REJUVENATION RESEARCH
Volume 17, Number 2, 2014

DOI: 10.1089/rej.2013.1541

SHIP2: A "NEW" Insulin Pathway Target for Aging Research

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Abstract

Strong evidence suggests that systemic inflammation and central adiposity contribute to and perpetuate metabolic syndrome. All of these alterations predispose individuals to type 2 diabetes mellitus (T2DM), cardiovascular disease, as well as Alzheimer's disease (AD), all characterized by chronic inflammatory status. On the other hand, extensive abnormalities in insulin and insulin-like growth factor I (IGF-I) and IGF-II signaling mechanisms in brains with AD have been demonstrated, suggesting that AD could be a third form of diabetes. The Src homology domain-containing inositol 5-phosphatase 2 (SHIP2) has an important role in the insulin pathway because its over-expression causes impairment of insulin/IGF-1 signaling. Because some single-nucleotide polymorphisms (SNP) of the gene encoding SHIP2 were significantly associated in T2DM patients with metabolic syndrome and some related conditions, we decided to conduct a case–control study on this gene, analyzing AD and T2DM subjects as cases and young, old, and centenarians as controls. Our results suggest a putative correlation between the the rs144989913 SNP and aging, both successful and unsuccessful, rather than age-related diseases. Because this SNP is an insertion/deletion of 28 bp, it might cause an alteration in SHIP2 expression. It is noteworthy that SHIP2 has been demonstrated to be a potent negative regulator of insulin signaling and insulin sensitivity. Many studies demonstrated the association of the insulin/IGF1 pathway with aging and longevity, so it is tempting to speculate that the found association with SHIP2 and aging might depend on its effect on the insulin/IGF-1 pathway.

Introduction

Aging is an ineluctable process resulting from the interaction among genetic, epigenetic, stochastic, and lifestyle factors.[1,2] However, *in vivo* studies in model animals demonstrate that single genetic mutations are able to modulate life span. The insulin-like growth factor-I (IGF-I) pathway seems to be correlated to human life span, and its homologs are closely conserved in the main experimental models such as yeast, nematode, and fruit fly in which mutations in genes encoding proteins involved in this pathway affect life span.[3]

Insulin is the most potent anabolic hormone and is essential for appropriate tissue development, growth, and maintenance of whole-body glucose homeostasis. Insulin resistance (IR) reflects impairments in the insulin signaling pathway, but the molecular mechanisms implicated are not so clear, although the inflammatory process is involved. IR is one of the features of metabolic syndrome, a pre-diabetic status.[4,5]

Interestingly, strong evidence suggests that systemic inflammation and central adiposity contribute to and perpetuate metabolic syndrome. All of these alterations predispose individuals to type 2 diabetes mellitus (T2DM), cardiovascular disease, as well as Alzheimer's disease (AD), all characterized by chronic inflammatory status.[6–12] In 2005, a group of American scientists hypothesized that AD could be a third form of diabetes. They demonstrated extensive abnormalities in insulin and IGF-I and IGF-II signaling mechanisms in brains with AD, showing that although each of the corresponding growth factors is normally made in central nervous system neurons, the expression levels are markedly reduced in AD.[13]

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Today, evidence demonstrates the presence of IR in subjects with neurodegeneration, such as AD or Parkinson's patients.[14] AD, the most common form of dementia, is characterized by accumulation of senile plaques constituted by deposits of the abnormal amyloid protein (Aβ40–42 amino acids) and neurofibrillary tangles originating from hyperphosphorylation of microtubular tau protein. The amyloid hypothesis is not unique for the pathogenesis of AD. Indeed, different pathophysiological theories exist that focus attention on inflammation, vascular changes, and metabolic disorders. The most plausible hypothesis is that all of these theories are not mutually exclusive and could be taken together. Actually, inflammation plays a relevant role in both vascular lesions and metabolic disorders and could be the link between AD and T2DM.[15–17] Moreover, some authors proposed the concept of ''metabolic cognitive syndrome'' based on the co-occurrence of AD and metabolic syndrome. Indeed, dementia and metabolic syndrome present some overlaps both in predisposition factors, such as diet, smoking, socio-economic status, and lifestyle, and in altered signaling cascades, *i.e.*, nutrient-sensing pathways such as the insulin pathway.[18]

