**Table 1**
Characteristics of personal care product samples and compounding agents.

(a) Personal care products (PCP) samples

| Sample No. | Type of PCP | Specific features | Application body site | Fluorinated ingredients (Ingredients listed in the labels on each product) | Sampling year | Country of origin | Manufacturing company |
|---|---|---|---|---|---|---|---|
| *Cosmetics* | | | | | | | |
| 1 | Manicure | – | Nail | PAPs[c] | 2009 | Japan | A |
| 2 | Manicure | – | Nail | PAPs[c] | 2009 | Japan | B |
| 3 | Foundation | – | Face | PAPs[c] | 2009 | France | C |
| 4 | Lip rouge | – | Lip | PAPs[c] | 2009 | Japan | D |
| 5 | Manicure (base coat) | – | Nail | PAPs[c] | 2009 | Japan | A |
| 6 | Powder fundation | – | Face | PAPs[c] | 2011 | Japan | E |
| 7 | Powder fundation | – | Face | PAPs[c] | 2011 | France | F |
| 8 | Powder fundation | – | Face | PAPs[c], polyfluorooctylmethyl trimethoxysilane | 2011 | Japan | G |
| 9 | Powder fundation | – | Face | PAPs[c], perfluorooctyl triethoxysilane | 2011 | Japan | B |
| 10 | Liquid foundation | – | Face | PAPs[c], perfluoroalkyl ethoxydimethicone | 2011 | Japan | E |
| 11 | Liquid makeup base | – | Face | PAPs[c], perfluoroalkyl ethoxydimethicone | 2011 | Japan | E |
| 12 | Foundation | – | Face | Polyfluoroalkyl silylated mica | 2009 | Korea | H |
| 13 | Foundation | – | Face | Polyfluorooctyl trimethoxysilane | 2009 | Japan | A |
| 14 | Lip rouge | – | Lip | Stearyl methacrylate/perfluorooctylethyl methacrylate copolymer | 2009 | Japan | E |
| 15 | Powder fundation | – | Face | Grapeseed fatty acid perfluorononyl octyldodecyl glycol | 2011 | United States | I |
| *Sunscreen* | | | | | | | |
| 1–1[b] | Skin milk | Waterproofness | Face and body | PAPs[c] | 2007 | Japan | J |
| 1–2[b] | Skin milk | Waterproofness | Face and body | PAPs[c] | 2009 | Japan | J |
| 1–3[b] | Skin milk | Waterproofness | Face and body | PAPs[c] | 2011 | Japan | J |
| 1–4[b] | Skin milk | Waterproofness | Face and body | PAPs[c] | 2011 | Japan | J |
| 2 | Skin milk | Waterproofness | Face and body | PAPs[c] | 2011 | Japan | J |
| 3 | Skin milk | – | Face | PAPs[c] | 2011 | Japan | J |
| 4 | Powder foundation | Waterproofness | Face | PAPs[c] | 2011 | Japan | B |
| 5 | Liquid foundation | Waterproofness | Face | PAPs[c], PEG-8 trifluoropropyl dimethicone copolymer | 2011 | Japan | B |
| 6 | Foundation | – | Face | Polyperfluoroethoxymethoxy difluoroethyl PEG phosphate | 2011 | Japan | J |
| *Control samples (does not list any fluorinated ingredients in their labels)*[a] | | | | | | | |
| 1 | Cosmetic (manicure) | – | Nail | – | 2009 | Japan | A |
| 2 | Sunscreen (skin milk) | – | Face and body | – | 2011 | Japan | J |

(b) Compounding agents of PCPs

| Sample No. | Component | Composition[d] (Weight%) | Sampling year | Country of origin |
|---|---|---|---|---|
| Compounding agents | | | | |
| 1 | Mica treated with PAPs[c] | Mica: 95 (%), PAPs[c]: 5 (%) | 2012 | Japan |
| 2 | Talc treated with PAPs[c] | Talc: 95 (%), PAPs[c]: 5 (%) | 2012 | Japan |

[a] The cosmetic and sunscreen samples that did not list any fluorinated ingredients in their labels were used as control samples.
[b] Sunscreen nos. 1-1, 1-2, 1-3 and 1-4 are the same product with different lot numbers.
[c] PAPs; polyfluoroalkyl phoshate esters.
[d] Composition listed in their raw material specification sheet.

