Genetic correlations: falls, muscle, bone and fracture

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ABSTRACT

Both extrinsic and intrinsic factors predispose older people to fall. We performed a genome-wide association analysis to investigate how much of an individual's fall susceptibility can be attributed to genetics in 89,076 cases and 362,103 controls from the UK Biobank Study. The analysis revealed a small, but significant SNP-based heritability (2.7%) and identified three fall-associated loci ($P \leq 5x10^{-8}$). Polygenic risk scores in two independent settings showed patterns of polygenic inheritance. Risk of falling had positive genetic correlations with fracture risk, identifying for the first time a pathway independent of bone mineral density. There were also positive genetic correlations with insomnia, neuroticism, depressive symptoms, attention deficit hyperactivity disorders, and different medication traits. Negative genetic correlations were identified with muscle strength, intelligence and subjective well-being. Brain, and in particular cerebellum tissue, showed the highest gene expression enrichment for fall-associated variants. Overall, despite the highly heterogenic nature underlying fall risk, a proportion of the susceptibility can be attributed to genetics.

INTRODUCTION

Falls are a growing healthcare problem in older adults. They are a major contributor to immobility and premature nursing home placement[1]. Furthermore, they are a leading cause of unintentional injuries, which require medical treatment[2], and increase the demand on healthcare resources. At present, between 0.85-1.50% of the total health care expenditures in Europe, North America, and Australia are fall-related costs[3]. As the global population continues to grow and become older, healthcare costs related to falls will grow accordingly[4].

There are numerous extrinsic and intrinsic factors predisposing older adults to fall that have been intensively studied in the past decades[5,6,7]. A number of the intrinsic ones, particularly postural balance, gait speed, muscle function and cognition have a recognized heritable component[8,9,10], suggesting that investigation into the genetic influence on falls may be warranted. Pharmacogenetic variability may also contribute to drug-induced falls as a result on the variability of drug responses and risk of adverse effects of medications[11,12]. Twins studies have found that familial factors, consisting of genetic and shared environmental influences, explain about 35% of the variability in the likelihood of experiencing at least one incident fall and 45% of the variability in the risk for recurrent falls[13]. Genetic variation is stable across the human lifespan and identification of genetic factors for falling may help optimizing effective fall prevention programs i.e., improved fall risk stratification; while also, providing biological insight into their etiology. So far, few studies have been performed to identify genetic factors underlying fall risk, likely due to lack of a well-powered discovery setting. In a candidate-gene study without replication (N_{cases}=955; N_{total}=4,163), Judson *et al* [14] reported that female carriers of the *ACTN3* genetic variant (rs1815739), which is associated with reduced muscle mass and force, had 33% higher risk of falling compared to non-carriers. However, to date, no genome-wide associations studies (GWAS) have been performed to identify genetic variants associated with increased fall risk in a hypothesis-free context.

To better understand the genetic architecture of falls, we undertook the first large-scale GWAS to determine the heritability of fall risk, identify genetic variants associated with falling, their underlying biology, and to investigate the relationship with fall-associated conditions and traits.

RESULTS

Genome-wide Association Study of Falling

Our study included data from 451,179 (89,076 cases) white European individuals (40-69 years) from the UK Biobank. We tested 7,745,390 million variants (minor allele frequency (MAF) >0.01, imputation quality >0.3) for association with fall risk. LD-score regression showed no sign of genomic inflation (Intercept=1.01) compatible with a polygenic architecture of the trait and no evidence for stratification. We identified two loci associated with fall risk mapping to 7p21.3 near *PER4* (rs2709062-A, OR=1.03, $P=3.4x10^{-8}$) (**Figure 1A**) and 19q12 near *TSHZ3* (rs2111530-G, OR=1.03, $P=1.2x10^{-8}$) (**Figure 1B**). Moreover, 58 SNPs were associated at $P<5x10^{-6}$ of which 15 were associated at genome-wide suggestive level (sGWAS, $P<5.0x10^{-7}$) (**Table 1, Supplementary Figure 1**). LD-score regression showed no sign of genomic inflation (Intercept=1.01) compatible with a polygenic architecture of the trait and no evidence for stratification (**Supplementary Figure 2**). Using a genome-wide gene-based approach, implemented by MAGMA, we identified nine fall associated genes (**Supplementary Figure 3**); the MAGMA gene-set results were later served as an input for the tissue expression analysis. The majority of the SNPs with $P<5x10^{-6}$ (**Supplementary Figure 4A**) were located in intergenic or intronic regions, and >70% of the variants overlapped chromatin state annotations (**Supplementary Figure 4B**) of potential involvement in gene regulation however, only 3.3% (regulomeDB score≤2) possessed strong regulatory potential (**Supplementary Figure 4C**). The 7p21.3 risk locus did not harbor genes with relevant eQTL and/or chromatin interactions (**Supplementary Figure 5A**). The *TSHZ3* gene at the 19q12 locus was annotated by eQTLs in the thyroid tissue and was also implicated by chromatin interactions in the mesendodermal tissue and the mesenchymal stem cells (**Supplementary Figure 5B**). However, none of the lead SNPs showed any evidence for eQTL effects. Finally, the lead SNP on locus 5q21.2 was previously associated with variety of traits such as insomnia, depression and neurotism. In addition, several suggestive SNPs were associated with body composition measures such as BMI, fat mass and fat free mass (**Supplementary Table 1**).

Replication

We followed for replication the 17 suggestive GWS SNPs ($P<5.0x10^{-7}$) from the discovery sample in two smaller and older prospective population-based studies, namely the Rotterdam Study (1,009 cases and 4,925 controls) and B-PROOF (1,206 cases and 1,364 controls) cohorts. The B-PROOF Study is a clinical trial on B-vitamin supplements in older adults of advanced age (mean age 74.1±6.5 years) in which fall risk was assessed using retrospective questionnaires at baseline and prospective fall calendars; while the Rotterdam Study is a population-based cohort with fall infor-

