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Pharmacometabolomic Signature of Ataxia SCA1 Mouse Model and Lithium Effects

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Abstract

We have shown that lithium treatment improves motor coordination in a spinocerebellar ataxia type 1 (SCA1) disease mouse model (Sca1154Q+/+). To learn more about disease pathogenesis and molecular contributions to the neuroprotective effects of lithium, we investigated metabolomic profiles of cerebellar tissue and plasma from SCA1-model treated and untreated mice. Metabolomic analyses of wild-type and Sca1154Q+/+ mice, with and without lithium treatment, were performed using gas chromatography time-of-flight mass spectrometry and BinBase mass spectral annotations. We detected 416 metabolites, of which 130 were identified. We observed specific metabolic perturbations in Sca1154Q+/+ mice and major effects of lithium on metabolism, centrally and peripherally. Compared to wild-type, Sca1154Q+/+ cerebellum metabolic profile revealed changes in glucose, lipids, and metabolites of the tricarboxylic acid cycle and purines. Fewer metabolic differences were noted in Sca1154Q+/+ mouse plasma versus wild-type. In both genotypes, the major lithium responses in cerebellum involved energy metabolism, purines, unsaturated free fatty acids, and aromatic and sulphur-containing amino acids. The largest metabolic difference with lithium was a 10-fold increase in ascorbate levels in wild-type cerebella (p<0.0002), with lower threonate levels, a major ascorbate catabolite. In contrast, Sca1154Q+/+ mice that received lithium showed no elevated cerebellar ascorbate levels. Our data emphasize that lithium regulates a variety of metabolic pathways, including purine, oxidative stress and energy production pathways. The purine metabolite level, reduced in the Sca1154Q+/+ mice and restored upon lithium treatment, might relate to lithium neuroprotective properties.

Introduction

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease that is caused by the expansion of a translated CAG repeat in ATAXIN1 (ATXN1). SCA1 is characterized by progressive loss of balance and coordination, mild cognitive impairments, speaking and swallowing difficulties, and eventually respiratory failure leading to premature death [1]. The toxic effects of the glutamine-expanded protein result in variable degrees of neurodegeneration, predominantly in the cerebellum, brainstem and spinocerebellar tracts [2]. A knock-in mouse model of SCA1 (Sca1154Q+/+) recapitulates many aspects of the human disease [3], enabling us to study SCA1 pathophysiology and test therapeutic candidates. Molecular mechanisms that underlie the pathophysiology of SCA1 are slowly becoming understood [4]. The dysregulation of several neuronal genes that has been observed in the tissue of humans with SCA1 has also been found in the Purkinje cells of SCA1 transgenic mice, even at the pre-symptomatic stage [4]. In light of this notion, Watase et al. [5] treated Sca1154Q+/+ mice with lithium—which exerts neuroprotective effects possibly by affecting gene transcription—and demonstrated that lithium rescues several SCA1 phenotypes in this model.

Lithium has been the standard pharmacological treatment for bipolar disorder for over fifty years. During the past two decades, attention has been drawn to the neuroprotective properties of lithium against diverse insults, including glutamate-induced excitotoxicity and endoplasmic reticulum stress [6]. Multiple molecular pathways such as phosphoinositides [7,8], protein kinase C signaling pathway [9] and glycogen synthase kinase 3 activity could explain some of the neuroprotective properties of lithium [10–13].

It is unclear how lithium improves the SCA1 disease model and what metabolic changes might be modified. There is no biological marker for following the SCA1 disease course or for measuring the effects of lithium or any other therapy on patients under the...
treatment. To seek potential markers and a deeper understanding of the disease pathogenesis and response to lithium, we applied techniques from the rapidly-evolving field of metabolomics, which enables the identification and quantification of hundreds of small molecules in cells, tissues and body fluids. The metabolic profile of an individual captures a metabolic state at a certain point in time and is regulated by net interactions between gene products and environmental influences [14]. Metabolic profiles could provide valuable insights into disease mechanisms, and can lead to the development of diagnostic and intervention assessment markers [15–17]. Metabolic signatures have been found for a variety of diseases, including neurodegenerative disorders [15,17], substance abuse [18,19] and drugs used for the treatment of these disorders [15,20].

Differences between individuals make it difficult to find a homogeneous group of human participants for studying the final metabolic output of the treatment and disease. Having an authentic mouse model of SCA1 disease [3] together with a carefully controlled lithium treatment provides the opportunity to discover biochemical changes in SCA1 disease and lithium treatment which can then be specifically explored in human patients.

In this study, we used a non-targeted mass-spectrometry-based metabolomics platform to map biochemical changes in the cerebellum and plasma of Sca1154Q/+ mice with and without lithium treatment compared to their wild-type littermates.

