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Review Article

Genetic Epidemiology in Aging Research

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Over the last two decades, aging research has expanded to include not only age-related disease models, and conversely, longevity and disease-free models, but also focuses on biological mechanisms related to the aging process. By viewing aging on multiple research frontiers, we are rapidly expanding knowledge as a whole and mapping connections between biological processes and particular age-related diseases that emerge. This is perhaps most true in the field of genetics, where variation across individuals has improved our understanding of aging mechanisms, etiology of age-related disease, and prediction of therapeutic responses. A close partnership between gerontologists, epidemiologists, and geneticists is needed to take full advantage of emerging genome information and technology and bring about a new age for biological aging research. Here we review current genetic findings for aging across both disease-specific and aging process domains. We then highlight the limitations of most work to date in terms of study design, genomic information, and trait modeling and focus on emerging technology and future directions that can partner genetic epidemiology and aging research fields to best take advantage of the rapid discoveries in each.

Key Words: Aging—Genetics—Epidemiology.

THE field of aging research now spans multiple frontiers that have begun to map the connections between biological processes and particular age-related diseases. This is certainly true in the field of genetics, where variation across individuals has improved our understanding of aging mechanisms, etiology of age-related disease, and even prediction of therapeutic responses (1). Traditional genetic approaches have led to new models of aging mechanisms and disease pathogenesis through identification of mutations related to aging phentoypes. Studies of large families with multiple affected members identified mutations in lamin A (LMNA) and helicase (RECQL2) genes that cause premature aging in Werner's syndrome and Hutchinson-Gilford progeria (2–4). Missense mutations in the amyloid precursor protein and presenilin 1 and presenilin 2 genes cause early-onset familial Alzheimer's disease (AD) in a rare set of families (5–8). Other examples exist for Parkinson's disease and cancers (9–15). Most of these findings characterize rare early-onset manifestations of later onset diseases and have been extremely important in understanding the biological pathways relevant for these diseases and for the aging process more generally. Unfortunately, approaches that were successful for these rare familial forms have not been as successful for more common late-onset traits that are considered to be of more complex etiology. Yet, even these complex forms of age-related disease and aging per se have a significant genetic contribution including late-onset AD: 50%–80% (16), prostate cancer: 58% (17), heart rate: 13%–23%, (18) systolic blood pressure: 38%–46% (19), bone mineral density in premenopausal women: 46%–92% (20), and general life span: 20%–50% (21–24).

These heritability estimates argue for a greater effort to identify genetic contributions to disease and aging processes. So far, variants of the Apolipoprotein E (APOE) gene have shown the most consistent associations with age-related diseases and longevity (25–41), although several other genes have accumulating, yet currently inconsistent evidence (42). The promising future of genetic epidemiology in aging research relies on the potential for novel findings in genes such as these to direct areas of prediction, prevention, and therapy. For example, variants of methylenetetrahydrofolate reductase gene have been associated with cognitive decline, cardiovascular disease outcomes, osteoarthritis, and longevity, implicating this pathway, which includes folate and homocysteine metabolism, in aging processes (43–48). In particular, folate and vitamin B_{12} supplementation have been shown to significantly improve baseline serum homocysteine levels in individuals homozygous for the C677T polymorphism (49–52), implicating genetic associations in better targeting and preventive therapy. Candidate genes for age-related chronic conditions, including CVD, AD, Parkinson's disease, and cancers have been well studied, but few genes have been established as consistent genetic risk factors for these diseases and fewer have been related to aging more generally. This is likely due to the limitations in design and technology discussed in this article. As these methods improve, genetic studies of age-related diseases will yield consistent results and ultimately help target specific biological mechanisms and lead to better treatment and prevention. Although not a formal meta-analysis per gene, Table 1 provides a snapshot of the current literature for genetic associations with longevity and highlights age-related diseases or phenotypes that are known to be associated with these genes.

