Note

Replication Study for the Association of Five SNPs Identified by GWAS and Trastuzumab-Induced Cardiotoxicity in Japanese and Singaporean Cohorts

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Trastuzumab (herceptin) is an effective drug for human epidermal growth factor receptor type 2 (HER2)-positive cancer. However, cardiotoxicity remains a serious complication. In our previous genome-wide association study (GWAS), we identified potential associations for five single nucleotide polymorphisms (SNPs) with trastuzumab-induced cardiotoxicity in a Japanese population. To validate this association, here we performed replication studies using Japanese and Singaporean case-control cohorts (Japan: 6 cases and 206 controls; Singapore: 22 cases and 178 controls). Although none of the SNPs showed a statistically significant association with trastuzumab-induced cardiotoxicity, we show that three (rs8032978, rs7406710 and rs9316695) and four (rs8032978, rs7406710, rs28415722 and rs11932853) SNPs had an effect in the same direction in the Japanese and the Singaporean cohort, respectively, as that in our previous study. Combining the previous study with the current replication studies, we find a strong association for two SNPs, rs8032978 and rs7406710, with trastuzumab-induced cardiotoxicity ($P_{combined} = 4.92 \times 10^{-5}$ and 5.50×10^{-5}, respectively). These data suggest that rs8032978 and rs7406710 could be predictive markers of trastuzumab-induced cardiotoxicity in Japanese and Singaporean populations, and support their potential use in clinical risk assessment. These findings offer a first step toward the development of clinically available markers for the potential risk of trastuzumab-induced cardiotoxicity as well as an improved understanding of the pathogenesis of this complication.

Key words cardiotoxicity; trastuzumab; pharmacogenomics; human epidermal growth factor receptor type 2 (HER2); single nucleotide polymorphism (SNP)

INTRODUCTION

Trastuzumab (Herceptin), a molecular targeted drug, prevents tumor growth by inhibiting human epidermal growth factor receptor type 2 (HER2) signaling and induces antibody-dependent cellular cytotoxicity (ADCC) by selectively binding to the extracellular domain of HER2.[1–3] Trastuzumab is therefore used in the treatment of patients with HER2-positive breast or gastric cancer.[4,5] Moreover, since November 2021, trastuzumab is now available for the treatment of salivary gland cancer in Japan.[6] Although trastuzumab is an effective drug for HER2-positive cancer, cardiotoxicity remains a serious complication,[7,8] with some patients needing to suspend their treatment temporarily or indefinitely.[9] However, whereas anthracycline-induced cardiotoxicity is irreversible and dose-dependent, trastuzumab-induced cardiotoxicity is typically reversible and dose-independent following the cessation of the therapy, suggesting that genetic factors are likely to be involved in trastuzumab-induced cardiotoxicity.[10–12] Although the precise mechanism by which trastuzumab induces cardiotoxicity is still unclear, it is recommended that patients planning to receive the drug monitor their cardiac function *via* left ventricular ejection fraction (LVEF) evaluation both at baseline (before treatment) and during treatment.[8,13]

Germline polymorphisms associated with trastuzumab-induced cardiotoxicity have been reported; however, these results have not yet been sufficiently validated.[14–16] Through genome-wide association study (GWAS), we previously identified five single nucleotide polymorphisms (SNPs) as possible genetic susceptibility factors for the risk of trastuzumab-induced cardiotoxicity among Japanese patients.[17] To build on this, here we investigated whether these associations could be further validated in another Japanese population and in a Singaporean population.

PATIENTS AND METHODS

Patients For the Japanese replication cohort, we selected 212 patients (6 cases and 206 controls) treated with trastuzumab from the samples registered in the National Cancer Center