**Materials and Methods**

**Mouse maintenance, diet and dissection**

Mouse generation and treatment were performed as described in Watase et al. [5]. In brief, Sca1154Q/+ mice and their wild-type littermates were obtained from crosses between male Sca1154Q/+ mice and wild-type female mice. All mice were on pure C57Bl/6 background. The mice were randomly assigned to two groups at the time of weaning (presymptomatic), when treatment was initiated. Animals were housed in groups of 3 to 5 per cage in a 12 hour light/dark cycle, with free access to food and water. For 10–12 weeks, each group was fed either chow containing 0.2% lithium carbonate or control Purina rodent chow (Harlan Teklad, http://www.teklad.com). At 13–15 weeks of age, the animals were anesthetized. Their blood was obtained via cardiac puncture and used for the treatment which can then be specifically explored in human patients.

**Blood sample preparation analysis**

Plasma samples (30 µl) were thawed on ice and vortexed for 10 seconds. Aliquots (15 µl) were extracted with 1 ml of a carefully degassed solvent mixture of 3:3:2 (v/v/v) acetonitrile/isopropanol-water at −20°C to perform protein precipitation and metabolite extraction. The plasma/solvent mixture was vortexed for 10 seconds and shaken for 4–6 minutes at 4°C. A subsequent centrifugation step removed insoluble proteins and cell membrane components. The supernatant was dried down using a speed vacuum concentration system (Labconco Centrivap cold trap). To remove most of the membrane lipids and triglycerides, which may interfere with the analysis of amino acids in gas chromatograph mass spectroscopy, 500 µl of a degassed mixture of 1:1 (v/v) acetonitrile/water was added and the sample underwent vortexing for 10 seconds, ultrasonication for 20 seconds and centrifugation at 13,000×g for 2 minutes. Afterward, 450 µl were decanted, which was dried down by speed vac for derivatization and analysis. A mixture of internal retention index markers was prepared using fatty acid methyl esters of C8, C9, C10, C12, C14, C16, C18, C20, C22, C24, C26, C28 and C30 linear chain length, and was dissolved in chloroform at a concentration of 0.8 mg/ml (C8–C16) and 0.4 mg/ml (C18–C30). One µl of this retention index mixture was added to the dried extracts. Ten µl of a solution of 20 mg/ml of 98% pure methoxyamine hydrochloride (Sigma, St Louis MO) in pyridine (silylation grade, Pierce, Rockford IL) was added and shaken at 30°C for 90 minutes to protect aldehyde and ketone groups. Ninety µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (1 ml bottles, Sigma-Aldrich) was added for trimethylsilylation of acidic protons and shaken at 37°C for 30 minutes. The reaction mixture was transferred to a 2 ml clear glass autosampler vial with microinsert (Agilent, Santa Clara CA) and closed using an 11 mm T/S/T crimp cap (MicroLiter, Suwanee GA).

**Gas Chromatograph Time-of-Flight mass spectrometry**

A Gerstel MPS2 automatic liner exchange system was used to inject 0.5 µl of sample at 50°C (ramped to 250°C) in splitless mode with 25 seconds splitless time. An Agilent 6890 gas chromatograph (Santa Clara CA) was used with a 30 m long, 0.25 mm i.d. Rtx5Sil-MS column with 0.25 µm 5% diphenyl film and an additional 10 m integrated guard column (Restek, Bellefonte PA). Chromatography was performed at a constant flow of 1 ml/minute, ramping the oven temperature from 50°C to 330°C with 22 minutes total run time. Mass spectrometry was performed using a Leco Pegasus IV time-of-flight mass spectrometer with a 280°C transfer line temperature, electron ionization at −70 V and an ion source temperature of 250°C. Mass spectra were acquired from m/z 85–500 at 17 spectra/s-1 and 1850 V detector voltage.

Result files were exported to the Fiehnlab servers and further processed by the in-house metabolomics BinBase database. All database entries in BinBase were matched against the Fiehn mass spectral library of 1,200 authentic metabolite spectra using retention index and mass spectrum information or the National Institute of Standards and Technology, version 5 commercial library. Identified metabolites were reported if they were present in at least 50% of the samples per study design group (as defined in the SetupX database). Quantitative data were normalized to the sum intensities of all known metabolites and used for statistical investigation.

**Statistical analysis**

Multivariate statistics and one-way analysis of variance (ANOVA) models were calculated using Statistica data miner V8 (Statsoft, Tulsa OK, USA). The ANOVA results were used for box-whisker plots and tables, and were subsequently converted into a Cytoscape node attribute file which was utilized to visualize the differential statistics on network graphs. Multivariate partial least square analysis was performed using the non-linear iterative partial least squares algorithm using unit standard deviation scaling, 50 iterations with 0.0001 as convergence criterion and 7-fold cross validation of the resulting partial least square (PLS) models.

