 11 acute myeloid leukemia (AML) cases, were evaluated in Japan using next generation sequencing (NGS). Flinders Technology Associates (FTA) cards used to transfer samples from Iraq, were proved to be good enough to yield an adequate dried blood spot-derived DNA suitable for NGS analysis. Iraqi childhood ALL and AML samples were successfully analyzed. B-ALL represented 49 (89.1%) of ALL cases, and they were categorized into subsets according to the molecular driver, such as chromosomal aneuploidy, rearrangements, and point mutations. Several of these subsets were comparable to the results elsewhere in terms of frequency, except for the striking findings of a highly recurrent TCF3-PBX1 fusion gene of (22.4%) in B-ALL, also a highly frequent RAS signaling pathway mutations of about (45%) was disclosed in each of ALL and AML Iraqi childhood cohorts.

研究分野：Pediatric Oncology

キーワード：ALL　FTA　AML　Iraq　RAS　TCF3-PBX1

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Background: Definition of prognostic algorithms of pediatric acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), helps in stratifying patients. Our successful genetic analysis in Japan, using dried blood spots (DBS) of pediatric cases of acute leukemia, transferred from underdeveloped countries lacking molecular diagnosis, like Iraq, and further implementation of results in management of those patients, have motivated us to perform more comprehensive analysis.

２．研究の目的

Objectives: Using Flinders Technology Associates (FTA) filter papers, our aim was to detect the genetic prognostic markers for Iraqi pediatric ALL/AML through advanced DNA sequencing, by performing the next generation sequencing (NGS), on the DBS-derived DNA. Disclosure of detailed genetic data will improve diagnosis, management, and survival of enrolled cases. Moreover, results may reveal geographic/environmental impacts.

３．研究の方法

Methods: FTA cards were used to transfer the DBS of newly diagnosed children with ALL/AML, from several hospitals in Iraq to Japan (Shinshu University), and NGS was done in Nagoya University. The study protocol was approved by the Ministry of Health in Iraq, and the institutional review board of Shinshu University School of Medicine, as well as the institutional review board of Nagoya University School of Medicine. Five main pediatric oncology centers had participated in this study from Iraq, including Children's Welfare Teaching Hospital (CWTH) in Baghdad (the major referral center for childhood cancers in the country), Ibn Al-Atheer Children Hospital at Mosul (IAH), Basra Children's Specialty Hospital (BCSH) at Basra (the main center in southern part of Iraq), Hiwa Cancer Hospital (HCH) at Sulaymaniyah, and Jin pediatric hematology-oncology center (JPHOC) at Duhok. CWTH and BCSH are in Arab provinces, while HCH and JPHOC are in Kurdistan, the area inhabited by the Kurdish ethnicity in the northern part of Iraq. Patients in the above centers were treated according to the Medical Research Council in the United Kingdom (MRC-UK) protocols for pediatric acute leukemia, including Modified UKALL 2011 for ALL, and AML-MRC15 for AML. Written informed consent was obtained from patients or parents according to the guidelines of the Helsinki. We collected bone marrow (BM) samples at diagnosis (day 0) (tumor status), and BM samples at day 30 or 60 (remission status), from Iraqi patients aged ≤ 16 years, newly diagnosed with ALL, and AML, until December 2020. A total of 127 cases were recruited for the study from Iraq, 53 from CWTH, 26 from IAH, 25 from BCSH, 15 from HCH, and 8 cases from JPHOC. However, a total of 66 patients with paired samples were evaluated, 36 were from CWTH, 17 from BCSH, 8 from HCH, and 5 samples from JPHOC.

