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Identification of compounds in red wine that effectively upregulate aquaporin-3 as a potential mechanism of enhancement of skin moisturizing

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ABSTRACT

In a previous clinical study, the moisture content in the stratum corneum of healthy Japanese women who consumed a beverage rich in oligomeric proanthocyanidins (OPCs) made from red wine extract was found to be higher than that in the control group. This finding suggested that OPCs can increase skin moisture content. In this study, we determined the expression level of aquaporin-3 (AQP3) in keratinocytes to elucidate the mechanism by which compounds in red wine grape increase moisture content in stratum corneum. Through in vitro studies, we confirmed that normal human epidermal keratinocytes (NHEK) incubated with red wine induced *AQP3* expression. Furthermore, the supplementation of red wine fractions enriched in OPC was shown to increase *AQP3* expression. Besides, the component of OPC-rich fractions that upregulated AQP3 expression was found to be a gallic acid (GA)-binding flavan-3-ol, particularly oligomeric compounds. We found that GA-binding OPC were able to upregulate *AQP3* expression and that these compounds were enriched in red wine. Our findings might suggest that the mechanism of enhancement of moisture content in stratum corneum by red wine might be via the upregulation of *AQP3* expression in the epidermal keratinocytes.

1. Introduction

Red wine is a rich source of polyphenols, such as anthocyanins and proanthocyanidins (PCs). PCs are polymerized polyphenols composed of flavan-3-ol subunits. Red wine PCs are known to contribute to astringency due to their affinity to salivary proteins [1]. They can also interact with anthocyanins to stabilize the color of red wine [2]. Red wine PCs are mainly extracted from the seeds and epicarp of grapes. The degree of polymerization of grape epicarp PCs is higher than that of grape seed PCs. Moreover, a higher number of PC–gallic acid conjugates are produced in grape seeds than in the grape epicarp [3,4]. Oligomeric proanthocyanidins (OPCs), which are PCs with a low degree of polymerization, have been reported to be associated with various activities. For example, OPCs in hawthorn extract were found to produce an antihypertensive effect in humans [5]. Grape seed derived Leucoselect® (Indena S.p.A. Milan, Italy), which is enriched in low-molecular-weight OPCs, was shown to exhibit human serum antioxidant activity [6].

The enormous demand for skincare products to improve the moisture content in the skin has prompted significant time and effort to be devoted new technologies in this area.

Aquaporins (AQPs) water channels are regulators of transcellular water flow. The AQP family in mammals consists of 13 unique members, namely, AQP0 through AQP12. AQPs are associated with diverse physiological and pathophysiological processes. These channels perform versatile functions, including urine concentration, digestive secretion, protection against dryness, and cellular adhesion [7].

The human skin is composed of three layers: the epidermis—the outermost surface—under which the dermis and various other subcutaneous tissues lie. The epidermis is further subdivided into four layers: cornified (stratum corneum), granular, spinous, and basal. The stratum

Abbreviations: OPC, oligomeric proanthocyanidin; AQP3, aquaporin-3; NHEK, normal human epidermal keratinocytes; GA, gallic acid; C, (−)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; EGCG, (−)-epigallocatechin; EC-C, procyanidin B1; EC-EC, procyanidin B2; EC-EC-EC, procyanidin C1; C–C, procyanidin B3; C-EC, procyanidin B4; LC-TOF-MS, liquid chromatography/time-of-flight/mass spectrometry; RA, retinoic acid; PPARγ, peroxisome proliferator-activated receptor γ; HIF-1α, hypoxia-inducible factor 1α.

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corneum prevents water loss from the skin. AQP3 is abundantly expressed in cell membranes of the basal epidermal cell layer in mammals [8]. Blood vessels are responsible for transporting water to all parts of the human body. However, the entire epidermis does not contain blood vessels. Instead, AQP3 is expressed in the keratinocytes of the epidermis and is responsible for water and glycerol transport throughout the stratum corneum layer. A previous study showed that the corneal water content and elasticity of the skin epidermis of AQP3-null mice was considerably lower than that of wild-type mice, indicating that AQP3 is required for the maintenance of skin moisture [9]. In addition, the downregulation of AQP3 expression can delay wound healing [10,11]. Conversely, the upregulation of AQP3 expression may result in increased moisture content of dermal tissues, improved skin texture, increased skin elasticity, as well as the ability to heal wounds and other injuries [12].