**Network representation**

Molfile-encoded chemical structures were retrieved from the PubChem database. A pair-wise similarity matrix of Tanimoto similarity coefficients among all the structures was performed using the online structural clustering tool hosted at the PubChem Web
site. The matrix was subsequently converted into a Cytoscape simple interaction format (SIF) formatted network using an in-house Javascript (script available upon request). Kyoto Encyclopedia of Genes and Genomes (KEGG) reaction pair relationships were downloaded from the KEGG ftp site and parsed for the main reactant pairs information. Using MS Excel, a single-step reaction network was constructed for the identified metabolites and saved as a SIF-formatted network. Both networks were imported into Cytoscape [21] and merged using the ‘merge network’ plug-in. Network graphs were visualized using an organic layout. Results of differential ANOVA statistics and relative abundance changes were mapped onto the node size (for magnitude of change), node color (for up/downregulation) and ANOVA p-value (color intensity), respectively.

Results

The overall study design has eight classes (2 genotypes×2 treatments×2 tissues) as shown in Table 1. Four hundred and sixteen metabolites (416) were quantified, of which we identified 130 non-redundant identified compounds for analysis of impact on metabolic networks. Structural identification of the 286 yet- unidentified markers is a time-consuming and costly process and thus beyond the scope of the work presented here. Raw data are downloadable at http://fiehnlab.ucdavis.edu:8080/m1/main_public.jsp [22–24] and attached as processed result files with quantification ions, full mass spectra, retention indices, database identifiers, names and external database references.

Plasma and cerebellar metabolic signature for Sca1<sup>154Q/+</sup> mice

We compared metabolic profiles of cerebellum and blood in Sca1<sup>154Q/+</sup> and wild-type mice. Figure 1A shows good separation of the four groups (plasma wild-type, plasma Sca1<sup>154Q/+</sup>, cerebellum wild-type and cerebellum Sca1<sup>154Q/+</sup>). The overall variance was clearly dominated by the differences in metabolite levels between plasma and cerebellar tissue, indicated by the contribution of vector 1 (48% of the overall metabolic variance). Differences that separated the two genotypes in both organs (vector 3 and 4) used only 5% of the overall metabolic variance, while vector 2 (not shown) was related to within-group variance. The clear separation of metabolic clusters in both blood plasma and cerebellum demonstrated that the disease-causing mutation is responsible for metabolic differences that underlie the disease phenotype in a systemic manner and were not confined to the brain. Using one-way ANOVA tests for each comparison, 41 metabolites were found to be differentially regulated at \( p, 0.05 \) between wild-type and Sca1<sup>154Q/+</sup> mice in cerebellum, out of which 18 were structurally identified using authentic standards.

We next set out to define the metabolic changes induced by polyglutamine (polyQ) expansion in Sca1<sup>154Q/+</sup> mice in plasma and cerebellum. Table 2 shows all compounds found in plasma or cerebellum at \( p, 0.05 \) with the magnitude of difference in metabolite levels, and shows the ratios of metabolites in Sca1<sup>154Q/+</sup>/wild-type that differ between these two groups. A wide range of metabolites were down-regulated in Sca1<sup>154Q/+</sup> mice and few were up-regulated. Metabolites that changed in the cerebellum included intermediates in glucose, lipid, tricarboxylic acid (TCA) cycle and purine metabolism, as well as a number of amino acids including cysteine, beta-alanine and serine. The plasma of Sca1<sup>154Q/+</sup> mice showed higher levels of tryptophan and threonin.

| Test Group | Plasma | Cerrebellum |
|------------|--------|------------|
|            | Wild-Type Sca1<sup>154Q/+</sup> | Wild-Type Sca1<sup>154Q/+</sup> |
| Control    | 11     | 16         |
| Lithium treated | 12     | 18         |

Table 1. Overall study design with the number of tested animals.
acids, and lower levels of phosphate and amino adipic acid (Table 2). None of the identified metabolites were significantly different in both organs, which points to specific impact of the polyQ-expanded ATXN1 on both cerebellar biochemistry and whole-body physiology. The difference in metabolite levels between organs and the specific impact of the SCA1 gene on plasma and cerebellum metabolism is visualized for nine selected metabolites in Figure 2 (see Figure S1 for box-whisker plots of other significant compounds from Table 2).

Comparison of central and peripheral biochemical changes caused by lithium treatment

We investigated the effect of lithium treatment for both genotypes, investigating individually for each organ. Eighty-five metabolites significantly differed under lithium treatment ($p < 0.05$, one-way ANOVA) in at least one of the genotypes. Forty-two of these compounds were structurally identified and are listed in Table 3 with their respective $p$-values and magnitude of alteration. In cerebellar tissue, lithium treatment caused the largest impact on metabolic variation, affecting 42 of 44 significantly-affected compounds. This led us to conclude that gender, weight and age (other factors evaluated) of the mice were not relevant parameters in this study.

