Original article

A study of the role of DIO1 and DIO2 polymorphism in thyroid cancer and drug response to therapy in the Saudi population

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ABSTRACT

Background: Deiodinases comprise a group of selenoproteins that regulate the bioavailability of active thyroid hormones (TH) in a time and tissue specific fashion. They increase the hormonal activity by metabolizing their inactive precursors to active forms or terminate their activity by deactivating active hormones. The role of the deiodinase (DIO) gene polymorphisms in thyroid cancer is not fully understood yet. This study evaluated the potential association of the DIO1 and DIO2 genes with differentiated thyroid cancer and differential thyroxine dose requirement in thyroidectomized patients in a Saudi cohort.

Methods: We selected four variants (one DIO1 and three DIO2) for the association studies using Taqman assays in 507 DTC patients undergoing treatment with thyroxin against 560 disease-free individual, all of Saudi Arab origin.

Results: None of the studied variants was linked to differentiated thyroid cancer. The rs1388378 G > T was initially linked to thyroxine dose requirement (p = 0.035) when all patients were considered together, but this association was lost when the patients were classified into either near suppressed (0.1 < TSH < 0.5) or suppressed (TSH < 0.1) TSH group.

Discussion: Although the results suggest only a weak relationship with differentiated thyroid cancer, they strongly indicate that the DIO2 polymorphism influences the hormonal dose requirement in patients undergoing treatment with thyroxine. This probably points to a distinction in the way this gene influences disease as compared to therapy thereof.

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1. Introduction

Deiodinase (DIOs) are a family of selenoproteins that regulate the bioavailability of active thyroid hormones (THs) in a time and tissue specific manner (Arrojo et al., 2013; Bianco, 2011). These enzymes stimulate the hormone activity by metabolizing inactive precursors to their active forms, and reduce their activity by deactivating active hormones. The role of the deiodinase (DIO) gene polymorphisms in thyroid cancer is not fully understood yet. This study evaluated the potential association of the DIO1 and DIO2 genes with differentiated thyroid cancer and differential thyroxine dose requirement in thyroidectomized patients in a Saudi cohort.

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Abbreviations: DIO (1,2,3), deiodinase (1,2,3) gene; D (1, 2), deiodinase (1,2) protein; FT4, free thyroxin; TH, thyroid hormone; TSH, thyroid-stimulating hormone-b; T3, triiodothyronine; T4, tetraiodothyronine; UGT1A, UDP glucuronosyltransferase family 1 member A; WSB-1, WB repeat and SOCs box-containing; UDP, uridine phosphorylase.

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well-being in hypothyroid patients (Young Cho et al., 2017), while a number of studies have recently pointed to the probability of DIO2 polymorphism influencing FT4 metabolism and function (Hoftijzer et al., 2011; Santoro et al., 2014; Torlontano et al., 2008). Thus, a reduction in the negative feedback of FT4 on TSH has been linked to homozygosity for the D2-rs12885300 T allele compared to either the heterozygotes or the wild-types (Hoftijzer et al., 2011), while the need for higher T4 intake in thyroidectomized patients has been linked to its Thr92Ala polymorphism (Torlontano et al., 2008). Put together, these findings point to the likelihood that genetic alterations in DIO2 may predict the T4 requirement to suppress TSH. In this study, we therefore elected to evaluate the role of the DIO1 and DIO2 in differentiated thyroid cancer manifestation and possible involvement in the adjustment requirement in treating thyroidectomized patients with the thyroxine (T4).

2. Materials and methods

2.1. Study patients and blood sampling

The study included a total 1067 Saudi individuals comprising 507 patients with differentiated thyroid cancer (DTC) and 560 disease-free controls of Saudi origin. Candidate patients had undergone total thyroidectomy, received radioiodine ablation and were on L-thyroxine suppressive therapy (Euthyrox, Merck Pharmaceuticals, NJ, USA). This therapy aimed to attain either suppressed (TSH < 0.1 mU/L) or near-suppressed (0.1 ≤ TSH < 0.5 mU/L) serum TSH levels with FT4 in the normal range (12–22 pmol/L). The study cases comprised 97.8% papillary thyroid cancer (PTC), 88.9% classic subtype, 9.1% follicular variant PTC, 1.2% tall cell variant, 0.4% diffuse sclerosing subtype, 0.4% insular subtype and 2.2% follicular thyroid cancer. Furthermore, 29.6% of the PTC patients presented with positive family history of the disease. Healthy controls were recruited from the general population and known not to have thyroid disorders, family history of thyroid cancer or to have been previously exposed to therapeutic levels of external radiation. The cases did not differ significantly from the healthy controls in the important confounding variables, such as age and body-mass index (Table 1).