In our previous study, test beverages reconstituted with OPC-rich red wine extract (with ethanol removed) were given to Japanese healthy women at 200 mg OPC per day. After 4, 8, and 12 weeks of continuous intake, the experimental group had significantly higher corneal moisture content than the control group [13]. In this study, we aimed to determine whether the mechanism of the skin moisturizing effects of red wine are correlated with AQP3 expression. Thus, we sought to identify the compounds responsible for this upregulation.

2. Materials and methods

2.1. Materials

(−)-Catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Procyanidin B1 (EC-C) was purchased from Extrasynthese (Lyon, France). Procyanidin B2 (EC-EC) was purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Procyanidin C1 (EC-EC-EC) and retinoic acid (RA) were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Gallic acid (GA) was purchased from Funakoshi Co. Ltd. (Tokyo, Japan). All chemicals were used without further purification. Procyanidin B3 (C–C), procyanidin B4 (C-EC), procyanidin B4-GA (C-ECG), EC-CG, C–C–C, and C–C-ECG were synthesized according to our previous study [14].

2.2. Preparation of samples from red wine (wine polyphenol fraction)

Ancellotta wine (12 L) was evaporated to remove ethanol, after which the resulting material was subjected to 10 L Diaion HP-2MG column chromatography (Supelco, Bellefonte, PA, USA). After elution with 20 L H2O, fractions eluted with 70% (v/v) ethanol were collected and evaporated to obtain a red powder (wine polyphenol fraction: 108.18 g). A portion of the 500 mg wine polyphenol fraction was applied to a Sephadex LH-20 column (1 L, GE Healthcare, UK Ltd., Little Chalfont, UK); the column was eluted with 4 L of 10%, 20%, 30%, 40%, 50%, and 60% methanol/H2O with 1% formic acid, and 50% acetone. Each fraction was evaporated, lyophilized, and used for further experiments.

2.3. LC-TOF-MS analysis

Each fraction of Ancellotta wine was analyzed using reversed-phase liquid chromatography/time-of-flight/mass spectrometry (LC-TOF-MS) with Q-TOF Premier (Micromass, Manchester, UK) analysis, according to our previous study [14].

2.4. Vitis vinifera juice

Frozen Ancellotta grapes were thawed by natural thawing and squeezed to obtain juice A. Juice B was obtained by freezing Ancellotta grapes in liquid nitrogen, homogenizing at 10,000 rpm for 5 min, and squeezing the homogenized fragments.

2.5. Cell lines and cell culture

Normal human epidermal keratinocytes (NHEK) were obtained from Kurabo Industries Ltd. (Osaka, Japan). These cells were seeded in 60 mm dishes at a density of 1×10^5 and cultured in HuMedia-KG2 (Kurabo Industries Ltd.) at 37 °C in a humidified 5% CO2 atmosphere. After cells were cultured to semi-confluency, the medium was replaced with fresh medium containing each fraction or compound and incubated further for 24 h for RT-PCR or for 48 h for ELISA. RA (Sigma-Aldrich Co. LLC) was used as a positive control.

2.6. Enzyme-linked immunosorbent assay

Cell membrane proteins were extracted from NHEK using sucrose buffer (250 mM sucrose with 1 mM EDTA and 1% protease inhibitor cocktail). AQP3 protein expression levels of each cell membrane fraction was quantified by Enzyme-linked immunosorbent assay (ELISA) using the human AQP3 ELISA kit (MyBioSource, San Diego, USA). The protein expression level was normalized to Na/K-ATPase protein expression levels as an internal standard for cell membrane protein which also quantified by ELISA using human Na/K-ATPase ELISA Kit (MyBioSource, San Diego, USA).

2.7. Real-time PCR analysis

RNA was extracted from NHEK using ISOGEN (NIPPON GENE Co. Ltd., Tokyo, Japan). For reverse transcription, cDNA was synthesized from 1 g of RNA using the Superscript VILO master mix (Life Technologies, Carlsbad, CA, USA). Target gene expression was analyzed by real-time PCR with KOD SYBR Green qPCR mix (TOYOBO Co. Ltd., Osaka, Japan) using the primers listed in Table 1. The mRNA levels were normalized to GAPDH mRNA levels.

2.8. Statistical analysis

Statistical analysis was performed using JMP ver.14.0.0 (SAS Institute Inc., Cary, NC, USA). Numerical data were expressed as mean ± SD. Significant differences between the groups were determined using Dunnett's test, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Red wine fractions

We investigated the ability of various red wine fractions at the equivalent concentration of 1:400 dilution to affect AQP3 expression. The following fractions significantly increased AQP3 expression: 10% methanol/water +1% formic acid (Fr.1), 20% methanol/water + 1% formic acid (Fr.2), 30% methanol/water + 1% formic acid (Fr.3), 40% methanol/water + 1% formic acid (Fr.4), 50% methanol/water + 1% formic acid (Fr.5), and 60% methanol/water + 1% formic acid (Fr.6) (Fig. 1A).