### *2.5. Method detection limits (MDLs), method quantification limits (MQLs), blank contamination, extraction recovery and total analyte recovery*

PFCAs were dissolved in 100 μL of a 0.1 M benzyl bromide/acetone solution and derivatized at 60 °C for 60 min with 1 ng of 11H-perfluoroundecanoic acid as external calibration standard. After benzylation, the stability was investigated by monitoring the peak area ratio over 24 h. Milli-Q water (Merck KGaA Millipore, Billerica, MA) was used as the procedural blank control, and was analyzed after every three samples ($n = 9$). The procedural blank was extracted using the process described above, and five replicate procedural blanks were prepared independently. In this study, we observed blank contamination for all PFCAs except PFTeDA (Table 2). The MDL and MQL were defined by the following equation; $\mathrm{MDL} = \alpha + 3\beta$, $\mathrm{MQL} = \alpha + 10\beta$, where $\alpha$ is the mean of the blank measurements and $\beta$ is the standard deviation of the blank measurements (ACS Committee, 1980). For PFTeDA, because there was no blank signal, the MDL and the MQL were defined as the mass of the analyte producing a peak with a signal-to-noise ratio of three and ten, respectively. The MDL and MQL varied according to the sample volume being used in chemical analysis. Using the established MDL and MQL values, we calculated the sum of the PFCAs concentrations using the following upper and lower boundary rules. (1) Upper boundary: concentrations lower than the MQL (and higher than MDL) were assigned the MQL for the calculation.

**Table 2**
Recoveries, method detection limits and method quantification limits for PFCAs analysis.

| Compound (carbon atoms) | | Quantification ions (confirmation ions) m/z | Instrument detection limit[a] (pg) (S/N = 3) | Extraction recovery of PFCAs [b]% (SD%) | | Total analyte recovery of PFCAs [c]% (SD%) | | Procedural blank (SD) (pg, $n = 9$) | Method detection limit[d,e] (for 1 mg samples)[f] (ng g$^{-1}$) | Method quantification limit[d,e] (for 1 mg samples)[f] (ng g$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 10 ng spiked ($n = 6$) | 0.1 ng spiked ($n = 6$) | 10 ng spiked ($n = 6$) | 0.1 ng spiked ($n = 6$) | | | |
| PFHxA | (C6) | 313 (294) | 0.004 | 82(11) | 79(6) | 63(9) | 60(5) | 19(15.6) | 66 | 176 |
| PFHpA | (C7) | 363 (344) | 0.004 | 79(8) | 94(26) | 61(11) | 73(18) | 40(6.8) | 60 | 108 |
| PFOA | (C8) | 413 (394) | 0.003 | 106(10) | 101(13) | 82(20) | 78(9) | 26(10.5) | 57 | 131 |
| PFNA | (C9) | 463 (444) | 0.003 | 96(8) | 102(10) | 74(19) | 79(7) | 11(3.2) | 21 | 43 |
| PFDA | (C10) | 513 (494) | 0.004 | 85(5) | 91(7) | 65(17) | 70(4) | 13(4.1) | 25 | 54 |
| PFUnDA | (C11) | 563 (544) | 0.004 | 86(4) | 78(6) | 66(15) | 60(8) | 9(2.0) | 15 | 29 |
| PFDoDA | (C12) | 613 (594) | 0.005 | 97(7) | 107(7) | 75(15) | 83(11) | 10(4.8) | 24 | 58 |
| PFTrDA | (C13) | 663 (644) | 0.005 | 95(9) | 92(12) | 73(16) | 71(14) | 3(2.4) | 10 | 27 |
| PFTeDA | (C14) | 713 (694) | 0.007 | 94(6) | 103(7) | 72(16) | 79(10) | <0.7 | 0.7[d] | 2.3[d] |
| 11H-PFUnDA | – | 554 | – | – | – | 77(19) | 75(8) | – | – | – |

