A Plasma Metabolomic Signature Involving Purine Metabolism in Human Optic Atrophy 1 (OPA1)-Related Disorders

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PURPOSE. Dominant optic atrophy (DOA; MIM [Mendelian Inheritance in Man] 165500), resulting in retinal ganglion cell degeneration, is mainly caused by mutations in the optic atrophy 1 (OPA1) gene, which encodes a dynamin guanosine triphosphate (GTPase) involved in mitochondrial membrane processing. This work aimed at determining whether plasma from OPA1 pathogenic variant carriers displays a specific metabolic signature.

METHODS. We applied a nontargeted clinical metabolomics pipeline based on ultra-high-pressure liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) allowing the exploration of 500 polar metabolites in plasma. We compared the plasma metabolic profiles of 25 patients with various OPA1 pathogenic variants and phenotypes to those of 20 healthy controls. Statistical analyses were performed using univariate and multivariate (principal component analysis [PCA], orthogonal partial least-squares discriminant analysis [OPLS-DA]) methods and a machine learning approach, the Biosigner algorithm.

RESULTS. A robust and relevant predictive model characterizing OPA1 individuals was obtained, based on a complex panel of metabolites with altered concentrations. An impairment of the purine metabolism, including significant differences in xanthine, hypoxanthine, and inosine concentrations, was at the foreground of this signature. In addition, the signature was characterized by differences in urocanate, choline, phosphocholine, glycercate, 1-oleoyl-rac-glycerol, rac-glycerol-1-myristate, aspartate, glutamate, and cysteine concentrations.

CONCLUSIONS. This first metabolic signature reported in the plasma of patient carrying OPA1 pathogenic variants highlights the unexpected involvement of purine metabolism in the pathophysiology of DOA.

Keywords: dominant optic atrophy, metabolomics, mitochondria, OPA1, optic neuropathy, purine metabolism

Since the first report of optic atrophy 1 (OPA1) mutations in 2000, 1,2 358 unique variants have been recorded in the locus-specific database dedicated to OPA1; 3 among them, more than two-thirds are considered as pathogenic variants. 4 The clinical spectrum of OPA1-related disorders is highly variable, 5,6 including isolated dominant optic atrophy (DOA, Kjer type; Mendelian Inheritance in man [MIM] 165500), DOA with neurosensory deafness (DOAD), 7 a syndromic form of DOA named DOA+ (MIM 125250), 8,9 recessive forms of syndromic optic atrophy including Behr-like syndrome (MIM 210000), 10,11 and a syndrome associating optic atrophy to cardiomyopathy and encephalopathy. 12 In DOA+, extra ophthalmologic signs typically appear during young adulthood after the onset of the optic neuropathy, and include sensorineural deafness, ataxia, myopathy, chronic progressive external ophthalmoplegia, and peripheral neuropathy. 13 Behr-like syndrome is characterized by severe early-onset optic neuropathy, occurring during the first years of life, associated with spinocerebellar degeneration, pyramidal signs, peripheral neuropathy, gastrointestinal dysmotility, and developmental delay. 11 Isolated DOA is mainly caused by heterozygous OPA1 variants leading to the lack of expression of one allele, a situation referred as haploinsufficiency, whereas DOAD and DOA+ are mainly due to heterozygous OPA1 missense variants resulting in a dominant negative effect. 5,15

