ADNP differentially interact with genes/proteins in correlation with aging: a novel marker for muscle aging

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Abstract Activity-dependent neuroprotective protein (ADNP) is essential for embryonic development with *ADNP* mutations leading to syndromic autism, coupled with intellectual disabilities and motor developmental delays. Here, mining human muscle gene-expression databases, we have investigated the association of *ADNP* transcripts with muscle aging. We discovered increased *ADNP* and its paralogue *ADNP2* expression in the vastus lateralis muscle of aged compared to young subjects, as well as altered expression of the *ADNP* and the *ADNP2* genes in bicep brachii muscle of elderly people, in a sex-dependent manner. Prolonged exercise resulted in decreased *ADNP* expression, and increased *ADNP2* expression in an age-dependent manner in the vastus lateralis muscle. *ADNP* expression level was further correlated with 49 genes showing age-dependent changes in muscle transcript expression. A high degree of correlation with *ADNP* was discovered for 24 genes with the leading gene/protein being NMNAT1 (nicotinamide nucleotide adenylyl transferase 1). Looking at correlations differentiating the young and the old muscles and comparing protein interactions revealed an association of *ADNP* with the cell division cycle 5-like protein (CDC5L), and an aging-muscle–

related interactive pathway in the vastus lateralis. In the bicep brachii, very high correlation was detected with genes associated with immune functions as well as mitochondrial structure and function among others. Taken together, the results suggest a direct association of *ADNP* with muscle strength and implicate *ADNP* fortification in the protection against age-associated muscle wasting.

Keywords Aging · Sex · Sarcopenia · Transcription profile · Endurance exercise

Introduction

Activity-dependent neuroprotective protein (ADNP) was originally discovered and characterized 19 years ago in the laboratory of Professor Illana Gozes as a glial-derived protein. Its expression is regulated by vasoactive intestinal peptide (VIP), a neuropeptide that possesses an important role in brain development and neuroprotection (Bassan et al. 1999). *ADNP* is essential for both mammalian brain formation and function (Pinhasov et al. 2003, Mandel et al. 2007), neurite outgrowth (Mandel et al. 2008), and glial-derived neuroprotection (Pascual and Guerri 2007, Vulih-Shultzman et al. 2007). *Adnp*-deficient mice embryos exhibit a dramatic increase in gene transcripts associated with lipid metabolism, coagulation, reduction in organogenesis, and neurogenesis-related transcripts (Mandel et al. 2007). *ADNP* contains cellular export and import sequences as well as a nuclear localization sequence,

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suggesting a secreted factor role that can affect cellular processes (Furman et al. 2004). Moreover, it contains a homeobox profile and 9 zinc fingers motifs, indicating transcription factor activity (Zamostiano et al. 2001). The *ADNP* gene reveals high evolutionary conservation in the human, mouse, and rat (90% homology) (Bassan et al. 1999, Sigalov et al. 2000, Zamostiano et al. 2001). The highly conserved *ADNP* gene is abundantly expressed in the brain (hippocampus, cerebral cortex, and cerebellum) and the body (skeletal muscle, heart, kidney, and placenta) (Bassan et al., 1999, Zamostiano et al. 2001). In addition, ADNP was identified as part of the chromatin remodeling complex SWI/SNF (mating type switching/sucrose nonfermenting) (Mandel and Gozes 2007). ADNP regulates the expression of more than 400 genes during brain formation (Mandel et al. 2007), thus controlling intracellular signaling cascades, angiogenesis, heart development, neuronal migration, and vital cellular functions (Vulih-Shultzman et al. 2007, Gozes 2011).

Another ADNP paralogue protein discovered in the Gozes laboratory is ADNP2, shares 33% identity and 46% similarity to ADNP, while also providing cell protection (Zamostiano et al. 2001, Kushnir et al. 2008).

In normal healthy conditions, *ADNP* mRNA levels positively correlate with *ADNP2* mRNA levels, whereas in postmortem hippocampal samples from schizophrenia patients, this correlation is decreased ($r = 0.931$, controls, $r = 0.637$, schizophrenia subjects, $p = 0.03$). This implies that maintaining the balance between *ADNP* and *ADNP2* is important in healthy conditions (Dresner et al. 2011). In lymphocytes derived from patients suffering from schizophrenia, *ADNP* and *ADNP2* transcripts were significantly increased (in females at the initial disease stages compared to healthy controls lymphocytes. This increase may be attributed to a compensatory effect (*ADNP*) and neuroleptic treatment (*ADNP2*) (Merenlender-Wagner et al. 2015). In Alzheimer's disease (AD) patients, the positive correlation between *ADNP* and *ADNP2* levels in lymphocytes was preserved, but with an increased expression of both proteins in relation to healthy controls. Furthermore, ADNP serum levels were positively correlated with IQ measures in elderly subject. This suggests that ADNP may serve as a potential biomarker for AD disease onset and development (Malishkevich et al. 2016).

In agreement with prior findings of the Gozes laboratory discovering and characterizing ADNP and attesting to its essential role in brain development and

cognition (Bassan et al. 1999, Zamostiano et al. 2001, Pinhasov et al. 2003, Mandel and Gozes 2007, Mandel et al. 2007, Vulih-Shultzman et al. 2007), the ADNP syndrome was discovered. Thus, in 2014, the Kooy and Eichler laboratories (Helsmoortel et al. 2014), using whole-exome sequencing (WES), identified *ADNP* to be de novo mutated in at least 0.17% of autism spectrum disorder (ASD) cases associated with intellectual disabilities, making it one of the most frequent ASD genes known to date. The prevalence of currently diagnosed children with ADNP syndrome is 190 worldwide (<https://www.adnpfoundation.org/map.html>) with estimated prevalence of approximately 13,200 in the developed world (Hacohen-Kleiman et al. 2018).

The phenotypical presentation of the syndrome includes other impairments such as global developmental delays, speech impediments, and motor dysfunctions (Gozes et al. 2015, Gozes et al. 2017a, b, Arnett et al. 2018, Hacohen-Kleiman et al. 2018, Van Dijck et al. 2019). Some *ADNP* mutations have been reported to affect myelin structure and function; motor impairment is envisaged. ADNP is further suggested to be involved in oligodendrocyte development and myelin formation, the generation of which is important for learning and maintaining motor skills (Malishkevich et al. 2015b).

As seen in cases of children with developmental delays, findings display the importance of ADNP in muscle and bone (Gozes et al. 2017b) not only during embryogenesis, but also far beyond embryonic development. ADNP children display signs of atrophy at the age of 2, low tone, hypotonic throughout the body, abnormal gait, and extreme muscle tightness, thus severely lagging behind in developmental progress compared to normally developed children of the same age (Gozes et al. 2017a). In our most recent publication (Hacohen-Kleiman et al. 2018), we showed that *Adnp*-deficient mice mimic the above-mentioned developmental delays in children with ADNP syndrome through presenting impaired muscle tone and grip strength (as observed in the hanging wire and grip strength tests), as well as gait deficits (exhibited by the CatWalk apparatus).

