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Whole genome expression analyses of single- and double-knock-out mice implicate partially overlapping functions of alpha- and gamma-synuclein

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Abstract

Alpha-synuclein has been implicated in the pathogenesis of Parkinson's disease. The function of α -synuclein has not been deciphered yet, however, it might play a role in vesicle function, transport or as a chaperone. Alpha-synuclein belongs to a family of three proteins, which includes β - and γ -synuclein. Gamma-synuclein shares 60% similarity with α -synuclein. Similar to α -synuclein, a physiological function for γ -synuclein has not been defined yet but it has been implicated in tumorigenesis and neurodegeneration. Interestingly, neither alpha- ($SNCA^{-/-}$), gamma- ($SNCG^{-/-}$) nor alpha/gamma ($SNCA_G^{-/-}$) deficient mice present with any obvious phenotype. Using microarray analysis we thus investigated whether deficiency of alpha- and gamma-synuclein leads to similar compensatory mechanisms at the RNA level and whether similar transcriptional signatures are altered in the brain. Sixty-five genes were differentially expressed in all mice. $SNCA^{-/-}$ mice and $SNCG^{-/-}$ mice shared 84 differentially expressed genes, $SNCA^{-/-}$ and $SNCA_G^{-/-}$ 79 genes, and $SNCG^{-/-}$ and $SNCA_G^{-/-}$ 148 genes. For many of the physiological pathways such as dopamine receptor signaling (down-regulated), cellular development, nervous system function, and cell death (up-regulated), we found groups of genes that were similarly altered in $SNCA^{-/-}$ and $SNCG^{-/-}$ mice. In one of the pathways altered in both models we found *Mapk1* as the core transcript. Other gene groups, however, such as TGF- β signaling and apoptosis pathways genes were significantly up-regulated in the $SNCA^{-/-}$ mice, but down-regulated in $SNCG^{-/-}$ mice. Beta-synuclein expression was not significantly altered in any of the models.

Keywords

Parkinson's disease; alpha-synuclein; gamma-synuclein; knock-out animals; microarray analysis

Introduction

The pathology of Parkinson's disease (PD) is characterized by a loss of dopaminergic neurons in the substantia nigra and the formation of intracellular inclusions, known as Lewy bodies [1,2]. One of the key proteins in the pathogenesis of PD is α -synuclein, which is encoded by the *SNCA* gene. *SNCA* duplications and triplications, as well as N-terminal

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A30P, E46K, and A53T missense mutations, have been linked to autosomal dominantly inherited PD [3,4,5,6,7]. *In vivo* studies in transgenic mice and flies overexpressing wildtype and mutant *Snca* further demonstrated the pathogenic effect of α -synuclein in neurons [8,9]. In contrast to the well-defined pathogenic effects of α -synuclein its biological function is far less understood. It is thought that α -synuclein might be responsible for regulating the size of the presynaptic vesicle pool in primary hippocampal neurons [10].

Alpha-synuclein is part of a family of proteins that includes β - and γ -synuclein [11]. At the amino acid level γ -synuclein shares 60% similarity with α -synuclein [12]. Both proteins are expressed in thalamus, substantia nigra, caudate nucleus, amygdala and the hippocampus [13]. Gamma-synuclein is abundant in spinal cord and sensory ganglia [14]. Interestingly, it is more widely distributed within the neuronal cytoplasm than α - and β -synuclein, being present throughout cell bodies and axons [14]. The presence of synucleins has also been detected in other cell types: α -synuclein was found in platelets [15] whereas γ -synuclein is present in the epidermis [16] and in metastatic breast cancer tissue [17], respectively. Gamma-synuclein is also known as persyn and BCSG-1 (breast cancer-specific gene 1) [18] and is significantly up-regulated in >70% of late stage breast and >85% of ovarian carcinomas [19]. Gamma-synuclein has multiple actions. It stimulates the ligand-dependent breast cancer cell and cell proliferations, and up-regulates matrix metalloproteases, which are enzymes implicated in tumorigenesis and neurodegeneration [20,21]. SNCG contains similar N-terminal and core domains as SNCA which cause nuclear exclusions. However, SNCG lacks the C-terminal domain of SNCA which supports its transport into the nuclear compartment [22] suggesting that the two synucleins might have different roles in the nucleus.