SD: relative standard deviation.
[a] 1 μL injection.
[b] Extraction recovery; all native PFCAs were spiked into control samples (see Table 1) before extraction. Extraction recovery is observed by comparing the response signal of native PFCAs and 11H-PFUnDA, which were added after extraction. This extraction recovery does not include derivatization efficiency.
[c] The total analyte recovery is obtained by multiplying extraction recovery rate by derivatization efficiency. We obtained the derivatization efficiency by comparing the response signals of 11H-PFUnDA and CB111.
[d] The values for MDL and MQL are given by MDL = $\alpha + 3\beta$ and MQL = $\alpha + 10\beta$ ($\alpha$ is the mean of the blank measures; $\beta$ is the standard deviation of the blank measures).
[e] No blank response was observed for PFTeDA. Its MDL was calculated from the instrument detection limit (signal-to-noise ratio of 3) and its MQL was calculated from a signal-to-noise ratio of 10.
[f] The sample size was approximately 1–200 mg and varied according to sample concentration.

Concentrations lower than the MDL were assigned the MDL for the calculation. (2) Lower boundary: concentrations lower than the MQL (and higher than MDL) were given the MDL for the calculation. Concentrations lower than the MDL were given zero for the calculation.

For method validation, we have checked extraction recovery and total analyte recovery. First, native PFCAs were added in the control PCP samples before extraction. Then, after extraction, we added underivatized 11H-PFUnDA as the external calibration standard. In addition to that, we added $^{13}C_{12}$-labeled PCB (CB111) to check the derivatization efficiency of 11H-PFUnDA. We chose CB111 because it was not affected by the derivatization and was not a naturally-occurring chemical. Then, we derivatized the compound with benzyl bromide/acetone solution and directly injected the derivative into the GC–MS. Extraction recovery and total analyte recovery were calculated based on the following assumptions: (1) Extraction recovery was observed by comparing the response signal of native PFCAs and 11H-PFUnDA and (2) The total analyte recovery was obtained by multiplying extraction recovery rate by derivatization efficiency. We obtained the derivatization efficiency by comparing the response signal of 11H-PFUnDA and CB111. Derivatization efficiency of all PFCAs and 11H-PFUnDA was assumed to be the same. The recoveries of the PFCAs were examined by spiking 10 and 0.1 ng of each native PFCA standard into control sunscreen samples ($N = 3$, for each spiking levels) and control cosmetic samples ($N = 3$, for each spiking levels) before extraction. In total, six samples were used for each spiking level. The results are summarized in the Table 2.

## 3. Results and discussion

### 3.1. The levels of PFCAs in PCPs

In the present study, we successfully quantified PFCAs in consumer-ready PCPs with total analyte recoveries ranging from 60% to 83% (Table 2). The concentrations of the PFCAs in the PCP samples are presented in Table 3. Twenty-one of the 24 samples that listed fluorinated compounds in their INCI labels contained detectable levels of PFCAs (13 of 15 cosmetic samples, 8 of 9 sunscreen samples). The maximum concentrations of total PFCAs were 5.9 μg g$^{-1}$ for cosmetics and 19 μg g$^{-1}$ for sunscreens. There was a large variation in PFCAs concentrations. For example, the levels of PFCAs in cosmetics samples nos. 4 and 5 and sunscreen sample No. 3 were two orders of magnitude lower than those in the other samples. The concentrations of PFCAs were highest in sunscreen sample no. 1–1, which contained 6.5 μg g$^{-1}$ PFHxA, 5.7 μg g$^{-1}$ PFOA, 2.9 μg g$^{-1}$ PFDA, and 1.4 μg g$^{-1}$ PFDoDA. Although PFHxA and PFOA were present at the highest concentrations, many different PFCAs were detected. The levels of PFOA in the PCPs were higher than those previously reported for end consumer products (Washburn et al., 2005). The contribution of PCPs to human PFCAs exposure is still unclear. The relatively high concentration of PFCAs in consumer-ready PCPs suggests that they could represent an exposure route for humans. If so, PCPs may explain the exposure source to females who have higher PFCAs levels in serum. Although the concentrations of PFCAs in female serum are similar or lower than that in males in the National Health and Nutrition Examination Survey in the US (Calafat et al., 2007), a recent study in China revealed PFOA levels in female serum have been increasing (Jin et al., 2007). The contribution of the PCPs to the total human PFCA exposure could be wide range of variations due to individual preferences as well as market supplies. Further studies are needed to investigate the relationship between the use of PCPs and exposure to PFCAs.