Interestingly, VIP an ADNP regulator was shown to play an important role in modulating normal and atrophying skeletal muscle mass and force in rodent models via the VPAC2R receptor that is expressed in skeletal muscle. This observation suggests that VIP, which is the agonist of VPAC2R, may have clinical efficacy for the treatment of sarcopenia, muscle-

wasting diseases, and acute skeletal muscle atrophy (Hinkle et al. 2005).

As people advance with age, they exhibit declines in muscle mass, strength, and function, a condition clinically known as sarcopenia (Raue et al. 2012). Since sarcopenia affects a gradually growing population worldwide, both healthy and ill, it has become a topic of major significant research (Siparsky et al. 2014). That said, there is not enough knowledge about the etiology of sarcopenia, thus raising the significance for a better understanding of its mechanisms, which affect a largely growing population. An effective way to ameliorate age-related sarcopenia is by resistance exercise, which helps strengthening individuals in their 60s and 70s (Raue et al. 2012). Based on the presented data and involvement of ADNP in skeletal muscle function and development, we were highly motivated to investigate the expression levels and potential role of ADNP in the aging muscle. For this purpose, we searched the Gene Expression Omnibus (GEO) for data sets of human muscles tissues affected by aging including, quadriceps (e.g., vastus lateralis), bicep brachii, deltoid, gastrocnemius, and tibialis. A number of data sets were identified. However, when aging effects were examined in data set (GSE4667) including quadriceps (e.g., vastus lateralis), deltoid, gastrocnemius, and tibialis, no effects were found and this data set was excluded. Four data sets showing differential expression with aging were identified and these data sets included mostly vastus lateralis and bicep brachii data. Importantly, data set (GSE28422) was selected as it included the most elderly participants and focused on vastus lateralis; this data set included 49 mRNA transcripts exhibiting age-dependent expression levels (Raue et al. 2012). Figure 1 details the interaction among the 49 protein products of these genes with the addition of ADNP and ADNP2 showing common pathways regulating proteoglycan binding and P53 signaling. Ten of these genes were further described in Table 1, including genes involved in muscle disease, like myosin-8 (*MYH8*) and cytosolic purine 5'-nucleotidase (*NT5C2*) as well as other debilitating diseases encompassing complement C1q sub-component subunit B (*C1QB*), DNA damage-binding protein 2 (*DDB2*), and nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1 (*NMNAT1*).

In our current study, we report for the first time that *ADNP* and its paralogue *ADNP2* were significantly upregulated in elderly muscle. *ADNP* was further down-regulated after prolonged endurance training while

ADNP2 was upregulated. Additionally, extensive correlations were discovered for *ADNP* and 24 (of the 49) muscle-aging-associated genes. These findings imply a function for *ADNP* in the aging muscle.

Materials and methods

GEO microarray analyses

The NCBI GEO was searched for expression data sets of human muscles tissues (vastus lateralis and bicep brachii). The following four data sets were collected: (1) data set GSE28422 (Raue et al. 2012) from vastus lateralis representing study A. Our cohort included two groups of participants: $n = 14$ young (8 men, 6 women) at the age of 24 ± 1 year old and $n = 12$ old adults (6 men, 6 women) at the age of 84 ± 1 year old, with the latter being the oldest cohort to date. All participants were healthy and underwent a physical examination, excluding those who had any acute or chronic illnesses. (2) Data set GSE674 (Welle et al. 2004) from seven young women's vastus lateralis at the age of 20–29, and from eight older women's vastus lateralis at the age of 65–71. The participants did not perform any type of regular exercises involving strenuous activity for more than 2 h per week. (3) Data set GSE38718 (Liu et al. 2013) from $n = 14$ young participants' bicep brachii at the age of 19–28 years (7 men, 7 women), and $n = 8$ old participants' bicep brachii at the age of 65–76 years (4 men, 4 women). The participants were recruited according to the same inclusion and exclusion criteria mentioned above. (4) Data set GSE9103 (Lanza et al. 2008) from $n = 18$ young participants' vastus lateralis at the age of 18–30 years (9 sedentary, 9 trained), and $n = 19$ old participants' vastus lateralis at the age of 59–76 years (10 sedentary, 9 trained). All participants were healthy. The trained group was subjected to physical activity in the past 4 years or more, which included at least 1 h of cycling or running 6 days per week. The sedentary group exercised less than 30 min per day, twice a week. *ADNP* and *ADNP2* were tested for age-based differential expression between young and old populations. Subsequently, the 49 most significantly differentiated genes expressed, influenced by age from GSE28422, were analyzed for correlation to *ADNP* expression. Before performing correlation analysis, we used two-way ANOVA repeated-measures followed by Fisher's LSD as post hoc, to examine whether the *ADNP*

Fig. 1 Functional enrichment and network analysis of 49 proteins that were affected by age in the vastus lateralis muscle plus ADNP and ADNP2. String analysis (<https://string-db.org>) revealed an interaction network map. The proteins are represented as nodes

Molecular Function (GO)			
GO-term	description	count in gene set	false discovery rate
GO:0043394	proteoglycan binding	3 of 32	0.0231
Cellular Component (GO)			
GO-term	description	count in gene set	false discovery rate
GO:0005581	collagen trimer	4 of 88	0.0183
Reference publications			
publication	(year) title	count in gene set	false discovery rate
PMID:22676806	"Premature expression of a muscle fibrosis axis in chronic HIV infection." (2012)	5 of 22	0.00027
PMID:9875645	"Immediate onset of DNA synthesis in remnant rat liver after 90% hepatectomy by an administration of follistatin." (1998)	3 of 6	0.0258
KEGG Pathways			
pathway	description	count in gene set	false discovery rate
hsa04115	P53 signaling pathway	3 of 68	0.0181
INTERPRO Protein Domains and Features			
domain	description	count in gene set	false discovery rate
IPR038861	Activity-dependent neuroprotector homeobox protein	2 of 2	0.0065

expression levels changed upon resistance training. As we did not find a significant effect of training on *ADNP* expression levels, we collapsed the data from four time points as follows: T1—pre training, T2—4 h post an acute bout of high-intensity resistance exercise (1st training session), T3—prior to last session (36th training session as part of a 12-week–resistance training program), T4—4 h after final session, in each age group. In addition, most genes differentially expressed in GSE38718 were divided into 5 different categories (mitochondrial structure and function, gene transcription and translation, lipid synthesis, immune function, and ECM remodeling), and were assessed to evaluate the correlation to *ADNP* gene expression levels. Protein interactions were studied by the String tool (<https://string-db.org/>).