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(NCC) Biobank (https://www.ncc.go.jp/jp/biobank/) from July 2016 to July 2019 (except the samples used in our previous GWAS).[17] For the Singaporean cohort, 200 patients with breast cancer (22 cases and 178 controls) treated with trastuzumab were collected at the Tan Tock Seng Hospital (TTSH) from September 2007 to September 2019. We used Singaporean patient data to investigate the reproducibility of our previous GWAS data because Singapore is multiethnic Asian society (Chinese, Malay, Indian, and others). The clinical characteristics of these patients are summarized in Table 1. In this study, "cases" were defined as those who developed trastuzumab-induced cardiotoxicity during trastuzumab treatment, whereas "controls" were those who showed no trastuzumab-induced cardiotoxicity, as previously reported by Nakano *et al.*[17] We defined trastuzumab-induced cardiotoxicity as left ventricular ejection fraction (LVEF) $<45\%$ or LVEF $<50\%$ with an absolute decrease of 10% from baseline according to the criteria in HERceptin Adjuvant (HERA) trial.[18,19] Transthoracic echocardiography was performed for an assessment of LVEF at baseline and during follow-up (every 1.2–28.5 months, median; 2.8 months). All participants provided written informed consent. This study was approved by the Institutional Review Board of the NCC (Tokyo, Japan) and TTSH (Jalan Tan Tock Seng, Singapore).

SNP Genotyping A total of five SNPs identified in our previous study[17] were chosen for the replication studies: rs9316695 at chr13q14.3, rs28415722 and rs8032978 at chr15q26.3, rs7406710 at chr17q25.3, and rs11932853 at chr4q25. These SNPs were genotyped using TaqMan Genotyping Assays (Thermo Fisher Scientific, MA, U.S.A.) according to the manufacturer's instructions.

Statistical Analysis Differences in the distribution of age, gender, primary cancer site, pretreatment with anthracycline, and hormone status were evaluated by Fisher's exact test or Mann–Whitney *U* test (Table 1). According to the previous study,[17] a case-control association analysis using Fisher's exact test was applied to an allelic frequency model for rs9316695, rs11932853 and rs8032978, or a recessive-inheritance model for rs28415722 and rs7406710. The odds ratios (ORs) and confidence intervals (CIs) were calculated using a non-risk allele or a non-risk genotype as a reference. Significance levels after Bonferroni correction were $p = 1.00 \times 10^{-2}$ [0.05/5] in the replication studies. In the combined analysis, the genotype counts from the replication studies were added to the count from our previous study.[17] All statistical analyses were carried out using R statistical environment v4.0.3, or the BellCurve for Excel v3.21 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

RESULTS

Patient Characteristics We previously identified five SNPs as possibly associated with trastuzumab-induced cardiotoxicity in a Japanese cohort. To validate these associations,

Table 1. Patient Demographics and Clinical Characteristics

Characteristic	Replication 1 (Japanese: $N=212$)		P	Replication 2 (Singaporean: $N=200$)		P	Total ($N=412$)		P
	Number of patients (%)			Number of patients (%)			Number of patients (%)		
	Case ($N=6$)	Control ($N=206$)		Case ($N=22$)	Control ($N=178$)		Case ($N=28$)	Control ($N=384$)	
Age (years)			0.33			0.73			0.64
Median	53.5	58		59	62		57.5	57.5	
Range	39–73	27–86		38–70	27–80		38–73	27–86	
Gender			0.60			1.00			0.25
Female	6 (100)	175 (85.0)		22 (100)	178 (100)		28 (100)	353 (91.9)	
Male	0 (0)	31 (15.0)		0 (0)	0 (0)		0 (0)	31 (8.1)	
Primary cancer site			0.02			1.00			0.01
Breast	5 (83.3)	165 (80.1)		22 (100)	178 (100)		27 (96.4)	343 (89.3)	
Gastric	0 (0)	41 (19.9)		0 (0)	0 (0)		0 (0)	41 (10.7)	
Pancreas	1 (16.7)	0 (0)		0 (0)	0 (0)		1 (3.6)	0 (0)	
Pretreatment with anthracycline			0.09			0.07			0.56
Yes	1 (16.7)	118 (57.3)		16 (72.7)	90 (50.6)		17 (60.7)	208 (54.2)	
No	5 (83.3)	88 (42.7)		6 (27.3)	88 (49.4)		11 (39.3)	176 (45.8)	
Her-2			1.00			0.30			0.25
Negative	0 (0)	0 (0)		0 (0)	1 (0.5)		0 (0)	1 (0.3)	
Positive	6 (100)	205 (99.5)		21 (95.5)	176 (98.9)		27 (96.4)	381 (99.2)	
Equivocal	0 (0)	1 (0.5)		1 (4.5)	1 (0.5)		1 (3.6)	2 (0.5)	
ER status (Breast cancer)			1.00			1.00			0.67
Negative	1 (20.0)	36 (21.8)		8 (36.4)	65 (36.5)		9 (33.3)	101 (29.4)	
Positive	4 (80.0)	129 (78.2)		14 (72.7)	113 (63.5)		18 (66.7)	242 (70.6)	
PR status (Breast cancer)			1.00			0.18			0.43
Negative	1 (20.0)	49 (29.7)		8 (36.4)	94 (52.8)		9 (33.3)	143 (41.7)	
Positive	4 (80.0)	116 (70.3)		14 (72.7)	84 (47.2)		18 (66.7)	200 (58.3)	

