

## BRIEF COMMUNICATION OPEN



# IMPDH2: a new gene associated with dominant juvenile-onset dystonia-tremor disorder

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The aetiology of dystonia disorders is complex, and next-generation sequencing has become a useful tool in elucidating the variable genetic background of these diseases. Here we report a deleterious heterozygous truncating variant in the inosine monophosphate dehydrogenase gene (*IMPDH2*) by whole-exome sequencing, co-segregating with a dominantly inherited dystonia-tremor disease in a large Finnish family. We show that the defect results in degradation of the gene product, causing *IMPDH2* deficiency in patient cells. *IMPDH2* is the first and rate-limiting enzyme in the de novo biosynthesis of guanine nucleotides, a dopamine synthetic pathway previously linked to childhood or adolescence-onset dystonia disorders. We report *IMPDH2* as a new gene to the dystonia disease entity. The evidence underlines the important link between guanine metabolism, dopamine biosynthesis and dystonia.

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## INTRODUCTION

Dystonias are rare movement disorders characterised by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements and/or postures. Dystonia can manifest as an isolated symptom or combined with e.g. parkinsonism or myoclonus [1]. While many pathogenic pathways are associated with dystonia, dopamine signalling is a commonly altered one [reviewed in [2]]. The first dopamine-related gene identified for dystonia was *GCH1*, encoding the rate-limiting enzyme in the pathway that converts guanosine triphosphate (GTP) to tetrahydrobiopterin (BH4) [3], which is an essential cofactor for dopamine biosynthesis (Fig. 2A). Heterozygous *GCH1* variants decrease dopamine synthesis in nigrostriatal neurons, leading to childhood-onset, progressive, dopa-responsive dystonia [4]. Variants in *HPRT1*, another purine metabolic enzyme, result in generalised dystonia with neuro-behavioural manifestations [5]. *HPRT1* pathophysiology involves a guanine metabolic defect and dopamine deficiency in the midbrain, without neurodegeneration [6], underscoring the importance of dopamine metabolism for posture and movement control.

Next-generation sequencing has uncovered numerous novel dystonia genes, furthering mechanistic knowledge. However, a large portion of dystonia patients still lack a genetic diagnosis. Here, we report inosine monophosphate dehydrogenase 2 (*IMPDH2*) as a novel gene for autosomal dominantly inherited dystonia.

## RESULTS

### Clinical description of the patients

A family of Finnish descent presented an autosomal dominantly inherited disease (Fig. 1A) characterised by dystonia and tremor.

The disease-onset was between 9 and 20 years of age. Table 1 summarises the clinical presentations.

### Case report

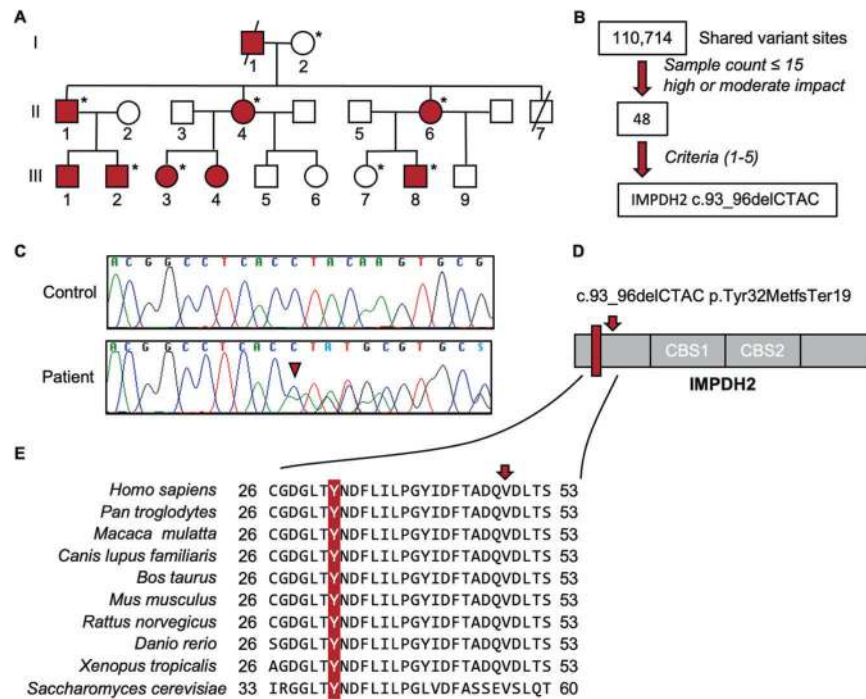
Patient II-6 is a 46-year-old woman. As a teenager she experienced episodic hand and head tremor attacks with flushing, considered as panic attacks. Constant handwriting problems and muscle cramps progressively worsened from age 20. Orthopaedic and rheumatological examination (wide-spread joint-associated pain without rheumatological disease signs) led to fibromyalgia diagnosis. The patient adapted her employment because of fatigue and muscle symptoms.

