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Body site-specific genetic effects influence naevus count distribution in women

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Abstract

Body site is highly relevant for melanoma: it affects prognosis and varies according to the patient's sex. The distribution of naevi, a major risk factor for melanoma, at different body sites also varies according to sex in childhood. Using naevus counts at different body sites in 492 unrelated adults from both sexes, we observed that women have an increased number of naevi on the lower limbs compared to men ($P=8.5x10^{-5}$), showing that a high naevus count on this site persists from childhood throughout life. Then, using data from 3,232 twins, we observed, in women, the lowest naevus count heritability on the trunk (26%), and the highest on the lower limbs (69%). Finally, we showed that, in

2,864 women, six genomic loci previously associated with both naevus count and melanoma risk (*IRF4, DOCK8, MTAP, 9q31.2, KITLG,* and *PLA2G6*) have an effect on naevus count that is body site-specific, but whose effect sizes are predominantly stronger on the lower limbs. Sex-specific genetic influence on naevus count at different sites may explain differences in site-specific melanoma incidence as well as prognosis between sexes.

Significance

The distribution of naevi, a major risk factor for melanoma development, at different body sites varies according to sex in childhood, mirroring the melanoma distribution observed in adulthood. Here, we observe an increased naevus count on the legs of adult women compared to men. In women, we also observe a different genetic contribution for naevus counts at different body sites, with a stronger effect on the legs, where known naevi and melanoma genomic loci also show, in general, a higher influence. Body site-specific genetic influence on naevus count may contribute to explain differences in site-specific melanoma incidence in women.

Introduction

Melanoma incidence at different body sites varies according to sex, with a higher incidence on the head and neck and trunk in males whilst females have a higher incidence on the lower limbs (Claus Garbe & Leiter, 2009; Krüger et al., 1992; Youl, Youlden, & Baade, 2013). Melanoma prognosis is also influenced by sex, with female patients showing longer survival than male ones (Joosse et al., 2011), and by the primary melanoma site, with upper trunk, upper arm, neck, and scalp being connected with higher risk of death, and the lower trunk, lower arms, legs, and face being linked to higher survival (C. Garbe et al., 1995).

Melanoma and naevus count share multiple genetic and environmental influences (Bauer et al., 2005; Duffy et al., 2018; Volkovova et al., 2012), and between 30-50% of melanomas arise from a naevus (Shitara et al., 2015; Shitara et al., 2014). The total body naevus count is the most powerful phenotypic marker to predict melanoma risk (Gandini et al., 2005), while naevus counts on the trunk in males and on the lower limbs in females are good predictors of sex-associated differences in melanoma risk (Krüger et al., 1992; Randi et al., 2006; Rodenas et al.,, 1997; Weinstock et al., 1989). Based on the existing epidemiological data and clinical observations, a theory of site-dependent susceptibility of melanocytes to malignant transformation has been proposed (Green, 1992), and studies suggest that different causal pathways may act at different body sites (Olsen et al., 2009; Siskind et al., 2005).This is further supported by the divergent pathway hypothesis (Whiteman et al., 2003) which suggests that in subjects with high melanocytic proliferative capacity (*i.e.,* with high naevus count), the risk of melanoma is increased at those sites with the larger melanocytic population.

Paralleling the sex-associated differences in melanoma incidence, sex-associated differences in the distribution of naevi has been observed in childhood: boys and girls by the age of 10 already show different naevus distribution, with girls having more naevi on the limbs, especially on the legs, and boys having more naevi on the head and neck and on the trunk (Autier et al., 2004; Dodd et al., 2007; Gallagher et al., 1990). Whether these sex-specific differences in naevus distribution are genetically determined or driven by environmental exposure is under debate. The idea that both acute and cumulative sun-exposure contribute to the emergence of naevi is supported by multiple studies assessing naevi number in schoolchildren living at different latitudes, which concordantly observe that children living at the lowest latitudes had significantly more naevi than those in the highest (Green et al., 1988; Fritschi et al., 1994; Sancho-Garnier et al.,1997). Additionally, a study of adolescent twins from the UK suggested that the higher number of naevi in sun-exposed sites in males could be due to a greater recreational sun exposure early in childhood (Wachsmuth et al., 2001), while a study of white young women living in England showed that holidays in countries hotter than the UK were associated with an increased number of naevi, particularly for sites intermittently exposed to sunlight, such as the lower limbs (dos Santos Silva et al., 2009). However, a recent study of Danish outdoor and indoor workers showed that the study participants, despite having different sun exposure, did not show any difference on the number of naevi in the sun-exposed left forearm (Grandahl, et al., 2019). Furthermore, a study of boys and girls in Queensland, Australia, observed a sex-specific difference for the number of naevi in the lower limbs that was not supported by a sex-specific difference in sun exposure (MacLennan et al., 2003).