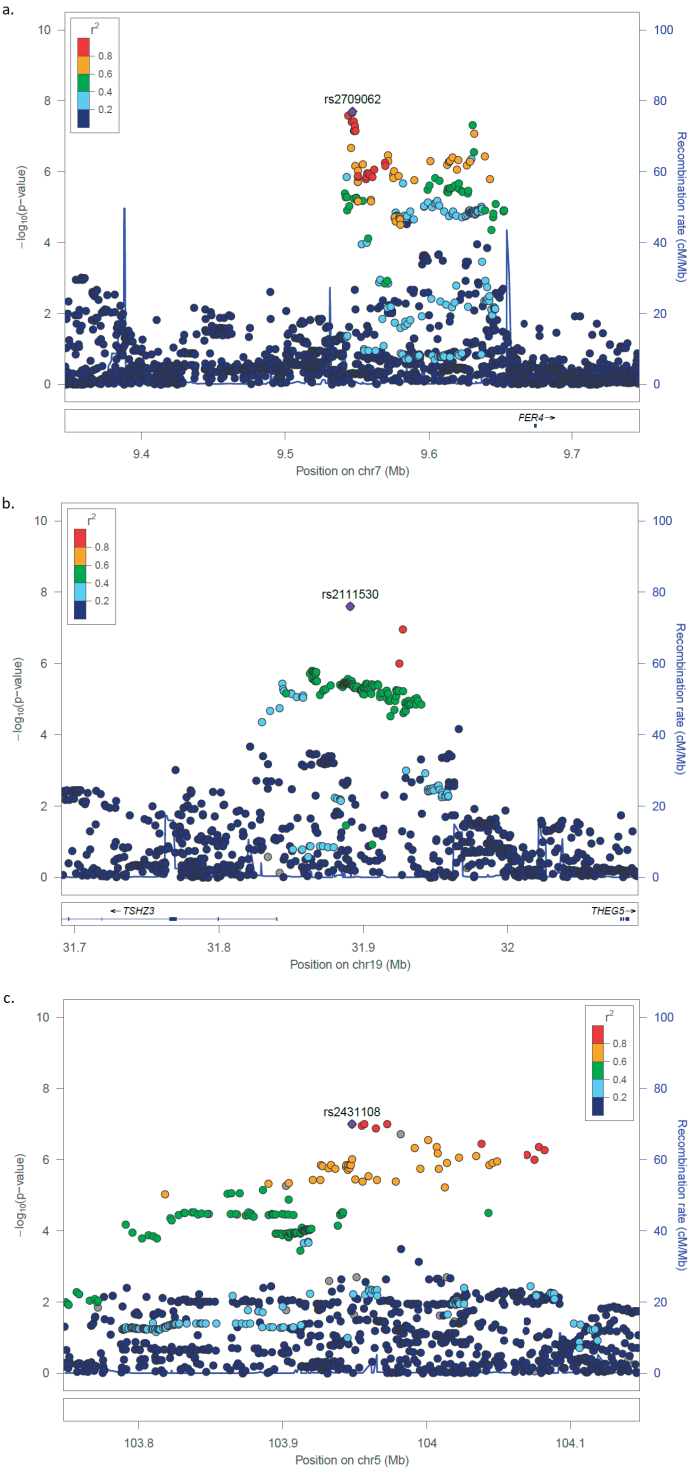

Figure 1 | Regional association plots displaying 7p21.3 (A), 19q12 (B) and 5q21.2 (C) loci. In each plot, the $-\log_{10}$ of p values are on the left y-axis; the SNP genomic position (hg19) on the x-axis; the estimated recombination rate from 1000 genomes (March 2015 EUR) are on the right y-axis and plotted in blue. SNPs are colored red to reflect linkage disequilibrium (LD) with the most significant SNP in purple (pairwise r^2 from 1000 genomes March 2014 EUR).

Table 1 | Lead SNPs of loci associated with increased risk of falling ($p<5x10^{-7}$)

Locus	Annotation	Closest gene	Position	SNP	EA	NEA	EAF	UKBB N=89,076/362,103		Rotterdam Study N= 1,009/4,925		B-PROOF N=1,206/1,364		Combined N=91,219/368,392	
								OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	p
Genome-wide significant loci ($P\leq5x10^{-8}$)															
5q21.2		*RP11-6N13.1*	103947968	rs2431108	C	T	0.33	1.03 (1.02-1.04)	$9.9x10^{-8}$	1.13 (1.03-1.25)	0.01	0.98 (0.88-1.10)	0.77	1.03 (1.02-1.04)	$4.20x10^{-8}$
7p21.3	intergenic	*PER4*	9546806	rs2709062	A	G	0.50	1.03 (1.02-1.04)	$2.4x10^{-8}$	1.08 (0.98-1.19)	0.12	1.13 (1.01-1.26)	0.03	1.03 (1.02-1.04)	$4.04x10^{-9}$
19q12	intergenic	*TSHZ3*	31891006	rs2111530	G	A	0.39	1.03 (1.02-1.04)	$2.5x10^{-8}$	1.00 (0.91-1.11)	0.88	1.08 (0.86-1.18)	0.20	1.03 (1.02-1.04)	$1.82x10^{-8}$
Genome-wide suggestive loci ($P<5x10^{-7}$)															
1p13.3	intergenic	*NTNG1*	107666942	rs76259395	A	G	0.03	1.03 (1.02-1.05)	$6.6x10^{-8}$	1.03 (0.90-1.19)	0.64	1.02 (0.82-1.26)	0.86	1.03 (1.02-1.05)	$6.46x10^{-8}$
1p13.2	intronic	*FAM212B*	112274162	rs6658723	T	C	0.02	1.02 (1.01-1.03)	$2.6x10^{-7}$	1.00 (0.91-1.11)	0.96	1.01 (0.90-1.11)	0.93	1.02 (1.01-1.03)	$3.14x10^{-7}$
2p16.1	intergenic	*EIF3FP3*	59295476	rs67174662	A	G	0.02	1.02 (1.01-1.03)	$5.0x10^{-7}$	0.99 (0.90-1.10)	0.91	1.02 (0.91-1.14)	0.77	1.02 (1.01-1.03)	$5.56x10^{-7}$
2p16.1	intergenic	*BCL11A*	60333030	rs974135	T	C	0.33	1.02 (1.02-1.03)	$3.9x10^{-7}$	0.96 (0.87-1.07)	0.51	0.94 (0.83-1.06)	0.34	1.02 (1.01-1.03)	$9.45x10^{-7}$
3p14.2	intronic	*FHIT*	60138226	rs7616516	A	G	0.07	1.05 (1.03-1.06)	$2.3x10^{-7}$	0.95 (0.79-1.14)	0.56	0.99 (0.85-1.16)	0.92	1.04 (1.03-1.06)	$3.49x10^{-7}$
5p35.2	intergenic	*DRD1*	174888896	rs2471020	C	T	0.59	1.02 (1.01-1.03)	$4.8x10^{-7}$	1.07 (0.97-1.17)	0.20	1.03 (0.92-1.16)	0.61	1.02 (1.01-1.03)	$2.43x10^{-7}$
6p21.1	intronic	*TRERF1*	42360455	rs72857666	T	C	0.03	1.07 (1.04-1.10)	$1.5x10^{-7}$	0.83 (0.61-1.14)	0.26	0.96 (0.68-1.36)	0.81	1.07 (1.04-1.09)	$2.85x10^{-7}$
7p21.3	intergenic	*NXPH1*	9629549	rs12666565	C	T	0.18	1.03 (1.02-1.04)	$4.7x10^{-7}$	1.06 (0.93-1.21)	0.41	1.13 (0.98-1.31)	0.10	1.03 (1.02-1.04)	$1.77x10^{-7}$

Table 1 | Lead SNPs of loci associated with increased risk of falling ($p<5x10^{-7}$) (*continued*)

Locus	Annotation	Closest gene	Position	SNP	EA	NEA	EAF	UKBB		Rotterdam Study		B-PROOF		Combined	
								N=89,076/362,103		N= 1,009/4,925		N=1,206/1,364		N=91,219/368,392	
								OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	p
11p14.1	intergenic	*BDNF*	27643725	rs11030084	C	T	0.03	1.03 (1.02-1.04)	$1.1x10^{-7}$	1.10 (0.96-1.25)	0.16	0.96 (0.91-1.20)	0.55	1.03 (1.02-1.04)	$8.92x10^{-8}$
11p14.1	intronic	*MPPED2*	30492581	rs494221	A	G	0.03	1.03 (1.02-1.04)	$6.7x10^{-8}$	0.98 (0.88-1.08)	0.68	0.88 (0.78-0.98)	0.02	1.02 (1.01-1.03)	$3.01x10^{-7}$
11p15.5	intronic	*TSPAN4*	855372	rs28672671	A	G	0.03	1.03 (1.02-1.04)	$5.1x10^{-8}$	0.95 (0.83-1.07)	0.27	0.89 (0.78-1.01)	0.08	1.03 (1.02-1.04)	$2.23x10^{-7}$
14.q21.2	intergenic	*RPL10L*	46984874	rs12884871	C	T	0.03	1.03 (1.02-1.04)	$1.4x10^{-7}$	1.05 (0.94-1.14)	0.37	0.97 (0.86-1.09)	0.59	1.03 (1.02-1.04)	$1.44x10^{-7}$
19q12		*ZNF536*	30772256	rs28633123	T	C	0.21	1.03 (1.02-1.04)	$9.4x10^{-8}$	1.65 (0.93-1.20)	0.37	1.00 (0.87-1.16)	0.96	1.03 (1.02-1.04)	$7.29x10^{-7}$
20q11.23	intronic	*CTNNBL1*	36382855	rs6063547	G	T	0.03	1.03 (1.02-1.04)	$6.8x10^{-8}$	1.06 (0.92-1.21)	0.44	0.99 (0.85-1.16)	0.92	1.03 (1.02-1.04)	$6.45x10^{-8}$

Footnote: lead SNP is defined as SNP with the lowest p-value. EA=effect allele; NEA: non-effect allele; EAF=effect allele frequency; OR= odds ratio; CI= confidence interval; p= p value of the SNP-falls association.

mation from participants (mean age 69.5±9.2 years) collected retrospectively from baseline questionnaires. We considered loci to replicate if they passed the nominal ($P<0.05$) significant threshold in the replication effort or the GWS ($P<5x10^{-8}$) in the combined meta-analysis. The top two SNPs from the discovery phase remained GWS significant in the combined meta-analysis, which also brought one additional locus (5q21.3) mapping to *RP11-6N13.1* above the GWS threshold (**Table 1; Figure 1C**). The new locus did not harbour genes with relevant eQTL and/or chromatin interactions (**Supplementary Figure 5C**).