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Behr-like syndrome is mostly caused by biallelic mutations associating one pathogenic variant to an hypomorphic allele.11 Finally, OPA1 pathogenic variant carriers have also been rarely reported in individuals affected with spastic paraplegia, multiple sclerosis-like syndrome, parkinsonism with dementia, and encephalopathy with cardiomyopathy.5,13 Despite this diversity of clinical phenotypes, the vast majority of individuals are commonly affected by an optic neuropathy, characterized by a progressive childhood onset and bilateral visual impairment.13 Ophthalmologic explorations disclose a temporal disc pallor at fundus examination, visual field defect such as centrocecal scotoma, and color vision defect. The visual impairment is irreversible, but highly variable within and between families, ranging from a subclinical expression to legal blindness.14 Optic atrophy is due to the specific degeneration of retinal ganglion cells (RGC) and their axons forming the optic nerve, which transmits the visual action potentials from the retina to the lateral geniculate nucleus, and further to the visual cortex. RGCs are highly sensitive to mitochondrial energetic defects, particularly in the proximal, prelaminar unmynelinated portion of the axons, which requires a higher amount of energy.15 Adenosine triphosphate (ATP) production proved to be highly reliant on mitochondrial dynamics and cristae organization and maintenance, which are controlled among others by the dynamin guanosine triphosphate (GTP)ase, OPA1. Pathogenic OPA1 variants lead to a fragmentation of the mitochondrial network as well as to cristae disorganization. However, many other pathomechanisms have been associated with OPA1 defects such as an increased susceptibility to apoptosis, altered calcium fluxes, energetic impairment with lower ATP production, increased oxidative stress, mitophagic dysfunction, and mitochondrial DNA instability.5 Nevertheless, the precise mechanisms by which OPA1 alterations lead specifically to RGC death remain unknown.

Clinical metabolomics is a “without a priori” multistep approach aimed at detecting various classes of metabolites in biological samples from cohorts of affected and control individuals, followed by statistical analyses and mathematical modeling, to infer metabolomic signatures. These signatures are useful not only in the identification of new diagnostic and prognostic biomarkers, but also in the investigation of pathophysiological mechanisms of diseases, thereby opening new therapeutic avenues. Although considered as a potentially powerful tool to explore ophthalmologic diseases, it has been so far scarcely used in retinal and optic nerve inherited diseases. Using a targeted metabolomics approach, we have recently reported a presymptomatic signature showing axonopathy and myelopathy in the optic nerves of an Opa1+/− mouse model.16 However, this targeted approach was restricted to a limited number of metabolites including hexoses, amino acids, biogenic amines, acyl-carnitines, and several phospholipid families. Here, we used a recently validated nontargeted metabolomics workflow17 with broader metabolite coverage, allowing the analysis of 500 polar metabolites in the plasma of individuals affected with an OPA1-related disorder, in comparison to a control cohort.

**METHODS**

**Chemicals and Reagents**

Methanol (MeOH), water, and formic acid (Optima LC/MS grade) were purchased from Fisher Scientific (Illkirch, France). Isotone metabolite standards including 17α-hydroxyprogesterone-d₈ (2,2,4,6,6,21,21-d₈), L-thyroxine-C₃H₂, succinic acid-2,2,3,3-d₄, pyruvic acid-1,3-C, and DL-alanine-15N with >98% purity were acquired from Sigma Aldrich (St. Quentin Fallavier, France).

**Human Plasma Sampling**

This study was approved by the Ethical Committee of the University Hospital of Angers (Comité de Protection des Personnes CPP Ouest II, Angers, France; identification number: CPP CB 2014/02; declaration number: DC-2011-1467; authorization number: AC-2012-1507), and all patients gave their written informed consent before participating in the study, which followed the tenets of the Declaration of Helsinki. Twenty-five plasma samples from individuals bearing OPA1 pathogenic variants and various phenotypes were compared to 20 plasma samples obtained from healthy subjects. The OPA1 population was recruited in the Department of Ophthalmology of the University Hospital of Angers after molecular diagnosis and ophthalmologic examination. The asymptomatic carriers of OPA1 mutations had normal ophthalmologic examination, including normal visual acuity, visual fields, and retinal nerve fiber thickness measured with optical coherence tomography. Apart from the OPA1-associated phenotypes, no other disease or comorbidity was found to affect more than two individuals in both groups. Blood samples from fasting patients and controls were collected in heparin tubes and immediately transported on ice to the laboratory where plasmas were recovered after centrifugation at +4°C prior to storage at −80°C in aliquots until metabolomics analysis.

