Association of a Polymorphism in the Ornithine Decarboxylase Gene with Whole Blood Polyamine Concentrations in a Non-smoking Healthy Population

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Ornithine decarboxylase (ODC) is the rate-limiting key enzyme in the biosynthesis of polyamines which have been confirmed to possess potent antioxidant and antiglycating properties. The aim of the present study was to examine the relationship of the *ODC* polymorphism and circulating whole blood polyamine concentrations. The study subjects were non-smoking, healthy men (n = 30) and women (n = 30) aged 55–69 years with equal numbers of AA, GA, and GG genotypes of the *ODC* gene, who were randomly recruited from 607 health checkup examinees. The whole blood polyamines (spermidine and spermine) were determined by high-performance liquid chromatography and were adjusted for hematocrit. The difference in the adjusted polyamines across the *ODC* gene was statistically tested using analysis of covariance. Women homozygous for the A-allele showed significantly higher spermidine levels than those with other genotypes. No such association was found among men, and spermine showed no association with the *ODC* genotype. In conclusion, the *ODC* polymorphism is significantly associated with whole blood polyamines in women. The *ODC* gene seems to be expressed more actively among female A-allele homozygotes than women carrying the G-allele.

Key words —— polyamine, ornithine decarboxylase, single nucleotide polymorphism, lifestyle-related disease, covariance analysis

INTRODUCTION

Two major polyamines, spermidine and spermine, both of which are widespread in all organisms, have been shown to play a significant role in the regulation of cell growth and differentiation.[1)] The key enzyme in mammalian polyamine biosynthesis is ornithine decarboxylase (ODC) which catalyzes the formation of putrescine, the precursor of spermidine and spermine, via decarboxylation of ornithine usually produced by arginase.[1–3)] In humans, the *ODC* gene is known to have a restriction fragment length polymorphism when cleaved with *Pst* I (Hickok), and two *ODC* alleles as A or G were determined depending on the base at +317 in intron I.[4,5)]

This single nucleotide polymorphism (SNP) site was identified in the human *ODC* promoter, and the association of the SNP with the risk for cancer has been extensively investigated.[5–8)] A recent study demonstrated a pronounced reduction in recurrence of colon adenoma associated with the *ODC* polymorphism,[9)] thereby hypothesizing that the A/G SNP of the *ODC* gene is a genetic marker for colon cancer risk. In contrast, no statistically significant association of advanced precancerous gastric lesions with *ODC* genotype was found in a cross-sectional study of subjects with mild chronic atrophic gastritis.[10)] While experiments using genetically-altered animals overexpressing *ODC* suggested that the *ODC* gene is involved in the development of a number of epithelial cancers,[11)] the role of the *ODC* polymorphism and

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carcinogenesis is yet to be established in humans.

Previously, the range of roles which polyamines play has been controversial.[3] Among a number of molecular functions performed by polyamines, the role as a direct free radical scavenger is critical in protecting DNA damage from reactive oxygen species.[12] More recently, polyamines were demonstrated to be potent antioxidant and anti-inflammatory agents most likely due to their prevention of superoxide generation.[13] In addition, polyamines were confirmed to have antiglycating properties by inhibiting the formation of advanced glycation end products, which appear to be linked to a number of pathophysiological processes potentially involved in the development of long-term diabetic complications.[3, 14, 15] This cumulative body of evidence suggests that polyamines might serve for risk evaluation in the etiology of chronically degenerative illnesses such as neoplasms, cardiovascular disease, and diabetic disorders in general populations.

Although *ODC* is the first and rate-limiting enzyme in the polyamine biosynthesis pathway, little is known about the relationship of the polymorphism of the gene encoding *ODC* and blood polyamine levels. Since information is not available in the literature as to whether the *ODC* genotype influences circulating polyamine levels in healthy people in the general healthy population, we conducted a cross-sectional study to examine such a relationship.

MATERIALS AND METHODS

A total of 607 residents (210 men and 397 women) living in a rural area of Hokkaido attended a health checkup program conducted in August 2005 and gave written informed consent to answering a lifestyle questionnaire and providing residual blood for *ODC* genotyping. The Hardy-Weinberg equilibrium,[16] which indicates an absence of discrepancy between genotype and allele frequency, was checked for the 607 subjects using a χ^2 test.