We also found lithium treatment to be a significant parameter for separating all cerebellar samples using PLS analysis (Figure 1B) with vector 1 explaining 23% of the group-dependent variance. Vectors 2 and 3 were not significant by themselves, but taken together were able to classify the distinct effects of the wild-type and $Sca1^{154Q/+}$ mice under lithium treatment and control conditions (explaining 29% of the group-dependent metabolic variance). While even unsupervised multivariate analysis (principal components analysis) could readily distinguish samples between lithium-treated and untreated animals (graph not shown), the within-group variance was too high to distinguish the effects of $Sca1^{154Q/+}$ without the classification power of supervised PLS.

To pinpoint the significance level for each metabolite individually, we used univariate statistics to compare lithium-treated versus control conditions in wild-type and $Sca1^{154Q/+}$ mice—separately for both—in the cerebellum and plasma (see below). Using multivariate ANOVA and using metabolites as dependent variables and study parameters as covariates (lithium, genotype, sex, age and weight), we found only 20 metabolites to be significantly impacted by at least one parameter. Gender was found to be significant for isoleucine, allo-inositol and aminoadipic acid. As these metabolites were not significantly different per lithium treatment, subsequent statistical analyses were performed by combining data from both sexes. Using one-way ANOVA tests, 59 plasma metabolites were significantly differentially regulated under lithium treatment when comparing both genotypes (wild-

### Table 2: Genotype effect on metabolic profiles: Significantly different metabolites comparing wild-type versus $Sca1^{154Q/+}$ mice under control conditions.

| Compounds                        | Average level SCA1/Wild-Type | p-value |
|----------------------------------|------------------------------|---------|
|                                  | Cerebellum | Plasma | Cerebellum | Plasma |
| Glucose-6-phosphate              | 0.5        | 1.1    | 0.032      | 0.614  |
| Cysteine                         | 0.6        | 1.6    | 0.0004     | 0.057  |
| Hypoxanthine                     | 0.6        | 1.3    | 0.0004     | 0.291  |
| Phosphoric acid                  | 0.7        | 0.9    | 0.019      | 0.270  |
| Beta-Mannosylglycerate           | 0.7        | 0.9    | 0.014      | 0.647  |
| Phosphate                        | 0.7        | 0.7    | 0.145      | 0.047  |
| 2-monopalmitin                   | 0.7        | 1.0    | 0.005      | 0.790  |
| Xanthine                         | 0.7        | 1.1    | 0.007      | 0.873  |
| Galactinol                       | 0.7        | 2.0    | 0.003      | 0.361  |
| Citric acid                      | 0.8        | 1.1    | 0.030      | 0.378  |
| Monopalmitin-1-glyceride         | 0.8        | 0.9    | 0.030      | 0.542  |
| Hydroxy carbamate NIST           | 0.8        | 1.4    | 0.021      | 0.088  |
| Inosine                          | 0.8        | 1.2    | 0.003      | 0.241  |
| Beta-alanine                     | 0.8        | 0.6    | 0.012      | 0.136  |
| 2-amino adipic acid              | 0.8        | 0.6    | 0.137      | 0.015  |
| Serine                           | 0.8        | 1.1    | 0.010      | 0.353  |
| Glycerol-3-galactoside           | 0.8        | 1.5    | 0.014      | 0.372  |
| Inositol allo-                   | 0.8        | 0.8    | 0.043      | 0.225  |
| Tryptophan                       | 1.0        | 1.5    | 0.840      | 0.012  |
| Idonic acid NIST                 | 1.1        | 2.5    | 0.426      | 0.001  |
| Threonic acid                    | 1.2        | 2.6    | 0.209      | 0.001  |
| Glycolic acid                    | 1.2        | 0.9    | 0.028      | 0.414  |
| Hexuronic acid                   | 1.2        | 1.0    | 0.017      | 0.839  |

Notes: Bold indicates statistical significance. One-way analysis of variance performed separately for cerebellum and blood plasma (see Figure 2 and supplemental Figure 1 for box-whisker plots). Abbreviation: NIST, National Institute of Standards and Technology. doi:10.1371/journal.pone.0070610.t002
Plasma and cerebellar metabolic signature for lithium treatment in wild-type mice

Lithium treatment in wild-type mice led to significant changes in 33 metabolites in cerebellum and 14 in plasma. Malic acid, fumaric acid, citric acid, methionine and pseudo uridine showed changes in both tissues.

Lithium effects seen in wild-type mice (Table 3 and Figure 3 panel B, orange circles) were clustered to the purine biochemical pathway (increased levels of xanthine, inosine, hypoxanthine), sulphur and aromatic amino acids (increased levels of cysteine, methionine, tryptophan, phenylalanine) and central energy metabolism (increased concentrations for fructose, glucose, fructose-6-phosphate, glucose-6-phosphate and TCA metabolites citrate, isocitrate, malate and fumarate with concomitant decreased levels of adenosine monophosphate [AMP]). There was also a dramatically increased level of ascorbic acid which was accompanied by reduction of threonate, and slightly increased levels of unsaturated free fatty acids (elaidic acid, oleic acid and linoleic acid).