Genotype and phenotype features of patients are summarized in Tables 1 and 2. There was no statistical significance between patients and control subjects in terms of sex and age (α² and Wilcoxon tests). All OPA1 pathogenic variants are described according to the OPA1 transcript variant 8 (RefSeq: NM_130837.2), representing the longest transcript including exon 4b and exon 5b, encoding an isoform of 1015 amino acids (aa). For standardization, the exons are now numbered 1 through 30.3 Variants originally reported according to variant 1 (Refseq: NM_015560.2) are also shown.

| TABLE 1. Features of the OPA1 Patient and Control Cohorts |
|-----------------|-----------------|-----------------|-----------------|
|                 | OPA1            | Control         |
|                 | Patients, n = 25| Subjects, n = 20|                 |
| Sex, M/F        | 15/10           | 11/9            | 1               |
| Age mean (range) | 56 (11–64)      | 25 (9–60)       | 0.08            |
| Clinical phenotype | 4 asymptomatic carriers | 3 moderate isolated DOA | 14 severe isolated DOA | 5 severe DOAD | 1 DOA- Behr-like |
| Pathogenic variants | 10 missense | 15 haploinsufficiency |

**Metabolomics Analyses**

We used a validated nontargeted reverse-phase (RP) metabolomics method that we had recently published.1 Briefly, cold MeOH was added to 30 μL plasma fortified with the isotope metabolite standards mixture (10 μg/mL in MeOH). After centrifugation, supernatants were evaporated to dryness. Then, samples were reconstituted with an aqueous solution (2% MeOH) prior to ultra-high-pressure liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) analysis. Sample injections and acquisitions were performed on Dionex Ultimate 3000 UHPLC system (Dionex, Sunnyvale, CA, US).
## Table 2. Genotype of the OPA1 Patients

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<th>Patient</th>
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<th>Age</th>
<th>Pathogenic Variant</th>
<th>Transcript Variant 8 (Location DNA Change, cDNA, Protein Consequence)</th>
<th>Transcript Variant 1 (Location DNA Change, cDNA, Protein Consequence)</th>
<th>Published as</th>
<th>Reference, to First Publication</th>
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<th>Probable Mutation Effect</th>
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<td>HI</td>
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<td>4</td>
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<td>Asymptomatic</td>
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<td></td>
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<td>32</td>
<td>2470C&gt;T</td>
<td>Exon 26 c.2635C&gt;T p.(Arg879?)</td>
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<td>Unpublished</td>
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</table>

HI, haploinsufficiency; MS, missense.
USA) coupled to a Thermo Scientific Q Exactive HRMS (Thermo Fisher Scientific, Bremen, Germany) equipped with a heated electrospray (HESI II). Metabolite identification was facilitated by our in-house database of 500 accurately identified molecules (carboxylic acids, aa, nucleotides, saccharides, fatty acids, lipids, and hormones) built using the Mass Spectrometry Metabolite Library of Standards (IROA Technology, Bolton, MA, USA) for mass spectrometry metabolomics. The same chromatographic and mass spectrometry conditions were used in the current investigation, thus allowing comparison of retention time (RT), MS/MS fragmentation spectra, accurate m/z, and isotopic pattern of metabolites.

A TraceFinder 3.3 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) processing method based on our in-house library was then used, and detected ions were filtered and integrated relative to the following criteria: quality control coefficient of variation (CV) area below 30%, an accurate m/z measurement under 10 ppm, an isotopic pattern matching the chemical formula, RT drift lower than 10 seconds, a linearity of dilution with a $r^2$ close to 1 and, when possible, MS/MS fragmentation matching. For unequivocal identification, at least three out of the four following conditions were required: an accurate m/z measurement (under 5 ppm), a perfect isotopic pattern, RT drift lower than 5 seconds, and two identical fragments. When either the identification was not firmly established with these criteria (e.g., isomers, no fragmentation ions, more than one possibility of chemical structure) or similarities to the data stored on our library were observed (i.e., an accurate m/z measurement, perfect isotopic pattern, fragment matching but not the correct RT), molecules were named by their chemical formula and RT.