Few drops of blood from BM aspirate were applied to the FTA classic card (Cat No. WB120205, GE Healthcare UK Limited, Buckinghamshire, UK) at the 5 Iraqi hospitals. After the blood spots were dried for 1 hour at room temperature, the FTA cards were kept in a special FTA envelope in a refrigerator for several weeks, and then transported by airplane to Japan. Two mm disks (8 disks) were

punched out from the dried material on FTA cards using a sterile hole puncher (Harris Micro-Punch, Shunderson Communications Inc., Ottawa, Canada). Genomic DNA was extracted from DBS on samples of FTA cards and purified by the QIA amp DNA Blood Mini Kit (Cat. No. 56304, Qiagen, Ltd., Tokyo, Japan) according to the manufacturer's instructions. After the extraction of DNA, it was measured using Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Life Technologies, USA) according to the manufacturer's instructions.

We performed whole-exome sequencing (WES), targeted sequencing-based structural variation detection, and whole-genome sequencing (WGS).

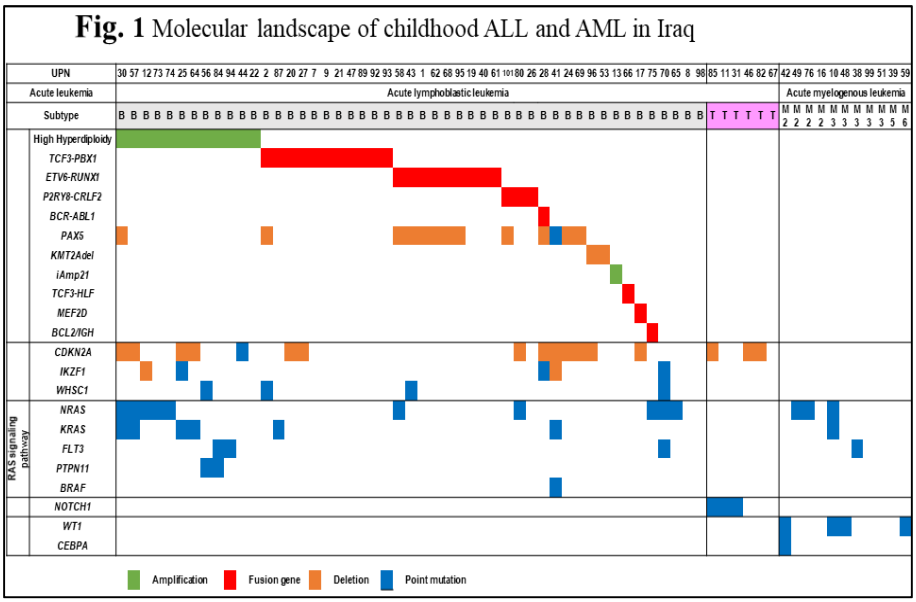
4．研究成果

Results: DBS-derived DNA samples of Iraqi children with ALL were comparable to those fresh DNA samples of Japanese children with ALL, in terms of sequencing coverage. For the matched remission-status samples especially those with diluted BM, we consumed more DBS disks (up to 40) to increase the yield of the DNA. A minimum of 20% tumor cells estimation based on mutations in a tumor sample is needed for a successful NGS, however >50% is desirable for good analysis, and at least 50 ng of DNA is needed. Notably, FTA derived-DNA tumor cells percentage was ranging from 60%-80%, in our study. Of interest, this is the first time to use NGS for studying the landscape of mutations in Iraqi childhood acute leukemia.

ALL cases, paired samples from 55 children with ALL from Iraq were evaluated including 49 (89.1%) precursor B (B-ALL), and 6 (10.9%) precursor T (T- ALL). The median age among the total ALL cases was 4.7 (1-13) years, with males to female's ratio of 2.1, and a median white blood cells count (WBC) of 22.4 (2.4-700) x10^9/l. The median age among B-ALL cases was 4.2 (1-13) years, with a ratio of male to female of 1.9, while the median age among T-ALL cases was 9.3 (3.5-12.8) years, and 5/6 of them were males. The median WBC in B-ALL, and T-ALL, was 16.4 (2.4-181) x10^9/l, and 280.5 (4.2-700) x10^9/l, respectively. The average age and WBC were significantly higher in T-ALL vs. B-ALL, with p-values of (0.007), and (<0.001), for age and WBC, respectively.

