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| タイトル<br>Title | Natural course and characteristics of cutaneous neurofibromas in neurofibromatosis 1 |
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| 掲載誌・巻号・ページ<br>Citation | JOURNAL OF DERMATOLOGY , 45 (1) : 53 - 57 |
| 刊行日<br>Issue Date | 2018 |
| 資源タイプ<br>Resource Type | 学術雑誌論文 / Journal Article |
| 版区分<br>Resource Version | 著者版 / Author |
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| DOI | 10.1111/1346-8138.14025 |
| URL | https://repository.lib.tottori-u.ac.jp/8439 |

**Title: Natural course and characteristics of cutaneous neurofibromas in neurofibromatosis 1.**

Short title: Cutaneous neurofibroma in NF1

The total number of words 1685, figures 6 (1, 2, 3a, 3b, 4, and 5) and tables Ⅰ and references 19.

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Conflicts of interest: The authors have no conflict of interest to declare.

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(Key words) neurofibromatosis 1, NF1, cutaneous neurofibroma, distribution, predilection body site

Funding source: This work was partly supported by Health Labour Sciences Research Grant (Y.Y) from the Ministry of Health, Labour and Welfare and the Platform Project for supporting in Drug Discovery and Life Science Research from the Japan Agency for Medical Research and Development (Y.Y and K.K) and Tottori University Faculty of Medicine Alumni Association (Y.E).

**ABSTRACT**

Neurofibromatosis 1 (NF1) is characterized by cutaneous, neurological and osseous manifestations. Most NF1 patients develop cutaneous neurofibromas. However, time-dependent change with aging and the predilection site of cutaneous neurofibromas remain unclear. To clarify the natural course and characteristics of cutaneous neurofibromas, a retrospective study was conducted for 57 NF1 patients who were treated at the Department of Dermatology of Tottori University Hospital between January 2007 and April 2016. For each patient, we investigated the time-dependent changes and the numbers of cutaneous neurofibromas in four body surface regions. There was a positive correlation between age and number of cutaneous neurofibromas ($r = 0.75$, $p < 0.001$). Cutaneous neurofibromas were located on the trunk (60.2%), lower limbs (16.1%), upper limbs (14.4%), and head and neck (9.2%). There was no significant relationship between each body type (e.g., obese or thin) and cutaneous neurofibromas. With respect to the year-to-year percentage change in cutaneous neurofibromas, the average annual rate of increase was 0.21 (range, −0.71-1.2). The number of cutaneous neurofibromas had increased in about 61% of the patients one year later. Our data will enable physicians to estimate the overall state of cutaneous neurofibromas in NF1 and will be useful for handling cutaneous manifestations before

they become a serious condition.

**Key words**: cutaneous neurofibroma, distribution, neurofibromatosis 1, NF1, predilection body site

## Introduction

Neurofibromatosis 1 (NF1) is an autosomal dominant genetic disease characterized by café-au-lait spots, neurofibromas, freckling, optic gliomas, Lisch nodules and bone deformity.[1-3] Cutaneous neurofibromas are soft, dome-shaped and flesh-colored nodules.[2-4] NF1 patients with cutaneous neurofibromas have serious cosmetic and social problems.[5,6] Generally, the number of cutaneous neurofibromas increases with aging.[7] However, natural course and characteristics of cutaneous neurofibromas remain unclear. The purpose of this study was to clarify the time-dependent changes and the predilection site of cutaneous neurofibromas in NF1.

## Patients and methods

### Study population (Patients)

A retrospective study was conducted. We investigated 57 NF1 patients (25 men and 32 women [no pregnancy]; median age, 34 years; age range, 0-79 years) at the Dermatology Department of Tottori University Hospital over a 9-year period from January 2007 to April 2016.

All of the patients were evaluated by expert dermatologists and met the diagnosis criteria by National Institutes of Health in 1988.[1,8] Mosaic NF1 (localized NF1)[9,10] was

excluded in our study. The study protocol was approved by the Ethics Committee of Tottori University Hospital (No. 2671). Demographic and clinical information on age, sex, number of cutaneous neurofibromas and any diagnosed complications was obtained from medical records. For each patient, we counted the number of cutaneous neurofibromas (> 5mm in diameter) except for subcutaneous neurofibromas by visual judgement at the 4 divisions of body surface area: head and neck, trunk, upper limbs and lower limbs during a physical examination. In addition, we calculated ratios of the number of cutaneous neurofibromas per body surface area. We also investigated the time-dependent change in cutaneous neurofibromas after 1 year.

**Genetic analysis**

After obtaining informed consent, we performed genetic analysis in 20 NF1 patients by next-generation sequencing as previously described.[11] Simply, genomic DNA was extracted from peripheral blood according to standard procedures. In-solution hybridization-based enrichment was performed using the SureSelect Target Enrichment system (Agilent Technologies, Santa Clara, CA, USA). When the next-generation sequencing protocol revealed truncating mutations, the variants were validated by direct capillary sequencing. The exon deletions were screened using a multiple

ligation-dependent probe amplification method (SALSA P081/082-B2 NF1MLPA assay kit; MRC-Holland).