The Src homology domain-containing inositol 5-phosphatase 2 (SHIP2), has an important role in the insulin pathway. It leads to the activation of AKT, acting on glycogen synthase kinase-3 (GSK3) (Fig. 1).[19,20] Dysregulation of GSK3 activity determines neuronal cell death, hyperphosphorylation of tau protein, and the production of amyloid protein with an involvement in the neuropathology of AD.[21,22] Many studies underline the role of SHIP2 as a probable negative regulator of insulin signaling.[19,23–25] A study conducted by Kaisaki et al. in T2DM subjects demonstrated a significant association between single-nucleotide polymorphisms (SNPs) of INPPL1 (rs2276047, rs9886, and rs144989913) and metabolic syndrome or correlated features,[26] a finding partly confirmed by another study.[27] Moreover, a study conducted in non-T2DM subjects with hypertension (one of the features of metabolic syndrome previously associated with the SNPs), found no association, identifying the T2DM as condition probably necessary for the association.[28]

Starting from all of these studies and observations, we decided to conduct a case–control study on this gene, analyzing AD and T2DM subjects as cases and young, old, and centenarians as controls, with the aim to strengthen the association between the above-mentioned age-related diseases. In particular, we studied two polymorphisms of INPPL1 (which encodes inositol polyphosphate-5 phosphatase-like 1), rs9886 and the rs144989913.