We recruited on a random basis 60 study subjects comprised of 30 age-matched men and 30 age-matched women. There were 10 of each *ODC* genotype in the male and female groups. Eligible subjects were 55–69 years of age who were not taking medication for lifestyle-related diseases, did not have a history of malignancy, and had quit smoking for over 5 years or had never smoked. The age- and genotype-specific distributions of subjects who met these criteria are shown in Table 1. Fasting whole blood samples were drawn in tubes containing sodium EDTA as an anticoagulant, and stored at 4°C and frozen at −85°C until being assayed for *ODC* genotyping and polyamine measurement, respectively.

Table 1. Age Distribution of Subjects by *ODC* Genotype

Age range	*ODC* genotype	Men	Women
55–59	GG	5	5
	GA	5	5
	AA	5	5
	subtotal	15	15
60–64	GG	1	1
	GA	1	1
	AA	1	1
	subtotal	3	3
65–69	GG	4	4
	GA	4	4
	AA	4	4
	subtotal	12	12

DNA was extracted from blood samples using a BioRobot EZ1 (QIAGEN Group, Tokyo, Japan). The *ODC* polymorphism was genotyped by polymerase chain reaction with confronting two-pair primers (PCR-CTPP).[17] The primers were F1: GGC CGA GCG CTC CTG CG, R1: GCT CGG CGA CCA CGG TCT CC, F2: GCC TCG CCG GCC TGC A, and R2: GCC CGG ATC ACC CTT ATC CAG C. Genomic DNA (30 ng to 100 ng) was used in a volume of 25 μl with 0.18 mM dNTPs, 25 pmol of each primer, 0.5 units of AmpliTaq Gold (Perkin-Elmer Corp., Foster City, CA, U.S.A.), 10% glycerol, and 2.5 μl GeneAmp 10 × PCR Buffer including 15 mM $MgCl_2$. The amplification condition was 5 min of initial denaturation at 95°C, followed by 30 cycles of 1 min each at 94°C (denaturation), 66°C (annealing), and 72°C (extension), and then 5 min at 72°C for the final extension. The DNA products of G-allele with a 212-bp and A-allele with a 106-bp, as well as a common band with 280-bp, were visualized on 2% agarose gels with ethidium bromide staining. For the determination of whole blood polyamine (putrescine, spermidine, and spermine) concentrations, thawed blood samples were eluted and assayed using a high-performance liquid chromatographic method based on postcolumn derivatization with o-phthalaldehyde.[18]

In preparation for statistical analysis, we divided the polyamine levels by the hematocrit (Hct)

because the polyamines circulating in blood are mostly localized in erythrocytes, whereas they are found in plasma or serum at extremely low pmol/ml ranges.[2,3] Analysis of covariance (ANCOVA) was used to test the difference in the concentrations in Hct-adjusted spermidine (Spd) and Hct-adjusted spermine (Spm) according to the 3 categories of the *ODC* genotype, with age, body mass index, and smoking habit (former, never) as covariates. For women, menopausal status (premenopausal, postmenopausal) was also added as a covariate. Furthermore, we performed tests to determine any trends by forming a linear contrast on the assumption of equal spacing between categories in the ANCOVA modeling. All statistical analyses were performed using SPSS (Chicago, IL, U.S.A.) for Windows version 11.0, and 2-sided p values < 0.05 were considered statistically significant. The study protocol was approved by the Ethics Committee of Nagoya University Graduate School of Medicine.

RESULTS

The observed frequency of the three genotypes did not deviate from the Hardy-Weinberg equilibrium ($\chi^2 = 2.6$, $p = 0.11$); 16.8% for GG, 52.2% for GA, and 31.0% for AA. Table 2 summarizes the demographic, clinical, and biochemical profiles of the subjects. Their average age was 61.2, ranging from 55–68 years in men, and 61.3, ranging from 55–68 years in women. Total cholesterol and high-density lipoprotein (HDL) cholesterol were significantly lower, and fasting plasma glucose was significantly higher in men than in women. Two women in the 55–59 age range self-reported themselves as premenopausal, and the rest as postmenopausal. The distribution of Spd and Spm among men and women is depicted in Fig. 1, demonstrating a number of outliers beyond the 5–95% ranges in both men and women. Men had significantly lower Spd and higher Spm than women. The putrescine level was much lower than 0.4 nmol/ml in all subjects.

