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Br J Dermatol 2021 Feb;184(2):289-295.
doi: 10.1111/bjd.19171. Epub 2021 Feb 1.

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Summary

Background

Naevi number changes with age. Thus, a better understanding of naevus biology will shed more light on the genetic and environmental factors involved in melanoma development.

Objectives

To use a two-wave study to better understand the evolution of naevi in healthy adults.

Methods

This study is a prospective two-wave study based on adult twins from the TwinsUK registry (n = 414) who underwent total body naevus counts with an interval of at least 15 years. A negative binomial hierarchical model with two levels, the individual and the twin pair, was used to estimate expected changes in naevus count between the first and second visit, at any specific body site and on the whole body. The model was adjusted for age, calendar year at the first visit, height and skin type.

Results

The mean age of participants was 46 years at the first visit and 63 years at the second visit (the mean elapsed time between visits was 17 years). An increase in naevus count was observed in 235 (57%) participants and a decrease was observed in 166 (40%). The mean difference in total naevus count between the two visits was nine. The expected total body naevus count increased, on a logarithmic scale, by 0·28 [95% confidence interval (CI) 0·16–0·40] with a change in the incidence rate of total body naevus count of 32% (95% CI 17–49%). However, the observed increase in naevus count over time was observed only on the upper parts of the body, whereas there was no evidence of an increase on the lower parts.

Conclusions

Naevus counts increased slightly over time at older ages, but this was dependent on body site. The overall decrease in naevus counts previously reported in cross-sectional studies has not been confirmed by this longitudinal study.

The risk of developing melanoma increases with increasing number of common naevi.1 Total body naevus count (TBNC) is one of the most important risk factors for melanoma development, with much higher relative risks than environmental exposure.2 Sunlight may be involved in naevogenesis, but naevi are mostly under genetic control, as shown by family and twin studies.3 To date, cross-sectional studies have shown a lower prevalence of naevi in older age groups, yet they do not address the natural history of naevi, which can be only loosely inferred from their findings.4-6 Understanding naevus biology would help in tailoring secondary prevention strategies for melanoma. Based on previous cross-sectional studies, it has been speculated that naevi typically involute after the fourth decade of life in white populations and are rarer in elderly people. However, individuals with susceptibility to melanoma often have a large number of common and atypical naevi, which persists until middle age or later.1 Almost all longitudinal studies, with smaller numbers of participants, have been undertaken in high-risk groups such as patients with melanoma, their relatives or patients with the atypical mole syndrome phenotype.7-9 Therefore, their findings are unlikely to be applicable to the population at large. To understand the evolution of naevi in healthy adults, we performed a two-wave study in the TwinsUK cohort, counting total body naevi twice with a minimum interval of 15 years.

Patients and methods

Participants and skin examination

Guy's and St Thomas' Hospital NHS Trust Research Ethics Committee approved the study, and all twins provided informed written consent. The TwinsUK resource is the biggest UK adult twin registry, comprising 12 000 twins aged 16–100 years. Clinical, physiological, behavioural and lifestyle data are collected either at twin visits to the hospital or via self-administered questionnaires, which volunteers complete either once or twice a year via post or email. Volunteers in the TwinsUK cohort are not recruited on the basis of any specific trait or disease and have been shown to have diseases and lifestyle characteristics similar to the general population.10 Twins are periodically asked to attend for different diagnostic purposes, and they are not aware of the diagnostic test that will be performed or the phenotype that will be collected at the specific visit.

Skin examination and data collection were undertaken for 3694 female twins between January 1995 and December 2003 as part of the TwinsUK study protocol, which has been published previously.11 For historical reasons, the TwinsUK database mainly includes female participants. The naevus counts were performed by research nurses trained by the same dermatologist (V.B.) during both benches and using a validated protocol. The second count was performed on 1987 twins

between January 2014 and February 2017 using the same protocol. Overall, 460 of the 1987 twins had been counted twice and are therefore included in this study as mentioned above.