ER, estrogen receptor; PR, progesterone receptor.

we performed two replication studies using another Japanese patient cohort and a Singaporean patient cohort. In replication 1 (the Japanese cohort), we selected 6 cases and 206 controls whereas, in replication 2 (the Singaporean cohort), we selected 22 cases and 178 controls. Cases were classified as those patients who demonstrated signs of trastuzumab-induced cardiotoxicity, whereas control patients did not. Table 1 shows the characteristics of these 412 patients. There were no statistically significant differences in terms of age, gender, pretreatment with anthracycline, or hormone status (Her-2, estrogen receptor (ER) and progesterone receptor (PR)) between the case and control groups; only primary cancer site was significantly different (Table 1).

Association between Five SNPs and Trastuzumab-Induced Cardiotoxicity We genotyped the samples for five SNPs and conducted a case-control association analysis using the Japanese cohort (replication 1) and the Singaporean cohort (replication 2). As shown in Supplementary Table S1, we did not observe large differences in the SNP allelic frequencies between this and previous studies, aside from rs9316695 and rs8032978. Overall, none of the SNPs showed a significant association. However, three SNPs (rs8032978, rs7406710 and rs93116695) in replication 1 had an effect in the same direction as that in our previous study; the uncorrected *p*-values for rs8032978, rs7406710, and rs93116695 were 0.39, 0.41, and 0.99, respectively. In replication 2, four SNPs (rs8032978, rs7406710, rs28415722 and rs11932853) showed an effect in the same direction as that in our previous study (uncorrected *p* value range = 0.34–1.00); however, again, there was no significant association. The effects were limited: the difference in risk allele frequency (RAF) for rs28415722 and rs11932853 were limited in the case and control groups. Finally, we sought to combine the replication study data with that of our previous study in a combined analysis. For the five SNPs, we found two SNPs, rs8032978 and rs7406710, to have a much stronger association with trastuzumab-induced cardiotoxicity, as shown in Table 2 ($P_{combined} = 4.92 \times 10^{-5}$, OR = 3.49, and $P_{combined} = 5.50 \times 10^{-5}$, OR = 3.47, respectively).

DISCUSSION

We previously reported five SNPs as potential predictive markers of the risk of trastuzumab-induced cardiotoxicity within a Japanese population.[17] However, no replication studies have been conducted to date. To establish the reproducibility of our primary results, in this study we undertook replication studies using an additional Japanese population and a Singaporean population.

No significant association was observed for any of the five SNPs with trastuzumab-induced cardiotoxicity in these independent cohorts. The differences in allelic frequencies between this and previous studies might be responsible for the replication failure. Furthermore, these cohorts might be underpowered to detect statistically significant associations of the two SNPs (rs8032978 and rs7406710) with trastuzumab-induced cardiotoxicity, or other common variants not identified in our

