Experimental Gerontology 84 (2016) 96–100

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Short report

Soluble sortilin is present in excess and positively correlates with progranulin in CSF of aging individuals

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ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form 12 August 2016
Accepted 5 September 2016
Available online 7 September 2016

Section Editor: Christian Humpel

Keywords:
Frontotemporal lobar degeneration
Progranulin
Sortilin
Single nucleotide polymorphism
ELISA
Decoy receptor
Biomarker
Aging

ABSTRACT

Mutations in progranulin are a major cause of frontotemporal lobe degeneration (FTLD). Hence, plasma progranulin is an attractive biomarker in FTLD but poorly reflects levels in cerebrospinal fluid (CSF), suggesting tissue-specific regulation of progranulin levels. Sortilin was recently identified as a progranulin scavenger receptor that destines it for lysosomal degradation. Proteolysis or alternative splicing generates soluble sortilin variants that retain progranulin binding and potentially functions as a decoy receptor. In the present study, we analyzed soluble sortilin and progranulin in plasma and CSF in 341 aging individuals. We found that soluble sortilin exists in CSF in ten-fold molar excess compared to progranulin and observed a highly significant positive correlation between soluble sortilin and progranulin levels in CSF but not in plasma. However, carriers of the minor allele of SNP rs646776 in SORT1 encoding sortilin displayed significantly increased soluble sortilin and reduced progranulin specifically in plasma but not in CSF. Taken together, our findings suggest that soluble sortilin may affect progranulin levels in both a tissue-specific and genotype-dependent manner.

1. Introduction

A major cause of familial frontotemporal lobar degeneration (FTLD), with TAR DNA-binding protein-43 (TDP-43) positive inclusions, is *GRN* haploinsufficiency causing decreased expression of the secreted growth factor progranulin (Baker et al., 2006; Cruts et al., 2006). Hence, sustaining or elevating progranulin levels is considered a potential strategy for treating FTLD. Recently, sortilin, encoded by the *SORT1* gene, was shown to bind and target progranulin for endocytosis and lysosomal degradation (Hu et al., 2010). Accordingly, sortilin knockout mice display markedly increased levels of progranulin in brain tissue as well as in plasma (Hu et al., 2010), and drug-induced reduction of sortilin levels increases progranulin in FTLD patient IPSC-derived neurons, displaying sortilin as a promising drug target in FTLD (Lee et al., 2014). Sortilin belongs to the Vps10p domain receptor family (sortilins) also encompassing SorLA (Lr11) and SorCS1, -2, and -3 (Glerup et al., 2014). The five receptors are involved in the regulation of neuronal survival, differentiation, and synaptic plasticity and have been implicated in a number of neurological and metabolic disorders, suggesting that sortilins play key roles in the establishment and maintenance of neuronal circuits (Breiderhoff et al., 2013; Glerup et al., 2013, 2014, 2016; Gustafsen et al., 2013). Mature sortilin consists of an extracellular part (675 residues), a single transmembrane domain (23 residues), and a short cytoplasmic tail (53 residues). The extracellular part comprises a large ten-bladed β-propeller domain with a central ligand binding cavity and two smaller supporting domains (10CCa and 10CCb) (Glerup et al., 2014). Progranulin binds to the β-propeller while adaptors interact with sorting motifs in the cytoplasmic tail and convey endocytosis of the sortilin-progranulin complex, resulting in subsequent lysosomal degradation of progranulin (Hu et al., 2010). Interestingly, the metalloproteinases ADAM10 and ADAM17 cleave sortilin at a site close to the plasma membrane, resulting in the release of its extracellular domain (soluble sortilin) from the cell surface (Evans et al., 2011). A similar soluble sortilin variant was recently reported to be generated by

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http://dx.doi.org/10.1016/j.exger.2016.09.002
0531-5565/

alternative splicing in a process inhibited by TDP-43 (Prudencio et al., 2012). Conversely, loss of TDP-43 promotes both the generation of the soluble sortilin variant but also increases the expression of full length sortilin (Prudencio et al., 2012). Soluble sortilin displays high affinity for progranulin (Hu et al., 2010) and competes with the full length receptor for binding (Prudencio et al., 2012), thereby potentially serving as a decoy receptor that may protect progranulin from being sorted to lysosomes. However, so far the putative relationship between soluble sortilin and progranulin levels has not been established in humans or animal models, and we, therefore, studied this in cerebrospinal fluid (CSF) and plasma of an aging human cohort.

2. Methods

2.1. Study population

We measured levels of soluble sortilin in cerebrospinal fluid (CSF) and plasma from a total of 341 participants between 71 and 95 years of age upon plasma and CSF sampling. Participants were recruited from Olmstead County, Minnesota as part of the Mayo Clinic Study of Aging (MCSA). Please see Nicholson et al. (2014) and Roberts et al. (2008) for detailed description of the study sample. All subjects were normal at the study entry but 62 converted to mild cognitive impairment (MCI) at the time of plasma and CSF sampling. The distribution of males and females and age at inclusion in the study can be found in Table 1.

