様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　 ６ 月 ２１ 日現在

機関番号：８２６１２
研究種目：基盤研究(B)（一般）
研究期間：2018～2021
課題番号：１８Ｈ０３０４４

研究課題名（和文）小児白血病の発症要因および生存者の医学的・社会的予後に関する包括的な疫学研究

研究課題名（英文）Epidemiological investigation of risk factors for childhood leukemia onset and adverse effects among survivors

研究代表者
浦山　ケビン（Urayama, Kevin）

国立研究開発法人国立成育医療研究センター・社会医学研究部・シニアフェロー

研究者番号：６０７２６８５０

交付決定額（研究期間全体）：（直接経費）　　13,200,000円

研究成果の概要（和文）：本研究ではまず、胎児期からの環境要因が小児血液がんの発症リスクに影響を持つことを示唆する欧米での知見を踏まえて、日本人において胎内環境・生活習慣等の要因と遺伝素因がそれぞれどのように影響するかの症例対照研究を実施した。 母子健康手帳から収集したデータと唾液検体DNAを抽出し、解析を行った又は進行中である。 症例と対照の比較によると、母親の年齢、出産方法、出生時体重、および年長の兄弟を持つこととの関連を示している。 国際共同研究で、妊娠中の感染は小児白血病のリスクと関連していることが明らかになった。遺伝子解析の結果、非アジア系集団でも報告されている小児白血病リスクに関連する遺伝子を8つ特定した。

研究成果の学術的意義や社会的意義
Without a greater consensus on causes of childhood leukemia, effective disease prevention will not be possible. The results of this study informs what types of exposures during pregnancy and the child's early development, as well as genetic factors in Japanese, contributes to this disease.

研究成果の概要（英文）：We established the first epidemiological study of childhood hematological cancers (Epi-HCC) focusing on the examination of risk factors during pregnancy of the mother, birth characteristics, and early childhood exposures. We collected data from the Maternal and Child Health Handbook and saliva samples as a source of DNA, and several analyses have been completed and are also ongoing. Case and control comparisons show an association with maternal age, delivery methods, birthweight, and having older siblings. Through international collaboration (16 studies across 8 countries), we found that infection during pregnancy is associated with an increased risk of childhood leukemia. In genetic analyses, we identified 8 genetic loci associated childhood leukemia risk which have also been reported in non-Asian populations. We found two additional risk-associated genes which may be specific to the Japanese population.

研究分野：Epidemiology

キーワード：epidemiology　leukemia　genetics　pregnancy　perinatal factor　risk

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Leukemia is the most common cancer in children, with about 700 diagnoses each year in Japan. Despite vast improvements in survival, progress in understanding its causes has been slow, thus, delaying efforts for prevention. Descriptive evidence has provided clues about the causes of childhood leukemia (*Greave, Lancet, 1997, 349:344-349*). Firstly, acute lymphoblastic leukemia (ALL), comprising over 80% of childhood leukemias, has a peak incidence at age 2 to 5 years, a time when children begin formal schooling and increase their exposure to infectious antigens. The more rare subtypes, acute myeloid leukemia (AML, ~15%) and the chronic subtypes do not exhibit this same pattern. Secondly, this peculiar age distribution for childhood ALL is only observed in the more economically developed regions of the world often associated with improved hygienic environments. These observations were instrumental in developing the "*delayed infection*" hypothesis in 1989 (*Greaves, Nat Rev Cancer, 2006, 6:193-203*) which describes that the lack of sufficient immune priming of the child in early life and subsequent infectious exposures during pre-school age (2-5 years) may lead to an adverse immunologic response that contribute to ALL risk. Epidemiological studies support this hypothesis based on analysis of proxy measures of early life immune modulation such as infectious exposures through daycare attendance (*Urayama et al., Int J Epidem, 2010, 39:718-32*), exposures from older siblings, and breastfeeding (*Urayama et al., Blood, 2012, 120:3039-47*). Recent evidence also suggests that exposures during the fetal and perinatal developmental stages may influence the trajectory of risk conferred by postnatal exposures and point towards the potential involvement of maternal exposures during pregnancy and circumstances at birth.

