

# Strategic Methods for Recruiting Grandparents: The Tohoku Medical Megabank Birth and Three-Generation Cohort Study

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Involvement of family members, especially grandparents, in genome epidemiological research is important to investigate both genetic and environmental factors of common diseases. The aim of the present study was to establish strategies to obtain enough number of family recruitment, especially focusing on grandparents, for the Tohoku Medical Megabank Birth and Three-Generation Cohort Study. Our main strategies are summarized below. 1) We standardized informed consent process with reference materials to help people understand the consent form, 2) we created an invitation letter to contact family members, and 3) we recruited family members in several settings. To obtain informed consent, we were careful of explaining clearly the complex reasons as well as drawing people's attention. By the end of March 2017, the number of invitation letters distributed to family members through the pregnant women was 23,806, including 18,702 grandparents. Among the grandparents who received invitation letters, 2,935 (15.7%) responded to us. Furthermore, some grandparents were asked to provide informed consent with other family members by staff at maternal clinics or Community Support Centers, and others directly booked Community Support Centers without responding to the invitation letter. Grandparents joined the study anytime during mother's maternal check-ups or delivery. Overall, 8,054 grandparents participated in our birth cohort study. The setting in which most grandparents were recruited was our own facilities. Importantly, both paternal and maternal grandparents more frequently participated in the study if the father also participated. In conclusion, we are able to recruit not only pregnant women but also fathers and grandparents.

**Keywords:** epidemiological studies; genome research; grandparents' involvement; interview; strategic recruitment  
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## Introduction

Family involvement in genome epidemiological research is very powerful, especially for analyzing rare

variants (Wang et al. 2016), which is gathering attention as a method of finding mechanisms or factors of common diseases. Including not only siblings or parents but also grandparents in the study participants provides the

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Part of the results was presented at 10th World Congress 15-18 October 2017 Rotterdam, The Netherlands.

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advantage of investigating both genetic and environmental factors for common diseases (Stolk et al. 2008).

Prospective cohort studies are often conducted to explore disease-related genes and to clarify the interaction of genetic and environmental factors for common diseases because this study design avoids recall bias and unnecessary harm or accidents, which can occur in intervention analysis. Furthermore, based on the Developmental Origins of Health and Disease (DOHaD) theory (Barker 2007), prospective cohort studies provide more possibilities to break through the mechanism of common diseases when investigating fetuses or children. However, there were no large prospective birth cohort studies with genome analysis involving grandparents before the Tohoku Medical Megabank Birth and Three-Generation (BirThree) Cohort Study launched in northern Japan in July 2013.

The purpose of the BirThree Cohort Study is to investigate the effects of the Great East Japan Earthquake on health and to establish precision medicine through both genome and lifestyle analyses. The participants are pregnant women and fetuses as well as family members such as the fetuses' fathers, siblings and grandparents. Because this study is innovative and the first of its kind, we interviewed some local residents before starting the cohort

study to determine the attitudes and opinions of the public and to create a suitable design and strategy for recruiting participants. We heard several opinions about genome analysis including positive interest in and concerns about participants receiving the results; however, we decided not to report the results to the participants for the time being. We also organized a team to introduce the importance of the study to potential participants and to recruit grandparents. In 3 years, we have recruited more than 70,000 people, including more than 8,000 grandparents, to participate in the study. This paper describes strategies we used to recruit family members for our prospective birth cohort study, focusing on grandparents in particular.

## Materials and Methods

The BirThree Cohort Study is one of the main activities of the Tohoku University Tohoku Medical Megabank Organization (ToMMo), which is funded by the Ministry of Education, Reconstruction Agency and Japan Agency for Medical Research and Development (AMED) to reconstruct the area most damaged by the Great East Japan Earthquake. More information and protocol details about the BirThree Cohort Study are described elsewhere (Kuriyama et al. 2016). It has been approved by the Ethics Committee of Tohoku University Graduate School of Medicine and the Ethics Committee of

Table 1. History of strategic approach to recruitment.

