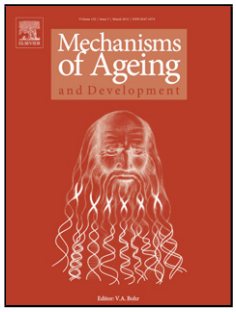


IP6K3 and *IPMK* variations in LOAD and longevity: evidence for a multifaceted signaling network at the crossroad between neurodegeneration and survival

Serena Dato (Writing - original draft) (Writing - review and editing), Paolina Crocco (Methodology) (Writing - review and editing), Francesco De Rango (Formal analysis) (Data curation), Francesca Iannone (Methodology), Raffaele Maletta, Amalia C. Bruni, Adolfo Saiardi (Conceptualization) (Writing - review and editing), Giuseppina Rose (Conceptualization) (Writing - original draft) (Writing - review and editing) (Funding acquisition), Giuseppe Passarino (Conceptualization) (Writing - review and editing)

PII: S0047-6374(21)00011-7

DOI: https://doi.org/10.1016/j.mad.2021.111439

Reference: MAD 111439

To appear in: *Mechanisms of Ageing and Development*

Received Date: 21 October 2020

Revised Date: 23 December 2020

Accepted Date: 18 January 2021

Please cite this article as: Dato S, Crocco P, De Rango F, Iannone F, Maletta R, Bruni AC, Saiardi A, Rose G, Passarino G, *IP6K3* and *IPMK* variations in LOAD and longevity: evidence for a multifaceted signaling network at the crossroad between neurodegeneration and survival, *Mechanisms of Ageing and Development* (2021), doi: https://doi.org/10.1016/j.mad.2021.111439

IP6K3 and *IPMK* variations in LOAD and longevity: evidence for a multifaceted signaling network at the crossroad between neurodegeneration and survival.

Serena Dato[1, *], Paolina Crocco[1, *], Francesco De Rango[1], Francesca Iannone[1], Raffaele Maletta[2], Amalia C. Bruni[2], Adolfo Saiardi[3], Giuseppina Rose[1, a], Giuseppe Passarino[1, a]

1) Department of Biology, Ecology and Earth Sciences, University of Calabria, Rende, Italy

2) Regional Neurogenetic Centre, ASP Catanzaro, Lamezia Terme, Italy.

3) Medical Research Council Laboratory for Molecular Cell Biology, University College London, London, U.K.

[a]Corresponding authors and co-senior authors

*These authors equally contributed

Email addresses of co-authors:

S. Dato: serena.dato@unical.it

P. Crocco: paolina.crocco@unical.it

F. De Rango: f.derango@unical.it

F. Iannone: francesca.iannone@unical.it

R. Maletta: maletta@arn.it

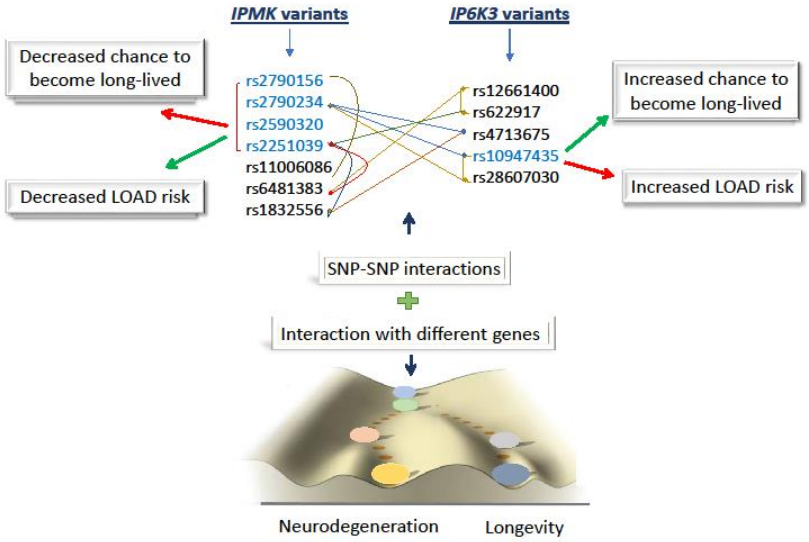
A.C. Bruni: bruni@arn.it

A. Saiardi: a.saiardi@ucl.ac.uk

G. Rose: pina.rose@unical.it

G. Passarino: giuseppe.passarino@unical.it

Graphical Anstract



Highlights:

Multifunctional Inositol-kinase *IP6K3* and *IPMK* affect LOAD and longevity

Risk alleles for LOAD act as pro-longevity variants

Protective alleles for LOAD act as risk factor for longevity

Interactions between *IP6K3* and *IPMK* account for phenotype-specific associations

Mitochondria as crossroad of pathways leading to neurodegeneration and/or longevity

Abstract

Several studies reported that genetic variants predisposing to neurodegeneration were at higher frequencies in centenarians than in younger controls, suggesting they might favor also longevity.

IP6K3 and IPMK regulate many crucial biological functions by mediating synthesis of inositol poly- and pyrophosphates and by acting non-enzymatically via protein–protein interactions. Our previous studies suggested they affect Late Onset Alzheimer Disease (LOAD) and longevity, respectively. Here, in the same sample groups, we investigated whether variants of *IP6K3* also affect longevity, and variants of *IPMK* also influence LOAD susceptibility. We found that: i) a SNP of *IP6K3* previously associated with increased risk of LOAD increased the chance to become long-lived, ii) SNPs of *IPMK*, previously associated with decreased longevity, were protective factors for LOAD, as previously observed for *UCP4*. SNP-SNP interaction analysis, including our previous data, highlighted phenotype-specific interactions between sets of alleles. Moreover, linkage disequilibrium and eQTL data associated to analyzed variants suggested mitochondria as crossroad of interconnected pathways crucial for susceptibility to neurodegeneration and/or longevity.

Overall, data support the view that in these traits interactions may be more important than single polymorphisms. This phenomenon may contribute to the non-additive heritability of neurodegeneration and longevity and be part of the missing heritability of these traits.

Keywords: IP6K3, IPMK, Aging, longevity, Alzheimer, SNP-SNP interaction

1. Introduction

The heterogeneity in age-related functional decline is a highly debated topic (Field et al, 2018; Lowsky et al, 2014). The understanding of genetic and non-genetic factors that affect such heterogeneity is fundamental for the development of strategies to attenuate age related decline and prolong a healthy life. The study of centenarians, exceptionally long-lived individuals that in most cases experienced a delayed aging, has arisen growing interest for its potential to reveal information on the combination of genes and lifestyle factors that can prevent or postpone age-related diseases. Numerous studies in this field demonstrated that longevity is a highly plastic and dynamic trait being the result of a lifelong remodeling process which depends on a complex genetic architecture, influenced by extensive genotype-by-genotype and genotype-by-environment interactions (Dato et al, 2017). Undeniably, centenarians represent an extreme phenotype of good health and they could be considered as super-controls to compare the distribution of risk alleles with respect to patients with age related diseases, such as type two diabetes (T2D), assuming that such alleles should have the highest frequency among patients, an intermediate frequency among healthy subject and the lowest among centenarians (Garagnani et al, 2013). On the other hand, the systematic analysis of the 'gerontome', the collection of over 2000 genes shown to modulate aging in model organisms and human, suggested complex relationships between aging-related genes and age-related diseases. (Fernandes et al, 2016). For instance, many genetic variants associated with increased risks of diseases are found in genomes of long-lived people, suggesting they might be risk factors or protective factors according to other (genomic or environmental) concurrent factors (Beekman et al, 2010; Mooijaart et al, 2011; Raule et al, 2014; Sebastiani et al, 2013; Freudenberg-Hua et al, 2014). Similarly, some genetic alleles show tradeoff-like effect on mortality risk during life course (i.e., risk factors at adult age and pro longevity at advanced ages), or the same

variant shows opposite effects on different age-related diseases, differently affecting the individual mortality risk (Ukraintseva et al, 2016).

