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Leukopenia, macrocytosis, and thrombocytopenia occur in young adults with Down syndrome

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ABSTRACT

Down syndrome (DS) is a common congenital disorder caused by trisomy 21. Due to the increase in maternal age with population aging and advances in medical treatment for fatal complications in their early childhood, the prevalence and life expectancy of DS individuals have greatly increased. Despite this rise in the number of DS adults, their hematological status remains poorly examined. Here, we report that three hematological abnormalities, leukopenia, macrocytosis, and thrombocytopenia, develop as adult DS-associated features. Multi- and uni-variate analyses on hematological data collected from 51 DS and 60 control adults demonstrated that young adults with DS are at significantly higher risk of (i) myeloid-dominant leukopenia, (ii) macrocytosis characterized by high mean cell volume (MCV) of erythrocytes, and (iii) lower platelet counts than the control. Notably, these features were more pronounced with age. Further analyses on DS adults would provide a deeper understanding and novel research perspectives for multiple aging-related disorders in the general population.

1. Introduction

Down syndrome (DS) is among the world's most prevalent congenital disorders caused by one extra copy of *Homo sapiens* chromosome 21 (HSA21), namely trisomy 21. Besides their widely recognized physical traits, intellectual disability, and internal organ anomalies including heart defects, DS individuals also suffer from many hematological abnormalities such as leukopenia, leukocytosis, anemia, polycythemia, macrocytosis (enlargement of red blood cells), thrombocytopenia, and thrombocytosis. Some of these non-malignant hematological defects are directly caused by the increased dosages of multiple hematopoietic and non-hematopoietic genes located on HSA21 (Osato and Ito, 2005; Yanagida et al., 2005; Antonarakis et al., 2020), while others may be indirect consequences of concurrent heart, thyroid, or infectious diseases. In addition, malignant diseases, such as transient abnormal myelopoiesis (TAM), acute megakaryocytic leukemia (AMKL), and acute lymphoblastic leukemia, are also common in DS subjects (Hasle et al., 2000). Notably, all of these blood abnormalities are almost exclusively

Abbreviations: AD, Alzheimer's disease; AMKL, acute megakaryocytic leukemia; DS, Down syndrome; Hb, hemoglobin; HSA21, Homo sapiens chromosome 21; HSC, hematopoietic stem cell; Ht, hematocrit; MCV, mean corpuscular volume; MDS, myelodysplastic syndrome; PLT, platelet; PB, peripheral blood; RBC, red blood cell; TAM, transient abnormal myelopoiesis; WBC, white blood cell.

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described for childhood DS patients, as the median life expectancy of DS subjects was 20 years old or younger till 1990 due to their life-threatening complications. However, significant advances in medical treatment for these fatal childhood complications have remarkably increased the median life expectancy of DS subjects to 55 years old in 2010 (Presson et al., 2013). Furthermore, as a result of worldwide changes in lifestyle and population aging, the rise in maternal age, the most significant risk factor leading to DS, has resulted in an accelerated increase in the prevalence of DS adults (Penrose, 1933; Allen et al., 2009). Despite this significant rise, the hematological conditions of DS adults are still understudied.

In this study, we analyzed the hematological parameters of 51 DS young adults and 60 control cases. Our multivariate analysis revealed leukopenia in the DS cohort, with a particular decrease in myeloid cells but not lymphocytes, and a decrease in platelets, in addition to macrocytosis, which is already present in childhood. We present the largest study to date for hematological abnormalities in adults with DS.

2. Methods

2.1. Study design

As a retrospective observational case-control study, hematological and demographic data of DS and healthy (control) individuals were collected from clinical records at the Misakaenosono Mutsumi Developmental Medical and Welfare Center. The DS data were collected from health examinations conducted between March 2010 and December 2019, while the non-DS healthy control data were recorded in the 2018 health examination. The DS group consisted of DS patients who were residents or outpatients who visited the center regularly. The control group consisted of employees working at the center, including the caregivers. The inclusion criteria include those who gave their consent to participate in this study. The exclusion criteria include those who were 20 years of age and below. The participants were recruited on a voluntary basis without any monetary compensation. The DS subjects were generally healthy, and their clinical features were mostly stable during the study period. In some cases, comorbidities were observed during the study period, and information about the comorbidities was collected from medical records of the institution. The data collected include age, sex, white blood cell (WBC), myeloid cells, lymphoid cells, red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), and platelets (PLT). Individual abnormalities in blood cell parameters were defined as follows: leukopenia, WBC < 4000 /μL; neutropenia, myeloid cells < 1500 /μL; macrocytosis, MCV > 100 fL; and thrombopenia, PLT $< 150 \times 10^3$ /μL. No symptoms of infectious diseases were present during the collection of these hematological data. We also conducted a questionnaire survey on each subject to assess their physical characteristics of aging. The primary caregivers who supported the DS subjects on a daily basis provided most of the information. The aging-related features assessed in the survey were as follows: long eyebrows, alopecia, canities, missing teeth, humpback, cataracts, flabby skin under the eyes, longitudinal groove along nails, senile freckles, and skin wrinkling. Informed consent was obtained from all participants, in accordance with the principles of the Helsinki Declaration. The study was approved by the Institutional Review Board of the above-mentioned center.

2.2. Statistical analysis

To identify DS-associated hematological features, the collected hematological and demographic data from DS and controls were first subjected to univariate analysis. Unpaired student *t*-test (two tailed), Mann-Whitney test, and χ^2 test were employed for continuous parametric, continuous non-parametric, and categorical variables, respectively.