**Statistical analysis**

Spearman rank correlation was used to determine the correlation between number of cutaneous neurofibromas and age. P-values $< 0.05$ were considered statistically significant. A correlation coefficient (r) of more than 0.7 was considered to indicate strong correlation and $0.5 < r \leq 0.7$ was considered to indicate moderate correlation. All statistical analyses were conducted using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0). More precisely, it is a modified version of R commander (version 1.32) designed to add statistical functions frequently used in biostatistics.[12]

**Results**

**Relationship between age and number of cutaneous neurofibromas**

We evaluated the numbers of cutaneous neurofibromas in 57 NF1 patients (25 men and 32 women [no pregnancy]; median age, 34 years). Demographic and clinical

information is shown in Table I . The number of cutaneous neurofibromas increased after puberty. The average number of cutaneous neurofibromas in patients with a family history was larger than that in patients without a family history ($p < 0.0113$). There was no difference in the number of cutaneous neurofibromas between men and women (not shown).

A representative case is shown in Fig. 1. There were 226 cutaneous neurofibromas on the trunk. There was a strong positive correlation between age and number of cutaneous neurofibromas ($r = 0.75$, $p < 0.001$) (Fig. 2). The number of neurofibromas increased with aging.

**Predilection site of cutaneous neurofibromas**

Since there are few cutaneous neurofibromas before puberty (as shown in Table I ), patients under 20 years old were excluded.

Forty-one of the 57 patients (17 men and 24 women [no pregnancy]; median age, 40 years, age range, 21-79 years) could be evaluated because there were records of the number of neurofibromas at each site. In the cases evaluated, 60.2% of the cutaneous neurofibromas were on the trunk, 16.1% were on the lower limbs, 14.4% were on the upper limbs and 9.2% were on the head and neck (Fig. 3a). The number was large on

the trunk and was small on the lower limbs.

We also evaluated the ratio of the number of cutaneous neurofibromas per body surface area. The Lund and Browder Chart is commonly used for measuring the percentage of total body-surface area (TBSA, %).[13] According to the Lund and Browder Chart, the ratios of neurofibromas based on the total body-surface area were 1.9 on the trunk, 1.0 on the head and neck, 0.8 on both upper limbs and 0.4 on both lower limbs (Fig. 3b). Thus, the ratio was also high on the trunk and was low on the limb.

In addition, we investigated the number of cutaneous neurofibromas in association with body constitutional differences (e.g., obese or thin) in each patient by using DuBois formula. There was no significant difference between each body type and number of cutaneous neurofibromas ($r = -0.23$, $p = 0.181$) (Fig. 4). The number of cutaneous neurofibromas was not related to body constitution.

**Time course change in number of cutaneous neurofibromas**

In order to determine the time course change in neurofibromas, we investigated the number of neurofibromas in 36 NF1 patients (14 men and 22 women; median age, 36 years; age range, 4-79 years) 1 year later.

The year-to-year percentage change in the number of cutaneous neurofibromas is shown

in Fig. 5. The number of cutaneous neurofibromas had increased in about 61% of the patients one year later. The average annual rate of increase was 0.21 (range, −0.71-1.2). There was a linear association between patients and year-to-year percentage change in the number of cutaneous neurofibromas.

**Relationship between type of *NF1* mutation and cutaneous neurofibromas**

NF1 mutation was detected in 16 of 20 patients (7 (35%) frameshift, 6 (30%) missense, 3 (15%) deletion and 4 (20%) negative). There was no significant difference between each type of *NF1* mutation and number of cutaneous neurofibromas (not shown). However, numerous cutaneous neurofibromas (≧4500) were recognized in a patient with whole *NF1* deletion.

## DISCUSSION

NF1 has cutaneous, neurological and osseous complications.[1,2,14] One of the major features of NF1 is cutaneous neurofibromas.[2,3,14] Huson et al. indicated that cutaneous neurofibromas increased with aging.[7] However, to our knowledge, there has been no statistical analysis of an association between cutaneous neurofibromas and age. Therefore, we investigated the natural course of cutaneous neurofibromas. In our study,

we revealed for the first time positive correlation between cutaneous neurofibromas and age statistically.

We also investigated the differences in the number of cutaneous neurofibromas in relation to family history. There was a significant relationship between family history and number of cutaneous neurofibromas (Table I ) ($p < 0.05$). Clinical symptoms in patients with a family history of NF1 seem to be more serious than those in patients without a family history of NF1.

Palmer et al. investigated associations between the presence of diffuse plexiform neurofibromas, cutaneous neurofibromas, and café-au-lait spots in individual body segments of NF1 patients.[15] They concluded that there was no significant correlation among them. Kaufmann et al. reported that the number of visible cutaneous neurofibromas was larger in a high temperature area (trunk) than in low temperature areas (arms/legs).[16] They compared the distributions of cutaneous neurofibromas by thermographs. However. it was difficult to assess the relation by their methods precisely because cutaneous neurofibromas have abundant blood supply themselves. In our study, we made it clear that the ratio of cutaneous neurofibromas was high on the trunk and was low on the limbs significantly.