**Data Statistical Analyses**

Before performing statistical analysis, data were normalized to avoid instrumental drift due to the large number of processed samples using the LOESS regression model present in galaxy.workflow-imetabolomics.org (in the public domain). To prevent any other uncontrolled variation, an additional normalization step was applied, dividing each ion by the total ion current (TIC) of the sample, using Microsoft Excel software (Microsoft Corp., Redmond, WA, USA). Moreover, the dataset was log$_{10}$ transformed and when appropriate, data were mean-centered and scaled by the square root of the standard deviation of each variable (Pareto scaling) to reduce the contribution of the most intense ions. Statistical analyses were performed following the workflow shown in Figure 1.

Univariate analyses were performed on MetaboAnalyst 3.0 using its Volcano plot module to highlight only metabolites that had an important fold change (>1.5) combined with a Wilcoxon test at a 0.05 threshold. Then, to minimize the error rate, Benjamini-Hochberg correction was applied and only molecules that remained significant were kept.

Multivariate analyses were performed using unsupervised principal component analysis (PCA) to investigate the population structure, and supervised orthogonal partial least-
squares discriminant analysis (OPLS-DA) was carried out purposefully to search for relevant metabolomic signatures. These statistical models were executed on Simca-P+ v 14.0 (Umetrics, Umea, Sweden) allowing the achievement of an optimization by variable exclusion, keeping only metabolites that showed a robust discrimination and high statistical reliability. The aim of this approach was to limit the risk of overfitting and to reduce the prediction variability, thereby simplifying the molecular signature interpretation. Selection of metabolites of interest was made through the combination of several kinds of information from different plots: S-plot (visualization of intensity and reliability), loading column plot with jackknife confidence intervals, coefficient plot, and variable importance in the projection (VIP) plot. Only metabolites with a VIP value larger than 1 were considered “highly important” in the metabolomic footprinting. OPLS-DA simulations were cross-validated by leaving out a one-seventh fraction of the samples and replicated seven times. The qualities and performances of OPLS-DA models were assessed using the R²Ycum (goodness of prediction), the R²Ycum qualities and performances of OPLS-DA models were assessed by simulations were cross-validated by leaving out a one-seventh combination of resampling (bootstraps), feature importance dataset, the Biosigner algorithm was constructed on a support vector machines (SVM). As the performances of these machine learning algorithm implemented as a module in Galaxy/Workflow4metabolomics, was used with the following criteria: 50 bootstraps, feature selections by S and A tiers (S tier = final signature, metabolites passed all the selection iterations; A tier = metabolites discarded during the last iteration), and a P value threshold of 0.05. This algorithm is based on three approaches: PLS-DA, random forest (RF), and support vector machines (SVM). As the performances of these statistical methodologies may be limited by the structure of the dataset, the Biosigner algorithm was constructed on a combination of resampling (bootstraps), feature importance ranking, significance assessment by permutation, and half-interval search. The resulting approach is a fast, automatic, highly restrictive and stable generator of metabolomic signatures.

RESULTS

Detected Plasma Metabolites

In the current investigation, the application of our metabolomics workflow led to the detection and relative quantification of 135 metabolites, in both positive and negative ionization modes. The MS/MS fragmentation matching was possible for more than 75% of the molecular features. For the remaining 25%, the molecule recognition was based on the isotopic pattern compatibility with the chemical formulas and on the RT comparable to the ones recorded in our library. The most important classes of detected molecules were aa and secondary amines (over 20%), followed by other carboxylic acids (more than 15%). Sugars, purines, and small fatty acids were also well represented, among which some were organo-oxygen compounds (over 5%). Other classes such as keto acids (less than 4%), pyridines (around 2%), benzenes (around 2%), and hormones (less than 2%) were less detected.