Multiple linear regression analysis was performed to evaluate the relationship between the hematological data and DS. To remove the influence of covariates, age and sex were included in this analysis. All statistical analyses were conducted using IBM SPSS version 24.0 and R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). p-value < 0.05 were considered statistical significant.

Age-dependent changes in hematological data, WBC, myeloid cells, lymphoid cells, MCV, and PLT, were also compared between adult DS and control groups.

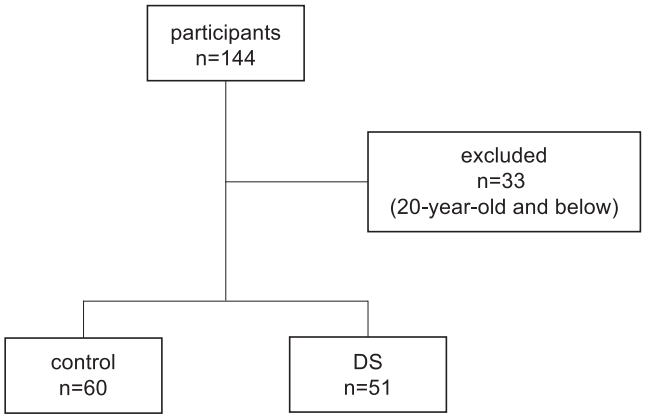

Fig. 1. A total of 111 people participated in this study. A total of 144 were originally recruited to the present study. 33 people who met the exclusion criteria were excluded. Eventually, data from 51 Down syndrome and 60 control individuals were subjected to the analyses.

Table 1
Comparison of demographic and hematological data between control and DS groups, employing univariate analyses.

	Control (n = 60)	DS (n = 51)	*p*-value
Age (year)	28.3 (3.5)	27 (4.0)	0.1
Sex (male), n (%)	19 (31)	27 (52)	0.02
WBC ($\times 10^3$/μL)	6.6 (1.4)	5.3 (1.6)	<0.001
myeloid cells ($\times 10^3$/μL)	4.3 (1.2)	3.2 (1.1)	<0.001
lymphoid cells ($\times 10^3$/μL)	2.1 (0.5)	2.0 (1.0)	0.25
myeloid cells/lymphoid cells	2.0 (0.8)	1.9 (1.1)	0.54
RBC ($\times 10^9$/μL)	4.5 (4.2–4.8)	4.5 (4.2–4.9)	0.47
Hb (g/dL)	14 (13–15)	15 (13–16)	0.01
Ht (%)	40 (38–43)	44 (41–47)	< 0.001
MCV (fL)	89 (87–92)	97 (92–98)	< 0.001
PLT ($\times 10^3$/μL)	250 (230–300)	230 (210–270)	0.01

Normally distributed variables are expressed by mean (SD), whereas not normally distributed variables are expressed by median (interquartile range). The former includes age, WBC, myeloid cells, lymphoid cells and myeloid / lymphoid cells, and were analyzed by unpaired student *t*-test (two-tailed), whereas the latter includes RBC, Hb, Ht, MCV and PLT, and were analyzed by Mann-Whitney test. A categorical variable, sex, is expressed by number (percentage) and was analyzed by χ^2 test. p-values represent the results of univariate analyses. Abbreviations: WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; PLT, platelet.

3. Results

A total of 144 individuals participated in the study, consisting of 79 DS and 65 healthy controls (Fig. 1). Thirty-three individuals who met the exclusion criteria were excluded, and the data from the remaining 111 participants (51 DS and 60 control subjects) were analyzed. The mean age of the DS group was 27 years old (range, 21–34 years old), and that of the control group was 28.3 years old (range, 21–36 years old) (Table 1). Comorbidities observed in the DS patients during the above-mentioned study period were liver dysfunction in 26 cases (50.9%), obesity 20 (39.2%), neurosis 17 (33.3%), cataract 16 (31.4%), developmental regression 16 (31.4%), dysuria 10 (19.6%), schizophrenia 10

Table 2
Comparison of frequencies of hematological abnormalities between control and DS groups.

hematological abnormalities*	Control (n = 60)	DS (n = 51)	*p*-value
Leukopenia, n (%)	0 (0)	11 (21.57)	<0.001
Neutropenia, n (%)	0 (0)	0 (0)	–
Macrocytosis, n (%)	0 (0)	9 (17.64)	0.001
Thrombocytopenia, n (%)	0 (0)	1 (1.96)	0.459

* Definitions for indicated hematological abnormalities are as follows: leukopenia, white blood cells (WBC) < 4000/μL; neutropenia, myeloid cells < 1500/μL; macrocytosis, mean corpuscular volume (MCV) > 100 fL; and thrombocytopenia, platelet (PLT) < 150 × 10^3/μL. Categorical variables in the individual abnormalities are expressed by number (percentage) and analyzed by χ^2 test. p-values represent the results of univariate analyses.