It is well known that mast cells and Schwann cells play an important role in the

pathogenesis of cutaneous neurofibromas in NF1.[17] Therefore, the difference in distributions of mast cells or Schwann cells at each body site may be associated with the frequency of cutaneous neurofibromas. Weber et al. reported that the mast cell population was large at peripheral skin sites (hands and feet).[18] We previously studied the number of mast cells on cutaneous neurofibromas at various body sites. In that study, the number of mast cells was not related to the number of cutaneous neurofibromas.[19] This means that the distribution of mast cells is not related to the large number of cutaneous neurofibromas on the trunk. On the other hand, there has been no report on the distribution of Schwann cells in human skin. The relationship between Schwann cells and number of cutaneous neurofibromas is not clear because it is difficult to evaluate the accurate distribution of Schwann cells in peripheral nerves. The distribution of peripheral nerves might be one of the reasons why there is a definite difference in the number of neurofibromas among body areas.

In daily medical examinations, NF1 patients are reluctant to show their whole body. Physicians can observe only lesions located on the head and neck or the limbs. Therefore, it is often difficult to estimate the overall state of cutaneous neurofibromas in patients with NF1. Once numerous cutaneous neurofibromas develop, much effort is needed to improve impaired quality of life for NF1 patients due to cutaneous

manifestations. In this study, we revealed natural course and characteristics of cutaneous neurofibromas. Based on our findings, physicians can estimate the total number of cutaneous neurofibromas on the whole body and predict the state of cutaneous neurofibromas, which is helpful to prevent serious cosmetic condition by the development of cutaneous neurofibromas. We believe that our findings will be useful for treatment of patients with NF1. In this study, we investigated the number of cutaneous neurofibromas by visual judgement. However it might be a time-consuming task for physicians. In order to save the time and effort for counting, we are trying to develop image analysis system to estimate the number of cutaneous neurofibromas in the future.

**ACKNOWLEDGMENTS**

This work was partly supported by Health Labour Sciences Research Grant (Y.Y) from the Ministry of Health, Labour and Welfare and the Platform Project for supporting in Drug Discovery and Life Science Research from the Japan Agency for Medical Research and Development (Y.Y and K.K) and Tottori University Faculty of Medicine Alumni Association (Y.E).

Conflict of interest: none.

REFERENCES


1. National Institutes of Health Consensus Development Conference. Neurofibromatosis: Conference statement. *Arch Neurol* 1988; **45**: 575-578.
2. Anderson JL, Gutmann DH. Neurofibromatosis type 1. *Handb Clin Neurol* 2015; **132**: 75-86.
3. Korf BR. Diagnosis and management of neurofibromatosis type 1. *Curr Neurol Neurosci Rep* 2001; 1: 162-167.
4. Yoshida Y. 71 Neurofibromatosis. In: Kelly, A. P., Taylor, S. C., editors. Taylor and Kelly's Dermatology for skin of Color 2nd eds. McGrawHill Press; 2016: p. 499-504.
5. Kodra Y, Giustini S, Divona L, et al. Health-related quality of life in patients with neurofibromasis type 1. A survey of 129 Italian patients. *Dermatology* 2009; **218**: 215-220.
6. Page PZ, Page GP, Ecosse E, Korf BR, Leplege A, Wolkenstein P. Impact of neurofibromatosis 1 on Quality of Life: a cross-sectional study of 176 American cases. *Am J Med Genet A* 2006; **140**: 1893-1898.
7. Huson SM, Harper PS, Compston DA. Von Recklinghausen neurofibromatosis. A clinical and population study in south-east Wales. *Brain* 1988; **111**: 1355-1381.
8. Gutmann DH, Aylsworth A, Carey JC, et al. The diagnostic evaluation and

multidisciplinary management of neurofibromasis 1 and neurofibromatosis 2. *JAMA* 1997; **278**: 51-57.

9. Ruggieri M, Huson SM. The clinical and diagnosis implications of mosaicism in the neurofibromatoses. *Neurology* 2001; **56**: 1433-1443.
10. Tanito K, Ota A, Kamide R, Nakagawa H, Niimura M. Clinical features of 58 Japanese patients with mosaic neurofibromasis. *J Dermatol* 2014; **41**: 724-728.
11. Maruoka R, Takenouchi T, Torii C, et al. The use of next-generation sequencing in molecular diagnosis of neurofibromatosis type 1: a validation study. *Genet Test Mol Biomarkers* 2014; **18**: 722-735.
12. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. *Bone Marrow Transplantation* 2013; **48**: 452-458.
13. Lund CC, Browder NC. The estimination of areas of burns. *Surg Gynecol Obst* 1944; **79**: 352-358.
14. Niimura M. Neurofibromatosis in Japan, In: Ishibashi Y, Hori Y (eds): Tuberous sclerosis and neurofibromatosis epidemiology, pathophysiology, biology and management. Excerpta Medica, Amsterdam, the Netherlands: Elsevier Science Publishers; 1990: 22-31.
15. Palmer C, Szudek J, Joe H, Riccardi VM, Friedman JM. Analysis of

neurofibromatosis (NF1) lesions by body segment. *Am J Med Genet A* 2004; **125A**: 157-161.