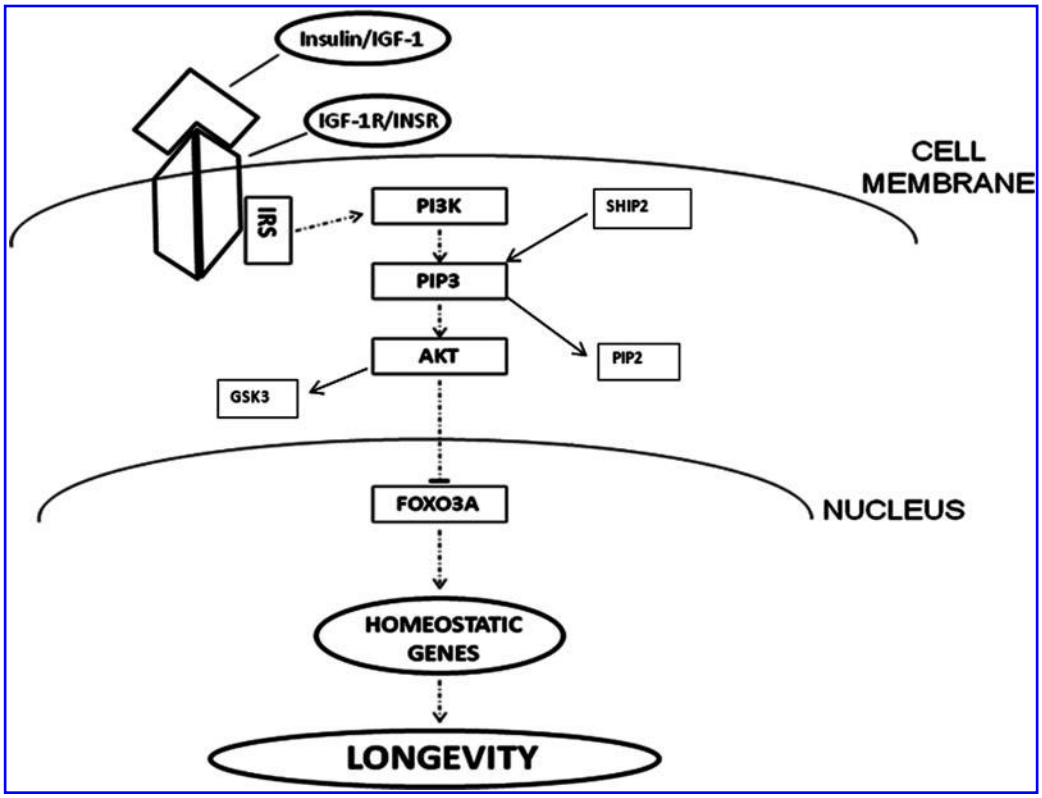

FIG. 1. Insulin-like growth factor I (IGF-1) pathway and Src homology domain-containing inositol 5-phosphatase 2 (SHIP2) action. This signaling pathway has a critical role in the determination of longevity. The bond of insulin/IGF-1 to the specific receptor (IGF-1R/INSR) activates the phosphatidylinositol-3′-kinase (PI3K) through the insulin-related substrate (IRS). It leads to the activation of AKT, through phosphatidylinositol(3,4,5)-trisphosphate (PIP3), which, in turn, inhibits Forkhead box O3A (FOXO3A). FOXO3A acts as transcription factor, activating the expression of many homeostatic genes. In the meantime, the downstream signal activated from PIP3 leads to the activation of AKT/protein kinase B (PKB), which phosphorylates and inactivates the glycogen synthase kinase 3 (GSK3). SHIP2 acts on the substrate lipid secondary messenger PIP3 to produce phosphatidylinositol 3,4-diphosphate (PIP2). Thus, SHIP2 is an antagonist of PI3K that phosphorylates PIP2 to obtain PIP3, attenuating the PI3K-mediated insulin signaling pathway.

Material and Methods

Sample collection

Informed consent was obtained from all cases of T2DM or guardians of AD patients and controls according to Italian law. In all, we collected 468 whole blood samples in EDTA Vacutainers.

Specifically, we enrolled 127 unrelated young subjects (mean age 35) randomly selected from blood donors and 105 old people (mean age 72), as controls. They were checked and judged to be in good health on the basis of their clinical history and on blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C-reactive protein, liver function test, iron, proteins, cholesterol, and triglycerides). Moreover, we selected 119 subjects probably affected by AD (mean age 77) as cases. AD patients were diagnosed according to standard clinical procedures and followed the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) and *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* (DSM-IIIR) criteria. Cognitive performance and alterations were measured according to the Mini-Mental State Evaluation and the Global Deterioration Scale. These cases were defined as sporadic because their family history did not mention any first-degree relative with dementia.[29,30] A total of 117 subjects affected by T2DM (mean age 68), diagnosed according to joint criteria of American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation, were also enrolled as cases. Moreover, we analyzed 20 DNA samples of centenarians belonging to our DNA bank.

Genetic analysis

Peripheral whole blood samples were collected and genomic DNA was extracted from leukocytes by a commercial kit. We genotyped the SNP rs144989913, which is an insertion/deletion (I/D) of 28 base pairs, by classic PCR and rs9886 by amplification-refractory mutation system (ARMS PCR). The size separation was conducted using agarose gel electrophoresis (3%).

Statistical analysis

The data were tested by a chi-squared test for the goodness of fit between the observed and expected genotype frequencies according to the Hardy–Weinberg equilibrium (HWE). Differences in allele and genotypic frequencies of the two SNPs among the groups were evaluated by gene count and the chi-squared test.

Results

A total of 488 individuals have been genotyped for the two SNPs. The frequencies of the genotypes of all SNPs under investigation, both in cases and controls, are in HWE. Table 1 shows the genotype and allele frequencies in all subjects of the two SNPs of INPPL1. We did not find any association for the rs9886 polymorphism, both for genotypic and allelic frequencies (data not shown). The distribution of the rs144989913 genotype between T2DM and young, old and young, and young and centenarians is significantly different. The frequency of the heterozygous genotype was increased in T2DM and AD patients as well as in old and centenarians with respect to young subjects. According to the genotype, a significant difference in the rs144989913 allele frequencies between T2DM and young, AD and young, old and young, and young and centenarians was observed. There were no significant differences for genotype and allele frequencies between T2DM and old or centenarians, AD and old, or centenarians and old and centenarians. Focusing on allelic frequencies of the D allele of rs144989913, we highlighted, with a 3×2 table, a growing significant increase ($p=0.0016$) of D with increasing age (young = 0.39; old = 0.11; centenarians = 0.15).