At 30 years of age, she was examined due to dizziness and visual problems (struggle to focus and headache). Brain CT-scan and ophthalmological examinations were normal. The symptomatology worsened progressively from age 35, with sleeping problems, heart palpitations, limb numbness, overactive bladder, and progressive tensiogenic headache. By the age of 35, the patient had undergone three lumbar discectomies, and at age 36, she underwent a cervical discectomy. Depression was diagnosed.

At the age of 42, she was examined due to swallowing problems, and a neurological evaluation indicated cervical dystonia. Segmental dystonia in the back region as well as focal upper limb dystonia were suspected. She had essential hand tremor and tremor in the head and upper back. Walking, speaking, balance, and eye movements were normal. Sensory testing showed distal lower leg allodynia and signs of hyperalgesia. Cold hypaesthesia was evident in both legs (distal from the right ankle and left knee) and cold hyperaesthesia on the right shin. Brain MRI and DAT scans were normal, and spinal MRI showed cervical and

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**Fig. 1 Heterozygous truncating variant in *IMPDH2* segregates in the family.** **A** Pedigree of the family. Red symbols: patients. Asterisk: individuals genetically studied for *IMPDH2* variant. **B** Schematic presentation of exome data analysis and variant filtering. Variants found in 15 or less patients in our in-house database (532 patients) with an impact rating of high or moderate effect were selected for further analysis. Criteria 1–5 described in the Methods. **C** *IMPDH2* sequence at the deletion site (arrowhead). **D** Graphical representation of the deletion consequences for the protein. Red rectangle: deletion site; Arrow: early termination codon; CBS, cystathione-beta-synthase domains. **E** *IMPDH2* protein sequence conservations at tyrosine-32, multiple sequence alignment. Arrow, location of the early stop codon.

**Table 1.** Clinical presentations.

| Patient                   | II-1             | II-4             | II-6             | III-2            | III-3            | III-8      |
|---------------------------|------------------|------------------|------------------|------------------|------------------|------------|
| Sex                       | Male             | Female           | Female           | Male             | Female           | Male       |
| Age of onset              | 20               | 20               | 18               | 12               | 12               | 9          |
| Cervical dystonia         | +                | +                | +                | +(mild)          | –                | +(mild)    |
| Focal upper limb dystonia | +                | +                | +                | +                | +                | –          |
| Tremor (head)             | Horizontal       | Horizontal       | Vertical         | Horizontal       | Horizontal       | Horizontal |
| Tremor (hands)            | Postural, action | Postural, action | postural, action | Postural, action | Postural, action | Postural   |
| Scoliosis                 | Moderate         | Mild             | –                | Mild             | –                | –          |

lumbar degeneration without any nerve contact or medullopthy. Bone scintigraphy was normal. Electromyoneurography was normal, but histological analysis of a skin sample from the lower leg diagnosed clear small fibre neuropathy with no observed subcutaneous nerve fibres.