16. Kaufmann D, Tinschert S, Algermissen B. Is the distribution of dermal neurofibromas in neurofibromatosis type 1 (NF1) related to the pattern of the skin surface temperature? *Eur J Dermatol* 2001; **11**: 521-526.
17. Zhu Y, Ghosh P, Charnay P, BurnsDK, Parada LF. Neurofibromas in NF1: Schwann cell origin and role of tumor environment. *Science* 2002; **296**: 920-922.
18. Weber A, Knop J, Maurer M. Pattern analysis of human cutaneous mast cell populations by total body surface mapping. *Br J Dermatol* 2003; **148**: 224-228.
19. Yoshida Y, Adachi K, Yamamoto O. Local mast cell histamine and plasma histamine levels in neurofibromatosis type 1. *Acta Derm Venereol* 2010; **90**: 637-639.

**Figure Legends**

**Figure 1)** A representative case of NF1 in a 39-year-old patient. There were 226 cutaneous neurofibromas on the trunk.

**Figure 2)** Relationship between age and number of neurofibromas ($r = 0.75$, $p < 0.001$).

**Figure 3) (a)** Predilection site of cutaneous neurofibromas. **(b)** The ratios of neurofibromas based on the total body-surface area.

**Figure 4)** Between each body type used by the DuBois formula and number of cutaneous neurofibromas ($r = -0.23$, $p = 0.181$).

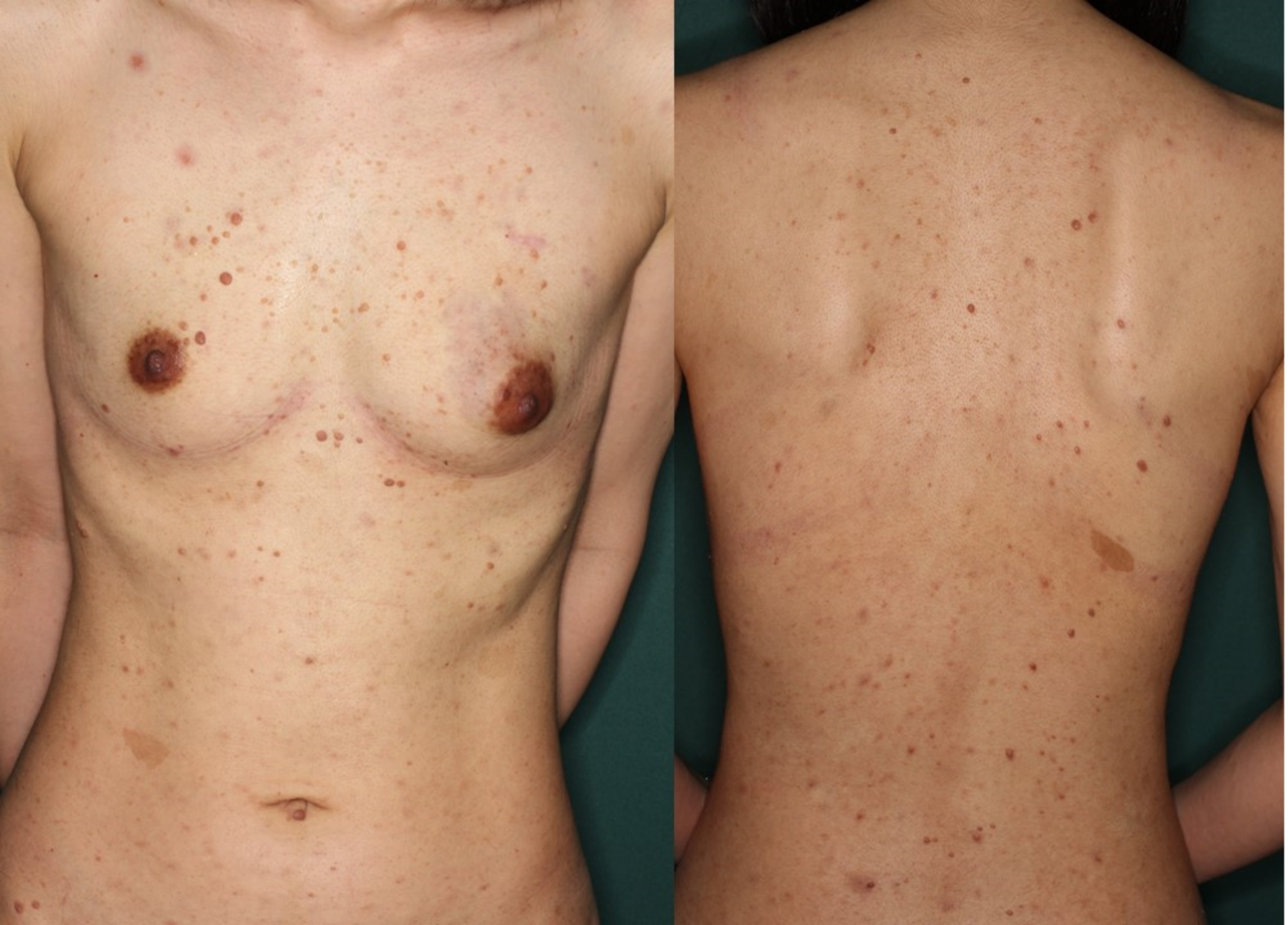
**Figure 5)** Year-to-year percentage change in the number of cutaneous neurofibromas.