Table 3 shows the ANCOVA results with multivariate-adjusted mean polyamine concentrations according to the *ODC* genotype. While no significant association was found in men, women showed a significant association of Spd with the genotype; this association was attributable mostly to a significant difference in the Spd levels between the AA genotype and GA genotype, but not between the GG genotype and the GA genotype, which was confirmed by Tukey's multiple comparison procedure. Furthermore, a significant trend for an increase in Spd from lowest in the G-allele homozygosity to highest in the A-allele homozygosity was confirmed in the linear contrast on the equal-spacing assumption. However, no such significant relationship was observed between the Spm or Spd/Spm ratio and the genotype among women.

Table 2. Basic Characteristics of Subjects

Characteristics	Men ($n = 30$)		Women ($n = 30$)	
	Mean	Range	Mean	Range
Age (years)	61.2	(55, 68)	61.3	(55, 68)
Body Mass Index (kg/m^2)	24.9	(21.2, 33.3)	24.4	(18.6, 29.9)
Systolic blood pressure (mmHg)	133.3	(108, 170)	138.3	(96, 188)
Diastolic blood pressure (mmHg)	83.8	(66, 108)	84.1	(60, 102)
Total cholesterol (mg/dl)	197.1	(136, 274)	214.9	(147, 298)
HDL cholesterol (mg/dl)	50.9	(35, 73)	59.4	(34, 89)
Triglyceride (mg/dl)	123.9	(38, 312)	123.8	(48, 286)
Fasting plasma glucose （mg/dl）	103.0	(83, 138)	94.8	(80, 133)
Spermidine[a] (nmol/ml)	22.5	(15.8, 31.1)	25.2	(15.9, 35.3)
Spermine[b] (nmol/ml)	12.4	(7.7, 22.8)	10.9	(7.3, 20.4)
	Percentage of subjects		Percentage of subjects	
Smoking status				
Never	36.7		93.3	
Quit for past 5 years or more	63.3		6.7	
Menopausal status				
Premenopausal	—		6.7	
Postmenopausal	—		93.3	

a) Hematocrit-adjusted whole blood spermidine concentration. *b*) Hematocrit-adjusted whole blood spermine concentration.

DISCUSSION

Previous evidence has indicated that, compared to the G-allele homozygosity, the homozygous minor A-allele of the *ODC* gene was associated with lower odds of colon adenoma recurrence, while no effect on recurrence was shown for heterozygous (GA) persons.[9] It has been suggested that either one or two G-alleles could be sufficient to facilitate *ODC* promoter activity and polyamine synthesis, whereas depressed polyamine levels were expected in the intestinal mucosa of individuals carrying two A-alleles.[9] However, no reports have previously revealed whether the intestinal polyamine contents were reflected in the levels of blood polyamines in healthy individuals. Blood polyamine concentrations increase significantly in cancer patients, and the quantification of blood polyamine levels is thought to be clinically important in monitoring the improvement of the patient's condition. In the presence of some confirmed beneficial effects of polyamines as antioxidant and antiglycating agents, we hypothesized that polyamines in the circulating blood might have potential for evaluating the risk for lifestyle-related diseases. Because the biosynthesis of polyamines is highly regulated by the key enzyme which the *ODC* gene encodes, we believed that an investigation into the correlation of the *ODC* genotype and whole blood polyamine concentrations in generally healthy persons could be of etiological importance.