Excluded from the study group were individuals on multiple drug treatment, or those who would have changed the thyroxine brand 3 months prior to the launching of the study. We also excluded patients who might have been on drugs that could potentially interfere with thyroxine treatment. These included (a) anti-epileptics and bile acid resins, (b) thyroid suppressors and other drugs that may alter thyroid hormone metabolism or production, and (c) medicines that could affect the pituitary-thyroid axis. Additionally, expectant females, individuals with mental illness, other types of cancer, as well as those having significant renal impairment (glomerular filtration rate < 60 ml/min) or chronic liver disease were also excluded. Compliance was determined through a questionnaire targeting each subject’s medical history, medication use, smoking, as well as measuring the TSH and FT4 levels.

2.2. DNA extraction

Genomic DNA was isolated according to the manufacturer’s protocol (Centra Puregene, Qiagen Sciences, Maryland, USA) from 5 ml peripheral blood drawn from each study individual into 6 ml vacutainer tubes containing K2EDTA (1 Becton Drive, Franklin Lakes, NJ USA). Briefly, 3 ml of blood were added to 9 ml red blood cell lysis buffer and incubated under continuous mixing for 5 min. The mixture was centrifuged at 2000g for 2 min, the leucocyte pellet re-suspended and vortexed in 3 ml cell lysis buffer solution and proteins were precipitated for 20 s by centrifuging at 2000g for 5 min. The supernatant was mixed with 3 ml isopropanol in a 15 ml tube and genomic DNA precipitated gently. The tube was then centrifuged at 2000g for 3 min, the supernatant discarded, and the DNA pellet washed twice in 3 ml of 75% ethanol. The pellet was air-dried, dissolved in 250 μl hydration solution at 65 °C for 1 h, quantified by Nanodrop ND-1000 spectrophotometer (Wilmington, DE, USA), aliquoted into 50 μl portions and stored at –20 °C.

2.3. Association studies

The study comprised 507 cases and 560 controls. Primers and TaqMan probes were designed using the Primer Express software V2.0 (Applied Biosystems, Foster City, CA, USA) and procured from Applied Biosystems (Foster City, CA 94404, USA). Genotyping was accomplished by real-time PCR using Taqman chemistry on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc., CA, USA). The fluorogenic probes bearing a suitable reporter dye on the 5′-end and a quencher dye on the 3′-end hybridized to the specific complementary sequence bearing the SNP of interest. Two probes were designed, one labeled with VIC dye and the other with FAM dye at the 5′-primer end. Accordingly, in the process of the primer extension and synthesis of the nascent strand by

| Table 1 |
| --- |
| Demographic data for the individuals in the population-based association study for cancer risk. |
| | Patients | Controls |
| | All (507) | Male (92) | Female (415) | All (560) | Male (159) | Female (401) |
| Age (years) | 45.6 ± 15.0 | 47.2 ± 15.2 | 44.6 ± 12.7 | 45.6 ± 15.2 | 52.2 ± 15.8 | 43.0 ± 14.2 |
| BMI | 30.3 ± 6.6 | 28.1 ± 5.7 | 30.8 ± 6.6 | 29.3 ± 6.7 | 28.6 ± 6.0 | 30.0 ± 7.0 |
| Smoking | 36 (7.1%) | 33 (35.6%) | 3 (0.7%) | 84 (15%) | 60 (37.7%) | 24 (6.0%) |