Biological markers of aging processes such as inflammation, oxidative stress, and DNA repair have been shown to be heritable, and these may therefore be good targets for genetic

Table 1. Summary of Chronic Disease-Related Genes With a Relationship to Aging and Longevity in Human Studies

Gene	Cited Polymorphism	Association with Longevity		Chronic Disease*
		Evidence For	Evidence Against	
ACE	intron16ins/del	(28, 29, 53–55)	(27, 56–61)	CVD, AD
AGT	M235T		(25, 61)	CVD
APOA1	G-75A	(62)		CVD/lipid metabolism
APOA4	Gln360His	(63)		CVD/lipid metabolism
	Asp127Ser		(62)	
APOB	3´-APOB VNTR allele	(64–67)	(28, 30, 68)	CVD/lipid metabolism
	Haplotype	(67)		
APOC1	HpaI site	(54)		CVD/lipid metabolism
APOC3	SstI cut site (G3238C)	(30)	(62)	CVD/lipid metabolism
	T-455C	(69)		
	C-641A	(70)	(41)	
APOE	E4/e2 isoforms	(25–41)	(53, 54, 58, 60, 71, 72)	CVD, AD, stroke
CETP	Ile405Val	(73, 74)	(41, 75–77)	CVD/lipid metabolism
FII	G20210A		(59)	CVD
FGB	G-455A		(61, 78, 79)	CVD
FVL	Arg506Gln		(61, 80–83)	CVD
Factor VII	R353Q	(25, 84, 85)	(61, 78, 85)	CVD
GPIa	C807T		(59)	CVD
GPIIIa	T1563C	(59)		CVD
MTHFR	C677T (Ala222Val)	(25, 83, 86–90)	(54, 59, 61, 91, 92)	CVD, AD, osteoporosis
MTR	D919G	(93)	(61)	CVD
MTP	G-493T and Q95H	(94, 95)	(41, 96–98)	CVD/lipid metabolism
PAI-1	4G/5G insertion	(78, 79)	(61, 99, 100)	CVD
PON1	Gln192Arg	(101-104)	(41, 105)	CVD/lipid metabolism
	T-107C	(105)	(101)	
	Ht combinations, GWAS SNPs	(102, 106, 107)		
REN			(108)	CVD
TAFI	G-438A	(79)	(61, 79)	CVD
TP53	Arg72Pro	(25, 109–112)		Cancer

Notes: Genes were included based on a Pubmed search with the following terms: gene | genetic & longevity | aging | age-related. Hits from this search were further investigated and additional references cited when appropriate. Final inclusion depended on relevance to particular disease processes versus aging mechanisms (Table 2). The search was first performed in fall 2006 and updated in March 2008. SNP = single nucleotide polymorphism; GWAS = genomewide association study.

*This column shows disease or biological processes previously related to the gene cited to provide biological context.

research as well (113,114). Several genes have already been implicated in these models of aging in either human or animal studies, with perhaps most consistency among the interleukin genes for inflammation and superoxide dismutase genes for oxidative stress. However, relatively little work compared with disease outcomes has been done, and no clear pattern has emerged given the limitations discussed below. Table 2 provides a summary of current findings for genetic associations with longevity and highlights biological mechanisms related to aging that are known to be associated with these genes.

Future directions

Although relationships between genes and aging are emerging, the field must still overcome critical limitations in sample sizes, genomic coverage, phenotype definitions, study design, and statistical approaches. As an example, the body of literature on the relationship between angiotensin converting enzyme (ACE) gene variation and stroke phenotypes has reported mixed results for many relatively small case-control studies for a variety of reasons (163–166). First, differences in study design may have been responsible for inconsistent results across studies, due to lack of precision (small sample sizes), underlying phenotypic heterogeneity (inappropriately mixing stroke subtypes), or varying ethnic compositions (166). Another major limitation may be that often only direct associations are considered, where well-studied polymorphisms within the ACE gene (eg, Alu insertion/deletion) are tested as potentially causal and no other variation in the gene is considered. Thus, unmeasured causal variants poorly correlated with these genotyped polymorphisms will be missed among detected associations. DNA variation catalogs such as the HapMap project (www.hapmap.org) now offer excellent single nucleotide polymorphism (SNP) correlation data that enable measurement of all common variation per gene or the selection of a particular variation that can serve as proxy information for all common variation within the gene. Finally, most published studies have focused on marginal associations, rather than modeling potential interactions. Some propose that the ACE effects may only be detectable through joint evaluation with important interacting factors. For example, Gao and colleagues reported no marginal associations between polymorphisms in ACE, APOE, and Fgβ and odds of ischemic stroke, yet found that individuals with "unfavorable" genotypes at all three loci had almost four times greater

Table 2. Summary of Genes Involved in Specific Biologic Processes With a Relationship to Aging and Longevity in Human Studies