To understand this genetic complexity, we need to consider a biological systems functioning as networks of biomolecules (Zhang et al, 2016; Cevenini et al, 2010); this implies that the individual effect of a gene can be negative or positive, or even neutral, depending on the interactions occurring with components of the different networks, which may change along life progression (Franceschi et al, 2020). Chiefly relevant to this scenario could be signaling molecules or proteins with multiple functions which may act as either signaling hubs or "switchers" connecting different pathways, influencing several cellular functions essential for survival (Wolfson et al, 2009). Energy production and storage may be crucial in this context. Indeed, the variability of mitochondrial DNA, affecting subunits of the oxidative phosphorylation chain, has been found to be correlated to longevity as well as diseases associated tissue (especially neuronal) degeneration. In several studies the same allelic variation in mtDNA was associated to both longevity and to degenerative disorders (Raule et al. 2014 and references therein). Similarly, mitochondrial uncoupling proteins 4 (*UCP4*) variation (rs9472817), affecting the management of energy, has been found to affect both longevity and neurodegenerative disease (Rose et al, 2011; Montesanto et al, 2016; Montesanto et al, 2018).

Inositol polyphosphates and specifically the pyrophosphate containing species are emerging as molecules playing key role in the management of energy homeostasis and with fundamental role in regulating multiple cellular process (Tsui and York, 2010; Livermore et al, 2016). The energy-rich inositol pyrophosphates IP_7 and IP_8, generated by sequential phosphorylation of the calcium releasing factor IP_3 or from the glycolytic intermediate glucose-6P (Desfougères et al, 2019), display pleiotropic effects acting as potential 'molecular switch' in the regulation of a wide spectrum of central processes such as phosphate homoeostasis and energetic

metabolism (Azevedo and Saiardi, 2017; Thota and Bhandari, 2015; Wilson et al, 2013; Wundenberg and Mayr, 2012). Hence, inositol pyrophosphates signaling may be critical and at the "crossroad" of age-related disease and longevity networks. In mammalian cells, the pathway of inositol pyrophosphates synthesis involves multiple enzymatic steps carried by different type of kinases; the ITPK1 type of multi kinase, the inositol polyphosphate multi kinase (IPMK), the inositol pentakisphosphate kinase IPPK, three different isoforms of inositol hexakisphosphate kinase (IP6K1–3) and two different isoforms of the diphosphoinositol pentakisphosphate kinase (PPIP5K1-2).

Noteworthy is the capacity of many of these kinases and especially of both IPMK and IP6K3 to, independently of their enzymatic ability, act non-catalytically via protein–protein interactions, influencing multiple different biological processes (Rojas et al, 2019; Kim et al, 2017; Fu et al, 2015). The multifunctionality of these proteins, is suggestive of a fine tuning of the inositol polyphosphates signaling during life progression. The complexity and adaptability of the inositol polyphosphate signaling pathways could contribute to the heterogeneity in the age-related functional decline. We hypothesize that the variability in *IPMK* and *IP6K3* genes could be leading either to neurodegeneration or to a long life.

Such hypothesis is supported by different evidence; a rare variant (rs12570088) near to *IPMK* locus is related to the susceptibility to Alzheimer's disease (Yokoyama et al 2016); IPMK acts as a regulator of fear extinction and synaptic plasticity (Park et al, 2019); furthermore, there are evidence that IP6K3 is linked to lifespan in mice (Moritoh et al, 2016).

We recently demonstrate that the genetic variability of *IPMK* affect human longevity, by reporting a six-SNPs haplotype that significantly influences female longevity (De Rango et al, 2019). Additionally, a study by Crocco et al (2016) showed that the variability of *IP6K3*, which is highly expressed in the brain, is associated with increased risk of late onset Alzheimer's disease (LOAD).

Considering that longevity and neurodegeneration share relevant pathways, in the same sample groups analyzed in Crocco et al (2016) and De Rango et al, (2019) here we investigated whether: a) the genetic variability of *IP6K3*, previously associated with LOAD can affect the ability to live longer, and b) the genetic variability of *IPMK*, previously correlated to longevity can affect the susceptibility to LOAD.

2. Materials and Methods

2.1 Study population

For this study we analyzed 848 unrelated subjects born in Calabria (South Italy) and recruited across the whole territory through several campaigns focused on the monitoring of the quality of aging in the region. The sample included 568 healthy subjects aged 64-105 years (55 % females), and 280 patients with LOAD aged 77.8 ± 5.0 years (63% females). Their Calabrian ancestry was ascertained up to the third generation.

The group of health subjects was divided in two age classes according to two "thresholds of longevity", 88 years for men and 91 years for women, corresponding to the point after which a significant negative change in the slope of the survival curve of the Italian population occurs (Passarino et al, 2007). Males younger than 88 and females younger than 91 years will be defined as controls (N =309, mean age 74 years); in accordance with this, the rest of the sample, males older than 88 and females older than 91 years, will be here defined as long lived samples (N =259, mean age 96.9 years). In the long-lived sample group females were 63%, while they were 49.5% in the adult controls. All subjects were free of the major age-related pathologies (e.g., cancer, type-2 diabetes, neurodegenerative and cardiovascular diseases), and were carefully assessed using a rigorous clinical history evaluation and a general/neurological examination, to exclude the presence of any neurological disorder.

LOAD patients were from the same geographical region and were enrolled by the Regional Neurogenetics Center (Lamezia Terme, Cz, Italy). Clinical diagnosis for LOAD was performed through the criteria of the National Institute on Aging, and the Alzheimer's Association workgroup (McKhann et al, 2011). All patients were fully characterized from a clinical point of view and a set of physical and biochemical parameters were measured. Cognitive status was investigated through Mini Mental State Examination (MMSE) (Folstein

et al, 1975). MMSE scores were adjusted for age and educational level according to Magni et al (1996).

It might be worth mentioning that long lived subjects were parts of the birth cohorts 1900-1915. Control samples were parts of the birth cohorts 1925-1945, as well as LOAD patients.

An informed written consent was signed by all subjects or their legal representative. This study was performed according to the Declaration of Helsinki with appropriate ethics committee approval.

2.2 SNP selection and genotyping

A panel of 17 SNPs within approximately 30 kb encompassing the entire *IP6K3* gene and its 5′ and 3′ flanking regions were genotyped in all subjects included in the study and chosen based on those genotyped in the previous study by Crocco et al (2016). Similarly, 14 SNPs were investigated for *IPMK*, mapping within and nearby the gene and prioritized by a tagging approach (De Rango et al, 2019). Genotyping was performed by iPlex Gold Genotyping Assay and Sequenom MassArray (Sequenom, San Diego, CA, USA) technology, following the manufacturer's instructions. SNP assays were designed using Sequenom's MassARRAY Assay Design v3.0 Software. Spectra were analyzed using MassARRAY Typer v3.4 Software (Sequenom). For quality control, to assess the reliability of the genotype identification protocols, about 10% of the samples were reanalyzed and the concordance rate of the genotypes was higher than 99%. For additional quality control, genotypes were excluded if Hardy-Weinberg equilibrium among controls $p < 0.05$ or call rates < 90%.

2.3 Linkage disequilibrium and functional annotation analysis of associated SNPs

Linkage disequilibrium analysis, LD, was explored using information from HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) and considering SNPs

associated with r2 ≥ 0.8. To explore the potential function of the candidate SNPs, functional annotation analysis was performed by interrogating GTEx (Genotype–Tissue Expression) dataset (https://gtexportal.org/), a comprehensive survey of the functional consequences of genetic variation in non-coding regions at the transcript level from various human tissues samples (The Genotype- Tissue Expression (GTEx) Consortium, 2013). Positive or negative effects of the allele on the gene expression were estimated by considering the normalized effect size (NES) of the eQTLs, defined as the effect of the alternative allele (ALT) relative to the reference allele (REF) in the human genome reference GRCh38/hg38. For each gene indicated to be regulated by the candidate SNPs, a nominal p-value threshold of $p < 0.05$ was considered; top significant genes and relative tissues were finally included in the list of variant-gene pairs.

2.4 MDR analysis of epistatic interactions

For testing the epistatic interaction between pairs of SNPs, multifactor dimensionality reduction (MDR) was applied (Moore, 2004; Ritchie et al, 2001). This approach allows to estimate high-order interactions among genes collaborating with respect to a given phenotype and thus multilocus genotype combinations associated with high or low risk of disease. The entropy-based clustering algorithm used by MDR sets a contingency table for k SNPs and calculates case–control ratios for each of the possible multilocus genotypes. The MDR interaction model describes percentage of entropy (information gain or IG) by each factor (values in the nodes indicate independent main effect) or 2-way interaction. Graphical visualization is made through connections among the markers and help to interpret additive and non-additive interactions effects on phenotype: positive values of entropy indicate synergistic or non-additive interactions, while negative entropy values indicate redundancy between the markers or lack of any synergistic interaction between the markers.