(19.6%), thyroid dysfunction 9 (17.6%), allergic rhinitis 7 (13.7%), insomnia 7 (13.7%), Alzheimer's disease (AD) 7 (13.7%), atopic dermatitis 4 (7.84%), orthostatic hypotension 4 (7.84%), hyperuricemia 3 (5.88%), hyperlipidemia 3 (5.88%), diabetes 2 (3.92%), depression 2 (3.92%), epilepsy 2 (3.92%), iron deficiency anemia 1 (1.96%), fatty liver 1 (1.96%), hypogonadism 1 (1.96%), anorexia 1 (1.96%), hydronephrosis 1 (1.96%), and parkinsonism 1 (1.96%). Before the recruitment to the study, the DS patients had the following diseases: ventricular septal defect in 7 cases (13.7%), pulmonary hypertension 2 (3.92%), gout 2 (3.92%), atrial septal defect 1 (1.96%), atrioventricular septal defect 1 (1.96%), Kawasaki disease 1 (1.96%), cholelithiasis 1 (1.96%), megacolon 1 (1.96%), thyroid dysfunction 1 (1.96%), avascular necrosis of the femoral head 1 (1.96%), duodenal atresia 1 (1.96%), cardiac hypertrophy 1 (1.96%), pancreatitis 1 (1.96%), bronchial asthma 1 (1.96%), and hemophagocytic syndrome 1 (1.96%). Notably, none of the DS patients in this study had TAM, although 5–30% of DS infants are known to develop TAM (Osato and Ito, 2005; Antnarakis et al., 2020). At the time of blood data collection, all the diseases listed above had been medically controlled and no clinical symptoms were observed in the individual patients.

The collected demographic and hematological data were first individually subjected to univariate analysis. As summarized in Table 1, the results demonstrate significantly higher values in the DS group than in the control group for the percentage of males (31 % in control vs 52 % in DS, p = 0.02), Hb (14 g/dL in control vs 15 g/dL in DS, p = 0.01), Ht (40 % in control vs 44 % in DS, p < 0.001), and MCV (89 fL in control vs 97 fL in DS, p < 0.001), and conversely significantly lower values in the DS group than in the control group for WBC (6.6 × 10^3 /μL in control vs 5.3 × 10^3 /μL in DS, p < 0.001), myeloid cells (4.3 × 10^3/μL in control vs 3.2 × 10^3 /μL in DS, p < 0.001), and PLT (250 × 10^3 /μL in control vs 230 × 10^3 /μL in DS, p = 0.01). Macrocytosis (0 % in control vs 17% in DS, p = 0.001) and leukopenia (0 % in control vs 21 % in DS, p < 0.001) were both observed only in the DS group (Table 2).

The results of multiple linear regression analysis showed that WBC (partial regression coefficient, −1562.051; 95 % Cl, −2148.835 – −975.267; p < 0.001), myeloid cells (partial regression coefficient, −1207.351; 95 % Cl, −1646.588 – −768.133; p < 0.001), and PLT (partial regression coefficient, −23234.314; 95 % Cl, −42076.068 – −4392.560; p = 0.016) were negatively related to DS, while Ht (partial regression coefficient, 2.235; 95 % Cl, 1.080–3.391; p < 0.001) and MCV (partial regression coefficient, 5.947; 95 % Cl, 4.421–7.474; p < 0.001) were positively related to DS (Fig. 2). The scatterplots for hematological data showed no extreme bias or outliers in the variables (Fig. 3).

As premature aging in DS adults is well documented, age-dependent changes in hematological data were next analyzed. The results revealed a significant inverse correlation between age and WBC in the DS group, but not in the control group (Fig. 4). Spearman's rank correlation coefficient (r) and the corresponding p-value were r = −0.357 and p = 0.01, respectively, in the DS group, whereas r = −0.232 and p = 0.074 were for the control group. Likewise, an inverse correlation between age and myeloid cells (r = −0.0412, p = 0.03 in DS; r = −0.254, p = 0.05 in control) was observed. However, no such correlation was detected between age and lymphoid cells (r = −0.176, p = 0.216 in DS; r = −0.028, p = 0.831 in control). Therefore, myeloid dominant leukopenia, namely neutropenia, in the DS group is considered to be an accelerated aging-related abnormality.

Macrocytosis, on the other hand, was not pronounced with age,

	partial regression coefficient	standardized partial regression coefficient	95%CI	*p*-value
WBC	-1562.051	-0.463	-2148.835 - -975.267	<0.001
myeloid cells	-1207.351	-0.460	-1646.588 - -768.133	<0.001
lymphoid cells	-282.709	-0.169	-605.389 - 39.97	0.085
	partial regression coefficient	standardized partial regression coefficient	95%CI	*p*-value
RBC	-0.065	-0.072	-0.21 - 0.08	0.376
Hb	0.266	0.093	-0.151 - 0.684	0.208
Ht	2.235	0.272	1.080 - 3.391	<0.001
MCV	5.947	0.595	4.421 - 7.474	<0.001
	partial regression coefficient	standardized partial regression coefficient	95%CI	*p*-value
PLT	-23234.314	-0.228	-42076.068 - -4392.560	0.016

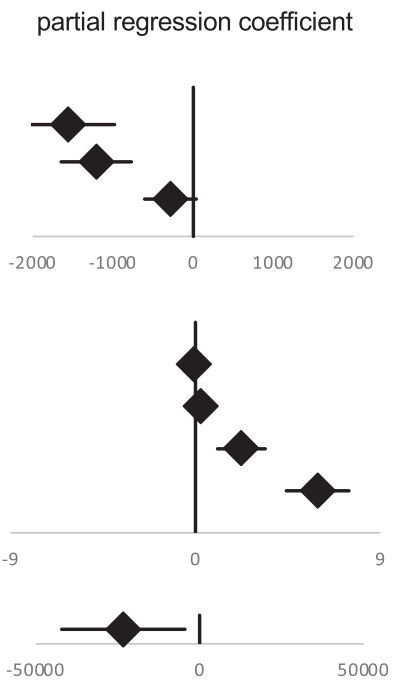

Fig. 2. Leukopenia, macrocytosis and decreased thrombopoiesis occur in DS young adults. The results of multivariate analyses are shown in a table and a forest plot. Lower WBC, lower myeloid cells, higher Ht, higher MCV, lower PLT were associated with Down syndrome. Abbreviations: WBC, white blood cell; RBC, red blood cell; PLT, platelet; MCV, mean corpuscular volume.