Gene	Related Biologic Process	Polymorphism	Association With Aging and/or Longevity	
			Evidence For	Evidence Against
CAT	Oxidative stress	C-262T		(115)
FOXO1A	Insulin resistance	T97347C		(116, 117)
		GWAS SNPs	(107)	
GH1	Insulin resistance	A1663T	(118)	
GHRHR	Insulin resistance	Intronic SNP		(118)
GSTP1	Oxidative stress	I105V		(119)
GSTM1	Oxidative stress	Deletion	(119, 120)	(121–123)
GSTT1	Oxidative stress	Deletion	(119, 120, 122, 124)	(121)
IGF-1R	Insulin resistance	G1013A	(116)	
IL6	Inflammation	C-174G	(125–129)	(79, 126, 130–132)
		C-634G		(133)
		VNTR	(134)	
IL2	Inflammation	T-330G	(135)	(128)
IL8	Inflammation	A->T intron 1		(128)
IL12	Inflammation	A->C exon 8		(128)
IL18	Inflammation	A-607C		(133)
IFNG	Inflammation	T874A	(135, 136)	(128, 132, 136)
IL1A	Inflammation	C'-889T		(131, 137)
IL1B	Inflammation	C-511T	(129)	(131, 137)
		+3953		(131)
IL1RN	Inflammation	VNTR		(131, 137)
IL10	Inflammation	G-1082A	(129, 135, 136, 138)	(128, 131–133, 138)
		T-819C	(133)	(136)
		C-592A		(136)
INS-R	Insulin resistance	2-SNP haplotype	(117)	
INS	Insulin resistance	VNTR		(118)
IRS-1	Insulin resistance	G972A	(118, 139)	(116, 117)
KL	Insulin resistance	KL-VS	(140, 141)	(41)
mtDNA	Energy metabolism	C5178A (D)	(142)	(142, 144)
		G9055A (K)	(143, 145)	
		C150T (J)	(146, 147)	
		Haplogroup J	(148, 149, 150)	
PARP	DNA metabolism/repair	Various mutations		(108, 151, 152)
PI3KCB	Insulin resistance	T-359C	(116)	(117)
PI3KCG	Insulin resistance			(117)
PPARGC1A	Insulin resistance			(117)
SIRT1	Caloric restriction	5 SNPs		(153)
SIRT3	Caloric restriction	G477T	(154)	
		VNTR- intron 5	(155)	
SOD1	Oxidative stress	Animal models	(156, 157)	
SOD2	Oxidative stress	Val16Ala	(25, 158, 159)	(108, 158)
TNFA	Inflammation	-308 G/A	(129)	(131, 138, 160, 161)
		T-1031C		(133)
TNFB	Inflammation	C869T		(133)
		G252A		(160)
TLR4	Inflammation	Asp299Gly	(162)	

Note: Genes were included based on a Pubmed search with the following terms: gene | genetic & longevity | aging | age-related. Hits from this search were further investigated and additional references cited when appropriate. Final inclusion depended on relevance to aging mechanisms versus particular diseases (Table 1). The search was first performed in fall 2006 and updated in March 2008. SNP = single nucleotide polymorphism; GWAS = genomewide association study; mtDNA = mitochondrial DNA.

odds of stroke compared with those with other genotype combinations (163).

These limitations are indicative of the need for new paradigms as the fields of genetic epidemiology and aging merge. New strategies to exploit the available genomic technology and to use more efficient study designs and increase sample sizes are needed. Further, different models of how genes influence aging beyond sequence variation alone, such as epigenetic contributions, should be pursued. Phenotype characterization, whether more precise definitions or new concepts of what constitute a genetic "trait," will be a key area for advancement. Yet, none of these can contribute without advanced statistical approaches to model the complexity that will result from expansion in each of these areas. We elaborate on each of these below.

Genetic Measurement

Genetic studies in aging are essentially based on general epidemiologic principles that relate an exposure to disease