While lithium treatment was the most significant study parameter affecting metabolite levels in plasma, it had only moderate effects on the identified metabolites (Figure 4). Similar to what was found in cerebellar tissues, TCA metabolites were increased with lithium treatment in wild-type mice (citric, aconitic, isocitric, malic and fumaric acid), albeit at low abundance differences.

Metabolic signature and therapeutic effect of lithium treatment in Sca1<sup>154Q/+</sup> mice

Of 29 significantly altered metabolites in Sca1<sup>154Q/+</sup> mouse cerebellum after lithium treatment, nine were specific to Sca1<sup>154Q/+</sup> mice. Moreover, lithium treatment affected 12 metabolites in wild-type mice only. Those compounds that only responded to lithium in one of the genotypes (Table 3 and Figure 3 Panel A, green circles) indicate metabolic pathways that might be disturbed by the action of the mutant Atxn1. For example, ascorbic acid (Vitamin C) exhibited the overall highest fold change in the cerebellum of wild-type mice (10-fold accumulation, one-way ANOVA p = 0.002) but not Sca1<sup>154Q/+</sup> mice. The
Pharmacometabolomic Signature of Ataxia SCA1 Mouse

Table 3. Effect of Lithium treatment on cerebellum metabolic profile: Significantly different metabolites comparing Lithium treatment versus control conditions.

| Compound                        | Average Level Li/control | p-value | Average Level SCA1 | p-value |
|---------------------------------|--------------------------|---------|-------------------|---------|
|                                 | SCA1 Wild-Type           |         | SCA1 Wild-Type    |         |
| Ascorbic acid                   | 1.0                      | 10.6    | 0.609             | 0.002   |
| Glucose-6-phosphate             | 4.3                      | 3.7     | 0.000             | 0.010   |
| Fructose-6-phosphate            | 2.7                      | 2.8     | 0.000             | 0.007   |
| Elaidic acid                    | 2.2                      | 2.3     | 0.001             | 0.009   |
| Glucose                         | 1.8                      | 2.3     | 0.017             | 0.021   |
| Hypoxanthine                    | 2.3                      | 2.2     | 0.000             | 0.000   |
| Fructose                        | 1.8                      | 1.6     | 0.118             | 0.001   |
| Methionine                      | 1.5                      | 1.6     | 0.000             | 0.000   |
| Maltose                         | 2.9                      | 1.6     | 0.000             | 0.019   |
| Cysteine                        | 1.8                      | 1.6     | 0.000             | 0.000   |
| Xanthine                        | 1.8                      | 1.5     | 0.000             | 0.003   |
| Insitol-4-monophosphate         | 1.0                      | 1.5     | 0.952             | 0.015   |
| Citric acid                     | 1.8                      | 1.4     | 0.000             | 0.001   |
| Tyrosine                        | 1.2                      | 1.4     | 0.041             | 0.023   |
| Inosine                         | 1.5                      | 1.4     | 0.000             | 0.001   |
| Fumaric acid                    | 1.1                      | 1.3     | 0.509             | 0.009   |
| Oleic acid                      | 1.2                      | 1.3     | 0.001             | 0.008   |
| Isocitric acid                  | 1.5                      | 1.3     | 0.000             | 0.004   |
| Icosenoic acid                  | 1.3                      | 1.3     | 0.038             | 0.109   |
| Linoleic acid                   | 1.2                      | 1.3     | 0.033             | 0.010   |
| Tryptophan                      | 1.3                      | 1.3     | 0.015             | 0.039   |
| Malic acid                      | 1.2                      | 1.2     | 0.027             | 0.008   |
| Phenylalanine                   | 1.2                      | 1.2     | 0.020             | 0.019   |
| Pseudo uridine                  | 1.2                      | 1.2     | 0.009             | 0.016   |
| Pantothenic acid                | 1.3                      | 1.2     | 0.006             | 0.087   |
| Valine                          | 1.2                      | 1.2     | 0.134             | 0.017   |
| Ornithine                       | 1.3                      | 1.2     | 0.009             | 0.132   |
| Glycine                         | 1.1                      | 1.1     | 0.166             | 0.037   |
| Monopalmitin-1-glyceride        | 1.3                      | 1.1     | 0.008             | 0.300   |
| Methionine sulfide              | 1.3                      | 1.1     | 0.007             | 0.470   |
| 2-aminoacidic acid              | 1.3                      | 1.0     | 0.043             | 0.799   |
| Glycerol-alpha-phosphate        | 0.7                      | 1.0     | 0.043             | 0.907   |
| 2-monopalmitin                  | 1.4                      | 1.0     | 0.003             | 0.698   |
| Allanoic Acid NIST               | 0.7                      | 0.9     | 0.001             | 0.324   |
| Ethanolamine                     | 0.9                      | 0.8     | 0.475             | 0.032   |
| Ribonic acid                    | 0.9                      | 0.8     | 0.400             | 0.030   |
| Theonic acid                    | 1.0                      | 0.8     | 0.948             | 0.007   |
| Nicotinamide                    | 0.9                      | 0.8     | 0.512             | 0.042   |
| Glyceric acid                   | 1.0                      | 0.8     | 0.813             | 0.007   |
| Phytol                          | 1.1                      | 0.6     | 0.664             | 0.002   |
| Adenosine-5-monophosphate       | 0.5                      | 0.5     | 0.001             | 0.011   |
| Cholestan-3-ol                  | 0.9                      | 0.5     | 0.566             | 0.015   |

