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Search for SCA2 blood RNA biomarkers highlights Ataxin-2 as strong modifier of the mitochondrial factor *PINK1* levels

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ABSTRACT

Ataxin-2 (ATXN2) polyglutamine domain expansions of large size result in an autosomal dominantly inherited multi-system-atrophy of the nervous system named spinocerebellar ataxia type 2 (SCA2), while expansions of intermediate size act as polygenic risk factors for motor neuron disease (ALS and FTLD) and perhaps also for Levodopa-responsive Parkinson's disease (PD).

In view of the established role of ATXN2 for RNA processing in periods of cell stress and the expression of ATXN2 in blood cells such as platelets, we investigated whether global deep RNA sequencing of whole blood from SCA2 patients identifies a molecular profile which might serve as diagnostic biomarker.

The bioinformatic analysis of SCA2 blood global transcriptomics revealed various significant effects on RNA processing pathways, as well as the pathways of Huntington's disease and PD where mitochondrial dysfunction is crucial. Notably, an induction of *PINK1* and *PARK7* expression was observed. Conversely, expression of *Pink1* was severely decreased upon global transcriptome profiling of *Atxn2*-knockout mouse cerebellum and liver, in parallel to strong effects on *Opa1* and *Ghitm*, which encode known mitochondrial dynamics regulators. These results were validated by quantitative PCR and immunoblots. Starvation stress of human SH-SY5Y neuroblastoma cells led to a transcriptional phasic induction of *ATXN2* in parallel to *PINK1*, and the knockdown of one enhanced the expression of the other during stress response.

These findings suggest that ATXN2 may modify the known PINK1 roles for mitochondrial quality control and autophagy during cell stress. Given that PINK1 is responsible for autosomal recessive juvenile PD, this genetic interaction provides a concept how the degeneration of nigrostriatal dopaminergic neurons and the Parkinson phenotype may be triggered by ATXN2 mutations.

1. Introduction

The protein Ataxin-2 (ATXN2) on chromosome 12q24 was named after its causal role for autosomal dominant spinocerebellar ataxia type 2 (SCA2) (Gispert et al., 1993, 1995; Hernandez et al., 1995; Pulst et al., 1996). Later on, gene variants at this chromosomal locus were implicated in several additional diseases (Auburger et al., 2014b) and as modifier of exceptional longevity in centenarians (Sebastiani et al., 2012, Sebastiani et al., 2010). The association of this human locus with obesity, hypertension, and type 1 diabetes (Auburger et al., 2014b) might be explained by ATXN2 deficiency, given that the knock-out (KO) of the *Atxn2* gene leads to obesity, insulin resistance and dyslipidemia in mouse (Lastres-Becker et al., 2008a). Conversely, the expansion mutations in the polyglutamine (polyQ) domain of ATXN2 trigger the atrophy of various neuronal populations in SCA2 via a toxic gain-of-function mechanism. PolyQ expansions of large size in ATXN2 affect preferentially the olivo-ponto-cerebellar circuitry and motor neurons leading to a clinical manifestation as spinocerebellar ataxia (Almaguer-Mederos et al., 2010; Auburger, 2012; Estrada et al., 1999; Lastres-Becker et al., 2008b; Riess et al., 1997; Rodriguez-Labrada et al., 2011; Schols et al., 1997; Velazquez-Perez et al., 2009, 2014, 2016a, 2016b). In later disease stages they involve many pathways of the spinal cord, brainstem, midbrain, basal ganglia, and cortex