Month and year	Strategy
April 2014	Start distributing invitation letters
June 2014	Start visiting homes to obtain informed consent from family members
June, July, September 2014	Perform examinations in Iwate Prefecture where ToMMo is conducting another adult cohort study
October 2014	Start posting invitation letters to mothers 4 months after childbirth
January 2015	Start distributing flyers through a door-to-door sales company
February 2015	Deliver flyers to homes
February - August 2015	Improve GMRCs' working environment in facilities where the number of participating grandparents is less than 20% the number of participating pregnant women
March 2015	<ul style="list-style-type: none"> <li>Start calling mothers who already finished the 1-month after childbirth check-up to ask them to invite their family members to participate</li> <li>Start enclosing invitation letters in blood test results for mothers</li> </ul>
May - July 2015	Deliver flyers to homes
June 2015	Start enclosing invitation letters in blood test results for fathers
July 2015	<ul style="list-style-type: none"> <li>Start enclosing invitation letters in blood test results for mothers and fathers who had tests performed at Community Support Centers</li> <li>Post flyers to all participating grandmothers</li> <li>Present promotional materials to pregnant women who agreed to give an invitation letter to their family members</li> <li>Enclose a car sticker in the blood test results for pregnant women</li> </ul>
August 2015	<ul style="list-style-type: none"> <li>Family recruitment campaign (open Community Support Centers frequently)</li> <li>Post a newsletter and invitation letter to mothers with no participating family members</li> </ul>
September 2015 - March 2016	Apply improvements and best practices in all facilities
November 2015	Post letters to fathers and grandparents who have not had bio specimen samples taken asking them to attend Community Support Centers with other family members who are not yet participating
November - December 2015	Invite grandparents and other family members to public facilities for health check-ups (outside of prefectural capital)
January 2016	Shift the target to invite potential participants of ToMMo's another adult cohort study to Community Support Centers on weekends
February 2016	Invite grandparents and other family members to public facilities for health check-ups (in the prefectural capital)
March 2016	<ul style="list-style-type: none"> <li>Call family members living outside of Miyagi Prefecture and willing to participate in the study to see</li> <li>Invite participants who are already involved in ToMMo's another adult cohort study</li> </ul>
April 2016	<ul style="list-style-type: none"> <li>Allow more booking for health check-ups at Community Support Centers since ToMMo's another adult cohort study completed recruitment in March 2016</li> <li>Introduce additional reference materials for grandparents</li> </ul>
June 2016	<ul style="list-style-type: none"> <li>Set the opening days of each Community Support Center until October 2016 and start accepting booking for health check-ups</li> <li>Modify flyers for fathers who have already invited their parents to participate</li> </ul>
July - October 2016	Invite grandparents and other family members to public facilities for health check-ups (outside of prefectural capital)
August 2016	<ul style="list-style-type: none"> <li>Set the operating hours of each Community Support Center until December 2016 and start accepting booking for health check-ups</li> <li>Post the operation calendar of each Community Support Center to families living outside of Miyagi Prefecture and willing to participate in the study</li> </ul>
September - November 2016	Perform examinations in Iwate Prefecture where ToMMo is conducting another adult cohort study
October 2016	Call fathers and grandparents who have not had bio specimen samples taken asking them to attend Community Support Centers with other family members who are not yet participating
November 2016	Recruit family members in a public facility near their house
December 2016	<ul style="list-style-type: none"> <li>Post the operation calendar of each Community Support Center to families living inside and outside of Miyagi Prefecture, and willing to participate in the study</li> <li>Stop distributing invitation letters at obstetric facilities</li> </ul>
January - February 2017	Recruit at home in Miyagi Prefecture
March 2017	Recruit at home out of Miyagi Prefecture

What we did for recruiting grandparents and other family members are described here.

Tohoku University Tohoku Medical Megabank Organization. History of all strategic approach to recruitment is shown in Table 1. In particular, our main strategies were; 1) We standardized informed consent process with reference materials to help people understand the consent form, based on the interview to some local residents, 2) We created an invitation letter to contact family members, and asked mothers to give the letter to her family members, 3) We recruited family members in clinical settings or our own facilities, and also visited their home or near their home.

#### *Standardizing informed consent*

In 2012, before starting the cohort study, we performed semi-structured interviews not only with pregnant women but also with grandparents to find a way to explain the context of the BirThree Cohort Study explicitly as well as to determine what might motivate people to participate in the study. One obstetric hospital and two municipalities in Miyagi Prefecture, Japan introduced us to potential interview candidates, whom we asked to participate in the interview. Finally, two nulliparous and two multiparous women, and four men and four women over 60 years old who might have had grandchildren in coastal and inland areas were interviewed.

Based on the results of these interviews, we created reference materials to help people understand the contents of the consent form. We also created a promotional video for fathers and grandparents and had it played at maternal clinics, hospitals and Community Support Centers in the target study area.