２．研究の目的

Using a well-established network of hospitals and pediatric oncologists, we assemble a hospital-based case-control study of childhood leukemia comprising DNA sample collection from children and their parents and a comprehensive questionnaire to examine the role of *in utero* and early postnatal immune-related exposures on risk of leukemia in the offspring. Through this new epidemiological study and integrating an international collaboration and existing resources and genomic data, we addressed 3 primary research objectives as follows: (1) identify the pregnancy-related exposures and birth characteristics associate with risk of childhood leukemia in the Japanese population, (2) engage in international collaboration and pursue a robust evaluation of the role of infections during pregnancy and risk of childhood leukemia in the offspring, and (3) confirm previously reported genetic risk loci and identify new genetic variants associated with the risk of childhood in the Japanese population.

３．研究の方法

In order to achieve the various research objectives, we assembled a new epidemiological study that comprised original data and sample collection in Japanese cases and controls, epidemiological datasets from international collaborators, and expanded our genetic analysis capability by integrating existing genomic data from the Japanese population.

(1) We initiated a new study called the Epidemiological Study of Hematologic Cancers in Children (Epi-HCC), which is a case-control study with patients (diagnosed aged <19 years with leukemia or lymphoma) recruited from 7 collaborating hospitals from the Tokyo Children's Cancer Study (TCCSG) network (n=270), and population controls recruited through the resident registration system and population survey panels (n=400) matched to cases on age, sex, and municipality of residence. Data were collected using a self-administered questionnaire that included content recorded in the Maternal and Child Health Handbook about the

mother and child during pregnancy, delivery, and first 3 years of the child's life. Saliva samples were collected with DNA extracted and stored for genomic analyses. Regression analyses are underway that address specific research questions related to the involvement pregnancy complications, birth outcomes, early exposure of the child to infections, and the risk of childhood hematological cancers.

(2) To inform and corroborate the findings from the Epi-HCC, in parallel, we have pursued an international collaborative analysis including 16 epidemiological studies of childhood leukemia conducted in various parts of the world (e.g. US, Australia, New Zealand, France, Egypt, Greece, Denmark, Brazil, and Japan) examining the association between infection during pregnancy and risk of childhood leukemia. The analysis includes about 12,000 cases and 25,000 controls. Most studies used a questionnaire to obtain data related to infectious disease episodes experienced by the mother during pregnancy; three studies assembled case-control data through record-linkage to medical records. Multivariable logistic regression analyses were pursued separately for each study, and risk estimates were combined using random effects meta-analysis.

(3) Genome-wide single nucleotide polymorphism (SNP) data were assembled for 540 childhood leukemia cases through the TCCSG, including for participants enrolled in the Epi-HCC Study. Control data comprised adult participants enrolled in two ongoing epidemiological studies of lifestyle-related chronic diseases in Japan, the Nagahama Study) (n=1,846) and the (HERPACC) Study (n=2,170). Genome-wide association analysis was performed using PLINK software.

4．研究成果

Using data collected through the newly assembled Epi-HCC Study, as well existing resources through the Childhood Leukemia International Consortium and Tokyo Children's Cancer Study Group, we report the following results.

(1) Epi-HCC Study cases and controls were successfully recruited over a 3-4 year period in which informed consent and data collection was conducted by phone and postal mail (Figure 1). Over half of the cases enrolled were diagnosed between 2 and 6 years of age and about 55% were male. Saliva samples were collected for over 90% of cases and about 70% of controls. Age and education level of mothers of childhood leukemia cases tended to be lower than controls. While the full set of data will continue to be analyzed, current activities have focused on three primary risk factors which have been implicated in previous literature, birthweight, delivery method, and having older siblings.

