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Genetic Burden Analyses of Phenotypes Relevant to Aging in the Berlin Aging Study II (BASE-II)

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Key Words
Genetic association · Genome-wide association study · Leukocyte telomere length · Bone mineral density · Body mass index · Weighted genetic risk · Aging · Genomic profile score

Abstract
Background: Body mass index (BMI), bone mineral density (BMD), and telomere length are phenotypes that modulate the course of aging. Over 40% of their phenotypic variance is determined by genetics. Genome-wide association studies (GWAS) have recently uncovered >100 independent single-nucleotide polymorphisms (SNPs) showing genome-wide significant ($p < 5 \times 10^{-8}$) association with these traits. ***Objective:*** To test the individual and combined impact of previously reported GWAS SNPs on BMI, BMD, and relative leukocyte telomere length (rLTL) in ~1,750 participants of the Berlin Aging Study II (BASE-II), a cohort consisting predominantly of individuals >60 years of age. ***Methods:*** Linear regression analyses were performed on a total of 101 SNPs and BMI, BMD measurements of the femoral neck (FN) and lumbar spine (LS), and rLTL. The combined effect of all trait-specific SNPs was evaluated by generating a weighted genomic profile score (wGPS) used in the association analyses. The predictive capability of the wGPS was estimated by determining the area under the receiver operating curve (AUC) for osteoporosis status (determined by BMD) with and without the wGPS. ***Results:*** Five loci showed experiment-wide significant association with BMI (*FTO* rs1558902, $p = 1.80 \times 10^{-5}$) or BMD (*MEPE* rs6532023, $p_{FN} = 5.40 \times 10^{-4}$, $p_{LS} = 1.09 \times 10^{-4}$; *TNFRSF11B* rs2062377, $p_{LS} = 8.70 \times 10^{-4}$; *AKAP11* rs9533090, $p_{LS} = 1.05 \times 10^{-3}$; *SMG6* rs4790881, $p_{FN} = 3.41 \times 10^{-4}$) after correction for multiple testing. Several additional loci showed nominally significant ($p < 0.05$) association with BMI and BMD. The trait-specific wGPS was highly significantly associated with BMD ($p < 2 \times 10^{-16}$) and BMI ($p = 1.10 \times 10^{-6}$). No significant association was detected for rLTL in either single-SNP or wGPS-based analyses. The AUC

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for osteoporosis improved modestly from 0.762 (95% CI 0.733–0.800) to 0.786 (95% CI 0.756–0.823) and 0.785 (95% CI 0.757–0.824) upon inclusion of the FN- and LS-BMD wGPS, respectively. ***Conclusion:*** Our study provides an independent validation of previously reported genetic association signals for BMI and BMD in the BASE-II cohort. Additional studies are needed to pinpoint the factors underlying the proportion of phenotypic variance that remains unexplained by the current models.

Introduction

The human aging process is highly variable, and many, often distinct, phenotypes modulate its course and the burden of illness, e.g. body mass index (BMI), bone mineral density (BMD), and leukocyte telomere length (LTL). The interindividual variability of these phenotypes is governed by a complex interplay of a multitude of genetic and environmental factors. A growing body of literature suggests that genetic factors play a particularly important etiologic role in this network. This is evidenced by recent estimates suggesting that the proportion of phenotypic variance determined by genetics (i.e. the heritability) likely exceeds 40% for BMI [1], BMD [2, 3], as well as LTL [4]. To decipher the underlying genetic architecture, large-scale genome-wide association studies (GWAS) – which allow a simultaneous assessment of several million variable sites in the genome – have been performed for each of these traits [5–7]. For all three traits combined, these studies have uncovered more than 100 independent single-nucleotide polymorphisms (SNPs) showing genome-wide significant association ($p < 5 \times 10^{-8}$) [5–7]. Despite their strong statistical support, the individual effect size of the SNPs associated with BMI, BMD, or LTL is comparatively small [5–7], similar to what is observed in most other genetically complex traits. It is likely via their combined (e.g. additive) action that these variants elicit relevant effects on the traits under study.

