Low prevalence of maternal microchimerism in Japanese children with type 1 diabetes

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1. Introduction

- Vertical transfer of maternal cells to a fetus via the placenta leads to maternal microchimerism (MMc) in the child [1,2]. Increased prevalence of MMc has been associated with various autoimmune diseases, such as juvenile dermatomyositis and systemic sclerosis [3,4], suggesting that MMc may alter immune tolerance of the children.
- In 2007, Nelson et al. investigated the possible association between MMc and type 1 diabetes by the use of quantitative polymerase chain reaction (q-PCR) targeting non-transmitted maternal HLA alleles [5]. As a result, circulating microchimeric cells were detected in 51% of people with type 1 diabetes, 33% of their unaffected siblings, and 17% of control individuals. Importantly, strikingly high levels of MMc were almost exclusively observed in the people with type 1 diabetes.
- However, because the vast majority of the participants of that study were Caucasian, the general applicability of these findings is yet to be examined.
- This study aimed to clarify the prevalence and degree of MMc in Japanese children with type 1 diabetes.

2. Methods

Subjects

We obtained peripheral blood samples from 153 Japanese children with type 1 diabetes and their 71 unaffected siblings. They satisfied the following criteria: (i) participants who did not have the same HLA genotype as his/her mother, (ii) participants whose mother was heterozygous for one of the common HLA alleles in the Japanese general population, i.e., DRB1*01, DRB1*15, DRB1*16, DRB1*04, DRB1*08, DRB1*09, DRB1*14, DQB1*03:01, and DQB1*03:04.

HLA-specific q-PCR

MMc levels, expressed as the genomic equivalent per 10^5 host cells, were determined by q-PCR. The experiments were performed according to the previous report [4], with slight modifications. We examined the relative genomic copy number of non-transmitted maternal HLA alleles to that of a control locus in the genome using TaqMan RNase P Control Reagents Kit. Q-PCR was performed using Applied Biosystems 7500 Fast Real-Time PCR System. Each sample was analyzed in triplicate and all assays were repeated at least two times.

Table 1. Characteristics of the participants.

	Children with type 1 diabetes		Unaffected siblings
	Autoantibody-positive	Autoantibody-negative	
Number of cases	124	29	71
Male : Female	50:74	11:18	13:34
Age at diagnosis, years	6.5 (3.6-9.8)	5.5 (3.4-9.0)	N.A.
Age at examination, years	12.7 (10.0-15.9)	13.6 (10.7-17.6)	N.A.
Birth order (First : Second or later : Unknown)	46:55:23	10:7:12	0:27:44
BMI SD score at diagnosis	-0.8 ± 1.2	-0.7 ± 1.2	N.A.
HbA1c (NGSP) at diagnosis, %	12.0 (10.2-13.7)	11.6 (9.9-13.9)	N.A.
DKA at diagnosis	40 / 103	13 / 24	N.A.
Autoantibody for GAD	100 / 124	0 / 29	N.A.
Autoantibody for IA2	92 / 124	0 / 29	N.A.
Autoantibody for ZnT8	19 / 49	0 / 15	N.A.
Susceptible HLA-DRB1 alleles	181 / 248	30 / 58	71 / 142
Protective HLA-DRB1 alleles	9 / 248	1 / 58	25 / 142

Data are represented as median (interquartile range) or mean ± SD.

3. Results

Table 2. The prevalence and degree of MMc.

	Children with type 1 diabetes		Unaffected siblings
	Autoantibody-positive	Autoantibody-negative	
Number of cases	124	29	71
The number of tested cells	104,103.5 ± 44,407.3	89,660.8 ± 39,696.7	100,137.9 ± 36,674.0
The prevalence of MMc	18 / 124	8 / 29	11 / 71
Degree of MMc per 10^5 host cells	0.0 [0.0-15.4]	0.0 [0.0-12.6]	0.0 [0.0-18.2]

Data are represented as median [minimum-max] or mean ± SD.

Figure 1. The prevalence of MMc in autoantibody-positive and -negative children with type 1 diabetes and their unaffected siblings.