### 3.2. The levels of PFCAs in compounding agents

We further searched sources of PFCAs in end consumer products. In this study, the PCPs that listed PAPs and the other fluorinated compounds in their INCI labels were collected (Table 1). All the samples that had PAPs in their labels contained detectable levels of PFCAs (11 cosmetic samples, five sunscreen samples), while some of PCPs that had other fluorinated compounds in their labels did not contain detectable concentrations of PFCAs (cosmetic sample nos. 14 and 15, and sunscreen sample No. 6) (Table 3). By contrast, cosmetic and sunscreen samples that were produced by the same manufacturers and did not list PAPs or other fluorinated

**Table 3**
Levels of PFCAs in personal care products.

| Sample No. | | Compound (carbon atoms), concentration (ng g$^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PFHxA (C6) | PFHpA (C7) | PFOA (C8) | PFNA (C9) | PFDA (C10) | PFUnDA (C11) | PFDoDA (C12) | PFTrDA (C13) | PFTeDA (C14) | ΣPFCAs Actual[e] | Upper[f] | Lower[g] |
| (In a concentration order) | | | | | | | | | | | | | |
| *Cosmetics* | | | | | | | | | | | | | |
| 8 | Powder fundation | 1100 | 170 | 1700 | 240 | 1000 | 150 | 940 | 67 | 590 | 5900 | – | – |
| 10 | Liquid foundation | 2100 | 290 | 1500 | 230 | 610 | 110 | 260 | 35 | 84 | 5100 | – | – |
| 3 | Foundation | 410 | 150 | 1400 | 250 | 880 | 130 | 430 | 56 | 210 | 3900 | – | – |
| 6 | Powder fundation | 800 | 200 | 1100 | 380 | 650 | 180 | 360 | 71 | 140 | 3900 | – | – |
| 9 | Powder fundation | 910 | 160 | 390 | 110 | 320 | 67 | 160 | 28 | 59 | 2200 | – | – |
| 1 | Manicure | 140 | 45 | 910 | 140 | 450 | 62 | 230 | 16 | 44 | 2000 | – | – |
| 12 | Foundation | 180 | 150 | 430 | 340 | 270 | 150 | 140 | 56 | 62 | 1800 | – | – |
| 2 | Manicure | 24 | Trace (<29)[a] | 204 | 42 | 108 | 21 | 58 | 8.2 | 12 | – | 506 | 492 |
| 7 | Powder fundation | 110 | 25 | 91 | 22 | 74 | 14 | 43 | 8.0 | 18 | 410 | – | – |
| 13 | Foundation | 360 | 25 | 7.9 | Trace (<1.2)[a] | 3.6 | Trace (<0.80)[a] | Trace (<1.6)[a] | n.d. (<0.28)[b] | n.d. (<0.019)[b] | – | 402 | 400 |
| 11 | Liquid makeup base | 55 | 13 | 43 | 3.4 | 12 | 2.1 | 5.9 | 0.91 | 1.8 | 140 | – | – |
| 5 | Manicure (base coat) | 4.7 | Trace (<2.0)[a] | 15 | 2.5 | 7.0 | 1.2 | 3.3 | Trace (<0.50)[a] | 1.5 | – | 38 | 37 |
| 4 | Lip rouge | Trace (<3.8)[a] | Trace (<2.4)[a] | 4.1 | 1.0 | 2.8 | 0.76 | 2.4 | Trace (<0.59)[a] | 0.75 | – | 19 | 15 |
| 14 | Lip rouge | n.d. (<5.7)[b] | n.d. (<5.1)[b] | n.d. (<4.9)[b] | n.d. (<1.8)[b] | n.d. (<2.1)[b] | n.d. (<1.3)[b] | n.d. (<2.1)[b] | n.d. (<0.87)[b] | n.d. (<0.060)[b] | – | 24 | 0 |
| 15 | Powder fundation | n.d. (<1.9)[b] | n.d. (<1.7)[b] | n.d. (<1.7)[b] | n.d. (<0.60)[b] | n.d. (<0.73)[b] | n.d. (<0.43)[b] | n.d. (<0.71)[b] | n.d. (<0.30)[b] | n.d. (<0.020)[b] | – | 8.1 | 0 |
