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Design and Progress of Oral Health Examinations in the Tohoku Medical Megabank Project

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In order to assess the long-term impact of the Great East Japan Earthquake on the oral health of disaster victims and to evaluate gene-environmental interactions in the development of major oral diseases and oral-systemic associations, the oral part of two large-scale genome cohort studies by the Tohoku Medical Megabank Organization (ToMMo), including the Community-based cohort (CommCohort) study and the Birth and Three-Generation cohort (BirThree) study, have been conducted. The study population comprised 32,185 subjects, including 16,886 participants in the CommCohort study and 15,299 participants in the BirThree cohort study, recruited from 2013 to 2017. The oral studies consist of a questionnaire regarding oral hygiene behavior, clinical examinations by dentists, and oral plaque and saliva sampling for microbiome analyses, which were carried out at seven community support centers in Miyagi prefecture. The median age of all participants was 55.0 years, and 66.1% of participants were women. Almost all participants reported that they brushed their teeth more than once a day. The median number of present teeth was 27.0, and the decayed, missing and filled tooth number was 16.0, with a significant difference according to age and sex. The median periodontal pocket and clinical attachment level was 2.48 mm and 4.00 mm, respectively. Periodontal parameters increased significantly according to age, except for the accumulation of dental calculus. The oral part of these extensive cross-sectional studies provides a unique and important platform for future studies on oral health and diseases that elicit through interactions with systemic diseases, lifestyles, life events and genetic backgrounds, and contributes to researches clarifying the long-term effects of disasters on oral health.

Keywords: dental caries; genome cohort study; Great East Japan Earthquake; oral health care; periodontal disease
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Introduction

After the Great East Japan Earthquake (GEJE) in 2011, the regional and community medical systems in the Tohoku district of Japan collapsed (Ishigaki et al. 2013). To foster creative reconstruction after the disaster and establish an advanced medical system aimed toward personalized medicine and healthcare, we established the Tohoku Medical Megabank Organization of Tohoku University (ToMMo) and the Iwate Tohoku Medical Megabank Organization (IMM) (Kuriyama et al. 2016). Both organizations set up a common framework, the Tohoku Medical Megabank Organization (TMM), and in collaboration developed a common TMM biobank that combines medical and genomic information under the concept of an integrated biobank (Minegishi et al. 2019). The TMM has been conducting two large-scale, prospective, genome cohort studies, the Community-based cohort (CommCohort) study, consisting of a population-based cohort with approximately 88,000 participants (Hozawa et al. 2020), and the Birth and Three-Generation (BirThree) cohort study, with approximately 73,500 participants, including babies and their parents, siblings, grandparents, and extended family members (Kuriyama et al. 2019). Both cohorts started recruit in 2013 and completed recruitment by 2017. Detailed follow up examinations of participants in both cohorts have been conducted since 2017 at an interval of approximately 5 years.

To date, there have been a number of investigations on the impacts of this major disaster on the general and oral health conditions of the inhabitants in the affected area, including physical and mental stressors caused by the earthquake. For instance, there are reports on myocardial infarction (Hao et al. 2014), stroke (Omama et al. 2013), depression (Sakuma et al. 2015), diabetes (Tanaka et al. 2015), pneumonia (Ohkouchi et al. 2013), and periodontitis (Tsuchiya et al. 2015). We surmise that oral hygiene deterioration due to devastation of the living environment, especially water shortage, could lead to various dental problems in the aftermath. Most dental problems, such as dental caries and periodontitis, that emerge in the inhabitants of a disaster-affected area are common chronic infectious diseases occurring in the human oral cavity and are major causes of missing teeth.

Recent population-based studies have revealed strong associations between oral conditions and systemic diseases, such as diabetes mellitus (Kim et al. 2018), arteriosclerosis (Aarabi et al. 2015), aspiration pneumonia (Taylor et al. 2000), and pregnancy complications (Michalowicz et al. 2006). A remarkable coincidence among oral and systemic diseases has also been noted for lifestyle-related diseases (Lira-Junior and Figueredo 2016; Aarabi et al. 2017; Chapple et al. 2017; Strauss et al. 2018). The following explanations may be pertinent to explain these links between oral and systemic diseases: 1) the inflammatory burden of oral infectious diseases may influence general conditions; 2) genetic risk factors common affect both oral and systemic diseases may reside; and 3) unexplained confounding factors related to both oral and systemic diseases may exist.

Based on these observations, we designed the scope of the oral health studies of the ToMMo cohort projects. We aimed to establish a database on the oral health of the people who suffered from the 2011 GEJE by assessing dental caries and tooth loss, restorative treatments, periodontitis, oral mucosa conditions, signs of temporomandibular joint (TMJ) function, oral care habits and oral health-related quality of life in the Miyagi prefecture. We also aimed to gather evidence of the links between oral health/diseases and general/systemic conditions through examinations of the population's lifestyle and genetic predisposition. In the present article, the design of the oral health studies is documented along with the examination methods, study populations, and questionnaire content. We also documented some of the baseline results related to oral health, such as dental and periodontal conditions.

Materials and Methods

Data collection methods and management

The examination was conducted with a plane dental mirror (11B1X1000625D001, YDM Corporation, Tokyo), and a sharp-pointed dental explorer (11B1X1000664D103, YDM Corporation) was used to detect caries or other defects, as necessary. No radiographs were collected from any participant.

Dentition status

The dentition status was obtained for all teeth, including primary teeth and third molars, in all participants. Before the oral examination, dentures were removed, but teeth were not air-dried or cleaned. The tooth status was recorded at ten (1-10) or eleven (1-11) levels. The levels were determined as follows, sound (level 1); decayed, with/without filling (2); filled, no decay (3); missing, need for prosthesis (4); missing, no need for prosthesis, or unerupted (5); artificial tooth of fixed prosthesis (6); artificial tooth of removable prosthesis (7); unrestored stump covered with removable prosthesis (8); restored stump covered with removable prosthesis (9); dental implant (10) and sealed fissure (11). The level 11 was assessed for only under-20 aged participants.

Abnormal tooth growth

The following abnormal tooth growth was recorded for each tooth: hyperdontia; microdontia; fused tooth; enamel hypoplasia (molar-incisor hypomineralization; MIH); and congenital anodontia.

Tooth wear

When a tooth was visually examined for the presence of exposed dentine, tooth wear was recorded for each tooth according to the simplified scoring criteria for the tooth wear index (Bardsley et al. 2004), because the correlation of the accuracy of visual and histological examinations is poor (Ganss et al. 2006).

Periodontal examination

The periodontal examination was performed at several locations around the entire circumference of all present teeth, including the third molars, using a disposable WHO periodontal probe furnished with a ball tip (11B1X 1000664D004, YDM Corporation). Several clinical measures of periodontal conditions were recorded in millimeters regarding the deepest periodontal pocket site of each tooth, including the probing depth (PD), defined as the distance from the gingival margin to the base of the gingival sulcus, and the clinical attachment level (CAL), defined as the distance from the cemento-enamel junction (CEJ) to the base of the sulcus, although we did not record the CAL if the CEJ was not visible. Bleeding on probing (BOP) indicated whether bleeding from within the periodontal pocket of each tooth occurred when performing a probing operation. The mobility of each tooth was examined using dental forceps (27B3X00017000012, Nichiei Co., Ltd., Osaka) and one finger to move the tooth in the horizontal and vertical directions, and tooth mobility (TM) was scored according to Miller's criteria (Salvi et al. 2015). Identification of the presence of dental calculus (DC) on the supra- and subgingival surfaces of teeth was carried out using a disposable WHO probe and recorded as "absent" or "present" for teeth #11, 16, 17, 26, 27, 31, 36, 37, 46, and 47, numbered using the Zsigmondy-Palmer system.

Oral hygiene status

Plaque deposition on the tooth surface was classified as small, medium, high, or extremely high. The tongue coating was assessed using the following codes (Miyazaki et al. 1995): absence of plaque on tongue (code 0); thin plaque covering less than one-third of the tongue surface (code 1); thin plaque covering less than two-thirds of the tongue surface or thick dental plaque covering less than one-third of the tongue surface (code 2); thin plaque covering more than two-thirds of the tongue surface or thick dental plaque covering less than two-thirds of the tongue surface (code 3); and thick plaque covering more than two-thirds of the tongue surface (code 4).

Soft tissue conditions in the orofacial area

The location, size and color of oral mucosa lesions and the soft tissue conditions in the orofacial area were recorded in all participants.

Frenal attachment

The presence of abnormalities in the attachment of the tongue and superior labial frenum was assessed.

Oral tori

The location and size of palatal and mandibular tori, buccal exostoses and any bone outgrowths in the oral cavity were recorded. Participants with these bony outgrowths were asked whether they had oral habits, such as tooth clenching and grinding, and the answers were recorded as "present with self-awareness," "present without self-awareness," "absent," or "unknown."

TMJ function

Voluntary maximum mouth opening was measured as the distance between the edges of the lower and upper incisors using a digital caliper (AD-5765-100, A&D Company, Limited, Tokyo, Japan) to the nearest 0.1 mm. TMJ noise was examined on each side, as follows: "absent" or "present," including clicking, popping, or crepitus. Pain or tenderness in the TMJ and associated masticatory muscles was checked during mandibular functions, including mouth opening, mouth closing, and chewing, or spontaneously. TMJ and associated masticatory muscle pain were also assessed by means of manual palpation.

