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Molybdenum cofactor deficiency

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18 **Molybdenum cofactor deficiency: Mutations in *GPHN*, *MOCS1* and *MOCS2***
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ABSTRACT

All molybdenum-containing enzymes other than the bacterial nitrogenase share an identical molybdenum cofactor (MoCo), which is synthesized via a conserved pathway in all organisms and therefore also is called “universal molybdenum cofactor”. In humans, four molybdoenzymes are known: aldehyde oxidase, mitochondrial amidoxime reducing component (mARC), xanthine oxidoreductase and sulfite oxidase. Mutations in the genes encoding the biosynthetic MoCo pathway enzymes abrogate the activities of all molybdoenzymes and result in the “combined” form of MoCo deficiency, which is clinically very similar to isolated sulfite oxidase deficiency, caused by mutations in the gene for the corresponding apoenzyme. Both deficiencies are inherited as an autosomal-recessive disease and result in progressive neurological damage and early childhood death in most cases. The majority of mutations leading to MoCo deficiency have been identified in the genes *MOCS1* (type A deficiency), *MOCS2* (type B deficiency), with one reported in *GPHN*. For type A deficiency an effective substitution therapy has been described recently.

KEYWORDS: molybdenum cofactor, MoCo, *MOCS1*, *MOCS2*, *MOCS3*, *GEPH*

Historical Background

The existence of a molybdenum-containing cofactor had been first postulated in 1964 (Pateman, et al., 1964) after the discovery of a series of pleiotropic mutations in the fungus *Aspergillus nidulans*, all of them affecting the enzymes nitrate reductase and xanthine dehydrogenase (hence the gene symbol *cnx* for cofactor of nitrate reductase and xanthine dehydrogenase, Table 1). The biosynthetic pathway leading to the cofactor and shared among all free-living organisms (Fig. 1) was elucidated mainly in the bacterium *Escherichia coli*, where its absence leads to chlorate resistance under anaerobic conditions (Rajagopalan and Johnson, 1992). This resulted in the gene symbol *chl*, which later in an attempt to unify the different nomenclature (Shanmugam, et al., 1992) was changed to *moa* through *mog* (see also Table 1).

The first case of a MoCo deficiency (MIM# 252150) has been described no earlier than in 1978 (Duran, et al., 1978) and the precise nature of the disease was only recognized in 1980 (Johnson, et al., 1980). The observation of two complementation groups among fibroblasts from different MoCo-deficient patients (Johnson, et al., 1989) was the basis to the understanding of the molecular basis of the human disease, which finally resulted in the identification of the gene *MOCS1* (Reiss, et al., 1998a; Reiss, et al., 1998b) mutated in the type A deficiency (MIM# 603707), and the gene *MOCS2* (Stallmeyer, et al., 1999a) and its mutations (Reiss, et al., 1999b) resulting in MoCo deficiency type B (MIM# 603708). Subsequently a mutation in the *GEPH* gene, resulting in the only described case of a type C deficiency (MIM# 603930), was reported (Reiss, et al., 2001).

Molybdenum deficiency versus MoCo deficiency

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10 Anecdotic reports on molybdenum toxicity in humans might originate from the well-documented
11 adverse effects of elevated molybdenum levels on ruminants. This effect, however, is strictly
12 limited to the special conditions in the rumen (Clarke and Laurie, 1980). In fact, the human body
13 can adapt to a wide range of molybdenum uptake (Novotny and Turnlund, 2007). Due to the
14 ubiquitous presence of molybdenum in organic food a dietary-induced deficiency of
15 molybdenum cannot be induced in animal models (Rajagopalan, 1988). Accordingly, only one
16 case of molybdenum deficiency has so far been described worldwide, which has been observed
17 after prolonged total parenteral nutrition of a Crohn's disease patient under omission of
18 molybdenum supplementation (Abumrad, 1984). The possibility of a beneficial effect of
19 drastically elevated levels of molybdenum on type C MoCo deficiency patients will be discussed
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40 In contrast to molybdenum or molybdate, respectively, MoCo or its precursors cannot be
41 recycled from nutritional sources. The simultaneous absence of endogenous rescue pathways
42 requires all MoCo molecules to be synthesized *de novo*. All proteins involved in MoCo
43 biosynthesis are encoded by autosomal genes and heterozygous mutations do not result in a
44 noticeable phenotype. Homozygosity for non-functional mutant forms of any of the involved
45 proteins, however, abolishes MoCo biosynthesis completely and thereby the activity of the
46 MoCo-dependent enzymes sulfite oxidase, xanthine oxidoreductase, mARC (Havemeyer, et al.,
47 2006) and aldehyde oxidase.
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Phenotype and diagnosis

Four MoCo-dependent enzymes are known in humans: aldehyde oxidase, mARC, xanthine oxidoreductase and sulfite oxidase. No isolated form of aldehyde oxidase or mARC deficiency are known, which might originate from overlapping substrate specificities of the enzymes. Classical xanthinuria type I is the result of an isolated xanthine oxidoreductase deficiency (Ichida, et al., 1997), while classical xanthinuria type II is caused by a defect in the molybdenum cofactor sulfuration by HMCS (Fig. 1) prior to incorporation into xanthine oxidoreductase and aldehyde oxidase (Ichida, et al., 2001). In symptomatic patients xanthine stones due to elevated levels of xanthine are the most prominent effects, although they are not life-threatening. Isolated sulfite oxidase deficiency (MIM# 606887) is also an autosomal-recessive genetic disease even rarer than the “combined” MoCo deficiency. 21 cases worldwide have been summarized previously (Tan, et al., 2005).