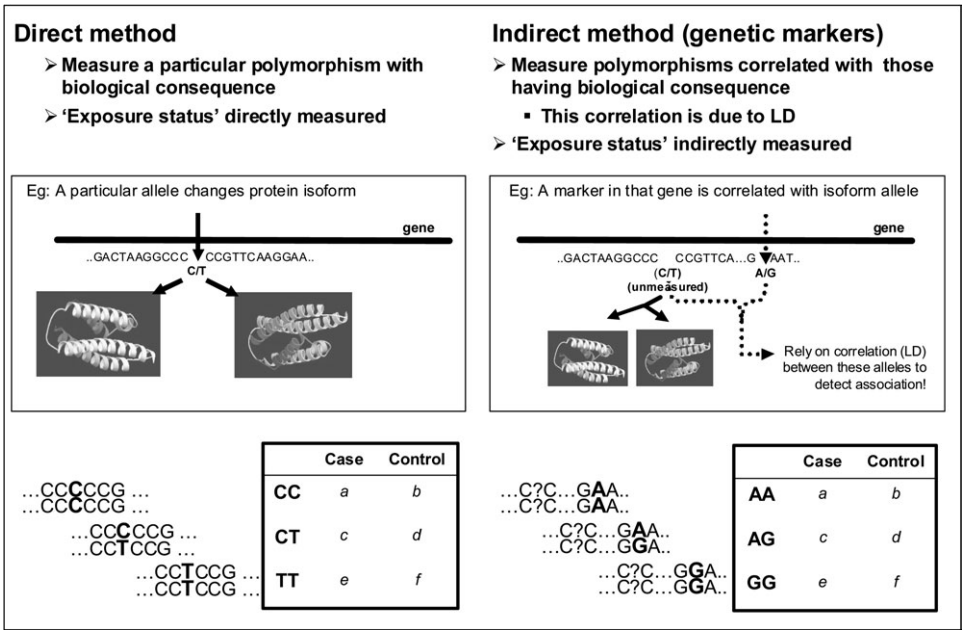

Figure 1. Direct versus indirect genetic association tests.

risk or quantitative values. The measurement of exposure can correspond directly to the factor of interest (eg, amount of alcohol consumed in a week) or can serve as an indirect proxy of that exposure (eg, reported drinks per week). Genetic exposures similarly can be thought of as direct, if the polymorphism genotyped in the study has a direct consequence on the biology and risk for disease. For example, the APOE ε4 variant codes for an isoform that is directly related to biological consequences that lead to vascular and cognitive diseases. However, most polymorphisms in the genome with direct consequences are not yet known, and studies typically rely on polymorphic "markers" in the genome that can serve as correlated proxies for causal variants. These markers often have no known biological consequences themselves but are highly correlated with the genomic sequence surrounding them and therefore serve as indirect measures of the rest of the nearby sequence that may contain a biologically relevant polymorphism (Figure 1). Continuing the APOE example, if other polymorphisms in the APOE gene were genotyped instead of the ε4 variant, those correlated with that variant would indirectly show association with diseases such as AD and CVD (167,168). A major limitation of previous genetic studies in aging has been the failure to measure enough polymorphisms per gene to achieve adequate coverage of all common variation within that gene.

Genetic markers at known locations in the genome act as surrogates for surrounding sequence by exploiting a population genetic property called "linkage disequilibrium" (LD). Under this property, genetic variation in very small genomic distances remains together as one unit over several generations and appears correlated at the population level. Past studies that have only genotyped one, or very few, polymorphisms have not fully exploited the underlying LD structure of a particular gene; thus, only the sequence correlated with genotyped polymorphisms has been represented in studies, leaving the rest of the gene or genome unmeasured. Until recently, more complete gene coverage was difficult to achieve because this requires genotyping of all polymorphisms or prior knowledge of the correlation structure so that efficient surrogate markers can be chosen to represent the unmeasured variation. The most common type of marker is a SNP, where identical sequences in a population vary by only one base pair. There are approximately 10 million SNPs in the human genome, and the location, prevalence, and LD information between SNPs have been cataloged for more than 6 million of these in the International HapMap Project (169). Because these factors differ greatly by race, HapMap has examined these data separately in populations with European, African, and Asian ethnicity (170). Algorithms to identify the most efficient set of SNP markers (often called tagSNPs) are readily available (171–173). This has revolutionized the design opportunities for genetic studies, making it timely and cost effective to measure nearly all variation in a gene or even the whole genome.

The newly found ability to query the whole genome in this way has led many to question whether candidate gene-focused studies themselves are the main limitation of previous work. In these instances, not only may variation in chosen candidates be missed but also, in fact, entire genes with potential relevance are unmeasured. A growing trend is to query the entire genome using an efficient set of tagSNPs, in what is termed a genomewide association study (GWAS).