Figure 1

Cases tended to be born with higher birthweights than controls in which 14% of cases and 8% of controls were born at 3,500 grams or higher (P=0.04). Fewer cases (14%) were born by caesarean section than controls (22%) which is a pattern in contrast to previous studies which have reported an increased risk of childhood ALL associated with caesarean section. Regarding the presence of older siblings, fewer cases reported having an older sibling (45%) compared to controls (60%). This is consistent with the hypothesis that exposure to infections through older siblings may benefit the developing immune system and reduce the risk of leukemia.

(2) In collaboration with the Childhood Leukemia International Consortium, study-specific analysis examining the association between infection during pregnancy and risk of childhood leukemia were conducted using logistic regression and adjusting for confounding factors. Overall, the majority of studies showed a tendency for an increased risk of childhood leukemia with odds ratios greater than 1.00 (Figure 2). Meta-analysis showed a statistically significant increased risk of childhood leukemia associated with an infectious episode experienced during pregnancy (combine OR=1.23, 95% CI: 1.06-1.44). Sub-analyses examining the type of infection showed a stronger influence of viral infections (OR=1.27, 95% CI: 1.03-1.56) than bacterial infections (OR=1.18, 95% CI: 0.96-1.46). These results suggest that exposure to infections may influence the fetus in a way that increases oncogenic mechanisms, either through inflicting oncogenic damage or indirectly by altering the fetal immune system.

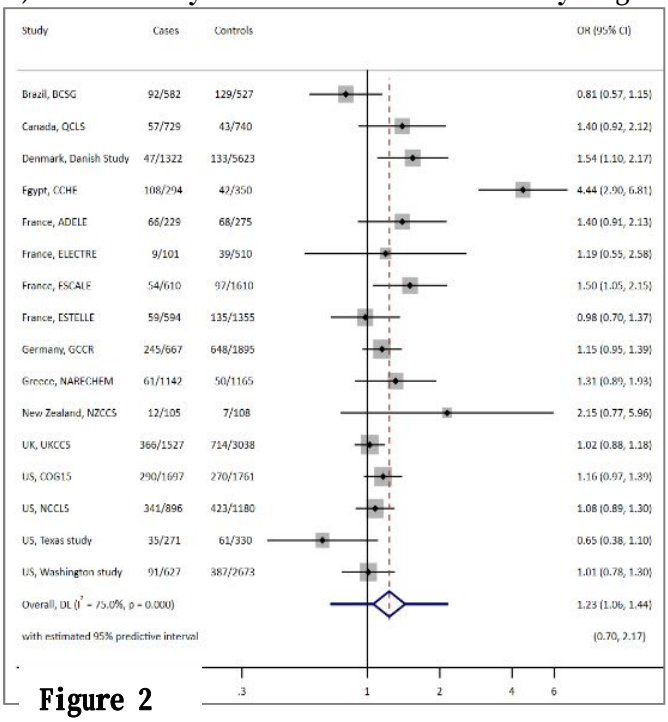

Figure 2

(3) In a series of 540 childhood ALL cases and about 3,000 controls, genome-wide SNP association analysis was conducted and showed minimal evidence of genomic inflation (lambda=1.09). Two genome-wide significant regions were observed represented by SNPs rs116977518 (OR=1.99, P=4.2x10^{-9}) located at 1q24.1 (intergenic, closest proximity to FM08P) and rs4245595 (OR=1.84, P=3.4 x 10^{-17}) located at chromosome 10q21.2 (ARID5B) (Figure 3). Suggestive associations were observed at 6p21.33 (rs2516643; OR=0.67, P=1.9 x 10^{-7}) and 15q12 (rs79649658, OCA2; OR=0.22, P=4.5 x 10^{-7}). Although not genome-wide significant, an association with the previously identified IKZF1 region was also observed (rs77563422, OR=1.62, P=9.5 x 10^{-8}). Our study confirmed an association with previously reported SNPs in ARID5B, IKZF1, DDC, CEBPE, PIP4K2A, GATA3, IKZF3, and the 8q24.21 locus. Among these risk-associated regions, a different SNP which showed a stronger association was observed for IKZF1 (rs77563422, OR=1.55), CEBPE (rs2239630, OR=1.19), PIP4K2A (rs10159730, OR=1.22), and the 8q24.21 locus (rs5003704, OR=1.77).