We performed LC-TOF-MS analysis for all polyphenol fractions, as described previously14. Anthocyanin was found to be concentrated in fractions 1 and 2. Moreover, malvidin 3-*O*-glucoside and petunidin 3-*O*-glucoside were found to be the most abundant and second-most abundant anthocyanins in red wine fraction 1 and 2 (Fig. 1A). OPCs were

Table 1
Primer sequences used for real-time PCR.

Gene	Primer	Sequence (5′–3′)
AQP3	Forward	CTGGGGACCCTCATCCTGGTGATG
	Reverse	CACGATAAGGGAGGCTGTGCCTATG
GAPDH	Forward	ACTGGTGTCTTCACCACCATGGAGAAGGC
	Reverse	CATGAGGTCCACCACCCTGTTGCTGTAGC

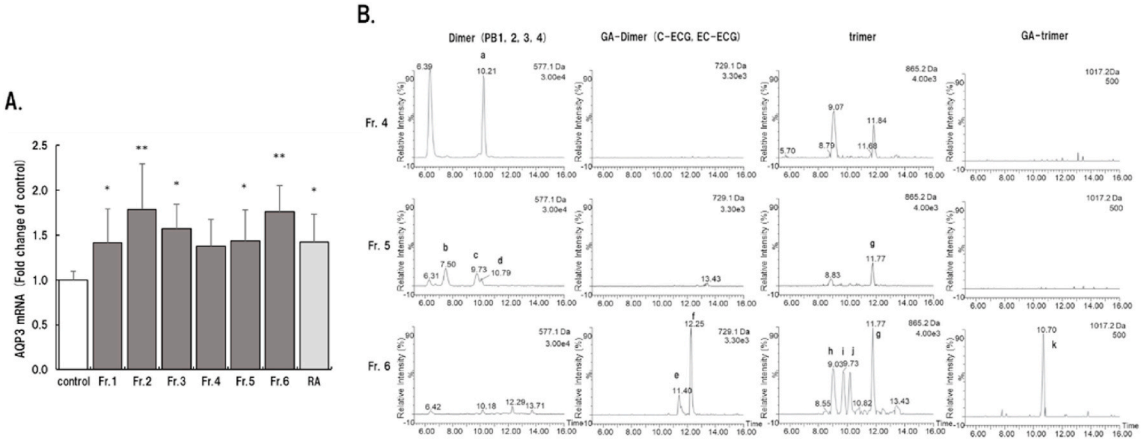

Fig. 1. Effect of polyphenol fractions of Ancellotta wine and the compounds in each fraction on APQ3 mRNA expression. (A) Effect of polyphenol fractions of Ancellotta wine on APQ3 mRNA expression. The mean levels of AQP3 mRNA expression of the control are indicated as 1.0. 10% methanol/water +1% formic acid (Fr.1), 20% methanol/water + 1% formic acid (Fr.2), 30% methanol/water + 1% formic acid (Fr.3), 40% methanol/water + 1% formic acid (Fr.4), 50% methanol/water + 1% formic acid (Fr.5), and 60% methanol/water + 1% formic acid (Fr.6). Each fraction was supplemented at the equivalent concentration of 1:400 dilution from the original wine. RA: retinoic acid as a positive control. The data are presented as the mean ± SD for six to eight experiments. Dunnett's test: $*P < 0.05$, $**P < 0.01$ vs. control. (B) Results of the MS analysis of LH-20 fractions of Ancellotta wine using LC-TOF-MS. *m/z* [M − H]-, 577.1; dimer (PB1,2,3,4), *m/z* [M − H]-, 729.1; GA-dimer (C-ECG,EC-ECG), *m/z* [M − H]-, 865.2; trimer, *m/z* [M − H]-, 1017.2; GA-trimer). a: PB2 (EC-EC), b: PB1 (EC-C), c: PB3 (C–C), d: PB4 (C-EC), e: C-ECG, f: EC-ECG, g: EC-EC-EC, h: Trimer-A [14], i:Trimer-B [14], j:Trimer-C [14], k: EC-EC-ECG. Fr.4 contains PB2 and unknown trimer and fragment ion peak of trimer (upper). Fr.5 contains PB1, PB3, PB4 and EC-EC-EC (middle). Fr.6 contains C-ECG, EC-ECG, Trimer-A, -B, –C, EC-EC-EC, EC-EC-ECG (lower).