There was no significant different between the two gender in both age and body mass index. However, there significantly more smokers among the male compared to the females in both the case and controls groups. Age and body mass index (BMI) are given as mean ± standard deviation.
Taq polymerase, the annealed probe is cleaved through exonuclease activity to facilitate the fluorescence emission by releasing the reporter dye from its proximity to the quencher. Optimal working probe concentrations were established by running serial dilutions. A 25 μl master-mix was each prepared by mixing 5 μl of 50 ng DNA, 12.5 μl of 2x Universal mix (Eurogentec, Liege Science Park, 4102 Seraing, Belgium), 1.25 μl of 20x probe assay mix in 6.25 μl DNase-free distilled water. Three controls (without a template) were included in each 96-well plate to normalize the emission signal. For the first cycle, the amplification profile for was set at 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 94 °C for 15 sec and 60 °C for 30 sec. The plates were then scanned for FRET signal using the 7900HT sequence detection system and data analyzed using SDS 2.0 software (Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analysis

Categorical variables were analyzed by Chi-Square test. Genotype and allele comparison between different groups for continuous dependent variables was accomplished by Analysis of Variance (ANOVA) or Student’s t-test as appropriate, and categorical variables were analyzed by Chi-Square test. Odds ratios and their 95% confidence intervals were computed by logistic regression analysis. Positive associations were entered into multivariate regression analysis with other conventional risk factors for thyroid cancer including age, sex, smoking as predictive variables for the risk of DTC. All other statistical analyses were performed using the SPSS software version 24 (SPSS Inc., Chicago, USA), and data are expressed as mean ± SEM. Associations with a two-tailed p value < 0.05 was considered statistically significant.

3. Results

3.1. Association of DIOs with thyroid cancer

In all one DIO1 SNP rs225013_G > T and three DIO2 SNPs (rs2294512_A > G, rs1388378_G > T, and rs12885300_C > T) were studied for their association with the disease. The schematic representations of the two genes are given in Figs. 1 and 2. The demographic data of the individuals involved in association analysis are displayed in Table 1. None of the three variants was related in any fashion with thyroid cancer (Table 2).

3.2. Association of DIOs with thyroxin dose requirement

Analysis of the relationship between thyroxine dose requirement and patient response to the therapy was evaluated in 453 DTC patients. The demographic data of individuals involved in the thyroxine dose are displayed in Table 3. The same four variants rs2294512_A > G, rs1388378_G > T, rs12885300_C > T were studied for their possible influence on the variability in thyroxine dose requirement. Initially, the analysis was performed for the whole patient (ALL) group. The results indicated an association for rs1388378_G > T (p = 0.035) with dose adjustment requirement. The patients were then grouped into the near suppressed (NS; 0.1 ≤ TSH < 0.5) and suppressed (SG; TSH < 0.1) TSH categories. The rs1388378 lost its association. Furthermore, no significant deviation from the normal doses required for therapy could be established for any of the other variants within a group (Table 4).

4. Discussion

The present study evaluated the potential role of the DIO1 and DIO2 gene polymorphisms in thyroid cancer disease as well as in explaining the differences in thyroxin dose requirement by patients undergoing treatment for differentiated thyroid cancer. In all, we elected to investigate one DIO1 and three DIO2 variants. None of these SNPs showed any association with the disease. To date, while polymorphisms of deiodinases have been described for some malignancies and several other forms of the diseases, information on their possible involvement in thyroid cancer remains scanty. Specifically, the DIO2 gene has been associated with diseases such autoimmune hypothyroidism disease (Carle et al., 2017), maternal thyroid status (He et al., 2009), grave’s disease (Inoue et al., 2018), drinking behavior (Lee et al., 2015), cognitive impairment (Luo et al., 2015), osteoarthritis (Waarsing et al., 2011; Meulenbelt et al., 2008), but not with thyroid cancer. On the other hand, it is widely acknowledged that, since serum TSH is a sensitive indicator of thyroid function, overt abnormalities in

Fig. 1. The figure represents a schematic diagram of the DIO1 (not to scale) showing the SNPs studied and their chromosomal loci.
this function lead to common endocrine disorders affecting a sizeable number of individuals over a life span. These malfunctions are likely to underlie genetic defects. Of the SNPs discussed herein, the rs2294512_A > G of the DIO1 gene has been found to interact with serum selenium in Crohn patients (Gentschew et al., 2012) and reduced psychological well-being in hypothyroid patients (Young Cho et al., 2017), while the rs1388378_G > T has been linked to mental retardation in the Chinese population (Zhang et al., 2012). Hence, although we were not able to establish any positive relationship for the SNPs with disease per se, genetic alterations in the TSH pathways are likely to play an important role in thyroid cancer manifestation.