2.2. Enzyme-linked immunosorbent assay (ELISA)

Progranulin levels were measured previously using the Human Progranulin ELISA Kit (Adipogen Inc., Seoul, Korea) (Nicholson et al., 2014). The sortilin-specific ELISA was carried out as recently described and shows no cross reactivity with other Vps10p domain receptors (Buttenschon et al., 2015). Briefly, 96-well Nunc Maxisorb F96 plates were coated with 10 μg/ml rabbit anti-sortilin antibody (5438) in 100 mM $NaHCO_3$, pH 9.8 for 1 h at 37 °C. After washing in 0.05% Tween-20 in PBS (washing buffer), coated wells were blocked with 2% bovine serum albumin in PBS for 30 min at RT. Purified sortilin extracellular domain was used for a standard dilution series. Samples were loaded and plates were incubated for 1 h at 37 °C. Plates were rinsed 5 times with washing buffer, and incubated with 1 μg/ml mouse monoclonal anti-sortilin (clone F11) (Gustafsen et al., 2013) antibody for 1 h at 37 °C before another 5 times washing procedure. Finally, plates were incubated with peroxidase-conjugated goat anti-mouse IgG (Dako) for 30 min at room temperature and rinsed 5 times before the addition of o-phenylenediamine dihydrochloride (Dako) mixed with hydrogen peroxide. The reaction was stopped using 0.5 M sulfuric acid and absorbance was subsequently measured at 490 nm. The performance of different sortilin antibodies used during optimization of the assay was shared at www.pabmabs.com. To assess whether the sortilin and progranulin ELISA signals were affected by increasing concentration of purified progranulin and sortilin, respectively, we compared the signal from standard curves of purified sortilin mixed with varying concentrations of progranulin (R&D Systems) and vice versa.

Table 1
Number of individuals measured for concentration of soluble sortilin and progranulin in plasma and serum, and their age and gender distribution.

		Mean age (std. dev)	Women/men
Sortilin			
Plasma	271	78.82 (±6.14)*	105/166
CSF	341	79.56(±5.19)	128/213
Progranulin			
Plasma	276	78.89(± 6.17)	107/169
CSF	342	79.59 (±5.22)	129/213

* information is missing for one person.

2.3. Genotyping

Genotyping of the 341 individuals used in this study is described elsewhere (Nicholson et al., 2014). Briefly, DNA was extracted from whole-blood using standard procedures and genotypes were determined using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA) for *APOE* (rs429358 and rs7412) and *SORT1* (rs646776) SNPs. TaqMan assays were performed using an ABI 7900 PCR system and genotyping calls were made using SDS version 2.2 software (Applied Biosystems).

2.4. Statistical analyses

The concentrations of sortilin and progranulin in CSF and plasma were not normally distributed and were therefore log10-transformed. However significant deviation from normality was still present in the data and, therefore, non-parametric tests were applied when relevant. Ordinary least squares regression was used to estimate the correlation between age (age at collection day of plasma and CSF) with sortilin concentration in plasma and CSF. Mann–Whitney test was used to test for a difference in sortilin concentration between males and females. The intra-individual correlation of sortilin concentrations in plasma and CSF was evaluated by Spearman's rank correlation test, which was also used to test for within individual correlation of sortilin concentration with progranulin concentration in both plasma and CSF.

Ordinary least square regression was performed in order to test for association of genotype with sortilin concentration in plasma and CSF. The *APOE* genotype is defined by the genotype of two SNPs (rs429358 and rs7412), which affects the isoform of the APOE protein. We tested for association of sortilin concentration with the following genotypes: no *ApoE4* alleles; *ApoE2 ApoE4; ApoE3 ApoE4 and ApoE4 ApoE4*. Additionally we tested for association of rs646776 with sortilin concentration, including gender, *APOE* genotype and age as covariates.

3. Results

We measured levels of soluble sortilin in cerebrospinal fluid (CSF) and plasma from 341 and 271 participants, respectively, from the Mayo Clinic Study of Aging (MCSA) (Roberts et al., 2008) using a previously developed sandwich ELISA (Buttenschon et al., 2015). Soluble sortilin levels in the CSF have not previously been reported and were found to be 14.7 ng/ml (±5.04) with a median of 14.0 ng/ml. CSF values were on average 23% lower than plasma values (mean = 19.0 ng/ml, $p = 6.43 \times 10^{-13}$) (Fig. 1 and Table 1). The levels measured in individuals diagnosed with MCI were 14.7 and 19.2 ng/ml in CSF and plasma, respectively, and were not significantly different from those of normal individuals. These samples were therefore included in the subsequent analyses. In the CSF, soluble sortilin correlated positively with age ($p_{CSF} = 0.049$, $r^2_{CSF} = 0.01$, slope = 0.0029 (std. error 0.0015)) whereas sortilin in plasma did not ($p_{plasma} = 0.86$; $r^2_{plasma} = 0.0001$, slope = 0.0003 (std. error 0.002)). The soluble sortilin concentration did not relate to gender, neither in plasma nor in CSF ($p_{plasma} = 0.31$, $p_{CSF} = 0.83$). Additionally the intra-individual concentrations of soluble sortilin in plasma and CSF did not correlate ($p = 0.55$), suggesting that the levels of soluble sortilin are differentially regulated inside and outside the CNS (Fig. 1A). The progranulin concentrations measured in this sample of individuals were previously reported to be correlated with several factors (age, sex and the intra-individual concentrations in plasma and CSF) (Nicholson et al., 2014). Furthermore, we did not observe an association of *APOE* genotypes with sortilin concentration in plasma and CSF ($p_{plasma} = 0.46$, $p_{CSF} = 0.63$), indicating that a general risk of neurological disorders does not influence soluble sortilin levels in these tissues. Additionally, we found that no correlation was found between the