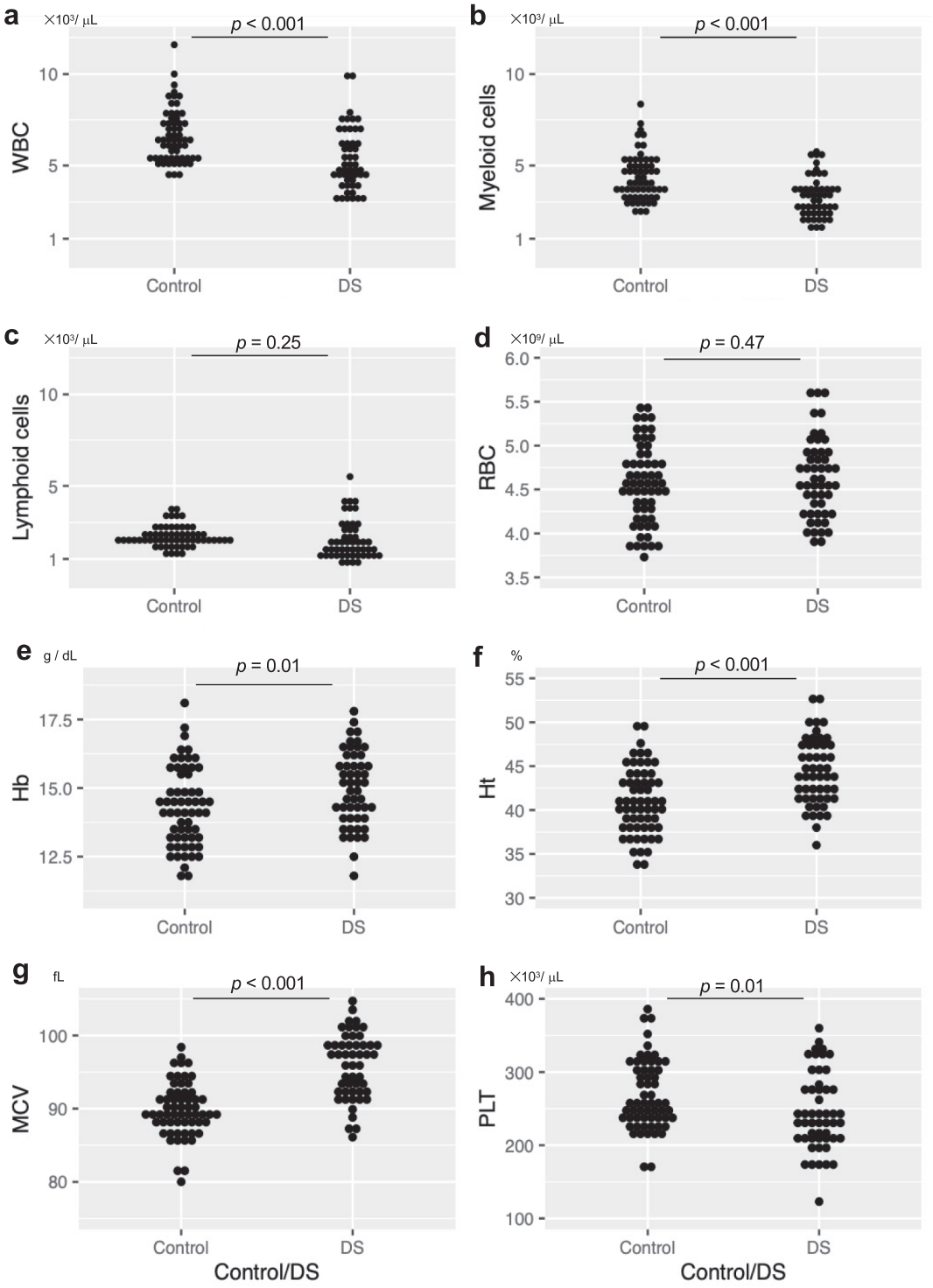

Fig. 3. Myeloid-dominant leukopenia, macrocytosis and lower platelet counts in DS young adults. Scatter plots of WBC, myeloid cells, lymphoid cells, RBC, Hb, Ht, MCV and PLT are shown. Abbreviations: see Fig. 2 legend.

despite a trend toward an age-dependent increase in MCV (r = 0.192, p = 0.176 in DS; r = −0.212, p = 0.022 in control) (Fig. 4d). MCV was observed to decrease with age in the control group. As the female dominance was seen in this particular control group, the correlation between age and MCV was further examined by sex (Fig. 5a). The result clearly exhibits a female-specific decline in MCV. Therefore, the declining MCV trend with age in the control group might be attributed to female dominance in the cohort. On the other hand, MCV was observed to increase with age in DS adult patients (Fig. 4d). To further explore the correlation between MCV and age, we reanalyzed the data after including the 33 participants who were initially excluded due to age (Aged 20 and below) and observed a clearer age-dependent progression of macrocytosis in DS (r = 0.299, p = 0.07 in DS; r = −0.180, p = 0.152 in control) (Fig. 5b).