Note: Bold indicates statistical significance. One-way ANOVA performed separately for wild-type and Sca1154Q/+ mice (see supplemental Figure 2 for box-whisker plots). Abbreviation: Li, Lithium.

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Table 4. Effect of Lithium treatment on blood plasma metabolic profile: Significantly different metabolites comparing Lithium treatment versus control conditions.

| Compound                        | Average Level Li/control | p-value |
|---------------------------------|--------------------------|---------|
|                                 | SCA1 Wild-Type           |         |
| Icosenoic acid                  | 2.0                      | 2.1     |
| Malic acid                      | 1.8                      | 1.8     |
| 2-aminoacidic acid              | 1.7                      | 1.1     |
| Fumaric acid                    | 1.5                      | 1.5     |
| Glyceric acid                   | 1.4                      | 1.1     |
| Phosphate                       | 1.3                      | 0.9     |
| Dehydroascorbate                | 1.3                      | 2.2     |
| Ascorbic acid                   | 1.3                      | 1.1     |
| Aconitic acid                   | 1.3                      | 1.7     |
| Glycycolamine                   | 1.3                      | 3.7     |
| Citric acid                     | 1.3                      | 1.5     |
| Methionine                      | 1.2                      | 1.4     |
| Isocitric acid                  | 1.2                      | 1.3     |
| Searic acid                     | 1.2                      | 1.6     |
| Pseudo uridine                  | 1.2                      | 1.4     |
| Indole-3-lactate                | 1.1                      | 1.5     |
| Palmitic acid                   | 1.1                      | 1.4     |
| Indole-3-acetate                | 1.1                      | 1.5     |
| N-acetylaspartic acid           | 0.9                      | 1.3     |
| Sucrose                         | 0.9                      | 1.6     |
| Aminomelonic acid               | 0.9                      | 1.6     |
| Hydroxycarbomate                | 0.9                      | 1.5     |
| Oxoproline                      | 0.8                      | 1.0     |
| 1-monostearin                   | 0.8                      | 0.9     |
| Pyrazine 2,5-dihydroxy          | 0.8                      | 1.1     |
| Cysteine                        | 0.6                      | 1.1     |
| Idonic acid NIST                | 0.5                      | 1.2     |
| Thronic acid                    | 0.4                      | 1.2     |

Note: Bold indicates statistical significance. One-way ANOVA performed separately for wild-type and Sca1154Q/+ mice (see supplemental Figure 2 for box-whisker plots). Abbreviation: Li, Lithium.

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increase of ascorbate in wild-type mice was accompanied by a reduction of threonate (Figure 3 and Figure 5), which is an oxidative catabolite of ascorbate (reaction pair KEGG RP01024). Interestingly, the threonic acid/ascorbate reaction pair and dehydroascorbate were differentially regulated in the plasma of Sca1154Q/+ mice but not wild-type mice, which was opposite to the finding in cerebellum. In a different metabolite module, phytol and cholesterol-3-ol were also affected by lithium treatment in wild-type mice but not in Sca1154Q/+ mice, albeit with a lower p-value (Table 3). Box-whisker graphs for selected metabolites (Figure 5) highlight common and specific responses to lithium treatment in the cerebellum (see Figure S3 for box-whisker plots of other compounds from Table 3).

To assess the therapeutic effects of lithium, we looked at the altered metabolites in Sca1154Q/+ mice that were corrected by lithium treatment (Table 5). In the plasma of Sca1154Q/+ mice, the levels of phosphate and 2-aminoacidic acid were down and the levels of idonic acid NIST (National Institute of Standards and
Threonate is a product of ascorbate oxidative cleavage (KEGG reaction pair RP01024). The changes in ascorbate and threonate—might all be related to the disease or to benefit from treatment.