#### Whole-exome sequencing identifies a heterozygous *IMPDH2* variant

We sequenced the exomes of three patients (II-1, II-6 and III-3). The identified shared variants were assessed based on rarity, predicted severity, conservation and gene function (Fig. 1B), yielding a single candidate gene, *IMPDH2*. A heterozygous four-base deletion led to an early termination in the first exon of *IMPDH2* (NM\_000884.2: c.93\_96del p.(Tyr32MetfsTer19), NC\_000003.12:g. 49066688\_49066691del) (Fig. 1D). No homozygotes for the variant allele and two heterozygous carriers of Finnish descent, potential undiagnosed or presymptomatic subjects, were found in the gnomAD database (<http://gnomad.broadinstitute.org/>) making the variant extremely rare. Multiple sequence alignment showed a high conservation of *IMPDH2*

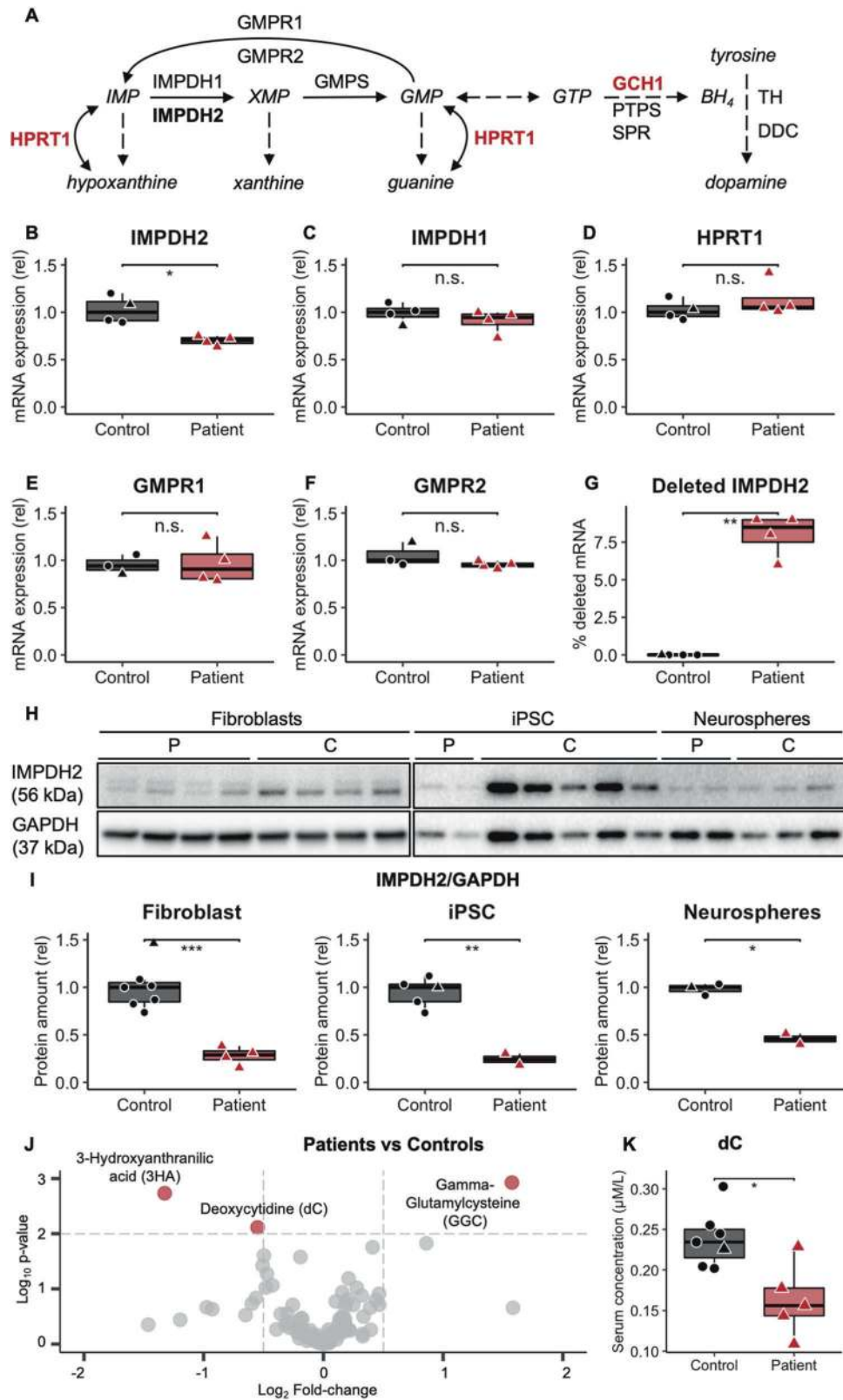
from human to yeast (Fig. 1E). Sanger sequencing of samples from six affected family members and two nonaffected family members confirmed the complete co-segregation of the dominant variant in the dystonic subjects (Fig. 1A, C).