Isolated sulfite oxidase deficiency and the combined deficiency of all MoCo-dependent enzymes due to MoCo deficiency are clinically indistinguishable and can be differentiated only by biochemical parameters like elevated xanthine and lowered uric acid as a result of the simultaneous loss of xanthine oxidoreductase deficiency in the combined form. They should be considered in the differential diagnosis of hypoxic-ischemic encephalopathy (Topcu, et al., 2001) and neonatal hyperekplexia (Macaya, et al., 2005). Both forms can simultaneously be detected by the use of commercial strip tests originally developed to measure sulfite concentrations in

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3 wine and other beverages. False-negative results, however, are frequent, when the test is used
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5 shortly after birth (when the sulfite has not accumulated sufficiently for a positive test
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8 (Carragher, et al., 1999)) or when urine samples are not tested immediately and the sulfite is
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10 oxidized non-enzymatically. Sulfur-containing drugs can cause false-positive results. Metabolic
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12 changes are summarized in Fig. 2.
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19 Elevated sulfite levels lead to progressive neurological damage (Sass, et al., 2003; Zhang, et al.,
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21 2004), while the contribution of other metabolites such as taurine, S-sulfocystein and thiosulfate
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23 to the disease progression is not yet fully understood (Abbas, et al., 2008). Usually seizures start
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25 shortly after birth and feeding difficulties remind of amino acid intolerance. Those patients who
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27 survive the neonatal period in most cases show severe mental retardation as well as dislocated
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29 lenses and usually do not learn to sit or speak. The first 15 patients have been summarized
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31 elsewhere (Johnson and Wadman, 1989). Further reports followed (Appignani, et al., 1996;
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33 Arnold, et al., 1993; Coskun, et al., 1998; Hahnewald, et al., 2006; Ichida, et al., 2006; Ichida, et
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35 al., 2001; Kavukcu, et al., 2000; Leimkuhler, et al., 2005; Macaya, et al., 2005; Parini, et al.,
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37 1997; Per, et al., 2007; Pintos-Morell, et al., 1995; Salman, et al., 2002; Salvan, et al., 1999;
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39 Sass, et al., 2010; Schuierer, et al., 1995; Serrano, et al., 2007; Slot, et al., 1993; Teksam, et al.,
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41 2005; van Gennip, et al., 1994).
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52 In general, the disease is not apparent at birth. Within the first week, feeding difficulties are
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54 observed accompanied by tonic/clonic seizures refractory to anticonvulsants with a prominent
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56 opisthotonus and an exaggerated startle reaction. A cerebral atrophy results in microgyria,
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3 bilateral ventricular enlargement and is probably also responsible for facial dysmorphic features.
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5 A few patients with relatively mild symptoms have been described with presumably at least one
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7 mutation allowing for significant residual enzyme activity. Although uric acid levels here
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9 occasionally were found to be normal (Arenas, et al., 2009; Johnson, et al., 2001), the
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11 biochemical parameters apparently do not correlate with the severity of the disease (Hughes, et
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13 al., 1998; Mize, et al., 1995; Ngu, et al., 2009).
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21 It had been suggested that the observed brain anomalies could at least in part be also attributable
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23 to a sulfate deficiency rather than sulfite toxicity (Rajagopalan and Johnson, 1992), but
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25 neuropathological investigation (Salman, et al., 2002) and studies in animal models (Reiss, et al.,
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27 2005) did not support this hypothesis. The disease has been found in a variety of ethnic groups
28
29 and all over the world, although it might be under-recognised in many countries. *De novo*
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31 mutations so far have not been described, but recently the first case of an uniparental disomy
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33 responsible for MoCo deficiency has been reported (Gumus, et al., 2010). Obligate heterozygotes
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35 display no symptoms. Prenatal diagnosis by enzyme activity assays has been described using
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37 amniotic cells and chorionic villi (Johnson, 2003; Johnson, et al., 1991), but nowadays can be
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39 substituted by DNA analysis (Reiss, et al., 1999a; Shalata, et al., 2000).
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52 **The *MOCS* genes and alternative splicing**