The skin examination included recording of skin type, hair, eye colour and freckles in addition to naevus count on 17 body sites performed by trained research nurses at St Thomas' Hospital in London.12 A naevus was defined as a melanocytic lesion ≥ 2 mm in diameter. Skin type was assessed according to the Fitzpatrick classification.

Statistical analyses

Preliminary analyses were performed to test differences in the distribution of naevus count for any specific body site over time by matched-pairs signed-rank test. The Benjamini–Hochberg method was used to control the false discovery rate, i.e. the expected proportion of falsely rejected hypotheses in the presence of multiple comparisons.13 In the univariate analysis, a linear model was used to predict changes in naevus count according to age at first visit, the calendar year at first visit, and the elapsed time between the two visits, modelled by restricted cubic splines with four knots placed at fixed percentiles (5%, 35%, 65%, 95%). Confidence intervals (CIs) were calculated by taking into account the correlation of individuals within a twin pair by robust variance. As the time interval between the two visits was uniformly distributed within a range of 5 years, which did not affect changes in naevus count, we modelled the data according to a longitudinal analysis model. To quantify the average change in naevus count between the first and second visit at any specific body site and also on the whole body, a negative binomial hierarchical model with two levels, i.e. the individual (first level) and the twin pair (second level), was used. The model was adjusted for age and calendar year at first visit, height and skin type. Continuous variables were centred at their mean value. The model including the age at first visit modelled by restricted cubic splines was compared with the negative binomial hierarchical model, but no difference was observed. Effects modification for the variables included in the model was checked using a likelihood-ratio test. A sensitivity analyses was performed by excluding outliers in the naevus count according to the criteria based on interquartile range.

Results

Of 460 participants, 414 female participants had complete data on naevus count and covariates at the two visits; 173 were twin pairs and the remaining 68 were singletons. The characteristics of recruited participants are reported in Table 1. The average age of the participants was 46 years at the first visit and 63 years at the second visit (SD 10·1 at both visits). The mean elapsed time between the two visits was 17 years (SD 1·13), with a range from 15 years to 20 years. There was

no difference in the elapsed time according to the age at first visit (P = 0·27). The distribution of naevus count at any of the 17 specific body sites is reported in Table 2.

The mean difference in the TBNC between the two visits was nine (SD 47·4, P < 0·001). Overall, the TBNC was slightly higher at the second visit compared with the first visit for all specific body sites, excluding the foot. The increase in naevus count over time was most pronounced for the neck, back and arms. After adjustment for multiple comparisons, the evidence remained for the neck, back and left arm above the elbow (Q = 0·027, Q < 0·001 and Q = 0·027, respectively).

When looking at cross-sectional data for visit 1, TBNC decreased with age at the first visit up to age 50 years and then increased slightly (Figure 1a). However, it remained positive or closed to null value over the complete range of ages (Figure 1b).

Calendar year and elapsed time between the two visits did not seem to influence the pattern of change in the naevus count (Figures S1, S2; see Supporting Information). The estimated adjusted difference, on a logarithmic scale, in the expected TBNC between the first and second visit, is reported in Table 3. The expected TBNC increased, on a logarithmic scale, by 0·28 (95% CI 0·16–0·40). Hence, for example, the expected TBNC in a woman who was 44 years old and 163 cm tall with skin type 1 increased from 13 to 17 after approximately 17 years since the first visit in 1998. On the nonlogarithmic scale, the percentage change in the incidence rate of the total count of naevi was a 32% (95% CI 17–49%) increase between the two visits, holding the other variables constant. On a logarithmic scale, the estimated adjusted difference in naevus counts at each body site between the first and second visit adjusted for all covariates is reported in Table 4. The expected naevus count increased over time in the upper part of the body. In particular, it increased, on a logarithmic scale, by 0·16 (95% CI 0·01–0·32) for the face, 0·32 (95% CI 0·13–0·50) for the neck, 0·31 (95% CI 0·11–0·51) for the chest, 0·16 (−0·01–0·33) for the abdomen and 0·66 (95% CI 0·51–0·81) for the back. An increase in naevus count was also observed in the left and right arm (0·20, 95% CI 0·05–0·35 and 0·18, 95% CI 0·03–0·34, respectively) but was less pronounced on the right leg (0·16, 95% CI −0·02–0·34), but not on the left leg (0·04, 95% CI −0·12–0·21). When applying adjustment for multiple CIs for selected parameters,14 the evidence of an increase remained for the neck, chest, back and both arms. The estimated effect of time on naevus counts did not change according to skin type, height, age and calendar year at the first visit. Calendar year and elapsed time between the two visits did not seem to influence the pattern of change in the naevus count (Figures S1, S2; see Supporting Information).