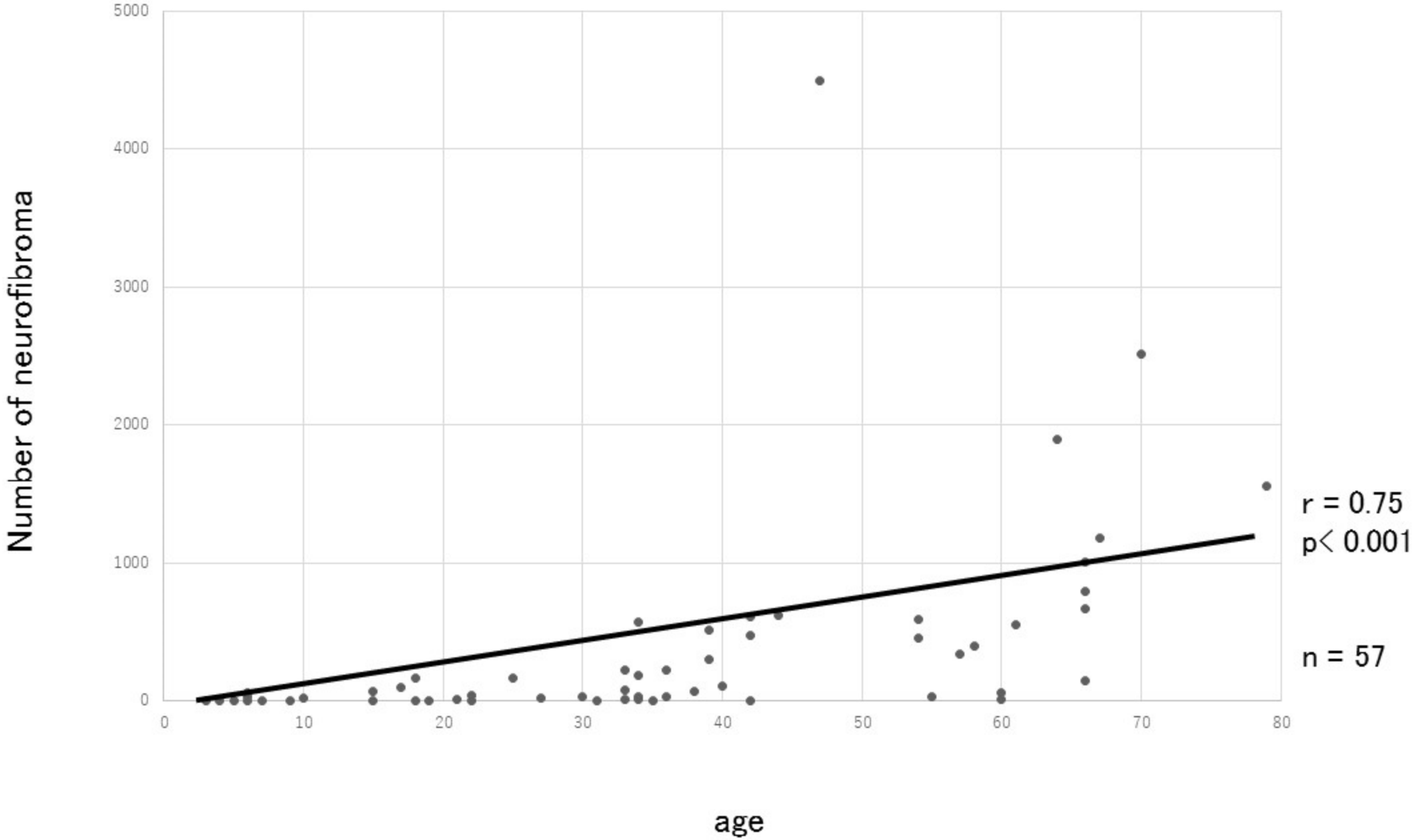
**Table I.** Dermographic information

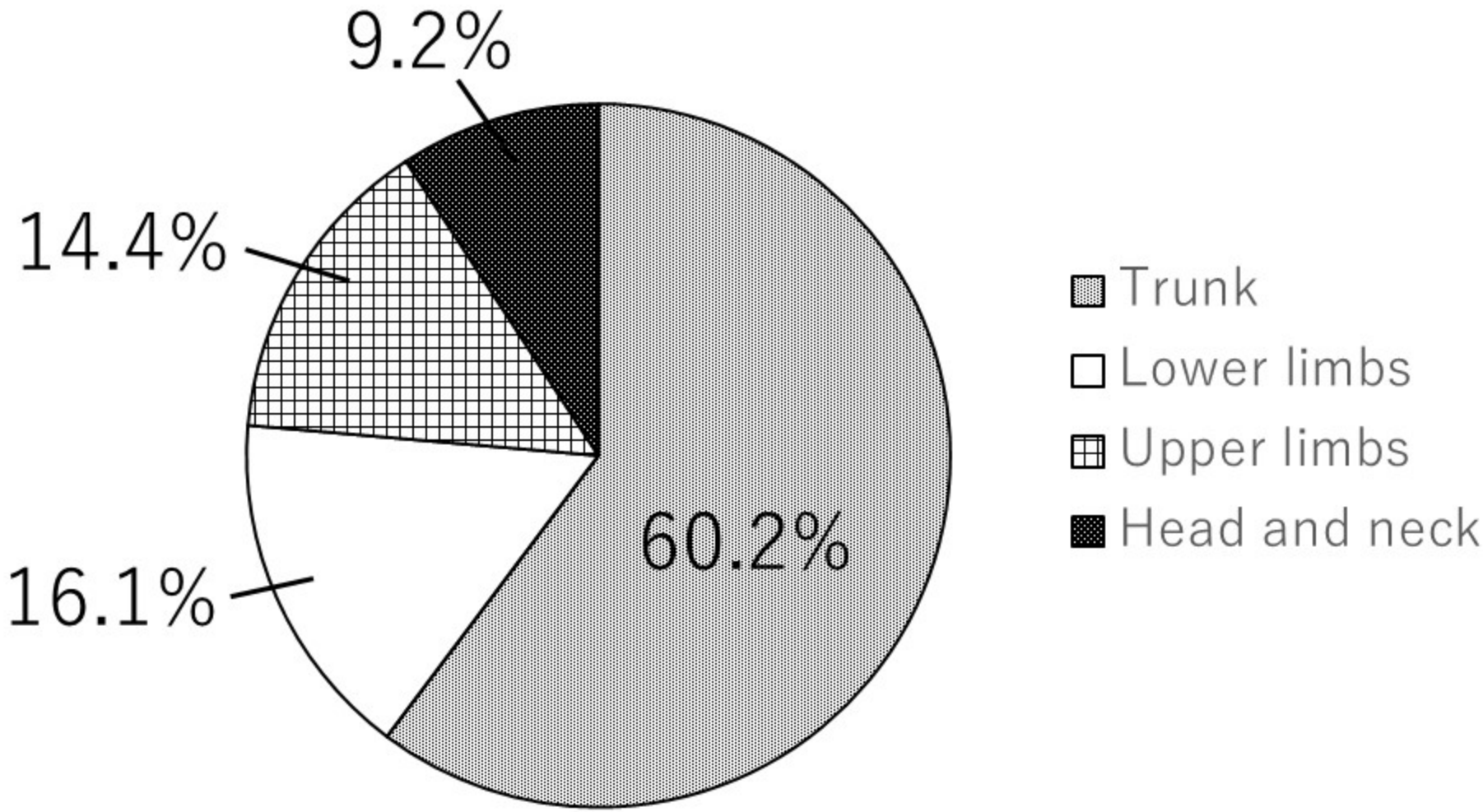
| Sex | The number of patients(ratio %) |
|---|---|
| men | 25(43.9%) |
| women | 32(56.1%) |

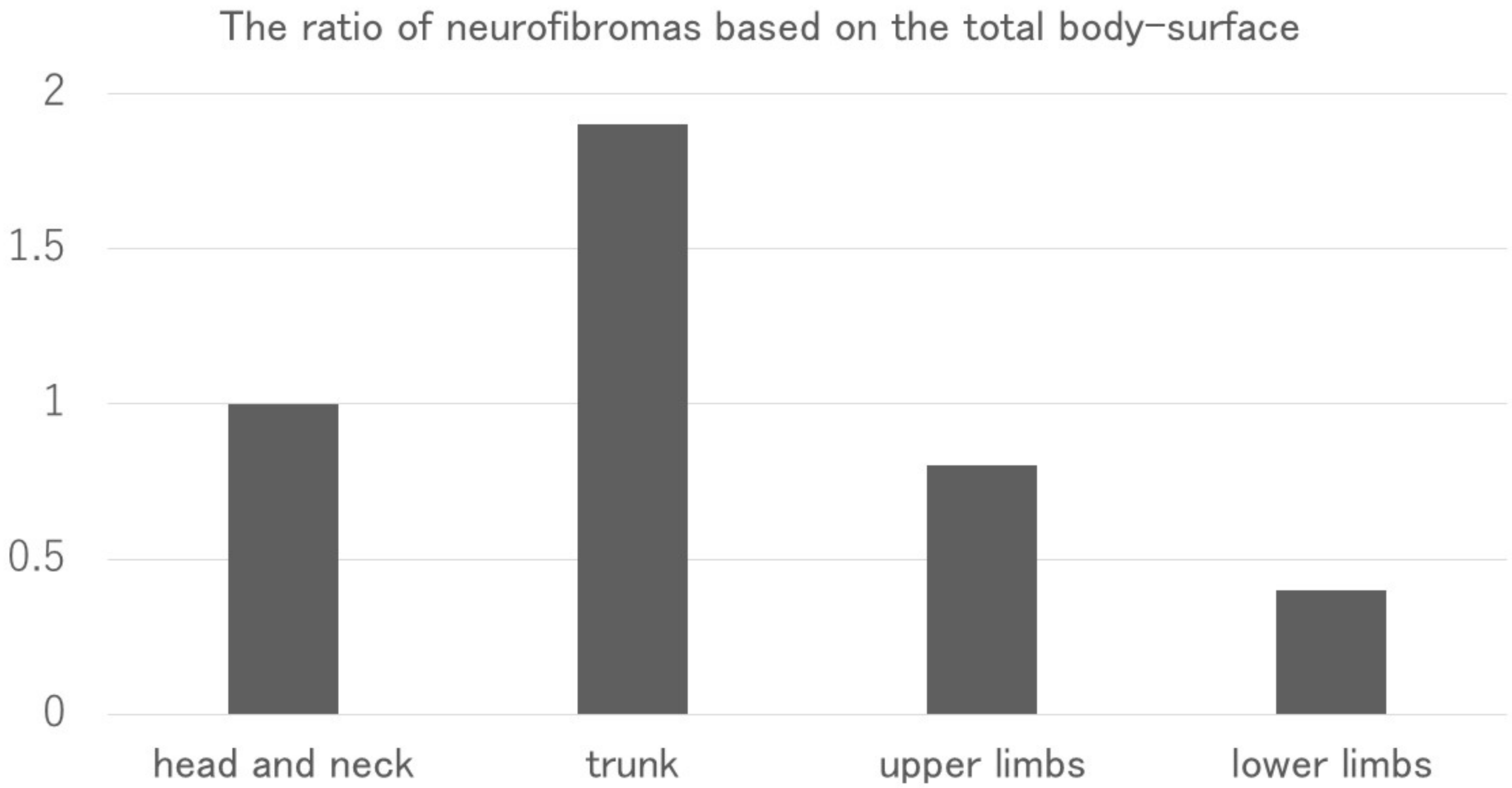
| Age (years-old) | The number of patients(ratio %) | The average amount(number) of neurofibromas |
|---|---|---|
| <10(0-9) | 8(14.0%) | 11.6 |
| <20(10-19) | 7(12.3) | 52.4 |
| <30(20-29) | 5(8.8%) | 48 |
| <40(30-39) | 15(26.3%) | 155.3 |
| <50(40-49) | 6(10.5%) | 1053.5 |
| <60(50-59) | 5(8.8%) | 363.2 |
| <70(60-69) | 9(15.8%) | 704.1 |
| <80(70-79) | 2(3.5%) | 2040 |
| | 57 | |