For figures, networks were plotted by setting two-three-way combinations (Fig. 1a and 1b) and two-five-way combinations (Fig. 1S and 2S) of the attributes. Connections in red and orange indicate nonlinear or epistatic interactions, connections in green and brown indicate independence or additivity and redundancy (blue lines). For significance, permutation testing is applied, dividing the dataset into 10 portions, and using nine portions as a training data set, and the remaining as a testing data set. Missing genotypes were imputed with the MDR data tool software (version 0.4.3), by imputing the data from existing data set. MDR analyses were implemented in the open-source MDR software package version 3.0.2 (available on https://omictools.com/mdr-tool).

2.5 Statistical analyses

For each polymorphism, allele and genotype frequencies were estimated by gene counting from the observed genotypes. Hardy–Weinberg equilibrium was tested by Fisher's exact test. Pairwise measures of linkage disequilibrium (LD) between the analyzed loci was estimated by Haploview. (https://www.broadinstitute.org/haploview/haploview). The association between the analyzed genetic variants and the phenotypes under study was evaluated by logistic regression models. In the analyses, controls (adult controls) were coded as 0 and cases (LOAD patients and long-lived individuals) were coded as 1.

In order to identify any relationship between alleles in affecting the phenotypes, different genetic models (dominant, additive and recessive) were used to test association, using for each SNP the minor allele as reference. For each SNP, the most likely genetic model was then estimated on the basis of minimum level of statistical significance (Wald test p-value). For SNPs with rare homozygous genotype < 3%, only the dominant model was considered.

Since SNPs were selected based on prior evidence of associations with the tested phenotypes, no Bonferroni correction was applied, as suggested by several authors (Armstrong 2014; Reuben et al, 2020).

3. Results

3.1 Experimental samples

The sample groups analyzed in this study is presented in Table 1. These samples were previously tested for SNPs of *IP6K3* (Adult control group vs LOAD patients), and for SNPs of *IPMK* (Adult control group vs long-lived subjects). In the current study we checked the same groups for potential cross-phenotype associations, by testing the variants of *IP6K3*, previously studied in LOAD patients, in long-lived subjects to compare them with the group of the adult control. Similarly, we studied the IPMK variants previously studied in long lived subjects in LOAD patients to compare them with the group of adult control.

3.2 Analysis of the association of IP6K3 variants with Longevity

Results for the analyzed *IP6K3* variants are presented in Supplementary Table 1S. There was no association between sex and genotype frequency for any of the SNPs tested. Conversely, Table 2 report the SNPs statistically associated with longevity in the present study, together with those associated with LOAD as reported by Crocco and colleagues (2016).

Our analysis found that the minor A allele of rs10947435 has a positive effect on longevity with a dominant model better fitting the data (OR=1.73, 95% CI 1.19-2.51; p=0.004). The additive model fit the data best for rs4713675 (OR=1.36, 95% CI 1.03-1.77; p=0.028), also with a positive effect of the minor T allele on longevity. The same allele (A) of rs10947435 has been associated with an increased risk of LOAD (Table 2), whereas no association has been detected between rs4713675 and LOAD risk (Crocco et al, 2016). Conversely, the variant rs28607030 did not result associated with longevity in this study, but instead has been associated with LOAD in the previous one. Therefore, we observed either phenotype-specific (rs4713675 and rs28607030) or cross-phenotype (rs10947435) associations of SNPs with different direction of risk.

3.3 Analysis of the association of IPMK variants with LOAD

We then investigated the *IPMK* variants previously studied in relation to longevity (De Rango et al, 2019), in patients affected by LOAD. In consideration of the sex-specific effect of *IPMK* SNPs on longevity, the data were analysed according to gender. Similarly, to what was seen for longevity, we found SNPs significantly associated with LOAD in females, but not in males. Results for the SNPs associated significantly to one or both traits are summarized in Table 3. Complete results are reported in Supplementary Table 2S. Out of the six SNPs negatively associated with female longevity, four SNPs, namely rs2790156, rs2790234, rs2590320 and rs2251039, conferred a protective effect on LOAD risk in the same gender ($p < 0.05$), with ORs (95% CI) of respectively 0.25 (0.06–0.92), 0.52 (0.27–0.99), 0.23 (0.06–0.83) and 0.61 (0.39–0.95). As before, we found cross-phenotype and phenotype-specific effects of SNPs.

We also performed haplotype analysis by including all the SNPs reported in Table 3. We identified the haplotype made of all the minor SNP alleles (namely A-G-A-t-a-T in Table 4) associated with reduced LOAD risk. The results also support a major effect of rs2790234-G allele on the trait; in fact, the A-G-A-t-a-T is significantly associated, while the A-C-A-t-a-T is not. Importantly, this is the same allele, lying in the six-SNPs haplotype, that exerts a major negative effect on longevity.

3.4 SNP-SNP interaction analyses

In view of the above findings, we evaluated SNP-SNP interactions both in LOAD and longevity of *IP6K3* and *IPMK* variants analyzed in the present and previous studies (Crocco et al, 2016; De Rango et al, 2019), by applying a Multi-Dimensional Reduction (MDR) method. In the interaction analysis, we included a SNP of *UCP4*, previously tested in the same

sample groups, that negatively impacted on the probability to attain longevity (Rose et al., 2011), while acted as a protective factor for LOAD (Montesanto et al., 2016).

This analysis has reported phenotype-specific SNP-SNP interactions, as shown in Supplementary Figures 1S and 2S, which plot the interaction network for longevity and LOAD, using one to five-way attributes combinations. As for longevity, the analysis also showed the contribution of sex as one of the factors influencing the entropy of the system.

For longevity, as shown by the entropy graph and dendrogram in Figure 1A and 1B, we found an epistatic interaction between rs2790234-*IPMK* and rs4713675-*IP6K3.* As shown by red line, combining these two SNPs using MDR gives a positive information gain, evidence of an increased contribution to the phenotype respect to the single variants, which were in any case associated to the phenotype. This interaction is significant and consistent (9/10 cross-validation consistency $p<0.0001$). Weaker interactions (orange lines) were found between rs2790234-*IPMK* and two SNPs, one being rs10947435-*IP6K3* and rs9472817-*UPC4*, both associated with longevity in single-SNP analysis (Rose et al, 2011). As for the blue line, such as for green one, these connections indicate a redundancy of the correspondent SNP pairs on the phenotype: this is particularly true here for rs4713675-*IP6K3* and rs10947435-*IP6K3*, which showed single associations with longevity.

In LOAD dataset, as shown by the entropy graph and dendrogram in Figure 2A and 2B, the analysis shows a strong single effect of rs9472817-*UCP4*, explaining alone the 9.28% of the entropy of the system; this SNP was previously reported to be associated with LOAD (Montesanto et al, 2016). This SNP epistatically interacts with rs1832556-*IPMK*, with a 7/10 cross-validation consistency and high training-balanced accuracy ($p < 0.0001$).

3.5 Functional annotation of the associated SNPs

We performed functional annotation for better understanding the associations found with longevity and LOAD. We first queried the Haploreg database for SNPs in linkage disequilibrium (LD) (r^2 >= 0.8) with those significantly associated with our phenotypes (Supplementary Table 3S). For *IP6K3,* rs10947435 resulted in LD with SNPs in the same gene and with several markers of *UQCC2* (ubiquinol-cytochrome c reductase complex assembly factor 2, alias *MNF1,* Mitochondrial nucleoid factor 1); rs4713675 tags only another variant in the *IP6K3* gene, while rs28607030 is not in LD with other SNPs. As for *IPMK* markers, searches yielded all the significant SNPs in LD with a large number of variants in the same gene and with *CISD1* (CDGSH Iron Sulfur Domain 1, also termed *mitoNEET*) markers.

To further evaluate allele- and/or tissue-specific differences in gene expression we performed expression quantitative trait loci (eQTL) analysis by interrogating GTEX database. This analysis reported both cis-regulatory and trans-regulatory allele-specific effects for all the tested SNPs (Supplementary Table 3S); in particular, the analysis showed allele- and tissue-specific effects on genes involved in several cellular functions including mitochondrial activity.