| *Sunscreen* | | | | | | | | | | | | | |
| 1-1[d] | Skin milk | 6500 | 670 | 5700 | 670 | 2900 | 330 | 1400 | 140 | 600 | 19,000 | – | – |
| 2 | Skin milk | 3300 | 530 | 1700 | 350 | 1100 | 210 | 640 | 100 | 300 | 8200 | – | – |
| 1-4[d] | Skin milk | 2900 | 450 | 1500 | 300 | 950 | 190 | 570 | 89 | 260 | 7200 | – | – |
| 1-2[d] | Skin milk | 2700 | 390 | 1400 | 280 | 970 | 170 | 590 | 88 | 280 | 6900 | – | – |
| 1-3[d] | Skin milk | 1300 | 210 | 680 | 140 | 440 | 81 | 250 | 39 | 107 | 3200 | – | – |
| 4 | Powder foundation | 350 | 62 | 270 | 73 | 210 | 47 | 160 | 29 | 76 | 1300 | – | – |
| 5 | Liquid foundation | 180 | 53 | 380 | 170 | 170 | 76 | 78 | 28 | 28 | 1200 | – | – |
| 3 | Skin milk | Trace (<4.6)[a] | n.d. (<1.6)[b] | 3.7 | Trace (<1.1)[a] | 1.9 | Trace (<0.77)[a] | Trace (<1.5)[a] | n.d. (<0.27)[b] | n.d. (<0.018)[b] | – | 15 | 8.8 |
| 6 | Foundation | n.d. (<2.3)[b] | n.d. (<2.1)[b] | n.d. (<2.0)[b] | n.d. (<0.72)[b] | Trace (<1.9)[a] | Trace (<1.0)[a] | n.d. (<0.90)[b] | n.d. (<0.36)[b] | n.d. (<0.025)[b] | – | 11 | 1.4 |
| *Control samples (do not list any fluorinated ingredients in their labels)* [c] | | | | | | | | | | | | | |
| 1 | Cosmetics (manicure) | n.d. (<0.61)[b] | n.d. (<0.56)[b] | n.d. (<0.53)[b] | n.d. (<0.19)[b] | n.d. (<0.23)[b] | n.d. (<0.14)[b] | n.d. (<0.23)[b] | n.d. (<0.094)[b] | n.d. (<0.0065)[b] | – | 2.6 | 0 |
| 2 | Sunscreen (skin milk) | n.d. (<0.32)[b] | n.d. (<0.29)[b] | n.d. (<0.28)[b] | n.d. (<0.10)[b] | n.d. (<0.12)[b] | n.d. (<0.071)[b] | n.d. (<0.12)[b] | n.d. (<0.049)[b] | n.d. (<0.0034)[b] | – | 1.3 | 0 |

Each sample was quantified once.
MDL: method detection limit; MQL: method quantification limit.
[a] If the concentration levels are under MQL (and upper MDL), the levels are descrived as "Trace (<the value of MQL)". MQL varied according to the sample volume used in chemical analysis.
[b] If concentration levels are under MDL, the levels are descrived as "n.d. (<the value of MDL)". MDL varied according to the sample volume used in chemical analysis.
[c] The cosmetic and sunscreen samples that did not list any fluorinated ingredients in their labels were used as control samples.
[d] Sunscreen Nos. 1-1, 1-2, 1-3 and 1-4 are the same product with different lot numbers.
[e] Actual; If all PFCA compounds are quantified, actual sum of PFCAs were calculated.
[f] Upper boundary; concentrations lower than the MQL and higher than MDL were given the MQL for the calculation. Concentrations lower than the MDL were given the MDL for the calculation.
[g] Lower boundary; concentrations lower than the MQL and higher than MDL were given the MDL for the calculation. Concentrations lower than the MDL were given zero for the calculation.