Occlusion (tooth contact)

Each contacted tooth was recorded during intercuspal occlusion. Overjet and overbite were recorded as the distance, in millimeters, between the ridge of the upper and lower central incisors in the intercuspal position. Crossbite, which is a form of malocclusion in which there is a reversal of the normal relationship of the mandibular and maxillary teeth, with lateral displacement of opposing teeth, was recorded as "absent" or "present."

Collection of dental plaque, tongue coating, and saliva samples

Supragingival plaque was obtained from two molars (#16 and #26) by swiping the tooth surface with a sterile Gracey Curette (ROUND SAKURA H #G11-12, 11B1X1000662D102, YDM Corporation). If the index first molar was missing and the second molar in the same quadrant was present, the sample was obtained from the second molar instead. If both the first and second molars were absent, the sample were collected from the premolar closest to the missing index teeth. If all maxillary molars in the quadrant were absent, the sample was obtained from mandibular molars on the same side. A tongue coating specimen was obtained from the dorsum of the tongue by swabbing with a plastic spatula. After collection, the plaque and tongue coating samples were immediately immersed in saline in a polypropylene tube (CryoELITE, 2 ml, W985865, Weathon, NJ). Unstimulated saliva samples were collected in sterilize centrifuge tubes (50 ml, VIO-50 #150065, AS ONE Corp., Tokyo). The samples were immediately stored at –80°C until transportation to our laboratory. Then, the saliva samples were thawed at room temperature and separated into the following three portions: 1) whole saliva (1 ml); 2) solid components of remaining saliva (1 ml), including pelleted oral microbiota, after centrifugation at 20,000 g for 10 min at 4°C; and 3) the supernatant separated from the same saliva by centrifugation.

Each portion was dispensed into a Matrix storage tube (1.4 ml, Thermo Fisher Scientific, Inc., MA) and stored at –80°C until use in future experiments (Yamagishi et al. 2016).

Statistical analysis

Numerical data are presented as the median and interquantile range. The interquartile range (IQR) represents the range from the 25th percentile to the 75th percentile, or the middle 50 percent as median, of each data set. The distributions of dental and periodontal parameters were tested for normality using the Kolmogorov-Smirnov (KS) test. The Kruskal-Wallis test was used to examine differences among age groups. Differences in dental and periodontal parameters among age groups were tested by chi-squared test. All statistical analyses were performed using SAS software (SAS 9.4, SAS Institute, NC, USA) and SAS enterprise guide. P values less than 0.05 were considered to indicate statistical significance.

Results

Populations of the oral health study and age distribution of participants

Participants who visited seven ToMMo community support centers in Miyagi prefecture from October 28, 2013, to June 30, 2017, and received an oral examination and/or general health check were registered for this oral health survey. As shown in Fig. 1, a total of 37,575 residents, including 13,459 males (median age 45.0 years (IQR: 32.0-65.1)), and 24,116 females, (median age 52.1 years (IQR: 34.0-63.1)) were included in either the CommCohort or BirThree cohort study. Among the participants, 4,695 who discontinued oral examinations were excluded. We also excluded 90 participants who withdrew consent and 222 died before March 05, 2020. In addition, 383 under 20 years old participants were excluded. Consequently, there was a total of 32,185 participants, including 16,886 partici-

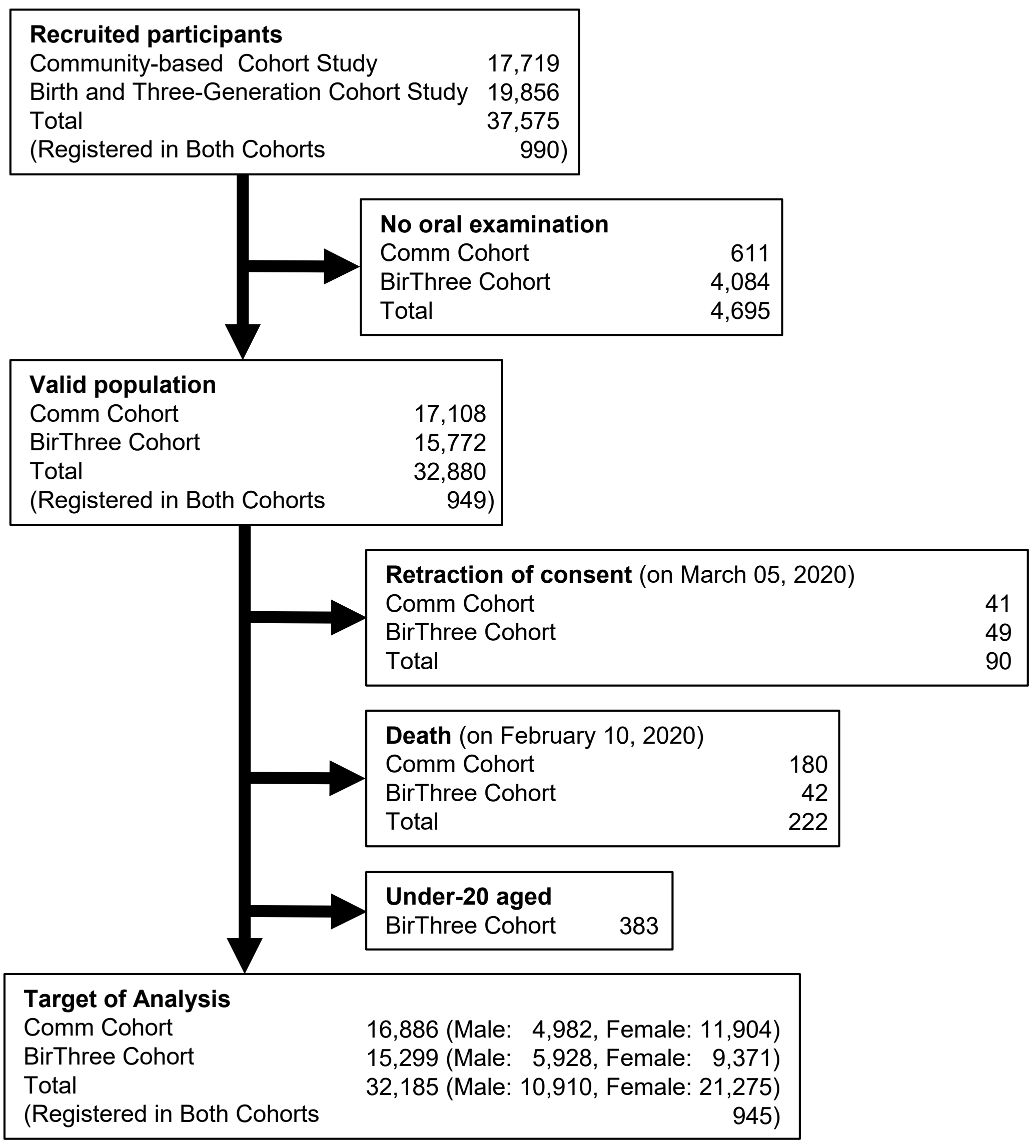

Fig. 1. Baseline survey participants with dental examination data.

pants (males, 4,982; median age 65.1 years (IQR: 55.0-77.1); females, 11,904; median age 60.1 years (IQR: 48.6-66.1)) in the CommCohort study, 15,299 participants (males, 5,928; median age 38.0 years (IQR: 32.0-59.1); females, 9,371; median age 37.1 years (IQR: 31.1-58.1)) in the BirThree cohort study, who served as the subjects of the baseline oral health survey in this cohort (Fig. 1). Note that 945 participants, who were recruited in both cohort studies, were received oral health examination for either or both cohort studies. Among them, 42 participants were examined oral health for both cohorts. In order to avoid the potential inclusion of duplicate data, in the present study we divided the 945 participants into either cohort (CommCohort; 634, BirThree cohort; 311) according to their initial oral health examination.

We examined the age distribution of 32,185 participants who underwent oral examinations for the CommCohort and BirThree cohort studies (Fig. 2). It should be noted that the participants in these cohort studies showed a wide age distribution. The CommCohort participants showed a peak age in the 60s in the age distribution (Hozawa et al. 2020), while the BirThree participants showed two peaks, one in the 30s and the other in 60s, reflecting the two major generations *i.e.,* parents and grand-

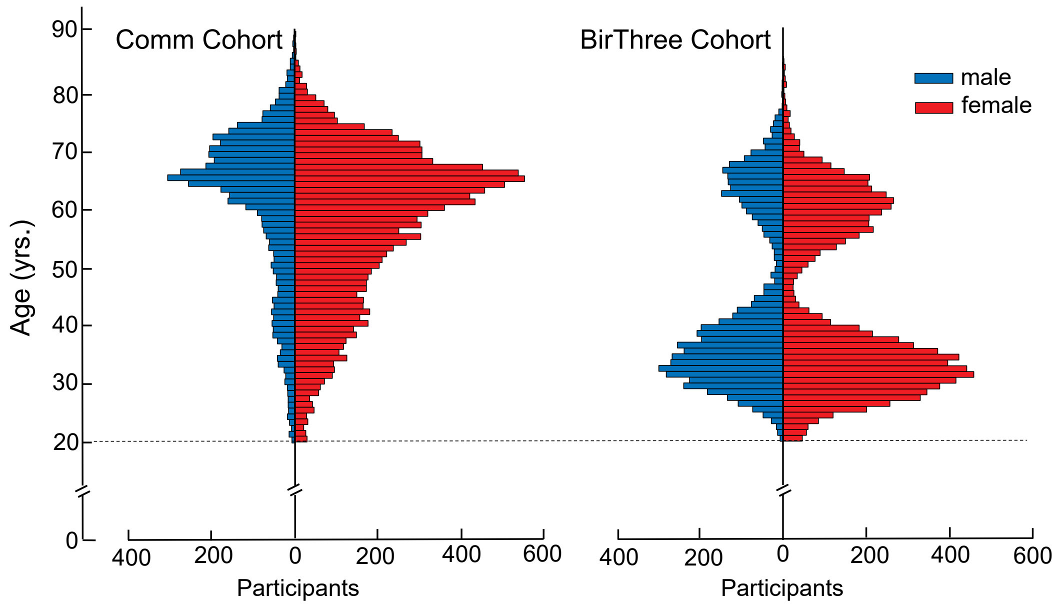

Fig. 2. Age distribution of participants undergoing oral examination.
The age distribution of participants who underwent an oral examination in each cohort study, where the ordinate denotes age and the abscissa denotes the number of participants. Each bin shows 1 year.