concentrated in fractions 4, 5 and 6, as shown in Fig. 1B. Fraction 4 mainly contained procyanidin B2 (PB2, EC-EC) and unknown trimer and fragment ion peak of trimer (Fig. 1B upper). Fraction 5 contained dimeric flavan-3-ols, including procyanidin B1 (PB1, EC-C), procyanidin B3 (PB3, C–C), procyanidin B4 (PB4, C-EC), and EC-EC-EC (Fig. 1B middle), while fraction 6 contained dimeric and trimeric flavan-3-ols with galloyl group such as C-ECG, EC-ECG, EC-EC-ECG, and trimers without GA such as Trimer-A,B,C [14] and EC-EC-EC (Fig. 1B lower). From this result, GA-attached dimer and GA-attached trimer were mainly found in fraction 6 (Fig. 1B).

3.2. Structure-activity relationship of OPCs

After incubation with fraction 5 or 6 containing OPCs, *AQP3* gene expression increased (Fig. 1A). Therefore, we examined the structure–activity relationship of flavan-3-ol monomers, dimers, and trimers, which form the OPC skeleton. Flavan-3-ol monomers with GA-attached compounds such as ECG and EGCG were found to significantly increase *AQP3* mRNA expression at concentrations of 30 μM (Fig. 2A). Similarly, flavan-3-ol dimers with GA-attached C-ECG and EC-ECG significantly increased *AQP3* mRNA expression (Fig. 2B). Flavan-3-ol trimers with the EC-EC-EC, which lacks a GA group, also significantly increased *AQP3* mRNA expression (Fig. 2C). Furthermore, GA-attached trimers with C–C-ECG were found to upregulate *AQP3* mRNA expression (Fig. 2C). For all GA-binding compounds, dimers and larger polymers caused greater upregulation of *AQP3* mRNA expression than flavan-3-ols without GA at 30 μM concentration (Fig. 4A). However, it is possible that degradation products of GA were produced in these experiments. It is unclear whether GA itself or a byproduct produced during incubation was the active ingredient. While GA alone might not produce this effect, further studies will determine whether OPCs binding to GA can upregulate *AQP3* mRNA expression. AQP3 protein expression was also partially evaluated by ELISA using membrane fraction of NHEK after 48-h incubation with C-EC and C-ECG (not shown). There was a 1.2 ± 0.5-

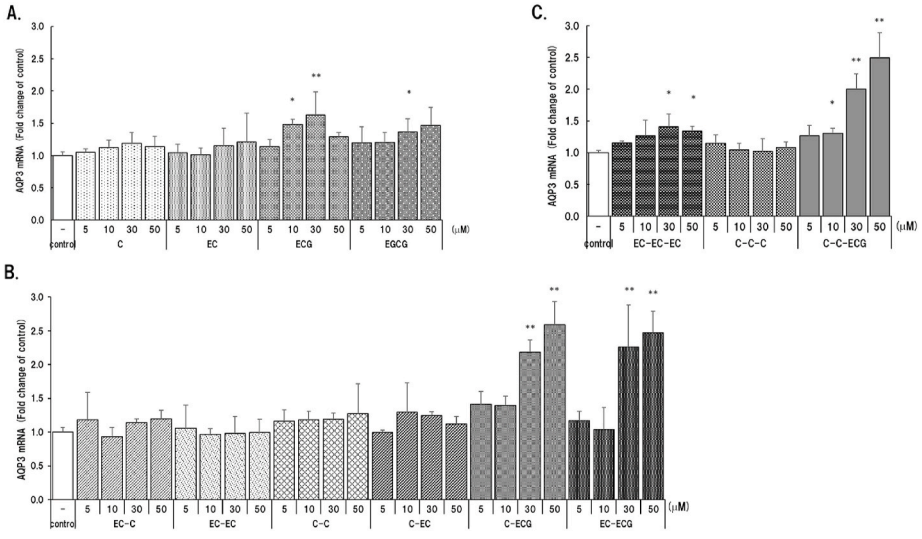

Fig. 2. *AQP3* mRNA levels in NHEK at different structural configurations of flavan-3-ol. The mean levels of *AQP3* mRNA expression of the control are indicated as 1.0. (A) Monomer, (B) dimer, (C) trimer. The data are presented as the mean ± SD for three to seven experiments. Dunnett's test: $*P < 0.05$, $**P < 0.01$ vs. control.