#### *Invitation letter to family members*

Once the number of pregnant women involved in the study was steadily increasing, we became focused on accelerating family involvement. We created an invitation letter (Fig. 1) and flyers, which were originally written in Japanese, to contact family members directly through the pregnant women (Fig. 2). First, a genome medical research coordinator (GMRC) explained the importance of family participation in the BirThree Cohort Study to a mother, and asked her to pass an invitation letter to her family members. Then, the mother explained the study to her family, and pass them the invitation letter. The invitation letter contained the following five options for family members to choose from: 1) I would like to participate, 2) Please send me more detailed material, 3) I would like to receive an explanation by telephone, 4) I am already participating in another adult cohort study, and 5) I am not interested at present. If family members were interested in participating in the study, they could also call or email us directly. After receiving responses from the family members who were interested in the study, we contacted them to obtain their informed consent in person.

The timing of distributing the invitation letter varied. Letters to family members were distributed to pregnant women in the first trimester, third trimester and 4 months after childbirth. We also enclosed the letter in the results of blood tests for mothers and fathers. When family members visited the study settings, we asked them if any other family members might be interested in participating in the study as well, and asked them to pass the invitation letter to those family members.

#### *Settings of family recruitment*

We obtained informed consent from pregnant women mainly at the maternal clinics or hospitals where they planned to give birth. If any family members accompanied the pregnant woman for maternal

check-ups or delivery, we invited them to participate in the study at that time, but we also utilized other settings to recruit family members. We built seven Community Support Centers in Miyagi Prefecture capable of performing detailed examinations such as carotid ultrasound imaging and respiratory function, and asked GMRCs to recruit and obtain informed consent from family members. However, sometimes the people attending the centers have already been registered as participants. Moreover, the centers could not be always open; therefore, we invited family members willing to participate in the study to some public facilities located in the neighborhood of their homes in case that they are ten or more in number. We also visited their homes in case that nine or less family members were willing to participate. The Tohoku Medical Megabank Project is conducting another adult cohort study in Iwate Prefecture, and Community Support Centers are located there as well. We therefore invited family members living in Iwate Prefecture to those centers.

#### *Other tips*

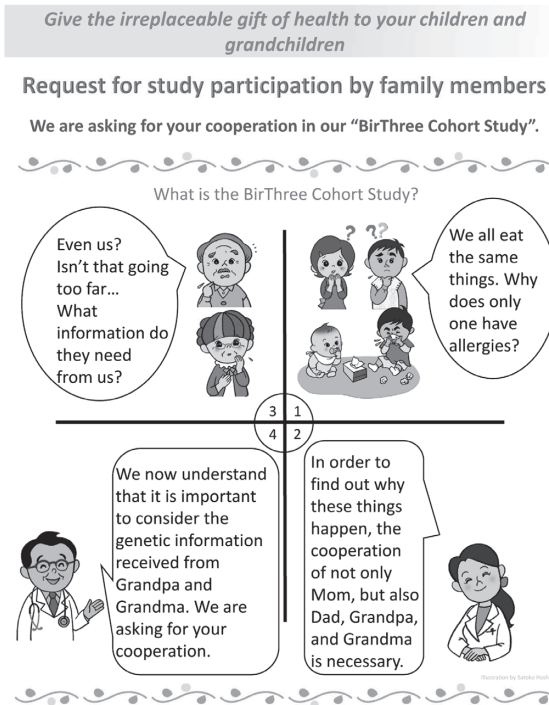
Since ToMMo dispatched GMRCs to each maternal hospital and clinic in the study area, we needed to standardize the basic approach of distributing the invitation letter to family members through pregnant women. We visited the hospitals and clinics once every 1-4 weeks to have a meeting with GMRCs to improve their routine activity more efficiently so that they could have enough time to ask pregnant women to pass the invitation letters to their families. After all facilities adapted the standardized approach, we then focused on participating pregnant women in the regions where the number of participating grandparents was less than 20%, and asked GMRCs what difficulties they encountered in recruiting family members. We then worked with GMRCs to increase the number of times they asked pregnant women to contact their families, or asked directly family members to participate in the study when they accompanied pregnant women for maternal check-ups or delivery. Furthermore, GMRCs became devoted to recruiting family members by revising reference materials to suit their own facilities and calling mothers to ask them to invite their families to participate in the study again. We applied these improved strategies and best practice to all facilities in the middle phase of our recruitment period.