Table 1. Assessments, collections, and measurements in participants who are 5, 10, 16 and 20 years old or over.

	Adult	Adolescent (around 16 yrs. old)	Children (around 5-10 yrs. old)
Dentition status	○	○	○
Abnormal tooth growth	○	○	○
Tooth wear	○	○	○
Periodontal examination	○	○	
Oral hygiene status	○	○	○
Oral mucosa lesion	○	○	○
Frenal attachment			○
Oral tori	○	○	
Temporomandibular joint (TMJ)	○	○	
Tooth contact and occlusion	○	○	○
Sample collection			
Supragingival plaque	○	○	○
Tongue coat	○	○	○
Saliva	○		

○: assessed or measured.

parents (Kuriyama et al. 2019). Thus, a significant difference between the CommCohort and BirThree cohort studies was found in the median age (62.0 years and 38.0 years, respectively; $p < 0.001$). In addition, there is another significant difference in the female-to-male ratio (2.39 and 1.58, respectively; $p < 0.001$).

We further examined and collected data and samples from children in the BirThree cohort study who were elder siblings of newborns or adults. Table 1 shows the extent of examinations performed for the children, adolescents, and adults. While we executed this plan for studying children, in the current baseline analysis we excluded 383 of the children, as our recruitment of children and adolescents is ongoing to increase the sample size. Analyses of the children and adolescents will be reported elsewhere.

Examiners, oral health questionnaire, and ethical considerations

We wished to conduct a comprehensive oral examination of participants by dentists. To this end, we organized a team of 133 examiners, which comprised 7 assistant professors in the ToMMo and 126 dentists/graduate students who belong the Graduate School of Dentistry, Tohoku University. All examiners retained a dentist's license in Japan and not only were given specific instructions in an orientation meeting regarding the oral examination methods in the ToMMo cohort studies but also received on-the-job training before participation in the oral examination program. Through this design, a high level of quality control for the collection of plaque and saliva samples was attained.

Participants in the present oral health survey completed a questionnaire consisting of 8 questions (Table 2). The questionnaire asked about oral health status, oral health problems, oral hygiene habits and the utilization of dental care. Almost all (98.6%) participants answered they brushed their teeth more than once a day (male; 96.7%, female; 99.4%). However, males brushing their teeth more than twice a day were significantly fewer than females (male: 65.8%, female: 86.5%, $p < 0.0001$, Chi-square-test).

Our genome medical research coordinator (GMRC),

Table 2. Questionnaire on oral health behavior.

Question	Answers
Q1. How often do you brush your teeth?	A1. 2 or more times a day A2. once a day A3. 4-5 times a week A4. 1-2 times a week A5. once a month or less A6. No
Q2. How long do you brush your teeth each time?	A1. 3 minutes or less A2. 3-5 minutes A3. 5 minutes or more
Q3. Which tool do you use for brushing teeth? (multiple choices allowed)	A1. Toothbrush A2. Dental floss A3. Interdental brush A4. Toothpick A5. Others
Q4. Do you clean your mouth (i.e. tongue, cheeks) other than teeth?	A1. 2 or more times a day A2. once a day A3. 4-5 times a week A4. 1-2 times a week A5. once a month or less A6. No
Q5. Which tool do you use for cleaning your mouth? (multiple choices allowed)	A1. Toothbrush A2. Tongue brush A3. Gauze A4. Others
Q6. Do you use dentures?	A1. Yes A2. No
Q7. How often do you clean your dentures?	A1. 2 or more times a day A2. once a day A3. 4-5 times a week A4. 1-2 times a week A5. once a month or less A6. No
Q8. What items do you use for cleaning your dentures? (multiple choices allowed)	A1. Washing with water A2. Tooth brush A3. Denture brush A4. Denture cleanser A5. Others

who was specially trained in house and licensed, informed eligible persons of the aims and protocols of the ToMMo oral health study and obtained their informed consent (Sakurai-Yageta et al. 2019). We adopted general and continuing consent to participate in the TMM studies. At baseline, we specifically explained the protocols of the cohort studies, biobanking, and general research methods for the genomic analyses, other omic analyses, and immortalized cell preparation.

Approval was obtained from the Institutional Review Board (IRB) and Ethics Committee of Tohoku University Graduate School of Medicine. This study was conducted in accordance with the Declaration of Helsinki, Ethical Guidelines for Human Genome/Gene Analysis Research, and other appropriate guidelines.

Present tooth number and tooth loss

Data regarding the dental examinations, questionnaires, oral samples, basic information, blood samples, and numbers of participants in our oral health cohort study are shown in Table 3. Especially, our participants almost fully finished genome analysis using Japanese-specific full custom array, which is referred to as Japonica Array™ (JPA) (Fuse et al. 2019; Yasuda et al. 2019). Details of the other surveys will be published elsewhere. Utilizing the data collected from the CommCohort and BirThree cohorts, we conducted a series of analyses of the basic dental health care data.

We first examined how many natural teeth the participants of the CommCohort and BirThree cohort studies retained, in reference to sex, age group and geographical location (Table 4) The locations of the community support centers were described previously (Kuriyama et al. 2019). In both cohort populations, most adults had 20 or more teeth (CommCohort: 83.7%, BirThree cohort: 93.2%). The number of teeth present decreased with age, and significant differences were observed in the number of teeth present among the age groups ($p < 0.001$). A minority (CommCohort: 1.5%, BirThree cohort: 0.5%) of the present study population had no natural teeth, and most of the edentulous persons were 55 years old or older. Significant variation in edentulousness was observed in relation to sex ($p < 0.001$), geographical location ($p < 0.001$) and cohort ($p < 0.001$).

The median number of natural teeth present in the BirThree cohort was 28.0, which was significantly higher than that of 26.0 in the CommCohort ($p < 0.001$). We surmised that this difference in the number of natural teeth reflected the difference in the age distribution of the cohorts (Fig. 2). Geographical variation was also observed regarding the number of teeth among the participants ($p < 0.05$). Participants in heavily Tsunami devastated area (Ishinomaki and Kesennuma) tended to show fewer numbers of natural teeth than those in inland area (Osaki and Shiroishi).

Caries and caries expression

We then examined the burden of caries in the participants of the CommCohort and BirThree cohort studies in reference to sex, age group and geographical location. The caries burden is expressed for the whole study population according to the following components: decayed teeth (DT); missing teeth (MT); and filled teeth (FT). These components were grouped and are referred to as DMFT (Table 5). The median DMFT value was 18.0 (IQR: 13.0-22.0) for the CommCohort and 14.0 (IQR: 9.0-19.0) for the BirThree cohort, indicating a significant difference in the median DMFT value between these two cohorts ($p < 0.001$). The difference in the median DMFT value was reproducible when the data were examined separately by sex ($p < 0.001$). Participants in heavily Tsunami devastated area (Ishinomaki and Kesennuma) tended to show higher DMFT values than those in inland area (Osaki and Shiroishi).

We found that the median DMFT was increased in both cohorts gradually with age and was increased by over 10 teeth in those aged 55-64 years when compared with those aged 20-24 years. This increase persisted in the oldest age group (Table 5). On the other hand, the occurrence of untreated caries (the DT component) was very low, with a median value of almost 0.0 DT for all age groups except for younger groups. In both cohorts, the median MT component increased with age ($p < 0.001$), and in the oldest age group, the value was 9.0 in the CommCohort (IQR: 4.0-17.0) and in the BirThree cohort (IQR: 4.0-15.0). In contrast, the FT component started decreasing in those aged 55-64 years and continued decreasing to the oldest age

Table 3. Number of examination data, basic information, oral and blood samples.

	Dental examination	Questionnaire for oral health	Oral samples			Basic information	Blood samples	Actual number of participants with data/samples
			Dental plaque	Tongue coating	Saliva			
CommCohort	16,886	16,883	16,778	16,851	16,780	16,886	16,867	-
	○	○	○	○	○	○	○	16,689
	○	○				○	○	16,864
BirThree Cohort	15,299	14,862	7,460	7,471	7,463	15,294	15,278	-
	○	○	○	○	○	○	○	7,441
	○	○				○	○	14,840

○: samples collected.

Table 4. Number of natural teeth for the participants vs. gender, age group and geographical location.