Statistical analysis

Results were analyzed for statistical significance between two groups by using two-tailed Student’s *t* test, and for multiple comparisons, by ANOVA two-way analysis of variance or a two-way ANOVA repeated-

and the connecting lines represent the interactions. Furthermore, the biological processes and pathways are summarized in the adjacent table

measure. This was then followed by Fisher’s LSD as post hoc using Sigma Plot for Windows software version 11 (Chicago, IL, USA). Significance was considered at *p* < 0.05 level, **p* < 0.05, ***p* < 0.01, and ****p* < 0.001. Before performing statistical analysis, the data outlier values were excluded using the GraphPad QuickCalcs outlier calculator (<https://graphpad.com/quickcalcs/Grubbs1.cfm>). Prior to correlation analysis, the data was checked for normal distribution, followed by the Pearson (for normally distributed data) or Spearman (for abnormally distributed data) correlation analysis. The data was analyzed using Sigma Plot, as above.

Results

GEO data mining indicates increased *ADNP* and *ADNP2* expression in the vastus lateralis muscle of aged compared to young patients

To address the question if *ADNP* is playing role in aging muscles, we searched GEO for data sets

Table 1 Selected muscle genes showing age-dependent expression

Gene name/protein	Function	Phenotype/MIM number
<i>C1QB</i> (complement C1q subcomponent subunit B)	C1QB associates with C1QA and C1QC subunits to form functional C1Q complex which is the recognition component of the classical pathway of complement activation (Sontheimer et al. 2005).	613,652 C1q deficiency (mesangial proliferative glomerulonephritis, autoimmune diseases (lupus))
<i>DAAM2</i> (disheveled-associated activator of morphogenesis 2)	The DAAM2 protein is used as an effector for disheveled (DVL) in canonical Wnt signal transduction during spinal cord development (Lee and Deneen 2012; Cui and Xie 2016). The Wnt signaling pathway is downregulated with aging and contributes to the progressive reduction in muscle regeneration and repair capacity (Conboy and Rando 2012). Wnt abnormalities lead to many neurodegenerative and mental diseases (Okerlund and Cheyette 2011; Mulligan and Cheyette 2012; Stamatakou and Salinas 2014). During the formation of the neuromuscular junction (NMJ), there is an involvement of both, positive and negative Wnt signaling pathways (Koles and Budnik 2012).	Not applicable
<i>DDB2</i> (DNA damage-binding protein 2)	Part of the UV-DDB complex (together with DDB1) which participates in repair of ultraviolet light-damaged DNA (Hwang et al. 1999; Inoki et al. 2004).	278,740 xeroderma pigmentosum, group E, DDB-negative subtype (Variety of skin abnormalities including photosensitivity, early onset of cancer, atrophy. Eye abnormalities: conjunctivitis, entropion, ectropion, keratitis and photophobia)
<i>MYH8</i> (Myosin-8)	MYH8 is a perinatal myosin heavy chain, a developmental isoform of skeletal muscle. Its expression considered as a hallmark of muscle regeneration after birth as well as for muscular dystrophies (Haslett et al. 2002a; Schiaffino et al. 2015a).	158,300 trismus-pseudocamptodactyly syndrome (Skeletal abnormalities: short gastrocnemius, reduced elbow supination, hip dislocation, flexion of fingers. Soft tissue abnormalities: shortening of various muscle-tendon groups in legs and feet. Feeding problems. Facial asymmetry).
<i>KLF5</i> (Krueppel-like factor 5)	KLF5 is a zinc-finger transcription factor. Similar to ADNP, KO in this gene is embryonically lethal. KLF5 is also important for skeletal muscle regeneration and myogenic differentiation. Additionally, it is highly expressed in skeletal muscles (Hayashi et al. 2016).	Not applicable
<i>NMNAT1</i> (nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1)	Key enzyme in NAD ⁺ biosynthesis, which is necessary for a variety processes in the cell: metabolic redox reactions, protein ADP-ribosylation, histone deacetylation, Ca(2+) signaling pathways (Berger et al. 2005b; Fang et al. 2017b). During the course of aging, there is a systemic decrease in NAD ⁺ across multiple tissues, implicating its crucial role in the pathophysiology of multiple diseases, including age-associated metabolic disorders, neurodegenerative diseases and mental disorders (Juel and Halestrap 1999).	608,553 Leber congenital amaurosis 9 (visual impairment that progress with age).
<i>NT5C2</i> (cytosolic purine 5'-nucleotidase protein)	NT5C2 is an enzyme located in the cytoplasmic matrix of cells. NT5C2 plays a role in the maintenance of purine/pyrimidine nucleotides composition. It has phosphotransferase active site, which is responsible in the catalysis of the	613,162 spastic paraplegia 45 Symptoms include, delayed motor development, hyperreflexia, contractures, hyperextension of knees, extensor plantar responses, and spastic

Table 1 (continued)

Gene name/protein	Function	Phenotype/MIM number
	dephosphorylation process of 6-hydroxypurine nucleoside 5'-monophosphates. In addition, it has a hydrolytic function on inosine monophosphate (IMP) and guanosine monophosphate (GMP), which regulates the IMP/GMP levels inside cells, especially maintaining the balance in the brain and spinal cord (Itoh et al. 1967; Tozzi et al. 1991; Pesi et al. 1994, Randitelli et al., 1996).	gait. Mental retardation and learning problems are also present (in some patients).
<i>PLAG1</i> (pleiomorphic adenoma gene 1 protein)	Plays a role as a transcription factor as well as an oncogene associated with certain types of cancer, most notably pleomorphic adenomas of the salivary gland (Juma et al. 2016).	181,030 adenomas, salivary gland pleomorphic (pleomorphic benign salivary gland adenoma (PSA)).
<i>Rab11FIP3</i> (Rab11 family-interacting protein 3)	Rab11FIP3 is one of the proteins interacting with and regulating Rab GTPase which is encoded by the <i>Rab11</i> gene (Hales et al., 2001). During interphase, FIP3 localizes to the endosomal-recycling compartment (ERC) and interacts with a component of the dynein motor complex. This molecular interaction serves to drive membrane trafficking from peripheral sorting endosomes to the ERC. Thus, Rab11FIP3 is required for structural integrity and pericentrosomal positioning of the ERC (Horgan et al. 2004; Horgan et al. 2007; Inoue et al. 2008).	Not applicable
<i>SLC16A6</i> (monocarboxylate transporter 7)	<i>SLC16A6</i> gene is one of 14 members of the SLC16 gene family that encodes the monocarboxylate transporters (MCTs) (Fisel et al. 2018). MCTs mediate the absorption and distribution of monocarboxylic compounds across plasma membranes (Fisel et al. 2018). Lactic acid in the body is produced mainly in the skeletal muscle but can also be used as a respiratory fuel by the skeletal oxidative fibers. Monocarboxylic compounds' transport is essential in skeletal muscle for pH regulation and homeostasis (Juel and Halestrap 1999). It is also necessary for metabolic communication between cells (Juel and Halestrap 1999).	Not applicable