We used several types of media to inform the general public about the study. We aired an advertisement on television and radio, and placed an advertisement in the local newspaper and magazines. We also posted flyers on doors in areas where the number of participating grandparents was low. A door-to-door sales company supported our study and distributed flyers when they visited their customers. We also prepared remuneration (500 yen voucher to each participant when they answered a questionnaire or provided their blood) or goods such as pens, seals, clear plastic folders, car stickers and so on as promotional materials for participation and decided which study results should be disclosed to the participants.

Through the years, we have advertised the study at local festivals or events held by municipalities or hospitals to make the study widely known to the public and to increase the number of participants.

#### *Analyzing the characteristics of grandparent participants*

To investigate the characteristics of grandparent participants, we compared the proportion of paternal grandparents with maternal grandparents. We also investigated whether they lived with mothers



This study can help make the children of the future smile. Your efforts today are needed to bring smiles to the children of tomorrow.

Please participate in our BirThree Cohort Study

Fig. 1. A. Invitation letter (surface).

Attach ID sticker

Circle one of the five choices below.

Three-generation cohort study

1. I would like to participate.
2. Please send me more detailed materials.
3. I would like to receive an explanation by telephone.
4. I am already participating in another adult cohort study.
5. I am not interested at present.

Your relationship to baby (circle one)

Father Sibling Other (    )

Grandfather (maternal, paternal)

Grandmother (maternal, paternal)

Name: \_\_\_\_\_

Date of birth: Year    Month    Day \_\_\_\_\_

Address: (Zip code    ) \_\_\_\_\_

Contact telephone number: \_\_\_\_\_

Email address: \_\_\_\_\_

Note: Please ensure that your email settings allow email messages from XXX@XXXXX.  
Please affix the personal information protection sticker before returning.

Fig. 1. C. Invitation letter (response form).

**I don't mind participating in the study, but what should I do? When and where should I go?**

Study participants can select from among the following:  
 Time: If you are related to a participating pregnant woman, you can participate in the study at any time.  
 Place: Community Support Center<sup>1</sup> Delivery hospital (some)<sup>2</sup> Your home (or near your home)  
 Test contents: (1) Saliva collection (2) Blood collection (3) Urine collection (4) Other detailed tests Tests (3) and (4) may be added to (1) or (2) above.  
 Survey form: Full version / Simple version  
 We also request your cooperation in follow-up studies by mail every 6 months to 1 year  
 Other: Please bring the Maternal and Child Health Handbook from when you yourself were born. (If you do not have this, it is not necessary.)

<sup>1</sup>Community Support Centers are in Sendai (Seiryō-machi), Kesennuma, Osaki, Ishinomaki, Tagajo, Iwanuma and Shiroishi.  
<sup>2</sup>Please telephone to find out which delivery hospitals are participating in the study. People living outside of Miyagi Prefecture may also participate.

**I don't like having blood taken. I dislike pain.**

For those who are uncomfortable with blood collection or who do not have time, saliva collection is OK.  
(Please note, however, that blood test results can only be returned to those who have a blood sample taken.)

**I know it's for future children, but... is there also some advantage for us?**

If you have blood or urine sampled after completing the questionnaire, the enclosed test results will be returned to you free of charge.  
 You can also have other detailed tests. You can use the free 24-hour health consultation service. Remuneration will also be given depending on the parts of the study you participate in.

**You can have these tests done**

- Blood and urine tests (In addition to general blood tests, allergy tests for pollen, mites, and other allergens are available)
- Body composition, grip strength measurements
- Pulmonary function/airway resistance tests
- Ocular fundus, hearing, and bone density tests
- Estimated central blood pressure
- Home blood pressure measurement, pedometer (We will lend you instruments to take measurements at home)
- Carotid ultrasonography
- Leg extension strength test
- Intraoral examination
- Tablet type survey
- MRI examination
- Cognitive and psychological tests

Note: The tests indicated in blue are available at the Sendai Center only

Note: All tests can be taken free of charge

**Inquiries**

Fig. 1. B. Invitation letter (back).

Fig. 1. Invitation letter for family members. A. The surface of the invitation letter used for family members. B. The back of the invitation letter. C. Response form, a post card. Family members who received the invitation letter returned the response form (C) to us. Note that the invitation letter and the response form were presented at 10th World Congress, October 15-18, 2017, Rotterdam, The Netherlands.