Gender analysis demonstrated that the significant difference in the rs144989913 genotypic and allele frequencies between T2DM and young, AD and young, and old and

Table 1. rs144999813 Genetic Distribution and Allele Frequency for Cases Represented by Alzheimer Disease Subjects and Type 2 Diabetes Mellitus Subjects and Controls Represented by Young, Old, and Centenarians Subjects and Association of the rs144999813 Between Cases and Controls and Young and Aged People

Genotype	*T2DM* (n = *117*) *(A)*	*AD* (n = *119*) *(B)*	*Young* (n = *127*) *(C)*	*Old* (n = *105*) *(D)*	*Centenarian* (n = *20*) *(E)*
I/I	97 (0.83)	97 (0.81)	117 (0.92)	81 (0.77)	14 (0.70)
I/D	20 (0.17)	22 (0.19)	10 (0.08)	24 (0.13)	6 (0.30)
D/D	0	0	0	0	0
P	AvsC = 0.028*	BvsC = 0.013*	CvsD = 0.0013*	DvsE = ns*	
	AvsD = ns*	BvsD = ns*	CvsE = 0.0031*		
	AvsE = ns*	BvsE = ns*			
ALLELE					
I	214 (0.91)	216 (0.91)	244 (0.96)	186 (0.88)	34 (0.85)
D	20 (0.09)	22 (0.09)	10 (0.039)	24 (0.11)	6 (0.15)
P	AvsC = 0.034**	BvsC = 0.017**	CvsD = 0.0020**	DvsE = ns**	
	AvsD = ns**	BvsD = ns**	CvsE = 0.004**		
	AvsE = ns**	BvsE = ns**			

*The significance of the different genotype distribution among groups was calculated by chi-squared test (3×2 table).
**The significance of the different allele distribution among groups was calculated by chi-squared test (2×2 table).
T2DM, type 2 diabetes mellitus; AD, Alzheimer's disease; I, insertion; D, deletion; ns, not significant.

young are present only in males rather than in both males and females (data not shown). Due to small number of centenarians, we could not study the gender effect in this population.

Discussion

Our study concerned the association between INPPL1 SNPs and age-related diseases, aging, and longevity. The results indicate a significant association of the rs144989913 polymorphism with both successful and unsuccessful aging. In a previous report, rs9886 and rs144989913 were shown to be associated, in haplotype, with rs2276047, to hypertension, obesity, metabolic syndrome, and T2DM, but no association with only rs2276047 was shown.[26] Thus, we exclusively analyzed the two above-mentioned SNPs but we obtained significant results only for rs144989913.

Both genotypic and allelic frequencies of rs144989913 showed significant association of this SNP between young and old in general, rather than between elderly and the specific age-related diseases. The frequency of the D allele increased from young to centenarians. Therefore, in a further step, the life expectancy should be analyzed in aged patients with the D allele in comparison with the I allele. Moreover, the specific association with males in not surprising because it has been claimed that males and females follow different strategies to attain longevity, and several case–control studies have been positive only in males.[31–33]

Concerning the function of SHIP2, it is noteworthy that its acts inside the signaling cascade of insulin, hence its alteration in terms of function and expression may cause insulin pathway impairment. Indeed, *in vivo* studies, demonstrated that SHIP2 is a potent negative regulator of insulin signaling and insulin sensitivity.[18,22–24] Many studies have demonstrated the association of the insulin/IGF1 pathway with aging and longevity. The replication of specific results in model organisms led to conducting studies also in human.[3,33] It is tempting to speculate that rs144989913 alleles may differently influence gene expression because they consist of a variation of 28 base pairs. They may differently modulate the insulin pathway involved in aging and longevity, hence functional studies are mandatory to confirm this suggestion.