4. Discussion

IP6K3 and *IPMK* gene products are crucial in the generation of inositol poly- and pyrophosphates and, also, interact with other cellular components to control numerous aspects of cell metabolism in response to distinct cellular signals (Mukherjee et al, 2020; Kim et al, 2017; Wilson et al, 2013). Data from previous studies (Crocco et al 2016; De Rango et al 2019) show that *IP6K3* and *IPMK* loci harbour variants that are associated with LOAD and longevity phenotype, respectively. As many reports have highlighted that genetic variation affecting neurodegeneration may affect longevity as well (Nygaard et al, 2019), and vice versa, we investigated the variation of these genes for both phenotypes.

We found that an allele within *IP6K3* locus (rs10947435-A), previously reported to be associated with increased risk for LOAD (Crocco et al, 2016), increased the chance to become long-lived, while a subset of alleles at *IPMK* locus (rs2790156-A, rs2790234-G, rs2590320-A, rs2251039-T), that were found to decrease the chance to become long-lived (De Rango et al, 2019), decreased the risk for LOAD.

Several scenarios fit these puzzling associations: diverse genetic mechanisms of pleiotropy, from the different forms of biological pleiotropy to spurious pleiotropy (Hodgkin, 1998; Solovieff et al, 2013); epistatic SNP-SNP interactions whereby the effect of an allele towards on trait is modifiable by alleles at other loci; genetic buffering, a type of epistatic interaction in which a favorable genotype attenuates the effect of one or more deleterious variants. In this model, interactions between longevity genotypes (buffering genes) and age-related disease genotypes (buffered genes) may account for the increased prevalence of deleterious disease alleles in long-lived individuals (Aviv et al, 2007; Tindale et al, 2017). Having regard to the above consideration, the finding that for most associated SNPs the best performing genetic model was different between the LOAD and longevity analyses may be explained by the fact that the different forms of interaction (i.e., different genotype-genotype combinations) may

have different genetic effects (either in magnitude or direction) depending on the genetic architecture of the trait itself (Mackay and Moore, 2014). Also, the phenotype-specific SNP-SNP interactions we observe reasonably support the hypothesis that genetic interactions could be major contributors to the complex observed associations.

A paradigmatic example of the complex relationships among alleles in different physiological contests is provided by the mtDNA variability, with evidence that while mutations in subunits of the complex I of the electron transport chain have a beneficial effect on longevity, the co-occurrence of mutations in complex I and III or in complex I and V seem to be detrimental (Raule et al, 2014). It is not surprising then that high frequency of mutations in complex I can be found in both mtDNA linked diseases (Man et al, 2004) and in long lived subjects (Tanaka et al, 1998).

Puzzling genetic associations may also represent the effects of co-localizing, that is causative, common, or rare, variants at the same locus (or proximal loci) tagged by the same SNP due to LD. It is also worth noting that an allele may differently affect the expression of near (cis-eQTL) or distant (trans-eQTL) genes or even it may have different effects on different tissues, thus likely exhibiting context-specific effects with different phenotypic consequences. This could be particularly true for genes, such as *IP6K3* and *IPMK*, endowed with more than one molecular function, or participating in diverse biological processes that could require the coordinate action of distinct signalling network.

The LD analysis performed, demonstrated that the associated SNPs are in LD not only with variants on the gene where they reside but also with those of other genes; thus, they may act as potential proxy markers for other SNPs in the same chromosomal region. Moreover, data extracted from GTEx database revealed allele-specific cis- and trans-eQTL effects across different tissues. We found that *IP6K3* rs10947435-A and rs4713675-T alleles also regulate, in a tissue specific manner, *BAK1* and *UQCC2* genes, both of which are related to

mitochondrial function (Gross, 2016; Tucker et al, 2013). This is also the case for the associated *IPMK* variants, which are all cis-eQTL for *CISD1*, which encodes for a mitochondrial Fe-S proteininvolved in the regulation of mitochondrial lipid oxidation (Yuan et al, 2016). It is also quite interesting that the differential effects on gene expression of *IPMK* and *IP6K3* variants distinguish neuronal tissues with respect to the other tissues (see Table 3S).

Likely the mechanistic basis of our observation may reside in mitochondria. The numerous evidence of the involvement of mitochondria in promoting neurodegeneration and in mediating longevity (Golpich et al, 2017; Rose et al, 2017) give plausibility to this hypothesis, that is supported by findings showing that inositol pyrophosphates act as energy sensors able to affect the cellular level of ATP, thus modifying the balance between mitochondrial oxidative phosphorylation and glycolytic flux (Gu et al, 2017; Szijgyarto et al, 2011). Besides, IPMK overexpression is reported to rescue deficits in mitochondrial metabolic activity in transgenic models of Huntington's disease (Ahmed et al, 2015) and also binds, in a glucose-mediated manner, the AMP-activated protein kinase (AMPK), a sensor of intracellular ATP levels that is rapidly activated after nearly all mitochondrial stresses (Bang et al, 2012). Additionally, IPMK appears to be a physiologic cofactor of mTOR (Kim et al 2011), which regulates many aspects of mitochondrial function and one of the central modulators of lifespan (Wei et al, 2015). Further evidence, of the role on inositol polyphosphate in bioenergetic control come through the Inositol (1,4,5) Trisphosphate Receptor Type 3 (ITPR3). This receptor determines an increase of Ca^{2+} release from Endoplasmic Reticulum and a concomitant increase of the Ca^{2+} uptake in mitochondria (Càrdenas et al, 2010), negatively affecting its activity. Notably, besides affecting the IP6K3 expression, the tested markers also differentially regulate the ITPR3 gene expression (Supplementary Table 3S).

Remarkably, a behaviour like the one observed in the present study was reported for the polymorphism (rs9472817) of the uncoupling protein 4 gene (UCP4), a neuron specific mitochondrial membrane protein that uncouple biofuel oxidation from ATP. Indeed, the rs9472817-C allele was found to increase the risk of LOAD and the penetrance of APOE-ε4 allele (Montesanto et al, 2016), the risk for Frontotemporal Dementia (FTD) (Montesanto et al, 2018), and, at the same time, was found overrepresented among centenarians (Rose et al, 2011). Noteworthy, UCP4 has multifunctional properties on the neuronal system which include thermogenesis, neuronal plasticity, neuroprotection against oxidative stress, regulation of mitochondrial membrane potential and ATP level and Calcium homeostasis (Ramsden et al, 2012).

The highly orchestrated intracellular signaling pathways are often integrating and modulating mitochondrial physiology essential to the cellular metabolic and energetic needs. It therefore seems likely that mitochondria functions are important determinants of both diseases state and longevity. Mitochondria appear to be central hubs for neurodegeneration (Anderson et al, 2019). On the other hand, the central nervous system is significantly enriched with hubs (about 73% of the whole human interactome) among pathways which act at the crossroad of longevity and age-related disease networks (Wolfson et al, 2009). A further support to this hypothesis is given by the genetic variability of mtDNA, and in particular by the 4336T>C mtDNA mutation which was found associated with Alzheimer's disease (AD) risk in a large number of studies and twice more frequent in ultra-nonagenarians than in younger controls (Brown et al, 1996; Santoro et al, 2010 and references therein).

Overall, these data support the idea that in neurodegenerative diseases and longevity, such as in other complex traits interactions may be more important than single polymorphic variations (Moore and Williams, 2002; Gilbert-Diamond and Moore, 2011) and that they play an important role on their non-additive heritability. Interactions may account for the missing

heritability of these traits and for their lack of replicability, as previously suggested based on studies on model organisms (Mackay, 2014). On the other hand, it has been shown that using whole genome data to find interactions affecting different phenotypes is extremely difficult due to huge number of interactions to be tested, the so-called curse of dimensionality (Bellman, 1961; Gilbert-Diamond and Moore, 2011), which leads to great standard errors or to a reduction of power (Concato et al, 1993; Hosmer and Lemeshow; 2000; Freitas, 2001; Gilbert-Diamond and Moore, 2011). On the contrary, to highlight some polymorphisms which appear to be correlated to phenotypes, emerging with appreciable additive effects (Mackay, 2014), may represent a starting point for interaction analyses and functional studies which avoid blind data mining.

5. Conclusions

Although additional large studies are warranted to validate our findings, some important conclusions emerge from this study. First, it supports a direct or indirect (i.e. mediate by the action of interacting partners) role of the multifunctional inositol-kinases IPMK and IP6K3 in both neurodegeneration and longevity. These kinases are recognized to be crucial in inositol-mediated transduction pathways and metabolic routes essential for cell homeostasis and survival. Second, it supports the view that the contribution of a gene promoting survival can be considered as an aggregated outcome of the multiple influences of its variants in the interactome network of the cell. Therefore, genes promoting longevity and/or affecting disease risks may be found in hubs interconnecting several signaling pathways. The final outcome will depend on the net effect of their interactions with other variants of proteins networking with them.