**Table 4**
Levels of PFCAs in compounding agents of PCPs.

| Sample No. | Component | Composition[b] (Wt%) | Compound (carbon atoms), concentration (ng $g^{-1}$) | | | | | | | | | | PFCAs/PAPs[b,c] ($\mu g\ g^{-1}{}_{\text{-PAPs}}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | PFHxA | PFHpA | PFOA | PFNA | PFDA | PFUnDA | PFDoDA | PFTrDA | PFTeDA | ΣPFCAs | |
| | | | (C6) | (C7) | (C8) | (C9) | (C10) | (C11) | (C12) | (C13) | (C14) | | |
| *Compounding agents* | | | | | | | | | | | | | |
| 1 | Mica treated with PAPs[a] | Mica: 95, PAPs:5 | 8400 | 3100 | 6000 | 3200 | 5700 | 2300 | 3800 | 1100 | 1900 | 35,000 | 700 |
| 2 | Talc treated with PAPs[a] | Talc: 95, PAPs:5 | 500 | 130 | 350 | 190 | 600 | 140 | 350 | 62 | 150 | 2500 | 50 |

Each sample was quantified once.
[a] PAPs: polyfluoroalkyl phoshate esters.
[b] Composition listed in their raw material specification sheet.
[c] On the basis that the specified amounts of PAPs are reliable, the levels of PFCAs in PAPs were estimated.

compounds in their INCI labels did not contain any detectable levels of PFCAs (control samples nos. 1 and 2). These results suggested that PAPs may be a major source of PFCAs in consumer-ready PCPs. To confirm this possibility, we analyzed commercially available compounding agents for PCPs, mica and talc, which were treated with PAPs. As anticipated, high concentrations of PFCAs were detected in these compounding agents (total PFCAs 2.5 $\mu g\ g^{-1}$ for talc treated with PAPs, 35.0 $\mu g\ g^{-1}$ for mica treated with PAPs) (Table 4).

### *3.3. The possible source of PFCAs to PCPs and compounding agents*

The contamination pathway of PFCAs in PCPs and compounding agents is still unknown. As mentioned above, FTOHs are important intermediates for the synthesis of PAPs. The starting material, perfluoroalkyl iodide ($CF_3(CF_2)_nI$, Telomer A) is manufactured by telomerization with a telogen such as pentafluoroethyl iodide ($CF_3CF_2I$) and a taxogen such as tetrafluoroethylene ($CF_2{=}CF_2$) (Lehmler, 2005). Telomer A is then coupled with ethylene ($CH_2{=}CH_2$) and converted to perfluoroalkyl ethyl iodide ($CF_3(CF_2)_n CH_2CH_2I$, Telomer B). Hydrolysis of Telomer B forms FTOH ($CF_3(CF_2) CH_2CH_2OH$: Telomer BA). FTOHs unintentionally contain perfluoroalkylate ester ($CF_3(CF_2)_nCOO(CF_2)_nCF_3$) and unreacted Telomer A (DuPont Company, 2006). Hydrolysis and oxidation of these impurities could result in the formation of PFCAs (DuPont Company, 2006). Thus we speculate that the origin of PFCAs in PCPs and compounding agents are impurities of PAPs. In addition to that, biotransformation of PAP to PFCA has been observed in rats (D'eon and Mabury, 2007, 2011) and in a microbial system (Lee et al., 2010). However, degradation of PAPs in finished consumer products is still not well-characterized. Future studies are needed to reveal the exact origin and pathway of PFCAs in PCPs.