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3 The first cDNAs derived from *MOCS1* (Reiss, et al., 1998b) carried two open reading frames
4 (ORFs) each coding for a protein homologous to different proteins known to be involved in
5 MoCo synthesis in bacteria (Supp. Fig S3; Rajagopalan and Johnson, 1992) [TO
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10 **COPYEDITOR: QUERY AUTHOR ON PLACEMENT OF CITE FOR SUPP. FIG. S3].**
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12 Later, an alternative splicing pattern was described leading to different monocistronic domain-
13 specific transcripts (Gray and Nicholls, 2000). A full length transcript contains a stop codon in
14 exon 9 (Fig. 3A) and encodes the protein MOCS1A with 385 amino acids homologous to MoaA
15 in *E. coli*, both carrying the glycine-glycine C-terminus conserved across all examined species
16 except Archea (Supp. Fig. S1A) and shown to be functionally important (Hanzelmann, et al.,
17 2002; Reiss, et al., 1998a). Splice forms 2 and 3 omit the stop codon by skipping exon 9
18 completely or partially (Fig. 3A), thus elongating the ORF into exon 10, which encodes the
19 MOCS1B domain exclusively expressed as a MOCS1AB fusion protein (Supp. Fig. S1B). The
20 physiological role of the alternative first exons and start codons (Gross-Hardt and Reiss, 2002) as
21 well as other alternative splicing patterns (Arenas, et al., 2009) so far is unknown (Fig. 3B). A
22 processed pseudogene (*MOCS1P1*) is located on chromosome 16 (NCBI NG_001271).
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43 The *MOCS2* gene encodes the small and large subunit of molybdopterin synthase in two
44 overlapping ORFs, i.e. the start codon of the second frame lies within the first frame (Fig. 4 and
45 Supp. Fig. S4) [TO COPYEDITOR: QUERY AUTHOR ON PLACEMENT OF CITE FOR
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48 **SUPP. FIG. S4].** Both ORFs are shifted relative to each other and thus do not share identical
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3 1b) was identified, which is located between exon 1(a), carrying the start codon of the first ORF,
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5 and the original exon 2. The use of the corresponding transcription start leads to a mature
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7 transcript rendering the start codon of the second ORF the first translation initiation site on the
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9 mRNA (Hahnewald, et al., 2006) thus allowing translation of MOCS2B according to a regular
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11 scanning mechanism (Kozak, 1978). MOCS2A, encoded by the first ORF, again carries a
12
13 conserved glycine-glycine C-terminus (Supp. Fig. S2), whose free accessibility appears to be
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15 essential for catalytic function as in the case of MOCS1A (Unkles, et al., 1999).
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24 The gene *MOCS3* encodes a protein believed to catalyze both the adenylyl transfer and subsequent
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26 thiocarboxylation at the C-terminus of the small subunit of molybdopterin synthase (Matthies, et
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28 al., 2004), thus providing the sulfur to be incorporated into cPMP (Fig. 1). This gene is located
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30 on chromosome 20 and contains no introns. A pseudogene (*MOCS3P1*) is located on
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32 chromosome 14 (NCBI NG_002557). No disease-causing mutation has so far been described in
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36 *MOCS3*.
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45 **The *GEPH* gene**

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52 The protein Gephyrin was originally identified by copurification with the mammalian inhibitory
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54 glycine receptor and named “bridging protein” due to its proposed function as a receptor-
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56 microtubule linker in subsynaptic receptor-clustering (Kirsch, et al., 1993; Prior, et al., 1992). Its
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homology to MoCo biosynthesis genes was described in comparison to corresponding sequences from bacteria, fruitflies and plants (Kamdar, et al., 1994; Stallmeyer, et al., 1995). Later, a dual function in receptor clustering and MoCo biosynthesis was confirmed by the investigation of a murine *Geph* knockout model (Feng, et al., 1998), reconstitution assays (Stallmeyer, et al., 1999b) and by the detection of a disease-causing *GEPH* mutation in a deceased MoCo-deficient patient (Reiss, et al., 2001). The Gephyrin protein has two distinct domains, whose function is depicted in Table 1 and Fig. 1.

Mutations in MoCo deficiency

With the exception of the above described *GEPH* mutation in a single patient (Reiss, et al., 2001), disease-causing mutations in MoCo deficiency have been described in *MOCS1* and *MOCS2* only (Reiss, 2000; Reiss and Johnson, 2003). Table 2 gives an update of the hitherto identified pathological mutations. The detection rate in biochemically confirmed cases (i.e. elevated sulfite and lowered uric acid levels) is > 99 %. Due to multiple start codons in different exons plus additional alternative splicing events, the nomenclature of mutations in the *MOCS* genes is not trivial. The first mutation reports preceded elucidation of the correct expression mechanisms.

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3 As we know now, the MOCS1B domain is not expressed as a distinct protein but rather as a
4 fusion protein “MOCS1AB” (Fig. 3A) rendering some previously used numbers of
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6 corresponding mutation designations on the protein level not conform to nomenclature
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8 guidelines. This should be changed as listed in Table 2. For both *MOCS1* and *MOCS2*, we
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10 suggest to use the start codon of the first open reading frame for cDNA numbering throughout
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12 the complete gene on the basis of a full length transcript (Supp. Figures S1 and S2). In case of
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14 multiple first exons the first described should be used to keep changes in nomenclature to a
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16 minimum. In case of *MOCS1*, the “Larin” exon 1a upstream of the first described start codon (in
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18 exon 1b) should be addressed explicitly (Table 2), while an identical length of 41 amino acids for
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20 the “Larin” trailer (encoded by exon 1a) and the “Reiss” trailer (encoded by exons 1b and 1c)
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22 does not interfere with downstream amino acid numbering (Fig. 3B). For the MOCS1AB fusion
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24 protein, splice form II should be the basis, i.e. the longer form in comparison to the splice form
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26 III protein (Fig. 3).

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29 In case of the *MOCS2* gene the nucleotide numbering upstream of the start codon will depend on
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31 the specification of the affected protein, i.e. into exon 1a in case of MOCS2A and into exon 1b in
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33 case of MOCS2B (Fig. 4). It is noteworthy that compound heterozygous patients carrying one
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35 mutation in the A domain and one in the B domain have not been observed in type A (*MOCS1*)
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37 neither in type B (*MOCS2*), indicating intragenic complementation via the different above
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39 described transcripts. An exception is the frameshift mutation c.1000insT located in the
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41 MOCS1A ORF (Table 2) and found together with c.1826T>C affecting the MOCS1B domain,
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43 which could be explained by a polar effect of the insertion (Fig. 3A). Within Europe a founder
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45 effect for most of the repeatedly identified mutations is still visible (Supp. Figure S5).
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Animal models and experimental therapies

Knockout mice have been described for *Geph* (Feng, et al., 1998) and *Mocs1* (Lee, et al., 2002). Homozygous *Geph* *-/-* mice die within 1 day after birth, but the comparison with *Mocs1* *-/-* mice suggests that this is due to neurological deficits caused by the loss of synaptic clustering of glycine receptors – the second function of Gephyrin besides MoCo biosynthesis. “Pure” MoCo-deficient mice such as the *Mocs1* *-/-* mice die approximately 1 week after birth and are an excellent model for therapeutical studies since they truly reflect the biochemical characteristics of the human disease. The absence of neuropathological findings in the MoCo-deficient mice might be explainable by (i) an improved maternal clearance due to reduced spatial conditions for biochemical gradients and/or (ii) the drastically accelerated progression of the sulfite poisoning (Reiss, et al., 2005). In this animal model, a substitution therapy with cPMP (Fig. 1) had already been described some years ago (Schwarz, et al., 2004).