The current study demonstrated a significant relationship of the AA genotype of the *ODC* gene and the whole blood Spd levels in healthy women, which we are inclined to attribute to *ODC* gene ac-

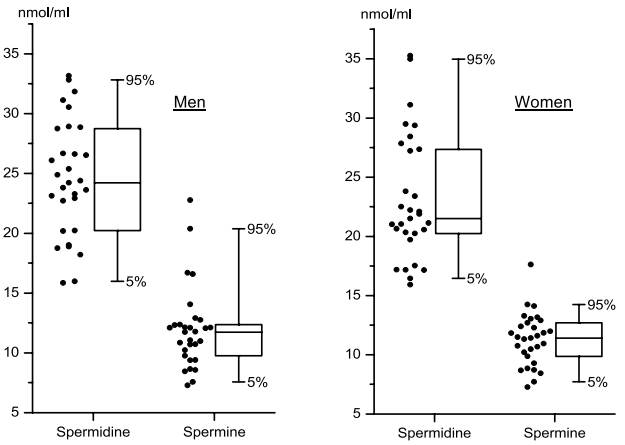

Fig. 1. Concentrations of Hematocrit-Adjusted Blood Polyamines

Table 3. Least-squares Means [a] of Polyamine Concentrations by *ODC* Genotype

ODC genotype	Men		Women	
	Mean	(95% CI)	Mean	(95% CI)
		Spd[d]		Spd[d]
GG	22.9	(19.7, 26.1)	22.0	(16.4, 27.6)
GA	22.2	(19.0, 25.3)	22.4	(16.8, 28.0)
AA	21.3	(18.0, 24.6)	27.5	(22.3, 32.6)
p for effect[b]	0.74		0.027	
p for trend[c]	0.44		0.016	
		Spm[e]		Spm[e]
GG	11.4	(9.2, 13.5)	10.1	(7.2, 13.0)
GA	12.4	(10.3, 14.5)	8.9	(5.9, 11.8)
AA	12.8	(10.6. 15.0)	10.3	(7.6, 13.0)
p for effect[b]	0.56		0.38	
p for trend[c]	0.30		0.87	
		Spd/Spm		Spd/Spm
GG	2.1	(1.7, 2.5)	2.3	(1.7, 2.8)
GA	1.8	(1.4, 2.2)	2.5	(1.9, 3.1)
AA	1.8	(1.4, 2.2)	2.6	(2.1, 3.1)
p for effect[b]	0.52		0.29	
p for trend[c]	0.33		0.12	

a) Adjusted for age, body mass index, smoking habit, and menopausal status (women only). *b*) *p* value by one-factor analysis of covariance. *c*) Test for trend was performed using a linear contrast on the assumption of equal spacing between categories from GG to AA. *d*) Hematocrit-adjusted whole blood spermidine concentration. *e*) Hematocrit-adjusted whole blood spermine concentration.

tivation associated with the homozygous A-allele. To date, the *ODC* gene is known to be one of the transcriptional targets of *c-myc*, which binds to specific *myc*-binding elements, known as E-boxes, of genes affecting both proliferation and apoptosis.[19] It is suggested that, for a given level of *myc* expression, the transcriptional activation of the *ODC* gene depends on the *ODC* genotype in a way that the AA genotype is associated with more *ODC* mRNA expression than the GG genotype.[5]

With respect to colon adenoma, the variant *ODC* A-allele was found to be associated with a lower risk for adenoma recurrence in a previous report, implying reduced polyamine synthesis in the intestinal mucosa of A-allele homozygous adenoma patients.[9] In our results, however, *ODC* AA genotype demonstrated higher Spd than the other two genotypes among women, likely reflecting a prompted biosynthesis of polyamines. This inconsistency might be partly attributable to the difference in the pathological conditions of the study subjects; though stimulation of tumor tissue ODC is responsible for the increase in polyamines in neoplasms, the eligibility criteria for subject selection precluded those who had a history of diseases likely to affect polyamine metabolism. Consequently, the polyamine concentrations determined in our subjects were mostly distributed in the physiological range, though the Spd levels appeared to deviate toward relatively high values. It is likely that, depending on the pathophysiological conditions, the *ODC* expression associated with the A-allele homogeneity varies in the opposite directions; underexpression in the neoplastic tissues where polyamine synthesis is highly activated, and overexpression under physiological conditions, both of which are beneficial in terms of the disease vulnerability of individuals homozygous for the A-allele.