Table 2. Summary of Association Results for Five SNPs

Chromosomal region	Position[a)]	SNP	Gene	Allele [1/2][b)]	Risk allele	Stage	Case				Control				*p*-Value	Odds ratio (95% CI)
							Genotype			RAF	Genotype			RAF		
							11	12	22		11	12	22			
13q14.3	54615773	rs9316695	No gene	C/A	A	Our previous study[c)]	10	11	4	0.38	352	98	6	0.12	6.00E − 06	4.46 (2.30–8.47)
						Replication 1	4	2	0	0.17	148	53	5	0.15	1.00E + 00	1.11 (0.24–5.18)
						Replication 2	13	8	1	0.23	78	82	17	0.33	2.29E − 01	0.60 (0.26–1.30)
						Combined	27	21	5	0.29	578	233	28	0.17	3.70E − 03	1.99 (1.28–3.08)
15q26.3	98609746	rs28415722	No gene	G/A	A	Our previous study[c)]	7	5	13	0.62	177	204	75	0.39	8.88E − 05	5.48 (2.21–13.69)
						Replication 1	0	6	0	0.50	86	92	28	0.36	1.00E + 00	0.00
						Replication 2	9	8	5	0.41	68	75	34	0.40	7.76E − 01	1.24 (0.33–3.82)
						Combined	16	19	18	0.52	331	371	137	0.38	2.34E − 03	2.64 (1.45–4.79)
17q25.3	79505624	rs7406710	No gene	C/T	C	Our previous study[c)]	21	3	1	0.90	201	212	43	0.67	1.07E − 04	6.64 (2.19–27.01)
						Replication 1	4	2	0	0.83	88	91	27	0.65	4.07E − 01	2.68 (0.48–14.97)
						Replication 2	15	7	0	0.84	106	62	10	0.77	4.95E − 01	1.45 (0.53–4.43)
						Combined	40	12	1	0.87	395	365	80	0.69	5.50E − 05	3.47 (1.83–6.58)
4q25	112024388	rs11932853	No gene	T/C	T	Our previous study[c)]	11	11	3	0.66	58	228	170	0.38	1.42E − 04	3.20 (1.70–6.23)
						Replication 1	1	2	3	0.33	29	99	78	0.38	1.00E + 00	0.81 (0.24–2.74)
						Replication 2	4	13	5	0.48	40	87	51	0.47	1.00E + 00	1.03 (0.52–2.03)
						Combined	16	26	11	0.55	127	414	299	0.40	2.98E − 03	1.83 (1.23–2.72)
15q26.3	101799790	rs8032978	No gene	A/G	G	Our previous study[c)]	17	7	1	0.18	424	31	1	0.04	1.60E − 04	5.83 (2.30–13.51)
						Replication 1	5	1	0	0.08	190	16	0	0.04	3.92E − 01	2.25 (0.27–18.51)
						Replication 2	16	4	2	0.18	143	24	10	0.12	3.40E − 01	1.56 (0.59–3.71)
						Combined	38	12	3	0.17	757	71	11	0.06	4.92E − 05	3.49 (2.01–6.03)

RAF, risk allele frequency; CI, confidence interval. *a*) Based on GRCh37 genome assembly. *b*) Reference allele (GRCh37) was defined as allele 1. *c*) Nakano *et al.*, A Genome-Wide Association Study Identifies Five Novel Genetic Markers for Trastuzumab-Induced Cardiotoxicity in Japanese Population. *Biol Pharm Bull*. **42**: 2045–2053.

previous GWAS due to small sample size that might strongly influence cardiotoxicity. However, in both cohorts, rs7406710 and rs8032978 had an effect in the same direction as that found in the previous study. Moreover, when we used a combined analysis, the associations were stronger than those in the previous study. We performed a scoring analysis for two SNPs (rs8032978 and rs7406710) using a strategy similar to that described in our previous study.[17] We found that the incidence of trastuzumab-induced cardiotoxicity among patients with a risk score ≥ 2 was higher (9.1%) than that for patients with score ≤ 1 (4.2%) ($p = 5.11 \times 10^{-2}$, OR = 2.31, Supplementary Table S2). Therefore, these two SNPs might be useful predictive markers for trastuzumab-induced cardiotoxicity not only in Japanese but also in a Singaporean population.

At present, there are but a few reports regarding the function of these SNPs. Selenoprotein S (*SELENOS*), small nuclear ribonucleoprotein polypeptide A' (*SNRPA1*), and proprotein convertase subtilisin/kexin type 6 (*PCSK6*) are located on chromosome 15q26.3, where rs8032978 is localized. The findings from genome-wide linkage analyses indicate that these genes are associated with congenital heart defects.[20] Others have shown the mRNA and protein expression levels of *PCSK6* to be upregulated in hypoxic cardiomyocytes, with an increase in serum PCSK6 levels also confirmed in patients with acute myocardial infarction.[21] In a mouse model, *PCSK6* overexpression results in impaired left ventricular function through enhanced collagen production and cardiac fibrosis.[21] These lines of evidence support our findings of the potential association between rs8032978 and trastuzumab-induced cardiotoxicity.