The table depicts 10 selected genes

comparing transcriptional signature/profiling between old and young populations. One of the data sets that we found to be interesting included the most elderly participants to date. Analysis of the data set GSE28422 showed a significant increase in the expression levels of *ADNP* in old males and females' vastus lateralis muscle in comparison to the young group ($p = 0.042$, $p < 0.001$, respectively) (Fig. 2a). Furthermore, sex differences were observed within an old group, with higher levels of *ADNP* seen in old women ($p = 0.004$) (Fig. 2a). *ADNP2*, a

paralogue to *ADNP*, showed a significant increase in expression in old females compared to the young group ($p < 0.001$); however, we did not observe the same pattern in the old male group (Fig. 2b). Comparisons of the basal level gene expression of young and old adults' vastus lateralis muscle indicated 49 differentially expressed genes (Raue et al. 2012). Next, a correlation analysis was performed in which we examined whether *ADNP* expression levels correlated with the highly differentially expressed genes, thus indicating a common pathway

Fig. 2 *ADNP* is differentially expressed with age and sex in vastus lateralis muscle. **a**, **b** Microarray expression levels of *ADNP* and *ADNP2* in 14 young (8 men, 6 women) and 12 old adults (6 men, 6 women) from vastus lateralis muscle (Data set GSE28422). Two-way ANOVA analysis with Fisher's LSD as post hoc was performed using SigmaPlot. **a** *ADNP*: a significant main effect of age ($F(1, 99) = 15.488, p < 0.001$) and sex ($F(1, 99) = 10.023, p = 0.002$) was observed. Old males and females showed a significant increase in *ADNP* expression compared to young groups ($p = 0.042, p < 0.001$, respectively). Furthermore, sex differences were

observed within the old group ($p = 0.004$). **b** *ADNP2*: significant main effects of age ($F(1, 96) = 12.523, p < 0.001$) and age $\times$ sex ($F(1, 96) = 6.321, p = 0.014$) were observed. Old females showed a significant increase in *ADNP2* expression compared to the young age group ($p < 0.001$). **c** A corroborating second data set was used (GSE674, 7 young women and 8 old women) was subjected to analysis and a two-tailed Student's *t* test revealed a significant upregulation in *ADNP* expression in old females compared to the young age group ($p < 0.05$)

(Table 2). For each of the analyzed genes, we first measured the effect of exercise, and compared either the baseline values to *ADNP* values, if there was an exercise effect, or the collapsed values, if there was no exercise effect.

The highest correlative values for *ADNP* in the young group were for *NMNAT1* (baseline females, $r = 0.980, p = 0.0006$), *PLAG1* (baseline males, $r = 0.761, p = 0.0282$; baseline females, $r = 0.891, p = 0.0425$), *EPB41L4B* (baseline females, $r = 0.858, p = 0.0289$), and *KLF5* (baseline females, $r = 0.848, p = 0.0331$), (Table 2). In contrast, *ADNP* was inversely correlated with *RAB11FIP3* (baseline females $= -0.964, p = 0.0019$), *SLPI* (baseline females, $r = -0.920, p = 0.0093$), and *NT5C2* (baseline females, $r = -0.912, p = 0.0112$). In the old group, *ADNP* was highly correlated with *SLC16A6* (baseline males, $r = 0.860, p = 0.0281$), *SYNPO2* (baseline females, $r = 0.820, p = 0.0456$), and *SKAP2* (trained males, $r = 0.602, p = 0.0023$; trained females $r = -0.592, p = 0.00291$), and inversely correlated with *DAAM2* (baseline females, $r = -0.924, p = 0.00851$) and *FLRT2* (baseline males, $r = -0.883, p = 0.0197$). As women have less muscle mass and strength than men do, the phenomenon of sarcopenia is more prevalent in women. Hence, we used another data set GSE674 that specifically examined the effects of aging on women's vastus lateralis. Similar to the first data set, the levels of *ADNP* gene transcripts were significantly upregulated in elderly women (Fig. 2c).

Expression alterations of the *ADNP* and *ADNP2* genes in the bicep brachii muscle of elderly people

We further aimed to find out whether the changes in *ADNP* and *ADNP2* expression that were seen in vastus lateralis were evident in bicep brachii as well. Analysis of the GEO data set GSE38718 showed a significant increase in the expression levels of *ADNP* in older men compared to young men ($p = 0.006$, Fig. 3a), whereas *ADNP2* expression levels were significantly upregulated in the old female group. Furthermore, significant sex differences were seen in the old group ($p = 0.011$, Fig. 3b). We next evaluated the correlation of *ADNP* and genes that were differentially expressed between young and old adults (males and females) divided in to 5 different categories: mitochondrial structure and function, gene transcription and translation, lipid synthesis, immune function, and ECM remodeling. The highest correlative values for *ADNP* in the young group were for *PLAT* (immune function, females, $r = 0.958, p = 0.00267$), *MYH10* (immune function, females, $r = 0.910, p = 0.0118$), and *SDCBP* (ECM remodeling, females, $r = 0.853, p = 0.0309$), and inversely correlated with *OFD1* (ECM remodeling, males, $r = -0.929, p = 0.0000002$), *DHRS4-AS1* (mitochondrial structure and function, males, $r = -0.894, p = 0.0066$), and *CAVI* (lipid synthesis, males, $r = -0.805, p = 0.0291$). In the elderly group, *ADNP* was found to be highly correlated with *PPARG* (lipid synthesis, females, $r = 1.000, p = 0.0182$),

Table 2 Correlation analysis between *ADNP* gene and 49 differentially expressed genes in young and old adults' vastus lateralis