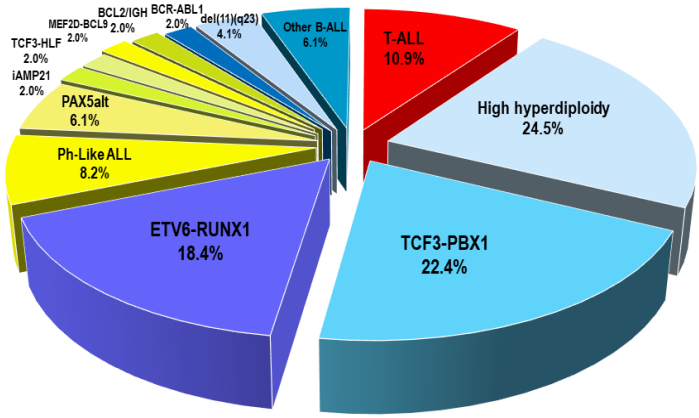
NGS results (**Fig. 1**) aid in identifying the molecular driver in B-ALL cases and thus categorizing them into several groups, including, high hyperdiploidy (HHD) (12, 24.5%), *TCF3-PBX1* (11, 22.4%), *ETV6-RUNX1*(9, 18.4%), *P2RY8-CRLF2* (3, 6.1%), *PAX5*-altered (*PAX5alt*) (3, 6.1%), and *del(11)(q23)* (2, 4.1%). In addition to 1 case

Fig. 1 Molecular landscape of childhood ALL and AML in Iraq



(2%) of each of *BCR-ABL1*, *MEF2D-BCL9*, *TCF3-HLF*, intrachromosomal amplification of chromosome 21 (*iAMP21*), Ph-like (*FLT3*), and *BCL2-IGH*. There were 3 (6.1%) remaining cases with unidentified driver referred as Other B-ALL (**Fig. 2**).

Fig. 2 Childhood ALL in Iraq including B-ALL (49) and T-ALL (6) cases.



Blue wedges refer to B- ALL, yellow wedges to recently identified subtypes of B-ALL and red wedge to T- ALL

Thus, *TCF3-PBX1* fusion gene associated with the translocation t(1;19)(q23;p13), with the frequency of (22.4%) in our cohort, was unprecedently recurrent than reported elsewhere. Our frequency was higher than those reported in African American, and Mexican B-ALL cases, of 16.3%, and 14.6%, respectively. Of note, the frequency of *TCF3-PBX1* transcript is known to be low in different ethnicities and countries, for example it is (3% in white American and in Malaysia-Singapore studies, 4.8% in Hong Kong, 6.1% in China, and 7.2% in Japan). Among our cases with *TCF3-PBX1*, the median age was 5.7 (2-12) years, with a ratio of males to females of 4.5, and a median WBC of 52.5 (4.6-152) $x10^9$/l. The average WBC was significantly higher in patients who possessed *TCF3-PBX1*, compared to those B-ALL cases without it, (63.4 vs. 31.2) $x10^9$/l, respectively, (p=0.03), whereas the average number of somatic mutations per patient associated with *TCF3-PBX1* cases was significantly lower than those B-ALL cases without *TCF3-PBX1*, (5.6 vs 11.9), respectively, (p=0.02). Thus, 7/11 *TCF3-PBX1*-positive cases had the high-risk criteria, including 6 patients with initial WBC > $50x10^9$/l and 3 of them > $100x10^9$/l, along with one patient > 10 years old, in line with some reports that point out to the association of *TCF3-PBX1* with high risk ALL and less somatic mutations. However, none of those who possessed *TCF3-PBX1* transcript had central nervous system or testicular disease at presentation. In total, we detected 589 somatic alterations in 55 ALL cases including (350 missense, 155 silent, 28 nonsense, 24 frameshift, 17 splice site, and 15 in-frame) mutations. The average number of the detected somatic mutations was 9 (range 0~38)/patient, and it was higher in T-ALL (12.3) compared to B-ALL (10.5). No correlation was found between the number of somatic mutations and age, gender, initial WBC, or ALL subtype, which could be related to the limited sample size. C>T single-base substitution was the predominant type of mutations (164/515, 31.8%). Potentially pathogenic non-silent mutations in ALL that we detected in our series include *RAS* signaling pathway mutations as the commonest, along with less frequent mutations such as *PAX5*, *IKZF1*, *IKZF3*, *KMT2D*, *CDKN2A*, *JAK2*, *IDH1*, *WHSC1*, *ARID1A*, *SETD2*, *TP53*, *GATA3*, *U2AF1*, *ZEB2*, *ARID5B*, *BCORL1*, and *CSF3R*, in B-ALL cases. A significantly higher mutations of frameshift, in-frame and splice site somatic mutations were identified in T-ALL (16/74, 21.6%) vs. B-ALL (40/515, 7.8%), (p<0.001). In B-ALL most of the *RAS* signaling pathway mutations occur in *NRAS*, *KRAS*, *FLT3* and *PTPN11*, reflecting a central role of these genes concerned with regulating cellular processes involved in