Using the same animal model, a gene therapy option with AAV vectors has been demonstrated (Hahnewald, et al., 2009; Kugler, et al., 2007). Although the MoCo deficiency phenotype could be completely reverted by just a single injection of AAV capsids carrying a *MOCS1* expression cassette, these long term experiments revealed an increased risk for malignancies after recombinant AAV injections. Similar observations have been described by others albeit with different interpretations. While the first corresponding report could not offer an explanation at all

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3 (Donsante, et al., 2001), specific transgenes such as the *lacZ* reporter have been held responsible
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5 for tumorigenesis (Bell, et al., 2006).
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12 Our data do not support a transgene hypothesis since we introduced the same expression cassette
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14 that was used for AAV studies also into the germline of MoCo wild-type and deficient mice via
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16 microinjection of fertilized oocytes and observed a tumor frequency of only 9 % over 2 years in
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18 46 animals. In contrast, over 20 % of 44 AAV-treated animals, injected either neonatally or 40
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20 days after birth, developed tumors in different organs. After intrahepatic injections
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22 predominantly hepatocellular carcinomas were seen, while intravenous applications resulted in a
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24 variety of tumors (data not shown).
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43 **Clinical studies**

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49 Early attempts to ameliorate the symptoms of MoCo deficiency included repeatedly the
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51 administration of high levels of inorganic molybdenum, which was found to be inefficient
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53 (Bamforth, et al., 1990; Endres, et al., 1988; Johnson, et al., 1980). It has been demonstrated in
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55 cell cultures, that this approach is sensible for type III patients with *GEPH* mutations (Reiss, et
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3 al., 2001). The above cited molybdate treatment of patients, however, preceded gene and
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5 mutation identification, which revealed that *GEPH* mutations as the cause of MoCo deficiency
6
7 are extremely rare. Most likely, patients with other mutations have been treated with molybdate
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9 in the published studies. The only patient with *GEPH* mutations described so far deceased before
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11 molecular analysis. It should be kept in mind, however, that this type of MoCo deficiency indeed
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13 might be curable with high molybdate concentrations. A diet low in sulfur amino acids has in
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15 some cases resulted in a significant reduction of abnormal sulfur metabolites although no
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17 neurological improvement could be observed (Johnson, et al., 1980).
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26 For decades it had been speculated that at least a part of the MoCo-deficient patients might profit
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28 from substitution with precursor Z (now called cPMP, Fig. 1). This pursuit culminated in the
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30 decision of an Australian court to allow experimental treatment of “baby Z” (as it was called in
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32 the popular press) with a cPMP preparation tested before exclusively in mice (Veldman, et al.,
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34 2010). Before, animal studies had ruled out convincingly any adverse effects (Schwarz, et al.,
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36 2004). The affected baby was homozygous for the MOCS1B mutation G588R (Table 2) and
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38 thereby classified as type A defective in cPMP synthesis (Fig. 2). Cranial ultrasound
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40 examinations at 1 month showed cerebral atrophy and loss of gray/white-matter differentiation,
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43 Intravenous replacement therapy with cPMP started on day 36 by extrapolating the dose that had
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45 been most efficient in the mouse model (Schwarz, et al., 2004). Since the cPMP infusions were
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47 well tolerated and triggered no observable adverse effects, they were continued on a daily basis
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49 with occasional dosage adjustment. Within a few days normalization of the previously markedly
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51 elevated levels of S-sulfocysteine and thiosulfate were achieved. Within 2 days the infant
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53 became more alert and at an age of 18 months she is clinically free of seizures. In summary, the
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3 substitution therapy cannot reverse cerebral injury observed before commencing cPMP
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5 supplementation. However, ongoing neurotoxicity and further deterioration definitely can be
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7 excluded. Four more type A patients are currently cPMP-treated with similar outcome (Günter
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9 Schwarz, personal communication).
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20 **Future prospects**

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26 The availability of a causative treatment immediately raised the question about screening
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28 possibilities for MoCo deficiency. A practicable (and affordable) way to the early identification
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30 of curable patients might be the routine determination of the ratio of uric acid and xanthine
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32 concentrations, which in case of lowered values and in the absence of other immediate
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34 explanations should be followed by a test for elevated sulfite levels. If the latter confirms a lack
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36 of active MoCo, the precise type of the deficiency must be determined either biochemically or by
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38 genetic testing. Although the ethnic origin of a patient often renders specific mutations to be
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40 found more likely than others (Supp. Figure S5), the speed of sequencing complete genes (if not
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42 genomes) reached by now suggests a complete analysis of *MOCS1* and *MOCS2* in a first step,
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44 which results in a mutation detection rate of more than 99 percent. Only in the case of negative
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46 results up to here, sequencing of the genes *GEPH* and *MOCS3* appears to be indicated.
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3 Despite the demonstration of successful treatment possibilities for type A in clinical studies and
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5 for type C in cell culture experiments, type B deficiencies so far appear to be incurable. The
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7 relative instability of molybdopterin (with or without molybdenum) hampers substitution therapy
8
9 analogue to cPMP supplementation. Theoretical options still to be explored are enzyme
10
11 replacement therapy with MOCS2 proteins or functional sulfite oxidase and safe avenues of gene
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13 therapy. The latter might include the use of AAV vectors, if parameters such as titer, serotype
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15 and route of application are optimized in terms of a minimal malignancy risk.
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For Peer Review