Threonate is a product of ascorbate oxidative cleavage (KEGG reaction pair RP01024). The changes in ascorbate and threonate—might all be related to the disease or to benefit from treatment.
**Figure 4. Lithium treatment effect on blood plasma metabolome. Metabolic network of wild-type and Sca1^{154Q/+} plasma phenotypes.**

**A.** Wild-type mice. **B.** SCA1 knock-in mice. Red nodes: Increased metabolite levels under Lithium treatment; blue nodes: decreased levels. Node shades indicate ANOVA significance levels, node size reflect differences in magnitude of regulation. Red lines: reactant pair relationships obtained from the KEGG reaction pair database. Yellow solid lines: chemical similarity >0.5 Tanimoto score (Tanimoto scores range between 0 to 1, where 1 reflects identical structures). Yellow broken lines: chemically closest structure at <0.5 Tanimoto scores.

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preparation was proper and homogenous. Lithium treatment appeared to boost levels of both glycolytic and TCA intermediates, and may therefore alleviate the reported inefficiency of glycolysis in neurological diseases [42–44]. Lithium treatment increases the level of several molecules involved in glycolysis, which is a critical pathway for energy production in the cell. None of these differential compounds appear directly associated with essential unsaturated fatty acids—such as linoleic acid—in membranes [41], and linoleic acid was increased after lithium treatment. Other neurodegenerative diseases have been associated with inefficient glycolysis. We found that lithium treatment increases the level of several molecules involved in glycolysis, which is a critical pathway for energy production in the cell. Linoleic acid was increased after lithium treatment, thus associating purine to motor neuron disease [39]. Further, recent studies have shown that ascorbate and purine metabolites that were regulated metabolites of purine metabolism pathway. The whiskers encompass 1.5 of the interquartile range (IQR). Median value is indicated with a line in the box. Abbreviations: Ctl, Control; KI, SCA1 knock-in; Li, Lithium; WT, Wild-type.

**Table 5.** Level of metabolites in SCA1 mice relative to wild-type non-treated mice: the treatment effect of lithium on impaired metabolites in SCA1 knock-in mice.

| Compounds                  | Wild-type control | Lithium | ScAl154Q/+ control | ScAl154Q/+ lithium |
|----------------------------|-------------------|---------|-------------------|-------------------|
| **Cerebellum**             |                   |         |                   |                   |
| Glucose-6-phosphate        | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Cystein                    | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Hypoxanthine               | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| 2-monopalmitin             | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Xanthine                   | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Citric acid                | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Monopalmitin-1-glyceride   | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Inosine                    | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| **Blood Plasma**           |                   |         |                   |                   |
| Phosphate                  | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| 2-aminoacidic acid         | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Tryptophan                 | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Iodonic acid NIST          | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |
| Threonine acid             | 1.0±0.2            | 1.0±0.3 | 1.0±0.3           | 1.0±0.3           |

Note: All values are relative to wild-type untreated animals. Abbreviations: Avg, Average; NIST, National Institute of Standards and Technology; SD, Standard deviation.

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In conclusion, our findings corroborate previous reports that lithium has multiple effects in neurological diseases. Our data further reveal that lithium affects metabolic intermediates in the cerebellum to a larger degree than in blood plasma. It also has a controlling role in a variety of metabolic pathways, including purine metabolism, oxidative stress reduction and energy production.

**Supporting Information**

**Figure S1** Effect of introducing the ScAl154Q/+ gene into the wild-type genetic background for plasma and cerebellum. This figure includes individual box-whisker plots for other significantly regulated metabolites (i.e., compounds from Table 2 not shown in Figure 2). The whiskers encompass 1.5 of the interquartile range (IQR). Median value is indicated with a line in the box. Abbreviations: Ctl, Control; KI, SCA1 knock-in; Li, Lithium; WT, Wild-type.

**Figure S2** Box-and-whisker plots: genotype-dependent metabolites in plasma samples with significant differences between lithium treatment and controls. (p-value <0.05; see Table 4). The whiskers encompass 1.5 of the interquartile range (IQR). Median value is indicated with a line in the box. Abbreviations: KI, SCA1 knock-in; WT, Wild-type.

**Figure S3** Box-and-whisker plots: genotype-dependent metabolites in cerebellum samples with significant differences between lithium treatment and controls. (p-value <0.05; see Table 3). The whiskers encompass 1.5 of the interquartile range (IQR). Median value is indicated with a line in the box. Abbreviations: Ctl, Control; KI, SCA1 knock-in; Li, Lithium; WT, Wild-type.

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**Author Contributions**
Conceived and designed the experiments: PJ JRG HYZ RKD OF. Performed the experiments: PJN JRG JC-B OF. Analyzed the data: BP LW OF WWK RKD. Contributed reagents/materials/analysis tools: DB OF HYZ. Wrote the paper: BP OF PJN HYZ WWK RKD.