leukemogenesis, including cell growth, survival, differentiation, and cell cycle regulation. *RAS* signaling pathway mutations, including 10 *NRAS*, 6 *KRAS*, 3 *FLT3*, 2 *PTPN11*, and 1 *BRAF*, with a total of 22 (44.9%) mutations were detected in (17/49, 34.7%) of B-ALL patients, including double *RAS* mutations in 5 cases (**Table 1**). Interestingly, (13/22, 59.1%) of *RAS* signaling pathway mutations were disclosed in (10/12, 83.3%) patients carrying HHD. A trend toward more somatic mutations and lower WBC were associated with *RAS*-mutated B-ALL vs. wild-*RAS* B-ALL, while no correlation was found in *RAS*-mutated vs. wild-*RAS* B-ALL in terms of sex or age, considering that only *KRAS*/*NRAS* mutated cases were evaluated. Of note, no *RAS* mutation was detected in our 6 patients with T-ALL. Our high incidence of *RAS* mutations was comparable to those reported by I. S. Jerchel *et al.* of (44.2%), and M. Case *et al.* of (30/86, 34.8%). Furthermore, *RAS* mutations in Iraqi childhood ALL was significantly higher when compared to USA, Japan, China, Taiwan and Sweden.

Table 1 B-ALL classification and concomitant somatic mutations including RAS signaling pathway mutations

B-ALL Subtype	No. of patients	(%)	Average number of somatic mutations/patient	*RAS* signalling pathway mutations		Important other somatic mutations
				number of cases	number of mutated genes	
High Hyperdiploidy (HHD)	12	24.5%	14	10 (3)*	4 *KRAS* 5 *NRAS* 2 *PTPN11* 2 *FLT3*	2 *CDKN2A*, 2 *KMT2D* 1, *IKZF1*, 1 *IKZF3*, 1 *GATA3*
TCF3-PBX1	11	22.4%	5.6	1	1 *KRAS*	1 *PAX5*, 1 *IKZF3*
ETV6-RUNX1	9	18.4%	13.7	1	1 *NRAS*	
PAX5alt	3	6.1%	12	1 (1)*	1 *KRAS* 1 *BRAF*	1 *PAX5*, 1 *TP53*
P2RY8-CRLF2	3	6.1%	10.7	1	1 *NRAS*	2 *JAK2*
del(11)(q23)	2	4.1%	9	0	0	
iAMP21	1	2.0%	23	0	0	
MEF2D-BCL9	1	2.0%	13	0	0	
BCR-ABL1	1	2.0%	7	0	0	1 *IKZF1*
TCF3-HLF	1	2.0%	8	0	0	
Ph-like *(FLT3)*	1	2.0%	13	1(1)*	1 *NRAS* 1 *FLT3*	1 *IKZF1*
BCL2/IGH	1	2.0%	3	1	1 *NRAS*	
Other B-ALL	3	6.1%	3	1	1 *NRAS*	1 *PAX5*