To define the biological functions of α - and γ -synuclein *in vivo*, knock out mice have been generated. Inactivation of the *Snca* gene in mice by homologous recombination does not lead to a severe neurological phenotype [23]. *SNCA*^{-/-} mice are viable, fertile and do not display any gross pathological abnormalities. Dopaminergic neurons and nerve terminals are normally possessed and the CNS of *SNCA*^{-/-} mice appears morphologically intact [23]. Nevertheless, they display a reduction in total striatal dopamine levels, as well as an attenuated locomotor response to amphetamine.

Similar to *SNCA*^{-/-} mice, absence of γ -synuclein in mice does not lead to any obvious phenotypical changes [24]. *SNCG*^{-/-} null mutant mice are also viable, fertile and do not display any gross pathological abnormalities. The number of neurons is not changed and sensory reflex thresholds were also intact in *SNCG*^{-/-} mice. A relatively small but statistically significant reduction in the number of tyrosine hydroxylase (TH)-positive neurons has been found in the substantia nigra pars compacta (SNpc) of adult *SNCG*^{-/-} mice [25]. This reduction in the number of TH-positive neurons was also seen in *SNCA*^{-/-}/*SNCG*^{-/-} (*SNCA_G*^{-/-}) double knock-out mice [25]. It was shown that dopaminergic neurons of the SNpc of *SNCA*^{-/-}, *SNCG*^{-/-} and *SNCA_G* knock-out mice are resistant to MPTP toxicity [25,26,27].

Except for expression analysis of the synuclein genes, there are basically no studies on the transcriptional dysregulation in *SNCA*^{-/-}, *SNCG*^{-/-} and *SNCA_G* knock-out mice. In *SNCA*^{-/-} mice the expression levels for β - and γ -synuclein mRNA and protein appeared normal indicating that the absence of α -synuclein has not been compensated by other synuclein family members [23]. Likewise, lack of γ -synuclein RNA and protein in *SNCG*^{-/-} null mutant mice was not accompanied by compensatory increases of α - or β -synuclein mRNA levels [27,28].

Considering these results, we initially hypothesized that α -synuclein and γ -synuclein participate in different pathways. To confirm this hypothesis we performed transcriptome analysis of brain tissue of SNCA^{-/-}, SNCG^{-/-} and SNCA_G knock-out mice and investigated whether or not altered expression pathways do overlap in the respective models.

Materials and Methods

Animals

A colony of α -synuclein mutant mice on C57BL/6 background was established from well-described mice [23]. SNCA^{-/-} and SNCG^{-/-} mice were bred to produce double heterozygous and subsequently double γ -synuclein/ α -synuclein null mutant mice (SNCA_G^{-/-}), respectively [24,25]. All mice had been maintained on an ad libitum diet with a 12 h dark to 12 h light cycle. Brains of three male 3-month-old SNCA^{-/-}, SNCG^{-/-} and SNCA_G^{-/-} mice as well as of C57BL/6-wildtype mice were dissected, snap-frozen and kept at -80°C.

RNA preparation for Microarray analysis

Isolation of RNA from whole mouse brain was performed using RNeasy® Kit (Qiagen, Germany) according to the manufacturer's recommendations. The quality of isolated RNA was controlled by Lab-on-Chip-System Bioanalyser 2100 (Agilent). Double-stranded cDNA was synthesized from the total RNA of each brain tissue using a Superscript choice kit (Invitrogen) with a T7-(dT)24 primer incorporating a T7 RNA polymerase promoter (Metabion). cRNA was prepared and biotin labeled by *in vitro* transcription (Enzo Biochemical). Labeled RNA was fragmented by incubation at 94°C for 35 min in the presence of 40 mM Tris-OAc (pH 8.1), 100 mM KOAc, and 30mM MgOAc.