Legends to Figures

Figure 1. Biosynthesis of MoCo via an ancient pathway common to all free-living species and types of diseases as a consequence of mutations in the different genes. MOCS1A belongs to the superfamily of S-adenosylmethionine-dependent radical enzymes, members of which catalyze the formation of radicals by a protein-bound [4Fe-4S] cluster (Hanzelmann, et al., 2004). It is speculated that the B domain of the MOCS1AB fusion protein might function as a radical acceptor (Teschner, et al., 2010). The two MOCS2 proteins A and B are subunits of molybdopterin synthase incorporating sulfur groups delivered by the MOCS3 sulfurylase (Matthies, et al., 2004). Finally, molybdenum is attached to these sulfur groups in a two step-reaction catalyzed by the *GEPH*-encoded two domain-protein Gephyrin (Kuper, et al., 2004; Llamas, et al., 2006).

Figure 2. Metabolites influenced by MoCo deficiency.

Figure 3. Alternative splicing of the *MOCS1* transcript. (A) Use of the first stop codon in exon 9 leads to an active MOCS1A protein with the strictly conserved and functionally obligate two glycine residues at the C-terminus in splice form I. Splice forms II and III lead to a fusion protein with an inactive A domain and the functional B domain. (B) The exons 1a and 1b are mutually exclusive. The two different trailers of the most common Larin and Reiss variants have an identical length of 41 amino acids.

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7 **Figure 4.** Expression of the *MOCS2* gene. Exons 1a and 1b are mutually exclusive and lead to
8 the expression of two distinct proteins via spliceforms I and III, respectively. Spliceform II
9 cannot be found in the databases anymore and probably was an artifact. The open reading frames
10 of the small and large subunit of molybdopterin synthase overlap in exon 3. The essential Gly-
11 Gly C-terminus of MOCS2A is in red color.
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Table 1. Nomenclature of homologous genes or protein domains, respectively, involved in MoCo synthesis among different species

Synthesis of	Bacteria, old	Bacteria,new	Fungi/Plants	Human/Mice	NCBI (human)
cPMP	<i>chlA</i>	MoaA	<i>cnxA/2</i>	MOCS1A	NG_009297
cPMP	<i>chlA</i>	MoaC	<i>cnxB/3</i>	MOCS1B	NG_009297
molybdopterin	<i>chlA</i>	MoaD	<i>cnxG/7</i>	MOCS2A	NM_176806
molybdopterin	<i>chlA</i>	MoaE	<i>cnxH/6</i>	MOCS2B	NM_004531
molybdopterin	<i>chlE</i>	MoeB	<i>cnxF/5</i>	MOCS3	NM_014484
MoCo	<i>chlE</i>	MoeA	<i>cnxE/1(E)</i>	Gephyrin E domain	NG_008875
MoCo	<i>chlG</i>	MogA	<i>cnxE/1(G)</i>	Gephyrin G domain	NG_008875
S-MoCo	-	XdhC/NifS4	<i>HxB/ABA3</i>	HMCS/MOCOS	NM_017947

Table 2. Mutations causative of MoCo deficiency as described through September 2010