One important subject of interest of the present study was the likelihood that changes in the DioS may explain why DTC patients tend to respond in various fashions to the therapy with thyroid hormones, with some patients requiring thyroxine dose adjustments. When we looked at the cases as a whole, we were able to link these differences to rs1388378_G > T. However, when patients were classified as being in either the near-suppressed or in the suppressed group, this variant lost its link with dose requirement in ether category. Like in the disease manifestation, there is currently lack of information on the role of the DioS in differential response to hormonal therapy of thyroid cancer. On the other hand, the observation that D2 metabolizes T4 into T3 and reverse rT3 into T2, while D1 functions as a scavenger by removing iodo groups means that functional changes in either of the encoding genes is likely to trigger perturbations in the hormone metabolism. To date the genes have been linked to TSH suppression (Santoro et al., 2014), shift in pattern of secretion of thyroid hormone (Peltsverger et al., 2012) and TRH-mediated acute rise in TSH (Luo et al., 2015). Among the variants discussed in the present paper, the rs12885300 is perhaps the most well-studied. It has been linked to TSH suppression (Santoro et al., 2014), TRH-

Table 2
Association of DIO1 and DIO2 variants with differentiated thyroid cancer risk.

|   |   |   |   |
|---|---|---|---|
| Gene | SNP ID | Genotypes | Patients f(%) | Control f(%) | P | OR(95%CI) |
| DIO1 | rs2294512_A > G | A | 252 (25.0) | 285 (25.7) | 0.701 | 0.962(0.791–1.171) |
| | | G | 758 (75.0) | 825 (74.3) | 0.872 | 0.980(0.768–1.252) |
| DIO2 | rs1388378_G > T | G | 854 (85.4) | 925 (85.6) | 0.096 | 0.850(0.702–1.030) |
| | | T | 146 (14.6) | 155 (14.4) | 0.799 | 0.978(0.822–1.163) |
| DIO2 | rs12885300_C > T | C | 659 (68.2) | 735 (71.6) | 0.096 | 0.850(0.702–1.030) |
| | | T | 307 (31.8) | 291 (28.4) | 0.799 | 0.978(0.822–1.163) |
| DIO2 | rs225013_G > T | G | 409 (40.5) | 458 (41.0) | 0.096 | 0.850(0.702–1.030) |
| | | T | 601 (59.5) | 658 (59.0) | 0.799 | 0.978(0.822–1.163) |

The table compares the real-time PCR data for the 507 studied DTC patients versus 560 healthy controls. SNP ID gives the single nucleotide identification number denoted with the “rs” nomenclature. f(%) gives the frequencies of the genotypes of alleles as a percentage. The letters A, C, G and T are the codes for the nucleotides adenine, cytosine, guanine and thymine respectively. OR, odds ratio; CI, confidence Interval; NS, non-significant; P, P value. Each SNP was entered in a multivariate analysis including age, sex, and smoking.

Table 3
Demographics data of individuals involved in the thyroxine dose association study.

|   | All | Male | Female |
|---|-----|------|--------|
| N | 453 | 371/453(81.9%) | 371/453(81.9%) |
| AGE (years) | 45.57 ± 12.90 | 47.74 ± 14.57 | 45.09 ± 12.47 |
| BMI | 30.34 ± 6.57 | 28.11 ± 5.72 | 30.84 ± 6.65 |
| TSH (mIU/l) | 0.16 ± 0.44 | 0.21 ± 0.46 | 0.15 ± 0.43 |
| FT4 (pmol/l) | 20.70 ± 1.70 | 20.69 ± 1.82 | 20.70 ± 1.67 |
| L-T4dose (µg/kg) | 2.05 ± 0.45 | 2.09 ± 0.51 | 2.04 ± 0.44 |