In order to improve performance of skin cancer prevention policies and campaigns, and to inform cancer research, it is of utmost importance to unravel the relative importance of genetic *vs* environmental factors influencing naevus development at different body sites.

In this study, we analysed a large cohort of healthy twins of European ancestry, predominately female, to investigate factors underlying naevus count variation at four body sites (head and neck, trunk, and upper and lower limbs). First, we tested whether the sex-associated difference in naevi distribution at different body sites observed in childhood is also maintained in adulthood. Then, we estimated the genetic and environmental contributions influencing naevus count variation at the different body sites. Finally, we studied the site-specific association of naevus count distribution with common DNA variants at five loci previously associated, in genome-wide association studies, with both naevus count and melanoma susceptibility, *i.e., IRF4, DOCK8, MTAP*, 9q31.2, *KITLG,* and *PLA2G6* (Duffy et al., 2010, 2018; Falchi et al., 2009).

Materials and Methods

Naevus count in the TwinsUK cohort

The TwinsUK adult twin registry includes about 14,000 subjects, predominantly females, unselected for any specific disease and with similar disease and lifestyle characteristic to the general population (Andrew et al., 2001). St. Thomas' Hospital Research Ethics Committee approved the study, and all twins provided informed written consent. Data on TwinsUK twin participants are available to bona fide researchers under managed access due to governance and ethical constraints. Raw data should be requested via the TwinsUK website (http://twinsuk.ac.uk/resources-for-researchers/access-our-data/), and requests are reviewed by the TwinsUK Resource Executive Committee (TREC) regularly.

3,923 twins with European ancestry underwent a skin examination, which included naevus count, and was performed by trained nurses at St Thomas' Hospital, London. Briefly, the naevus count at different body sites was defined as the sum of all naevi larger than 2mm in diameter. The body sites analysed were: head and neck (H&N), trunk (back abdomen and chest), upper limbs (including shoulder), and lower limbs. The whole-body naevus count was defined as the sum of all naevi at the four aforementioned sites. We excluded from the analysis twins which were reared apart or adopted (N=20), whose zygosity was unknown (N=4), and with no naevus observed in any body sites (N=295), and outliers (N=80, individuals having a whole-body naevus count greater than 3 standard deviation the dataset mean), resulting in 3,524 individuals (N=3,296 and 228, females and males, respectively). The female (male) subset included 1,077 (79) dizygotic twin pairs, 435 (25) monozygotic twin pairs, and 272 (20) singletons (**Supplementary Table 1, Supplementary Figure 1**). We further removed outliers (*i.e.,* measurements 3 standard deviation further than the dataset mean) from each of the tested naevus count and, to ensure the normality of their distribution, naevus count used for the heritability analysis and the genetic association study were quantile normalized.

Difference in sex-associated naevi distribution

From the 228 male twins we randomly selected 123 unrelated individuals which we age-matched to 369 unrelated female individuals using the *matchit* R package (Ho et al., 2011) (version: 3.0.2). We used the Wilcoxon's test to assess whether there was a different distribution of naevi at different body sites between males and females, and considered this difference significant if the obtained P value passed a conservative Bonferroni-derived threshold of $P<0.05/4=0.0125$. To ensure the robustness of our results, we evaluated an empirical p-value using further 1,000 randomly selected sets of age-matched unrelated individuals.

Heritability analysis

We used the classic twin ACE model to estimate the contribution of additive genetic (A), common (C) and individual-specific environment (E) effects on age-adjusted naevus count variation (ACE model) in 1,512 female twin pairs (435 monozygotic and 1,077 dizygotic pairs) and 104 male twin pairs (25 monozygotic and 79 dizygotic pairs). We then compared, using the Akaike's information criterion (AIC), the ACE model with the most parsimonious AE model, which does not include the effect of the common environment (C), and the CE and E models, which hypothesises that the trait variation is completely dependent on the effects of the environment. The models along with their 95% confidence intervals and AIC, were estimated using the *mets* R package (Scheike et al., 2014) (version: 1.2.5).