When excluding outliers, TBNC increased, on a logarithmic scale, by 0·22 (95% CI 0·10–0·34). When stratifying the analyses according to TBNC at the first visit, there was indication of

regression to the mean as the naevus count for women whose baseline TBNC was unusually high tended to decrease and vice versa (Figure S3; see Supporting Information). An increase in TBNC was observed in 235 (57%) participants and a decrease occurred in 166 (40%) participants. Among those who lost naevi over time, the median TBNC at visit 1 was 38 (range 15–68) compared with those who gained new naevi with a median TBNC of 11 (range five to 25) at visit 1. For those who did not have any change over time, the median TBNC was seven (range one to eight).

We did not adjust, a priori, for body mass index because TBNC has been shown to be associated with height rather than weight.15 When adjusting for weight gain during the elapsed time between the two visits, the estimates did not change [the expected TBNC increased over time, on a logarithmic scale, by 0·27 (95% CI 0·14–0·40)].

Discussion

TBNC is an independent and powerful risk factor for melanoma. There is a well-established, positive, dose-dependent relationship between total number of melanocytic naevi and the risk of developing melanoma. This is explained, in part, by a reduced senescence of naevi with age in those at risk.3 This increased risk is distinct from the risk of progression of any single naevus to melanoma.16 Thus, understanding melanoma risk during the ageing process is important17 as the biology and evolution of naevi throughout a lifetime is likely to shed light on melanocyte senescence.

Naevi are growth-arrested, clonal neoplasms of melanocytes initiated by well-defined oncogenic mutations in the mitogen-activated protein kinase pathway, most commonly by *BRAF* V600E-activating mutation. TBNC in any given individual is thought to peak during the fourth decade of life.12 This peak is due to reduced formation of new naevi combined with the clinical regression of some existing naevi. Clinical regression of naevi is a poorly understood process during which naevi involute and eventually disappear entirely. The frequency of naevus regression increases with advancing age, especially for junctional naevi.18

The natural biology and evolution of naevi with age can also be examined using dermoscopy; globular naevi appear in childhood, while in adulthood, naevi are usually dermoscopically reticular. In late adulthood, naevi become dermal or tend to disappear in elderly people, especially on areas other than the head and neck.19 Genes involved in this senescence process are not well established, but *BRAF*, *CDKN2A* and telomere genes are likely to play a role20 in addition to immune-related genes.21, 22 Previous studies on naevus counts have shown that genetic factors are important for naevus patterns.23 Moreover, different genes seem to be involved in the appearance of naevi at different body sites.24

For historical reasons, the TwinsUK database mainly includes female participants. The naevus counts were performed by research nurses trained by the same dermatologist (V.B.) during both

benches and using the same validated protocol. The second count was performed on 1987 twins between January 2014 and February 2017, but only 460 twins had been counted twice and were therefore included in this study. Previous studies on naevus counts have mainly used a cross-sectional design. Hence, changes in naevus counts with age may be due to a cohort effect rather than a true age effect. Indeed, the age-related decrease in naevus count may be explained by a secular trend if younger generations tended, for example, to be more exposed to the sun.