PLT-age correlation analysis revealed a trend of age-dependent decline in PLT, though not statistically significant (r = −0.135, p = 0.347 in DS; r = 0.096, p = 0.464 in control) (Fig. 4e).

As many blood parameters exhibited age-dependent changes, we

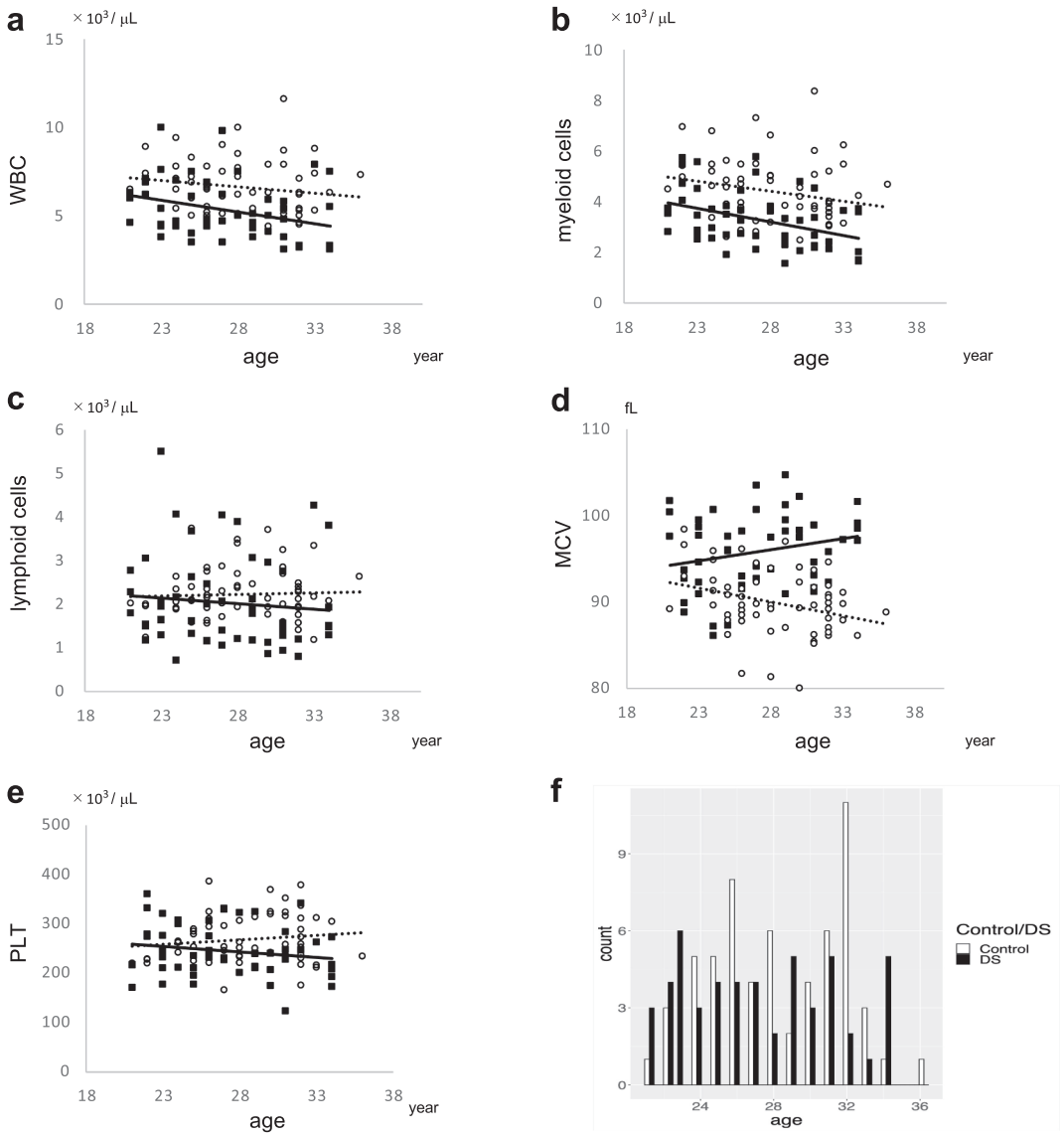

Fig. 4. Leukopenia progresses in an age-dependent manner in DS adults. Age-dependent changes in (a) WBC [Spearman's rank correlation coefficient ($r = -0.357$, and $p = 0.01$ in DS; $r = -0.232$, $p = 0.074$ in control)], (b) myeloid cells ($r = -0.0412$, $p = 0.03$ in DS; $r = -0.254$, $p = 0.05$ in control), (c) lymphoid cells ($r = -0.176$, $p = 0.216$ in DS; $r = 0.028$, $p = 0.831$ in control), (d) MCV ($r = 0.192$, $p = 0.176$ in DS; $r = -0.212$, $p = 0.022$ in control), and (e) PLT ($r = -0.135$, $p = 0.347$ in DS; $r = 0.096$, $p = 0.464$ in control), are plotted in distribution diagrams. Open circles and dotted lines represent data of the control group, whereas closed squares and solid lines represent data of the DS group. (f) Age distribution of the participants. Abbreviations: see Fig. 2 legend.

conducted a questionnaire survey on aging-related physical features. The result demonstrated that DS individuals frequently exhibited cataracts (31.4%), canities (25.6%) and longitudinal groove along nails (25.5%) (Table 3). The frequency difference between the DS and control groups was observed in the following aging-related physical features: cataracts (0% in control vs 25.6% in DS, $p < 0.001$), flabby skin under the eyes (0% in control vs 7.8% in DS, $p = 0.042$), skin wrinkling (10.0% in control vs 0% in DS, $p = 0.03$).