Microarray analysis

Labeled, fragmented cRNA (15 µg) was hybridized for 16 h at 45°C to a Mouse Genome U74Av2 genome array (Affymetrix) containing more than 12.000 transcripts. After hybridization, the microarrays were automatically washed and stained with streptavidin-phycoerythrin using a fluidics station. The probe arrays were scanned at 3-µm resolution using a Genechip System confocal scanner made for Affymetrix by Agilent.

Each cRNA generated from whole brain was hybridized onto one microarray separately. In total, 12 cRNA samples were analyzed (3 SNCA^{-/-} mice, 3 SNCG^{-/-}, 3 SNCA_G^{-/-}, and 3 controls). For downstream analysis Affymetrix .Cel files were imported into ArrayAssist 3.3 (Stratagene) and GC-RMA normalized for further analysis. To filter for transcripts that are differentially expressed between two conditions, the signals were first filtered for an absolute change in signal level of 1.5 fold. The remaining transcripts were subjected to statistical analysis using a t-Test with Benjamini-Hochberg false discovery rate for multiple testing correction [29]. Transcripts with a fold change of 1.5 and a corrected p-value of 0.05 were considered as statistically significant regulated. Categorization was based on the NetAffx annotations (<https://www.affymetrix.com/analysis/netaffx/index.affx>).

Pathway analysis

A total of 548 genes, defined by the criteria's described above, were used for the pathway analysis with the Ingenuity Pathway Analysis software 3.1 (Ingenuity Systems). The identified genes were mapped to genetic networks available in the Ingenuity database and were then ranked by score. The score is defined by the probability that a collection of genes equal to or greater than the number in the respective network could be achieved by chance alone. In this context a value of 3 indicates that there is a 1/1000 chance that the focus genes

are in a network due to random chance. Therefore, scores of 3 or higher have a 99.9% confidence of not being generated by random chance alone.

RNA isolation and cDNA preparation for qRT-PCR

Cortex, cerebellum, midbrain and brainstem of 4 different mice genotypes (WT, SNCA^{-/-}, SNCG^{-/-} and SNCA_G^{-/-}) were dissected. Total RNA of two replicates was isolated using the RNeasy Mini Kit (Qiagen) including a DNase digest on the column with RNase-Free DNase Set (Qiagen). The RNA quality was controlled by Lab-on-Chip-System Bioanalyser 2100 (Agilent) and the concentration was determined using a BioPhotometer (Eppendorf). 0.5 µg total RNA was employed for the cDNA synthesis which was performed with the QuantiTect Reverse Transcription Kit (Qiagen). The negative control (RT-) consists of a sample without Quantiscript Reverse Transcriptase to exclude genomic DNA contamination of the sample. A 1:20 cDNA dilution was used in the q-RT-PCR.

Quantitative Real Time PCR

The quantitative Real Time PCR (q-RT-PCR) was executed with LightCycler® 480 SYBR Green I Master (Roche) in the LightCycler®480 system (Roche). Primer (listed in Table 1) with an average length of 80-160 bp, spanning an exon-exon boundary, were designed using the Primer3 Software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The q-RT-PCR reaction (volume 10 µl) contained 5 µM of each primer, 5 µl LightCycler® 480 SYBR Green I Master and 1 µl of the 1:20 diluted cDNA. The q-PCR conditions were 95°C for 10 min followed by 45 cycles of 95°C for 15 sec, 58°C for 10 sec, 72°C for 20 sec. The acquisition was performed after the 72°C step of each cycle. Melting curves were generated at the end of the run. Standard curves of each amplified gene were created, to obtain the PCR efficiency. PDHB, SDHA and HMBS were analyzed as reference (housekeeping) genes. PDHB was determined as the most adequate housekeeping gene using the GeNorm tool of the qBase software version 1.3.3. and applied for normalization.

The Cp-values of the reference gene and the target genes were detected by the LightCycler 480 software release 1.2.0.0625 applying the 2nd derivative maximum method. Relative expression levels of all genes were calculated using the REST-384© (version 2) [30].