Acknowledgments:

The work has been made possible by the collaboration with Gruppo Baffa (Sadel Spa, Sadel San Teodoro srl, Sadel CS srl, Casa di Cura Madonna dello Scoglio, AGI srl, Casa di Cura Villa del Rosario srl, Savelli Hospital srl, Casa di Cura Villa Ermelinda), and by funds of the Italian Ministry of University and Research (PRIN: Progetti di Ricerca di rilevante Interesse Nazionale – 2015, Prot. 20157ATSLF) to G.R. AS is supported by the Medical Research Council grant MR/T028904/1.

Conflict of interest statement:

No conflict of interest to declare.

Author contributions

Conceptualization, G.P., G.R. and A.S.; methodology, P.C. and F.I.; formal analysis, F.D.R.; data curation, F.D.R.; writing—original draft preparation, G.R. and S.D.; writing—review and editing, G.R., G.P., S.D., P.C. and A.S.; funding acquisition, G.R.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Ahmed, I., Sbodio, J. I., Harraz, M. M., Tyagi, R., Grima, J. C., Albacarys, L. K.,… Snyder, S. H. (2015). Huntington's disease: Neural dysfunction linked to inositol polyphosphate multikinase. Proceedings of the National Academy of Sciences of the United States of America, 112(31), 9751–9756. https://doi.org/10.1073/pnas.1511810112

Anderson, A. J., Jackson, T. D., Stroud, D. A., & Stojanovski, D. (2019). Mitochondria-hubs for regulating cellular biochemistry: emerging concepts and networks. Open biology, 9(8), 190126. https://doi.org/10.1098/rsob.190126

Armstrong, R.A. (2014). When to use Bonferroni correction. Ophthalmic & Physiological Optic., 34: 502–508. https://doi.org/10.1111/opo.1213

Aviv, B., Atzmon, G., Ye, K., MacCarthy, T., Barzilai, N. (2007). Buffering mechanisms in aging: a systems approach toward uncovering the genetic component of aging. PLoS Comput Biol., 3, e170. https://doi.org/10.1371/journal.pcbi.0030170

Azevedo, C., & Saiardi, A. (2017). Eukaryotic Phosphate Homeostasis: The Inositol Pyrophosphate Perspective. Trends in biochemical sciences, 42(3), 219–231. https://doi.org/10.1016/j.tibs.2016.10.008

Bang, S., Kim, S., Dailey, M. J., Chen, Y., Moran, T. H., Snyder, S. H., & Kim, S. F. (2012). AMP-activated protein kinase is physiologically regulated by inositol polyphosphate multikinase. Proceedings of the National Academy of Sciences of the United States of America, 109(2), 616–620. https://doi.org/10.1073/pnas.1119751109

Beekman, M., Nederstigt, C., Suchiman, H. E., Kremer, D., van der Breggen, R., Lakenberg, N., …& Slagboom, P. E. (2010). Genome-wide association study (GWAS)-identified disease risk alleles do not compromise human longevity. Proceedings of the National Academy of Sciences of the United States of America, 107(42), 18046–18049. https://doi.org/10.1073/pnas.1003540107

Bellman, R. (1961). Adaptive Control Processes. Princeton, N. J.: Princeton University Press.

Brown, M. D., Shoffner, J. M., Kim, Y. L., Jun, A. S., Graham, B. H., Cabell, M. F., Gurley, D. S., & Wallace, D. C. (1996). Mitochondrial DNA sequence analysis of four Alzheimer's and Parkinson's disease patients. American journal of medical genetics, 61(3), 283–289. https://doi.org/10.1002/(SICI)1096-8628(19960122)61:3<283::AID-AJMG15>3.0.CO;2-P

Cárdenas, C., Miller, R. A., Smith, I., Bui, T., Molgó, J., Müller, M., …Foskett, J. K. (2010). Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria. Cell, 142(2), 270–283. https://doi.org/10.1016/j.cell.2010.06.007

Cevenini, E., Bellavista, E., Tieri, P., Castellani, G., Lescai, F., Francesconi, M., Mishto, M., Santoro, A., Valensin, S., Salvioli, S., Capri, M., Zaikin, A., Monti, D., de Magalhaes, J. P., Franceschi, C. (2010). Systems biology and longevity: an emerging approach to identify innovative antiaging targets and strategies. Curr Pharm Des, 16, 802–81. https://doi.org/10.2174/138161210790883660

Concato, J., Feinstein, A.R., Holford, T.R. (1993). The risk of determining risk with multivariable models. Ann Intern Med, 118(3),201-210. doi: 10.7326/0003-4819-118-3-199302010-00009.

Crocco, P., Saiardi, A., Wilson, M. S., Maletta, R., Bruni, A. C., Passarino, G., & Rose, G. (2016). Contribution of polymorphic variation of inositol hexakisphosphate kinase 3 (IP6K3)

gene promoter to the susceptibility to late onset Alzheimer's disease. Biochimica et biophysica acta, 1862(9), 1766–1773.

Dato, S., Rose, G., Crocco, P., Monti, D., Garagnani, P., Franceschi, C., & Passarino, G. (2017). The genetics of human longevity: an intricacy of genes, environment, culture and microbiome. Mechanisms of ageing and development, 165(Pt B), 147–155. https://doi.org/10.1016/j.mad.2017.03.011

De Rango, F., Crocco, P., Iannone, F., Saiardi, A., Passarino, G., Dato, S., & Rose, G. (2019). Inositol Polyphosphate Multikinase (IPMK), a Gene Coding for a Potential Moonlighting Protein, Contributes to Human Female Longevity. Genes, 10(2), 125. https://doi.org/10.3390/genes10020125

Desfougères, Y., Wilson, M., Laha, D., Miller, G. J., & Saiardi, A. (2019). ITPK1 mediates the lipid-independent synthesis of inositol phosphates controlled by metabolism. Proceedings of the National Academy of Sciences of the United States of America, 116(49), 24551–24561. https://doi.org/10.1073/pnas.1911431116

Fernandes, M., Wan, C., Tacutu, R., Barardo, D., Rajput, A., Wang, J., … de Magalhães, J. P. (2016). Systematic analysis of the gerontome reveals links between aging and age-related diseases. Human molecular genetics, 25(21), 4804–4818. https://doi.org/10.1093/hmg/ddw307

Field, A. E., Robertson, N. A., Wang, T., Havas, A., Ideker, T., & Adams, P. D. (2018). DNA Methylation Clocks in Aging: Categories, Causes, and Consequences. Molecular cell, 71(6), 882–895. https://doi.org/10.1016/j.molcel.2018.08.008

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of psychiatric research, 12(3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Franceschi, C., Garagnani, P., Olivieri, F., Salvioli, S., Giuliani, C. (2020). The Contextualized Genetics of Human Longevity: JACC Focus Seminar. J Am Coll Cardiol., 75(8), 968-979. doi: 10.1016/j.jacc.2019.12.032.

Freitas, A.A. (2001). Understanding the crucial role of attribute interaction in data mining. *Artif. Intel. Rev.,16*, 177–199. https://doi.org/10.1023/A:1011996210207

Freudenberg-Hua, Y., Freudenberg, J., Vacic, V., Abhyankar, A., Emde, A. K., Ben-Avraham, D., … Davies, P. (2014). Disease variants in genomes of 44 centenarians. Molecular genetics & genomic medicine, 2(5), 438–450. https://doi.org/10.1002/mgg3.86

Fu, C., Xu, J., Li, R. J., Crawford, J. A., Khan, A. B., Ma, T. M., … Snyder, S. H. (2015). Inositol Hexakisphosphate Kinase-3 Regulates the Morphology and Synapse Formation of Cerebellar Purkinje Cells via Spectrin/Adducin. The Journal of neuroscience: the official journal of the Society for Neuroscience, 35(31), 11056–11067.https://doi.org/10.1523/JNEUROSCI.1069-15.2015

Garagnani, P., Giuliani, C., Pirazzini, C., Olivieri, F., Bacalini, M. G., Ostan, R., … Franceschi, C. (2013). Centenarians as super-controls to assess the biological relevance of genetic risk factors for common age-related diseases: a proof of principle on type 2 diabetes. Aging, 5(5), 373–385. https://doi.org/10.18632/aging.100562

Gilbert-Diamond, D., & Moore, J. H. (2011). Analysis of gene-gene interactions. Current protocols in human genetics, Chapter 1, Unit1.14. https://doi.org/10.1002/0471142905.hg0114s70

Golpich, M., Amini, E., Mohamed, Z., Azman Ali, R., Mohamed Ibrahim, N., & Ahmadiani, A. (2017). Mitochondrial Dysfunction and Biogenesis in Neurodegenerative diseases: Pathogenesis and Treatment. CNS neuroscience & therapeutics, 23(1), 5–22. https://doi.org/10.1111/cns.12655

Gross A. (2016). BCL-2 family proteins as regulators of mitochondria metabolism. Biochimica et biophysica acta, 1857(8), 1243–1246. https://doi.org/10.1016/j.bbabio.2016.01.017

GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013;45(6):580-5.