4. Discussion

Despite the recent growing prevalence of DS, the hematological abnormalities of adult DS individuals have not been well investigated. Here, we present a basic hematlogical comparative analysis of young adults with DS and healthy control subjects. The three lineage cells, WBC, RBC and PLT, exhibited clear abnormalities, leukopenia, macrocytosis, and to a lesser extent, thrombocytopenia in DS subjects. Macrocytosis is likely a continuation of conditions from their childhood, while leukopenia and thrombocytopenia have not been well described before in DS adults. Our study demonstrates statistically significant prevalence of leukopenia and decreased platelet counts in DS young adults.

The behavior of leukocyte counts in DS subjects is documented to vary with age even within the childhood period (Table 4). In 80% of DS neonates, neutrophil-dominant increase of leukocytes is reported in two distinct studies (Henry et al., 2007; Martínez-Macías et al., 2017). This elevation is rapidly normalized in a month, and WBC counts in DS children continue to stay relatively low, although the values are within the normal range and statistically not significant as compared to control subjects (Roizen and Amarose, 1993; Kivivuori et al., 1996). In adult DS, hematological parameters were only examined in nine cases in a single study to date (McLean et al., 2009). Six of them presented leukopenia of WBC 2.3–3.6 $\times 10^3/\mu L$, and two of these six cases were neutropenia. The two leukopenia cases revealed hypocellular bone marrow, suggesting that mild bone marrow failure underlies their leukopenia, although the mechanistic basis needs further investigation. Our study on 51 DS adults

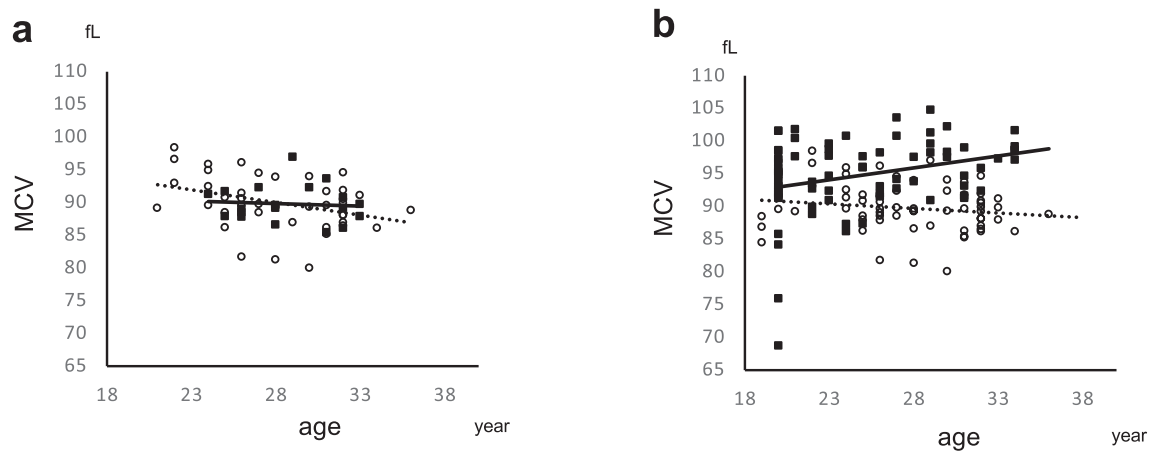

Fig. 5. Macrocytosis progresses in an age-dependent manner in DS young adults. (a) MCV declines with age in females in the control group. Age-dependent changes in MCV are plotted in a distribution diagram. Age-dependent changes in MCV within the control group are compared between males and females. Open circles and a dotted line represent data of females, whereas closed squares and a solid line represent data of males ($r = -0.059$, $p = 0.810$ in male; $r = -0.364$, $p = 0.019$ in female). (b) MCV increases with age in DS young adults. Age-dependent changes in MCV were reanalyzed by additionally including cases of 20 years and under, and are plotted in a distribution diagram. Open circles and a dotted line represent data of the control group, whereas closed squares and a solid line represent data of the DS group ($r = 0.299$, $p = 0.07$ in DS; $r = -0.180$, $p = 0.152$ in control). Abbreviations: see Fig. 2 legend.

Table 3
Comparison of frequencies of aging-related physical characteristics between control and DS groups.

Physical signs of aging	Control	DS	*p*-value
Long eyebrow, n (%)	1 (1.7)	1 (2.0)	1
Hump back, n (%)	0 (0.0)	1 (2.0)	0.459
Cataracts, n (%)	0 (0.0)	16 (31.4)	<0.001
Flabby skin under the eyes, n (%)	0 (0.0)	4 (7.8)	0.042
Longitudinal groove along nails, n (%)	25 (41.7)	13 (25.5)	0.108
Skin winkling, n (%)	6 (10.0)	0 (0.0)	0.03
Alopecia (Forehead), n (%)	6 (10.0)	1 (2.0)	0.215
subtle	5 (8.3)	1 (2.0)	
advanced	1 (1.7)	0 (0.0)	
Alopecia (Parietal region), n (%)	6 (10.0)	1 (2.0)	0.215
subtle	5 (8.3)	1 (2.0)	
advanced	1 (1.7)	0 (0.0)	
Canities, n (%)	22 (36.7)	13 (25.5)	0.322
<10%	19 (31.7)	11 (21.6)	
10≤, <20 %	3 (5.0)	1 (2.0)	
20%≤	0 (0.0)	1 (2.0)	
Missing teeth, n (%)	9 (15)	2 (3.9)	0.247
27–32 remaining teeth	51 (85.0)	49 (96.1)	
23–26 remaining teeth	6 (10.0)	2 (3.9)	
13–22 remaining teeth	2 (3.3)	0 (0.0)	
0–12 remaining teeth	1 (1.7)	0 (0.0)	
Total	60	51	