**References**

1. Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23: 217–247.
2. Zoghbi HY, Orr HT (1995) Spinocerebellar ataxia type 1. Semin Cell Biol 6: 29–35.
3. Watae K, Werber EJ, Xu B, Antalffy B, Yacu-Paylor L, et al. (2002) A long CAG repeat in the mouse Scn1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. Neuron 34: 905–919.
25. Rowe MK, Chuang DM (2004) Lithium neuroprotection: molecular mechanisms and clinical implications. Expert Rev Mol Med 6(21): 1–18.

26. Baselin M, Chang L, Rapoport SI (2006) Chronic lithium chloride administration to rats elevates glucose metabolism in wide areas of brain, while potentiation of negative effects on metabolism of dopamine D2-like receptor stimulation. Psychopharmacology (Berl) 187: 303–311.

27. Macko AR, Beneze AN, Teachey MK, Henriksen EJ (2008) Roles of insulin signaling and mTOR in the activation by lithium of glucose transport in insulin-resistant rat skeletal muscle. Arch Physiol Biochem 114: 331–339.

28. Shaltiel G, Deutsch J, Rapoport SI, Baselin M, Behnaker RH, et al. (2009) Is phosphohepoxigenase phosphate phosphatase a target of lithium's therapeutic effect? J Neural Transm 116: 1543–1549.

29. Knafo I, Chessex P, Rouleau T, Lavoie JC (2005) Association between hydrogen peroxide-dependent byproducts of ascorbic acid and increased hepatic acyl-CoA carboxylase activity. Clin Chem 51: 1462–1471.

30. Liu HL, Dixit SS, Xu S, Tin GS, Stock AM, et al. (2006) NPC2, the protein deficient in Niemann-Pick C2 disease, consists of multiple glycoforms that bind a variety of sterols. J Biol Chem 281: 36740–36753.

31. Saleen G, Polito A (1972) Biosynthesis of 3′,5′-cyclic AMP in cerebrocortical xanthomatosus. J Clin Invest 51: 134–140.

32. Skrede B, Bjorkman I (1985) A novel route for the biosynthesis of cholestanol, and its significance for the pathogenesis of cerebrotendinous xanthomatosis. Scand J Clin Lab Invest Suppl 177: 15–21.

33. Kastama T, Byun DS, Seyama Y (1987) Quantitative analysis of sterols in serum by high-performance liquid chromatography. Application to the biochemical diagnosis of cerebrotendinous xanthomatosis. J Chromatogr 400: 241–246.

34. Berridge MJ, Downes CP, Hanley MR (1989) Neural and developmental actions of lithium: a unifying hypothesis. Cell 59: 411–419.

35. Cordeiro ML, Gunderson CB, Umbach JA (2003) Dietary lithium induces individual suprachiasmatic nucleus neurons. Neuropeptide 11: 3261–3264.

36. Youdim KA, Martin A, Joseph JA (2000) Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci 18: 383–399.

37. Berridge MJ, Downes CP, Hanley MR (1989) Neural and developmental actions of lithium: a unifying hypothesis. Cell 59: 411–419.

38. Chiu CT, Chuang DM (2010) Molecular actions and therapeutic potential of lithium in preclinical and clinical studies of CNS disorders. Pharmacol Ther 128: 269–301.

39. Fiehn O, Wohlgemuth G, Scholz M (2008) Quality metadata. Lect Notes Comput Sc 3615: 224–239.

40. Killcoyne S, Carter GW, Smith J, Boyle J (2009) Cytoscape: a community-based framework for network modeling. Methods Mol Biol 563: 219–239.

41. Youdim KA, Martin A, Joseph JA (2000) Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci 18: 383–399.

42. Hoyer S, Oesterreich K, Wagner O (1988) Glucose metabolism as the site of the production of interleukin-1β in monocytes. Biochem J 257: 29–37.

43. Quinones MP, Kaddurah-Daouk R (2009) Metabolomics tools for identifying the primary abnormality in early-onset dementia of Alzheimer type? J Neurol Neurosurg Psychiatry 46: 255–260.

44. Kirwood JS, Lebold KM, Mirands CL, Wright CL, Miller GW, et al. (2012) Vitamin C deficiency activates the purine nucleotide cycle in zebrafish. J Biol Chem 287: 3833–3841.

45. Crespo-Barreto J, Fryer JD, Shaw CA, Orr HT, Zoghbi HY (2010) Partial loss of ataxin-1 function contributes to transcriptional dysregulation in spinocerebellar ataxia type 1 pathogenesis. PLoS Genet 6: e1001021.

46. Lakshmanan J, Seelan RS, Thangavel M, Vadnal RE, Jaukkola AJ, et al. (2012) Proteomic analysis of rat prefrontal cortex after chronic lithium treatment. J Proteomics Boinform 5: 140–146.