Gu, C., Nguyen, H. N., Hofer, A., Jessen, H. J., Dai, X., Wang, H., & Shears, S. B. (2017). The Significance of the Bifunctional Kinase/Phosphatase Activities of Diphosphoinositol Pentakisphosphate Kinases (PPIP5Ks) for Coupling Inositol Pyrophosphate Cell Signaling to Cellular Phosphate Homeostasis. The Journal of biological chemistry, 292(11), 4544–4555. https://doi.org/10.1074/jbc.M116.765743

Hodgkin J. (1998). Seven types of pleiotropy. The International journal of developmental biology, 42(3), 501–505.

Hosmer, D.W., Lemeshow, S. (2000). Applied Logistic Regression. New York: John Wiley & Sons.

Kim, E., Ahn, H., Kim, M. G., Lee, H., & Kim, S. (2017). The Expanding Significance of Inositol Polyphosphate Multikinase as a Signaling Hub. Molecules and cells, 40(5), 315–321. https://doi.org/10.14348/molcells.2017.0066

Kim, S., Kim, S. F., Maag, D., Maxwell, M. J., Resnick, A. C., Juluri, K. R., … Snyder, S. H. (2011). Amino acid signaling to mTOR mediated by inositol polyphosphate multikinase. Cell metabolism, 13(2), 215–221. https://doi.org/10.1016/j.cmet.2011.01.007

Livermore, T. M., Azevedo, C., Kolozsvari, B., Wilson, M. S., & Saiardi, A. (2016). Phosphate, inositol and polyphosphates. Biochemical Society transactions, 44(1), 253–259. https://doi.org/10.1042/BST20150215

Lowsky, D. J., Olshansky, S. J., Bhattacharya, J., & Goldman, D. P. (2014). Heterogeneity in healthy aging. The journals of gerontology. Series A, Biological sciences and medical sciences, 69(6), 640–649. https://doi.org/10.1093/gerona/glt162

Mackay T. F. (2014). Epistasis and quantitative traits: using model organisms to study gene-gene interactions. Nature reviews. Genetics, 15(1), 22–33. https://doi.org/10.1038/nrg3627

Mackay, T.F., Moore, J.H. (2014). Why epistasis is important for tackling complex human disease genetics. Genome Med 6, 42. https://doi.org/10.1186/gm561

Magni, E., Binetti, G., Bianchetti, A., Rozzini, R., & Trabucchi, M. (1996). Mini-Mental State Examination: a normative study in Italian elderly population. European journal of neurology, 3(3), 198–202. https://doi.org/10.1111/j.1468-1331.1996.tb00423.x

Man, P. Y. W., Howell, N., Mackey, D. A., Nørby, S., Rosenberg, T., Turnbull, D. M., & Chinnery, P. F. (2004). Mitochondrial DNA haplogroup distribution within Leber hereditary optic neuropathy pedigrees. Journal of medical genetics, 41(4), e41. https://doi.org/10.1136/jmg.2003.011247

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Jr, Kawas, C. H., … Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups

on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia: the journal of the Alzheimer's Association, 7(3), 263–269. https://doi.org/10.1016/j.jalz.2011.03.005

Montesanto, A., Crocco, P., Anfossi, M., Smirne, N., Puccio, G., Colao, R., … Rose, G. (2016). The Genetic Variability of UCP4 Affects the Individual Susceptibility to Late-Onset Alzheimer's Disease and Modifies the Disease's Risk in APOE-ε4 Carriers. Journal of Alzheimer's disease: JAD, 51(4), 1265–1274. https://doi.org/10.3233/JAD-150993

Montesanto, A., Crocco, P., Dato, S., Geracitano, S., Frangipane, F., Colao, R., … Rose, G. (2018). Uncoupling protein 4 (UCP4) gene variability in neurodegenerative disorders: further evidence of association in Frontotemporal dementia. Aging, 10(11), 3283–3293. https://doi.org/10.18632/aging.101632

Mooijaart, S. P., van Heemst, D., Noordam, R., Rozing, M. P., Wijsman, C. A., de Craen, A. J., … Slagboom, P. E. (2011). Polymorphisms associated with type 2 diabetes in familial longevity: The Leiden Longevity Study. Aging, 3(1), 55–62. https://doi.org/10.18632/aging.100250

Moore, J. H., & Williams, S. M. (2002). New strategies for identifying gene-gene interactions in hypertension. Annals of medicine, 34(2), 88–95. https://doi.org/10.1080/07853890252953473

Moore, J.H. (2004). Analysis of gene-gene interactions. *Curr Protoc Hum Genet.1,* 1.14. doi: 10.1002/0471142905.hg0114s70

Moritoh, Y., Oka, M., Yasuhara, Y., Hozumi, H., Iwachidow, K., Fuse, H., & Tozawa, R. (2016). Inositol Hexakisphosphate Kinase 3 Regulates Metabolism and Lifespan in Mice. Scientific reports, 6, 32072. https://doi.org/10.1038/srep32072

Mukherjee, S., Haubner, J., & Chakraborty, A. (2020). Targeting the Inositol Pyrophosphate Biosynthetic Enzymes in Metabolic Diseases. Molecules (Basel, Switzerland), 25(6), 1403. https://doi.org/10.3390/molecules25061403

Nygaard, H. B., Erson-Omay, E. Z., Wu, X., Kent, B. A., Bernales, C. Q., Evans, D. M., … Strittmatter, S. M. (2019). Whole-Exome Sequencing of an Exceptional Longevity Cohort. The journals of gerontology. Series A, Biological sciences and medical sciences, 74(9), 1386–1390. https://doi.org/10.1093/gerona/gly098

Park, J., Longo, F., Park, S. J., Lee, S., Bae, M., Tyagi, R., … Snyder, S. H. (2019). Inositol polyphosphate multikinase mediates extinction of fear memory. Proceedings of the National Academy of Sciences of the United States of America, 116(7), 2707–2712. https://doi.org/10.1073/pnas.1812771116

Passarino, G., Montesanto, A., De Rango, F., Garasto, S., Berardelli, M., Domma, F., … De Benedictis, G. (2007). A cluster analysis to define human aging phenotypes. Biogerontology, 8(3), 283–290. https://doi.org/10.1007/s10522-006-9071-5

Ramsden, D. B., Ho, P. W., Ho, J. W., Liu, H. F., So, D. H., Tse, H. M., Chan, K. H., & Ho, S. L. (2012). Human neuronal uncoupling proteins 4 and 5 (UCP4 and UCP5): structural properties, regulation, and physiological role in protection against oxidative stress and mitochondrial dysfunction. Brain and behavior, 2(4), 468–478. https://doi.org/10.1002/brb3.55

Raule, N., Sevini, F., Li, S., Barbieri, A., Tallaro, F., Lomartire, L., … Franceschi, C. (2014). The co-occurrence of mtDNA mutations on different oxidative phosphorylation subunits, not detected by haplogroup analysis, affects human longevity and is population specific. Aging cell, 13(3), 401–407. https://doi.org/10.1111/acel.12186

Reuben, A., Sugden, K., Arseneault, L., et al. (2020). Association of Neighborhood Disadvantage in Childhood With DNA Methylation in Young Adulthood. JAMA Netw Open, 3(6): e206095. https://doi:10.1001/jamanetworkopen.2020.6095

Ritchie, M. D., Hahn, L. W., Roodi, N., Bailey, L. R., Dupont, W. D., Parl, F. F., & Moore, J. H. (2001). Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. American journal of human genetics, 69(1), 138–147. https://doi.org/10.1086/321276