A recent systematic review of the literature,25 which screened 708 studies, found only two studies that met the criteria for population-based longitudinal studies in adults with a baseline and at least one follow-up naevus count.26, 27 These two studies reported conflicting results. Tindall *et al*.26 published a study based on 163 individuals in North Carolina who were aged 64 years and older. Naevi count was performed at baseline and after 10 years of follow-up on 69 living participants. The percentage of individuals with 10 or more naevi decreased from 15% at baseline to 7%. A major limitation of this study was the lack of information concerning participant selection, which makes it quite difficult to derive inferences with respect to the general population.

Koseoglu *et al*.27 conducted a cohort study that included 60 individuals in Turkey with an average age of 44 years, which described changes in dermoscopy over time. Naevi count was performed on the trunk, arms and legs at baseline and at 3 months, 6 months and 12 months, but the study reported only baseline and 12-month counts. No change in naevus count was observed after 12 months. However, the sample was not representative of the population as it included only immunosuppressed patients and the time interval between the two counts was too short. Furthermore, no information was provided about the examiners and the naevus count protocol.

To our knowledge, this is the largest two-wave naevus count study ever conducted in adults with a long enough interval to assess changes of naevi over time. The TwinsUK cohort is representative of the general female population of the UK as the participants were unaware of the phenotypes being collected before the visit in order to avoid bias.10 However, the results of this study cannot be extrapolated for a male population because naevi distribution and behaviour differ among sexes.

As naevi were counted on several body sites, this allowed us to distinguish body areas most affected by changes over time and assess sun-exposed vs. non-sun-exposed sites. The body area showing the most significant increase was the back, but the appearance of flat seborrhoeic keratoses and solar lentigines, which increase with age, may have been misclassified as naevi. The changes in naevi over time were not seen on the legs, and solar lentigines and seborrhoeic keratoses are rarer, so this also supports the possibility of misclassification.

Scope *et al*.28 demonstrated the concept of 'naevus volatility' in children, with an overall increase in naevus counts observed. Specifically, children with higher back naevus counts had greater naevus volatility, being more likely both to develop new naevi and have disappearing naevi during follow-up. In our study, the median naevus count at the first visit was higher among those who lost naevi over time compared with those who gained new naevi. This is due mainly to the regression to the mean phenomenon. Furthermore, this may suggest misclassification as phenotypic signs of sun damage with solar elastosis and solar lentigines are more evident in individuals with low naevus counts compared with those who have high naevus counts. The negative association between high naevus counts and solar keratoses, another marker of sun damage, has been shown previously.29 However, we cannot directly quantify the volatility because we do not have specific information on how many naevi appeared and disappeared individually.

Data on the naevus count at the second visit were collected on only just over 12% of the initial cohort. Although we cannot exclude the possibility that the twins attending the second visit were those with a higher mole count, the selection bias, if present, is likely to be small because twins are periodically asked to come for different diagnostic purposes, and they are not aware of the diagnostic test that will be performed or the phenotype that will be collected at the specific visit.

This study confirms the need for a skin check-up in patients with high naevus counts, especially in areas not clearly accessible by self-examination (such as the back), because any new melanocytic lesion has a higher probability of being a malignant lesion with increasing age. As around 70% of new melanoma appears as a new melanocytic lesion,30 an observation of any new melanocytic lesion in adulthood is important in terms of change in size, shape or colour, and dermoscopic monitoring of melanocytic naevi in high-risk groups with a family history of melanoma and/or the atypical mole syndrome is recommended.

In summary, TBNC seemed to decrease with age based on cross-sectional data at the first visit in this study. However, when the longitudinal data were examined we found that TBNC increased slightly over time at older ages. The age-related decrease in naevi count observed in cross-sectional studies could therefore be explained by a cohort effect as suggested by Plasmeijer *et al*.25 The main reason for this cohort effect could be attributed to lower sun exposure in older cohorts.31 Therefore, the evidence that naevi decrease with age in cross-sectional studies could not be confirmed by this two-wave study at a 15-year minimal interval. A decrease in naevus counts may occur earlier and other studies collecting longitudinal naevus data with similar protocols may need to include younger age groups. Further longitudinal studies that also include male participants should be carried out on three or more waves in order to provide more information about the structure and

form of the change process and allow for the testing of hypotheses that cannot be tested using a longitudinal study based on two waves in middle-aged adults.