Results

We compared whole transcriptome RNA expression profiles of total brain tissue of SNCA^{-/-}, SNCG^{-/-}, and SNCA_G^{-/-} mice. To avoid transcriptional changes due to the hormone cycle in females only male mice were analyzed. At the age of three months, none of the mouse models had any obvious behavioral or pathological phenotype [23,24,25]. However, gene expression was significantly altered in all mouse models. In SNCA^{-/-} mice, 369 genes were significantly differentially regulated (p < 0.05, SLR = 0.66 which is equivalent to 1.5 fold). In SNCG^{-/-} mice, we found 220 genes, and in SNCA_G^{-/-} mice 215 genes with altered expression. Sixty-five genes were differentially expressed in all mice, respectively (Figure 1). SNCA^{-/-} mice and SNCG^{-/-} mice shared 84 differentially expressed genes, SNCA^{-/-} and SNCA_G^{-/-} 79 genes, and SNCG^{-/-} and SNCA_G^{-/-} 148 genes (Figure 1), respectively. For many of the physiological pathways such as dopamine receptor signaling (down-regulated), cellular development, nervous system function and cell death (up-regulated), we found groups of genes that were similarly altered in SNCA^{-/-} and SNCG^{-/-} mice (Figure 2). On the other hand, other gene groups such as TGF-β signaling and apoptosis pathway genes were significantly up-regulated in the SNCA^{-/-} mice but down-regulated in SNCG^{-/-} mice (Figure 2).

The Ingenuity software delivered 29 networks in *SNCA*^{-/-} mice, but only one including *SNCA* (Figure 3a). Four of ten direct interacting proteins are up-regulated and one is down-regulated. Tyrosine hydroxylase (*Th*) and 14-3-3-zeta are direct interactors of *Snca* and have between 1.5 and 2.5 times higher expression levels than in wildtype mice. Investigating the expression profile of *SNCG*^{-/-} we received also 29 altered networks. *Snca* has only one known differentially regulated direct interactor which is *Grk5* (down-regulated, Figure 3b). The other direct interactor of *Grk5* in this network which is differentially regulated is *Snca* (down-regulated). Besides of *Grk5*, *Mapk1* is down-regulated and *Th* is up-regulated in *SNCG*^{-/-} mice as well as *14-3-3-zeta*, all similar to the changes seen in *SNCA*^{-/-} mice.

A complete list of all differentially regulated genes in *SNCA*^{-/-}, *SNCG*^{-/-} and *SNCA_G*^{-/-} mice can be received from the authors. In the following we will discuss selected genes according to their tentative assignment to groups of genes with specific function.

Transcription pathways altered in alpha- and gamma synuclein single knock-out mice

Applying Ingenuity software we found one pathway that overlaps between *SNCA*^{-/-} mice and *SNCG*^{-/-} mice strongly: the *Mapk1* pathway. In this pathway 12 genes are up-regulated and 8 genes down-regulated (Figure 3 a + b). This implies that α - and γ -synuclein have some common functions despite their rather weak similarity of 60% at the amino acid level [15].

In contrast, other pathways for instance with the core transcript *Tp53* were only altered in the *SNCG*^{-/-} mice but not affected in *SNCA*^{-/-} mice (Figure 3 c) suggesting also different functions of α - and γ -synuclein. Indeed, if both proteins would have entirely complementary functions one might expect a more pronounced phenotype of the double knock out mice and also more severe alterations of the regulatory pathways compared to single knock out mice.

In *SNCA*^{-/-} mice we found numerous genes up- or down-regulated which are known to be involved in development and function of the nervous system such as *Th* (up-regulated), *Gria1*, *Atm*, *Casp3*, and *Syt4* (all down-regulated). Some of these genes also contribute to cell death and apoptosis (*Atm*, *Casp3*, *Cd59*, *Mapk1* [all down-regulated] and *FosB* [up-regulated]). Furthermore, several genes encode proteins in vesicle function or transport such as *myocilin* [31], *synaptotagmin IV* [32] and *ataxia teleangiectasia mutated* [*Atm*, 33].