Rojas, T., Cheng, W., Gao, Z., Liu, X., Wang, Y., Malla, A. P., … Fu, C. (2019). Inositol hexakisphosphate kinase 3 promotes focal adhesion turnover via interactions with dynein intermediate chain 2. Proceedings of the National Academy of Sciences of the United States of America, 116(8), 3278–3287. https://doi.org/10.1073/pnas.1817001116

Rose, G., Crocco, P., De Rango, F., Montesanto, A., & Passarino, G. (2011). Further support to the uncoupling-to-survive theory: the genetic variation of human UCP genes is associated with longevity. PloS one, 6(12), e29650. https://doi.org/10.1371/journal.pone.0029650

Rose, G., Santoro, A., & Salvioli, S. (2017). Mitochondria and mitochondria-induced signalling molecules as longevity determinants. Mechanisms of ageing and development, 165(Pt B), 115–128. https://doi.org/10.1016/j.mad.2016.12.002

Santoro, A., Balbi, V., Balducci, E., Pirazzini, C., Rosini, F., Tavano, F., … Franceschi, C. (2010). Evidence for sub-haplogroup h5 of mitochondrial DNA as a risk factor for late onset Alzheimer's disease. PloS one, 5(8), e12037. https://doi.org/10.1371/journal.pone.0012037

Sebastiani, P., Bae, H., Sun, F. X., Andersen, S. L., Daw, E. W., Malovini, A., … Perls, T. T. (2013). Meta-analysis of genetic variants associated with human exceptional longevity. Aging, 5(9), 653–661. https://doi.org/10.18632/aging.100594

Solovieff, N., Cotsapas, C., Lee, P. H., Purcell, S. M., & Smoller, J. W. (2013). Pleiotropy in complex traits: challenges and strategies. Nature reviews. Genetics, 14(7), 483–495. https://doi.org/10.1038/nrg3461

Szijgyarto, Z., Garedew, A., Azevedo, C., & Saiardi, A. (2011). Influence of inositol pyrophosphates on cellular energy dynamics. Science (New York, N.Y.), 334(6057), 802–805. https://doi.org/10.1126/science.1211908

Tanaka, M., Gong, J. S., Zhang, J., Yoneda, M., & Yagi, K. (1998). Mitochondrial genotype associated with longevity. Lancet (London, England), 351(9097), 185–186. https://doi.org/10.1016/S0140-6736(05)78211-8

Thota, S. G., & Bhandari, R. (2015). The emerging roles of inositol pyrophosphates in eukaryotic cell physiology. Journal of biosciences, 40(3), 593–605. https://doi.org/10.1007/s12038-015-9549-x

Tindale, L. C., Leach, S., Spinelli J. J., Brooks-Wilson, A. R. (2017). Lipid and Alzheimer's disease genes associated with healthy aging and longevity in healthy oldest-old. Oncotarget., 8, 20612-20621. DOI: 10.18632/oncotarget.15296

Tsui, M.M., York, J.D. (2010). Roles of inositol phosphates and inositol pyrophosphates in development, cell signaling and nuclear processes. Adv Enzyme Regul. 50(1), 324-37. DOI: 10.1016/j.advenzreg.2009.12.002

Tucker, E. J., Wanschers, B. F., Szklarczyk, R., Mountford, H. S., Wijeyeratne, X. W., van den Brand, M. A., … Thorburn, D. R. (2013). Mutations in the UQCC1-interacting protein,

UQCC2, cause human complex III deficiency associated with perturbed cytochrome b protein expression. PLoS genetics, 9(12), e1004034. https://doi.org/10.1371/journal.pgen.1004034

Ukraintseva, S., Yashin, A., Arbeev, K., Kulminski, A., Akushevich, I., Wu, D., … Stallard, E. (2016). Puzzling role of genetic risk factors in human longevity: "risk alleles" as pro-longevity variants. Biogerontology, 17(1), 109–127. https://doi.org/10.1007/s10522-015-9600-1

Wei, Y., Zhang, Y. J., Cai, Y., & Xu, M. H. (2015). The role of mitochondria in mTOR-regulated longevity. Biological reviews of the Cambridge Philosophical Society, 90(1), 167–181. https://doi.org/10.1111/brv.12103

Wilson, M. S., Livermore, T. M., & Saiardi, A. (2013). Inositol pyrophosphates: between signalling and metabolism. The Biochemical journal, 452(3), 369–379. https://doi.org/10.1042/BJ20130118

Wolfson, M., Budovsky, A., Tacutu, R., & Fraifeld, V. (2009). The signaling hubs at the crossroad of longevity and age-related disease networks. The international journal of biochemistry & cell biology, 41(3), 516–520. https://doi.org/10.1016/j.biocel.2008.08.026

Wundenberg, T., & Mayr, G. W. (2012). Synthesis and biological actions of diphosphoinositol phosphates (inositol pyrophosphates), regulators of cell homeostasis. Biological chemistry, 393(9), 979–998. https://doi.org/10.1515/hsz-2012-0133

Yokoyama, J. S., Wang, Y., Schork, A. J., Thompson, W. K., Karch, C. M., Cruchaga, C., … Alzheimer's Disease Neuroimaging Initiative (2016). Association Between Genetic Traits for Immune-Mediated Diseases and Alzheimer Disease. JAMA neurology, 73(6), 691–697. https://doi.org/10.1001/jamaneurol.2016.0150

Yuan, H., Li, X., Zhang, X., Kang, R., & Tang, D. (2016). CISD1 inhibits ferroptosis by protection against mitochondrial lipid peroxidation. Biochemical and biophysical research communications, 478(2), 838–844. https://doi.org/10.1016/j.bbrc.2016.08.034

Zhang, Q., Nogales-Cadenas, R., Lin, J. R., Zhang, W., Cai, Y., Vijg, J., & Zhang, Z. D. (2016). Systems-level analysis of human aging genes shed new light on mechanisms of aging. Human molecular genetics, 25(14), 2934–2947. https://doi.org/10.1093/hmg/ddw145

Supporting information list:

Table 1S: Logistic regression analysis for the association between IP6K3 genotypes and longevity

Table 2S: Results of the logistic regression models for IPMK SNPs in the female LOAD samples

Table 3S: Summary of functional annotation for relevant SNPs at *IP6K3* and *IPMK* loci, as obtained by Genotype-Tissue Expression (GTEx) pilot analysis (https://gtexportal.org/home/).

Figure 1S. Interaction graph (a) and interaction dendrogram (b) in longevity data set, resulting from MDR analysis. In a, the network graph obtained by setting from two to five-way combinations of the attributes. For each SNP is reported in per cent the value of information gain (IG) and numbers in the connections indicate the entropy-based IG for the SNP pairs. Red bar and orange bar indicate the high-level synergies on the phenotype, while the brown indicate a medium-level interaction, green and blue connections with negative IG values indicate redundancy or lack of synergistic interactions between the markers. In b, the interaction dendrogram for the same dataset, obtained from the information gain values, organized in a distance matrix to carry out a hierarchical cluster analysis. Pairs of SNPs with stronger interactions have a smaller distance. The shorter is the line connecting two attributes, stronger is the interaction. As before, the color of the line indicates the type of interaction. Red and orange suggest there is a synergistic relationship (i.e. epistasis). Yellow suggests independence. Green and blue suggest redundancy or correlation.

Figure 2S. Interaction graph (a) and interaction dendrogram (b) in LOAD data set, resulting from MDR analysis. In a, the network graph obtained by setting from two to five-way combinations of the attributes. For each SNP is reported in per cent the value of information gain (IG) and numbers in the connections indicate the entropy-based IG for the SNP pairs. Red bar and orange bar indicate the high-level synergies on the phenotype, while the brown indicate a medium-level interaction, green and blue connections with negative IG values indicate redundancy or lack of synergistic interactions between the markers. In b, the interaction dendrogram for the same dataset, obtained from the information gain values, organized in a distance matrix to carry out a hierarchical cluster analysis. Pairs of SNPs with stronger interactions have a smaller distance. The shorter is the line connecting two attributes, stronger is the interaction. As before, the color of the line indicates the type of interaction. Red and orange suggest there is a synergistic relationship (i.e. epistasis). Yellow suggests independence. Green and blue suggest moderate and high redundancy respectively.

Table 1. Summary of the studied sample groups

	Subjects, n	Mean age (years, +/- SD)	Female, %
Adult control	309	74.06 ± 6.95	49.5
LOAD	280	77.8 ± 5.0	63.0
Long lived	259	96.92 ± 3.72	63.0

Table 2. Relevant comparisons of association results for *IP6K3* SNPs with longevity (present study) and LOAD (Crocco et al, 2016).