Gene/Protein	Nucleotide change ¹	Location	Predicted effect	Legacy name	Reference	
MOCS1A	c.99_100delGG	Exon 1a ²	p.E34fsLarin		Personal communication ³	
	c.195_212del18	Exon 1d	p.G66_Y71del	195del18	Reiss et. al. [1998a]	
	c.199C>T	Exon 1d	p.R67W	R67W	Leimkühler et. al. [2005]	
	c.217C>T	Exon 1d	p.R73W	R73W	Reiss et. al. [1998a]	
	c.218G>A	Exon 1d	p.R73Q		Personal communication ³	
	c.238T>G	Exon 1d	p.C80G	C80G	Leimkühler et. al. [2005]	
	c.250T>C	Exon 2	C84R		This study	
	c.251G>T	Exon 2	p.C84F	C84F	Leimkühler et. al. [2005]	
	c.253C>T	Exon 2	p.Q85X		This study	
	c.256T>G	Exon 2	p.Y86D		This study	
	c.291delC	Exon 2	p.A99fs		Reiss and Johnson [2003]	
	c.367C>T	Exon 2	p.R123W	R123W	Reiss and Johnson [2003]	
	c.377G>A	Exon 2	p.G126D	G126D	Reiss et. al. [1998a]	
	c.379G>A	Exon 2	p.G127R	G127R	Reiss et. al. [1998a]	
	c.394C>T	Exon 2	p.R132W		This study	
	c.418+1G>A	Intron 2	Skipping exon 2	418+1G>A	Reiss et. al. [1998a]	
	c.583+1G>A	Intron 3	Skipping exon 3	583+1G>A	Leimkühler et. al. [2005]	
	c.603_623del21	Exon 4	p.G202_E208del		This study	
	c.664C>T	Exon 5	p.R222X		This study	
	c.666_667insCGA	Exon 5	R222_G223insR	667insCGA	Leimkühler et. al. [2005]	
	c.721delC	Exon 5	p.L241fs		Personal communication ³	
	c.722delT	Exon 5	p.L241fs	722delT	Reiss et. al. [1998b]	
	c.956G>A	Exon 7	p.R319Q	R319Q	Reiss et. al. [1998a]	
	c.970G>A	Exon 7	p.G324R	G324R	Reiss and Johnson [2003]	
	c.971G>A	Exon 7	p.G324E	G324E	Reiss et. al. [1998a]	
	c.1000insT	Exon 8	p.S334fs		This study	
	c.1015_1018del4	Exon 8	p.R339fs	1015delCGGG	Leimkühler et. al. [2005]	
	c.1027C>T	Exon 8	p.R343X	L342X	Leimkühler et. al. [2005]	
	c.1064T>C	Exon 8	p.I355T		Macaya et. al. [2005]	
	c.1102+1G>A	Intron 8	Skipping exon 8	1102+1G>A	Reiss et. al. [1998a]	
	c.1150G>A	Exon 9	p.G384S	G384S	Reiss et. al. [1998a]	
	c.1165+6 T>C	Intron 9	Skipping exon 9		Arenas et. al. [2009]	
	MOCS1AB	c.1313_1314insG	Exon 10	p.P434fs	1313insG	Reiss et. al. [1998a]
		c.1502_1507del6 insCT	Exon 10	p.V496fs		This study
		c.1523_1524del2	Exon 10	p.E503fs	1523delAG	Reiss et. al. [1998b]
		c.1640delA	Exon 10	p.Q542fs		This study
		c.1777G>A	Exon 10	p.G588R	G175R	Leimkühler et. al. [2005]
		c.1798A>C	Exon 10	p.T595P	T182P	Reiss et. al. [1998a]
		c.1826T>C	Exon 10	p.L604P		This study

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3		c.1838_1843del6insCC	Exon 10	p.C608fs	Personal communication ³
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15	MOCS2A	c.-9_14del23	Exon 1a	Initiation failure	Hahnewald et. al. [2006]
16		c.1A>G	Exon 1a	Initiation failure	Personal communication ³
17		c.3G>A	Exon 1a	Initiation failure	M1I Reiss et. al. [1999]
18		c.16C>T	Exon 1a	p.Q6X	Johnson et. al. [2001]
19		c.19 G>T	Exon 2	p.V7F	Johnson et. al. [2001]
20		c.33T>G	Exon 2	p.Y11X	Y11X Leimkühler et. al. [2005]
21		c.45T>A	Exon 2	p.S15R	S15R Reiss and Johnson [2003]
22		c.88C>T	Exon 2	p.Q30X	Q30X Reiss and Johnson [2003]
23		c.106C>T	Exon 2	p.Q36X	Q36X Reiss and Johnson [2003]
24		c.130C>T	Exon 2	p.R44X	Per et. al. [2007]
25		c.220C>T	Exon 3	p.Q74X	This study
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30	MOCS2A/B	c.252_253insC	Exon 3	MOCS2A: I85fs MOCS2B: L23fs	252insC Reiss et. al. [1999]
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34	MOCS2B	c.413G>A	Exon 4	p.G76R	Reiss and Johnson [2003]
35		c.501delA	Exon 5	p.K105fs	This study
36		c.533_536del4	Exon 5	p.V116fs	533del4 Reiss et. al. [1999]
37		c.564G>C	Exon 5	p.G126A	Reiss and Johnson [2003]
38		c.564+1G>A	Intron 5	Skipping exon 5	This study
39		c.635_637del3	Exon 6	p.A150 del	Reiss and Johnson [2003]
40		c.658_664del7insG	Exon 6	p.L158_K159del	Reiss and Johnson [2003]
41		c.689G>A	Exon 7	p.E168K	E168K Reiss et. al. [1999]
42		c.714_718del5	Exon 7	p.G178fs	Reiss and Johnson [2003]
43		c.726_727delAA	Exon 7	p.K180fs	726del2 Reiss et. al. [1999]
44		c.754A>C	Exon 7	p.X189Y	X189Y Leimkühler et. al. [2005]
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49	Gephyrin	c.65-?_102+?del	Intron1-3	p.V22fs	Exon2/3del Reiss et al. [2001]
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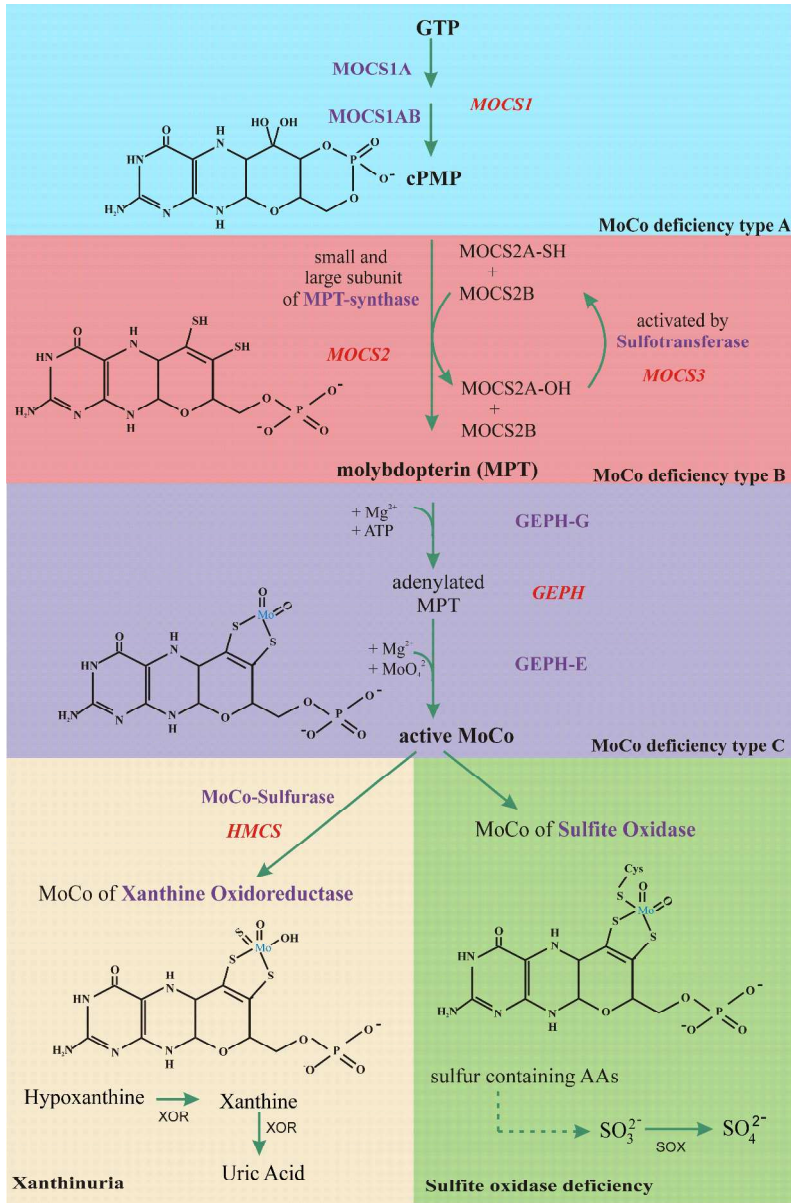
¹Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence GenBank AJ224328.1 for MOCS1 (see also Supp. Fig. S1) and Genbank AF091871.1 for MOCS2 (see also Supp. Fig. S2), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