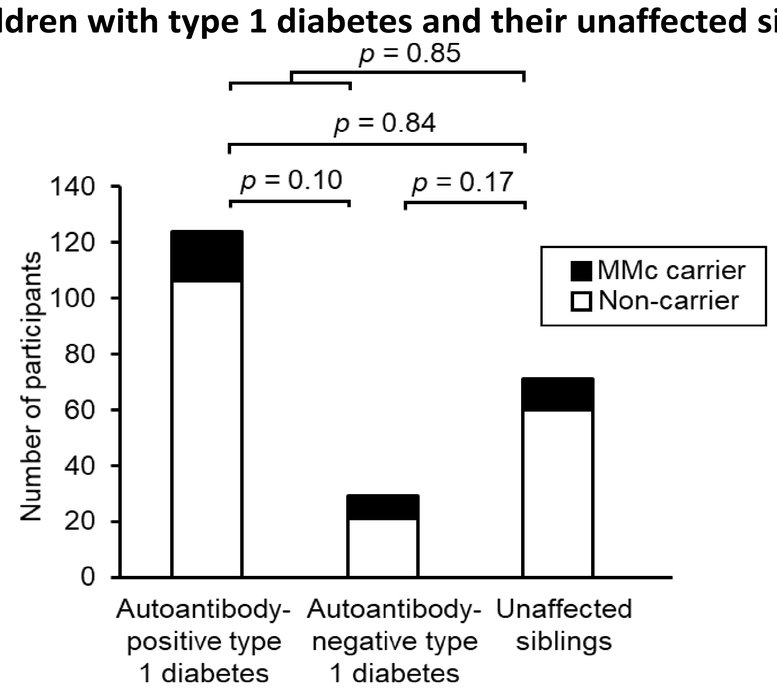
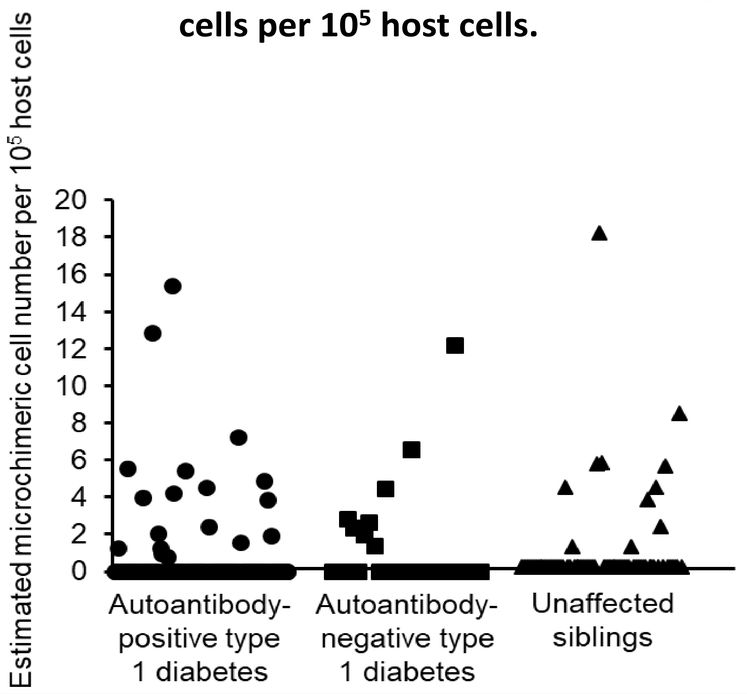


Figure 2. Estimated numbers of circulating microchimeric cells per 10^5 host cells.



The prevalence of MMc and the estimated numbers of microchimeric cells per 10^5 host cells were comparable among autoantibody-positive children, autoantibody-negative children, and unaffected siblings.

4. Discussion

- Our results are inconsistent with those of the previous study on Caucasian people, which demonstrated a significant increase in the number of circulating microchimeric cells in people with type 1 diabetes [5]. The difference in the results between the present and previous studies may reflect the difference in the genetic susceptibility of the participants. Indeed, Nelson et al. reported that MMc in Caucasian people with type 1 diabetes was frequently associated with the paternally-inherited HLA DQB1*03:02-DRB1*04 haplotype, a susceptible haplotype that accounts for substantial percentage of Caucasian people with type 1 diabetes [6]. These results imply that the functional significance of MMc may vary among people with type 1 diabetes, depending on their genetic susceptibility.
- Currently, there is a debate as to whether MMc has a beneficial or deleterious role in type 1 diabetes [7,8]. Nelson et al. speculated that microchimeric cells could contribute to the repair of damaged tissues, although it is also possible that these cells act as an effector in autoimmunity or constitute a target of autoimmune responses. Vanzyl et al. documented that insulin-positive microchimeric cells in the pancreas from people with type 1 diabetes appeared healthy and were unlikely to be subjected to immune attack [9]. In fact, our data exclude the possibility that MMc plays the major role in the development of type 1 diabetes in Japan. Further studies are necessary to clarify whether microchimeric cells contribute to the tissue repair in children with type 1 diabetes or are simple bystanders.

5. Conclusion

Circulating microchimeric cells are unlikely to be associated with type 1 diabetes in Japanese children.

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