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(Almaguer-Mederos et al., 2013; Gierga et al., 2005; Hoche et al., 2011; Hoche et al., 2008; Rodriguez-Labrada et al., 2016; Rub et al., 2003a, 2003b, 2004, 2005a, 2005b, 2006, 2007, 2013a, 2013b; Seidel et al., 2016; Tuin et al., 2006; Velazquez-Perez et al., 2004, Velazquez-Perez et al., 2011). In spite of severe midbrain dopaminergic neuron loss, the Parkinsonian symptoms in SCA2 usually are overshadowed by the effects of subthalamic neurodegeneration and by cerebellar ataxia, so that a typical Parkinsonian syndrome does not appear (Schols et al., 2015). However, particularly in Asian patients with Levodopa-responsive Parkinsonism, intermediate size polyQ expansions of AXTN2 were repeatedly found as the underlying cause of PD (Charles et al., 2007; Gwinn-Hardy et al., 2000; Infante et al., 2004; Kim et al., 2007; Lim et al., 2006b; Lu et al., 2004, 2006; Modoni et al., 2007; Park et al., 2015; Payami et al., 2003; Shan et al., 2001; Simon-Sanchez et al., 2005; Wang et al., 2015a, 2015b; Wilkins et al., 2004; Yamashita et al., 2014; Zhang et al., 2015).

Beyond these monogenic effects, polyQ expansions of intermediate size in ATXN2 act as risk factor for motor neuron diseases of spinal cord and of cerebral cortex such as ALS (amyotrophic lateral sclerosis) and FTLD (fronto-temporal lobar dementia) (Elden et al., 2010; Gispert et al., 2012; Lee et al., 2011). Possibly they have a similar role also for basal ganglia degenerations such as PSP (progressive supranuclear palsy) and PD (Parkinson's disease) (Gispert et al., 2012; Ross et al., 2011; Yamashita et al., 2014). Many studies reproduced this modifier gene role of ATXN2 intermediate expansions (Neuenschwander et al., 2014), and a risk haplotype of single nucleotide polymorphisms at the *ATXN2* locus was identified in 20% of ALS patients (Lahut et al., 2012), underlining its importance in polygenic neurodegeneration. *Drosophila melanogaster* fly studies came to the conclusion that Ataxin-2 acts as generic modifier that affects multiple if not all neurodegenerative diseases (Na et al., 2013).

Thus, on the one hand widespread neuronal atrophy appears to occur via ATXN2 overactivity, while on the other hand a weight gain occurs via ATXN2 deficiency. Both pieces of evidence are compatible with the notion that ataxin-2 plays a basal role in nutrient metabolism and cell growth. Indeed, global expression profiling efforts in the *Atxn2*-KO mice revealed very strong downregulations at transcript and protein level of mitochondrial amino acid and fatty acid metabolism pathways with prominent effects on the leucine homeostasis enzyme isovaleryl-CoA-dehydrogenase (IVD), accompanied by a transcriptional upregulation of the ribosomal translation machinery (Fittschen et al., 2015; Halbach et al., 2016; Meierhofer et al., 2016).

A role of ataxin-2 for nutrients and growth is also supported by recent reports showing that mammalian ATXN2 is transcriptionally induced during starvation stress via the mTOR phosphorylation pathway (Lastres-Becker et al., 2016) and relocalizes to stress granules during periods of cell stress (Ralser et al., 2005a). The *Caenorhabditis elegans* ortholog ATX-2 has its effects prominently in calorie-restricted conditions, for an overall control of fat content and cell size (Bar et al., 2016). The ATXN2 ortholog in *Saccharomyces cerevisiae* yeast, Pbp1, is responsible for the transient sequestration of the nutrient sensor TORC1 to stress granules in periods of heat stress or low energy, thus modulating growth and proliferation (DeMille et al., 2015; Takahara and Maeda, 2012). In parallel, Pbp1 and ATXN2 sequestrate mRNAs and the mRNA translation factor PABPC1 to stress granules, thus modifying the mRNA degradation at P-bodies (Damrath et al., 2012; Nonhoff et al., 2007; Satterfield and Pallanck, 2006). During normal growth, ATXN2 is localized at the rough endoplasmic reticulum (rER) in cosedimentation with the ribosomal S6 protein and ATXN2 is thought to ensure mRNA stability through direct interaction with uridine-rich elements of the 3′-untranslated region (3′UTR) (van de Loo et al., 2009; Yokoshi et al., 2014). The deficiency of ATXN2 reduces the maximal translation at ribosomes and leads to transcriptional inductions of global translation factors (Fittschen et al., 2015). Thus, ATXN2 appears to be important for mRNA stability, while adapting the rate of global translation and growth to the availability of energy and nutrients. Interestingly, ATXN2 can also be present at the plasma membrane to modulate growth factor receptor internalization and signaling via its proline-rich motifs (Drost et al., 2013; Nonis et al., 2008; Ralser et al., 2005b), and may shuttle to the nucleus to exert transcriptional effects (Hallen et al., 2011). The proline-rich motifs as modulators of endocytosis, the PAM2 domain for interaction with poly(A)-binding protein, and the Lsm-domains for direct RNA interaction were highly conserved during phylogenesis in ATXN2-orthologs throughout eukaryotes and even in plants (Jimenez-Lopez et al., 2015; Jimenez-Lopez and Guzman, 2014).