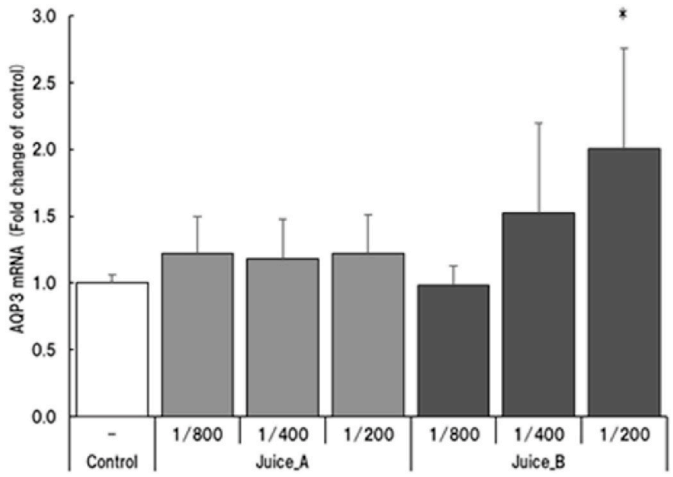

Fig. 3. *Vitis vinifera* (grape) juice. The mean levels of *AQP3* mRNA expression of the control are indicated as 1.0. Each juice was supplemented at 1:800, 1:400, and 1:200 dilution. The data are presented as the mean ± SD for three to seven experiments. Dunnett's test: $^{*}P < 0.05$, vs. control.

fold increase in C-EC and a 1.9 ± 0.9-fold increase in C-ECG compared to control. Although there was no significant difference in this study, there was a possibility that GA-binding flavan-3-ols could increase protein expression.

3.3. Grape juice

Previous studies have demonstrated that a 1:400 dilution of red wine can increase *AQP3* mRNA expression by 1.82 ± 0.19-fold (data not shown). While the juice obtained directly from naturally thawed grapes (juice A) had no effect on *AQP3* mRNA expression in NHEK, the supplementation of the juice squeezed from grapes frozen and homogenized in liquid nitrogen (juice B) increased *AQP3* mRNA levels in a dose-dependent manner (Fig. 3). In the process of wine production, ethanol elutes compounds such as OPCs from grape seeds. Juice B was produced by the homogenization of grapes; this resulted in the release of OPCs into the resultant juice from seeds—a phenomenon is similar to wine production. Hence, similar effects were observed.

4. Discussion

In this study, we determined the expression level of AQP3 in keratinocytes to elucidate the mechanism by which compounds in red wine grape increase human skin moisture content. In a previous clinical trial, the moisture content in the stratum corneum of healthy Japanese women who consumed 200 mg wine OPC-containing beverages daily for 12 weeks was found to be higher than that in the control group; the moisture content in the stratum corneum increased at 4, 8, and 12 weeks following the administration of OPC-containing beverages [13]. Here, we performed in vitro analysis using NHEK and found that the extract of red wine can upregulate *AQP3* mRNA expression in human keratinocytes. Thus, we sought to identify the compounds responsible for this upregulation.

First, we investigated the ability of red wine polyphenol fractions to regulate *AQP3* mRNA expression. Fractions containing high OPC or high anthocyanin levels were found to upregulate *AQP3* mRNA expression. We then analyzed flavan-3-ol monomers, dimers, and trimers for their effect on *AQP3* mRNA expression. Flavan-3-ol OPCs binding GA groups—particularly dimers and trimers—increased *AQP3* mRNA expression. *AQP3* mRNA expression was the highest at concentrations above 30 μM. These results suggest that GA-binding OPCs are active compounds that can upregulate *AQP3* expression. We also investigated other polyphenols found in red wine, including resveratrol and anthocyanin. However, they did not increase *AQP3* expression. In accordance with the previous study [15], resveratrol was found to downregulate *AQP3* expression. 10, 30, and 50 μM *trans*-resveratrol inhibited *AQP3* mRNA expression to 14, 31, and 46% of inhibition compared to control respectively. Malvidin 3-glucoside (Mal-3-Glc) and petunidin 3-glucoside (Pet-3-Glc) as the wine anthocyanin were examined the effect on *AQP3* mRNA expression. As the results, the ratio of 10 μM Mal-3-Glc to control was 1.12, and 10 μM Pet-3-Glc to control was 1.09.