Thus, a powerful approach to account for the polygenic nature of genetically complex traits (such as BMI, BMD, and LTL) is to assess the combined effects of trait-influencing SNPs in one statistical model. This can be achieved by constructing a summary variable incorporating the effects of each individual SNP in the form of a weighted genomic profile score (wGPS). The wGPS represents a single number calculated per individual that sums up the effective alleles of all relevant SNPs weighted by their effect sizes. While wGPS-based analyses have been previously applied to BMI [5] and BMD [6], this method has not been used for LTL.

The aim of this study was to investigate the individual (via single-SNP analyses) as well as the cumulative (via wGPS) impacts of all loci previously reported to show genome-wide significant association with BMI, BMD, and LTL in 1,752 participants of the Berlin Aging Study II (BASE-II) [8].

Methods

Participants

This study included a total of 1,752 participants of Caucasian ancestry from Germany whose genotype data and data on at least one of the four phenotypic traits of interest (see below) were available for analysis. All participants were part of the baseline recruitment of BASE-II, a multi-institutional project aimed at investigating factors modulating the aging process [8]. The dataset investigated here comprised a group of 1,376 older (aged 60–88 years) and 376 younger adults (aged 23–39 years; see table 1 for demographic details). Written consent was provided by all BASE-II individuals before participation. The study was approved by the institutional review boards of each participating research unit prior to participant recruitment.

Phenotypes

The phenotypes under study here, i.e. BMI, BMD [of the femoral neck (FN-BMD) and lumbar spine (LS-BMD)], and relative LTL (rLTL) were collected as described elsewhere [9, 10]. In brief, weight and height were assessed with an electronic weighing and measuring station (seca 764; seca, Hamburg, Germany) and rounded to the nearest 0.1 kg or 0.1 cm, respectively. BMI was calculated as weight (kg)/height $(m)^2$. FN-BMD and LS-BMD (representing the mean BMD of lumbar vertebrae 1–4) were recorded using DXA (Hologic Discovery Wi, Software APEX version 3.0.1). rLTL was measured by a modified quantitative PCR protocol [comprising telomere PCR and single copy gene (36B4) PCR] originally described by Cawthon [11]. All samples were measured in triplicate, and their mean was used for further analysis when the Ct (cycle threshold) values of both PCRs showed a variation coefficient <2%. The rLTL was subsequently calculated according to Pfaffl [12]. DNA from 10 randomly selected subjects was pooled and used as the reference (rLTL = 1).

Genotyping, Imputation and Quality Control

DNA was extracted from whole blood using standard procedures and then subjected to microarray-based SNP genotyping using the 'Genome-Wide Human SNP Array 6.0' (Affymetrix Inc.). This was followed by an extensive quality control [using the PLINK software v1.7 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml)] if not indicated otherwise). For quality control, we excluded SNPs that deviated from Hardy-Weinberg equilibrium at $p \leq 5 \times 10^{-6}$ or showed call rates <98%. In addition, we excluded samples with call rates <90%, evidence for sample duplication, relatedness (PLINK pi-hat >0.125) or contamination, inconsistencies between recorded and genotypic sex, excessive heterozygosity (FDR <1%), or those who represented population outliers [i.e. in-

Table 1. Demographic details of all 1,752 BASE-II participants assessed in the genetic association analyses

Variable	All (n = 1,752)	Younger subgroup (n = 376)	Older subgroup (n = 1,376)
Sex			
Female, %	52.0	53.2	51.7
Missing, n	0	0	0
Age			
Mean ± SD	60±17	29±3	68±4
Range	22–84	22–37	60–84
Missing, n	0	0	0
BMI			
Mean ± SD	26±4	23±4	27±4
Range	12–48	12–44	17–48
Missing, n	135	17	118
FN-BMD			
Mean ± SD	–0.72±1.07	–0.02±1.12	–0.91±0.96
Range	–7.65 to 3.69	–7.65 to 3.69	–3.56 to 3.07
LS-BMD			
Mean ± SD	–0.73±1.44	–0.58±1.09	–0.77±1.52
Range	–4.73 to 4.56	–3.90 to 4.03	–4.73 to 4.56
Missing (FN- and LS-BMD), n	167	30	137
Osteoporosis			
Yes/no	188/1,397	13/333	175/1,064
Missing, n	167	30	137
rLTL			
Mean ± SD	1.14±0.23	1.22±0.23	1.13±0.22
Range	0.17–2.13	0.42–2.13	0.17–1.93
Missing, n	134	26	108