Genetic association study

Genotyping was performed in 2,864 females from the TwinsUK cohort with a combination of Illumina arrays (HumanHap300, HumanHap610, 1M-Duo and 1.2M-Duo 1M) and imputation was carried out using the Michigan Imputation Server and the Haplotype Reference Consortium (HRC version r1.1) panel (the Haplotype Reference Consortium, 2016). We selected ten SNPs at the *IRF4, DOCK8, MTAP*, 9q31.2, *KITLG,* and *PLA2G6* loci which were previously associated with naevus count (Duffy et al., 2010, 2018; Falchi et al., 2009), and extended this set with 2,435 SNPs in high linkage disequilibrium with them (r^2>0.8, distance limit 500 kb; EUR populations), as detected using LDLink (Machiela & Chanock, 2015)**.** We then used for the association testing 1,836 out of the 2,435 SNPs, which were available in our panel and met the following conditions: call rate ≥95%, minor allele frequency (MAF) ≥1% and Hardy–Weinberg equilibrium test with $P \geq 1 \times 10^{-9}$ (**Supplementary Table 2**). To take into account the non-independence of the twin data, the association with naevus count on the whole body and on the four body sites was evaluated using GEMMA (Zhou & Stephens, 2014) (version 0.97), assuming an additive genetic model and including age at visit and the first five principal components assessed on the genomic data as covariates. Given the high correlation between both the site-specific naevus count and the SNPs in strong linkage disequilibrium, we used the method introduced by Conneely and Boehnke (Conneely & Boehnke, 2007) (P_{ACT}) to calculate adjusted P values accounting for multiple and correlated tests. P_{ACT} yields to the same accuracy attained by permutation tests, providing an unbiased test of the null hypothesis.

Results

The distribution of naevi on the lower limbs varies according to sex

Using data from 123 randomly-selected unrelated male and a subset of 369 age-matched unrelated female members of our cohort (**Supplementary Figure 2**), we observed a sex-associated difference in naevi distribution on the lower limbs, with females showing a statistically significantly higher number of naevi on the lower limbs (mean N=6.7 and 4.1, in females and males, respectively; P = 8.5×10^{-5},

Wilcoxon's test, **Figure 1**), which was confirmed by a permutation test (eP = $5.0x10^{-3}$). Males showed a higher number of naevi on the trunk (mean N=0.48 and 0.94, in females and males, respectively), although this was only nominally significant (P=0.02, Wilcoxon's test) and not supported by the permutation test in this small dataset (eP > 0.05).

The heritability of naevus count varies according to body sites in women

The best fitting model for site-specific heritability of naevus count in women was predominantly the full ACE model (**Supplementary Table 3**), suggesting that both unique (E) and shared environmental factors (C) play a role in its variability. Additive genetic effects accounted for 59% of the variance of the whole body naevus count, while environmental effects explained the remaining 41% (**Table 1**). A highly variable influence of environmental versus additive genetic factors accounted for the variability of naevus count at individual sites, with the trunk showing the lowest genetic influence (A=26%), and the lower limbs showing the largest (A=69%; **Table 1**).

In males, the AIC values indicated a better fit for the more parsimonious AE and CE models (**Supplementary Table 3**), most likely due to the small male sample size. Indeed, while the female subset included 1,512 twin pairs (435 monozygotic and 1,077 dizygotic pairs), the male subset was composed by 104 twin pairs (25 monozygotic and 79 dizygotic pairs). The additive genetic effects accounted for more than 67% of the naevus count variation in whole body and at all the studied sites, apart from the lower limbs, where the naevus count distribution was completely explained by environmental effects (**Table 1**).

The effect of known naevus loci varies according to body sites in females

Using data on 2,864 female individuals for whom both phenotypical and genomic data was available, we confirmed significant associations between naevus count on whole body and *MTAP*, *PLA2G6,* and the 9q31.2 region, but failed to identify associations with *IRF4, DOCK8,* and *KITLG*. We hypothesise that we were not able to replicate the association with *IRF4* because the effect of these variants on the H&N plus trunk and the lower limbs, which include 26% and 31% of the total naevus count, have an opposite direction, therefore nullifying the effect on the whole body. On the other hand, it is likely that *DOCK8* and *KITLG* exert a small effect on naevus count, detectable only by a large sample, as that reached in the recent meta-analysis study of 52,506 individuals that lead to their identification (Duffy et al., 2018).