In our previous study, the concentration of OPCs in Ancellotta wine was estimated to be 3030 μg/mL. Moreover, the OPC concentration in Ancellotta wine is the highest among other wines [14]. The concentrations of each type of OPC in Ancellotta red wine—PB1, PB2, PB3, PB4, and PB2-GA—were determined using reverse-phase LC/MS to be 32.9, 17.3, 6.7, 2.9, and 1.2 μg/mL, respectively [13]. PB1, PB2, PB3, and PB4 are found in high percentages in wine. Fractions containing four dimers (Fig. 1A, Fr.5) were found to upregulate *AQP3* expression, which was 1.4 ± 0.34 times upregulated compared to the control, but only in

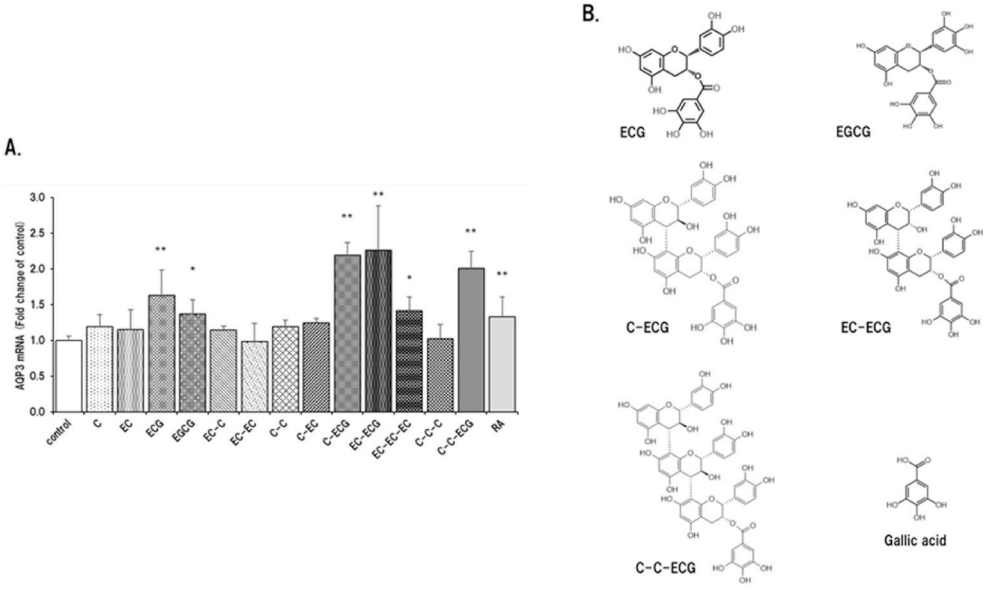

Fig. 4. Compound activity and structure. Compound activity at 30 μM. The mean levels of *AQP3* mRNA expression of the control are indicated as 1.0. (B) Structure of compounds. RA concentration is 2 μM. The data are presented as the mean ± SD for three to seven experiments. Dunnett's test: $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

conjugation which means each of PB did not have effect (Fig. 2B). Meanwhile, fractions containing dimers and trimers with GA-compounds (Fig. 1A, Fr.6) showed higher activity, which was 1.8 ± 0.29 times upregulated compared to the control, and each fraction containing dimer or trimer with GA-binding compounds was effective (Fig. 2). We did not determine whether Ancellotta red wine is richer in GA-binding compounds compared to other wine types. However, we believe that because Ancellotta red wine has higher OPC levels than other wines, it is richer in GA-binding compounds. It has been reported that polyphenols, dimers, and trimers are absorbed intestinally [16]. Therefore, the ingestion of these polyphenols might be effective in inducing skin moisturization via upregulating *AQP3*. We understand the concentration we use was relatively higher than biological. That was because the purpose of this study was investigation of the mechanism. Further studies are needed about bioavailability and pharmacokinetics of these compounds.

Whereas the grape juice obtained by squeezing naturally thawed Ancellotta grapes (juice A) had little effect on *AQP3* mRNA expression, supplementation of the grape juice obtained by freezing and homogenization of Ancellotta grapes with liquid nitrogen (juice B) increased *AQP3* mRNA expression in a dose-dependent manner. Squeezed juices contain compounds present in the grape pulp. However, homogenization breaks the grape seed into tiny fragments, allowing low-molecular-weight compounds such as OPCs to be extracted. We believe this to be the reason for juice B to stimulate *AQP3* expression. The grape seed is rich in GA-binding compounds, which are released during the wine-making process.