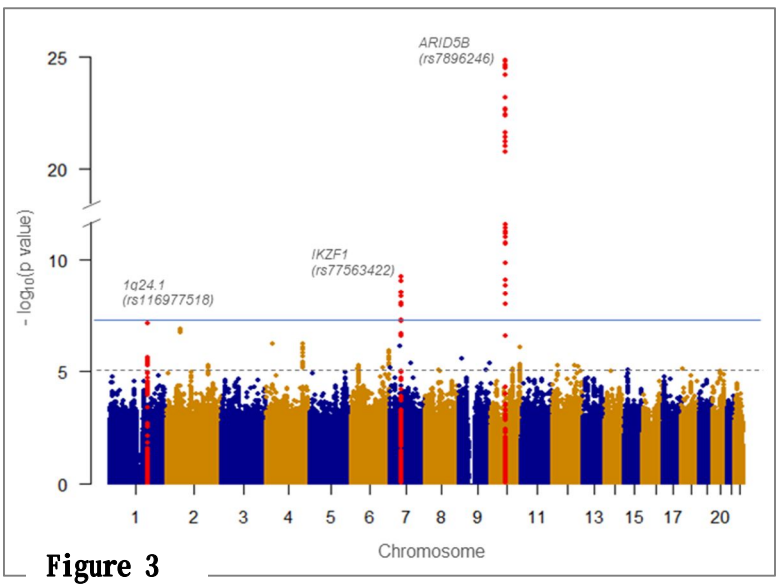

Figure 3

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Kevin Y. Urayama	４．巻 62
２．論文標題 Epidemiology of childhood leukemia: a targeted overview	５．発行年 2021年
３．雑誌名 Rinsho Ketsueki	６．最初と最後の頁 733-738
掲載論文のDOI（デジタルオブジェクト識別子） 10.11406/rinketsu.62.733	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 －

〔学会発表〕　計3件（うち招待講演　3件／うち国際学会　0件）

１．発表者名 Kevin Y. Urayama
２．発表標題 Maternal Infections and Childhood Leukemia Risk
３．学会等名 Annual Meeting of the Childhood Leukemia International Consortium（招待講演）
４．発表年 2022年

１．発表者名 Kevin Y. Urayama
２．発表標題 Genetic and Environmental Contributions in the Risk of Childhood Leukemia
３．学会等名 The 82nd Annual Meeting of the Japanese Society of Hematology（招待講演）
４．発表年 2021年

１．発表者名 浦山ケビン
２．発表標題 Epidemiology of Childhood Cancers: Perspectives from East Asia
３．学会等名 CLIC-I4C Joint International Symposium（招待講演）
４．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究分担者	高木　正稔 (Takagi Masatoshi) (10406267)	東京医科歯科大学・大学院医歯学総合研究科・准教授 (12602)	
研究分担者	真部　淳 (Manabe Atsushi) (20292849)	北海道大学・医学研究院・教授 (10101)	
研究分担者	清河　信敬 (Kiyokawa Nobutaka) (60195401)	国立研究開発法人国立成育医療研究センター・小児血液・腫瘍研究部・部長 (82612)	

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1件

国際研究集会	開催年
CLIC-I4C Joint International Symposium on "The Environment and Genetics in Pediatric Cancers: Epidemiological Advances through International Collaboration"	2018年～2018年

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			
米国	University of California, Berkeley	University of Minnesota	University of Southern California	他3機関
フランス	INSERM			
エジプト	Children's Cancer Hospital			
カナダ	McGill University			
デンマーク	Danish Cancer Registry			
米国	University of California, Berkeley	University of Minnesota	University of Southern California	他3機関

共同研究相手国	相手方研究機関			
フランス	INSERM			
エジプト	Children's Cancer Hospital			
カナダ	McGill University			
デンマーク	Danish Cancer Society			
				他3機関