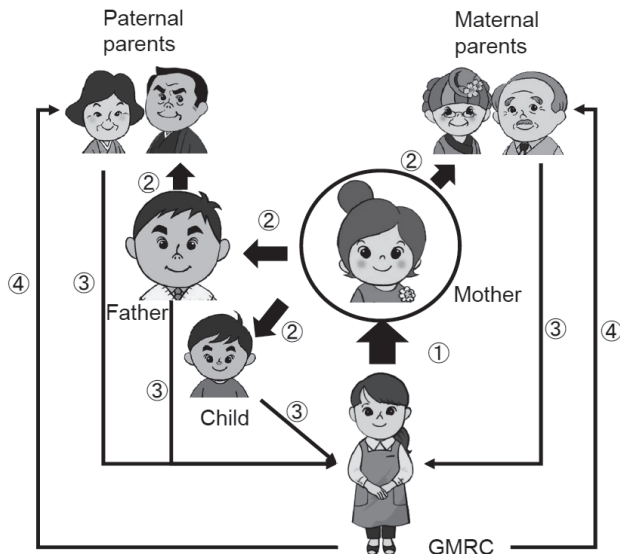


Fig. 2. An approach to contact family members through a pregnant woman. Flow of the approach that a genome medical research coordinator (GMRC) asks a pregnant woman to invite her family to the study is shown. Note that this figure was presented at 10th World Congress, October 15-18, 2017, Rotterdam, The Netherlands.

and whether they were alive. The data were obtained from questionnaires answered by mothers during pregnancy and at 12 months after delivery. Mothers who completely withdrew their consent, who participated in the study during multiple pregnancies, or whose sisters or other family members also participated in the study as pregnant women were excluded from this analysis. We did not analyze whether paternal grandparents were alive because the data were not available. The Chi-squared test was used to compare the percentage of participation among grandparents based on the father's participation. Statistical analyses were performed using the SAS package (version 9.4, SAS Institute Inc., Cary, North Carolina, USA).

## Results

### Process of standardizing informed consent

Interviewees, pregnant women and men/women living in coastal areas thought that studying the current health status of residents is important and/or necessary, whereas interviewees living in inland areas felt that establishing precision medicine was more attractive than studying their current health status. In particular, one pregnant woman living in a coastal area thought that the study was necessary for herself as well as for her parents.

However, because the BirThree Cohort Study has several purposes, it was difficult for the interviewees to capture the meaning of the study to themselves. To improve the flow of introducing the BirThree Cohort Study to participants, we considered the methods to explain clearly that the research to analyze the effects of the Great East Japan Earthquake on their current health status and the additional research for precision medicine are both

important to improve the health of people living in the disaster-stricken area.

Most of the interviewees were not concerned about having their genes analyzed. However, some were concerned that the results of genome analysis could negatively affect their family relationships. Therefore, we decided not to report the genome analysis results to the participants until the accuracy of the results is confirmed, whereas we decided to report the results only if the information is critical to the participant's health, the disease is treatable, and the results can be reported without hindering the study. We stated this information in the explanation of the BirThree Cohort Study given to participants.

As for receiving the results of their examination, the results of magnetic resonance imaging (MRI) were particularly attractive to interviewees. One pregnant woman strongly wanted to recommend her parents take an MRI examination. Although one pregnant woman was attracted by receiving vouchers or promotional materials for participating, elder interviewees thought that there was no relationship between remuneration and study participation.

### Distribution of invitation letters

As of the end of March 2017, the number of invitation letters distributed to family members directly through the pregnant women was 23,806, 18,702 of which were distributed to grandparents. Of these 18,702 grandparents who received the letters, 2,935 (15.7%) responded to ToMMo. The highest proportion of responses was for "1) I would like to participate" (67.0%). The proportion of responses marked "2) Please send me more detailed material" was 6.7%, and "3) I would like to receive an explanation by telephone" was 0.9%. The proportion of responses marked "4) I am already participating in another adult cohort study" was 3.3%, and "5) I am not interested at present" was 18.7%. Overall, 69.0% of the total respondents finally decided to go to the Community Support Center to give informed consent and to have health check-up.

The largest number of maternal grandmothers ( $n = 5,917$ ; 31.6% of the total distributed letters) received invitation letters, followed by maternal grandfathers ( $n = 5,222$ ; 27.9% of the total distributed letters) among 4 groups of grandparents as shown in Table 2. A smaller, but considerable number of paternal grandmothers ( $n = 3,928$ ; 21.0% of the total distributed letters) and paternal grandfathers ( $n = 3,635$ ; 19.4% of the total distributed letters) received invitation letters. The percentages of responses showed a similar tendency among 4 groups of grandparents (Table 2). In contrast, paternal and maternal grandmothers had higher percentages of booking Community Support Centers to have health check-up with informed consent than did paternal and maternal grandfathers (69.2% and 74.1% for paternal and maternal grandmothers, respectively vs. 62.9% and 65.1% for

Table 2. Distribution of invitation letters to grandparents.