Polygenic Risk Scores

Next, we evaluated the ability of polygenic risk scores (PRSs) constructed from the UK Biobank GWAS results to discriminate between fallers and non-fallers in two independent prospective cohorts. We hypothesized falling risk to follow a polygenic mode inheritance, i.e., is influenced by numerous genes with small individual effects and, hence, non-GWS SNPs may also contribute to the genetic component of falling risk. Therefore, PRS were constructed using PRSice[15] for a series of P-value thresholds ranging from $5x10^{-8}$ to 1. Variants ($P<0.05$, MAF>0.05 and imputation quality>0.3) were clumped before analysis ($r^2 < 0.1$, window: 300Kb) to obtain the most significant SNP in the locus. In line with polygenic inheritance, the PRS explained a small, but robust, proportion of the trait variance (max R^2=0.29%) along different P-value thresholds. In the BPROOF Study, the PRS derived from GWS variants ($P\leq 5x10^{-8}$) was associated with prospective falls (reported by fall calendar) and explained the largest fraction of the trait variance (max R^2=0.29%) (**Figure 2A**). In contrast, the PRSs constructed from variants in the lower significance thresholds ($P \leq 5x10^{-3}$) were the ones more strongly associated with retrospectively collected falls (reported by baseline questionnaires) (**Figure 2B and 2C**).

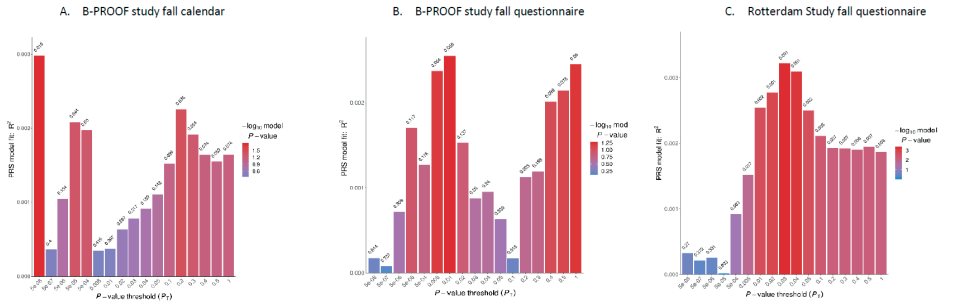

Figure 2 | Association of falls PRS adjusted for age and sex across several different p-value thresholds (x-axis) within two different populations (A. B-PROOF study falls calendar; B. B-PROOF Study falls questionnaire and C. Rotterdam Study falls questionnaire). The number on top of the bars represent p values of the association between the score and fall risk. Color scale: $-\log_{10}$ of P-value.

Individual and Shared Heritability of Falls

We then used LD-Score Regression (LDSR) to estimate the heritability (individual and shared) between falls and different diseases and traits[16]; We considered traits that are closely phenotypically related with fall risk such as musculoskeletal, neurological, psychological ones and use of a variety of medications. As expected, the SNP-based heritability of falls was low (h2=0.027, SE=0.002). In relation to other traits (**Figure 3**), falling had positive genetic correlation with fracture (r_g=0.45, SE=0.05) and was negatively genetically correlated (r_g=-0.17, SE=0.04) with muscle strength. Of particular note, there was no evidence of any genetic correlation between fall risk with bone mineral density or lean mass. We also used LDSR to explore whether falls are genetically correlated with a range of neurological, psychiatric and behavioral traits (**Figure 3**). Falls were strongly positively correlated with insomnia, neuroticism, depressive symptoms and attention deficit hyperactivity disorders (ADHD). We observed a small to moderate negative correlation with intelligence/IQ (r_g=-0.12, SE=0.04) and subjective well-being (r_g=-0.29, SE=0.05) (**Figure 3**). Medication use is a well-established risk factor for falls, either directly (affecting balance, attention or muscle tone) or indirectly (as proxies of underlying conditions influencing the risk of falling; joint pain, arthrosis, cardiovascular diseases among many others). A recent GWAS in approximately 320,000 individuals from the UK Biobank[17], identified 505 independent genetic loci associated with medication use grouped across 23 categories. Using this

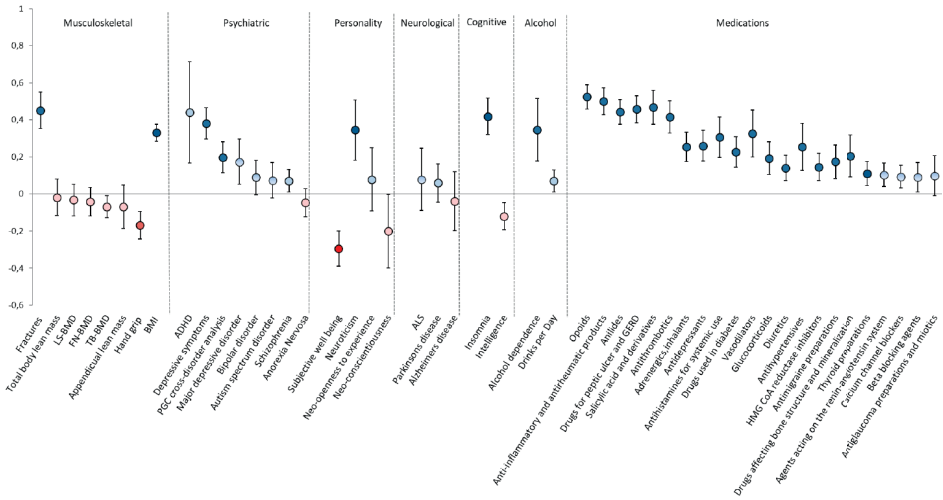

Figure 3 | Estimates of the genetic correlation between falls and different traits and medications. The bars around the point estimates represent confidence intervals. The blue colored point estimates indicate a positive whereas the red colored point estimates a negative genetic correlation. The lighter shades are indication of non-significant correlation after correcting for multiple testing (p=0.05/49=0.001).

data, we observed positive genetic correlations between fall risk and use of medication such as opioids, anti-inflammatory and anti-rheumatic drugs, anilids and drugs for peptic ulcer and gastro-esophageal reflux disease.

Enrichment of Gene Expression Across Tissues

Further, to quantify the enrichment of the falls-associated SNPs signals across different tissues we used an extension of LDSR, namely *Stratified Linkage Disequilibrium Score Regression* (LDSC-SEG) and identified significant enrichment confined to tissues from the central nervous system and particularly those derived from the cerebellum (**Figure 4A**). We then used *Generalized gene-set analysis of GWAS data* (as implemented in *MAGMA*[18]) and also identified significant enrichment of the signals confined to gene