When studying loci-specific effect on different body sites, we observed that all tested loci apart from *DOCK8, MTAP,* and *KITLG* where significantly associated with the number of naevi on the lower limbs, but only *PLA2G6* was exerting a significant effect on naevus on the trunk (adjP > 0.05, **Table 2**). The naevus distribution on the H&N was associated with DNA variants at the *IRF4* and *MTAP*

loci, while the naevus on the upper limbs was significantly associated with DNA variants at the *DOCK8, MTAP* and *PLA2G6* loci (**Table 2).**

Discussions

Genome-wide association studies have shown that melanoma and naevus count share a common genetic background (Duffy et al., 2018). According to Whiteman's "*divergent pathway hypothesis*", subjects with high melanocyte proliferation are more likely to develop melanoma at those body sites showing high naevus count (Whiteman et al., 2003).

We show, here, that the number of naevi on the lower limbs is almost double in females compared to males. These results are in line with what has been previously observed in children (Autier et al., 2004; Dodd et al., 2007; Gallagher et al., 1990) and adults (Krüger et al., 1992) thus suggesting that a sex-associated difference in naevi distribution on the lower limbs persists throughout life.

We confirmed a strong heritability of 60-70% on whole body naevus count in women (Wachsmuth et al., 2001), and observed sex-specific heritability at different sites. The genetic influence on naevus count was higher on the lower limbs and lower on the trunk (69% and 26%, respectively). In males, significant heritability was detected at all body sites (>67%) apart from the lower limbs, where we could not observe any genetic influence – perhaps due to the small male sample size. Our results support the hypothesis that the larger number of naevi on the female lower limbs is unlikely due to higher sun exposure alone (Bataille, 2013), and indicate that naevus development and persistence on the lower limbs with age is actually under significant sex-specific genetic control.

The association between variants at the *IRF4, DOCK8, MTAP*, 9q31.2, *KITLG,* and *PLA2G6* loci and both naevus count and melanoma development has been largely investigated (Duffy et al., 2010, 2018; Falchi et al., 2009). However, few studies explored whether these variants have a different contribution on melanoma development at different body site (Kvaskoff et al., 2011; Potrony et al., 2017) and none of them explored their effect on naevi distribution. Apart from a weak significant association between *DOCK8* and naevus count in the upper limbs (adjP=0.023), we could not find any other significant association (adjP < 0.05) between naevus count and both *DOCK8* and *KITLG*, most likely due to the small effect these genes exert on naevus count, and that would require a much larger dataset for its identification. However, we showed that, in females, all the other studied loci, apart from *MTAP,* concordantly affect naevus count on the lower limbs, while their effects on the other body sites was locus-specific (**Table 2**). It has been observed, in mixed-sex cohorts, that variants at the *IRF4* locus contribute to melanoma development both via chronic sun exposure and via melanocytic proliferative capacity (Gibbs et al., 2016), and that they influence melanoma survival as well as the site of the primary tumour, increasing the risk of developing melanoma on the H&N but

decreasing the risk of a melanoma on the trunk (Potrony et al., 2017). In this study, *IRF4* variants increased naevus counts on the lower limbs (β=0.21, adjP =$3.7x10^{-5}$) and decreased that on the H&N (β=-0.15, adjP =$1.7x10^{-3}$). A discordant direction of effects of *IRF4* variants on naevus count and melanoma at the H&N could be explained by a sex-associated difference in the incidence rate of tumour at this site, which seems to be more common in males (Kadakia et al., 2016; Sanlorenzo et al., 2014; Youl et al., 2013) who have not been included in our study. We failed to replicate the association between *IRF4* and naevus count on whole body, likely due to different direction of effects at lower limbs versus H&N plus trunk, which may nullify the effect on the total naevus sum. This could also explain the lack of association between *IRF4* and naevus counts observed in another UK study (Newton-Bishop et al., 2010). It has already been observed, in a mixed-sex cohort, that variants at the *MTAP* locus exert an effect on melanoma risk but no evidence of heterogeneity across sites has been detected (Kvaskoff et al., 2011). Here, we confirmed an association with whole body naevus count and with all sites apart from the trunk, which is a less common site for melanoma development in females (Garbe & Leiter, 2009), and the lower limbs. An association between variants in *PLA2G6* and site-specific melanoma has not been previously detected (Kvaskoff et al., 2011; Newton-Bishop et al., 2010). In this study, associations with *PLA2G6* were found for whole-body naevus count and for all body sites excluding H&N, one of the least heritable sites, therefore supporting the hypothesis that genetic variants at *PLA2G6,* at least for naevus count, have a greater effect on sites mostly under genetic influence. The effect of genetic variants at the 9q31.2 locus on neavus count and melanoma has only recently been discovered (Duffy et al., 2018), and no studies have assessed its sex- or body-site specific effect. In this study, we confirmed associations between genetic variants at this locus and the number of naevi in the whole body, and highlight association between this locus and the number of naevi in the lower limb alone.