Family members	Invitation letters		
	No. distributed (n = 18,702)	No. of responses (% of responses) <sup>a</sup> (n = 2,935)	No. of booking Community Support Centers <sup>b</sup> (% of booking) <sup>c</sup> (n = 2,025)
Paternal grandfather	3,635	456 (12.5)	287 (62.9)
Paternal grandmother	3,928	565 (14.4)	391 (69.2)
Maternal grandfather	5,222	793 (15.2)	516 (65.1)
Maternal grandmother	5,917	1,121 (18.9)	831 (74.1)

Number of each grandparent who received an invitation letter and how they react was described here.

No. distributed, the number of distributed letters.

<sup>a</sup>The percentage of responses was calculated as the number of responses/the number of distributed letters in each group.

<sup>b</sup>No. of booking Community Support Centers shows the number of grandparents who booked the visit to the Community Support Center to have health check-ups with informed consent.

<sup>c</sup>% of booking Community Support Centers were calculated as the number of booking Community Support Center / the number of responses in each group.

paternal and maternal grandfathers, respectively) (Table 2).

Mothers who were asked to contact family members about participating in the study 4 months after delivery had a higher percentage of response (6.9%) from their family members than that of pregnant women receiving examination results (5.5%). The percentage of response from their family members when fathers who already participated in the study were asked to recommend our study to family when fathers received the result of their examination was 2.0%. After introducing the invitation letter, the number of participating grandparents almost doubled in the following month (65 and 107 participating grandparents in April and May 2014, respectively).

#### *Grandparents' participation*

As of the end of March 2017, 8,054 grandparents, 22,493 mothers and 8,821 fathers were participating in the study. The number was higher than that of respondents of the invitation letters because some were directly asked to obtain informed consent with other family members by staff at maternal clinics or hospitals, or Community Support Centers, and others also booked Community Support Centers without responding to the invitation letter. More grandparents joined the study around August; in contrast, recruitment among grandparents was lower in May (Fig. 3).

#### *Characteristics of grandparent participants*

A total of 5,785 mothers completed the questionnaire at 12 months after delivery. In the families of those mothers, 2,429 fathers and 2,565 grandparents joined the study. More maternal grandparents participated in the study than did paternal grandparents (660 paternal and 1,905 maternal grandparents).

There was a wide variety of timing that grandparents participated in the study after the mother participated in (the mean  $\pm$  standard deviation of days when grandparents par-

ticipated in the study after the mother's participation: 212.9  $\pm$  186.6 days). The setting in which most grandparents were recruited was Community Support Centers (n = 1,541, 60.1%), followed by maternal clinics and hospitals (n = 877, 34.2%), and their houses or public facilities near their houses (n = 147, 5.7%). 9.7% and 4.6% of the mothers had already lost their father or mother, respectively.

Among the 5,785 mothers, 5,770 mothers completed the questionnaire during their first trimester. According to their responses, 679 paternal grandfathers (11.8%), 686 paternal grandmothers (11.9%), 418 maternal grandfathers (7.2%) and 527 grandmothers (9.1%) lived with the mothers. Among them, 21.6% of paternal grandfathers and 24.2% of paternal grandmothers participated in the study, whereas 9.6% of maternal grandfathers and 14.7% of grandmothers participated in the study.

The number of both paternal and maternal grandparents who participated in the study was significantly larger with the father's participation than without the father's participation (Table 3).

## **Discussion**

The BirThree Cohort Study recruited pregnant women and their families including grandparents through a variety of strategies, and we presented our recruiting strategies in this paper. To date, our recruitment of grandparents remains stable. The LifeLines Study, which recruited three generations of family members, is one of the most successful studies with respect to enrolling a large number of participants. Among the LifeLines participants, 49% were recruited by their general practitioners and 38% by their family members (Scholtens et al. 2015). In the BirThree Cohort Study, pregnant women were recruited by medical staffs at maternal clinics or hospitals, and their families were mostly recruited through invitation letters they received from pregnant women or other family