		CommCohort									BirThree Cohort									
		n	%	Number of natural teeth present						P	n	%	Natural teeth present (%)						p	P
				Median	(IQR)	20 =<	10-19	1-9	0				Median	(IQR)	20 =<	10-19	1-9	0		
						(%)	(%)	(%)	(%)						(%)	(%)	(%)	(%)		
	Total	16,886	100	26.0	(22.0-28.0)	83.7	10.9	3.8	1.5		15,299	100	28.0	(26.0-29.0)	93.2	4.8	1.5	0.5	***	
Gender	Male	4,982	29.6	26.0	(22.0-28.0)	80.8	11.9	4.9	2.4	< .0001	5,928	38.7	28.0	(26.0-29.0)	92.8	4.8	1.7	0.8	***	0.0028
	Female	11,904	70.4	26.0	(23.0-28.0)	85.0	10.5	3.4	1.1		9,371	61.3	28.0	(26.0-29.0)	93.5	4.8	1.4	0.4	***	
Age	20-24	190	1.1	28.0	(28.0-30.0)	100.0	0.0	0.0	0.0	< .0001	484	3.2	29.0	(28.0-30.0)	99.8	0.2	0.0	0.0		< .0001
	25-34	940	5.6	28.0	(28.0-30.0)	99.8	0.2	0.0	0.0		5,735	37.5	28.0	(28.0-30.0)	100.0	0.0	0.0	0.0		
	35-44	1,944	11.5	28.0	(27.0-29.0)	99.0	1.0	0.0	0.0		3,331	21.8	28.0	(28.0-30.0)	99.3	0.7	0.0	0.0	***	
	45-54	2,485	14.7	27.0	(26.0-28.0)	96.5	2.9	0.5	0.1		943	6.2	27.0	(25.0-28.0)	92.8	5.5	1.4	0.3	***	
	55-64	4,899	29.0	26.0	(23.0-28.0)	85.6	11.1	2.6	0.8		3,177	20.8	26.0	(22.0-28.0)	84.5	11.3	3.2	1.1		
	65-74	5,497	32.6	24.0	(19.0-27.0)	72.8	17.6	7.0	2.6		1,510	9.9	24.0	(19.0-27.0)	72.8	17.8	7.0	2.5		
	75-	931	5.5	21.0	(11.0-26.0)	53.5	25.4	12.8	8.4		119	0.8	21.0	(15.0-26.0)	63.0	20.2	13.5	3.4		
Geographical location	Ishinomaki	1,938	11.5	25.0	(20.0-28.0)	77.7	13.9	5.7	2.7	< .0001	1,792	11.7	28.0	(26.0-29.0)	90.9	6.3	1.9	1.0	***	< .0001
	Iwanuma	2,035	12.1	26.0	(22.0-28.0)	84.3	9.9	4.7	1.1		2,113	13.8	28.0	(26.0-29.0)	94.5	3.7	1.1	0.7	***	
	Kesennuma	1,526	9.0	25.0	(19.0-28.0)	74.5	14.9	6.4	4.3		626	4.1	28.0	(25.0-29.0)	89.0	6.4	3.4	1.3	***	
	Osaki	2,637	15.6	26.0	(23.0-28.0)	84.5	10.7	3.6	1.1		2,368	15.5	28.0	(27.0-29.0)	93.9	4.7	1.1	0.3	***	
	Sendai	4,811	28.5	27.0	(23.0-28.0)	86.7	9.6	2.9	0.9		5,294	34.6	28.0	(26.0-29.0)	93.3	4.9	1.5	0.3	***	
	Shiroishi	1,259	7.5	26.0	(23.0-28.0)	85.9	10.0	2.7	1.4		1,042	6.8	28.0	(26.0-29.0)	92.7	4.6	2.0	0.7	***	
	Tagajo	2,680	15.9	27.0	(23.0-28.0)	85.9	10.3	2.7	1.0		2,064	13.5	28.0	(26.0-29.0)	94.5	3.7	1.4	0.3	***	

P: compared within gender, age and geographical location group of each cohort study.
p: compared between same parameters of each cohort study.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

Table 5. Caries burden for the participants vs. gender, age group, geographical location and each cohort population.

		ComCohort						BirThree Cohort					
		n	%	DMFT	DT	MT	FT	n	%	DMFT	DT	MT	FT
				Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)			Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
	Total	16,886	100	18.0 (13.0-22.0)	0.0 (0.0-1.0)	4.0 (3.0-7.0)	11.0 (8.0-15.0)	15,299	100	14.0*** (9.0-19.0)	0.0*** (0.0-1.0)	3.0*** (2.0-5.0)	9.0*** (5.0-13.0)
Gender	Male	4,982	25.3	17.0 (13.0-22.0)[a]	0.0 (0.0-1.0)[a]	4.0 (3.0-8.0)[a]	10.0 (6.0-14.0)[a]	5,928	38.7	13.0*** (9.0-19.0)[a]	0.0*** (0.0-2.0)[a]	3.0*** (2.0-4.0)[b]	8.0*** (4.0-12.0)[a]
	Female	11,904	74.7	18.0 (14.0-22.0)	0.0 (0.0-0.0)	4.0 (3.0-7.0)	12.0 (8.0-15.0)	9,371	61.3	14.0*** (10.0-20.0)	0.0*** (0.0-1.0)	3.0*** (2.0-5.0)	10.0*** (6.0-13.0)
Age	20-24	190	1.1	7.0 (4.0-10.0)[a]	0.0 (0.0-2.0)[a]	2.5 (1.0-3.0)[a]	4.0 (1.0-6.0)[a]	484	3.2	9.0*** (6.0-12.0)[a]	1.0* (0.0-2.0)[a]	3.0 (1.0-3.0)[a]	4.0* (2.0-7.0)[a]
	25-34	940	5.6	10.0 (7.0-14.0)	0.0 (0.0-1.0)	2.0 (1.0-3.0)	7.0 (4.0-10.0)	5,735	37.5	10.0 (7.0-14.0)	0.0* (0.0-2.0)	3.0*** (2.0-3.0)	6.0** (4.0-10.0)
	35-44	1,944	11.5	14.0 (10.0-17.0)	0.0 (0.0-1.0)	3.0 (1.0-3.0)	10.0 (7.0-13.0)	3,331	21.8	13.0 (10.0-17.0)	0.0*** (0.0-1.0)	3.0*** (2.0-3.0)	9.0*** (6.0-13.0)
	45-54	2,485	14.7	17.0 (14.0-20.0)	0.0 (0.0-1.0)	3.0 (2.0-5.0)	12.0 (9.0-15.0)	943	6.2	18.0*** (14.0-22.0)	0.0*** (0.0-1.0)	4.0*** (3.0-6.0)	12.0* (9.0-15.0)
	55-64	4,899	29.0	19.0 (15.0-22.0)	0.0 (0.0-1.0)	5.0 (3.0-7.0)	12.0 (9.0-15.0)	3,177	20.8	20.0*** (15.0-23.0)	0.0*** (0.0-1.0)	5.0*** (3.0-8.0)	12.0 (8.0-15.0)
	65-74	5,497	32.6	20.0 (16.0-24.0)	0.0 (0.0-0.0)	6.0 (3.0-10.0)	11.0 (7.0-15.0)	1,510	9.9	21.0* (16.0-24.0)	0.0*** (0.0-1.0)	6.0*** (4.0-11.0)	11.0 (7.0-14.0)
	75-	931	5.5	23.0 (18.0-27.0)	0.0 (0.0-0.0)	9.0 (4.0-17.0)	10.0 (6.0-15.0)	119	0.8	22.0* (17.0-26.0)	0.0* (0.0-1.0)	9.0 (4.0-15.0)	10.0 (6.0-13.0)
Geographical location	Ishinomaki	1,938	11.5	19.0 (15.0-23.0)[a]	0.0 (0.0-1.0)[a]	5.0 (3.0-9.0)[a]	11.0 (7.0-14.0)[a]	1,792	11.7	14.0*** (9.0-19.0)[a]	0.0*** (0.0-1.0)[a]	3.0*** (2.0-5.0)[a]	8.0*** (5.0-13.0)[a]
	Iwanuma	2,035	12.1	18.0 (13.0-22.0)	0.0 (0.0-1.0)	4.0 (3.0-7.0)	11.0 (8.0-15.0)	2,113	13.8	14.0*** (10.0-19.0)	0.0*** (0.0-2.0)	3.0*** (2.0-5.0)	9.0*** (6.0-13.0)
	Kesennuma	1,526	9.0	19.0 (15.0-23.0)	0.0 (0.0-1.0)	5.0 (3.0-10.0)	11.0 (7.0-14.0)	626	4.1	16.0*** (11.0-21.0)	0.0 (0.0-1.0)	3.0*** (2.0-5.0)	10.0* (6.0-14.0)
	Osaki	2,637	15.6	17.0 (13.0-21.0)	0.0 (0.0-1.0)	4.0 (2.0-7.0)	11.0 (8.0-15.0)	2,368	15.5	13.0*** (9.0-18.0)	0.0*** (0.0-2.0)	3.0*** (2.0-4.0)	8.0*** (5.0-12.0)
	Sendai	4,811	28.5	18.0 (13.0-22.0)	0.0 (0.0-0.0)	4.0 (3.0-7.0)	11.0 (8.0-15.0)	5,294	34.6	14.0*** (10.0-20.0)	0.0*** (0.0-1.0)	3.0*** (2.0-5.0)	9.0*** (6.0-13.0)
	Shiroishi	1,259	7.5	17.0 (13.0-21.0)	0.0 (0.0-1.0)	4.0 (2.0-6.0)	11.0 (7.0-14.0)	1,042	6.8	13.0*** (9.0-18.0)	0.0*** (0.0-1.0)	3.0*** (2.0-5.0)	8.0*** (5.0-12.0)
	Tagajo	2,680	15.9	18.0 (13.0-22.0)	0.0 (0.0-0.0)	4.0 (2.0-7.0)	11.0 (8.0-15.0)	2,064	13.5	13.0*** (9.0-19.0)	0.0*** (0.0-1.0)	3.0*** (2.0-5.0)	9.0*** (5.0-13.0)

Statistical comparisson was performed between same parameters of each cohort study.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.
Statistical comparisson was performed within each parameters among gender, age and geographical location in each cohort study.
[a]$p < 0.001$, [b]$p < 0.05$.

group in both cohorts. This was inversely correlated with an increase in missing teeth, or the MT component. The FT component was larger in females than in males ($p < 0.01$). Regarding geographical differences, we did not find large differences among the seven support center regions.