Unfortunately, the TwinsUK cohort has recruited more females than males, therefore we could not reliably assess the influence of genetic effects on male-specific naevi distribution because of the small sample size for males.

Here, we paved the way for a better understanding of the genetic basis of naevus and melanoma body distribution in women of European ancestry. Our results showed that a high naevus count on the lower limbs, a site believed to be mostly driven by environmental exposure, is actually under genetic control, and suggested that these specific genetic influences on naevus count at different sites may explain differences in site-specific melanoma incidence. These findings are important for informing cancer prevention policymakers, campaigners, and cancer researchers. Indeed. stratification by sex and body site would be advisable for future research on neavus count and melanoma susceptibility, since the genes determining susceptibility to melanoma are highly likely to differ according to these characteristics.

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Figure Legends

Figure 1. Naevus count distribution by sex at different body sites. The mean number of naevi (μ) and the P values, evaluated by means of the Wilcoxon's test, are reported.

Supplementary Figure Legends

Supplementary Figure 1. Age distribution by sex in the entire study sample. Distribution were evaluated for 3,296 females and 228 of European ancestry. The P value was evaluated by means of the Wilcoxon's test.

Supplementary Figure 2. Age distribution by sex in the age-matched dataset. The P value, evaluated by means of the Wilcoxon's test, shows a perfect age-match between male and female set.

Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1. Estimates of the percentage of age-adjusted naevus count variance in different body sites due to additive genetic (A), common (C), and individual-specific (E) environmental effects along with their 95% confidence intervals (CI, in brackets). We reported the estimates for the best fitting model (ACE, AE, CE, E) as evaluated using the AIC criterion. Each model was generated using data collected in 1,512 female twin pairs (435 monozygotic and 1.077 dizygotic pairs) or 104 male twin pairs (25 monozygotic and 79 dizygotic pairs).

Sex	***Body site***	**Model**	**A (95% CI)**	**C (95% CI)**	**E (95% CI)**
	Whole body	ACE	59.0 (47.8, 70.2)	15.9 (6.1, 25.8)	25.1 (21.6, 28.6)
	H&N	ACE	33.5 (16.6, 50.3)	14.5 (2.1, 27.0)	52.0 (45.2, 58.8)
Females	Trunk	ACE	25.9 (11.4, 40.4)	29.6 (18.6, 40.6)	44.5 (38.7, 50.3)
	Upper limbs	ACE	54.4 (41.4, 67.3)	12.5 (1.8, 23.2)	33.1 (28.5, 37.7)
	Lower limbs*	AE	69.0 (65.0, 72.9)	-	31.0 (27.1, 35.0)
	Whole body	AE	70.8 (56.7, 84.9)	-	29.2 (15.1, 43.3)
	H&N	AE	72.1 (56.3, 87.9)	-	27.9 (12.1, 43.7)
Males	Trunk	AE	67.3 (50.6, 84.1)	-	32.7 (15.9, 49.4)
	Upper limbs	AE	76.1 (63.3, 89.0)	-	23.9 (11.0, 36.7)
	Lower limbs	CE	-	55.6 (42.1, 69.2)	44..4 (30.8, 57.9)

**Despite the ACE model being the best model according to the AIC, the estimate for the C component was not significative, and the AE model has been reported instead. For the sake of completeness, the ACE results for the model were: A=57.6% (95%CI: 44.7-70.5%), C=10.2% (95%CI: -0.6-21.1%), E=32.2% (95%CI: 27.8%-36.6%).*

Table 2. Results of the genetic association study 2,864 female twins. For each body site/locus, we report the top-associated SNP, along with its coordinates (build: GRCh37), effect and non-effect allele (A1/A0) and effect allele's frequency (AF), effect size (β), standard error (SE), and association P value evaluated via likelihood ratio tests (P). Adjusted-P values (adjP) were evaluated with P_{ACT} taking into account the number of tested SNPs and body sites (Conneely & Boehnke, 2007).