#### Mutant *IMPDH2* transcript is degraded leading to an *IMPDH2* deficiency

*IMPDH2* is the first and rate-limiting enzyme in de novo GMP biosynthesis, oxidising inosine monophosphate into xanthosine monophosphate [7] (Fig. 2A).

Cultured fibroblasts showed a reduced *IMPDH2* transcript amount in patients, with ~70% of residual mRNA (Fig. 2B), from which ~10% is mutant mRNA (Fig. 2G). The finding indicates almost complete degradation of the mutant transcript, consistent with nonsense-mediated mRNA decay due to the early stop codon.

To study a physiologically relevant cell type, we generated induced pluripotent stem cells (iPSCs) and induced them to a neural lineage. The total protein amount of *IMPDH2* and its highly homologous isoform *IMPDH1* were detected together, as specific antibodies against



IMPDH2 are unavailable to our knowledge [8]. The isoforms likely can heterotetramerize, but show different expression patterns, *IMPDH2* being the major form in the central nervous system (CNS).

Total IMPDH protein was decreased down to 70% in the patient fibroblasts, iPSCs and neurospheres compared to controls (Fig. 2H, I).

The reduction was more severe than expected, as *IMPDH2* mRNA was reduced by 30% and *IMPDH1* mRNA expression was control-like (Fig. 2C). The expression of the enzymes participating in alternative or *IMPDH2*-related pathways were unchanged (Fig. 2D–F). The *IMPDH2* variant leads to depletion of the total protein pool of

**Fig. 2** *IMPDH2* deletion transcript leads to *IMPDH* deficiency. Red: patients. Black: controls. Healthy family member: black triangle. **A** *IMPDH2*: rate-limiting enzyme and first reaction in de novo biosynthesis of guanine nucleotides. Enzymes previously associated with dystonia in red. BH tetrahydrobiopterin, DDC aromatic amino acid decarboxylase, GCH1 GTP cyclohydrolase I, GMPR guanosine monophosphate reductase, GMPS guanosine monophosphate synthetase, HPRT1 hypoxanthine phosphoribosyltransferase 1, PTPS pyruvoyl-tetrahydropterin synthase, SPR sepiapterin reductase, TH tyrosine hydroxylase. **B–F** Relative (rel) mRNA expression of *IMPDH2*, *IMPDH1*, *HPRT1*, *GMPR1* and *GMPR2*. **G** Quantification of *IMPDH2* transcript with deletion in patients and controls. **H, I** *IMPDH2* protein amount in fibroblasts, induced pluripotent stem cells (iPSC) and differentiated neurospheres. **J** Volcano plot: serum metabolic profile changes between patients and healthy controls. In red: significantly changed metabolites (Fold-change > 1.5 and *p* value < 0.01). **K** Deoxycytidine (dC) concentration in serum. Statistical significance: pairwise two-tailed *t* test (\**p* ≤ 0.05, \*\**p* ≤ 0.01, \*\*\**p* ≤ 0.001).

*IMPDH*, suggesting that *IMPDH2* amount and its metabolic consequences regulate protein stability of both *IMPDH2* and *IMPDH1*. These findings suggest that *IMPDH2* depletion causes reduction of both *IMPDH* isoforms post-transcriptionally.