Periodontal conditions

We also examined the periodontal conditions of the participants in the CommCohort and BirThree cohort studies in reference to sex and age group. Periodontal parameters were estimated, excluding the edentulous participants. We found that in both cohort populations, the median periodontal pocket depth (PPD) became deeper with age (Table 6). Except for those in the oldest age group (over 75 years old), the median PPD of males was significantly larger than that of females, at 2.65 vs. 2.46 in the CommCohort and 2.54 vs. 2.39 in the BirThree cohort (both $p < 0.001$). Posterior teeth showed a deeper PPD than anterior teeth (data not shown). The PPD in the total CommCohort and BirThree cohort populations also showed a significant difference, and this difference was reproducible in both the male and female participants in the two cohorts.

In this study, we measured the CAL for each tooth when the CEJ was visually recognized at the measurement site. Only 1.4-2.2% of the participants in the youngest age group (20-24 years old) had at least one tooth with a measurable CAL. The proportion of participants with a measurable CAL increased with age and reached 25.8-42.1% at 75 years old and older (Fig. 3). Table 7 shows the number of participants with at least one CAL and the median CAL (mm). A larger median CAL in males than in females ($p < 0.001$) and in older age groups than younger age groups ($p < 0.001$) was found in both cohorts. These results indicate that there are differences in periodontal regeneration according to sex and age.

We also measured BOP to assess periodontal inflammation in the participants. Table 8 shows the number of participants and the number of teeth with BOP. Consistent with previous observations, we observed more teeth with BOP in the youngest age group and in males than in the older age groups and in females in both the CommCohort and BirThree cohort (Table 8). With aging, the number of teeth with BOP decreased more significantly in males than females (males: 2.0-4.0, females: 0.5-3.0). The prevalence of teeth with BOP was higher in males than in females. Both cohorts showed almost the same tendency in these parameters.

We measured the nonphysiological tooth mobility (TM) of all teeth of the participants in both cohorts with Miller's criteria, and expressed the result as either negative or positive. Table 9 presents the median percentage of positive tooth among present teeth of participants.

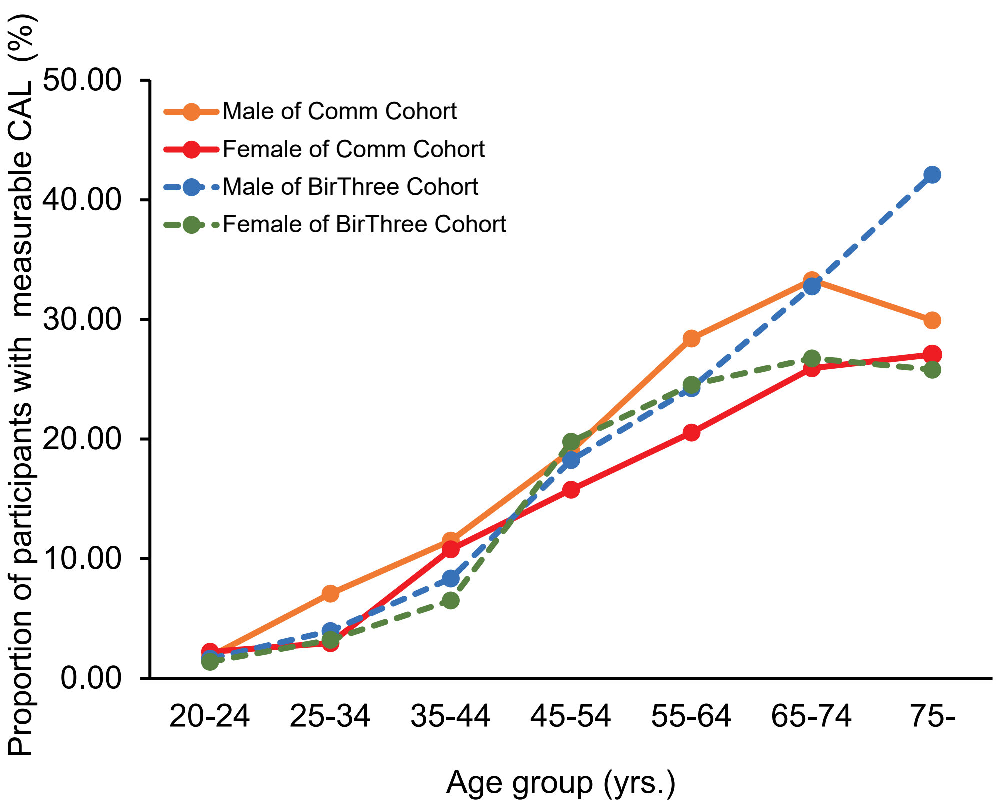

Fig. 3. Proportion of participants with measurable CAL for each age group.
The proportion of participants who had at least one tooth with a measurable CAL accompanied by a visible cemento-enamel junction (CEJ) plotted for each age group. Orange: males in the CommCohort; red: females in the CommCohort; blue: males in the BirThree cohort; green: females in the BirThree cohort.

Table 6. Periodontal probing depth (PPD) for the participants vs. gender and age group and cohort.

Age	PPD (mm) in CommCohort									PPD (mm) in BirThree Cohort								
	Total			Male			Female			Total			Male			Female		
	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)
Total	16,886 (16,915)	2.52^{a}	(2.15-3.00)	4,851 (4,982)	2.65^{a}	(2.25-3.16)	11,753 (11,904)	2.46$^{a, §§}$	(2.11-2.96)	15,211 (15,299)	2.45^{***,a}	(2.14-2.87)	5,880 (5,928)	2.53^{***,a}	(2.20-2.97)	9,331 (9,371)	2.39$^{***,a, §§}$	(2.11-2.81)
20-24	190 (190)	2.29	(2.10-2.64)	55 (55)	2.37	(2.14-2.75)	135 (135)	2.28	(2.07-2.54)	484 (484)	2.34	(2.10-2.75)	122 (122)	2.38	(2.09-2.83)	362 (362)	2.33	(2.10-2.71)
25-34	940 (940)	2.36	(2.06-2.75)	226 (226)	2.49	(2.14-2.93)	714 (714)	2.33§§	(2.04-2.69)	5,732 (5,735)	2.36	(2.09-2.72)	2,098 (2,098)	2.43	(2.14-2.79)	3,634 (3,637)	2.32§§	(2.07-2.68)
35-44	1,944 (1944)	2.38	(2.07-2.83)	469 (469)	2.56	(2.23-3.00)	1,475 (1,475)	2.32§§	(2.04-2.76)	3,330 (3,331)	2.42*	(2.14-2.80)	1,641 (1,641)	2.50**	(2.19-2.88)	1,689 (1,690)	2.36§§	(2.10-2.72)
45-54	2,482 (2,485)	2.48	(2.14-2.93)	493 (494)	2.63	(2.21-3.08)	1,989 (1,991)	2.44§§	(2.12-2.89)	940 (943)	2.54**	(2.21-3.04)	294 (296)	2.68	(2.29-3.17)	646 (647)	2.50**§§	(2.19-3.00)
55-64	4,853 (4,899)	2.53	(2.15-3.04)	1,231 (1,253)	2.64	(2.25-3.14)	3,622 (3,646)	2.50§§	(2.13-3.00)	3,138 (3,177)	2.56*	(2.21-3.04)	928 (948)	2.72*	(2.30-3.22)	2,210 (2,229)	2.50§§	(2.17-2.96)
65-74	5,344 (5,497)	2.58	(2.18-3.13)	1,997 (2,064)	2.70	(2.26-3.25)	3347 (3,433)	2.53§§	(2.15-3.05)	1,472 (1,510)	2.67**	(2.27-3.19)	740 (766)	2.75	(2.31-3.25)	732 (744)	2.59*§§	(2.20-3.08)
75-	851 (931)	2.75	(2.31-3.31)	380 (421)	2.81	(2.33-3.43)	471 (510)	2.72§	(2.29-3.21)	115 (119)	2.82	(2.41-3.29)	57 (57)	2.81	(2.48-3.38)	58 (62)	2.86	(2.30-3.19)

n: upper value shows number of subjects and lower shows population of each age and gender group.
Statistical comparisson was performed between same parameters of each cohort study.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.
Statistical comparisson was performed for age groups within gender of each cohort study.
$^{a}p < 0.001$.
Statistical comparisson was performed for gender within age groups of each cohort study.
$^{§§}p < 0.001$, $^{§}p < 0.05$.