The levels of ATXN2 are controlled firstly via degradation by the ubiquitin ligase components FBXW8 and PARK2, which are involved in mTOR/insulin signaling (Kim et al., 2012; Xu et al., 2008) and in mitochondrial autophagy, respectively (Auburger et al., 2014a; Youle and Narendra, 2011); secondly the ATXN2 levels are controlled via synthesis by ETS1, a proto-oncogene and transcription factor involved in the protection of brain from starvation (Halbach et al., 2015; Salnikow et al., 2008; Scoles et al., 2012) and by autofeedback onto its transcription in cooperation with ZBRK1, a tumor suppressor acting in DNA damage responses downstream of E2F1 (Hallen et al., 2011; Liao et al., 2010). The ATXN2 yeast ortholog Pbp1 is activated by the AMP kinase pathway (DeMille et al., 2015). Jointly, this experimental evidence delineates a molecular pathway upstream and downstream from ATXN2, which controls its activity in dependence on cell bioenergetics and nutrient status, to influence RNA quality control and damage response pathways.

It is important to note that the deficiency of ATXN2 orthologs was shown to delay or ameliorate the neuronal toxicity of the ATXN2 protein interactor ATXN1 in a fly model of spinocerebellar ataxia type 1 (SCA1), and of its protein interactor TDP-43 in a yeast model of ALS (Al-Ramahi et al., 2007; Elden et al., 2010), so the investigation of molecular ATXN2 deficiency effects has general therapeutic value for neurodegenerative diseases.

Before the molecular knowledge on ATXN2 can be exploited for neuroprotective experiments, it is urgent for medical diagnostics to define molecular biomarkers of altered ATXN2 function and of the progression of atrophy. According to expression data in public databases (www.genecards.org), ATXN2 abundance in peripheral blood mononuclear cells and in plasma is similarly strong as in brain, so ATXN2 mutations are likely to produce subtle molecular consequences also in blood. In a previous attempt to define a diagnostic molecular signature, a proteome study of blood plasma from SCA2 patients found several secreted proteins such as Apolipoproteins A1, C2, C3, E to exhibit dysregulated levels, possibly due to ATXN2 polyQ expansion effects on rER translation and the secretome. The same team also documented an elevation of circulating blood plasma DNA levels in SCA2 patients (Swarup et al., 2011, 2013). In view of the role of ATXN2 for mRNA processing and stability, we now sought to establish the global mRNA profile of whole blood after overnight fasting in advanced stages of SCA2, through the investigation of three closely matched case-control pairs within one large pedigree from Turkey. Overnight fasting was necessary to standardize the food intake among individuals and to maximize the ATXN2-triggered effects, given that fasting was a prerequisite to detect the ATXN2 involvement in endocytosis (Nonis et al., 2008), in transcriptional regulation (Lastres-Becker et al., 2016) and in cell size (Bar et al., 2016).

2. Materials and methods

2.1. SCA2 patients

From a large Turkish pedigree (Fig. 1), three patients with late-stage SCA2 and three non-SCA2 first degree relatives matched for age, sex and geographic background consented to undergo comparative studies. Individual details are provided in Table 1. Peripheral blood samples were collected on the same morning during a family visit, after overnight fasting of all individuals under study, with informed written consent and approval of the Ethics Commission of Boğaziçi University and