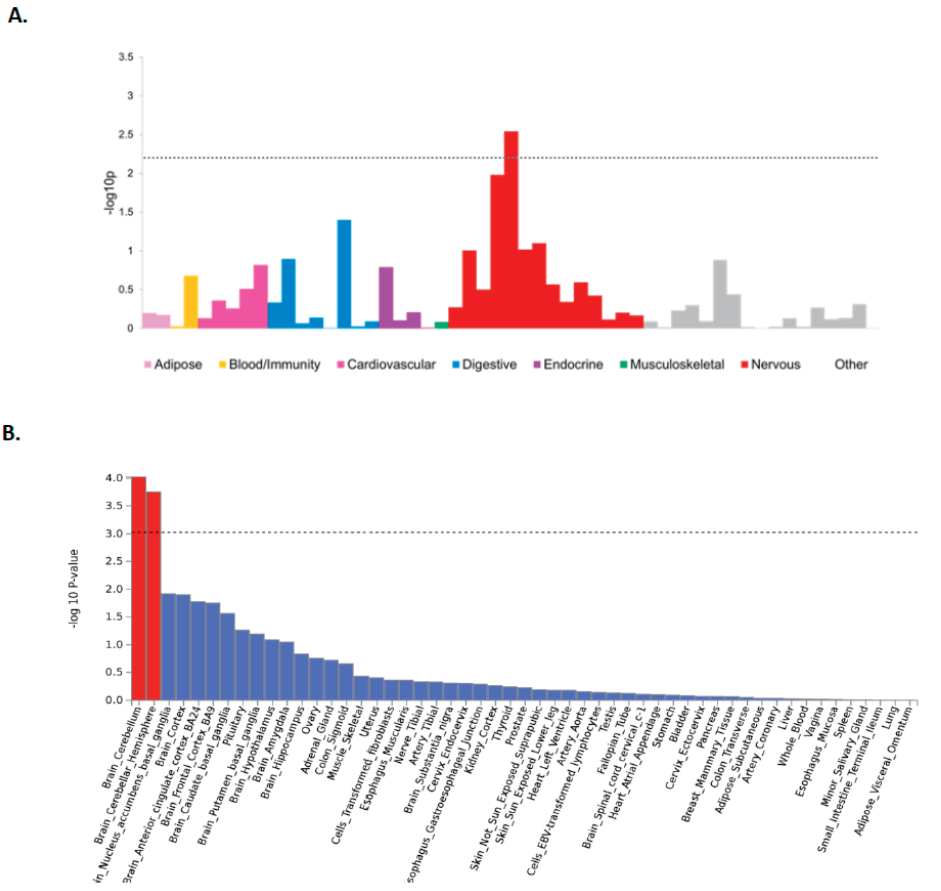

Figure 4 | GTEx brain tissues were positively enriched for falls-associated variants. A. Heritability partitioning enrichment estimates across tissue groups using LD-SEG; B. Tissue enrichment analysis using MAGMA. The most enriched brain tissue is the cerebellum

expression in cerebellar tissue (**Figure 4B**). The latter findings indicate that biological processes related to movement control of limbs, locomotion, adaptation of posture and dynamic regulation of balance originated at the cerebellum could play a role shaping the complex mechanisms underlying fall risk.

Mendelian Randomization

We then tested whether the association between seven risk factors and falls was causal by using genetic factors as instruments within a two-sample Mendelian Randomization approach. Although there are many determinants and conditions that influence the risk of falling, well-powered GWASs allowing the use of adequate genetic instruments were available for *alcohol consumption*[19], *alcohol dependence*[20], *body mass index* (*BMI*)[21], and *relative hand grip strength* defined as the average of measurements of right and left hand divided by weight [22]. In addition, we evaluated the effect of antihypertensive medication use on fall risk using genetic variants mapping to target genes for several antihypertensive drugs. For this purpose we used genetic instruments for antihypertensive drug use that were recently created by Gill et al[23]. These antihypertensive drug medications showed no significant evidence for a causal effect. Alcohol dependence was nominally associated with increased fall risk (OR=1.04, 95%CI=1.01 to 1.08, P=0.03) but not after Bonferroni correction. Alcohol consumption also showed no significant evidence for a causal effect on falls. We did find evidence for a causal effect of BMI (OR=1.13, 95%CI=1.06 to 1.20, P<0.0001) and relative hand grip strength (OR=0.41, 95%CI=0.23 to 0.41,P<0.0001) on fall risk (**Supplementary Table 2**), suggesting that interventions targeted at improving muscle function and weight control may be successful at decreasing falling risk.

DISCUSSION

To our knowledge, this is the first GWAS for fall risk performed to date. Our findings indicate that fall risk is an extremely heterogeneous polygenic trait with large environmental influence. Despite such complex genetic architecture, we were able to identify variants in three loci mapping to chromosomes 5q21.2, 7p21.3 and 19q12. Polygenic risk scores were associated with falling risk in two independent population-based settings. On aggregate, associated markers show significant enrichment for genes expressed in cerebellar tissue providing insight into potential mechanisms mediating fall risk. Shared genetic variation with fracture risk, muscle strength, medication use and other risk factors suggests potential pleiotropic relationships and common biological pathways could mediate diverse aspects of falling risk.

Overall, falls are multifactorial in origin and many different pathways can contribute to the individual propensity to fall. Given the polygenic nature of fall risk, it is expected that a large number of genes influence the risk of falling, each with a very small contribution. Therefore, large-scale analysis such as the present UK Biobank study, are required to discover such real, but weak genetic associations. Additionally, the low heritability of fall risk in our study indicates that there is a strong environmental component underlying the risk of falling. On the other hand, in twins studies 35% of the variability in the likelihood of at least one incident fall and 45% of the variability in the risk for recurrent falls was attributed to genetic factors[13]. Notably, it has been shown that heritability may not be constant during the lifespan[24], with heritability typically decreasing with increasing age as a consequence of accumulation of environmental influences with aging[25].

We demonstrated that risk for falling has a strong positive genetic correlation with fracture and low grip strength, while no significant correlation was observed with BMD or lean mass. This finding implicates a mechanistic pathway influencing fracture risk that is independent of bone mineral density. Fracture occurs when the force applied to a bone is greater than the overall bone strength. Low BMD is a key component of bone fragility, i.e. a necessary but not sufficient cause of fractures [26]. Although falls may be an independent predictor of fractures, the fall-related fracture risk dramatically increases in presence of low BMD. However, low BMD alone explains less than one-half of all non-vertebral fractures[27,28] and fractures occurring at higher BMD thresholds require the presence of other risk factors. Recent studies have postulated that falls and not osteoporosis per se constitute the strongest risk factor for fractures in extremely old individuals[29,30]. Therefore, falling may be a major contributing factor to overall fracture occurrence independent of, and in addition to age and BMD[31]. The strong genetic correlation of falls with fractures but not BMD corroborates the findings of epidemiological studies, while it also provides novel insights into the complex interplay between these traits.

The GWAS signal on chromosome 19q12 (rs2111530; MAF=0.39) maps in the vicinity (50.6 kb) of *TSHZ3*, gene encoding a zinc-finger transcription factor involved in diverse developmental processes. Recently, a GWAS meta-analysis found a variant (rs6510186) near this gene associated with total body bone mineral density (TB-BMD) exclusively in middle-aged adults (45-60 years old)[32]. Nevertheless, this variant is unlikely to arise from the same association signal, as it is not in LD with the top variant from our GWAS (distance 236.4 Kb, r^2=0.003). There is scarce information about the function of the *TSHZ3* gene, except that it is suggested to be part of a node of 24 genes with high degree of connectivity (i.e. hub genes) with strong levels of expression in early fetal cerebral cortical development[33,34]. Reduced expression of *TSHZ3* resulting in caspase upregulation has been proposed to be involved in the pathogen-

esis of Alzheimer's disease[35]. Moreover, genetic linkage[36,37] and association studies[38] have identified this gene as a potential candidate for autism susceptibility disorders. Altogether, this could suggest a role of *TSHZ3* in cortical development and in the pathogenesis of neurodevelopmental disorders. Pending additional evidence of its involvement in cerebellar biology, our findings suggest its plausible involvement in susceptibility to fall. Annotations relevant to *TSHZ3* include both eQTLs and chromatin interactions which could further support evidence for the gene involvement in falling susceptibility. The 7p21.3 variant (rs2709062; MAF=0.50) is also intergenic located 127.1 Kb upstream of *PER4*, a pseudogene, affiliated with lncRNA and of which little is known. Next, the combined meta-analysis of all participating cohorts yielded another signal (rs2431108 MAF=0.33) surpassing the GWS threshold. The SNP maps to 5q21.2 in RP11-6N13.1, a long intervening/intergenic noncoding RNA (lincRNA), which does not overlap protein-coding genes. This SNP have been previously reported in association with several psychiatric traits such as insomnia, anxiety, neurotism, and depression. Overall, none of the lead SNPs show any evidence for significant eQTLs and given the lack of information we cannot claim if the closes genes are also the causal genes.