In *SNCG*^{-/-} mice, *Snca* was also down-regulated which could possibly explain the overlap of genes of the *Mapk1* pathway which were differentially regulated in the *SNCA*^{-/-} mice. In none of the models, expression of *Snca*, the third member of the synuclein family, was significantly changed. Most interestingly, *Hap1*, an interactor of the huntingtin protein, was also up-regulated in *SNCG*^{-/-} mice adding to recent findings of tight interactions of disease pathways in PD and Huntington's disease. Similarly to *SNCA*^{-/-} mice, where *Gria1* was down-regulated, we found *Gria2*, the gene encoding the ionotropic AMPA receptor 2, down-regulated in *SNCG*^{-/-} mice. Other genes involved in cell death and apoptosis such as *Cd59*, *Cebpd*, *Fos*, and *JunB*, were down-regulated as well.

Altered transcriptional pathways in alpha-/gamma-synuclein double knock-out mice

We were interested how the transcriptional networks of the brain were changed in *SNCA_G*^{-/-} double knock-out mice in comparison to the *SNCA*^{-/-} or *SNCG*^{-/-} single KO mice. This analysis is interesting as α - and γ -synuclein belong to the same protein family, but a direct interaction or involvement in the same pathways of both proteins has not been demonstrated yet. Studying the networks we came to the following conclusions: (i) the level of expression of differentially regulated genes in the double KO mice compared to single KO mice as measured by the respective SLRs, was not potentiated (Table 2) indicating that

these proteins have no additive effects, (ii) some of the proteins such as myocilin, kinesin family member 5B, *Atm* (all down-regulated in *SNCA*^{-/-} mice), and guanine nucleotide binding protein beta 1 (up-regulated in *SNCA*^{-/-} mice) were not differentially regulated in double KO mice, and (iii) no regulatory pathway was altered in the double KO mice which was not already affected in the single KO mice which might explain that - as single *SNCA*^{-/-} mice and *SNCG*^{-/-} mice - double KO mice are perfectly viable.

In *SNCA_G*^{-/-} double-knock-out mice we found no network connecting *Snca* and *Sncg*, each of them was integrated in its own network.

q-RT-PCR validation in different brain areas

To validate the microarray data we selected 6 differentially regulated genes and analyzed their expression in cerebellum, cortex, midbrain, and brainstem (Figure 4a-e). The first gene of interest was *Snca*, which was knocked-out in all brain areas of *SNCA*^{-/-} and *SNCA_G*^{-/-} mice, and down-regulated in all brain areas of *SNCG*^{-/-} mice (Figure 4a). These data are in agreement with the microarray analysis. The *Sncg* gene was down-regulated in the *SNCG*^{-/-} and *SNCA_G*^{-/-} mice in all investigated brain areas as expected from the microarray data (Figure 4b). In cortex and midbrain of *SNCA*^{-/-} mice, the *Sncg* gene was up-regulated like in the microarray analysis, whereas it was down-regulated in cerebellum and not differentially regulated in brainstem (Figure 4b). The up-regulation of *Th* in all mouse models found by microarray analysis has also been confirmed by q-RT-PCR analyses studying the midbrain of these mouse lines. These latter analyses showed an extreme up-regulation of *Th* in the cortex of *SNCA*^{-/-} mice, whereas in all other brain areas the *Th* gene was down-regulated (Figure 4c). In *SNCA*^{-/-} mice the *Atm* gene was down-regulated in all brain areas, but was not found to be differentially regulated in whole brain by microarray analysis of *SNCG*^{-/-} and *SNCA_G*^{-/-} mice (Figure 4d). All four brain areas of *SNCG*^{-/-} mice had a down-regulated expression of *Atm*. Q-RT-PCR analysis showed its up-regulation in midbrain of the *SNCA_G*^{-/-} mice, whereas in the three other investigated brain areas the *Atm* gene was down-regulated. The q-RT-PCR data of *Syt4* in different brain areas confirmed the microarray data of whole brain. *Syt4* was down-regulated in whole brain and in all other investigated brain areas, except for midbrain of *SNCA_G*^{-/-} mice. The last gene we analyzed by q-RT-PCR was *Mapk8*, which was up-regulated in microarray data of *SNCA*^{-/-} mice, but not in *SNCG*^{-/-} and *SNCA_G*^{-/-} mice. *Mapk8* was the only gene investigated for which we did not confirm the microarray data by q-RT-PCR (data not shown).