N, number of individuals in the group; BMI, body mass index; TSH, thyroid stimulating hormone; FT4, free thyroxine level; L-T4, Dose is given as µg/kg.
mediated acute rise in TSH (Luo et al., 2015), a shift in pattern of secretion of thyroid hormone (Peltsverger et al., 2012) and TRH-mediated acute rise in TSH (Luo et al., 2015). Specifically, structural alterations in the DIO2 have been implicated in differential response to disease therapy (He et al., 2009). One study suggested that negative feedback of FT4 on TSH receptor is weaker in patients homozygous for the D2-rs12885300 T allele than in wild-type and heterozygous subjects (Hoftijzer et al., 2011). Furthermore, the Thr92Ala polymorphism was suggested to predict the need for higher T4 intake in thyroidectomized patients (Torlontano et al., 2008). Besides, UGT1A polymorphism has been associated with T4 dosage in DTC patients, but the effect appeared to account for only a very small proportion of individuals (Santoro et al., 2014). Ubiquitination is an essential step in the control of D2 activity by triggering its inactivation through T4 binding to and/or T4 signaling. Biochim. Biophys. Acta 1830, 3956–3964

Table 4

| Gene | SNP ID | Geno-types | All (TSH < 4.3) | P | NGS 0.1 ≤ TSH < 0.5 | P | SC(TSH < 0.1) | P |
|------|--------|------------|----------------|---|---------------------|---|----------------|---|
| DIO1 | rs2294512_A > G | A | 245 | 150.7 ± 32.3 | 0.78 | 59 | 149.4 ± 34.3 | 0.92 | 172 | 151.0 ± 31.0 | 0.78 |
|      |         | G | 735 | 151.4 ± 33.4 | 213 | 149.9 ± 33.1 | 466 | 151.8 ± 32.5 |
|      |         | rs1388378_G > T | 828 | 152.2 ± 33.1 | 0.035 | 229 | 150.9 ± 33.1 | 0.24 | 535 | 152.4 ± 32.2 | 0.17 |
|      |         | T | 144 | 145.9 ± 33.3 | 35 | 143.7 ± 36.7 | 103 | 147.6 ± 31.5 |
| DIO2 | rs12885300_C > T | C | 644 | 152.2 ± 32.8 | 0.29 | 183 | 151.3 ± 34.9 | 0.097 | 417 | 152.5 ± 31.6 | 0.76 |
|      |         | T | 296 | 149.8 ± 34.0 | 75 | 143.7 ± 29.1 | 195 | 151.3 ± 33.6 |
|      |         | rs225013_G > T | G | 399 | 151.6 ± 33.6 | 0.82 | 116 | 149.8 ± 35.7 | 0.92 | 255 | 152.1 ± 31.7 | 0.78 |
|      |         | T | 581 | 151.2 ± 32.8 | 154 | 150.2 ± 31.6 | 385 | 151.4 ± 32.3 |

5. Conclusion

Although it remains unclear as to whether DIO polymorphism exerts direct impact on thyroid cancer disease manifestation, it can be speculated that DIO2 plays an important role in determining thyroxine dose requirement in patients under hormonal therapy for differentiated thyroid cancer, which calls for further investigation to discern this link more clearly.

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References

Drigo, E., Arrojo, R., Fonseca, T.L., Werneck-de-Castro, J.P., Bianco, A.C., 2013. Role of the type 2 iodothyronine deiodinase (D2) in the control of thyroid hormone signaling. Biochim. Biophys. Acta 1830, 3956–3964.

Bianco, A.C., Kim, B.W., 2006. Deiodinases: implications of the local control of thyroid hormone action. J. Clin. Invest. 116, 2571–2579.

Carle, A., Faber, J., Steffensen, R., Lauberg, P., Nygaard, B., 2017. Hypothyroid patients encoded combined MCT10 and DIO2 gene polymorphisms may prefer LT4 over L-T4 combination therapy – data using a blind, randomized, clinical study. Eur. Thyroid J. 6, 143–151.

Darras, V.M., Houbrechts, A.M., Van Herck, S.L., 2015. Intracellular thyroid hormone metabolism as a local regulator of nuclear thyroid hormone receptor-mediated impacts on vertebrate development. Biochem. Biophys. Acta 1849, 130–141.