The polygenic risk score analyses performed in two independent prospective cohorts corroborated the polygenic architecture underlying fall risk. The joint effect of PRS could reliably determine some of the variation underlying fall risk, implicating variants associated up to a significance level of $5x10^{-3}$. The scores constructed from the two most significantly associated variants were more strongly associated when using the more sensitive prospective falling assessment (fall calendars, used in the B-PROOF study); while employing a retrospective definition of falling (as used in the discovery and in both replication studies) resulted in the strongest polygenic risk scores arising from the inclusion of variants below the genome-wide significant level. These discrepancies can be the consequence of many different factors, including differences in assessment methodology, as retrospective falls were self-reported and may underestimate the occurrence of falls as a result of recall bias[39]. On the other hand, fall calendars are considered the best tool available for reliable fall assessment in older adults, providing more accurate information on falls (i.e., prospective assessment) as participants report fall incidents each week. Nevertheless, the PRS results should be interpreted with caution given the low SNP heritability of fall risk[40].

Medication use is recognized as an important risk factor for falling, while polypharmacy among the elderly has increased dramatically in the past decades[41]. Epidemiologically, several types of psychotropic, cardiac and analgesic drugs are associated with a significant risk of falling, typically including sedatives and hypnotics such as benzodiazepines, antipsychotics, antidepressants, diuretics, antiepileptics and opioids[42,43,44]. In line with the epidemiological relationship, we found that falls had significant genetic correlation with use of most of these medication categories and in

the expected directions. The strongest genetic correlation was observed with opioids and anti-inflammatory drug use. These findings suggest that some of the genetic predisposition for falling risk is shared with use of medication associated with falling risk. Yet, the causal mechanistic pathways can differ across different pleiotropic (vertical vs horizontal) relationships. Vertical pleiotropy will be relevant for medications causally-related to fall risk, where the genetic correlation with a condition will also be related to the drug indication all together, pointing to a common biological pathway influencing all three components (fall risk, condition and medication). Another form of vertical pleiotropy will be expected to arise when the medication is not necessarily causally related to fall risk (e.g. NSAIDs), but the condition driving the drug indication (e.g. arthrosis or other musculoskeletal disorder affecting mobility) will be the causal factor leading to falling. True horizontal pleiotropy is much more difficult to ascertain in a largely polygenic trait[45] like falling risk, but we can expect it to arise with specific conditions through biological pathways influencing neurological/cerebellar function as further discussed below.

Reduced hand grip strength and increased BMI are risk factors identified by MR to be causally related to fall risk. A decreased in relative handgrip strength was observed to be causally associated with increased risk of falling. Therefore, muscle weakness is a clinically relevant risk factor for falls that should be assessed and treated in older adults at risk for falls[46]. Our MR results also support the evidence from recent observational studies that older obese individuals have greater risk of falling[47,48]. The exact mechanisms by which BMI increases the risk of falling in older adults remain unclear and require further exploration. One possible explanation is that obesity may alter balance control which is an important risk factor for falling[49].

Further, using grouped cell-type and tissue expression analysis, the cerebellum was the most significant enriched tissue. The cerebellum plays an important role in motor control and maintaining postural balance[50]. Cerebellar disorders can lead to orthostatic hypotension, vertigo and syncope, all important risk factors for falls[51,52]. All these factors can increase the fall risk, either individually or combined.

Some limitations of our study need to be noted. Although self-reported measures can appear robust for other phenotypes such as birthweight[53], it is possible that recall bias may have influenced the assessment of fall risk. Falling is a complex, heterogeneous trait and most of the cases it may be attributed to non-genetic factors (e.g., medication use, mobility disorders, and hazardous household environments). Also, given the UKBB age range (40-69 years) and inclusion of European ancestry individuals only, we cannot assume that our results generalize to other age groups in whom falls are more common, and/or ancestral populations. There are other factors explaining the large heterogeneity underlying fall risk. Different fall patterns are observed between young and old people; while on the other hand, the number

of co-morbid conditions and medication use associated with falls also increase with age[54]. The genetic susceptibility for falls may be of less importance in individuals with multiple fall risk factors regardless of age. Different factors can increase the risk of falling across different age decades. Next, the heritability and genetic susceptibility of fall risk might be higher in individuals with recurrent falls which we were not able to test in the current study. Similarly, a GWAS on medication use-related falls is warranted to address the causal role of medication and understand further the biological pathways underlying fall risk. Finally, participants from the UK Biobank were included in the GWASs of both relative handgrip strength and falls. This sample overlap might increase the probability of a Type I error resulting in false-positive findings; thus, it needs to be replicated in independent efforts. There is an ongoing collection of hospital admission data in the UK Biobank that may provide an improved and well-powered resource for future research. Lastly, in our GWAS study we only tested SNPs for association with falling risk. We were not able to take into account other genetic structural variations such as single number variations (CNVs) or inversions which may also contribute to the genetic landscape of falling risk. Similarly, we also did not test for potential epigenetic modifications.

In conclusion, our study demonstrated that fall risk is a heritable, heterogeneous and polygenic trait genetically correlated with fracture risk and grip strength, among other neuropsychiatric and medication traits. The cerebellum tissue enrichment of falls-associated variants supports the mediation of postural balance in the etiology of falls. Our study provides novel biological insight that can be used for optimizing strategies directed at preventing falls and their associated deleterious consequences in aging individuals.

SUBJECTS AND METHODS

Study Population

Our analyses were performed using data from the UK Biobank study. Briefly, the UK Biobank is a large prospective cohort study of approximately a half-million adult (ages 40-69) participants living in the United Kingdom (UK), recruited from 22 centers across the UK in 2006-2010[55]. We use a subsample of the total study who were identified as or white European ancestry using a combination of genetic principle components and self-reported ethnicity. Ethical approval was granted by the Northwest Multi-centre Research Ethics Committee, and written informed consent was obtained from all participants.

Assessment of Falls

The number of falls in the UK Biobank was self-reported via a touch screen questionnaire. In total 89,076 individuals have reported that they have had one or more falls answering to the question "In the last year have you had any falls?". Individuals who selected "prefer not to answer" or "do not know" were set to missing; the rest of the population were classified as controls (N=362,103).

GWAS Data and Imputation

The majority of UK Biobank participants were genotyped with the Affymetrix UK Biobank Axiom Array (Santa Clara, CA, USA), while 10% of participants were genotyped with the Affymetrix UK BiLEVE Axiom Array. Detailed quality control and imputation procedures are described elsewhere[56]. Imputation was performed using the Haplotype Reference Consortium panel[57]. Only participants of white European-ancestry (identified using a k-means clustering approach based on genetic principal components, as well as self-identification) were analyzed.

Association Analysis

Genetic association analyses were performed using BOLT-LMM[58]. Briefly, this method uses a linear mixed model to account for relatedness and population structure using a relationship matrix. Fall risk was corrected for age and sex in logistic regression models. SNP association was tested for all autosomal variants. Individuals were excluded based on unusually high heterozygosity or >5% missing genotype rate, a mismatch between self-reported and genetically-inferred sex. SNP exclusions were made based on, low minor allele frequency (<1%) and low imputation quality (info<0.3). SNPs with $P \leq 5 \times 10^{-7}$ were considered suggestive while SNPs with $P \leq 5 \times 10^{-8}$ were considered genome wide significant (GWS).

Replication and Meta-analysis

The suggestive SNPs ($P \leq 5 \times 10^{-7}$) were later followed for replication in smaller and older prospective population-based studies i.e. the Rotterdam Study (1,009 cases and 4,925 controls) and B-PROOF (1,206 cases and 1,364 controls) cohorts. The Rotterdam Study is an ongoing population bases cohort within a suburb in Rotterdam. Its design, objective and methods have been described in detail[59]. Briefly, the study was initiated in 1989 and 7,983 participants aged 55 and above were included. Participants were interviewed and underwent an extensive set of examination that were repeated every 4-5 years. B-PROOF has been also described in details elsewhere[60]. In short, it is a multi-center, randomized, placebo-controlled, double-blinded trial investigating the efficacy of vitamin B and folic acid supplementation on the prevention of fractures in people aged 65 and older. In total 2,919 participants were included and followed for

2-3 years. Both studies have been approved by the medical ethics committee. In the Rotterdam Study, fall history was assessed from baseline questionnaire. In B-PROOF, retrospective and prospective falls were reported. Prevalent falls were assessed using a baseline questionnaire while fall incidences during follow-up were reported prospectively using a falls' calendar in a period of 2-3 years. The baseline questionnaire in both cohorts consisted of a single question: "*Have you fallen in the past 12 months?*". In B-PROOF we used fall incidence for the GWAS analysis as it provided larger sample size (500 participants had missing information on prevalent falls). Both studies used commercially available genome-wide arrays to genotyped their participants. SNPs were imputed to the Haplotype Reference Consortium (HRC) reference panel[57] (build 37) using the Michigan Imputation Server (MIS).