Muscle type	Age group	GenesSymbol	Training			
			Baseline Males	Baseline Females	Trained Males	Trained Females
Vastus lateralis	Young 24 years $\pm$ 1 year	<i>NMNAT1</i> (nicotinamide nucleotide adenyltransferase 1) (223692_at)		$r = 0.980$, $p = 0.0006$ Pearson		
		<i>PLAG1</i> (pleiomorphic adenoma gene 1) (205372_at)	$r = 0.761$, $p = 0.0282$ Pearson	$r = 0.891$, $p = 0.0425$ Pearson		
		<i>EPB41L4B</i> (erythrocyte membrane protein band 4.1 like 4B) (223427_s_at)		$r = 0.858$, $p = 0.0289$ Pearson		
		<i>KLF5</i> (Kruppel-like factor 5 (intestinal)) (209211_at)		$r = 0.848$, $p = 0.0331$ Pearson		
		<i>EPB41L3</i> (erythrocyte membrane protein band 4.1-like 3) (206710_s_at)	$r = 0.738$, $p = 0.0287$ Pearson			
		<i>MYH8</i> (myosin, heavy chain 8, skeletal muscle, perinatal) (206717_at)				$r = 0.589$, $p = 0.0100$ Spearman
		<i>SLIT2</i> (slit guidance ligand 2) (228850_s_at, 230130_at)			$r = 0.452$, $p = 0.0094$ Pearson	$r = 0.458$, $p = 0.0278$ Pearson
		<i>PCDH9</i> (protocadherin 9) (219738_s_at)				$r = 0.451$, $p = 0.0310$ Pearson
		<i>SKAP2</i> (src kinase associated phosphoprotein 2) (216899_s_at)			$r = 0.419$, $p = 0.0169$ Pearson	
		<i>CDKN2B</i> (cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)), (207530_s_at)			$r = -0.468$, $p = 0.0071$ Spearman	$r = -0.483$, $p = 0.0196$ Spearman
		<i>GREB1</i> (growth regulation by estrogen in breast cancer 1) (210855_at)				$r = -0.499$, $p = 0.0155$ Spearman
		<i>DDB2</i> (damage-specific DNA binding protein 2, 48 kDa) (203409_at)		$r = -0.818$, $p = 0.0466$ Spearman		
		<i>COL5A3</i> (collagen, type V, alpha 3) (52255_s_at)	$r = -0.834$, $p = 0.019$ Pearson			
		<i>C1QB</i> (complement component 1, q subcomponent, B chain) (202953_at)	$r = -0.834$, $p = 0.0197$ Pearson			
		<i>NT5C2</i> (5'-nucleotidase, cytosolic II) (209155_s_at)		$r = -0.912$, $p = 0.0112$ Pearson		
		<i>SLPI</i> (secretory leukocyte peptidase inhibitor) (203021_at)		$r = -0.920$, $p = 0.0093$ Pearson		
		<i>RAB11FIP3</i> (RAB11 family-interacting protein 3 (class II)) (228613_at)		$r = -0.964$, $p = 0.0019$ Pearson		

Table 2 (continued)

Muscle type	Age group	GenesSymbol	Training			
			Baseline Males	Baseline Females	Trained Males	Trained Females
	Old 84 years $\pm$ 1 year	<i>SLC16A6</i> (solute carrier family 16, member 6) (230748_at)	$r = 0.860$, $p = 0.0281$ Pearson			
		<i>SYNPO2</i> (synaptopodin 2) (225720_at)		$r = 0.820$, $p = 0.0456$ Pearson		
		<i>SKAP2</i> (src kinase associated phosphoprotein 2) (216899_s_at, 225639_at)			$r = 0.602$, $p = 0.0023$ Pearson	$r = -0.592$, $p = 0.002$ - 91 Pearson
		<i>ATP1B4</i> (ATPase, (Na+)/K+ transporting, beta 4 polypeptide) (220556_at)			$r = 0.454$, $p = 0.0258$ Pearson	
		<i>ADNP2</i> (activity-dependent neuroprotector homeobox protein 2) (203322_at)				$r = 0.479$, $p = 0.0279$ Pearson
		<i>NT5C2</i> (5'-nucleotidase, cytosolic II) (241778_at)			$r = 0.546$, $p = 0.0057$ Pearson	
		<i>PCDH9</i> (protocadherin 9) (238919_at)			$r = 0.419$, $p = 0.0416$ Pearson	
		<i>FST</i> (follistatin) (204948_s_at)				$r = -0.414$, $p = 0.0441$ Spearman
		<i>FLRT2</i> (fibronectin leucine rich transmembrane protein 2) (204359_at)	$r = -0.883$, $p = 0.0197$ Pearson			
		<i>DAAM2</i> (disheveled associated activator of morphogenesis 2) (212793_at)		$r = -0.924$, $p = 0.008$ - 51 Pearson		

Genes and probes are described in the table. Pearson's (normal distribution) or Spearman's correlation coefficient (normal distribution failure) for the expression levels of each gene relative to *ADNP*, as well as the p value, are presented in the table. The values are distributed from the highest to the lowest correlative value in each group (young and old). For each of the analyzed genes, we first measured the effect of exercise. If there was an exercise effect on gene transcription levels, we then compared the baseline values to *ADNP* values. In case there was no exercise effect on transcription levels, we collapsed the data from four time points: T1—pre training, T2—4 h post an acute bout of high-intensity resistance exercise (1st training session), T3—prior to last session (36th training session as part of a 12 week resistance training program), T4—4 h after final session, in each age group

CAV2 (lipid synthesis, females, $r = 1.000$, $p = 0.00008$; males, $r = 1.000$, $p = 0.00008$), and *DHRS4I2* (mitochondrial structure and function, females, $r = 0.999$, $p = 0.0325$), and inversely correlated with *MRPS30* (mitochondrial structure and function, females, $r = -1.000$, $p = 0.0120$), *HSF2* (gene transcription and translation, females, $r = -0.999$, $p = 0.0236$), and *UCP3* (mitochondrial structure and function, females, $r = -0.996$, $p = 0.00366$).

Prolonged endurance exercise differentially affects *ADNP* and *ADNP2* expression levels

To find out whether prolonged endurance exercise has an impact on *ADNP* and *ADNP2* expression levels in young and old population, we looked for GEO data set comparing the transcriptome profile upon endurance exercise. Analysis of the data set GSE38718 showed a significant reduction in *ADNP*

Fig. 3 *ADNP* is differentially expressed with age in bicep brachii muscle. **a**, **b** Microarray expression levels of *ADNP* and *ADNP2* in 14 young (7 men, 7 women) and 8 old adults (4 men, 4 women) from bicep brachii muscle (data set GSE38718). Two-way ANOVA analysis with Fisher's LSD as post hoc was performed using SigmaPlot. **a** *ADNP*: a significant main effect of age ($F(1, 17) = 10.93, p < 0.001$) and no significant effect of sex ($F(1, 17) = 0.469, p = 0.503$) were observed. Old males showed a significant

increase in *ADNP* expression compared to young age group ($p = 0.006$). **b** *ADNP2*: significant main effects of age ($F(1, 17) = 6.376, p = 0.022$), sex ($F(1, 17) = 5.474, p = 0.032$), and age $\times$ sex ($F(1, 17) = 4.656, p = 0.046$) were observed. Old females showed a significant increase in *ADNP2* expression compared to the young age group ($p = 0.004$). Furthermore, sex differences were observed within the old group ($p = 0.011$)

expression upon prolonged endurance exercise in young and old groups (Fig. 4a, $p < 0.05$ and $p < 0.001$, respectively). Furthermore, age differences were observed within the trained old group of *ADNP* (probe 201773_at) and *ADNP2* transcripts (Fig. 4b, ($p < 0.05$) Fig. 4c ($p < 0.01$)).

Contrarily to *ADNP*, *ADNP2* showed upregulated expression levels upon training within an old group (Fig. 4c, $p < 0.001$).