In conclusion, our results are only a small contribution to aging research, but they do represent the first study that couples INPPL1/SHIP2 and aging. INPPL1 might be a ''new'' interesting gene in aging research, and this study represents the first step.

Acknowledgments

This work was supported by the authors' respective institutions (ex 60% to G.C. and C.C.). G.A. and C.V. are PhD students (Tutor G.C., Supervisor C.C.), and this paper is submitted in partial fulfillment of their PhD degree.

Author Disclosure Statement

The authors have no conflict of interest.

References

1. Caruso C, Passarino G, Puca A, Scapagnini G. ''Positive biology'': The centenarian lesson. Immun Ageing 2012;9:5.
2. Montesanto A, Dato S, Bellizzi D, Rose G, Passarino G. Epidemiological, genetic and epigenetic aspects of the research on healthy ageing and longevity. Immun Ageing 2012;9:6.
3. Ziv E, Hu D. Genetic variation in insulin/IGF-1 signaling pathways and longevity. Ageing Res Rev 2011;10:201–204.
4. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev 2005;26:19–39.
5. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793–1801.
6. Elks CM, Francis J. Central adiposity, systemic inflammation, and the metabolic syndrome. Curr Hypertens Rep 2010;12:99–104.
7. Ahtiluoto S, Polvikoski T, Peltonen M, Solomon A, Tuomilehto J, Winblad B, Sulkava R, Kivipelto M. Diabetes, Alzheimer disease, and vascular dementia: A population-based neuropathologic study. Neurology 2010;75:1195–1202.
8. Luchsinger JA. Insulin resistance, type 2 diabetes, and AD: Cerebrovascular disease or neurodegeneration? Neurology 2010;75:758–759.
9. Sanz C, Andrieu S, Sinclair A, Hanaire H, Vellas B; REAL.FR Study Group. Diabetes is associated with a slower rate of cognitive decline in Alzheimer disease. Neurology 2009;73:1359–1366.
10. Vanhanen M, Koivisto K, Moilanen L, Helkala EL, Hanninen T, Soininen H, Kervinen K, Kesaniemi YA, Laakso M, Kuusisto J. Association of metabolic syndrome with Alzheimer disease: A population-based study. Neurology 2006;67:843–847.
11. Razay G, Vreugdenhil A, Wilcock G. The metabolic syndrome and Alzheimer disease. Arch Neurol 2007;64:93–96.
12. Haslam DW, James WP. Obesity. Lancet 2005;366:1197–1209.
13. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J Alzheimer Dis 2005;7:63–80.
14. Domínguez RO, Pagano MA, Marschoff ER, González SE, Repetto MG, Serra JA. Alzheimer disease and cognitive impairment associated with diabetes mellitus type 2: Associations and a hypothesis. Neurologia, Barcelona, Spain, 2013;S0213–4853.
15. De la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. Lancet Neurol 2004;3:184–190.
16. Milionis HJ, Florentin M, Giannopoulos S. Metabolic syndrome and Alzheimer's disease: A link to a vascular hypothesis? CNS Spectr 2008;13:606–613.
17. Candore G, Bulati M, Caruso C, Castiglia L, Colonna-Romano G, Di Bona D, Duro G, Lio D, Matranga D, Pellicano' M, Rizzo C, Scapagnini G, Vasto S. Inflammation, cytokines, immune response, apolipoprotein E, cholesterol, and oxidative stress in Alzheimer disease: Therapeutic implications. Rejuvenation Res 2010;13:301–313.
18. Frisardi V, Solfrizzi V, Capurso C, Imbimbo BP, Vendemiale G, Seripa D, Pilotto A, Panza F. Is insulin resistant brain state a central feature of the metabolic-cognitive syndrome? J. Alzheimers Dis 2010;21:57–63.
19. Dyson JM, Kong AM, Wiradjaja F, Astle MV, Gurung R, Mitchell CA. The SH2 domain containing inositol polyphosphate 5 phosphatase-2: SHIP2. Int J Biochem Cell Biol 2005;37:2260–2265.