Table 7. Clinical attachment level (CAL) for the participants vs. gender and age group.

Age	CAL (mm) in CommCohort									CAL (mm) in BirThree Cohort								
	Total			Male			Female			Total			Male			Female		
	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)
Total	3,608 (16,915)	4.11[a]	(3.40-5.00)	1,334 (4,982)	4.50[a]	(3.75-5.50)	2,274 (11,904)	4.00[a]	(3.17-5.00)	1,903 (15,299)	4.00[a]	(3.44-5.00)	781 (5,928)	4.38[a]	(3.67-5.50)	1,122 (9,371)	4.00[a]	(3.14-5.00)
20-24	4 (190)	3.50	(2.50-4.00)	1 (55)	4.00	(4.00-4.00)	3 (135)	3.00	(2.00-4.00)	7 (484)	3.50	(3.00-4.00)	2 (122)	2.50	(2.00-3.00)	5 (362)	3.50	(3.50-4.00)
25-34	37 (940)	3.67	(3.00-4.25)	16 (226)	4.00	(3.25-5.00)	21 (714)	3.00	(3.00-4.00)	200 (5,735)	4.00	(3.00-4.00)	83 (2,098)	4.00	(3.00-4.33)	117 (3,637)	4.00	(3.00-4.00)
35-44	213 (1,944)	4.00	(3.00-4.50)	54 (469)	4.00	(3.00-4.25)	159 (1,475)	4.00	(3.00-4.50)	247 (3,331)	4.00*	(3.00-5.00)	137 (1,641)	4.00	(3.50-5.00)	110 (1,690)	4.00[§]	(3.00-4.67)
45-54	408 (2,485)	4.00	(3.23-5.00)	94 (494)	4.50	(3.50-5.50)	314 (1,991)	4.00[§]	(3.00-4.50)	182 (943)	4.00	(3.50-5.00)	54 (296)	4.13	(3.67-5.50)	128 (647)	4.00	(3.33-5.00)
55-64	1,105 (4,899)	4.00	(3.33-5.00)	356 (1,253)	4.50	(3.75-5.50)	749 (3,646)	4.00[§§§]	(3.17-5.00)	777 (3,177)	4.17	(3.50-5.00)	230 (948)	4.67	(4.00-5.90)	547 (2,229)	4.00[§§§]	(3.33-5.00)
65-74	1,577 (5,497)	4.33	(3.50-5.25)	687 (2,064)	4.75	(3.80-5.52)	890 (3,433)	4.00[§§§]	(3.30-5.00)	450 (1,510)	4.50*	(3.63-5.60)	251 (766)	4.73	(3.71-5.86)	199 (744)	4.33[§]	(3.50-5.33)
75-	264 (931)	4.59	(4.00-5.38)	126 (421)	4.75	(4.00-6.00)	138 (510)	4.50	(3.75-5.29)	40 (119)	4.55	(3.58-6.00)	24 (57)	4.48	(3.69-6.00)	16 (62)	4.55	(3.45-5.25)

n: upper value shows number of subjects and lower shows population of each age and gender group.
Statistical comparisson was performed between same parameters of each cohort study.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.
Statistical comparisson was performed for age groups within gender of each cohort study.
[a]$p < 0.001$.
Statistical comparisson was performed for gender within age groups of each cohort study.
[§§§]$p < 0.001$, [§§]$p < 0.01$, [§]$p < 0.05$.

Table 8. Median number of teeth with bleeding on probing (BOP) for each patient.

Age	BOP (teeth) in ComCohort						BOP (teeth) in BirThree Cohort					
	Male			Female			Male			Female		
	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)
Total	4,861 (4,982)	4.00[a]	(1.00-9.00)	11,768 (11,904)	4.00[a,§§]	(1.00-8.00)	5,882 (5,928)	6.00***[,a]	(2.00-12.00)	9,338 (9,371)	4.00***[,a,§§§]	(1.00-9.00)
20-24	55 (55)	8.00	(2.00-16.00)	135 (135)	5.00[§]	(1.00-9.00)	122 (122)	7.00	(3.00-15.00)	362 (362)	5.00[§]	(1.00-11.00)
25-34	226 (226)	6.00	(2.00-12.00)	714 (714)	4.00[§§]	(1.00-10.00)	2,098 (2,098)	7.00*	(3.00-13.00)	3,637 (3,637)	4.00[§§§]	(1.00-10.00)
35-44	469 (469)	7.00	(2.00-12.00)	1,475 (1,475)	4.00[§§§]	(1.00-10.00)	1,641 (1,641)	7.00	(2.00-13.00)	1,690 (1,690)	4.00[§§§]	(1.00-9.00)
45-54	493 (494)	4.00	(1.00-10.00)	1,990 (1,991)	4.00	(1.00-10.00)	294 (296)	7.00***	(2.00-13.00)	646 (647)	5.00**[§§]	(2.00-11.00)
55-64	1,235 (1,253)	4.00	(1.00-9.00)	3,627 (3,646)	4.00	(1.00-8.00)	930 (948)	5.00**	(2.00-10.00)	2,213 (2,229)	5.00***	(1.00-9.00)
65-74	2,002 (2,064)	3.00	(1.00-8.00)	3,355 (3,433)	3.00	(1.00-8.00)	740 (766)	5.00***	(1.00-10.00)	732 (744)	4.00***	(1.00-9.00)
75-	381 (421)	4.00	(1.00-7.00)	472 (510)	3.00	(1.00-8.00)	57 (57)	4.00	(1.00-10.00)	58 (62)	4.50	(1.00-12.00)

n: upper value shows number of subjects and lower shows population of each age and gender group.
Statistical comparisson was performed between same parameters of each cohort study.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.
Statistical comparisson was performed for age groups within gender of each cohort study.
[a]$p < 0.001$.
Statistical comparisson was performed for gender within age groups of each cohort study.
[§§§]$p < 0.001$, [§§]$p < 0.01$, [§]$p < 0.05$.

Table 9. Median ratio of tooth with nonphysiological mobility among present teeth for sex, age groups among each cohort population.

Age	Teeth with Nonphysiological TM in CommCohort						Teeth with Nonphysiological TM in BirThree Cohort					
	Male			Female			Male			Female		
	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)
Total	4,861 (4,982)	0.00[a]	(0.00-5.00)	11,768 (11,904)	0.00[a,§§]	(0.00-0.00)	5,882 (5,928)	0.00[a,***]	(0.00-0.00)	9,338 (9,371)	0.00[a,***,§]	(0.00-0.00)
20-24	55 (55)	0.00	(0.00-0.00)	135 (135)	0.00	(0.00-0.00)	122 (122)	0.00	(0.00-0.00)	362 (362)	0.00	(0.00-0.00)
25-34	226 (226)	0.00	(0.00-0.00)	714 (714)	0.00	(0.00-0.00)	2,098 (2,098)	0.00	(0.00-0.00)	3,637 (3,637)	0.00	(0.00-0.00)
35-44	469 (469)	0.00	(0.00-0.00)	1,475 (1,475)	0.00	(0.00-0.00)	1,641 (1,641)	0.00	(0.00-0.00)	1,690 (1,690)	0.00	(0.00-0.00)
45-54	493 (494)	0.00	(0.00-0.00)	1,990 (1,991)	0.00	(0.00-0.00)	294 (296)	0.00	(0.00-0.00)	646 (647)	0.00***	(0.00-0.00)
55-64	1,235 (1,253)	0.00	(0.00-4.55)	3,627 (3,646)	0.00	(0.00-4.55)	930 (948)	0.00	(0.00-7.69)	2,213 (2,229)	0.00§	(0.00-4.55)
65-74	2,002 (2,064)	0.00	(0.00-10.53)	3,355 (3,433)	0.00	(0.00-9.09)	740 (766)	0.00*	(0.00-15.00)	732 (744)	0.00§	(0.00-9.52)
75-	381 (421)	0.00	(0.00-15.38)	472 (510)	0.00	(0.00-18.75)	57 (57)	4.26	(0.00-17.65)	58 (62)	0.00	(0.00-21.74)

n: upper value shows number of subjects and lower shows population of each age and gender group.
Statistical comparisson was performed between same parameters of each cohort study.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.
Statistical comparisson was performed for age groups within gender of each cohort study.
[a]$p < 0.001$.
Statistical comparisson was performed for gender within age groups of each cohort study.
[§§]$p < 0.001$, [§]$p < 0.05$.

Table 10. Median ratio of dental calculus detected on the present index teeth for sex, age groups and each cohort population.