Potential mechanism: comparing protein interactions in young vs. old vastus lateralis muscle

We have first described changes in the vastus lateralis muscle, and then showed that muscle gene expression differences and correlations to *ADNP* expression were not limited to the vastus lateralis but were extended to the bicep brachii and not only to aging, but also to exercise. We wanted to extend the findings to potential age-dependent protein-protein interaction and chose the vastus lateralis muscle, looking at the most correlative genes for protein-protein interactions with *ADNP* without pre-segregation to different pathways. Results shown in Fig. 5a depict major age-dependent differences (almost 90% of the 24 *ADNP* correlated genes), with shared *ADNP* association at both the young and the old muscle including protocadherin 9 (PCDH9), src kinase-associated phosphoprotein 2 (SKAP2), and cytosolic purine 5'-nucleotidase (NT5C2). Notably, NT5C2 was associated with muscle disease (Table 1).

Taking the proteins specifically correlated to *ADNP* with aging further revealed proteins associated with SMAD and the overlapping transforming growth factor β (TGF β) signaling (Fig. 5b). Additionally, *ADNP* was associated with CDC5L as exemplified in the old and shared protein interactions (Fig. 5b, c).

Discussion

During our life span, the maximum of physical capacity of individuals comprises the second and third decade of life (Keller and Engelhardt 2013). After the fifth decade, we experience pronounced loss in muscle mass and strength at a rate of 1–2%/year and 1.5–5%/year, respectively (Buford et al. 2010, Keller and Engelhardt 2013). In a recent publication, it was demonstrated that the muscle loss starts even earlier around the fourth decade (Keller and Engelhardt 2013). The decline in muscle mass and strength is initially due to the progressive atrophy and loss of type II muscle fibers and motor neurons (Buford et al. 2010). Also, the repair capacity of muscle is reduced with aging (Keller and Engelhardt 2013). As the world population ages, the need to address age-related pathologies becomes more pressing. Sarcopenia, a hallmark disease of aging, is present in about 5–10% of persons over 65 years of age (Morley et al. 2014). Sarcopenia is characterized by a decline in walking speed or grip strength associated with low muscle mass (Morley et al. 2014) and it significantly impacts quality of life. In the coming decades, the total

Fig. 4 *ADNP* is downregulated upon endurance training whereas *ADNP2* is upregulated. **a**, **b** Microarray expression levels of *ADNP* and *ADNP2* in 18 young (9 sedentary, 9 trained) and 19 old adults (10 sedentary, 9 trained) from vastus lateralis muscle (data set GSE9103). Two-way ANOVA analysis with Fisher's LSD as post hoc was performed using SigmaPlot. **a** *ADNP* (probe 226426_at): a significant main effect of training ($F(1, 32) = 20.263, p < 0.001$) was observed. *ADNP* levels were significantly downregulated in the young and old age groups after endurance training ($p < 0.05$ and $p < 0.001$, respectively). **b** *ADNP* (probe 201773_at): significant main effects of training ($F(1, 33) = 9.418$,

$p = 0.004$) and age ($F(1, 33) = 10.178, p = 0.003$) were observed. *ADNP* levels were significantly downregulated in the young age group after endurance training ($p < 0.05$). Furthermore, *ADNP* levels were significantly upregulated in the old trained group in comparison to young trained group ($p < 0.05$). **c** *ADNP2*: significant main effects of training ($F(1, 33) = 6.698, p = 0.014$) and age $\times$ training ($F(1, 33) = 18.218, p = 0.007$) were observed. *ADNP2* levels were significantly upregulated in the old group after endurance training ($p < 0.001$). *ADNP2* levels were significantly upregulated in the old trained group in comparison to young trained group ($p < 0.01$)

number of persons over 65 years is expected to double (Federal Interagency Forum on Aging-Related Statistics 2009, (Buford et al. 2010)). In parallel, the prevalence and costs associated with sarcopenia are expected to rise sharply.

ADNP has been associated with various neurodegenerative diseases (Gozes and Ivashko-Pachima 2015) and was also found to be one of the most frequently mutated genes in ASD (see "Introduction" section). Mutations in the *ADNP* gene are responsible for a broad phenotypical presentation, including global developmental delays, intellectual disabilities, speech impediments, and motor dysfunctions (Hacohen-Kleiman et al. 2018). One of our latest publications demonstrated that our unique mouse model with a partial deficiency in the *Adnp* gene mimics the developmental delays as seen in children with the *ADNP* syndrome, presenting impaired muscle tone and grip strength (as observed in the hanging wire and grip strength tests), as well as gait deficits (exhibited by the CatWalk apparatus) (Hacohen-Kleiman et al. 2018). Together, these findings all point to the essential function of the *ADNP* molecule in motor function.

Here, the novel aspect of this investigation was to examine the role of *ADNP* and *ADNP2* in aging of skeletal muscle (vastus lateralis and bicep brachii) and the impact of endurance exercise on gene expression levels. For this purpose, we searched GEO data sets comparing transcriptional signature/profiling between old and young populations. The microarray data

(GSE28422) provided an insight in to basal level skeletal transcriptome on vastus lateralis of the oldest participants to date (Raue et al. 2012). Here, we demonstrated for the first time that *ADNP* and its paralogue *ADNP2* were associated with aging muscles. *ADNP* expression levels were significantly upregulated in old study subjects (males and females), while *ADNP2* exhibited a similar significant age-dependent increase only in old females. The increase *ADNP* expression might imply a potential compensatory effect toward protection against aging-accelerated loss of muscle mass and function. Furthermore, *ADNP* was differentially expressed in elderly males and females' vastus lateralis, with higher expression levels in females, and this is compatible with our previously published results showing sex-dependent differences in *ADNP* expression in the postmortem hippocampus (Malishkevich et al. 2015a). These observations suggest that there is a possibly compensatory effect due to loss of skeletal muscle mass, strength, and function. Next, we performed a correlation analysis between *ADNP* and 49 genes differentially expressed between young and old adults.

Twenty-four genes were found to correlate with *ADNP* expression as described in the results. Among the genes that *ADNP* was found to correlate with, the highest positive correlative value was with *NMNAT1* gene that encodes a nuclear NAD^+ enzyme and is highly expressed in skeletal muscles (Fang et al. 2017a). NAD^+ is an essential cofactor, playing a pivotal role in a variety of signaling pathways

Fig. 5 ADNP differentially interacts with proteins in the young and the old vastus lateralis muscle. The gene transcripts (Table 1) described in the “Results” section as differentiating between young and old muscles in correlation with ADNP were compared by a Venn diagram (<http://bioinfogp.cnb.csic.es/tools/venny>) (a). Further, protein interaction pathway analysis used the String tool (as in Fig. 1). **b** Protein correlated with ADNP in the old muscle. **c**

Proteins correlated with ADNP in both young and old muscles. Note, while different interactions were discovered in the young and the old muscle, the cell division cycle 5-like protein (CDC5L), a DNA-binding protein involved in cell cycle control, was found to be associated with ADNP at both the old and the shared protein groups

(Berger et al. 2005a). During the course of aging, there is a systemic decrease in NAD^+ across multiple tissues, implicating its crucial role in the pathophysiology of multiple diseases, including age-associated metabolic disorders, neurodegenerative diseases, and mental disorders (Johnson and Imai 2018). This suggests NAD^+ as a potential target for prevention and treatment age-associated diseases (Johnson and Imai 2018). Interestingly, *NMNAT1* is associated with Leber congenital amaurosis 9, a visual impairment that progresses with age (Table 1).