### *3.4. Possible health concerns from direct application of PCPs*

Cosmetics and sunscreens are applied directly to human skin, which is of concern because of dermal exposure to PFCAs. Recent data have indicated that statistically significant and dose-responsive increases in serum PFOA concentrations follow topical dermal application in rodent models, which suggests that PFOA is dermally absorbed (Franko et al., 2012). PFOA was also found to be immunotoxic following dermal exposure, with an enhancement of a hypersensitivity response to ovalbumin, which implies that PFOA exposure may augment the immunoglobulin E response to environmental allergens (Fairley et al., 2007). Because PFCAs are likely to cause similar effects in humans, health risk from PAP usage in PCPs needs to be assessed. In addition, measures need to be taken to reduce impurities in PCPs or promote use of alternative materials.

### *3.5. Possible effect of PCPs to the indoor environment*

In addition to the effect of direct application to human skin, residual PFCAs in the PCPs may be an important emission source in indoor spaces. The indoor environment is thought to be an important source of human exposure for PFCAs. Several studies have reported detection of PFCAs in indoor dust (Strynar and Lindstrom, 2008; Zhang et al., 2010; Liu et al., 2011; Shoeib et al., 2011) and associations between levels of fluorinated chemicals in indoor dust and the levels in human serum (Haug et al., 2011; Fraser et al., 2012). However, the exact source has not been identified. Previous studies have revealed that organic chemicals used in PCPs, like polycyclic musks, are detected in house dust (Fromme et al., 2004; Lu et al., 2011). Residual PFCAs in PCPs could also be a source of PFCAs in house dust. Indeed, PAPs were detected in house dust and positively correlated with the PFCAs level in a recent study (De Silva et al., 2012). Clearly detailed emission studies and exposure assessments are required.

### *3.6. Limitation of this study*

A major limitation of this study is that the concentrations of PAPs in PCP samples and the compounding agents were not determined. Therefore, the amounts of PAPs in PCPs are unclear. Thus our discussion at the present time remains speculative. For compounding agents, the composition is listed in their raw material specification sheet (95% of Mica or Talc and 5% of PAPs). On the basis that the specified amounts of PAPs are reliable, the levels of PFCAs in PAPs were estimated to 700 $\mu g\ g^{-1}{}_{\text{-PAPs}}$ and 50 $\mu g\ g^{-1}{}_{\text{-PAPs}}$ (Table 4). However compositional information such as chain length of the PAPs was not specified. Thus rigorous analyses of those PAPs and PFCAs are not possible. To investigate further, chemical analysis of PAPs in PCP and the compounding agents is strongly recommended. In terms of the sample size, we analyzed 21 samples for PCPs, but only 2 samples for compounding agents. Clearly a more extensive study is needed to properly define the relationship between PAPs and PFCAs.

## 4. Conclusion

In conclusion, we have quantified PFCA concentrations in PCPs and identified compounding agents as a major source of PFCAs. The limitations of this study are the small sample size and the fact that the concentrations of PAPs in the PCP samples and the compounding agents were not determined. To establish rational management policies for PAPs in PCPs, further studies are needed to ascertain the relationship between PAPs and PFCAs.

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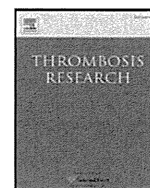

Letter to the Editors-in-Chief

**Protein S K196E mutation, a genetic risk factor for venous thromboembolism, is limited to Japanese**

*Dear Editors,*

Ethnic differences in thrombotic genetic risk have received much attention in recent years because the established thrombotic genetic variants, factor V Leiden and prothrombin G20210A mutation, are limited in Caucasian populations but not in East Asian populations [1]. We and others have identified the protein S K196E mutation (rs121918474) as a genetic risk factor for venous thromboembolism (VTE) in a Japanese population with odds ratios between 3.74 and 8.56 [2–5]. The allele frequency of this mutation is 0.0089 [5,6]. When overlapping with congenital protein C deficiency, this mutation facilitates the development of VTE [4,7]. The individuals heterozygous for the mutant E-allele had 16% lower protein S anticoagulant activity than wild-type subjects [8]. An *in vitro* study showed that the recombinant protein S with the K196E mutation lost activated protein C-dependent anticoagulant activity [9]. Since East Asians including Japanese, Chinese, and Koreans are geographically and genetically close, the protein S K196E mutation, which has so far been found only in Japanese, may be found in other East Asians. In this study, we have genotyped the protein S K196E mutation in Chinese and Korean populations to clarify the geographic distribution of this thrombotic mutation. The mutation was not found in Chinese or Koreans.