Age Effect of the Population Structure

To explore the data structure of the studied population, PCA were carried out. PCA is a nonsupervised approach allowing spontaneous clustering or separation of individuals on the basis of their global metabolite profiles. Each observation is situated on the plot according to the two first components (the axes t[1] and t[2]) that explain the largest variations of the dataset. PCA models taking into account only OPA1 patients (Fig. 2A), or both the patients and controls (Fig. 2B), revealed an important discrimination between them delimited by the red ovals. This separation was due to neither sex nor the type or severity of the OPA1 pathogenic variant, but was rather due to the individual’s age. This age effect had a predominant influence compared to that of the pathogenic OPA1 variants, since patients and controls were mixed in this first PCA analysis (Fig. 2B).

A PLS model (Fig. 2C) was performed to study the metabolomic profile leading to the age effect and to discriminate this profile from the one caused by the pathogenic OPA1 variants. PLS is a supervised method that allows the separation between groups of observations. A good model of predictability of this age effect was found with a Q²(cum) of 73.6% (a cutoff of 50% is widely accepted for discriminating predictive models) and a P value obtained by the CV-ANOVA analysis that was highly significant (P value = 5.08092e⁻⁸). The metabolites contributing to this model were visualized in the VIP plot (Fig. 2D). The features that mostly influenced the age effect (VIP > 1) were, by decreasing order of importance: aniline, quinate, 2-methylmaleate, C₅H₅NO₁.92, rhamnose, and pipecolate, all of them showing a trend toward an increased concentration with age. Other metabolites, such as caffeine and creatinine, although with a less important VIP score, were also associated to the age-related signature, as expected. Among these metabolites, variations of concentration of aniline, quinate, and caffeine had already been associated with aging in a French cohort.²⁴ It is clear that in metabolomics approaches, this kind of confounding effect has to be considered to avoid any bias that could lead to inappropriate conclusions.²⁵

Accordingly, we removed the six (VIP > 1) molecules mainly responsible for the age discrimination and repeated the unsupervised PCA exploration that revealed this time a spontaneous separation of individuals according to the OPA1 genotype (Fig. 2E).

Plasma Signature of OPA1 Pathogenic Variant Carriers

Plasma Signature of OPA1 Pathogenic Variant Carriers

To simplify the interpretation, we chose to associate each statistically relevant feature with an increase (↑) or decrease (↓) symbol correlated to the concentration detected in the OPA1 population compared to controls. Metabolites with a robust discrimination and high statistical reliability were used to optimize an OPLS-DA model (Fig. 3A) that led to an important predictive level (Q²(cum) = 80.8%), with excellent performances during the permutation and CV-ANOVA tests (P value = 9.20575e⁻¹⁶). OPLS-DA is a supervised method, which allows the identification of class discriminating variables.

The VIP plot related to this model was characterized by nine important metabolites (VIP > 1) (Fig. 3B, in red) associated with purine (hypoxanthine ↓, inosine ↑, and xanthine ↓), lipid (1-oleyl-rac-glycerol ↓ and rac-glycerol-1-myristate ↓) and aa (aspartate ↓ and glutamic acid ↓) metabolic pathways, as well as with two unidentified metabolites (C₄H₄N₂O₃.74 ↓ and C₄H₅N₂O₁.61 ↓). Seven other metabolites (Fig. 3B, in green) participating in the OPA1 signature with a 0.5 > VIP > 1 included phosphocholine (↑), choline (↑), glycylate (↓), cystine (↓), and uracil (↑) and two unidentified metabolites (C₄H₁₁O₃N₀.81 ↓ and C₄H₅O₂⁻₁.34 ↓).