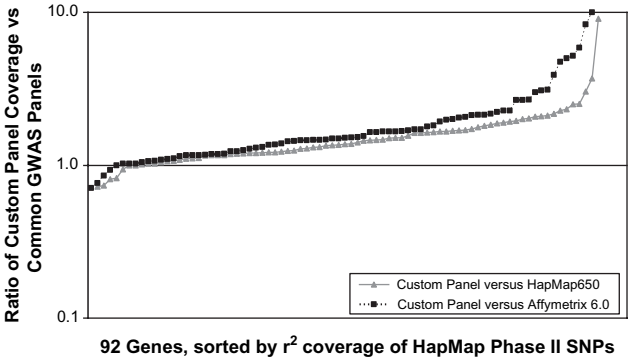

Figure 2. Coverage comparison between a custom-made single nucleotide polymorphism (SNP) panel for 92 candidate genes and current genomewide association panels. The *y* axis shows the ratio of coverage for the custom panel versus either the Illumina 650 panel or the Affymetrix 6.0 panel. Coverage is defined as the percent of common (>5%) Yoruban HapMap Phase II SNPs that are in high linkage disequilibrium with genotyped SNPs on the panel ($r^2 \geq .8$) and thus are "covered" by the panel even if they are not genotyped. Points falling under 1 indicated that genomewide association studies have better coverage at that particular gene, whereas points falling above 1 indicate that the custom-made panel yields better coverage at that gene.

A typical GWAS measures between 500,000 and 1 million SNPs, which act as correlated surrogates for 70%–92% of the remaining unmeasured common variation in European Caucasians depending on genotyping platform (169). These new advances allow us to identify genes implicating novel biological pathways to aging and age-related diseases that may not have been considered as a candidate gene. For example, six independent GWAS have recently reported an association between a 100-kb region on chromosome 9q21 and type 2 diabetes mellitus, coronary artery disease, myocardial infarction (MI), and coronary heart disease (174–179). This genomic region contains no annotated genes, yet lies adjacent to tumor suppressor genes CDKN2A and CDKN2B, which express proteins $p16^{INK4a}$ and $p16^{INK4b}$, respectively. This is potentially exciting for aging research because animal and human studies have shown increased expression of these proteins in aging tissue (180–182).

The emergence of GWAS has inevitably been accompanied by new challenges, given the multiplicity of genotypes measured. Most importantly, for one to detect association while maintaining precision and controlling type I error, sample sizes on the order of thousands to tens of thousands are needed (174). For most U.S. research studies, these large sample sizes are unrealistic and only possible via collaborative efforts. However, careful consideration of sampling schemes, source populations, and phenotype characterization is needed before the pooling of studies can occur. Measurement of nearly a million SNPs remains an expensive endeavor, and many strategies are currently employed to most efficiently use genotyping funds. For example, staged approaches where the entire genome is queried on a subset of participants and top signals are genotyped in the remaining set are now common (183). However, choice of proportion to use in initial versus follow-up stages can be critical for the ultimate power of the study (184,185). Statistical methods and computational resources to handle this amount of data are currently limited and are an emerging field of methodological research.

Although GWAS technology is an exciting new tool, even 1 million SNPs do not represent the entire genome because they rely on indirect association between markers and disease and therefore depend on the correlation between those markers and truly causal variants in the genome that are not directly measured. Current GWAS panels do not completely cover all variation within each gene and in fact miss some genes altogether. This is particularly true for populations or ethnicities not included in the original panel design because the markers are chosen based on genomic correlation structures specific to particular ethnicities. For example, Figure 2 shows gene coverage of common genetic variation, defined as any SNP with a minor allele frequency of at least 5%, for a study of muscle-related candidate genes among African Americans, using a tailored custom candidate gene SNP panel, versus the coverage of those same genes obtained via currently used GWAS panels. Several genes are completely unrepresented in the GWAS panel, and most are greatly underrepresented compared with a custom approach (often with several-fold lesser coverage). Therefore, focused candidate gene studies that choose optimal marker sets to represent strong biological candidate genes in particular populations remain the most powerful measurement approach for specific genes. Recent genotype imputation methods improve on the GWAS power by borrowing from known population-specific correlation structures to impute genotypes for SNPs that were not actually on the study panel, and thus increase the coverage of unmeasured SNPs that can be achieved (186–188). However, some truly associated genes may still be missed in GWAS, and among the discoveries that are made, careful replication and often additional genotyping is needed to capture and characterize the full genetic variation. The aging field will best benefit from a combination of GWAS and more finely tuned candidate gene studies with information flowing in both directions.