Gene-based Testing and Functional Mapping

Gene-based GWAS analysis was carried out with MAGMA 1.6[18] using the default settings implemented in FUMA[61]. According to the number of tested genes, the level of gene-wide significance was set at $0.05/18{,}615=2.7\times10^{-6}$. Functional annotation (i.e. prioritization, annotation and interpretation) of GWAS results was also performed using FUMA[61].

Polygenic Risk Scores (PRSs)

We used imputed genotype data from the B-PROOF and the Rotterdam Study cohorts to calculate PRSs for each participant using the PRsice software[15]. For the construction of the PRS we first selected SNPs with $P<0.05$, MAF>0.05 and imputation quality>0.3 from the UK Biobank GWAS. Next, we excluded SNPs with MAF<0.05 and imputation quality<0.3 in the Rotterdam study and B-PROOF. In total 436,130 SNPs passed these thresholds and were followed in the PRS analysis. PRSs were created for a series of *P*-value thresholds ranging from 5×10^{-8} to 1. In total, 17 PRSs were created. SNPs were pruned with PLINK (version 1.9) using stringent clumping thresholds based on both linkage-disequilibrium and distance using an r^2 of 0.1 and a distance of 300 Kb. We tested each of the 17 PRSs in relation to fall risk adjusted for age and sex stratified by cohort. In the B-PROOF study we tested the scores with both prevalent and incident falls. We reported the proportion of variance explained (based on R^2) by each fall PRS.

Linkage Disequilibrium Score Regression

SNP-based heritability and genomic inflation

To estimate the genomic inflation in the data and the SNP-based heritability of falls, we used LD-score regression (LDSR)[62]. The LD-score regression intercept provides estimate of inflation due to population stratification or model misspecification; im-

portantly, it is not unduly affected by polygenicity[63]. We used pre-calculated LD-score from the 1000 Genomes European reference population (https://data.broadinstitute.org/alkesgroup/LDSCORE/).

Shared genetic architecture of falls and other traits

To estimate the genetic correlation between falls and other complex traits and diseases, we used (cross-trait) LD-score regression[16] as implemented in the online web utility LDHub[64]. This method uses the cross-products of summary test statistics from two GWASs and regresses them against a measure of how much variation each SNP tags (its LD-score)[65]. The data base of the LDHub web utility contains a range of data relating to genetic effects on common diseases and phenotypes. In addition, locally we estimated the genetic correlation between fall risk and 22 medication classes from the latest GWAS on medication use [17]; where medications were self-reported and classified using the Anatomical Therapeutic Chemical (ACT) Classification System into 1,752 categories (with minimum 10 users) after careful evaluation of the medication record data.

Stratified heritability and functional enrichment and tissue specificity analyses of falls-associated variants

To identify tissues and cell types that are likely to be involved in falling susceptibility, we applied LD-score regression to specifically expressed genes (LDSC-SEG)[66]. Annotation data was obtained from the LD-score website (https://github.com/bulik/ldsc). For each tissue, we ranked genes by a t-statistic for differential expression, using sex and age as covariates and excluding all samples in related tissues[66]. For example, we compared expression in cerebellum samples to expression in all non-brain samples. We used the top 10% of genes by this ranking and used stratified LD-score regression to estimate the contribution of genomic annotations to per-SNP falls heritability, adjusting for 24 main annotations categories (eg. coding, UTR, promotor, intron) in the baseline model[67]. In addition, we performed MAGMA Tissue Expression analysis (using FUMA) to test relationships between tissue specific gene expression and disease-gene associations. MAGMA was performed using the result of gene-set analysis (gene-based P-value) and tested for one side (greater) with conditioning on average expression across all tissue types.

Mendelian Randomization

We used the largest previously published GWAS meta-analyses of the trait included in the MR analysis. The construction of genetic instruments for antihypertensive drug targets are explained in details by Gill et al [23]. To reduce potential bias due to population stratification, we restricted the analyses to studies with participants of

European descent. In addition, instrumental variables which were nominally ($p<0.05$) associated with fall risk were excluded from the analysis. The resulting individual SNP effect estimates were pooled using inverse-variance weighted (IVW) meta-analysis. We applied a conservative Bonferroni corrected threshold to account for the multiple testing (that is, $\alpha=0.007$, because seven exposures were assessed). To test the third assumption (a lack of pleiotropic effects of the SNPs on the outcome, independent of the exposure), we used Mendelian randomization-Egger regression. Moreover, as sensitivity analyses for robust causal inference, we additionally performed Mendelian randomization analyses using a weighted median estimator. The analyses were conducted with the R-package MendelianRandomization [68].

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Supplementary Table 1 | Previously reported SNP- and Gene-associations from the GWAS catalog

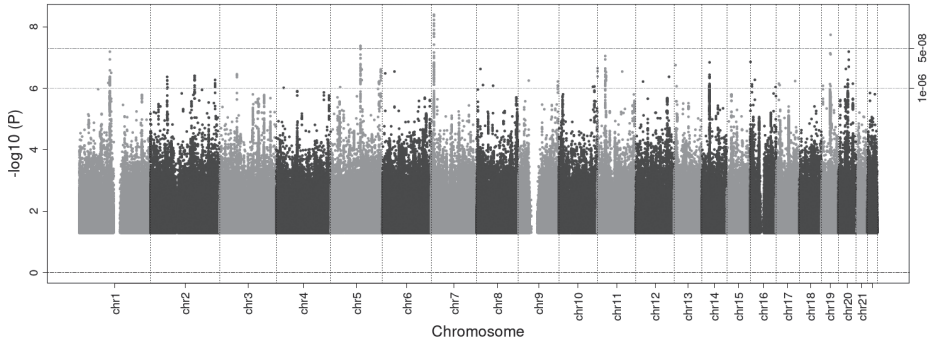
SNP	Know SNP-associations	Gene	Know Gene-associations
rs2431108	Insomnia, Anxiety, Nap during the day, Leg fat percentage, Neurotism, Well-being, Loneliness, Depression, Mornigness	*RP11-6N13.1*	-
rs2709062	-	*PER4*	-
rs2111530	Systolic blood pressure	*TSHZ3*	Lung function, Chronotype measurement, BMD
rs76259395	-	*NTNG1*	Blood proteins, LDL-cholesterol, BMI
rs6658723	BMI, Fat Mass, Waist Circumference, Waist-Hip ratio	*FAM212B*	BMI*
rs67174662	BMI, Fat Mass, Waist Circumference	*EIF3FP3*	Neuroticism, Mood swings
rs974135	Height	*BCL11A*	Blood traits, Education, Cognition
rs7616516	-	*FHIT*	BMI, Physical activity, Smoking, Depression
rs2471020	-	*DRD1*	Eye refractive error
rs72857666	-	*TRERF1*	Blood traits
rs12666565	-	*NXPH1*	chronotype measurement
rs11030084	BMI, Fat Mass, Fat-free Mass, Hip and Waist Circumference, Menarche, Smoking, Risk-taking behaviour	*BDNF-AS*	BMI*, Hip* and Waist circumference, Menarche*, chronotype measurement, smoking*, alcohol consumption
rs494221	Height, Hip circumference, Fat-Free Mass, Basal metabolic rate	*MPPED2*	Estimated glomerular filtration rate, height, Weight circumference, chronotype measurement
rs28672671	-	*TSPAN4*	
rs12884871	-	*RPL10L*	BMI
rs28633123	-	*ZNF536*	Height, BMI, education
rs6063547	-	*CTNNBL1*	-

*the lead SNPs for both traits are in LD

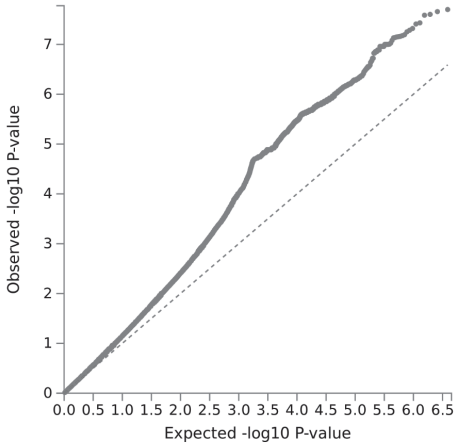
Supplementary Table 2 | Mendelian Randomization analyses of several potential risk factors for falls