According to the Genotype-Tissue Expression (GTEx) database, rs7406710 is associated with the expression of fascin actin-bundling protein 2, retinal (*FSCN2*) in the thyroid ($p = 1.2 \times 10^{-15}$).[22] *FSCN2* is a member of the fascin protein family and is a disease gene linked with autosomal dominant retinitis pigmentosa.[23–25] However, any functional association between *FSCN2* and trastuzumab-induced cardiotoxicity remains unclear. Further investigations will be needed to confirm the role of rs7406710 in trastuzumab-induced cardiotoxicity.

This study had several strengths and limitations. The main strength of our study is that this is the first replication study to seek to identify associations for the five SNPs identified by us previously[17] with trastuzumab-induced cardiotoxicity. Although no significantly associated SNP with trastuzumab-induced cardiotoxicity was found, rs7406710 and rs8032978 showed strong associations and may be useful predictive markers for trastuzumab-induced cardiotoxicity in Japanese and Singaporean populations. The main limitation is that the sample size is likely to be too small to have sufficient power to detect a statistically significant association between SNPs and trastuzumab-induced cardiotoxicity. Hence, our results will need to be confirmed by a prospective study with a larger sample size.

In conclusion, our replication studies using Japanese and Singaporean cohorts supports the potential association of two SNPs with trastuzumab-induced cardiotoxicity. These findings may help to contribute to our understanding of the mechanism of trastuzumab-induced cardiotoxicity and lead to better prognosis and QOL for patients with HER2-positive cancer.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials This article contains supplementary materials.

REFERENCES

1) Hudis CA. Trastuzumab--mechanism of action and use in clinical practice. *N. Engl. J. Med.*, **357**, 39–51 (2007).
2) Gennari R, Menard S, Fagnoni F, Ponchio L, Scelsi M, Tagliabue E, Castiglioni F, Villani L, Magalotti C, Gibelli N, Oliviero B, Ballardini B, Da Prada G, Zambelli A, Costa A. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. *Clin. Cancer Res.*, **10**, 5650–5655 (2004).
3) Collins DM, O'Donovan N, McGowan PM, O'Sullivan F, Duffy MJ, Crown J. Trastuzumab induces antibody-dependent cell-mediated cytotoxicity (ADCC) in HER-2-non-amplified breast cancer cell lines. *Ann. Oncol.*, **23**, 1788–1795 (2012).
4) Feldman AM, Lorell BH, Reis SE. Trastuzumab in the treatment of metastatic breast cancer: anticancer therapy *versus* cardiotoxicity. *Circulation*, **102**, 272–274 (2000).
5) Boku N. HER2-positive gastric cancer. *Gastric Cancer*, **17**, 1–12 (2014).
6) Chugai Pharmaceutical Co. Ltd. "Anti-cancer agent herceptin approved for additional indication of salivary gland cancer (2021).": ‹https://www.chugai-pharm.co.jp/english/news/detail/20211125160000_875.html›, accessed 22 February, 2022.
7) Seidman A, Hudis C, Pierri MK, Shak S, Paton V, Ashby M, Murphy M, Stewart SJ, Keefe D. Cardiac dysfunction in the trastuzumab clinical trials experience. *J. Clin. Oncol.*, **20**, 1215–1221 (2002).
8) Advani PP, Ballman KV, Dockter TJ, Colon-Otero G, Perez EA. Long-term cardiac safety analysis of NCCTG N9831 (alliance) adjuvant trastuzumab trial. *J. Clin. Oncol.*, **34**, 581–587 (2016).
9) Curigliano G, Cardinale D, Suter T, Plataniotis G, de Azambuja E, Sandri MT, Criscitiello C, Goldhirsch A, Cipolla C, Roila F. Cardiovascular toxicity induced by chemotherapy, targeted agents and radiotherapy: ESMO clinical practice guidelines. *Ann. Oncol.*, **23** (Suppl. 7), vii155–vii166 (2012).
10) Perez EA, Rodeheffer R. Clinical cardiac tolerability of trastuzumab. *J. Clin. Oncol.*, **22**, 322–329 (2004).
11) Gabani M, Castañeda D, Nguyen QM, Choi SK, Chen C, Mapara A, Kassan A, Gonzalez AA, Khataei T, Ait-Aissa K, Kassan M. Association of cardiotoxicity with doxorubicin and trastuzumab: a double-edged sword in chemotherapy. *Cureus*, **13**, e18194 (2021).
12) Yu AF, Yadav NU, Lung BY, Eaton AA, Thaler HT, Hudis CA, Dang CT, Steingart RM. Trastuzumab interruption and treatment-induced cardiotoxicity in early HER2-positive breast cancer. *Breast Cancer Res. Treat.*, **149**, 489–495 (2015).
13) Stone JR, Kanneganti R, Abbasi M, Akhtari M. Monitoring for