While further research is necessary to fully understand the mechanism by which OPCs upregulate *AQP3* mRNA expression, this study demonstrates the undeniable possibility that the transcription of *AQP3* mRNA is affected by polyphenols. We propose the following putative mechanism: the activity of peroxisome proliferator-activated receptor γ (PPARγ) is modulated by flavan-3-ol, and/or AQP3 transcription is activated via hypoxia-inducible factor 1 α (HIF-1α). Previous studies indicated that polyphenolic compounds [17] and flavan-3-ol- and flavonol-rich polyphenol fractions of partridge berry [18] can modulate PPARγ and HIF-1α activity. PPARs are fatty acid-activated transcription factors that belong to the nuclear hormone receptor family. While they are best known as transcriptional regulators of lipid and glucose metabolism, there is accumulating evidence to suggest their importance in skin homeostasis [19]. PPARγ is involved in the treatment of several skin diseases [19], including benign epidermal tumors, papillomas, acne vulgaris, and psoriasis [19]. Furthermore, PPARγ activators were demonstrated to stimulate AQP3 expression in keratinocytes/epidermis [20]. Therefore, we surmise that wine OPCs might affect AQP3 transcription via PPARγ.

HIF-1 is also a transcription factor. *AQP3* mRNA expression was found to be upregulated under hypoxic conditions in L929 fibrosarcoma cells [21]. Moreover, AQP3 expression was shown to be upregulated under hypoxic conditions in fibrosarcoma cells derived from HIF-1α knockdown mice [22].

Further research is required to investigate the effects of GA-binding dimers and trimers on the expression of transcription factors to elucidate the underlying mechanism. Moreover, studies are required to confirm whether or not the activation of these transcription factors is involved in the regulation of *AQP3* mRNA transcription.

Studies have shown that resveratrol inhibits NHEK proliferation by downregulating *AQP3* expression in an SIRT1/ARNT/ERK-dependent manner [15]. This result is consistent with that of our study, wherein resveratrol suppressed *AQP3* mRNA expression.

AQP3 is categorized as an aquaglyceroporin, a channel through which glycerol, urea, and other small compounds in addition to water can traverse [23]. AQP3 is involved in cell proliferation; therefore, it might be involved in the repair of damaged DNA caused by aging or UV exposure. Because AQP3 can enhance water content of the epidermis and increase cell proliferation due to its ability to transport glycerol [9], it is possible that the compounds identified here that upregulate *AQP3* mRNA levels can also upregulate glycerol transport. About protein expression, further study is needed because there was a possibility for AQP3 protein to increase with GA-binding flavan-3-ols, but no significant difference was found.

These results indicate that the mechanism of moisture content increase in the human stratum corneum by wine OPCs derived from Ancellotta grapes might be through the upregulation of AQP3 expression. Furthermore, the active ingredients that achieve this upregulation include GA-binding flavan-3-ols, particularly dimers and trimers.

Considering that the compounds that actively contribute to this process have been identified, further studies regarding the characterization of extracts that are abundant in these compounds, as well as improvements in extraction methods, would help advance products and technologies that increase AQP3 expression to moisturize the skin.

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Author contributions

Y.N., TT. and Y.F. designed study and conducted the research; Y.N. and T.T. conducted statistical analysis; and Y.N., T.T. and Y.F. wrote the manuscript. All authors take responsibility for the final content of the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] W. Ma, P. Waffo-Teguo, M. Jourdes, H. Li, P.L. Teissedre, Chemical affinity between tannin size and salivary protein binding abilities: implications for wine astringency, PloS One 11 (2016), e0161095.
[2] V.L. Singleton, E.K. Trousdale, Anthocyanin-tannin interactions explaining differences in polymeric phenols between white and red wines, Am J Enol Vitcic 43 (1992) 63–70.
[3] E. Capanoglu, R.C. de Vos, R.D. Hall, D. Boyacioglu, J. Beekwilder, Changes in polyphenol content during production of grape juice concentrate, Food Chem. 139 (2013) 521–526.
[4] J.A. Kennedy, Y. Hayasaka, S. Vidal, E.J. Waters, G.P. Jones, Composition of grape skin proanthocyanidins at different stages of berry development, J. Agric. Food Chem. 49 (2001) 5348–5355.
[5] G.N. Asher, A.J. Viera, M.A. Weaver, R. Dominik, M. Caughey, A.L. Hinderliter, Effect of hawthorn standardized extract on flow mediated dilation in prehypertensive and mildly hypertensive adults: a randomized, controlled cross-over trial, BMC Compl. Alternative Med. 12 (2012) 26.
[6] S.L. Nuttall, M.J. Kendall, E. Bombardelli, P. Morazzoni, An evaluation of the antioxidant activity of a standardized grape seed extract, Leucoselect, J. Clin. Pharm. Therapeut. 23 (1998) 385–389.
[7] R.E. Day, P. Kitchen, D.S. Owen, C. Bland, L. Marshall, A.C. Conner, R.M. Bill, M. T. Conner, Human aquaporins: regulators of transcellular water flow, Biochim. Biophys. Acta 1840 (5) (2014) 1492–1506.
[8] R. Sougrat, M. Morand, C. Gondran, P. Barre, R. Gobin, F. Bonte, M. Dumas, J. M. Verbavatz, Functional expression of AQP3 in human skin epidermis and reconstructed epidermis, J. Invest. Dermatol. 118 (2002) 678–685.
[9] M. Hara-Chikuma, A.S. Verkman, Roles of aquaporin-3 in the epidermis, J. Invest. Dermatol. 128 (2008) 2145–2151.