A diagnosis of osteoporosis was defined as a BMD measurement value <–2.5 in any of the two sites, i.e. FN and/or LS. SD = Standard deviation.

dividuals who exceed 6 standard deviations along one of the top 10 principal components (PCs)]. The latter was determined using the 'Eigenstrat' function in the EIGENSOFT program [13] with iterative outlier removal. Subsequently, the PCs of the quality-controlled GWAS data of all resulting 2,175 BASE-II participants were recomputed. Based on the examination of scree plots, the first four PCs were retained and used as covariates in the association analysis to account for subtle population admixture. Genome-wide imputation of unobserved genotypes was carried out on the quality controlled data using IMPUTE2 v2.2.2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) [12] based on precompiled 'ALL 1000G Phase1 integrated haplotypes' reference panels (December 2013 release) [14]. A total of 27,213,648 SNPs were imputed, but only autosomal SNPs with an IMPUTE info value ≥0.35 and minor allele frequencies ≥1% were retained for subsequent analyses. Overall, 1,752 samples with nonmissing data for at least one of the four phenotypic traits investigated here (i.e. BMI, FN-BMD, LS-BMD, and rLTL; see table 1 for details) were included in the subsequent genetic association analyses.

Calculation of the wGPS

For the construction of the wGPS, we selected autosomal SNPs previously reported to show genome-wide significant ($p < 5 \times 10^{-8}$) association with BMI, FN-BMD, LS-BMD, and rLTL. SNP selection was based on the largest available GWAS meta-analysis in populations of European ancestry for each trait. Only reports published before January 15th 2015 were considered [i.e. 5–7]. For the wGPS, 101 SNPs were included that showed genome-wide significance in the primary (n = 93 SNPs) as well as conditional (n = 8 SNPs) analyses (note that the latter were not assessed in the single-SNP analyses described below). SNPs that showed genome-wide significant association only after stratification for additional covariates or in other traits related but not identical to the traits of interest in this study were not considered. Following these criteria, 32, 50, 50, and 7 SNPs were identified for BMI [5], FN-BMD [6], LS-BMD [6], and rLTL [7], respectively. Thirty-eight of the BMD-SNPs were identical across both FN- and LS-BMD, suggesting a shared genetic background across these traits, as described in the original publication [6]. For all eligible SNPs, the β-coefficients,

Table 2. Genetic association results of SNPs showing nominally significant association with BMI and BMD in BASE-II