Acknowledgments

TwinsUK received funding from the Wellcome Trust (WT086904MF & WT081878MA), the European Community's Seventh Framework Programme (FP7/2007-2013), BioResource (funded by the National Institute for Health Research) and the Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London.

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Table 1. Characteristics of participants in the study ($N = 414$)

Demographic data	
Fitzpatrick skin type	
1	57 (13·8)
2	130 (31·40)
3	167 (40·3)
4	50 (12·1)
5	10 (2·4)
Height (cm)	163 (159–167)
Age at first visit (years)	46·0 (35·6–51·1)
Age at second visit (years)	62·9 (53·7–68·4)
Calendar year at first visit	1999 (1998–2000)
Calendar year at second visit	2016 (2016–2017)

Table 2. Description of number of naevi in the study participants (N = 414)

Body sites	Median (IQR) at T_1	Mean (SD)	Median (IQR) at T_2	Mean (SD)	Difference	Mean (SD)	*P*-values	*Q*-values[a]
Total count	18 (6–42)	31·35 (37·46)	25 (11–52)	40·45 (46·09)	4 (−11–24)	9·09 (47·42)	< 0·001	
Face	1 (0–3)	1·68 (2·11)	1 (0–3)	1·99 (2·51)	0 (−1–1)	0·32 (2·84)	0·263	0·405
Neck	1 (0–2)	1·24 (1·76)	1 (0–3)	2·02 (3·43)	0 (−1–1)	0·77 (3·52)	0·004	0·027
Chest	0 (0–2)	1·57 (2·70)	1 (0–3)	2·36 (4·77)	0 (−1–1)	0·79 (4·62)	0·350	0·500
Back	2 (0–6)	4·53 (6·74)	5 (1–11)	8·52 (11·22)	1 (0–7)	3·98 (10·78)	< 0·001	< 0·001
Abdomen	0 (0–2)	1·63 (2·96)	1 (0–2)	1·97 (3·91)	0 (−1–1)	0·34 (3·48)	0·132	0·240
Chest, back and abdomen	4 (1–11)	7·73 (10·52)	7·5 (2–16)	12·85 (17·66)	2 (−1–9)	5·12 (16·05)	< 0·001	< 0·001
Right whole arm	3 (1–7)	5·66 (7·95)	4 (1–9)	6·45 (8·19)	0 (−3–5)	0·93 (9·90)	0·037	0·123
Right forearm	1 (0–3)	1·92 (3·31)	1 (0–3)	2·23 (3·03)	0 (−1–2)	0·31 (4·11)	0·070	0·155
Right arm above elbow	2 (0–4)	3·54 (5·08)	2 (0–5)	4·16 (5·69)	0 (−1–3)	0·62 (6·73)	0·021	0·084
Left whole arm	3 (1–8)	5·42 (7·13)	4 (1–9)	6·40 (7·59)	0 (−2–5)	0·98 (8·79)	0·048	0·137
Left forearm	1 (0–3)	1·89 (2·92)	1 (0–3)	2·20 (2·87)	0 (−1–2)	0·31 (3·65)	0·048	0·137
Left arm above elbow	2 (0–5)	3·53 (4·75)	2 (1–5)	4·20 (5·29)	0 (−2–3)	0·67 (5·93)	0·004	0·027
Right whole leg	1·5 (0–6)	4·63 (7·62)	2 (0–6)	5·25 (8·50)	0 (−2–3)	0·62 (9·28)	0·203	0·338
Right leg below knee	0 (0–2)	1·88 (3·51)	1 (0–3)	2·12 (3·66)	0 (−1–1)	0·24 (4·27)	0·099	0·198
Right leg above knee	1 (0–3)	2·75(4·56)	1 (0–4)	3·13 (5·30)	0 (−1–2)	0·38 (5·67)	0·448	0·498
Left whole leg	2 (0–6)	4·92 (7·83)	2 (0–7)	5·24 (8·10)	0 (−2–3)	0·33 (8·65)	0·406	0·541
Left leg below knee	1 (0–3)	2·06 (3·67)	1 (0–3)	2·22 (3·87)	0 (−1–1)	0·17 (4·51)	0·413	0·486
Left leg above knee	1 (0–4)	2·86 (4·73)	1 (0–4)	3·02 (4·81)	0 (−1–1)	0·16 (5·01)	0·409	0·511
Right foot	0 (0–0)	0·13 (0·45)	0 (0–0)	0·16 (0·57)	0 (0–0)	0·04 (0·69)	0·526	0·554
Left foot	0 (0–0)	0·14 (0·46)	0 (0–0)	0·14 (0·46)	0 (0–0)	0·00 (0·56)	0·620	0·620