Locus	Body Site	SNP	Coordinate	A1/A0	AF	β	SE	P	adjP
	Whole body	rs12203592	chr6:396321	T/C	0.19	0.041	0.044	0.350	ns
	H&N	rs62389423	chr6:421281	A/G	0.16	-0.156	0.046	$6.0x10^{-4}$	0.013
IRF4	Trunk	rs3778607	chr6:403799	G/A	0.55	-0.070	0.031	0.024	ns
	Upper limbs	rs62389424	chr6:422631	A/C	0.17	0.064	0.043	0.142	ns
	Lower limbs	rs12203592	chr6:396321	T/C	0.19	0.211	0.045	$2.7x10^{-6}$	$7.5x10^{-5}$
	Whole body	rs581731	chr9:206838	C/A	0.47	-0.073	0.028	$9.4x10^{-3}$	ns
	H&N	rs581731	chr9:206838	C/A	0.47	-0.049	0.028	0.085	ns
DOCK8	Trunk	rs3008099	chr9:177034	G/T	0.36	0.094	0.034	$5.3x10^{-3}$	ns
	Upper limbs	rs581731	chr9:206838	C/A	0.47	-0.090	0.028	$1.2x10^{-3}$	0.023
	Lower limbs	rs471897	chr9:270224	T/G	0.34	-0.046	0.030	0.128	ns
	Whole body	rs7860576	chr9:21714920	C/T	0.48	-0.131	0.029	$4.2x10^{-6}$	$1.1x10^{-4}$
	H&N	rs7852450	chr9:21825075	C/T	0.52	-0.118	0.028	$2.9x10^{-5}$	$7.4x10^{-4}$
MTAP	Trunk	rs7029077	chr9:21682302	C/G	0.14	0.079	0.041	0.057	ns
	Upper limbs	rs6475574	ch9: 21736052	T/C	0.51	-0.112	0.028	$6.3x10^{-5}$	$1.5x10^{-3}$
	Lower limbs	rs7860576	chr9:21714920	C/T	0.48	-0.082	0.029	$5.0x10^{-3}$	ns
	Whole body	rs10816590	chr9:110700471	G/C	0.59	-0.089	0.029	$2.0x10^{-3}$	0.036
	H&N	rs10816591	chr9:110700994	A/G	0.59	-0.059	0.029	0.041	ns
9q31.2	Trunk	rs7026539	chr9:110707964	T/C	0.59	0.041	0.029	0.154	ns
	Upper limbs	rs10816597	chr9:110713179	C/T	0.39	-0.078	0.029	$6.2x10^{-3}$	ns
	Lower limbs	rs10816590	chr9:110700471	G/C	0.59	-0.105	0.030	$3.8x10^{-4}$	$8.4x10^{-3}$
	Whole body	rs1492349	chr12:88854647	A/G	0.92	0.132	0.052	0.011	ns
	H&N	rs78835149	chr12:88804217	C/G	0.27	0.095	0.038	0.013	ns
KITLG	Trunk	rs2639099	chr12:89008364	T/C	0.06	0.106	0.061	0.079	ns
	Upper limbs	rs7486790	chr12:88816594	G/T	0.84	0.107	0.038	$4.8x10^{-3}$	ns
	Lower limbs	rs7974506	chr12:88848006	G/A	0.92	0.138	0.052	$8.3x10^{-3}$	ns
	Whole body	rs132941	chr22:38545942	C/T	0.44	-0.145	0.029	$4.8x10^{-7}$	$1.4x10^{-5}$
	H&N	rs5756914	chr22:38502639	C/T	0.53	-0.077	0.029	$7.5x10^{-3}$	ns
PLA2G6	Trunk	rs2076372	chr22:38474965	T/C	0.28	-0.100	0.032	$2.0x10^{-3}$	0.037
	Upper limbs	rs132972	chr22:38562056	C/T	0.54	-0.104	0.028	$1.9x10^{-4}$	$4.4x10^{-3}$
	Lower limbs	rs132941	chr22::38545942	C/T	0.45	-0.141	0.029	$1.5x10^{-6}$	$4.3x10^{-5}$

ns=not significative (adjP > 0.05)