Trait	SNP	Location (hg38)	Locus	A1	A2	MAF	β	SE	p
BMI	rs10938397	4:45,180,510	*GNPDA2*	G	A	0.452	0.286	0.147	0.0259
	rs4836133	5:124,996,410	*LOC101927421*	A	C	0.492	0.313	0.146	0.0162
	rs3817334	11:47,629,441	*MTCH2*	T	C	0.41	0.28	0.145	0.0266
	rs12444979	16:19,922,278	*GPRC5B*	C	T	0.149	0.379	0.204	0.0319
	rs1558902	**16:53,769,662**	***FTO***	**A**	**T**	**0.431**	**–0.607**	**0.146**	**1.80E–05**
	wGPS (32 SNPs)	–	–	–	–	–	–	–	**1.10E–06**
FN-BMD	rs6426749[a]	1:22,384,980	*ZBTB40*	C	G	0.159	0.077	0.045	0.0431
	rs1346004[a]	2:165,744,536	*GALNT3*	A	G	0.491	–0.084	0.032	4.71E–03
	rs430727[a]	3:41,087,073	*CTNNB1*	T	C	0.45	–0.089	0.033	3.71E–03
	rs6532023[a]	**4:87,852,697**	***MEPE***	**T**	**G**	**0.338**	**–0.113**	**0.035**	**5.40E–04**
	rs1366594	5:89,080,244	*MEF2C-AS1*	A	C	0.441	0.1	0.034	1.53E–03
	rs4727338[a]	7:96,491,363	*C7orf76*	C	G	0.328	0.085	0.035	7.50E–03
	rs3801387[a]	7:121,334,711	*WNT16*	A	G	0.272	–0.099	0.038	4.58E–03
	rs2062377[a]	8:118,995,181	*TNFRSF11B*	A	T	0.432	–0.06	0.033	0.0345
	rs7084921	10:100,054,045	*CPN1*	T	C	0.396	0.061	0.034	0.0365
	rs7108738	11:15,688,538	*LOC102724957*	T	G	0.191	–0.074	0.042	0.0391
	rs7932354[a]	11:46,700,671	*ARHGAP1*	T	C	0.297	0.076	0.036	0.017
	rs3736228[a]	11:68,433,827	*LRP5*	T	C	0.131	–0.117	0.049	8.90E–03
	rs2887571[a]	12:1,529,005	*LINC00942*	A	G	0.261	–0.062	0.037	0.0499
	rs1286083[a]	14:90,976,435	*RPS6KA5*	T	C	0.214	–0.078	0.039	0.0233
	rs11623869[a]	14:103,417,296	*MARK3*	T	G	0.382	–0.064	0.033	0.0268
	rs10048146	16:86,677,054	*FOXL1*	A	G	0.190	0.106	0.042	5.60E–03
	rs4790881	**17:2,165,638**	***SMG6***	**A**	**C**	**0.312**	**–0.121**	**0.036**	**3.41E–04**
	wGPS (50 SNPs)	–	–	–	–	–	–	–	**<2E–16**
LS-BMD	rs6426749[a]	1:22,384,980	*ZBTB40*	C	G	0.159	0.175	0.064	3.31E–03
	rs430727[a]	3:41,087,073	*CTNNB1*	T	C	0.45	–0.115	0.048	0.0082
	rs6532023[a]	**4:87,852,697**	***MEPE***	**T**	**G**	**0.338**	**–0.184**	**0.05**	**1.09E–04**
	rs4869742[a]	6:151,586,613	*CCDC170*	T	C	0.277	–0.1	0.052	0.0276
	rs3801387[a]	7:121,334,711	*WNT16*	A	G	0.272	–0.096	0.055	0.0391
	rs2062377[a]	**8:118,995,181**	***TNFRSF11B***	**A**	**T**	**0.432**	**–0.149**	**0.048**	**8.70E–04**
	rs3736228[a]	11:68,433,827	*LRP5*	T	C	0.131	–0.197	0.071	2.72E–03
	rs9533090[a]	**13:42,377,313**	***AKAP11***	**T**	**C**	**0.473**	**–0.149**	**0.048**	**1.05E–03**
	rs1286083[a]	14:90,976,435	*RPS6KA5*	T	C	0.214	–0.121	0.056	0.0159
	rs10048146[a]	16:86,677,054	*FOXL1*	A	G	0.19	0.149	0.06	6.85E–03
	wGPS (50 SNPs)	–	–	–	–	–	–	–	**<2E–16**

This table displays the SNPs that show nominally significant ($p < 0.05$) association with BMI (effective sample size n = 1,617) and FN-/LS-BMD (n = 1,585) in BASE-II. Bold text indicates results surviving multiple testing correction. A1 is the effective allele. The alleles A1 and A2 are annotated on the forward strand according to human genome 38 (hg38) build. Locus = Nearest gene according to RefGene as annotated on the UCSC genome browser (on hg38); note that the nearest gene does not necessarily represent the functional element underlying the genetic association; MAF = allele frequency of the minor allele in BASE-II; SE = standard error.