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4 ²This mutation affects only the Larin variant (see also Fig. 3B), which is not included in the
5 reference sequence, but in Supp. Fig. S1.
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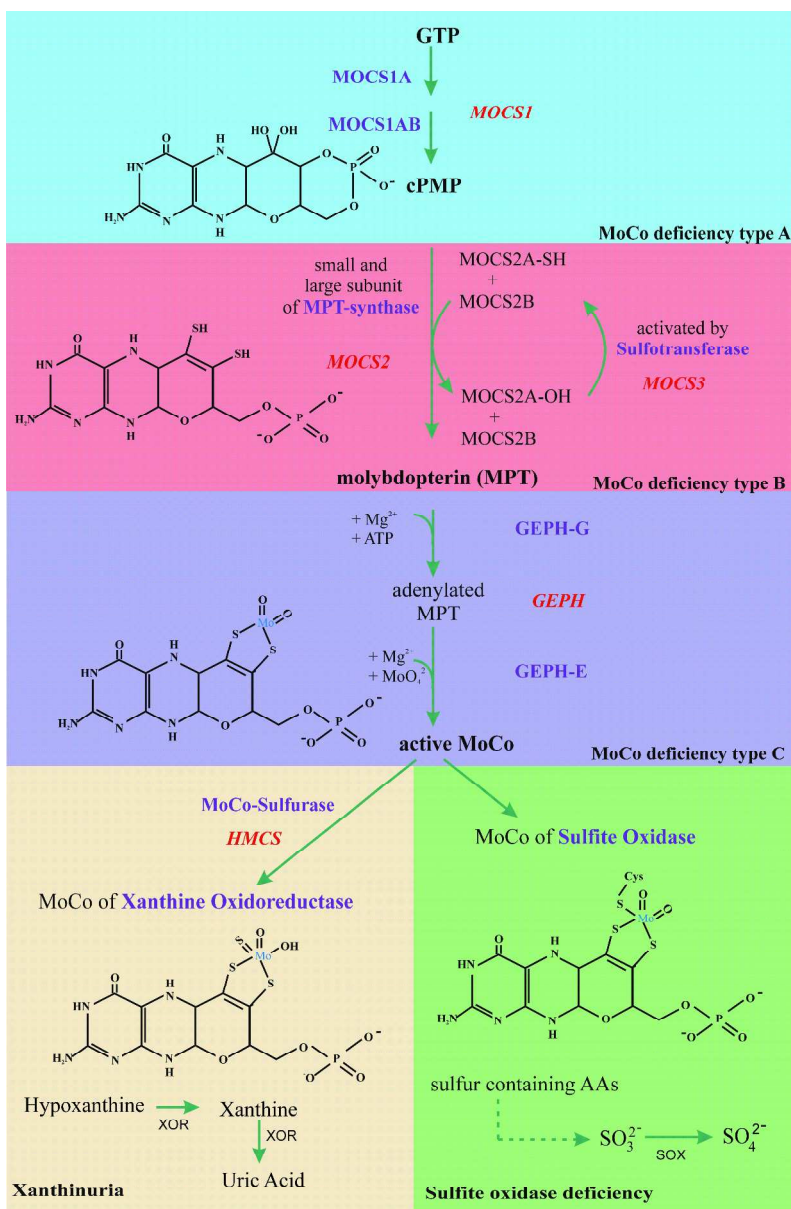
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8 ³ Cecile Acquaviva, Service Maladies Hereditaires du Metabolisme, Centre de Biologie Est –
9 Groupement Hospitalier Est, 59, bd Pinel – 69677 BRON cedex
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For Peer Review