Table 3. Estimated difference in the logs of expected count of naevi between the first and second visit by the negative binomial hierarchical model ($N = 414$)

Variable	Adjusted coefficient[a]	95% CI
Constant	2·56	2·25–2·87
Visit		
First visit	0·00	ref
Second visit	0·28	0·16–0·40
Skin type		
1	0·00	ref
2	0·26	−0·03–0·54
3	0·33	0·05–0·61
4	0·35	−0·02–0·71
5	0·39	−0·20–0·98
Height (centred at 163 cm)	0·00	−0·01–0·01
Age at first visit (centred at 44 years)	−0·02	−0·04 to −0·01
Calendar year at first visit (centred at 1998)	−0·02	−0·12–0·13

Table 4. Estimated difference in the logs of expected counts of naevi between the first and second visit, holding other variables constant by the negative binomial hierarchical model ($N = 414$)

	Adjusted coefficient[a]	95% CI
Face	0·16	0·01–0·32
Neck	0·32	0·13–0·50
Chest	0·31	0·11–0·51
Back	0·66	0·51–0·81
Abdomen	0·16	−0·01–0·33
Chest, back and abdomen	0·52	0·39–0·65
Left whole arm	0·20	0·05–0·35
Left arm above elbow	0·20	0·05–0·35
Left arm below elbow	0·17	−0·01–0·36
Right whole arm	0·18	0·03–0·34
Right arm above elbow	0·15	−0·02–0·31
Right arm below elbow	0·18	−0·01–0·37
Left whole leg	0·04	−0·12–0·21
Left leg above knee	0·03	−0·14–0·21
Left leg below knee	0·09	−0·12–0·29
Right whole leg	0·16	−0·02–0·34
Right leg above knee	0·14	−0·03–0·32
Right leg below knee	0·15	−0·06–0·36
Left foot	−0·04	−0·43–0·34
Right foot	0·23	−0·24–0·70

CI, confidence interval. [a]Adjusted for age and calendar year at the first visit, height and skin type.

Fig S1 Pattern of change in naevus count over calendar year, modelled by restricted cubic spline.