[a] Genome-wide significant for both FN- and LS-BMD in the original report [6].

corresponding effective alleles, and DNA-strand information were extracted from the corresponding publications [5–7]. Two SNPs (i.e. rs1864325 and rs12821008) were not available in the BASE-II GWAS dataset. For these, we selected suitable proxies (see online suppl. table 1 for details; for all online suppl. material, see www.karger.com/doi/10.1159/000438900). For each trait, the wGPS was calculated using PLINK v1.7 as previously described [e.g. 15, 16]. In brief, for each trait and BASE-II individual, the wGPS represents a sum of the number of effect alleles weighted by their β-coefficient (i.e. their effect size estimate). The β-coefficients used for the wGPS construction were obtained from published GWAS meta-analyses [5–7], which were estimated independently from the BASE-II dataset. Each wGPS was transformed to a z-score using the R language (http://www.r-project.org). Given the trait-specific nature of the approach – it is based on the specific alleles and their specific effect sizes estimated from previous association analyses – the wGPS calculations were performed for each trait separately. This specifically includes the two BMD traits which, although being correlated on the phenotype level, differed in their allelic architecture in the previous GWAS.

Statistical Analysis

Linear regression analyses correlating genotype status at single SNPs with BMI, FN-BMD, LS-BMD, and rLTL were performed using SNPTEST v2.5 (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html). This program takes into account the uncertainty of imputed genotypes. All results were adjusted for PCs 1–4, age, and sex. Analyses on FN-BMD and LS-BMD were also adjusted for weight. Resulting p values for each trait were one-tailed and were corrected for multiple comparisons using the Bonferroni method correcting for number of SNPs per trait (cutoffs: $p_{BMI} = 1.56 \times 10^{-3}$, $p_{FN\text{-}BMD} = 1.11 \times 10^{-3}$, $p_{LS\text{-}BMD} = 1.19 \times 10^{-3}$, $p_{rLTL} = 7.14 \times 10^{-3}$). One-tailed p values were used given the specific nature of our hypothesis, i.e. SNPs were not only probed for their general evidence of association with the trait of interest but also whether or not the direction of effect was the same as previously reported. Linear regression analyses of the z-score-transformed wGPS and each trait were computed using R language and adjusted for the same covariates as in the single-SNP analyses. The performance of the predictive capability of the FN- and LS-BMD wGPS for osteoporosis status was quantified by calculating the area under the receiver-operating characteristics curves (AUC) using R. The AUC was calculated based on age, sex, weight, and PCs 1–4 with and without the wGPS. Potential differences in the association findings across the 'old' versus 'young' BASE-II individuals were assessed by calculating the association statistics in each subgroup separately and testing for an interaction of age group with SNP genotype (last column in trait-specific analyses in online supplementary table 4). The power to detect the previously described effect sizes at the trait-specific Bonferroni-corrected α-levels (see above) was calculated using Monte-Carlo simulations (1,000 iterations) for each trait-SNP combination (see online suppl. table 3). To the best of our knowledge, there is currently no established method to estimate power for wGPS-based analysis.