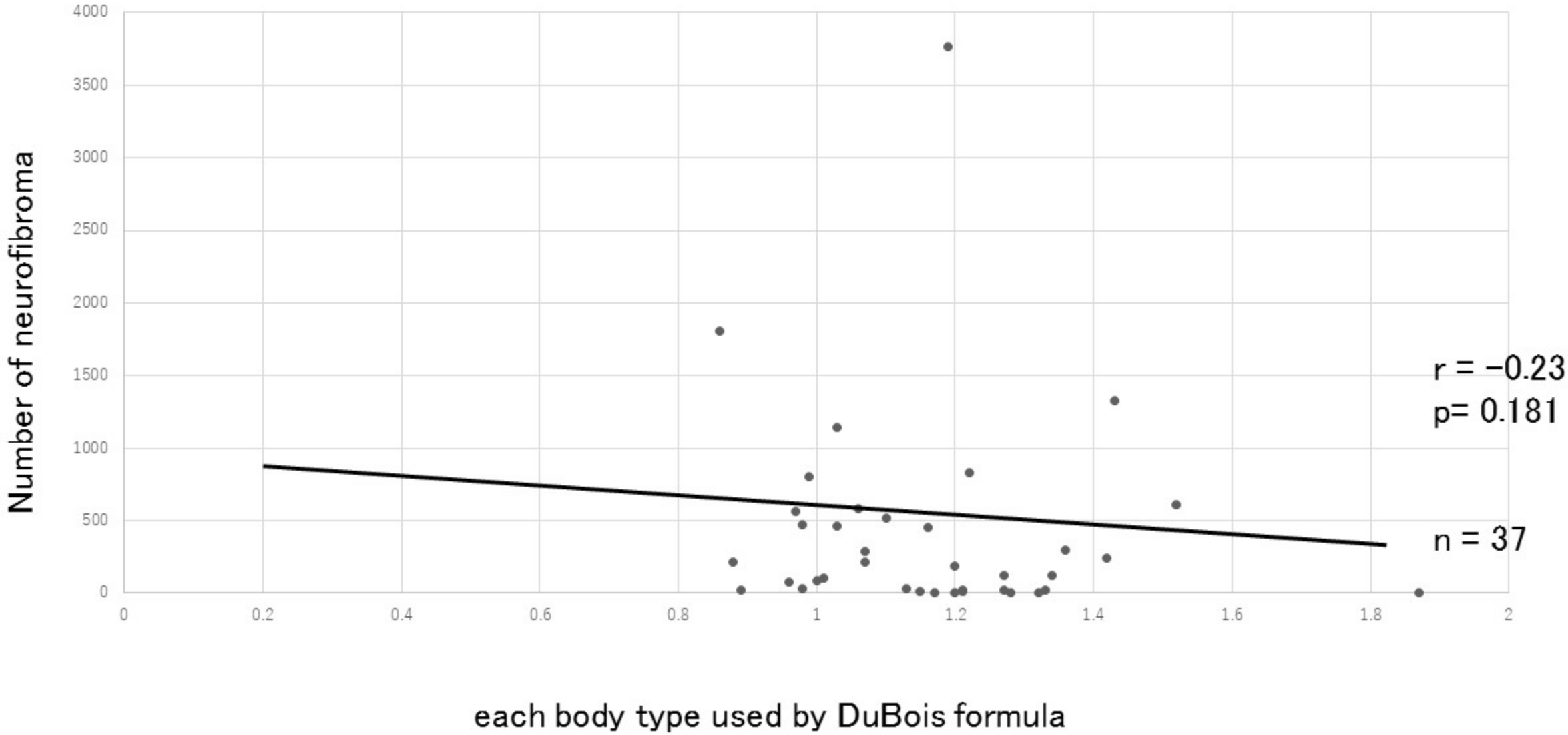
| Family history of NF1 | The number of patients(ratio %) | Average age | The average amount(number) of neurofibromas |
|---|---|---|---|
| yes(+) | 28(49.1%) | 39.9 | 590 |
| no(-) | 29(50.9%) | 32.3 (p=0.103) | 174 (p=0.0113) |