Discussion

This is the first study, which investigates the consequences of a total loss of function of α -synuclein, γ -synuclein and of combined α -/ γ -synuclein deficiency on expression networks in mouse brain. We found as well overlapping but also differing altered expression pathways in the two KO mouse models indicating partially overlapping but also individual functions of the two proteins. Sixty five genes were differentially regulated in the same direction in all three models, such as *Snca*, *Mapk1*, *Sp1*, *Syt4*, *Th*, and *14-3-3-zeta*. These proteins fit into one pathway which is implicated in cellular growth and proliferation indicating that α - and γ -synuclein are involved in neuronal growth (Figure 3 a).

A key protein in this pathway which was up-regulated in both KO models (SLR in *SNCA*^{-/-} = 1.281, and in *SNCG*^{-/-} = 1.139) is 14-3-3. 14-3-3 proteins have been implicated in many neurodegenerative diseases as key molecules in neurotransmitter synthesis, transcriptional control, apoptosis and regulators of protein kinases [34]. In particular for PD several molecular studies underline the involvement of 14-3-3 proteins in the pathogenesis. Initial studies suggest that 14-3-3 proteins bind α -synuclein [36]. Furthermore, 14-3-3 inhibits

parkin activity which is abolished by several ARJP-causing mutations [37]. We and others have shown that 14-3-3 proteins are components of the Lewy bodies [35] and although SNCA^{-/-} mice do not develop these intracellular protein aggregates our expression data support close functional interaction of α -synuclein and 14-3-3 proteins. This is further supported by analyzing SNCA_B^{-/-} double KO mice in which a mild reduction of 14-3-3zeta and a slight increase of 14-3-3e has been found at the protein level [38]. Our data, however, are not directly comparable to the ones of Chandra and coworkers [38] as RNA and protein levels are not always directly correlated. Also, there are age- and strain dependent differences in the expression of genes. Whereas we used 3 month old mice on a C57BL/6 background, 2 month old mice were used in the former study and the genetic background has not been mentioned. Unfortunately, Chandra et al. [38] did not study protein changes of the SNCA^{-/-} single KO mice which would have allowed a more direct comparison of the data.

It is assumed that α -synuclein and 14-3-3 regulate dopamine homeostasis through negative modulation of dopamine transporter activity [39,40] and by regulation of dopamine biosynthesis [41]. Both proteins bind to Th [41,42], the rate-limiting enzyme in dopamine biosynthesis. Whereas the dopamine transporter was not differentially regulated we found *Th* to be up-regulated, in both, SNCA^{-/-} and SNCG^{-/-} mice. We confirmed the upregulation of *Th* by q-RT-PCR and found an increase in particular in the midbrain in all models. In SNCA^{-/-} mice a striking but consistent increase has been found in the cortex (Figure 4). A compensatory upregulation of *Th* has also been observed in the retina of SNCA^{-/-} mice [43].

SNCA inhibits the activity of Th by decreasing its phosphorylation whereas interaction of 14-3-3 proteins with phosphorylated Th increased its activity [44]. One of the kinases phosphorylating Th is Mapk1 [45] which has already been implicated in PD [46,47]. *Mapk1* RNA is decreased in SNCA^{-/-} with a SLR of -3.016 and in SNCG^{-/-} mice with a SLR of -1.847, respectively. Close dependency of synuclein regulation and *Mapk* expression has already been demonstrated in γ -synuclein overexpressing retinoblastoma cells which led to an upregulation of *Mapk* and *Elk-1* [48]. In different cell lines it was shown that the suppression of Mapk phosphorylation increases with higher levels of Snca [47,49]. Mitogen-activated kinases mediate the transduction of external stimuli typically via receptor tyrosine kinase or G protein-coupled receptor activation [50]. Interestingly, Grk5 (G protein-coupled receptor kinase 5) protein has been found as a component of the Lewy bodies and phosphorylates α -synuclein at position Ser-129 [51,52]. Although not changed at the transcriptional level in SNCA^{-/-} mice, we found *Grk5* to be down-regulated (SLR = -0.909) in SNCG^{-/-} mice.