Results

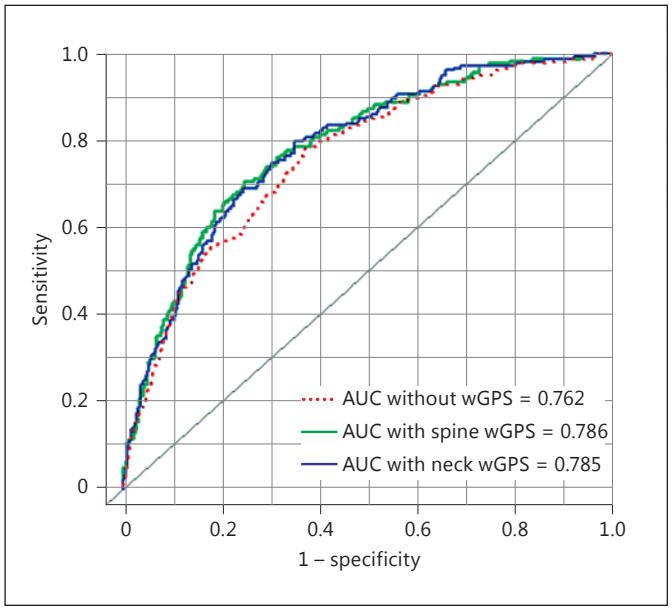
Across all loci that were genome-wide significantly associated with BMI, BMD, or rLTL in previous GWAS [5–7], five loci showed significant association with BMI or BMD in BASE-II after correction for multiple testing (table 2), despite generally relatively modest power to detect the previously reported effect sizes (online suppl. table 3). SNP rs1558902 in *FTO* (analyzed for association with BMI) showed the most significant association in these analyses ($\beta = 0.607$, $p = 1.80 \times 10^{-5}$). For BMD, four loci showed evidence for association after multiple testing correction. This included rs6532023 in *MEPE* associated with both BMD measurements ($\beta_{FN} = 0.113$, $p_{FN} = 5.40 \times 10^{-4}$ and $\beta_{LS} = 0.184$, $p_{LS} = 1.09 \times 10^{-4}$), and rs2062377 in *TNFRSF11B* with LS-BMD ($\beta_{LS} = -0.149$, $p_{LS} = 8.70 \times 10^{-4}$); this SNP was also nominally associated with FN-BMD. SNP rs9533090 in *AKAP11* showed significant association with LS-BMD ($\beta_{LS} = -0.149$, $p_{LS} = 1.05 \times 10^{-3}$), and rs4790881 in *SMG6* was associated with FN-BMD ($\beta_{FN} = 0.121$, $p_{FN} = 3.41 \times 10^{-4}$; table 2, online suppl. table 1). Several additional loci showed nominally significant ($p < 0.05$) association with BMI (4 SNPs) and FN-/LS-BMD (15 and 7 SNPs, respectively; table 2), with effect estimates pointing in the same direction as described in the original publications [5, 6]. In contrast to BMI and BMD, none of the seven loci that were previously reported to show genome-wide significant association with rLTL [7] elicited any statistically significant signals in BASE-II (online suppl. table 1). Interestingly, a substantial (i.e. 76%) proportion of the remaining (nonsignificant) SNPs across all traits showed effect estimates pointing in the same direction as in the original reports [5–7]; this included five of the seven rLTL loci (online suppl. tables 1, 2). Comparing each single-locus association result across both BASE-II age groups showed only sporadic evidence for differences in effect size estimates (online suppl. table 4). The most notable exception was observed in the rLTL analyses for SNP rs7675998, which showed a relatively pronounced effect size estimate in the subgroup of young ($\beta = -0.0578$, standard error = 0.01987) but not old ($\beta = 0.0173$, standard error = 0.0107) individuals ($p_{interaction} = 0.000296$).

The trait-specific wGPS, summarizing the overall genetic burden per individual, was highly significantly associated with both BMD traits ($p < 2 \times 10^{-16}$; table 2) and BMI ($p = 1.10 \times 10^{-6}$; table 2), although the proportion of variance explained by the respective wGPS was relatively small (BMI: 1.2%, FN-BMD: 3.9%, LS-BMD: 5.0%). No significant association was detected between the wGPS and rLTL ($p = 0.702$). This result did not change upon restricting the analyses to the 1,376 BASE-II participants aged 60 and older ($p = 0.669$).

Finally, we assessed the predictive capability of the BMD wGPS on osteoporosis status across 188 BASE-II participants with a clinical diagnosis of osteoporosis (defined as FN- and/or LS-BMD ≤–2.5) and 1,397 unaffected BASE-II controls. The AUC was 0.762 [95% confidence interval (CI) 0.733–0.800] for osteoporosis when including only age, sex, weight, and the first four PCs into the model. It increased to 0.786 (95% CI 0.756–0.823) and 0.785 (95% CI 0.757–0.824) after including the FN- and LS-BMD wGPS, respectively (fig. 1), although this difference was not statistically significant.

Discussion

Our study represents the first genetic assessment of BMI, BMD, and LTL in the BASE-II cohort, and one of the first studies of its kind in a predominately elderly pop-



Color version available online

Fig. 1. Receiver-operating characteristics (ROC) curve for osteoporosis status in BASE-II with and without inclusion of the wGPS. This figure displays the ROC curves for osteoporosis status across 188 BASE-II participants with a clinical diagnosis of osteoporosis and 1,397 unaffected BASE-II controls. The red line displays the ROC curve with inclusion of age, sex, weight, and the first three PCs. The green and blue lines represent the ROC curves after addition of the wGPS for SNPs known to impact LS-BMD and FN-BMD. As can be seen, the AUC improves modestly after addition of either wGPS.