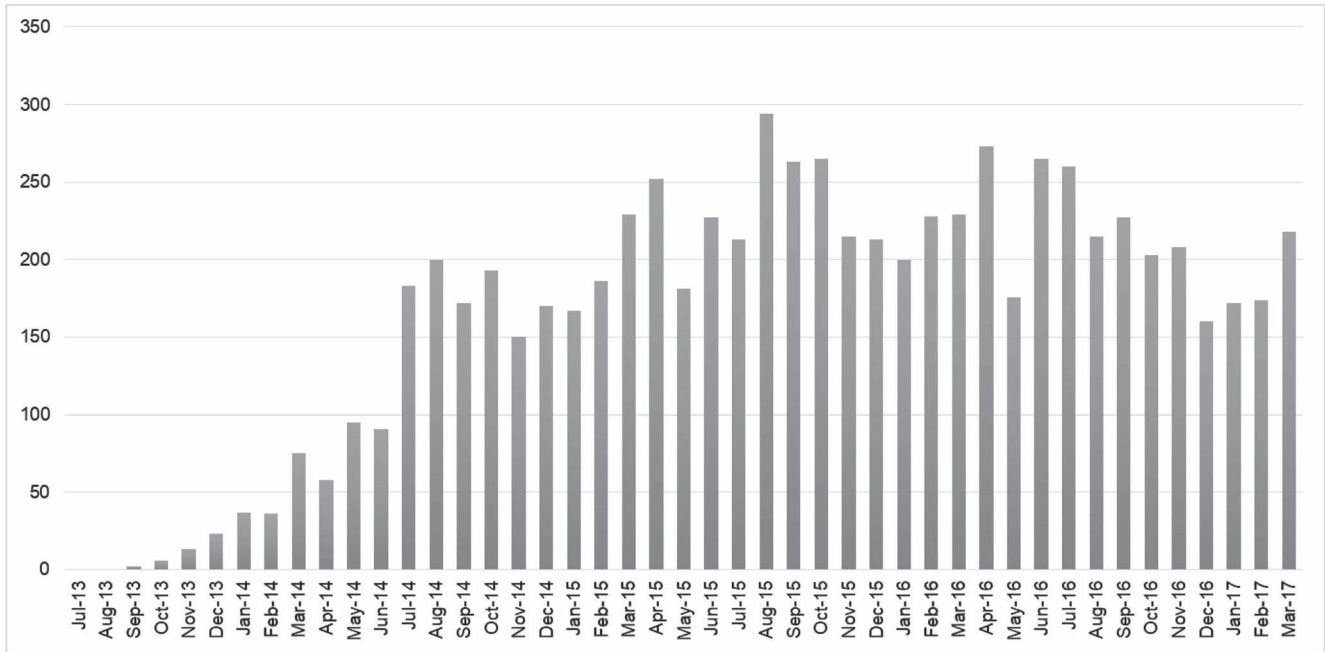


Fig. 3. Monthly number of the participation of grandparents. The numbers of the grandparents who participated in the study each month from July 19, 2013 to March 31, 2017 are shown.

Table 3. Grandparents’ participation based on the father’s participation.

Grandparents	Without father’s participation (n = 3,155)	With father’s participation (n = 2,215)	P
Paternal grandfathers	3 (0.09)	267 (11.0)	< 0.0001
Paternal grandmothers	3 (0.09)	387 (15.9)	< 0.0001
Maternal grandfathers	192 (5.7)	384 (15.8)	< 0.0001
Maternal grandmothers	568 (16.9)	761 (31.3)	< 0.0001

Analysis of effects on grandparents’ participation due to father’s participation are described here.  
n, (%). The percentages were the proportion of each number of grandparent per fathers who participated in the study or fathers who did not participate in.

members who had already been participating in the study. Standardizing the informed consent process and creating reference materials to help potential participants understand the purpose and details of the BirThree Cohort Study based on the results of an interview survey might have been effective for recruitment. Several studies have shown that obtaining the consent of study participants requires clarifying the purpose and contents of the study, as well as the benefits and risks of participation (Beskow et al. 2001; Barata et al. 2006). All other strategies, such as advertising through mass media, providing vouchers and promotional materials, and educating and changing the working environment of GMRCs may also explain our success in the recruitment process.