confirms that their WBC counts are lower than those in control individuals, and its decrease is largely due to the depletion of myeloid cells, but not lymphoid cells.

The magnitude of leukopenia in our study seems more pronounced with age (Fig. 4a and b). As DS individuals are known to experience accelerated aging, such as AD (Kondoh et al., 2011), the leukopenia in DS adults might also be due to premature aging. The two leukopenia cases with hypocellular bone marrow mentioned earlier may support the notion of segmental aging. Mouse studies employing Ts65Dn and other DS mouse models also showed a decrease in hematopoietic stem cells (HSCs) in flow cytometry and bone marrow transplantation assays (Adorno et al., 2013). Similar to other organs such as neuronal tissues, increased oxidative stress in DS may have damaged HSCs (Franceschi et al., 2019), thereby leading to bone marrow failure and leukopenia as an aging phenotype.

PLT counts in DS children are also shown to change with age. Thrombocytopenia occurs at the neonatal stage during the first week after birth, but thrombocytosis develops from 2-month-old onwards until 12-month-old throughout the infant stage (Table 4) (Henry et al., 2007; Kivivuori et al., 1996). Subsequently, PLT counts are normalized by 2-year-old and remain unchanged until 15-year-old (David et al., 1996). Indeed, in Fig. 4e in our study, there was no difference in PLT between the DS and control groups around 20-year-old. However, PLT counts were observed to decrease in DS subjects beyond 20-year-old, and its magnitude became clearer with age. In the earlier study by McLean et al., no obvious PLT abnormalities were observed in nine adult DS cases. One of nine cases presented thrombocytopenia, but this particular case simultaneously suffered from myelodysplastic syndrome (MDS) (McLean et al., 2009) (Table 4). As hematological malignancies can suppress normal hematopoiesis, including PLT production, the thrombocytopenia seen in this case is most likely due to the coexisting MDS rather than an intrinsic DS defect. Altogether, our study is the first to demonstrate the decrease of PLT in DS adults without hematological malignancies. Interestingly, this PLT reduction becomes more significant with age, suggesting that PLT production, like leukocyte production, is likely to be affected by bone marrow failure or premature aging in DS adults.

Unlike leukocytes and PLT counts, the behavior of erythrocyte in DS is reported to be rather stable throughout childhood and adult stages in DS individuals (Table 4). The macrocytosis appears as early as 6-month-old and persists in DS children. Our study cohort of young adult DS cases further suggests that macrocytosis remains through young adulthood. Hypothyroidism and hematinic deficiency are frequently present in DS cases with macrocytosis, but no such cases with obvious clinical symptoms were found in our cohort. This suggests that the macrocytosis in the DS group is likely caused by an increased copy number of the cystathionine β-synthase (CBS) gene on chromosome 21. The enhanced CBS activity has been shown to facilitate the elevation of folate remethylation pathway, decrease DNA synthesis, prolong S-phase and cell cycle, and eventual increase in cell size in erythrocytes, namely macrocytosis (David et al., 1996; Jackson et al., 2016; Neurohr et al., 2019). Alternatively, macrocytosis in DS adults may be caused by segmental aging, as MCV is also known to increase with age (Hoffmann et al., 2015).

Table 4
Summary of hematological status at distinct developmental stages and ages in DS.

Age category		Neonate		Infant	Childhood		Adult	
Age		0- 1 wk	0- 1 wk	0–1 yo	2–6 yo	2–15 yo	21–34 yo	24–60 yo
Study		Henry et al., 2007	Martínez-Macías et al., 2017	Kivivuori et al., 1996	Roizen and Amarose, 1993	David et al., 1996	This study	McLean et al., 2009
Country		USA	Mexico	Finland	USA	Italy	Japan	Ireland
Sample size	control	0	226	0	18	63	60	0
	DS	158	135	25	18	50	51	9
Study design		longitudinal	cross-sectional	longitudinal	cross-sectional	cross-sectional	cross-sectional	longitudinal
Adjusted for confounding factors? (If yes, which statistical method was employed?)		No	Yes (multiple logistic regression analysis)	No	Yes (case control study: age and gender matched)	Yes (age matched)	Yes (multiple linear regression analysis)	No
WBC		NA	leukocytosis (27.4%)	normal[a]	lower#	lower#	leukopenia (21.6%)	leukopenia (66.7%)
Myeloid cells		neutrophilia (80%)	normal	normal[a]	NA	normal	lower*	neutropenia (22.2%)
RBC/Hb/Hct		polycytemia (33%)	polycytemia (23.2%)	normal[a]	higher Hct*	higher Hb, Hct*	higher Hb, Hct*	erythrocytosis (22.2%)
MCV		normal	higher*	macrocytosis (9–12 mo, 12–44%)	macrocytosis (66%)	higher*	macrocytosis (17.6%)	macrocytosis (77.7%)
PLT		thrombocytopenia (66%)	thrombocytopenia (61.5%)	thrombocytosis (80%)	NA	normal	lower*	thrrombocytopenia (11.1%)

Abbreviations: DS, Down syndrome; NA, not available; wk, weeks old; yo, years old; others, see footnote of Table 1.
* Individual values are within the normal range. But the mean value in DS is higher or lower than that in controls with statistical significance.
# Individual values are within the normal range. But the mean value in DS is higher or lower than that in controls without statistical significance.
[a] Elevated only in neonates.