20. Plum L, Schubert M, Bruning JC. The role of insulin receptor signaling in the brain. Trends Endocrino Metab 2005;16:59–65.
21. Lucas JJ, Hernández F, Gómez-Ramos P, Morán MA, Hen R, Avila J. Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. EMBO J 2001 15;20:27–39.
22. Jope RS, Johnson GV. The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 2004;29:95–102.
23. Clement S, Krause U, Desmedt F, Tanti JF, Behrends J, Pesesse X, Sasaki T, Penninger J, Doherty M, Malaisse W, Dumont JE, Le Marchand-Brustel Y, Erneux C, Hue L, Schurmans S. The lipid phosphatase SHIP2 controls insulin sensitivity. Nature 2001;409:92–97.
24. Soeda Y, Tsuneki H, Muranaka H, Mori N, Hosoh S, Ichihara Y, Kagawa S, Wang X, Toyooka N, Takamura Y, Uwano T, Nishijo H, Wada T, Sasaoka T. The inositol phosphatase SHIP2 negatively regulates insulin/IGF-I actions implicated in neuroprotection and memory function in mouse brain. Mol Endocrinol 2010;24:1965–1977.
25. Hori H, Sasaoka T, Ishihara H, Wada T, Murakami S, Ishiki M, Kobayashi M. Association of SH2-containing inositol phosphatase 2 with the insulin resistance of diabetic db/db mice. Diabetes 2002;51:2387–2394.
26. Kaisaki PJ, Delepine M,Woon PY, Sebag-Montefiore L, Wilder SP,Menzel S, Vionnet N,Marion E, Riveline JP, Charpentier G, Schurmans S, Levy JC, Lathrop M, Farrall M, Gauguier D. Polymorphisms in type II SH2 domain-containing inositol 5-phosphatase (INPPL1, SHIP2) are associated with physiological abnormalities of the metabolic syndrome. Diabetes 2004;53:1900–1904.
27. Kagawa S, Sasaoka T, Yaguchi S, Ishihara H, Tsuneki H, Murakami S, Fukui K, Wada T, Kobayashi S, Kimura I, Kobayashi M. Impact of SRC homology 2-containing inositol 5-phosphatase 2 gene polymorphisms detected in a Japanese population on insulin signaling. J Clin Endocrinol Metab 2005;90:2911–2919.
28. Marçano AC, Burke B, Gungadoo J, Wallace C, Kaisaki PJ, Woon PY, Farrall M, Clayton D, Brown M, Dominiczak A, Connell JM, Webster J, Lathrop M, Caulfield M, Samani N, Gauguier D, Munroe PB. Genetic association analysis of inositol polyphosphate phosphatase-like 1 (INPPL1, SHIP2) variants with essential hypertension. J Med Genet 2007;44:603–605.
29. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Service Task Force on Alzheimer's Disease. Neurology 1984;34: 939–944.
30. American Psychiatric Association. *American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders* (DSM-IIIR), 3rd revised ed. Washington DC: American Psychiatric Association, 1987.
31. Candore G, Caruso C, Colonna-Romano G. Inflammation, genetic background and longevity. Biogerontology 2010; 11:565–573.
32. Capri M, Salvioli S, Monti D, Caruso C, Candore G, Vasto S, Olivieri F, Marchegiani F, Sansoni P, Baggio G, Mari D, Passarino G, De Benedictis G, Franceschi C. Human longevity within an evolutionary perspective: The peculiar paradigm of a post-reproductive genetics. Exp Gerontol 2008;43:53–60.
33. Di Bona D, Accardi G, Virruso C, Candore G, Caruso C. Association between genetic variations in the insulin/insulin-like growth factor (IGF-1) signalling pathway and longevity: A systematic review and meta-analysis. Curr Vasc Pharmacol Dec 18. [Epub ahead of print]

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Received: December 8, 2013
Accepted: December 8, 2013