** Number of cases with double mutations*

AML cases, paired samples of 11 childhood AML cases from Iraq were analyzed. The median age was 8.1 (4-13) years, with a ratio of males to females of 2.7, and a median WBC of 39 (3.8-320) $x10^9$/l. A higher frequency of AML-M3 morphology or acute promyelocytic leukemia (APL); (5/11, 45.5%) was observed, followed by M2 (4/11, 36.4%), and one case of M5 and M6. In AML, 68 somatic mutations were illustrated including (30 missense, 20 silent, 8 nonsense, 8 frameshift, 1 splice site, and 1 in-frame). The average number of the detected somatic mutations was 6.2 (range 1~15)/patient. As in ALL, C>T was the most frequent type of mutations in AML, (16/68, 23.5%). Likewise, *RAS* mutations were frequently disclosed (5/11, 45.5%) mutations, in 4 of our AML cases (4/11, 36.4%). Other potentially pathogenic non-silent mutations in AML were *CEBPA*, *WT1*, and *MYC*.

It was found that RAS signaling pathway mutations can lead to growth factor-independent proliferation of hematopoietic progenitors that can trigger leukemogenesis of myeloid leukemia in mouse model.

In summary, this study identified distinct results in both ALL and AML among Iraqi children. *TCF3-PBX1* fusion gene was strikingly higher than the records elsewhere in the world. Compared to literature, and in agreement with our previous observation (Al-Kzayer LF, et al., Pediatr Blood Cancer., 2014, 2015), the overall somatic *RAS* signaling pathway mutations of around (~45%) in Iraqi children with acute leukemia, are among the highest reported frequencies whether in ALL or in AML. Our results suggest that the biology of childhood acute leukemia in Iraq is probably different, and the environment related to war and war-aftermath in Iraq may play a role along with the geographic or ethnic background. Further studies enrolling more Iraqi patients with acute leukemia are required to address that.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　4件）

１．著者名 Lika ' a Fasih Y. Al-Kzayer, Hanadi Munaf H. Al-Aradi, Tomonari Shigemura, Kenji Sano, Miyuki Tanaka, Motoharu Hamada, Kenan Hussien Ali, Yozo Nakazawa, and Yusuke Okuno	４．巻 114
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1186/s12881-019-0837-4	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Le Thanh Nha Uyen, Yuji Amano, Lika ' a Fasih Y. Al-Kzayer, Noriko Kubota, Jun Kobayashi, Yozo Nakazawa, Kenichi Koike, Kazuo Sakashita	４．巻 111
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s12185-019-02799-4	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

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掲載論文のDOI（デジタルオブジェクト識別子） 10.18632/oncotarget.23479	査読の有無 有
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２．論文標題 A Syrian Refugee in Iraq Diagnosed as a Case of IL12RB1 Deficiency in Japan Using Dried Blood Spots	５．発行年 2019年
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４．発表年 2018年～2019年

１．発表者名
Lika ' a Fasih Y. Al-Kzayer

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An Iraqi girl with a slowly growing jaw mass diagnosed in Japan as a case of DOCK8 deficiency

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２．発表標題
A SYRIAN GIRL DIAGNOSED WITH IL12RB1 DEFICIENCY IN JAPAN

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2018年～2019年

１．発表者名
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２．発表標題
AN IRAQI GIRL WITH A SLOWLY GROWING JAW MASS DIAGNOSED IN JAPAN AS A CASE OF DOCK8 DEFICIENCY

３．学会等名
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2018年～2019年

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４．発表年 2019年～2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関