Another interesting protein that was found to be in a high positive correlation with ADNP is KLF5 (Tables 1 and 2), a zinc-finger transcription factor. Similar to ADNP, KLF5 is a transcription factor and KO in this gene is embryonically lethal (Hayashi et al. 2016). KLF5 is also important for skeletal muscle regeneration and myogenic differentiation (Hayashi et al. 2016). Another family member KLF1 is an activator of β -globin gene, and this function is mediated by interaction of the acetylated K288 with the chromatin-remodeling SWI/SNF-related complex (E-RC1) through the BRG1 subunit (Siatecka and Bieker 2011; Viprakasit et al. 2014). Furthermore, KLF1 was showed to be central to erythropoiesis (Siatecka and Bieker 2011). In a similar way, we showed in 2012 that ADNP plays an important role in globin synthesis and erythropoiesis in two model systems of zebrafish and the murine erythroleukemia cell. Thus, we showed a direct interaction of ADNP with the locus control region of the beta globin gene (Dresner et al. 2012). Considering the high similarity between ADNP and KLF family member 1 and 5, we assumed that ADNP also has an important role during muscle regeneration and myogenic differentiation. Importantly, like KLF1, ADNP and ADNP2 interact with BRG1 and constitute components of the SWI/SNF chromatin remodeling complex (Mandel and Gozes 2007, Dresner et al. 2012). Table 1 further describes additional transcripts correlated with *ADNP* (Table 2) including *CIQB* (associated with mesangial proliferative glomerulonephritis, autoimmune diseases (lupus)), which negatively correlated with *ADNP* transcripts (young). In this respect, ADNP has been previously associated with immune regulation (Quintana et al. 2006). Disheveled-associated activator of morphogenesis 2 (*DAAM2*) showed high negative correlation in females (Table 2, old) and as a homeobox profile containing protein ADNP has been tightly associated with morphogenesis, influencing distinct facial features in the ADNP syndrome patients (Van Dijck et al. 2019). *DDB2* positively correlated with *ADNP* in young females is linked to xeroderma pigmentosum, a skin

disease, and our recent study showed a direct involvement of ADNP in skip development and impediments as a consequence of *ADNP* mutations (Mollinedo et al. 2019). *MYH8* was correlated with *ADNP* in trained young females. *MYH8* a perinatal myosin heavy chain influences skeletal muscle development (Haslett et al. 2002b, Schiaffino et al. 2015b). Mutations in *MYH8* result in the trismus-pseudocamptodactyly syndrome characterized by the inability to completely open the mouth (trismus), and the presence of abnormally short tendon units causing the fingers to curve (camptodactyly) (Veugelers et al. 2004; Sreenivasan et al. 2013; Schiaffino et al. 2015b). Perinatal *MYH8* supports muscle contractility and body movements in joint development and in shaping the form of the face (Schiaffino et al. 2015b). Cytosolic 5'-nucleotidase II (*NT5C2*) was correlated with *ADNP* in trained young males. This enzyme has a critical role in maintaining the balance of nucleotides, nucleosides, and free nucleobases in the brain and spinal cord (Elsaid et al. 2017). Specifically, mutations in *NT5C2* lead to the muscle disorder spastic paraplegia 45 (Straussberg et al. 2017). *ADNP* expression was also correlated with the pleiomorphic adenoma gene 1 protein transcript *PLAG1* in young males and females and we have shown before that ADNP is linked to cancer growth (Zamostiano et al. 2001). ADNP had further presented an inverse correlation with Rab11 family-interacting protein 3 (*RAB11FIP3*) in the young female vastus lateralis (Table 2) associated with muscle development (Table 1). Lastly, in old males, we discovered a correlation between *ADNP* expression and monocarboxylate transporter 7 (*SLC16A6*) that mediates the absorption and distribution of monocarboxylic compounds across plasma membranes including lactic acid, resulting from exercise (Fisel et al. 2018). The age differences in correlation are also depicted in Fig. 5, indicating aging specificity.

We also examined *ADNP* and *ADNP2* expression levels in bicep brachii muscle, in order to exclude the changes in lifestyle, that may have an effect on gene transcription and to better comprehend the molecular events due to the biology of aging. For this intent, we looked at the data set GSE38718. As seen in the vastus lateralis, in the bicep brachii muscle, the expression levels of *ADNP* were significantly upregulated in older men compared with young men, while *ADNP2* expression levels were significantly upregulated in the old female group. We also observed that *ADNP2* was expressed in a sex-dependent manner in the elderly group. We further performed a correlation analysis between the *ADNP* gene and genes that were differentially

Table 3 Correlation analysis between *ADNP* and genes that were differentially expressed between young and old adults bicep brachii divided in to 5 different categories

Age group	Entrez gene name	Mitochondrial structure and function		Gene transcription and translation		Lipid synthesis		Immune function		ECM remodeling	
		M	F	M	F	M	F	M	F	M	F
Young	<i>PLAT</i> plasminogen activator, tissue type 201860_s_at							$r=0.958$, $p=0.0026$ Pearson			
	<i>MYH10</i> myosin heavy chain 10212372_at							$r=0.910$, $p=0.0118$ Pearson			
	<i>SDCBP</i> syndecan binding protein 200958_s_at									$r=0.853$, $p=0.0309$ Pearson	
	<i>COX8A</i> cytochrome c oxidase subunit 8A 201119_s_at		$r=0.847$, $p=0.0335$ Pearson								
	<i>TGFBR2</i> transforming growth factor beta receptor 2208944_at							$r=0.835$, $p=0.0387$ Pearson			
	<i>MIF4GD</i> MIF4G domain containing			$r=0.804$, $p=0.0295$ Pearson							
	<i>CAV1</i> caveolin 1					$r=-0.805$, $p=0.0291$ Pearson					
	<i>DHRS4-AS1</i> DHRS4 antisense RNA 1224153_s_at	$r=-0.894$, $p=0.0066$ Pearson								$r=-0.929$, $p=0.0000002$ Spearman	
	<i>OFD1</i> OFD1, centriole and centriolar satellite protein 203569_s_at										
	<i>PPARG</i> peroxisome proliferator activated receptor gamma 208510_s_at							$r=1.000$, $p=0.0182$ Pearson			
Old	<i>CAV2</i> caveolin 2203323_at					$r=-0.978$, $p=0.0221$ Pearson		$r=1.000$, $p=0.00008$ Pearson			
	<i>DHRS4I2</i> dehydrogenase/reductase 4 like 2218021_at		$r=0.999$, $p=0.0325$ Spearman								
	<i>IKBKIP</i> inhibitor of nuclear factor kappa B kinase subunit beta 209341_s_at				$r=0.995$, $p=0.0051$ Pearson						
	<i>COL3A1</i> collagen type III alpha 1 chain 201852_x_at										