	IVW		Weighted median		MR-Egger		Egger Intercept	Number of SNPs
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	P	
Relative handgrip	0.41 (0.23-0.41)	<0.0001	0.44 (0.23-0.85)	<0.0001	0.24 (0.02-2.09)	0.197	0.63	103
Body mass index	1.13 (1.06-1.20)	<0.0001	1.16 (1.08-1.24)	<0.0001	1.25 (1.08-1.44)	0.002	0.12	77
Alcohol consumption	1.01 (0.99-1.04)	0.38	1.00 (0.97-1.02)	0.82	1.01 (0.94-1.08)	0.84	0.91	97
Alcohol dependence	1.04 (1.01-1.08)	0.029	-		-		-	1
Antihypertensive drugs								
ACE inhibitors	1.00 (0.97-1.03)	0.80	-		-		-	1
Beta-blockers	0.99 (0.97-1.01)	0.47	0.99 (0.98-1.01)	0.37	1.03 (0.96-1.10)	0.45	0.30	6
Calcium channel blockers	1.00 (0.99-1.01)	0.43	0.99 (0.97-1.01)	0.46	0.99 (0.97-1.00)	0.16	0.23	24

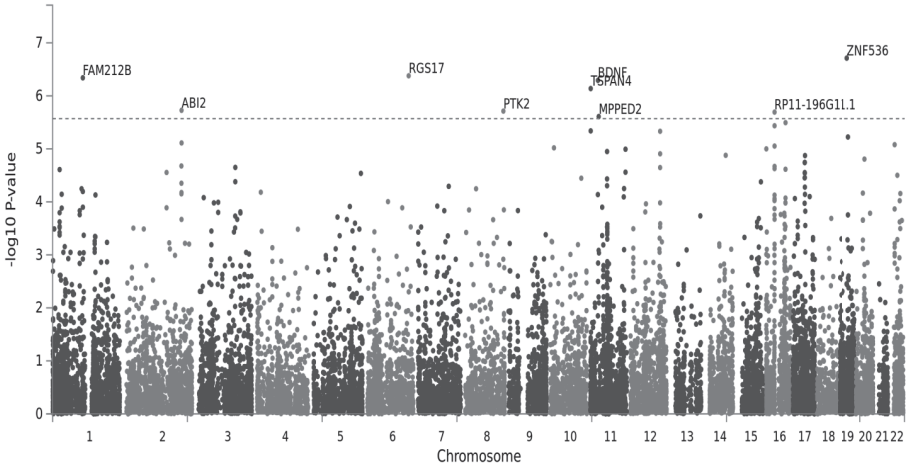
Footnote: The weighted median and the Egger regression MR analyses require at least 3 SNPs for implementation. The OR for relative hand grip (m^2). BMI (kg/m^2) and alcohol consumption (log transformed) are per 1unit increase in the exposure.



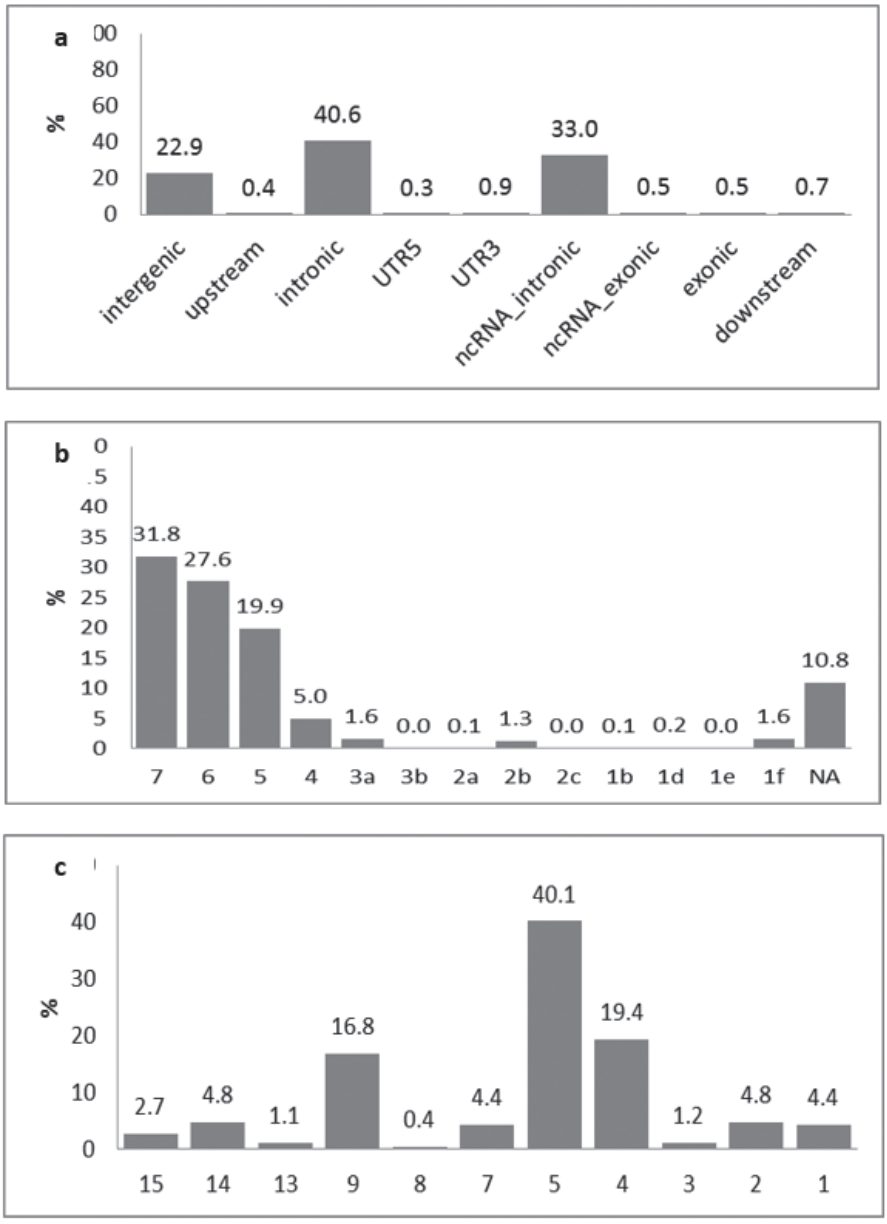
Supplementary Figure 1 | Manhattan Plot of Association Statistics ($-\log_{10}$ (P)) for falling risk for the combined meta-analysis. Each dot represents a SNP and the x axis indicates its chromosomal position (built 37 NCBI). Dashed horizontal red line marks the GWS threshold ($P \leq 5 \times 10^{-8}$).



Supplementary Figure 2 | Quantile-quantile (Q-Q) plot of observed versus expected P values of the GWAS results. The straight line in the Q-Q plot indicates the distribution of SNPs under the null hypothesis