[10] T. Ma, M. Hara, R. Sougrat, J.M. Verbvatz, A.S. Verkman, Impaired Stratum corneum hydration in mice lacking epidermal water channel aquaporin-3, J. Biol. Chem. 277 (19) (2002) 17147–17153.
[11] M. Hara-Chikuma, A.S. Verkman, Aquaporin-3 facilities epidermal cell migration and proliferation during wound healing, J. Mol. Med. 86 (2008) 221–231.
[12] M. Dumas, N.S. Sadick, E. Noblesse, M. Juan, N. Lanchmann-Weber, M. Boury-Jamot, R. Sougrat, J.M. Verbavatz, S. Schnebert, F. Bonte, Hydrating skin by stimulation biosynthesis of aquaporins, J. Drugs Dermatol. JDD 6 (2007) s20–s24.
[13] T. Tsuchiya, Y. Fukui, R. Izumi, K. Numano, M. Zeida, Effects of oligometic proanthocyanidins (OPCs) of red wine to improve skin whitening and moisturizing in healthy women - a placebo-controlled randomized double-blind parallel group comparative study-, Eur. Rev. Med. Pharmacol. Sci. 24 (3) (2020) 1571–1584.
[14] T. Fujimaki, S. Mori, M. Horikawa, Y. Fukui, Isolation of proanthocyanidins from red wine, and their inhibitory effects on melanin synthesis in vitro, Food Chem. 248 (2018) 61–69.
[15] Z. Wu, H. Uchi, S. Morino-Koga, W. Shi, M. Furue, Resveratrol inhibition of human keratinocyte proliferation via SIRT1/ARNT/ERK dependent downregulation of1aquaporin 3, J. Dermatol. Sci. 75 (1) (2014) 16–23.
[16] C. Tsang, C. Auger, W. Mullen, A. Bornet, J.M. Rouanet, A. Crozier, P.L. Teissedre, The absorption, metabolism and excretion of flavan-3-ols and procyanidins following the ingestion of a grape seed extract by rat, Br. J. Nutr. 94 (2005) 170–181.
[17] J.A. Dominguez-Avila, G.A. Gonzalez-Aguilar, E. Alvarez-Parrilla, L.A. de la Rosa, Modulation of PPAR expression and activity in response to polyphenolic compounds in high fat diets, Int. J. Mol. Sci. 17 (2016) E1002.
[18] K.S. Bhullar, H.P. Rupasinghe, Partridgeberry polyphenols protect rat primary cortical neurons from oxygen-glucose deprivation-reperfusion-induced injury via suppression of inflammatory adipokines and regulation of HIF-1 and PPAR, Nutr Neurocci 19 (2016) 260–268.
[19] L. Michalik, W. Wasli, Peroxisome proliferator-activated receptors (PPARs) in skin health, repair and disease, Biochim. Biophys. Acta 1771 (2007) 991–998.
[20] Y.J. jiang, P. Kim, Y.F. Lu, K.R. Feingold, PPARgamma activators stimulate aquaporin 3 expression in keratinocyte/epidermis, Exp. Dermatol. 20 (2011) 595–599.
[21] D. Hoogewijs, M. Vogler, E. Zwenger, S. Krull, A. Zieseniss, Oxygen-dependent regulation of aquaporin-3 expression, Hipoxia 4 (2016) 91–97.
[22] J.A. Stuart, O. Aibueku, O. Bagshaw, F. Moradi, Hypoxia inducible factors as mediators of reactive oxygen/nitrogen species homeostasis in physiological normoxia, Med. Hypotheses 129 (2019) 109249.
[23] M. Hara-Chikuma, A.S. Verkman, Aquaporin-3 functions as a glycerol transporter in mammalian skin, Biol. Cell. 97 (2005) 479–486.