Phenotype Measurement

Measurement error and misclassification always plague epidemiologic research, as even nondifferential misclassification can introduce noise and reduce power. The potential for error in phenotype measurement in age-related traits is high, given the reliance on measurement tools that are too blunt, such as self-reports, or very sensitive, but too expensive to carry out in large numbers. Further, many age-related traits, for example, sarcopenia, are often dichotomized for research and treatment purposes, neglecting the true continuous nature of the underlying phenotype and decreasing power to detect genes that contribute to this distribution. The phenotype problem is further exacerbated by the

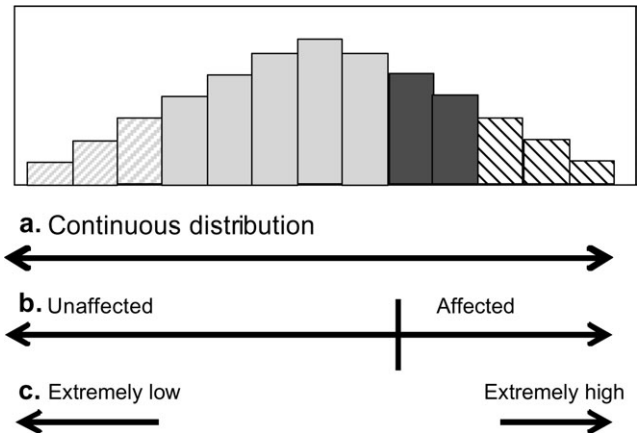

Figure 3. Possible phenotype definitions for an underlying continuous trait.

complex nature of most aging phenotypes that often present as a result of many underlying causes.

Considering the large sample sizes needed to accommodate the number of genomic measurements in current studies, lack of precision and validity on phenotype measurement is now a major limitation in estimating genetic effects because sample size requirements are further inflated with increasing misclassification. Movement to more precise phenotype measurement tools is necessary to minimize the sacrifice in power due to misclassification, yet these are often prohibitively expensive for large-scale use. For example, lower extremity muscle strength in older adults is commonly measured with a handheld dynamometer (HHD) that is inexpensive and portable, allowing use in large samples. However, HHD lacks precision and validity compared with stationary Biodex dynamometers, which are expensive and require participants to come into a clinic (189). Epidemiologic techniques such as regression calibration or direct adjustment of measurement error can be quite useful in these situations (190,191), although they have not been applied widely in aging or genetic epidemiologic research. For example, the known correlation between HHD measures and Biodex measures from a small set of participants can be used to correct odds ratios (or other risk ratios) estimated using the HHD on a much larger set of participants.

The practice of dichotomizing continuous phenotypes for treatment and research is another implicit source of misclassification. Continuing our muscle strength example, many classify individuals into "weak/not weak" categories, based on scores that populate a full distribution (Figure 3). This necessarily means that two individuals with very similar strength values may be classified differently, if they are just on either side of the threshold. Use of the full distribution, rather than this dichotomy, would remove this type of misclassification and take advantage of the actual measurements. One offshoot of this thinking has been to consider only those at the extremes of a continuous distribution (eg, top and bottom 10%) (192,193). Although this is appealing, finding these individuals, controlling for important confounders, and allowing for interactions in analysis become a daunting task. For this reason, extreme sampling or analysis techniques are often used only for screening, with the full distribution used to confirm and accommodate more sophisticated analyses (193).

Beyond these measurement concerns, it is possible that the genetic "trait" of interest does not correspond to a disease classification, but rather to particular features related to disease. For example, genetic risk may be confined to subcategories of disease, such as frail individuals whose clinical syndrome is driven by sarcopenia, rather than anemia or reduced energy metabolism. Alternatively, genetic variation may contribute to the continuous distribution of a biologically relevant measure across the general population. Often, such features are considered "endophenotypes," assumed to be more powerful given their continuous nature and proximity to the genetic cause (194). This has been the idea behind the study of "endophenotypes" in psychiatry and may help move the field of aging genetic epidemiology forward. For example, more effort is now focused on particular biological features or mechanisms related to frailty, such as serum levels of inflammatory markers.

Finally, the field may achieve the greatest advances by removing the disease paradigm all together and focusing on aging mechanisms, such as the efficiency of a biological system or the trajectory of an aging process that is quantitative in nature and may be more proximal to the genetic cause. Although practitioners may not immediately see the relevance of identifying genes with no specificity to disease, it is likely that such a paradigm is more true to the biology of aging and will more directly affect our broad understanding of aging. For example, identifying novel genes that have a role in free radical detoxification would inform mechanisms of oxidative stress and thus improve the understanding of a host of other disease-specific models of pathogenesis.

Other Genetic Frontiers

Genetic involvement in biological aging is not limited to inherited SNP variation of nuclear DNA. In fact, we all inherit multiple copies of mitochondrial DNA (mtDNA) from our mothers. Sequence variation of mtDNA, which codes for the critical energy production functions of the mitochondria, has been associated with longevity (148) (see Table 2). Methods to measure and analyze mtDNA in conjunction with nuclear DNA are needed at a much greater scale. Further, even nuclear DNA contains variation other than SNPs. The most common include repeat variants, where a particular segment of sequence is repeated multiple times in row and copy number variation (CNV) where more or less than the expected two parental copies exist for one person at a particular genomic region, due to nondysjunctions (>2 copies) or deletions (<2 copies), among other causes. CNVs are now easier to identify with whole-genome genotyping, panels and recent discoveries have shown large

variability across individuals (195,196). This can have downstream implications in the timing or amount of expression of particular genes. Methods to measure both genotype and CNV are now in development and will add an important component to genetic studies of aging.