It is believed that postmenopausal healthy women are more susceptible to the risks of lifestyle-related diseases than premenopausal ones, and thus physiologically depend on a preventive mechanism in place of estrogen. We hypothesized that women homozygous for the A-allele are likely to express the *ODC* gene more actively than women carrying the G-allele in response to exposure to those risks and that they would therefore benefit from the antioxidant or antiglycating effects of spermidine. The reason for no significant difference in Spm across the *ODC* genotype in women is unclear. Spermidine is known to be a poor inhibitor of monocyte cytokine synthesis as compared to spermine,[20] implying that the anti-inflammatory effect of spermidine is less inhibitory in the circulating blood of healthy individuals. As spermidine is the precursor of spermine in the polyamine biosynthetic pathway, higher concentrations of spermidine in circulating blood associated with the A-allele homozygosity might be readily catalyzed to spermine when exposed to environmental risk factors in order to perform potent physiological functions such as free radical scavenging.

Limitations of our study include the lack of data on ODC activity. Thus far, however, no assay method has been established for measurement of ODC in blood samples, whereas tissue-based experimental measurements of ODC have been reported in previous publications.[21,22] Moreover, we believe that the measurement of ODC is of little relevance to the current study considering its short half-life, which is less than 20 min.[1,23] Second, the statistical power of such a small sample size might be the cause of some concern. In addition, the randomization for subject selection to reflect balanced genotype proportions as used in this study did not represent the population genotype distribution, which might hinder the generalization of the results. Lastly, the cross-sectional design of this study indicated only limited public health significance; although our results indicated that healthy postmenopausal women homozygous for the A-allele had more activated *ODC* gene expression, the consequence of the *ODC* polymorphism in terms of the development of lifestyle-related diseases should be corroborated in a longitudinal observation. In another study of healthy male workers, we recently found a significantly high concentration of whole blood polyamines in the presence of low HDL cholesterol levels, high white blood cell counts, or high hemoglobin A1c levels,[24] suggesting the secondary elevation of circulating polyamines to quench the oxidative stress contributing to the evolution of lifestyle-related diseases. With an appropriate study using a cohort design, the relationship of the *ODC* genotype and the susceptibility to those diseases can be addressed.