Other differentially expressed genes found in both KO models have also been implicated in synuclein function. One of these proteins is myocilin which is an interactor of γ -synuclein. In cultured cells, γ -synuclein upregulates myocilin expression [53] suggesting that down-regulation of γ -synuclein might cause similar effects for myocilin as well. However, as γ -synuclein is not a transcription factor per se, these regulatory pathways are only indirectly linked. Surprisingly, myocilin was significantly down-regulated in SNCA^{-/-} mice (SLR -2.16; Table 2) but not in SNCG^{-/-} mice. However, it is too simple to assume that a reduction of one protein would lead to an up- or down-regulation of its direct interacting partners. Expression levels of other partners of α -synuclein, as synphilin-1 for instance, are not effected either. As long as the transcription factors regulating synuclein expression have not been defined yet, a straight comparison of complex pathways can not be done easily.

For SNCA^{-/-} mice our mRNA data indirectly support previous findings at the protein level that β -synuclein and γ -synuclein remain unchanged [23,27]. In SNCA_B^{-/-} double KO

mice [38], however, γ -synuclein was increased by 50%. It will be interesting to study these double KO mice at the mRNA level as well to correlate transcriptome data with the existing protein data. Ninkina et al. [24] found no compensatory increases of α -synuclein or β -synuclein in SNCG^{-/-} mice. In contrast, in our microarray analysis we did observe reduced expression of α -synuclein in SNCG^{-/-} mice. This might be due to different ages of the studied mice. On the other hand expression of β -synuclein which is a direct interactor of α -synuclein [Figure 3b, 54,55] and indirectly interacts with γ -synuclein [Figure 3b,51], is not differentially regulated (Figure 3b).

The down-regulation of the gene encoding the AMPA receptor glutamate receptor 1 (*Gria1*) in SNCA^{-/-} mice and of *Gria2* in SNCG^{-/-} mice is an interesting novel finding and might bring these models to the attention of behavioral scientist. *Gria1* and *2* knock out mice have been generated and display deficits in conditioned rewards [56,57] and hippocampus-dependent spatial working memory tasks [58], suggesting that these receptors are important for synaptic plasticity. Although robust NMDA-dependent long-term potentiation has been observed in SNCA^{-/-} mice [23] detailed analysis of AMPA receptors are lacking and in SNCG^{-/-} mice such experiments need still to be done. Behavioral scientists have now models in hand to study reduced AMPA receptor glutamate receptors in addition to the complete knock out mice. The definition of subtle phenotypes in the SNCA^{-/-} mice becomes even more important as deficiency of *synaptotagmin IV* leads to memory and motor deficits [59] and of *Atm* to a selective loss of dopaminergic nigrostriatal neurons in the respective knock out mice [60,61]. Although a clear motor phenotype and degeneration of dopaminergic neurons have not been observed for SNCA^{-/-} mice, an attenuation of dopamine-dependent locomotor response to amphetamine has been shown [23]. As α -synuclein, *Atm* [33] and *synaptotagmin IV* [61] bind to cytoplasmic vesicles suggesting that several gene products contribute to the subtle phenotype in the SNCA^{-/-} mice.

The data derived from the whole genome microarray analysis of the SNCA^{-/-} and SNCG^{-/-} mice also allow a comparison to toxic mouse models of PD. Basically, there is only minor overlap of the altered gene signatures between the knock outs and MPTP and 6-hydroxydopamine induced models [63,64,65], or to MPP⁺ treated dopaminergic cell lines [66,67]. However, these toxic models cause a clear degeneration of dopaminergic neurons with a rapid pathology and are therefore not directly comparable to the knock out models. Interestingly, both, SNCA^{-/-} and SNCG^{-/-} mice, are resistant to MPTP toxicity of dopaminergic neurons in the substantia nigra [25]. For the toxic models, it will be interesting to learn whether the altered expression pathways reflect more closely the situation in the transgenic PD models, and to study if resistance to toxicity is purely due to *Snca* and/or *Sncg* deficiency or rather due to a complex network of genes protecting neuronal cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Venn diagram showing the number of genes regulated in $SNCA^{-/-}$ mice (white circle), $SNCG^{-/-}$ mice (light grey circle) and in $SNCA_G$ double-knock-out mice (dark grey circle).