chemotherapy-related cardiotoxicity in the form of left ventricular systolic dysfunction: a review of current recommendations. *JCO Oncol. Pract.*, **17**, 228–236 (2021).
14) Beauclair S, Formento P, Fischel JL, Lescaut W, Largillier R, Chamorey E, Hofman P, Ferrero JM, Pagès G, Milano G. Role of the HER2 [Ile655Val] genetic polymorphism in tumorogenesis and in the risk of trastuzumab-related cardiotoxicity. *Ann. Oncol.*, **18**, 1335–1341 (2007).
15) Stanton SE, Ward MM, Christos P, Sanford R, Lam C, Cobham MV, Donovan D, Scheff RJ, Cigler T, Moore A, Vahdat LT, Lane ME, Chuang E. Pro1170 Ala polymorphism in HER2-neu is associated with risk of trastuzumab cardiotoxicity. *BMC Cancer*, **15**, 267 (2015).
16) Serie DJ, Crook JE, Necela BM, Dockter TJ, Wang X, Asmann YW, Fairweather D, Bruno KA, Colon-Otero G, Perez EA, Thompson EA, Norton N. Genome-wide association study of cardiotoxicity in the NCCTG N9831 (Alliance) adjuvant trastuzumab trial. *Pharmacogenet. Genomics*, **27**, 378–385 (2017).
17) Nakano MH, Udagawa C, Shimo A, Kojima Y, Yoshie R, Zaha H, Abe N, Motonari T, Unesoko M, Tamura K, Shimoi T, Yoshida M, Yoshida T, Sakamoto H, Kato K, Mushiroda T, Tsugawa K, Zembutsu H. A genome-wide association study identifies five novel genetic markers for trastuzumab-induced cardiotoxicity in Japanese population. *Biol. Pharm. Bull.*, **42**, 2045–2053 (2019).
18) Piccart-Gebhart MJ, Procter M, Leyland-Jones B, *et al.* Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N. Engl. J. Med.*, **353**, 1659–1672 (2005).
19) Seferina SC, de Boer M, Derksen MW, van den Berkmortel F, van Kampen RJ, van de Wouw AJ, Joore M, Peer PG, Voogd AC, Tjan-Heijnen VC. Cardiotoxicity and cardiac monitoring during adjuvant trastuzumab in daily dutch practice: a study of the southeast netherlands breast cancer consortium. *Oncologist*, **21**, 555–562 (2016).
20) Flaquer A, Baumbach C, Piñero E, García Algas F, de la Fuente Sanchez MA, Rosell J, Toquero J, Alonso-Pulpon L, Garcia-Pavia P, Strauch K, Heine-Suñer D. Genome-wide linkage analysis of congenital heart defects using MOD score analysis identifies two novel loci. *BMC Genet.*, **14**, 44 (2013).
21) Kuhn TC, Knobel J, Burkert-Rettenmaier S, Li X, Meyer IS, Jungmann A, Sicklinger F, Backs J, Lasitschka F, Müller OJ, Katus HA, Krijgsveld J, Leuschner F. Secretome analysis of cardiomyocytes identifies PCSK6 (proprotein convertase subtilisin/kexin type 6) as a novel player in cardiac remodeling after myocardial infarction. *Circulation*, **141**, 1628–1644 (2020).
22) Battle A, Brown CD, Engelhardt BE, Montgomery SB. Genetic effects on gene expression across human tissues. *Nature*, **550**, 204–213 (2017).
23) Kureishy N, Sapountzi V, Prag S, Anilkumar N, Adams JC. Fascins, and their roles in cell structure and function. *BioEssays*, **24**, 350–361 (2002).
24) Wada Y, Abe T, Takeshita T, Sato H, Yanashima K, Tamai M. Mutation of human retinal fascin gene (FSCN2) causes autosomal dominant retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.*, **42**, 2395–2400 (2001).
25) Wada Y, Abe T, Itabashi T, Sato H, Kawamura M, Tamai M. Autosomal dominant macular degeneration associated with 208delG mutation in the FSCN2 gene. *Arch. Ophthalmol.*, **121**, 1613–1620 (2003).