Besides the CBS gene, the increased dosages of other hematopoietic and non-hematopoietic genes located on HSA21 have been shown to underlie the above-mentioned hematological abnormalities in adult DS subjects. One of the most well-characterized hematopoietic genes on HSA21 is RUNX1, a transcription factor essential for the generation of HSCs and frequently mutated in human leukemia (Ng et al., 2010; Osato, 2004; Mok et al., 2014). RUNX1 is also responsible for the maintenance of HSCs as well as the differentiation and proliferation of myeloid and lymphoid cells at multiple stages. RUNX1′s involvement in DS-related hematological malignancies, such as TAM and AMKL, have also been well documented (Osato and Ito, 2005; Yanagida et al., 2005). Two ETS transcription factor family genes, ERG and ETS2, are also HSA21 genes and their involvement in normal and malignant hematopoiesis have been reported (Stankiewicz and Crispino, 2009). Other HSA21 genes which might be responsible for blood abnormalities in DS include BACH1, TIAM1, IFNAR1, GART, SON, SOD1, HMGN1 and USP16 (Robakis et al., 1987; Tanzi et al., 1987; Lott, 2012; Wang et al., 2012; Osato and Ito, 2005; Adorno et al., 2013).

Comorbidities in DS patients who participated in this study included aging-related diseases such as cataracts, dementia, developmental regression, and AD. These diseases are well reported to be commonly seen in DS patients (Congdon et al., 2004; Fraser, 1876; Malamud, 1972; Wisniewski et al., 1985). According to the results of our questionnaire survey for physical signs of aging, cataracts and flabby skin under the eyes were also frequently seen in DS as compared to the control group (Table 3). These signs have also been reported as features of premature aging in DS patients (Daneshpazhooh et al., 2007; Sureshbabu et al., 2011; Orentreich et al., 1979; Morifuji et al., 2014). Despite their young age, the DS participants in this study displayed various physical signs of aging, suggesting that the hematological abnormalities observed in the DS group may be attributed to DS-associated premature aging.

Trisomy 21, the cause of Down syndrome, is largely attributed to oocyte aneuploidy caused by unequal distribution of an HSA21 to daughter cells during mitotic cell division (Tsutsumi et al., 2014). In oocytes, cohesion between the sister chromatids is mediated by cohesins and established at the fetal stage. However, meiosis-specific cohesins, REC8 and SMC1B, are not replenished after birth and decrease with age. Therefore, this age-related decrease in cohesins is considered to underlie the increase of trisomy 21 and DS babies with maternal age. Indeed, the incidence of DS babies is significantly increased with maternal age, and it has become the standard care to offer prenatal screen for DS to all mothers aged 35 and older (Penrose, 1933; Allen et al., 2009; Hodges et al., 2005; Subramanian et al., 2008; Lister et al., 2010; Liu and Keefe, 2008; Chiang et al., 2010). New technological advancements have enabled non-invasive prenatal testing by employing cell-free fetal DNA in the maternal blood, allowing prenatal tests for DS to be increasingly widespread and readily available. As a result, decision making for DS babies based on such prenatal testing is currently an ethical issue which needs to be carefully addressed (Antonarakis et al., 2020).

The world is facing an issue of population aging: there will be more elderly people than ever before. Despite such a widespread healthcare problem, our understanding of biological aging remains limited. Heterogeneity and multilayered complexity have hampered the molecular dissection of the basis for aging in the general population. A stepwise approach focusing on a specific kind of accelerated aging, namely segmental progeria, in people carrying a predisposing genetic background might be an effective alternative to overcome such long-standing issues in aging research. DS is well-documented to be associated with an early onset and a higher incidence of aging-related phenomena such as AD. Notably, in contrast to AD in general, telomere shortening, a hallmark of aging, is clearly detected in the peripheral blood (PB) of individuals with DS-related ADs (Jenkins et al., 2016). Therefore, the PB abnormalities described in this study are likely to be caused by segmental aging. Conversely, these findings suggest that PB from adult DS may serve as a valuable resource for aging research. Further extensive analyses of DS adults might offer us with deeper insights and novel research directions for multiple aging-related disorders in both DS and non-DS adults.

CRediT authorship contrubution statement

Yo Hamaguchi: Conceptualization, Methodology, Formal analysis,

Investigation, Writing - Original Draft. **Tatsuro Kondoh:** Conceptualization, Resources, Data Curation, Supervision. **Masafumi Fukuda:** Resources. **Kazumi Yamasaki:** Formal analysis, Supervision. **Koh-ichiro Yoshiura:** Supervision. **Hiroyuki Moriuchi:** Supervision. **Mariko Morii:** Investigation. **Masashi Muramatsu:** Investigation. **Takashi Minami:** Supervision, Funding acquisition. **Motomi Osato:** Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding acquisition. All authors reviewed and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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