Somatic mutations that are not inherited, but rather accumulate over the life span, are likely a very important part of the aging process. Damage to DNA is inevitable during cellular replication and a clear relationship exists between aging and reduced DNA repair function (197,198). Thus, sequence variation in genes controlling DNA repair have been associated with aging, and genomic regions particularly susceptible to mutation may accumulate errors at a faster rate and therefore accelerate the aging process. For example, mtDNA has been shown to be more susceptible to oxidative damage leading to somatic mutations than nuclear DNA, leading to reduced energy production and accelerated aging (199–204). Another highly cited example of such susceptibility is the telomere region of each chromosome, which is maintained via telomerase enzyme activity. Studies have observed that increased oxidative stress may trigger loss of telomeres and telomerase function and can lead to genomic instability and single- and double-stranded DNA breaks and ultimately cause premature aging, whereas conservation of telomeres has been associated with longevity (205–207). Shortened telomere length has been associated with increased mortality and risk of cancer and CVD (208,209).

Finally, the amount of methylation, or other chemical modifications of the DNA, may be an important contributor to aging mechanisms. Such modifications, often termed epigenetic, do not involve the DNA sequence itself, but are rather chemical modifications on top of the sequence, such as methylation of cytosines and acetylation or phosphorylation of histones (210). These chemical signals are stable across DNA replication and control the timing and amount of gene expression in each tissue of the body by restricting or allowing access to promoter architecture. There is growing evidence that these modifications change with age, implying a role in aging biology (211–214). It is possible that accumulation of somatic DNA mutations does not occur at a high enough rate during the life span to induce common aging diseases, but epigenetic changes may occur at a frequency that could contribute to these effects. Very few studies have demonstrated epigenetic changes in humans with age, due to technical and biosample limitations, but as measurement tools improve, this will be yet another area of active research. A recent study has shown differences in local and global methylation by age by examining the similarity in methylation patterns between monozygotic (MZ) twins aged 3 years and MZ twins aged 50 years. Although these analyses were not in the same individuals (the same twins were not followed longitudinally), the similarity in methylation patterns between young twins compared with the dissimilar patterns among older twins argues strongly for age-related changes in the epigenome (215). Interestingly, this may be a way of connecting cumulative environmental damage with genetic mechanisms because it may be the epigenetic chemistry that is modified by environments, providing a biological connection between genetic and exposure-focused epidemiology. Efforts are now in place to improve measurement and to catalog variably methylated sites in the genome in several human tissues (216) to provide the foundations for monitoring age-related changes in the epigenome and connecting methylation to age-related phenotypes (210).

Analytic Complexity

Advances in genetic measurement and trait modeling bring new analytic challenges. Most analyses focus solely on the unadjusted marginal effects of one genotype at a time, using 1-degree of freedom score statistics such as the Cochran-Armitage trend test (217,218). This has several limitations including the potential misspecification of the genetic inheritance model, the lack of control of confounders, and the lack of modeling of important interactions. Because trend tests assume incremental effects of each allele on the trait (eg, additive or log-additive), they may not be appropriate for recessive, dominant, or other genetic inheritance models. Many have chosen to focus on 2-degree of freedom tests that estimate effects for each genotype separately; however, this may slightly reduce power, per test. More sophisticated modeling that is commonplace in other areas of aging epidemiology, such as controlling for confounders and assessing interactions, is often not pursued until after marginal effects in these simple unadjusted analyses are detected. It may be argued that confounding is not a grave concern for genetic risk factors because they are not time varying and were likely determined before any confounder. One important exception is genetic ancestry, which does determine marker genotype status as well as trait status and can therefore confound associations between markers and trait phenotypes. Thus, genetic ancestry is now commonly assessed using measured genotypes among study participants (219–222). Any genetic "outliers" can be removed from subsequent analyses, or, if subgroups of ancestry are observed, group membership can be adjusted for in the association analyses as a confounder or by adjusting the test statistic accordingly (220,223,224).