	Association with longevity (present study)				*Association with LOAD (Crocco et al, 2016)*		
	Adult controls vs Long-lived				*LOAD vs Adult controls*		
IP6K3 SNPs	*Minor allele*	*OR*	*95% C.I.*	*pModel*	*OR*	*95% C.I.*	*pModel*
rs10947435	A	1.73	1.19-2.51	**0.004**^{D}	1.90	1.15-3.14	**0.010** ^{D}
rs4713675	T	1.36	1.03-1.78	**0.028**^{A}	1.45	0.85-2.48	0.170 ^{D}
rs28607030	G	0.75	0.45-1.25	0.270^{R}	0.57	0.36–0.90	**0.011**^{D}

OR, odds ratio; CI, 95% confidence interval. OR adjusted for sex. PModel is the p-value of the best-fit genetic model. The choice of each genetic model was based on AIC value. D is dominant, R is recessive and ADD is the additive model.

Table 3. Relevant comparisons of association results for *IPMK* SNPs with LOAD (present study) and longevity (De Rango et al, 2019) in female samples.

	Association with LOAD (present study)				*Association with longevity (De Rango et al, 2019)*		
	LOAD vs Adult controls				*Adult controls vs Long-lived*		
IPMK SNPs	*Minor allele*	*OR*	*95% C.I.*	*pModel*	*OR*	*95% C.I.*	*pModel*
rs2790156	A	0.25	0.06-0.92	**0.042**^{R}	*0.61*	*0.38–0.98*	**0.042** ^{D}

rs2790234	G	0.52	0.27–0.99	**0.048^{D}**	*0.33*	*0.16-0.67*	**0.002 ^{D}**
rs2590320	A	0.23	0.06–0.83	**0.025^{R}**	0.57	0.36–0.91	**0.019 ^{D}**
rs6481383	T	0.96	0.55–1.47	0.697^{D}	0.59	0.37-0.94	**0.026 ^{D}**
rs1832556	A	0.40	0.13–1.19	0.101^{R}	0.59	0.37-0.94	**0.028 ^{D}**
rs2251039	T	0.61	0.39–0.95	**0.029^{D}**	0.61	0.38-0.97	**0.038 ^{D}**

OR, odds ratio; CI, 95% confidence interval. PModel is the p-value of the bestfit genetic model. The choice of each genetic model was based on AIC value. D and Rindicate the dominant and recessive model, respectivaly.

Table 4: Relevant comparisons of association results for *IPMK* haplotypes with LOAD (present study) and longevity (De Rango et al, 2019) in female samples.

	Association with LOAD (present study)			*Association with longevity (De Rango et al, 2019)*		
	LOAD vs Adult controls			*Adult controls vs Long-lived*		
Haplotype	Frequency	Score	*P value**	Frequency	**Score**	*P value**
A-G-A-t-a-T	**0.073**	**-2.000**	**0.044**	**0.067**	**-2.897**	**0.002**
A-C-A-t-a-T	0.135	-1.144	0.252	0.138	-0.668	0.483
G-C-C-t-g-C	0.186	1.259	0.207	0.161	-0.353	0.715
G-C-C-c-g-C	0.566	-0.170	0.864	**0.616**	**2.155**	**0.024**

* simulated p-value obtained by Monte Carlo replication up to 10,000 bootstraps

Figure legends:

Figure 1. Interaction graph (a) and interaction dendrogram (b) in longevity data set, resulting from MDR analysis. In a, the network graph obtained by setting from two to three-way combinations of the attributes. For each SNP is reported in per cent the value of information gain (IG) and numbers in the connections indicate the entropy-based IG for the SNP pairs. Red bar and orange bar indicate the high-level synergies on the phenotype, while the brown indicate a medium-level interaction, green and blue connections with negative IG values indicate redundancy or lack of synergistic interactions between the markers. In b, the interaction dendrogram for the same dataset, obtained from the information gain values, organized in a distance matrix to carry out a hierarchical cluster analysis. Pairs of SNPs with stronger interactions have a smaller distance. The shorter is the line connecting two attributes, stronger is the interaction. As before, the color of the line indicates the type of interaction. Red and orange suggest there is a synergistic relationship (i.e., epistasis). Yellow suggests independence. Green and blue suggest redundancy or correlation.

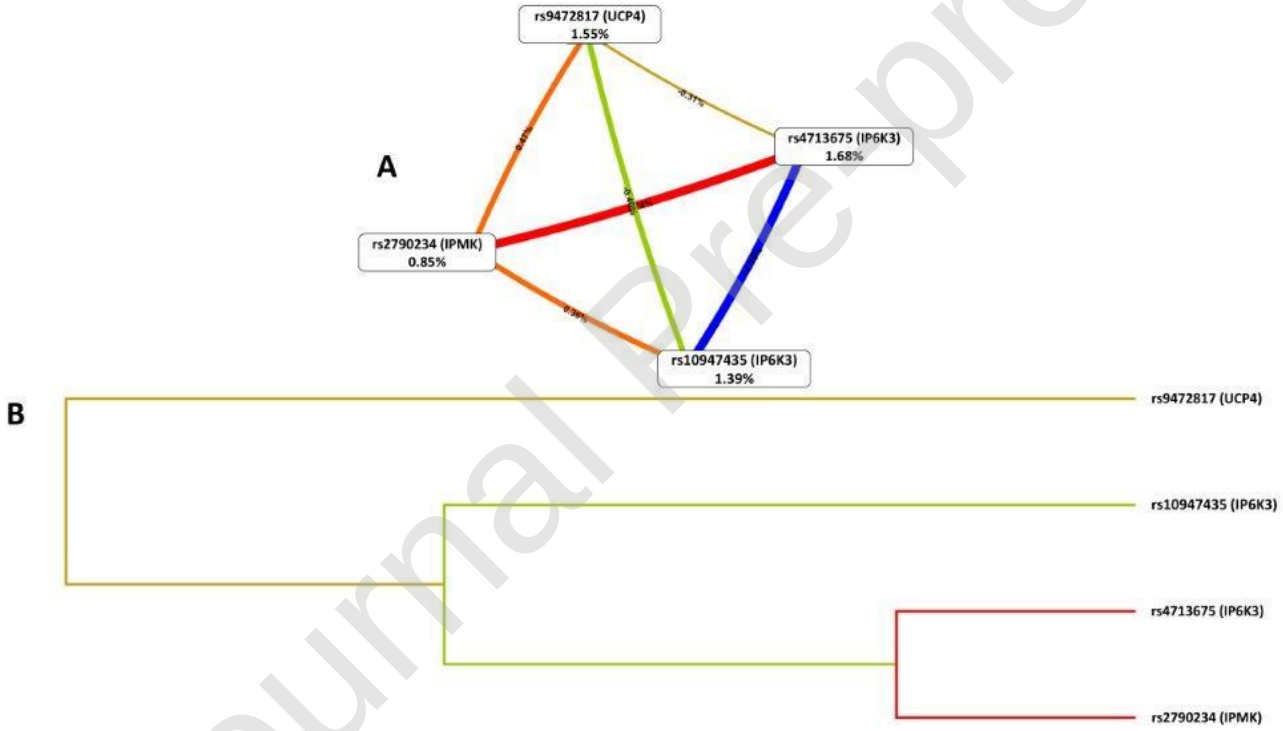


Figure 2. Interaction graph (a) and interaction dendrogram (b) in LOAD data set, resulting from MDR analysis. In a, the network graph obtained by setting from two to three-way combinations of the attributes. For each SNP is reported in per cent the value of information gain (IG) and numbers in the connections indicate the entropy-based IG for the SNP pairs. Red bar and orange bar indicate the high-level synergies on the phenotype, while the brown indicate a medium-level interaction, green and blue connections with negative IG values indicate redundancy or lack of synergistic interactions between the markers. In b, the interaction dendrogram for the same dataset, obtained from the information gain values, organized in a distance matrix to carry out a hierarchical cluster analysis. Pairs of SNPs with stronger interactions have a smaller distance. The shorter is the line connecting two attributes, stronger is the interaction. As before, the color of the line indicates the type of interaction. Red and orange suggest there is a synergistic relationship

(i.e., epistasis). Yellow suggests independence. Green and blue suggest moderate and high redundancy, respectively.