Figure 2.

Regulated pathways in the knock-out models. Selection of differentially regulated pathways in *SNCA*^{-/-}, *SNCG*^{-/-} and *SNCA_G*^{-/-} mice implicate their functions in PD, TGF-β, dopamine and glutamate signaling, and in apoptosis and ER stress.

Figure 3.

Altered expression networks in SNCA^{-/-} mice (a), and in SNCG^{-/-} mice including the synuclein family (b) and TP53 in a central position (c). Networks were created by Ingenuity software. Up-regulated genes are listed in red, down-regulated in green. Unfilled symbols represent genes which are not differentially regulated. Abbreviations at the lines between the proteins have the following meaning: A – Activation/Deactivation, B – Binding, E – Expression, I – Inhibition, L – Proteolysis, P – Phosphorylation/Dephosphorylation, and T – Transcription (full names of gene symbols are listed in supplementary table 1).

Figure 4.

Q-RT-PCR expression profiles of the *Snca* (a), *Sncg* (b), *Th* (c), *Atm* (d), and *Syt4* (e) genes in $\square$ cerebellum, $\square$ cortex, $\square$ midbrain and $\blacksquare$ brainstem of SNCA^{-/-}, SNCG^{-/-} and SNCA_G^{-/-}, and for comparison with microarray analysis from $\square$ whole brain. Data is presented in mean $\pm$ SEM.

Table 1

Oligonucleotides for the q-RT-PCR validation of the microarray experiment

gene	primer - sequences	fragment [bp]	efficiency
SNCA	accagttgggcaagaatgaa	104	2,03
	cccttcctcagaaggcattt		
SNCG	caacacagtggccaacaaga	86	1,96
	ggggttccaagtcctcctt		
TH	gcctcctcacctatgcactc	122	2,02
	cccagagatgcaagtccaat		
Mapk8	agaaactgttccccgatgtg	83	1,86
	ggataacaaatctcttgctga		
ATM	cgatggaagttatgcggagt	141	2,03
	tggaggtcggactcatcttc		
SYT4	tgattcctctttcagggattg	115	1,93
	cagagagagaccagaagttcacc		
PDH	gtagaggacacgggcaagat	89	1,90
	tgaaaacgcctcttcagca		
SDHA	gcagcacaggaggtatca	153	1,96
	ctcaaccacagaggcagga		
HMBS	aaagttccccaacctggaat	98	1,89
	ccaggacaatggcactgaat		

Table 2

Selection of genes differentially regulated in SNCA^{-/-}, SNCG^{-/-} and SNCA^{-/-} SNCG^{-/-} mice. (The entire list of differentially regulated genes of all mice can be received from the authors.)

Probe Set ID	p-value	SLR-SNCA	SLR-SNCG	SLR-SNCA_G	Title	Gene Symbol
94122_at	0.0000	-2,1635			myocilin	Myoc
160417_at	0.0000	-1,6746			kinesin family member 5B	Kif5b
101180_at	0.0004	-0,9571			ataxia telangiectasia mutated homolog (human)	Atm
161295_r_at	0.0005	1,3905	0,7114		mitogen-activated protein kinase kinase kinase 4	Map4k4
104280_at	0.0017		-2,2661	-2,3800	synuclein, gamma	Sneg
93273_at	0.0000	-7,4243	-0,7490	-7,7313	synuclein, alpha	Snca
93253_at	0.0001	-3,0160	-1,8466	-1,8403	mitogen activated protein kinase 1	Mapk1
160190_at	0.0001	-1,4594	-0,8709	-0,8074	synaptotagmin 4	Syt4
100690_at	0.0493	0,7422	0,7575	1,1153	tyrosine hydroxylase	Th
97544_at	0.0003	1,2811	1,1388	1,2541	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	Ywhaz
92631_f_at	0.0237	1,5290	0,8383	1,1073	calmodulin 3	Calm3