It is likely that many gene effects occur in complicated scenarios of gene-gene or gene-environment interactions. Estimation of effects without accommodating the underlying model is likely to attenuate estimates and contribute to false negatives. The field is now peppered with methods aimed at tackling this problem from the data mining, neural network, and classification perspectives, which all focus primarily on model building (225–229). The challenge becomes one of picking the best high-order interaction model from the almost-infinite number of possible combinations, realizing the large sample sizes needed in both discovery and validation phases. Many have resorted to only pursuing this kind of model building once a marginal genetic effect

has been identified, which is likely to guarantee the importance of such models by limiting false positives but will miss many interactive relationships that do not yield a marginal signal. Some who pursue these methods circumvent the false-positive concern by seeding model selection according to known biological pathways or gene ontology (230). This may be quite fruitful, but is again likely to miss new discoveries and it puts much weight on prior knowledge.

A major analytic challenge has become the appropriate treatment of the multiple comparisons incurred in modern genetic epidemiology, which may include thousands to millions of genetic markers, modeled independently, interactively, and often for multiple phenotypes. Traditional focus on controlling type I errors for each statistical test has pursued control of the familywise error rate (FWER) across a family of tests, such that the probability of a single significant finding across *M* tests when all are truly null does not exceed a specified level (eg, 5%). This is the concept behind the Bonferroni and Sidak methods (231), and strong FWER control prevents unnecessary follow-up of false positives, making replication in subsequent studies more likely. However, this has been considered too conservative for most genetic applications, resulting in very low power and missed true-positive associations, for at least two reasons. First, genetic markers are correlated in the genome due to population genetic history and therefore are not independent. Many circumvent this limitation while controlling FWER through permutation procedures that estimate empirical *p* values that accommodate multiple tests while keeping the original correlation structure of the observed data intact (232). However, this is computationally intensive and often not feasible for large samples with large numbers of markers. As an alternative, methods exist to control FWER by estimating the effective number of independent tests as a single parameter based on the observed genotype data (233) or by accounting for the asymptotic distribution of the test statistics via simulation or numerical integration (234,235).

The second criticism of FWER control as an approach to the multiple comparison problem is the notion that one actually desires to control the probability of at least one significant result, assuming all tests are null. In fact, many would argue that we do not want to make such an assumption and instead have a prior belief that some of our genetic markers are indeed true positives. With this in mind, many support simply judging each association estimate separately and focusing on biological plausibility and replication. Others choose to control the false discovery rate rather than FWER (236,237). In this context, one protects the proportion of tests considered to be significant that are false positives, similar to controlling the positive predictive value (or its inverse) in a diagnostic testing framework(236,237). Finally, Bayesian methods have also been proposed that place uninformative (or informative) priors on a set of genetic markers and estimate a common effect across those markers, thus reducing the multiplicity problem (238,239).

Emerging genetic associations have shown that many effects are of modest size and that most data sets (even with thousands of individuals) are underpowered to detect these effects, especially if one requires very stringent significance criteria. Therefore, inappropriate focus on type I errors will almost surely result in increased type II errors (loss of power), and this must be carefully considered when applying any of these multiple comparison approaches.

The future is wide open regarding how to best incorporate such a wealth of genetic information into complex model building. The overall goal is to incorporate measurements from each of the emerging areas discussed into convergent models of aging, and this is an analytic challenge on the frontier of statistical aging and genetics research.

Summary

This is an exciting time for aging research and for genetic epidemiology. Several advances have been made but were limited by the available human genome information, samples, and phenotype measures. Traditional genetic epidemiology designs focused on a single or very few genetic markers per gene. This approach relates to using a poor proxy for an unmeasured risk factor. It is likely that many true associations have been missed due to this incomplete marker information. Following the "indirect" association paradigm and using emerging human genome information, we are now able to adequately represent the common genetic variation in genes and across the whole genome using only a subset of well-chosen and easily genotyped SNPs. Many studies mentioned in earlier sections have been limited to relatively small samples, despite full-scale recruiting efforts, and have focused on disease outcomes with likely multiple causes, rather than on quantitative endophenotypes. Thus, detection of small marginal effects has been difficult, and replication across studies has been unlikely. We must focus on more precise measurements of continuous traits of interest and much larger and richer study samples that are adequately powered to detect small risk effects and interactions. The movement toward consortia focused on combined efforts, standardized measurements, and quantitative traits will provide a richer set of information as we move forward. Successful partnership of aging and genetic epidemiology can lead to discovery of new pathways for disease treatment and better identification of at-risk individuals, as well as new insights into the aging process that can guide behavior and treatment to maintain health with age.

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