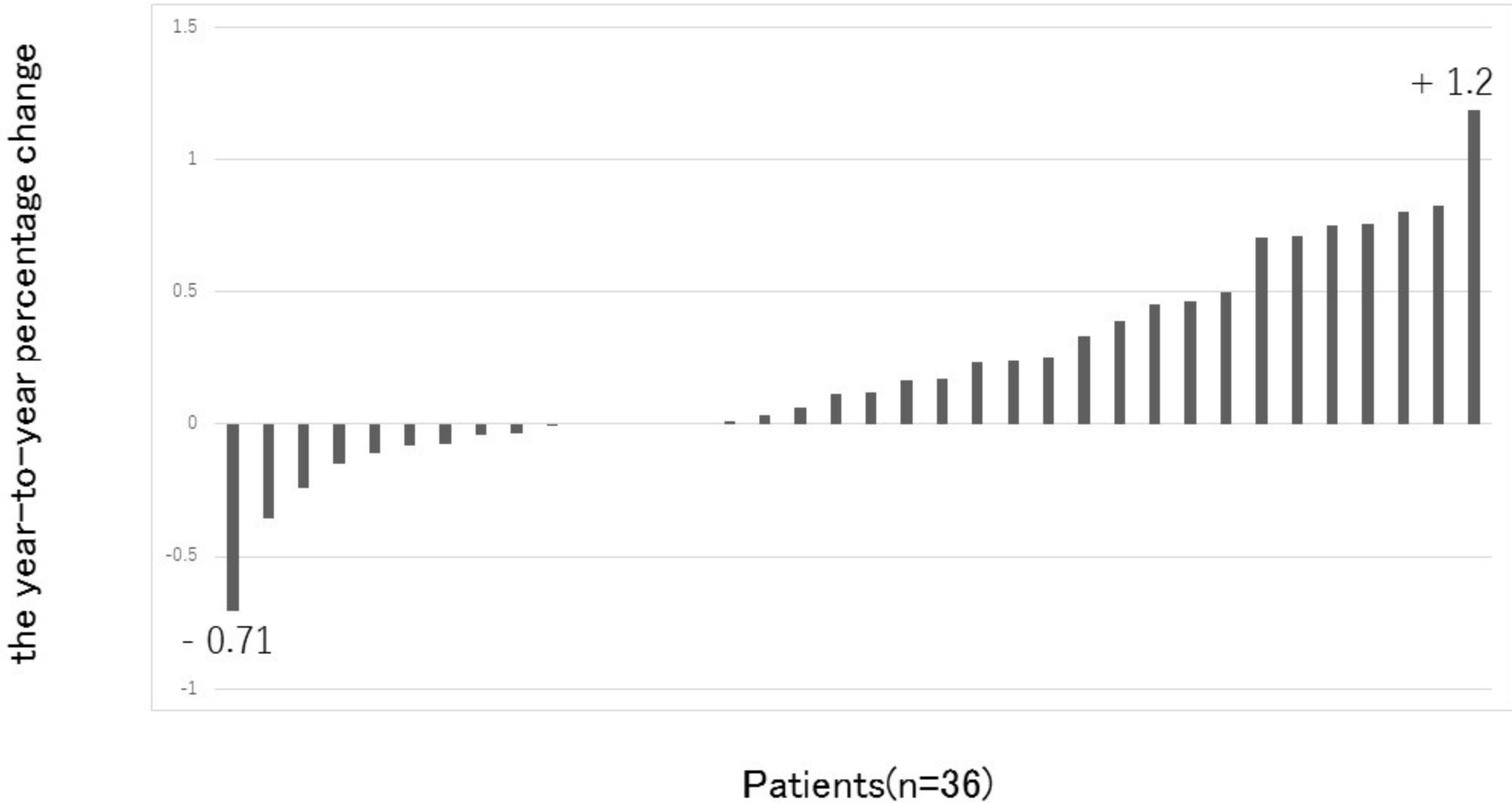



Number of neurofibroma
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
age
r = 0.75
p< 0.001
n = 57


9.2%
14.4%
16.1%
60.2%
Trunk
Lower limbs
Upper limbs
Head and neck


The ratio of neurofibromas based on the total body-surface
2
1.5
1
0.5
0
head and neck
trunk
upper limbs
lower limbs


Number of neurofibroma
4000
3500
3000
2500
2000
1500
1000
500
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
r = −0.23
p= 0.181
n = 37
each body type used by DuBois formula


the year-to-year percentage change
1.5
1
0.5
0
-0.5
-1
+ 1.2
- 0.71
Patients(n=36)