The first panel for the genotyping was a general Chinese population consisting of 509 individuals: 50 in Dehui, 49 in Huludao, 50 in Beijing, 49 in Jinan, 50 in Xi'an, 47 in Baoji, 50 in Shanghai, 50 in Changsha, 15 in Heping, 50 in Nanning, and 49 in Tainan. The second panel was a general Korean population consisting of 492 individuals including 105 in Seoul, 29 in Chonan, 46 in Haman, 247 in Pusan, and 65 in Jeju-do [10,11]. The third panel consisted of 122 Chinese patients with VTE and 112 Chinese control individuals [12]. Furthermore, from the 1000 Genomes Project (http://www.1000genomes.org/) we retrieved the genetic information of protein S K196E mutation in 197 Chinese consisting of 97 Chinese in Beijing and 100 Chinese in southern China, as well as in 89 Japanese [13].

The genotype of protein S K196E mutation in the first two panels was determined by the TaqMan genotype discrimination method [8] using the primers 5'- ACCACTGTTCCTGTAAAAATGGTTT/5'- TTTAATT CTACCATCCTGCTCTTACCT and the probes 5'-FAM - CAATCTTTCTtAT TTGAAAGC-MGB (the wild-type allele)/5'-VIC- AATCTTTCTcATTTGA AAGC (the mutant allele). In the third panel, we genotyped the mutation by the Homogeneous Mass Extend and iPLEX assays (Sequenom, Hamburg, Germany) and validated it with the SNaPshot assay (Applied Biosystems, USA). Primer sequences of the SEQUENOM assay are available on request. The PCR primers for the SNaPshot were AGACATAA ATGAACGCAAAGATC and TCTAACTGGGATTATTCTCACAC, and the sequence for the extension probe was TTTTTTTTTTTTTTCTTACCTTTACA GTCTTTCT. The SNaPshot products were resolved and analyzed using an ABI 3730 Automated Sequence Analyzer (Applied Biosystems). The study protocol was approved by the Institutional Review Boards and Ethics Committees of Kyoto University School of Medicine and by the General Hospital of the People's Liberation Army.

We genotyped the protein S K196E mutation in 509 Chinese and 492 Koreans and found that none carried the mutation. We did not identify the mutation in 122 Chinese patients with VTE or in 112 Chinese control individuals. Thus, the genetic analysis of three independent panels indicated that the protein S K196E mutation is present solely in Japanese and not in Chinese. The number of Korean individuals we genotyped was insufficient to yield conclusive evidence, and the 1000 Genomes Project does not include Koreans, but it is likely that Koreans also do not carry the mutation. The 1000 Genomes Project showed that none of the 197 Chinese carried the protein S K196E mutation, but one heterozygous carrier was present in 89 Japanese. We calculated a statistical power to detect a difference of minor allele frequency between Japanese and Chinese/Korean with the 0.05 level of significance. A one-sided statistical power more than 0.80 was considered statistically significant. Albeit a large enough statistical power for Chinese (0.82), a low statistical power, 0.67, for Korean may permit us to apply above argument to only Chinese.

The finding that the protein S K196E mutation is present only in Japanese was unexpected, because in thrombosis-related factors, two genetic mutations found in Japanese but not in Caucasians, plasminogen A620T and ADAMTS13 P475S, are both present in Chinese and Koreans [14,15]. The present results suggest that, even though Japanese, Chinese, and Koreans are geographically close to one another, the genetic background for thrombosis differs among them. The results also suggest that the protein S K196E mutation is a recent occurrence and fixed within the Japanese population.

## Conflict of Interest Statement

The authors state that they have no conflict of interest.

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