Age	DC (%) in CommCohort						DC (%) in BirThree Cohort					
	Male			Female			Male			Female		
	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)	n	Median	(IQR)
Total	4,761 (4,982)	16.67	(0.00-50.00)	11,636 (11,904)	12.50[a,§§§]	(0.00-28.57)	5,842 (5,928)	12.50[a,***]	(0.00-37.50)	9,290 (9,371)	12.50[a,***§§§]	(0.00-20.00)
20-24	55 (55)	12.50	(0.00-37.50)	135 (135)	12.50[§]	(0.00-12.50)	122 (122)	12.50	(0.00-37.50)	362 (362)	12.50[§§§]	(0.00-25.00)
25-34	226 (226)	12.50	(0.00-50.00)	714 (714)	12.50[§§§]	(0.00-25.00)	2,098 (2,098)	12.50*	(0.00-37.50)	3,637 (3,637)	0.00***[§§§]	(0.00-12.50)
35-44	469 (469)	14.29	(0.00-50.00)	1,476 (1,475)	12.50[§§§]	(0.00-25.00)	1,641 (1,641)	12.50***	(0.00-33.33)	1,690 (1,690)	0.00***[§§§]	(0.00-14.29)
45-54	492 (494)	16.67	(0.00-50.00)	1,988 (1,991)	12.50[§§§]	(0.00-25.00)	292 (296)	14.29	(0.00-50.00)	647 (647)	12.50[§§§]	(0.00-25.00)
55-64	1,221 (1,253)	16.67	(0.00-50.00)	3.597 (3,646)	12.50[§§§]	(0.00-33.33)	922 (948)	20.00	(0.00-50.00)	2,186 (2,229)	12.50[§§§]	(0.00-25.00)
65-74	1,935 (2,064)	16.67	(0.00-50.00)	3,279 (3,433)	12.50[§§§]	(0.00-33.33)	712 (766)	15.48	(0.00-42.86)	713 (744)	12.50[§§§]	(0.00-25.00)
75-	363 (421)	14.29	(0.00- 50.00)	448 (510)	6.25[§§]	(0.00-33.33)	55 (57)	16.67	(0.00-57.14)	56 (62)	14.29	(0.00-33.33)

n: upper value shows number of subjects and lower shows population of each age and gender group.
Statistical comparisson was performed between same parameters of each cohort study.
*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.
Statistical comparisson was performed for age groups within gender of each cohort study.
[a] $p < 0.001$.
Statistical comparisson was performed for gender within age groups of each cohort study.
§§§ $p < 0.001$, §§ $p < 0.01$, § $p < 0.05$.
No statistical significance was observed the Median DC rate within male age group in CommCohort, p = 0.9946.

Nonphysiological TM was not observed often in either cohort until the age of 45 years. However, after that age, the median number of teeth with nonphysiological TM progressively increased with age (Table 9). This trend in the prevalence of nonphysiological TM according to age was statistically significant in both the CommCohort and the BirThree cohort studies, but there was no difference according to sex.

The presence of DC on the present indexed teeth was measured and is expressed as a ratio. In both cohort populations, the number of index teeth positive for supra- and subgingival calculus was significantly lower in females than in males, indicating a strong difference according to sex (Table 10). Almost all participants in their 20s had every index tooth, whereas elderly participants had a small percentage of the index teeth. This loss of index teeth with aging was related to the tooth type; the lower incisors (94.7%) showed the highest survival rate, followed by the upper incisors (88.3%), upper molars (82.3%) and lower molars (74.3%). The presence of DC on each type of tooth is shown in Table 11. The frequency at which DC was detected on the lower incisors was 63.2% in males and 48.1% in females. DC was observed on the other nine index teeth at a similar low frequency (males, 17.7 to 24.1%; females, 10.4 to 14.8%).

Discussion

To clarify the interactive effects of genetic and environmental factors on the occurrence of common diseases, large-scale cohort studies with various genomic and other omic analyses have been conducted in a number of countries (Miyamoto et al. 2016; Kalsbeek et al. 2018; Rutten-Jacobs et al. 2018). Recent progress in the technical, ethical and social aspects of these genome cohort studies has allowed us to collect a large number of healthy residents using a questionnaire-based survey (Hayasaka et al. 2013). Although many reports from such studies suggested a close relationship between oral conditions and general health/diseases (Dorfer et al. 2017), large-scale genome cohort studies focusing on the association of oral conditions and general health/diseases have not yet been established. The ToMMo project aims to address this issue, and elaborate objective oral examinations have been designed within genome cohort studies. Here, as the first step to accomplish this goal, we describe the initial progress and findings of the oral examinations, as well as the design of the cohort studies, especially focusing on the oral examinations.

Of the participants in two ToMMo cohorts, *i.e.,* the CommCohort and the BirThree cohort, 32,185 participants underwent an oral examination performed by specially trained dentists. This number is equivalent to 25.3% of all participants in the two genome cohort studies. It should be noted that over 90% of the participants who were less than 60 years old retained more than 20 natural teeth. After the age of 60 years, the proportion of edentulous participants progressively increased; nonetheless, 55.0% of the participants aged 75 years or more had more than 20 natural teeth. These observations show very good agreement with the results of the previous survey of the Japanese adult population, with approximately 3820 participants (Ministry of Health, Labour and Welfare, Japan 2016). In collaboration

Table 11. Number of Dental Calculus (DC) detection and number of all idex teeth present.

Age (yrs.)	Index teeth	#11 Male	#11 Female	#11 p	#16 Male	#16 Female	#16 p	#17 Male	#17 Female	#17 p	#26 Male	#26 Female	#26 p	#27 Male	#27 Female	#27 p	#31 Male	#31 Female	#31 p	#36 Male	#36 Female	#36 p	#37 Male	#37 Female	#37 p	#46 Male	#46 Female	#46 p	#47 Male	#47 Female	#47 p
DC (teeth) in CommCohort																															
20-24	n	55	135	0.0454	54	135	0.0016	53	135	0.0043	53	134	0.0058	55	135	0.0243	55	134	0.2865	54	135	0.2199	53	134	0.3340	54	134	0.1787	55	133	0.0479
(M = 55, F = 135)	DC (+)	10	11		18	18		16	17		19	23		18	24		33	69		12	70		11	20		12	19		14	18	
25-34	n	222	712	0.0005	223	707	< 0.0001	225	699	0.0004	222	703	0.0004	224	695	0.0003	224	709	< 0.0001	218	705	0.0002	220	700	0.0002	222	697	< 0.0001	221	702	0.0005
(M = 226, F = 714)	DC (+)	39	65		58	92		57	105		56	105		59	109		148	303		49	87		51	90		57	99		54	101	
35-44	n	460	1,454	< 0.0001	450	1,433	< 0.0001	445	1,403	< 0.0001	454	1,430	< 0.0001	445	1,415	< 0.0001	463	1,463	< 0.0001	433	1,384	< 0.0001	429	1,416	< 0.0001	432	1,386	< 0.0001	434	1,418	< 0.0001
(M = 469, F = 1,475)	DC (+)	110	156		126	238		120	237		121	231		130	246		315	683		110	184		106	190		117	201		117	221	
45-54	n	450	1,878	< 0.0001	430	1,834	< 0.0001	430	1,763	< 0.0001	426	1,836	0.0002	438	1,754	0.0014	483	1,967	< 0.0001	365	1,599	0.0116	411	1,717	0.0001	376	1,611	0.1140	410	1,754	0.0002
(M = 494, F = 1,991)	DC (+)	87	219		119	314		126	298		108	323		107	311		315	1,016		71	227		90	244		73	229		93	265	
55-64	n	1,048	3,173	< 0.0001	942	2,930	< 0.0001	980	2,716	< 0.0001	945	2,962	< 0.0001	956	2,671	< 0.0001	1,160	3,508	< 0.0001	758	2,238	0.0035	816	2,494	< 0.0001	772	2,277	< 0.0001	845	2,572	0.0003
(M = 1,253, F = 3,646)	DC (+)	179	380		254	531		274	498		268	533		273	466		755	1,841		137	307		161	332		175	328		172	388	
65-74	n	1,611	2,671	< 0.0001	1,353	2,350	< 0.0001	1,335	2,121	< 0.0001	1,332	2,325	< 0.0001	1,317	2,112	< 0.0001	1,773	3,093	< 0.0001	1,181	1,765	< 0.0001	1,165	1,911	< 0.0001	1,209	1,786	< 0.0001	1,168	1,976	< 0.0001
(M = 2,064, F = 3,433)	DC (+)	313	298		353	414		331	371		349	455		353	396		1,081	1,573		269	262		245	284		281	287		266	328	
75-	n	73	322	0.8301	228	274	0.0525	229	236	0.0019	233	256	0.0634	220	240	0.0013	299	395	0.0078	215	226	0.0953	195	229	0.0138	223	242	0.2756	182	238	0.1632
(M = 421, F = 510)	DC (+)	34	42		56	48		63	37		66	54		62	38		166	179		40	29		39	26		44	38		37	36	
DC (teeth) in BirThree Cohort																															
20-24	n	121	359	0.0026	122	358	0.0025	122	358	0.0002	122	359	0.0024	121	356	0.0488	121	362	< 0.0001	120	355	0.0665	121	357	0.0021	117	351	0.4285	120	357	0.0573
(M = 122, F = 362)	DC (+)	22	30		32	51		34	48		32	51		27	52		81	158		27	54		29	44		22	55		26	51	
25-34	n	2,089	3,623	< 0.0001	2,061	3,601	< 0.0001	2,073	3,587	< 0.0001	2,061	3,581	< 0.0001	2,062	3,578	< 0.0001	2,089	3,614	< 0.0001	2,042	3,564	< 0.0001	2,055	3,580	< 0.0001	2,047	3,585	< 0.0001	2,053	3,595	< 0.0001
(M = 2,098, F = 3,637)	DC (+)	332	241		424	345		430	335		397	324		402	335		1,287	1,401		358	377		350	333		385	383		364	352	
35-44	n	1,610	1,674	< 0.0001	1,569	1,649	< 0.0001	1,573	1,637	< 0.0001	1,579	1,657	< 0.0001	1,265	1,460	< 0.0001	1,629	1,680	< 0.0001	1,521	1,618	< 0.0001	1,536	1,623	< 0.0001	1,544	1,630	< 0.0001	1,560	1,632	< 0.0001
(M = 1,641, F = 1,690)	DC (+)	266	133		311	184		308	187		309	158		315	179		1,033	711		246	162		245	170		264	175		281	175	
45-54	n	280	592	0.0009	257	565	0.0005	258	530	0.0026	251	553	0.0001	254	537	< 0.0001	285	634	< 0.0001	221	462	0.0059	235	517	0.0027	229	469	0.0004	242	514	0.0003
(M = 296, F = 647)	DC (+)	58	72		69	93		68	91		67	85		74	84		205	364		45	57		50	66		59	69		55	64	
55-64	n	781	1,915	< 0.0001	706	1,791	< 0.0001	714	1,663	< 0.0001	723	1,789	< 0.0001	702	1,644	< 0.0001	865	2,114	< 0.0001	555	1,343	< 0.0001	639	1,566	< 0.0001	595	1,392	< 0.0001	616	1,566	< 0.0001
(M = 948, F = 2,229)	DC (+)	150	209		199	257		196	242		197	251		186	244		580	1,142		111	153		126	183		133	183		143	203	
65-74	n	589	589	0.0006	496	527	0.0002	488	476	0.0003	477	511	0.0025	472	475	< 0.0001	654	677	0.0188	415	383	0.0032	413	422	0.0004	409	383	0.0041	426	421	< 0.0001
(M = 766, F = 744)	DC (+)	99	59		118	77		110	65		111	80		119	69		381	351		75	41		75	41		86	51		78	36	
75-	n	44	40	0.7305	40	32	0.2733	39	29	0.9187	36	33	0.0958	37	34	0.2333	50	46	0.7298	32	25	0.4795	23	28	0.1629	31	28	0.7279	26	25	0.3431
(M = 57, F = 62)	DC (+)	9	7		12	6		9	7		13	6		11	6		30	26		9	5		7	4		9	7		7	4	