ulation worldwide. Beyond highlighting several genetic loci individually associated with BMI and BMD in our dataset, our study demonstrates that many additional loci, though not reaching statistical significance on their own, still significantly influence phenotypic variance when combined in one statistical model by wGPS. Similar observations have been made for many other polygenic phenotypes, e.g. schizophrenia [15], intracerebral hemorrhage [17], or cardiovascular disease [18]. Except for rLTL, the statistical support resulting from the wGPS-based analyses exceeded that resulting from single-locus analyses in each case. Unsurprisingly, this was most pronounced for BMD where the largest number of SNPs was included to construct the wGPS. A reflection of this observation is the substantial proportion of BMI and BMD loci that showed the previously described [5, 6] direction of effect among all nonsignificant loci examined in this study. Altogether, our analyses provide compelling independent support for the genetic findings reported previously for BMI and BMD.

In contrast to the highly supportive results for BMI and BMD, we did not detect significant association signals with SNPs previously reported to show association with rLTL [7] in our dataset, neither in single-locus nor wGPS analyses. This lack of independent replication can be due to several reasons: first, limited power to detect the modest effects of previously described [7] SNPs on telomere length. However, the effect sizes reported for rLTL were comparable to those reported for BMI or BMD, which did not hamper our ability to confirm many of the previously reported associations reported for these latter traits. Also, as can be seen from our BMI and BMD analyses, power can be substantially improved by combining SNP effects via wGPS. Although wGPS-based analyses also failed to show association with rLTL in our cohort, it needs to be emphasized that, to date, only 7 SNPs have been reported to show genome-wide significant association with rLTL [7] and were thus included in the wGPS (compared to 32 for BMI and 50 for each BMD trait). Thus, the gain in power by utilizing the wGPS for rLTL is much more modest than for the other two traits. Finally, our inability to detect significant genetic signals with rLTL could be owing to differences in dataset composition between previous studies and BASE-II, e.g. in terms of age or general health status) and differences in the experimental procedures used to determine rLTL. Both factors could result in capturing different sets of genes influencing rLTL in our study compared to the original report [7]. Notwithstanding these considerations, we observed identical effect directions in 5 out of the 7 rLTL SNPs tested. It will be interesting to see how the results of our analyses (in particular those of the wGPS) change once additional SNPs are uncovered by larger GWAS.

Despite the strong statistical support underlying the wGPS-based analyses in BMD, the predictive capability of osteoporosis status improves only modestly over models not including genetics. This is quite typical for genetic studies of complex traits and suggests that our genetic models and knowledge for these traits are still very limited. This is in line with the observation that although heritability for all three traits analyzed here is high (possibly up to 88% for BMD [3]), the proportion of variance explained by genetics – even when considering all 'top' GWAS signals simultaneously via genetic burden analyses – remains small (here ~4–5% in BMD). This discrepancy (sometimes referred to as 'missing heritability of complex traits') [19] highlights the fact that much remains to be learned about the heritable genetic (and epigenetic and nongenetic) factors underlying susceptibility for disease and interindividual trait variation in humans.

In summary, our study is one of the first comprehensive genetic assessments of aging-relevant traits using genome-wide SNP data in a cohort predominantly consisting of individuals aged >60 years, and the first to assess genetic burden for telomere length. Our data not only provide compelling independent support of previous GWAS results in BMI and BMD, but also highlight the added value of performing genetic burden analyses in complex traits. It remains to be seen which other genetic, epigenetic, and nongenetic variables will lead to further improvements in our ability to predict disease status based on genetics. Planned extensions of the genetics aims of BASE-II include the application of next-generation sequencing and DNA methylation profiling to assess the role of other DNA variants (e.g. structural, rare) and epigenetics in the phenotypes of interest, analyses to characterize the genetic factors determining the rate of change over time using longitudinal phenotyping data, and assessments to delineate genetic pathways potentially shared across different phenotypes. The systematic evaluation of these and other topics, in combination with data from other similar-minded projects outside BASE-II, will eventually lead to a deeper general understanding of the genetic forces underlying the human aging process.

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