### Patients present normal serum metabolomic profile and nucleotide balance

The serum metabolomic analysis of five patients, one unaffected family member and five age and gender-matched controls was performed by a semi-quantitative targeted panel of 102 metabolites. Three significantly changed metabolites were found (Fig. 2J), out of which two were most enriched in the family members independent of the disease status (Fig. S1). Deoxycytidine, a nucleotide precursor, was reduced in the patients (Fig. 2K), suggesting nucleotide metabolic disbalance. In fibroblasts, our quantitative deoxynucleoside triphosphate (dNTP) pool concentration analysis showed normal dNTP pools in dividing and quiescent fibroblasts (Figs. S2, and S3).

### DISCUSSION

Here we report *IMPDH2* as a novel disease gene for dominantly inherited juvenile-onset dystonia-tremor disorder. The causative role of *IMPDH2* was supported by (1) the complete segregation and penetrance of the manifestation in a large pedigree; (2) high conservation of the protein and mutation site in species; (3) remarkably decreased gene product and (4) pathomechanistic similarity to previously reported dopamine-linked dystonia pathways. Furthermore, a recent large study focusing on neurodevelopmental disorders with dystonia raised attention to *IMPDH2* as a candidate gene [9], but direct evidence has been lacking.

*IMPDH2* and *IMPDH1* are tissue-specific enzyme isoforms, rate-limiting in the de novo guanine biosynthesis pathway. *IMPDH1* variants underlie autosomal dominant retinopathy [10, 11], but *IMPDH2* had not yet been assigned to a disease. We found a heterozygous truncating variant of *IMPDH2* to decrease protein levels of both *IMPDHs*, more severely than predicted by mRNA levels. The finding suggests that low *IMPDH2* amount or involved metabolites signal for high guanine, downregulating *IMPDH1* as well as preventing compensatory GTP-synthetic pathways. We propose that in post-mitotic cells of the CNS, with considerably lower nucleotide pools than in cultured fibroblasts [12] and high *IMPDH2* expression, the enzyme defect becomes rate-limiting, challenging guanine and dopamine synthesis and resulting in dystonia and tremor.

Clinically, *IMPDH2*-linked dystonia mimicks other dominantly inherited dystonias such as those caused by variants in *GCH1*, also an enzyme in the GTP-BH4 pathway. The *IMPDH2* reaction is flanked by *HPRT1* products—also a dystonia-linked protein—upstream from *GCH1*. The conversion of GTP into the dopamine biosynthetic cofactor BH4 is often affected in genetic dystonias (Fig. 2A) and also linked to pain sensitivity [13]. One of our patients showed sensory defects in the legs, whether this is linked to BH4 remains to be shown. A heterozygous variant in *GMPR* (GMP reductase), the enzyme catalysing the reverse reaction of *IMPDH2*, causes autosomal dominant progressive external ophthalmoplegia with muscle mitochondrial respiratory chain defect [14]. Our findings highlight the cell-

type-specific importance of metabolic pathways and their directionality and point towards a shared pathophysiological mechanism behind *IMPDH2* deficiency and other inherited dystonias.

In conclusion, *IMPDH2* is a novel dystonia gene linked to the dopamine synthesis pathway, implying that the symptoms may be L-DOPA responsive. Improved genetic knowledge is highly valuable for diagnosis and therapy choices for this complex and heterogeneous disease group.

Materials and methods are described in the supplementary information.

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### AUTHOR CONTRIBUTIONS

A.K., J.C.L. and A.S. designed the study; A.K. and J.C.L. performed the experimental work, analysed and interpreted data and wrote the manuscript. J.K. identified the patients; J.K. M.J., M.A. and T.T. examined the patients and characterised the clinical features of the disease. V.V. was responsible of the metabolomic analysis. A.S. and J.K. conceived the study; A.S. supervised the study, interpreted data and wrote the manuscript. All the authors edited or commented the manuscript.

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### COMPETING INTERESTS

The authors declare no competing interests.

### ETHICAL APPROVAL

The study was approved by the South Savo Social and Health Care Authority and performed in accordance with the Helsinki Declaration, with informed written consent from the participants.

### ADDITIONAL INFORMATION

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41431-021-00939-1>.

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