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REFERENCES

1) Teti, D., Visalli, M. and McNair, H. (2002) Analysis of polyamines as markers of (patho)physiological conditions. *J. Chromatogr. B*, **781**, 107–149.
2) Zhang, M., Wang, H. and Tracey, K. J. (2000) Regulation of macrophage activation and inflammation by spermine: a new chapter in an old story. *Crit. Care Med.*, **28**, N60–N66.
3) Gugliucci, A. (2004) Polyamines as clinical laboratory tools. *Clin. Chim. Acta*, **344**, 23–35.
4) Hickok, N. J., Seppanen, P. J., Gunsalus, G. L. and Janne, O. A. (1987) Complete amino acid sequence of human ornithine decarboxylase deduced from complementary 11 DNA. *DNA*, **6**, 179–187.
5) Guo, Y., Harris, R. B., Rosson, D., Boorman, D. and O'Brien, T. G. (2000) Functional analysis of human ornithine decarboxylase alleles. *Cancer Res.*, **60**, 6314–6317.
6) Matsubara, N., Yoshitaka, T., Hanafusa, H., Tanaka, N. and Shimizu, K. (2002) Genetic alterations of the ornithine decarboxylase gene in human colorectal cancers. *J. Exp. Clin. Cancer Res.*, **21**, 191–195.
7) Visvanathan, K., Helzlsouer, K. J., Boorman, D. W., Strickland, P. T., Hoffman, S. C., Comstock, G. W., O'Brien, T. G. and Guo, Y. (2004) Association among an ornithine decarboxylase polymorphism, androgen receptor gene (CAG) repeat length and prostate cancer risk. *J. Urol.*, **171**, 652–655.
8) Gerner, E. W., Ignatenko, N. A., Lance, P. and Hurley, L. H. (2005) A comprehensive strategy to combat colon cancer targeting the adenomatous polyposis coli tumor suppressor gene. *Ann. N. Y. Acad. Sci.*, **1059**, 97–105.
9) Martinez, M. E., O'Brien, T. G., Fultz, K. E., Babbar, N., Yerushalmi, H., Qu, N., Guo, Y., Boorman, D., Einspahr, J., Alberts, D.S. and Gerner, E. W. (2003) Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 7859–7864.
10) You, W. C., Hong, J. Y., Zhang, L., Pan, K. F., Pee, D., Li, J. Y., Ma, J. L., Rothman, N., Caporaso, N., Fraumeni, J. F., Jr., Xu, G. W. and Gail, M. H. (2005) Genetic polymorphisms of CYP2E1, GSTT1, GSTP1, GSTM1, ALDH2, and *ODC* and the risk of advanced precancerous gastric lesions in a Chinese population. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 451–458.
11) O'Brien, T. G., Megosh, L. C., Gilliard, G. and Soler, A.P. (1997) Ornithine 12 decarboxylase overexpression is a sufficient condition for tumor promotion in mouse skin. *Cancer Res.*, **57**, 2630–2637.
12) Ha, H. C., Sirisoma, N. S., Kuppusamy, P., Zweier, J. L., Woster, P. M. and Casero, R. A. (1998) The natural polyamine spermine functions directly as a free radical scavenger. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 11140–11145.
13) Farriol, M., Segovia-Silvestre, T., Venereo, Y. and Orta, X. (2003) Antioxidant effect of polyamines on erythrocyte cell membrane lipoperoxidation after free-radical damage. *Phytother. Res.*, **17**, 44–47.
14) Gugliucci, A. and Menini, T. (2003) The polyamines spermine and spermidine protect proteins from structural and functional damage by AGE precursors: a new role for old molecules? *Life Sci.*, **72**, 2603–2616.
15) Mendez, J. D. and Leal, L. I. (2004) Inhibition of in vitro pyrraline formation by L-arginine and polyamines. *Biomed. Pharmacother.*, **58**, 598–604.
16) Hardy, G. H. (1908) Mendelian proportions in a mixed population. *Science*, **28**, 49–50.
17) Hamajima, N., Saito, T., Matsuo, K., Kozaki, K., Takahashi, T. and Tajima, K. (2000) Polymerase chain reaction with confronting two-pair primers for polymorphism genotyping. *Jpn. J. Cancer Res.*, **91**, 865–868.
18) Fujita, K., Nagatsu, T. and Shinpo, K. (1983) Assay methods for polyamines. In *Methods in Biogenic Amine Research* (Pravez, S., Nagatsu, T., Nagatsu, I. and Pravez, H., Eds.), Elsevier, Amsterdam, pp.741–776.
19) Bello-Fernandez, C., Packham, G. and Cleveland, J. L. (1993) The ornithine decarboxylase gene is a transcriptional target of c-Myc. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 7804–7808.
20) Zhang, M., Caragine, T., Wang, H., Cohen, P. S., Botchkina, G., Soda, K., Bianchi, M., Ulrich, P., Cerami, A., Sherry, B. and Tracey, K. J. (1997) Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: a counterregulatory mechanism that restrains the immune response. *J. Exp. Med.*, **185**, 1759–1768.
21) Ngo, T. T., Brillhart, K. L., Davis, R. H., Wong, R. C., Bovaird, J. H., Digangi, J. J., Ristow, J. L., Marsh, J. L., Phan, A. P. and Lenhoff, H. M. (1987) Spectrophotometric assay for ornithine decarboxylase. *Anal. Biochem.*, **160**, 290–293.
22) Alhonen, L., Rasanen, T. L, Sinervirta, R., Parkkinen, J. J., Korhonen, V. P., Pietila, M. and Janne, J. (2002) Polyamines are required for the initiation of rat liver regeneration. *Biochem. J.*, **362**, 149–153.
23) Chabanon, H., Aubel, C., Larvaron, P., Villard, C., Carraro, V. and Brachet, P. (2005) Ornithine decarboxylase activity is inhibited by the polyamine pre-

cursor amino acids at the protein stability level in Caco-2 cells. *Biochim. Biophys. Acta*, **1723**, 74–81.
24) Kondo, T., Imai, R., Suzuki, T., Takagi, K., Hori, Y., Yatsuya, H., Tamakoshi, K. and Toyoshima, H. (2006) Relationship of blood polyamine concentrations with the risk factors of lifestyle-related diseases in a healthy population. *J. Epidemiol.*, **16** (Suppl.), 143.