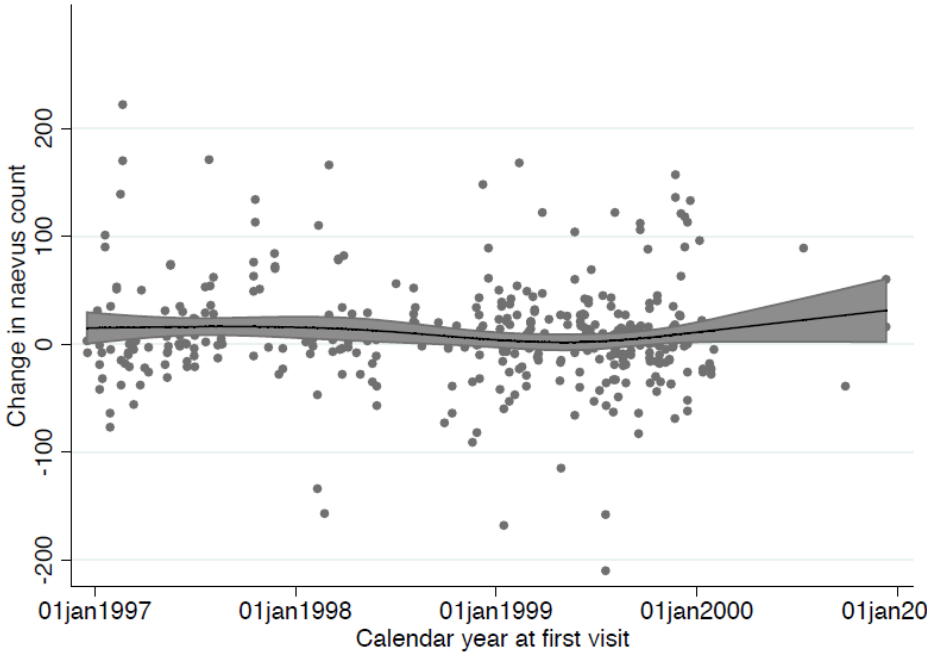

Fig S2 Pattern of change in naevus count according to the elapsed time between the two visits, modelled by restricted cubic splines.

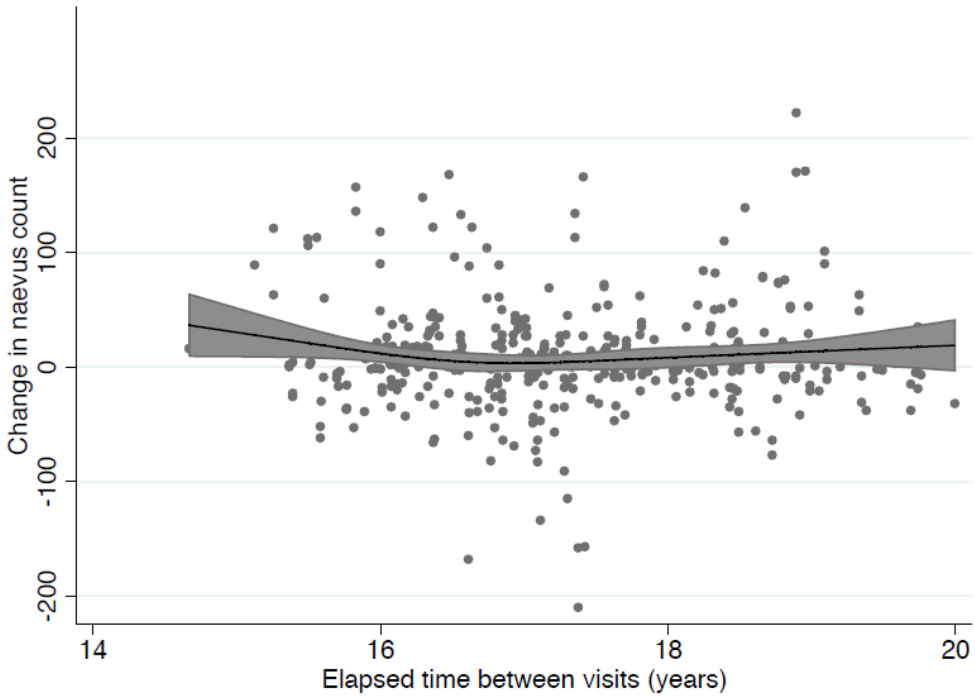

Fig S3 Scatter plot of paired and log-transformed naevi counts showing change [log(follow-up) minus log(baseline)] against log(baseline). The solid line represents perfect agreement (no change) and the dashed line is the fitted regression line. Naevus counts for women whose baseline results were unusually high have tended to decrease so that change values are likely to be below the solid line and naevus counts for women whose baseline results were unusually low have tended to increase so that change values are likely to be above the solid line.