The Biosigner machine learning algorithm is considered more stringent than the OPLS-DA analysis, mainly due to its ability to combine a large number of data permutations with three different statistical analyses. It generated a robust model
These results confirmed the implication of hypoxanthine (↑) and xanthine (↑) on purine metabolism and the involvement of 1-oleyl-rac-glycerol (↑). The SVM test suggested other metabolites (aniline [↑], quinate [↑], and 2-methylmaleate [↑]) that we had already described as implicated in the age fingerprinting and were consequently excluded from the disease signature.

After applying the Benjamini-Hochberg correction, 14 molecules were found significant using univariate analysis performed on the 133 compounds, with fold changes higher than 1.5 and P values lower than 0.05 (Table 3). These metabolites (xanthine ↑, hypoxanthine ↑, 1-oleyl-rac-glycerol ↑, aspartate ↑, \( \text{C}_9\text{H}_8\text{O}_2\)↑, inosine ↑, \( \text{C}_7\text{H}_{12}\text{N}_2\text{O}\)↑, \( \text{C}_7\text{H}_{15}\text{N}_3\)↑, glycerate ↑, phosphocholine ↑, and urocanate ↑) were common with the results of at least one of the previous multivariate analyses.

Due to the nature of the disease, which is rare and heterogeneous, it was not possible to obtain a robust statistical model able to discriminate subpopulations among our OPA1 cohort, either for the pathogenic variant types (OPA1 haploinsufficiency versus missense mutations) or for the clinical features (isolated DOA versus DOA+ and moderate versus severe optic neuropathy).

Thus, three statistical complementary methods were used in this study: univariate analysis with Benjamini-Hochberg correction and a fold change > 1.5, OPLS-DA performed with the Simca software, and the Biosigner machine learning algorithm using the combination of three different tests; the intersections between their respective results are summarized...
on a Venn diagram (Fig. 4). Sixteen molecules determining the OPA1 signature were finally retained. Three of them (hypoxanthine, xanthine, and 1-oleyl-rac-glycerol) were the most robust since they were found by all methods. Eleven compounds (aspartate, C6H12O7_1.34, inosine, C6H6N2O_1.61, rac-glycerol-1-myristate, glutamic acid, C5H4N4O_3.74, C7H19N3_0.81, glycerate, phosphocholine, and urocanate) were highlighted by OPLS-DA and univariate analyses, but not by the Biosigner algorithm that is the most selective method. Finally, two additional metabolites (cystine and choline) were specifically identified by the OPLS-DA signature but were excluded by the univariate analysis because of their fold change, which was lower than 1.5 but still higher than 1.2.

**DISCUSSION**

In this study, we found that 16 compounds provide a relevant metabolic signature in plasma from individuals affected with OPA1-related disorders. Several of these molecules are metabolically related, pointing out three main affected pathways: (1) the purine pathways (hypoxanthine, xanthine, and inosine), (2) the lipid metabolism pathways (1-oleyl-rac-glycerol, rac-glycerol-1-myristate and glycerate, phosphocholine, and choline), and (3) the aa pathways (cystine, aspartate, glutamic acid, and urocanate, a catabolite of histidine). Other detected metabolites are just informative at this stage, because they have not been univocally identified (C6H12O7_1.34, C6H6N2O_1.61, C3H2N4O_3.74, C7H19N3_0.81).
Alteration of plasma aa profiles has been regularly reported in mitochondrial diseases.\textsuperscript{26,27} For instance, the decreased plasma concentration of aspartate may be caused by the OPA1-related energetic defect. As a matter of fact, mitochondrial aspartate biosynthesis is sharply altered by oxidative phosphorylation (OXPHOS) dysfunctions, since mitochondrial respiration is essential to provide electron acceptors for aspartate synthesis.\textsuperscript{28} Our team has previously shown that OXPHOS coupling is impaired in DOA, and it is therefore not surprising to observe a diminution of aspartate levels in the plasma of affected individuals.\textsuperscript{29}