Gentzschew, L., Bishop, K.S., Han, D.Y., Morgan, A.R., Fraser, A.G., Lam, W.J., Karunasinghe, N., Campbell, B., Ferguson, L.R., 2012. Selenium, selenoprotein gene and Cohnheim’s disease in case-control population from Auckland, New Zealand. Nutrients 4, 1247–1259.

He, B., Li, J., Wang, G., Ju, W., Lu, Y., Shi, Y., He, L., Zhong, N., 2009. Association of genetic polymorphisms in the type II deiodinase gene with bipolar disorder in a subset of Chinese population. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 986–990.

Hoftijzer, H.C., Heemstra, K.A., Visser, T.J., le Cessie, S., Peeters, R.P., Corssmit, E.P., Smit, J.W., 2011. The type 2 deiodinase ORFa-GLY3Atp polymorphism (rs12885300) influences the set point of the hypothalamus-pituitary-thyroid axis in patients treated for differentiated thyroid carcinoma. J. Clin. Endocrinol. Metab. 96, E1527–E1533.

Inoue, N., Watanabe, M., Katsumata, Y., Ishido, N., Hidaka, Y., Iwata, Y., 2018. Functional polymorphisms of the type 1 and type 2 iodothyronine deiodinase genes in autoimmune thyroid diseases. Immunol. Invest. 47, 534–542.

Lee, M.R., Schwandt, M.L., Bollinger, J.W., Dias, A.A., Oet, E.N., Goldman, D., Hodgkinson, C.A., Leggio, L., 2015. Effect of functionally significant deiodinase single nucleotide polymorphisms on drinking behavior in alcohol dependence: an exploratory investigation. Alcohol. Clin. Exp. Res. Exp. Res. 39, 1665–1670.

Luo, M., Zhou, X.H., Zou, T., Keyim, K., Dong, L.M., 2015. Type II deiodinase polymorphisms and serum thyroid hormone levels in patients with mild cognitive impairment. Genet. Mol. Res. 14, 5407–5416.

Meulenbelt, I., Min, J.L., Bos, S., Ryuazi, N., Hovingh-Duistermat, J.J., van der Wijk, H.J., Kroon, H.M., Nakajima, M., Ikekawa, S., Uitterlinden, A.G., van Meurs, J.B., van der Deure, W.M., Visser, T.J., Seymour, A.B., Lakenberg, N., van der Breggen, R., Kremer, D., van Bergeijk, G.M., Kloppejnen, M., Slagboom, P.E., 2008. Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. Hum. Mol. Genet. 17, 1867–1875.

Peltsverger, M.Y., Butler, P.W., Alberobello, A.T., Smith, G., Guevara, Y., Dubox, O.M., Luzon, J.A., Linderman, J., Coli, F.S., 2012. The -258A/G (SNP rs12885300) polymorphism of the human type II deiodinase gene is associated with a shift in the pattern of secretion of thyroid hormones following a TRH-induced acute rise in TSH. Eur. J. Endocrinol. 166, 639–645.

Santoro, A.B., Vargens, D.D., Barros Filho Mde, C., Bulzico, D.A., Kowalski, L.P., Santoro, A.B., Vargens, D.D., Barros Filho Mde, C., Bulzico, D.A., Kowalski, L.P., 2015. The type 2 deiodinase ORFa-Gly3Asp polymorphism of the human type II deiodinase gene influences the set point of the hypothalamus-pituitary-thyroid axis in patients treated for differentiated thyroid carcinoma. J. Clin. Endocrinol. Metab. 96, E1527–E1533.

Young Cho, Y., Jeong Kim, H., Won Jang, H., Hyuk Kim, T., Ki, C.S., Wook Kim, S., Hoon Chung, J., 2017. The relationship of 19 functional polymorphisms in iodothyronine deiodinase and psychological well-being in hypothyroid patients. Endocrine 57, 115–124.

Zhang, K., Xi, H., Wang, X., Guo, Y., Huang, S., Zheng, Z., Zhang, F., Cao, X., 2012. A family-based association study of DIO2 and children mental retardation in the Qinba region of China. J. Hum. Genet. 57, 14–17.