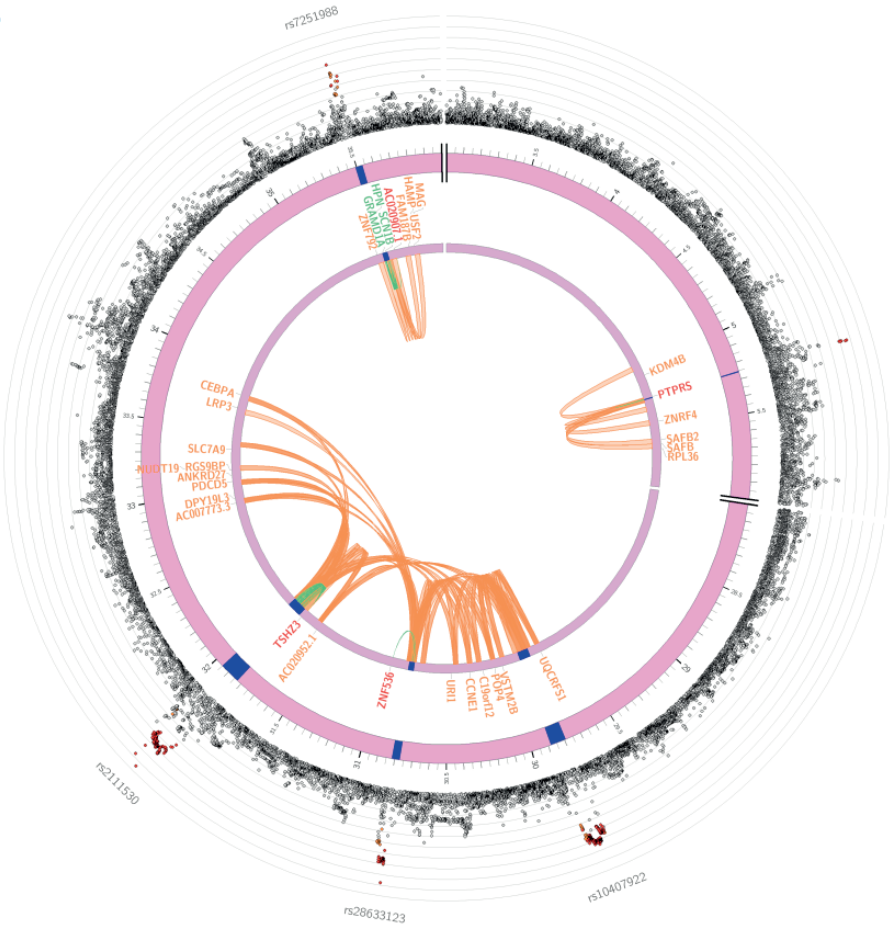


Supplementary Figure 3 | Manhattan plot of the gene-based test as computed by MAGMA. Each dot represents one gene and the x axis indicates its chromosomal position (built 37 NCBI). The dashed red horizontal line marks the gene-number-adjusted threshold (p=0.05/18,185 tested genes).



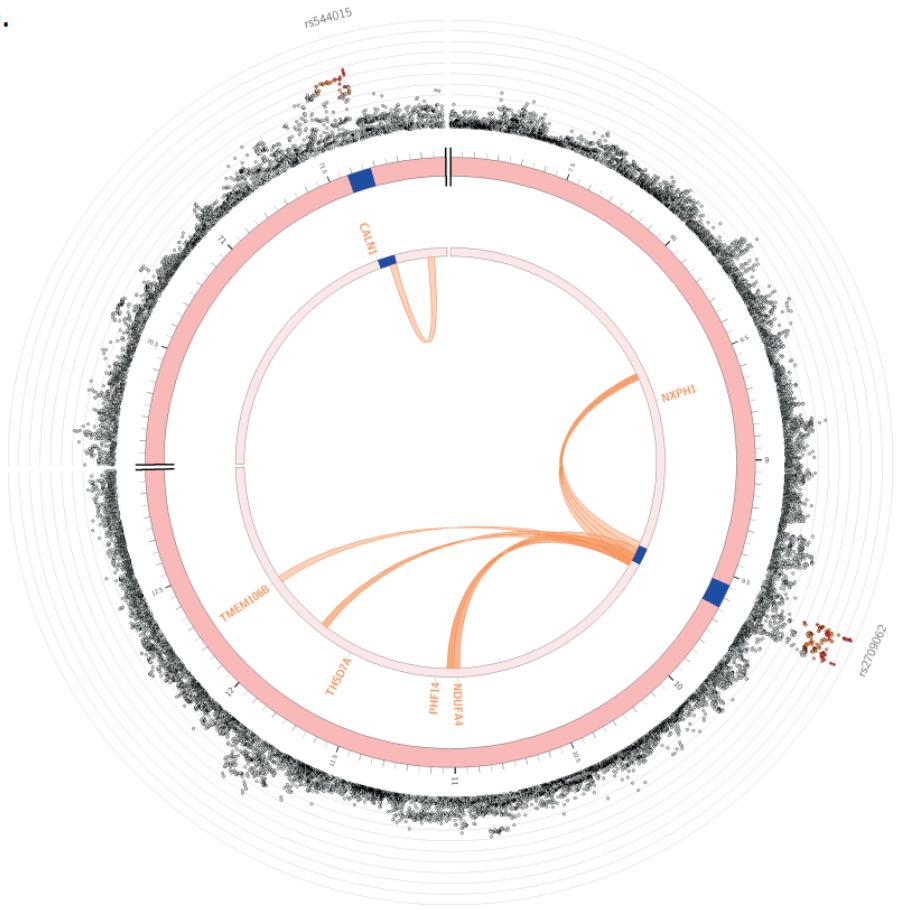
Supplementary Figure 4 | Functional annotation for all SNPs with $r^2 \geq 0.6$ with the top SNPs ($P<1x10^{-6}$). a) Percentage of SNPs according to their functional category; b) Percentage of SNPs according to their RegulomeDB score (x-axis). Lower score indicates a more likely regulatory role; c) Percentage of SNPs according to their minimum chromatin state across 127 tissues. Lower score (x-axis) indicates a more likely regulatory role. NA – not available in RegulomeDB.

a.



Supplementary Figure 5 | Circos plots demonstrating the results of eQTL and chromatin interaction mapping for loci on chromosome **a)** 7, **b)** 19 and **b)** 5. Genes mapped by Hi-C or eQTLs are colored orange and green, respectively. Genes that were mapped by both eQTL associations and chromatin interaction data are highlighted in red. The highlighted SNPs (blue box) represent the GWS SNPs ($P \leq 5 \times 10^{-8}$).

b.



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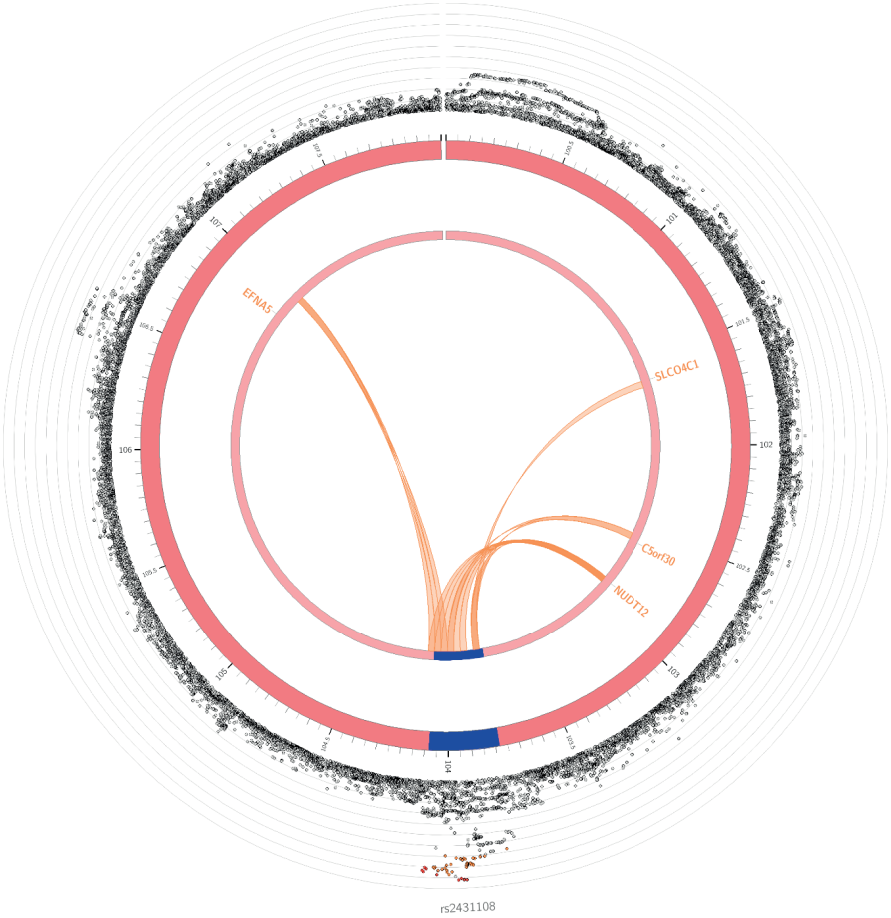
c.



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