Table 3 (continued)

Age group	Entrez gene name	Mitochondrial structure and function		Gene transcription and translation		Lipid synthesis		Immune function		ECM remodeling	
		M	F	M	F	M	F	M	F	M	F
	<i>GADD45A</i> growth arrest and DNA damage inducible alpha 203725_at									$r = 0.993$, $p = 0.00649$ Pearson	
	<i>SCD</i> stearoyl-CoA desaturase 200832_s_at						$r0.993$, $p0.0074$ Pearson				
	<i>RAPGEF3</i> Rap guanine nucleotide exchange factor 3210051_at							$r = 0.992$, $p = 0.0078$ Pearson			
	<i>CAV1</i> caveolin 1212097_at					$r0.964$, $p0.0363$ Pearson	$r0.992$, $p0.0077$ Pearson				
	<i>LPL</i> lipoprotein lipase 203549_s_at							$r0.989$, $p0.0109$ Pearson			
	<i>AOC3</i> amine oxidase, copper containing 3204894_s_at							$r0.985$, $p0.0148$ Pearson			
	<i>SLC25A25</i> solute carrier family 25 member 25225212_at	$r0.984$, $p0.0164$ Pearson									
	<i>CD36</i> CD36 molecule 242197_x_at, 228766_at					$r0.980$, $p0.0202$ Pearson	$r0.957$, $p0.0432$ Pearson				
	<i>SDCBP</i> syndecan binding protein 200958_s_at									$r0.977$, $p0.0225$ Pearson	
	<i>TGFBR2</i> transforming growth factor beta receptor 2208944_at							$r0.977$, $p0.0232$ Pearson			
	<i>FABP4</i> fatty acid binding protein 4203980_at						$r0.976$, $p0.0245$ Pearson				
	<i>JAM2</i> junction adhesion molecule 2229127_at							$r0.975$, $p0.0247$ Pearson			
	<i>ADIPOQ</i> adiponectin, C1Q and collagen domain containing 207175_at						$r0.974$, $p0.0262$ Pearson				
	<i>FBLN2</i> fibulin 2203886_s_at									$r0.970$, $p0.0299$ Pearson	

Table 3 (continued)

Age group	Entrez gene name	Mitochondrial structure and function		Gene transcription and translation		Lipid synthesis		Immune function		ECM remodeling	
		M	F	M	F	M	F	M	F	M	F
	<i>RPLP0</i> ribosomal protein lateral stalk subunit P0 208856_x_at				$r = -0.959$, $p0.0409$ Pearson						
	<i>RPL3L</i> ribosomal protein L3 like 206768_at				$r = -0.981$, $p0.0190$ Pearson						
	<i>GCV1L1</i> general control of amino-acid synthesis 1-like 1 yeast 216232_s_at				$r = -0.995$, $p0.0054$ Pearson						
	<i>CAV3</i> caveolin 3208204_s_at									$r = -0.951$, $p0.0487$ Pearson	
	<i>COX8A</i> cytochrome c oxidase subunit 8A 201119_s_at		$r = -0.955$, $p0.0451$ Pearson								
	<i>CYC1</i> cytochrome c 201066_at		$r = -0.973$, $p0.0267$ Pearson								
	<i>SLC25A11</i> solute carrier family 25 member 11207088_s_at		$r = -0.976$, $p0.0239$ Pearson								
	<i>ACADL</i> acyl-coenzyme A dehydrogenase, long-chain 206069_s_at										
	<i>NDUFS3</i> NADH:ubiquinone oxidoreductase core subunit S3 201740_at		$r = -0.987$, $p0.0127$ Pearson				$r = -0.983$, $p0.0173$ Pearson				
	<i>UCP3</i> uncoupling protein 3207349_s_at		$r = -0.996$, $p0.00366$ Pearson								
	<i>HSF2</i> heat shock transcription factor 2211220_s_at										
	<i>MRPS30</i> mitochondrial ribosomal protein S30 227600_at		$r = -1.000$, $p0.0120$ Pearson								

Pearson's or Spearman's correlation coefficient for the expression levels of each gene relative to *ADNP*, as well as the *p* value, is presented in the table. The values are distributed from the highest to the lowest correlative value in each group (young and old). M, males and F, females

expressed between young and old males and females divided into 5 different categories: mitochondrial structure and function, gene transcription and translation, lipid synthesis, immune function, and ECM remodeling; the summary of the findings is presented in Table 3. The correlations discovered, especially dealing with lipid synthesis (Table 3), were in agreement with previous findings of ADNP association with gene expression during embryonic development including for example the peroxisome proliferative activated receptor gamma (PPARG) (Mandel et al. 2007), correlating development with aging. Furthermore, the high correlation with immune related genes was also shown before for ADNP, as an anti-inflammatory gene in neuro-inflammatory diseases like multiple sclerosis (Quintana et al. 2006; Braitch et al. 2010), see also above.

An impact of exercise has been explored in aging to preserve muscle mass and strength (and found to be an effective and safe in old and frail individuals) (Kalyani et al. 2014). It was demonstrated that high-intensity resistance training caused a large increase in muscle size and strength in elderly men and women (Evans 1995) potentially by also decreasing skeletal muscle apoptosis and improving mitochondrial function (Evans 1995; Kalyani et al. 2014). In the current study, we also explored the impact of prolonged endurance exercise on *ADNP* and *ADNP2* expression levels. We found that *ADNP* expression levels were significantly downregulated in vastus lateralis after training while *ADNP2* expression levels were upregulated after training. Furthermore, an age effect was observed within the trained group. This finding may be explained by the fact that after training, there is increase in muscle size and strength and the necessity in protective proteins is decreased.

Taken together, our current findings pave the path to a better understanding of the molecular mechanisms underlying the pathogenesis of sarcopenia development. The major differences found in correlative expression and protein association of ADNP between the young and the old vastus lateralis with emphasis on energy metabolism in the young and neuronal system in the old suggest differential involvement of ADNP in muscle functionality with aging.

We introduce for the first time ADNP as a potential major player in muscle aging. The increase in ADNP with aging and decrease with exercise may not be sufficient for muscle strength maintenance and may require additional increases with the ADNP snippet peptide,

drug candidate NAP (CP201) which was shown to increase muscle strength in mice (Hacohen-Kleiman et al. 2018). Similar drug candidates may provide new prevention and treatment strategies that will improve the quality of life for millions of older adults worldwide.

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Compliance with ethical standards

Conflict of interest Professor Illana Gozes in the Chief Scientific Officer of Coronis Neurosciences, developing CP201 (under patent protection and license from Ramot at Tel Aviv University).

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