#11 upper right inciser, #16 upper right first molar, #17 upper right second molar, #26 upper left first molar, #27 upper leftsecond molar, #31 lower left inciser, #36 lower left first molar, #37 lower left second molar, #46 lower right first molar, #47 lower right second molar.

with the previous study, the rate of edentulism in our survey conclusively proves that the rate in Japan is much lower than that in most countries in the world (World Health Organization 2019). We surmise that the lower tooth mortality rate in our participants and in Japan is achieved by the high level of hygiene habits and development of the dental health care system. In fact, regarding the former, 98% of our participants answered in the questionnaire that they brush their teeth more than once a day.

The total caries experience among our participants aged 35-44 years, indicated by a median DMFT index of 14.0 in the CommCohort and 13.0 in the BirThree cohort, is slightly higher than that among adults in Japan (DMFT index, 12.1; 35-44 years) (Ministry of Health, Labour and Welfare, Japan 2016). Whereas several industrialized Western countries, including Sweden (12.5), Denmark (13.5), the UK (16.6) and the USA (10.9), showed comparable DMFT indices in those aged 35-44 years, the caries burden in our cohorts and in the Japan national survey were appreciably higher than those in neighboring countries in East Asia (China, 3.11; Korea 5.5; both 35-44 years) (World Health Organization 2019). These differences suggest that the etiology and progression of dental caries are influenced by both lifestyle and genetic factors. We noted that participants living in the heavily disaster-affected areas (Ishinomaki and Kesennuma) tended to show a higher DMFT index than those living inland areas (Osaki and Shiroishi). To further validate the relationship of stress/disaster experience and caries development, we are planning to analyze the other environmental factors recorded simultaneously in our cohort studies, which include changes in amounts of drinking and smoking and stressors caused by drastic changes in living environments.

Periodontal registrations in the present survey were performed at one site per tooth, during which the deepest PPD and CAL around each tooth was recorded. This simplified method for recording the pathology of the periodontal tissue is designed for cohort studies (Salvi et al. 2015). We envision that in large-scale epidemiological analyses, even if multiple-site measurements are conducted, the largest value of periodontal parameters will be considered the representative value of the tooth concerned (Zimmermann et al. 2015). The CAL is used to estimate the amount of tissue lost in periodontal diseases and to identify the apical extension of the inflammatory lesion. If the CEJ appears at the crown side of the gingival margin, the CAL measurement is simple. However, if the CEJ is located at the same level as the gingival margin or at the root side of the margin, the examiners have to detect the CEJ using a periodontal probe under blind conditions. In the latter case, accurate measurement of the CAL becomes difficult (Leroy et al. 2010). Therefore, in this study, if the CEJ was visible, we measured the CAL, but if the CEJ was not visible, we avoided measuring the CAL. A small proportion (1.4-2.2%) of participants with a visible CEJ was observed in the youngest age group (20-24 years), but the proportion increased with age and reached 25.8% in females and 42.1% in males in the BirThree cohort above the age of 75 years, indicating that the proportion of individuals with a visible CEJ is much smaller than previously expected (Holtfreter et al. 2010; Yin et al. 2017).

The median CAL in the present study was 4.38 mm in males in the CommCohort and 4.50 mm in males in the BirThree cohort, whereas the median CAL was 4.00 mm in females in the respective cohorts. These indices are higher than those reported in previous population-based studies, such as 1.9 mm in Japan (median age, 55.7 years) (Shimazaki et al. 2007), 1.59 mm in the USA (those aged 30 years or more) (Eke et al. 2012a), 3.7 ± 1.1 mm in China (nonpregnant women, 27.1 ± 4.0 years) (Wu et al. 2013), and 3.62 mm (females, 55-64 years) and 3.97 mm (males, 55-64 years) in Denmark (Kongstad et al. 2013). Upon the interpretation of these data, it is important to consider the fact that the assessment rule used in the present survey tends to exclude healthy subjects without gingival recession. Therefore, the comparison should not be evaluated directly.

TM represents the movement of loose teeth within their sockets. As a periodontal parameter, TM is an effective indicator of external force applied to teeth, including bite force. TM also serves as an effective parameter of periodontal tissue destruction by inflammation in the periodontal pocket (Reinhardt and Killeen 2015). In this survey, we found that TM increased sharply with age. TM was higher in males than in females. These changes are similar to those observed in the PPD and CAL (Wu et al. 2016). In contrast, in this study, we found that there was no significant change in BOP with increasing age. This point is controversial in the literature, as similar findings have been shown by several epidemiological reports (Eke et al. 2012b; Zimmermann et al. 2015), while one other report showed that the percentage of participants with BOP increased with age (Kongstad et al. 2013). Our results are similar to the former results and support the notion that BOP is an early sign of gingivitis rather than of the progression of periodontal disease (Greenstein 1984).

Our survey demonstrates that DC formed most abundantly on the surfaces of the mandibular incisors, detected at a rate of, 63.1% among males in the CommCohort and 63.2% among males in the BirThree cohort and, 50.3% and 45.5% among females in the respective cohorts, followed by the maxillary and mandibular molars. In contrast, the least amount of DC formed on the maxillary incisors, detected at a rate of, 18.8% among males in the CommCohort and 17.0% among males in the BirThree cohort and 11.3% and 8.5% among females in the respective cohorts. These results show very good agreement with the finding that supragingival calculus accumulates on the surface of teeth close to the orifices of major salivary glands in both the mandible and maxilla (White 1997; Jin and Yip 2002). These results support the contention that DC itself may not be pathogenic but serves as a contributing factor, leading to the accumulation of dental plaque on the rough surface generated by DC.

The present study reveals a higher prevalence and severity of periodontal disease in males than in females. This difference in periodontal disease has been considered to be associated with hormones, pregnancy in young females, and behavioral differences between the sex, such as higher rates of smoking and relatively poor oral hygiene in males (Shiau and Reynolds 2010; Eke et al. 2012a; Cepeda et al. 2017). Meanwhile, many studies have identified a higher prevalence and susceptibility to dental caries in females (Martinez-Mier and Zandona 2013; Shaffer et al. 2015). Our results provide further insights into their observations. For instance, we found a higher number of FT but lower numbers of DT and MT in females than in males, supporting the hypothesis that females compensate for their susceptibility to caries by more frequently visiting dental clinics (Ministry of Health, Labour and Welfare, Japan 2010).

In conclusion, we document the results of oral health examinations of more than 33,000 inhabitants who experienced the GEJE and participated in our two large-scale genome cohort studies. The prevalence and severity of dental caries and periodontal disease among the participants are higher than those in population-based surveys executed previously in Japan and several other countries. It is difficult to obtain oral health data of our participants from before the GEJE, and an accurate assessment of the impact of natural disaster-related stressors on oral health cannot be carried out. We hope that our present data will be useful for future disaster-based oral health studies in the world. The oral health data will be combined with general health data in our cohort studies. Oral health/disease and general conditions, including genetic background, should be longitudinally followed to elucidate lifestyle-related, common diseases. The causal relationships between oral and general health, including the long-lasting effects of the GEJE, should also be investigated.

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Conflict of Interest

The authors declare no conflict of interest.

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