The presence of five metabolites implicated in lipid metabolism can be connected to the involvement of OPA1 in the mitochondrial inner membrane and in lipid processing.\textsuperscript{31} They could be helpful to precisely decipher the mechanisms involved in the mitochondrial membrane fusion defect observed in cells from individuals bearing OPA1 pathogenic variants.\textsuperscript{31}

The involvement of purine metabolism in OPA1-related disorders is particularly relevant because the significant decrease of xanthine and hypoxanthine concentrations was detected by all statistical methods (Fig. 4) and reinforced by the increase of inosine level identified by two methods. As shown in Figure 5, xanthine, hypoxanthine, and inosine are at the center of the interplay between ATP and GTP metabolisms. Interestingly, a defect of ATP production has been evidenced in OPA1-related disorders, caused by both mitochondrial complex I or IV deficiencies and mitochondrial OXPHOS uncoupling.\textsuperscript{29,32} Furthermore, OPA1 is a dynamin GTPase using GTP hydrolysis to mechanically act on the mitochondrial inner membrane fusion. Such variations of ATP production and GTP consumption may lead to an alteration of purine metabolism in some cells and organs, which may become detectable in plasma through these three biomarkers. Unfortunately, our in-house library does not include all the metabolites involved in the purine pathways (see Fig. 5). We also noted the absence of other metabolites listed in the library (e.g., adenosine) due to both the intrinsic properties of the metabolite itself (the lability or the ability to diffuse in the plasma compartment, e.g.) and the limits of the technique. Moreover, the decreased purine metabolism could also be related to an alteration of nuclear factor-erythroid 2 (NRF2) observed in OPA1-haploinsufficiency models.\textsuperscript{33} NRF2 is a transcription factor whose activity, among others, influences the cellular redox status and regulates de novo purine biosynthesis.\textsuperscript{34,35}

The alteration of purine concentrations could be particularly relevant to explain the pathophysiology of OPA1-related disorders. These molecules are important to the maintenance of the nucleotide pools that play an essential role in the stability of mitochondrial DNA. An imbalance of the nucleotide pools may explain the presence of multiple mitochondrial DNA deletions in the skeletal muscles of most individuals carrying OPA1 pathogenic variants.\textsuperscript{13} Such mitochondrial DNA instability, due to an impairment of the mitochondrial nucleotide
pools, is well established in patients carrying mutations in the thymidine kinase, thymidine phosphorylase, and deoxyguanosine kinase genes.\textsuperscript{36,37}

Despite the heterogeneity of this disease and the small size of the cohort, a robust metabolomics signature has been obtained. One limitation of our metabolomics analysis on plasma, while interesting in the sense that it gives a general view of the individual's conditions, is in fact not suitable to identify the organs that are responsible of the compounds' secretion contributing to the signature. Nevertheless, the importance of purine metabolism has recently been highlighted by a metabolomics study exploring the impact of light on retina in mouse models.\textsuperscript{38} In that study, light exposure was found to greatly inhibit the de novo purine synthesis, resulting in a depletion of most purine metabolites including xanthine, hypoxanthine, and inosine. Interestingly, the proposed mechanism of this de novo purine synthesis inhibition involves the decrease of aspartate concentrations, a metabolite that we found significantly reduced in the plasma of individuals with OPA1-related disorders. Thus, our results point to a possible mechanism of an increased susceptibility of the retina to light damage, which would be interesting to further explore in models of OPA1-related optic neuropathy.

In conclusion, we report for the first time a metabolomic signature performed in individuals with OPA1-related disorder that highlights an unexpected involvement of purine metabolism. This signature is consistent with the disease, in connection with ATP and GTP metabolism and lipid processing. Further studies should emphasize whether and how the metabolites identified in this study contribute specifically to the RGC progressive degeneration.

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