Since March 2015, the number of participants recruited per month has usually been over 200. The time of year in which most grandparents joined the study was concentrated

around August. Japan has holidays called the Bon Festival in August in which family members get together and we speculate that this provided an opportunity for families to take the survey and examinations and to talk about the study. We also opened the Community Support Centers more frequently during this period, especially in 2015. In contrast, the number of participants joining the study was low in May. This might have happened because Japan has long public holidays in May. Although about 60% of grandparents were recruited at Community Support Centers, most of the centers were closed on at least one of three or more consecutive holidays in May because of issues related to human resources and management of transferring bio-specimens. In addition, grandparents attended childbirths or went to meet their children and grandchildren at maternal clinics or hospitals during the holidays; therefore, GMRCs could not meet grandparents at the clinics or hospitals

during their working shifts. The data of monthly number of the participation of grandparents shown in Fig. 3 support this hypothesis. For example, the number of grandparents whom we explained the study in April and May, 2016 were 288 and 194. In those people, 284 and 192 people agreed with participation in April and May, 2016. The percentages of the agreement were therefore 98.6% and 99.0% for each month. Although the percentages of the agreement were almost equal between April and May, the number of people whom we explained was lower in May than in April.

The study location was determined based on the estimated number of childbirths. We included the prefectural capital, where one-fifth of the grandparents lived. Including this area was therefore effective in recruiting grandparents. Maintaining a high follow-up rate is our next challenge.

In the BirThree Cohort Study, maternal grandparents participated more frequently than did paternal grandparents. Among maternal grandparents participated, only 9.6% and 14.7% of grandfathers and grandmothers lived with mothers, respectively. This tendency can be explained by the fact that pregnant women can easily ask their biological parents to participate in the study whereas it might be more difficult to ask their parents-in-law. In addition, the previous study by Jenkins et al. (2009) reported that paternal skepticism was a barrier to mothers' participation in DNA collection in an epidemiological study. We can speculate that this phenomenon occurred in the BirThree Cohort Study from the number of invitation letters distributed to grandparents. Our results showed that if fathers were involved in the study, paternal grandparents were also more likely to participate. According to the Framingham Heart Study's Third Generation Cohort, one of the factors contributing to high recruitment rates was that participants were familiar with the study because their parents and other family members had already participated in former studies (Splansky et al. 2007). Involving pregnant women and their partners might have enhanced the participation of both maternal and paternal grandparents in the BirThree Cohort Study.

The main analytical plan of the BirThree Cohort Study is to investigate disease-related genes and the interaction between genetics and environmental factors for diseases, which are especially affected or advanced in severity by the Great East Japan Earthquake. Three-generation families with both maternal and paternal lines enable analysis of genetic inheritance or investigation of *de novo* mutation. Indeed, other studies such as the Avon Longitudinal Study of Parents and Children, which involved three or more generations as participants, reported that the timing of female meiosis I during the grandmother's pregnancy affected the grandchild's genome by showing the association between the maternal grandmother's age and the prevalence of autism spectrum disorder among their grandchildren (Golding et al. 2010). Data collection of the follow-up study will be more intensive, especially for

children, and it might be therefore helpful to analyze traits related to their development in detail.

One limitation on recruiting grandparents in the BirThree Cohort Study was the limited budget. We could have held events for families or offered greater vouchers or promotional materials with a less restricted budget. The budget also influenced the recruitment of grandparents who lived outside of Miyagi Prefecture. Greater financial resources would have allowed us to reach out to grandparents outside the prefecture more frequently, or to obtain informed consent through video calls. However, we were able to contact most of the grandparents living in the same prefecture as the pregnant women; therefore, we could also collect information on environmental factors such as lifestyle, climate, culture and the aftermath of the disaster since the participants lived in the same region and encountered similar environmental circumstances.

The main limitation of the study itself is that we did not measure the effects of each of our strategies quantitatively, and could therefore not analyze which were actually effective. However, regarding the effects of the invitation letters, the percentages of responses did not include grandparents who were directly contacted by GMRCs by phone or in person, those who responded to media advertisements or those who did not show interest in participating. In addition, more than three-quarters of grandparents did not live with mothers. The effect of the invitation letters might be larger than the percentages of responses suggest. Further research is necessary to clarify the best practices of recruiting grandparents for birth cohort studies with genome analysis.

In conclusion, the BirThree Cohort Study recruited not only pregnant women and fetuses but also fathers and grandparents with many strategies created based on interviews with local people without any major complications. However, improving genome literacy in the general public and advancing information and communication technology are necessary for a more effective approach to recruiting extended family members for research studies. Including grandparents in birth cohort studies with genome analysis will contribute to determining the mechanisms and factors for diseases.

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### Conflict of Interest

The authors declare no conflict of interest.



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