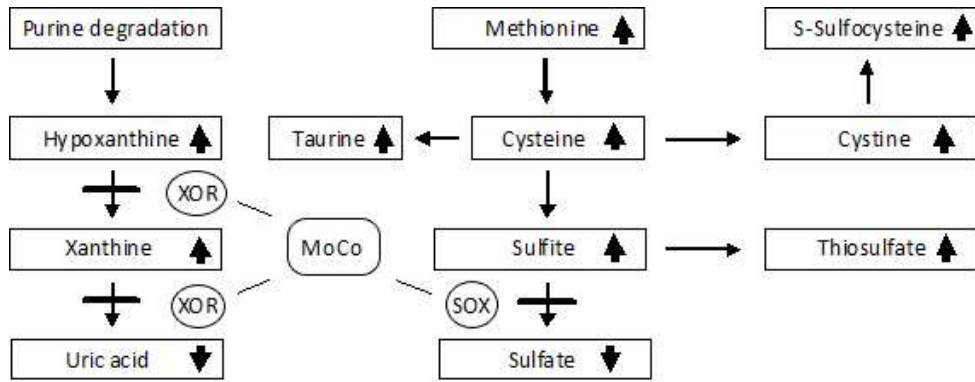
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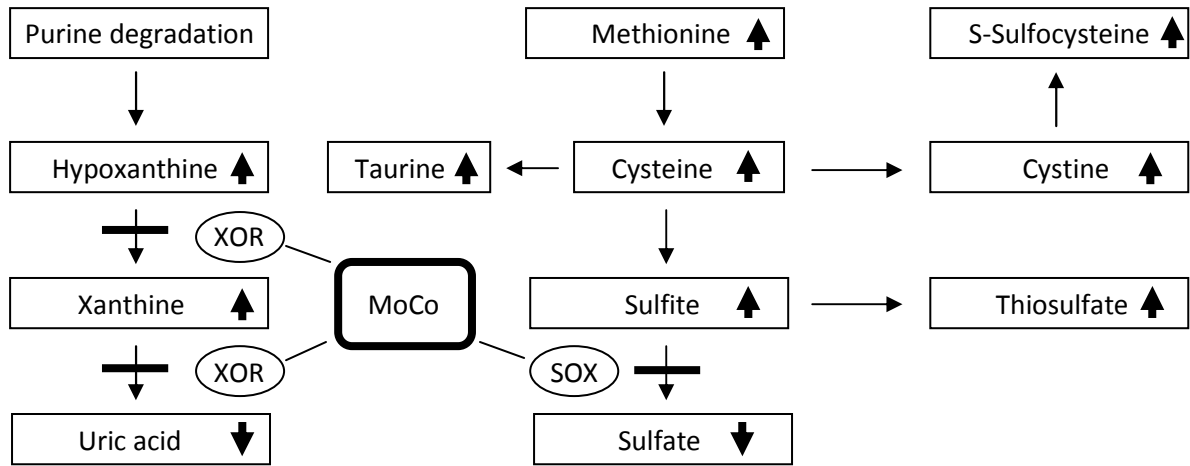
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207x81mm (72 x 72 DPI)

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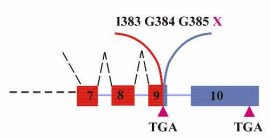


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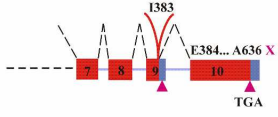
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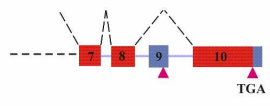
A spliceform I: bicistronic transcript - active MOCS1A



spliceform II: monocistronic transcript - inactive MOCS1A and active MOCS1B

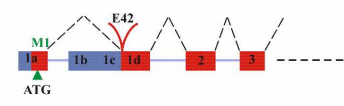


spliceform III: monocistronic transcript - inactive MOCS1A and active MOCS1B

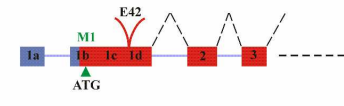


■ coding region ■ untranslated region

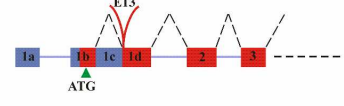
B Larin - variant



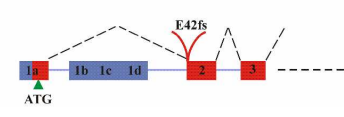
Reiss - variant



Gross-Hardt - variant



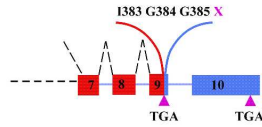
Arenas - variant



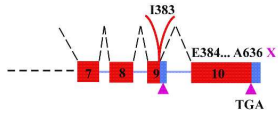
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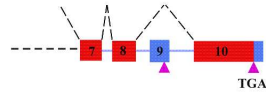
A spliceform I: bicistronic transcript - active MOCS1A



spliceform II: monocistronic transcript - inactive MOCS1A and active MOCS1B

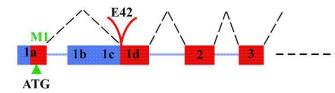


spliceform III: monocistronic transcript - inactive MOCS1A and active MOCS1B

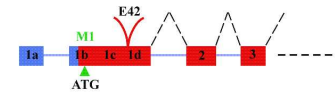


■ coding region ■ untranslated region

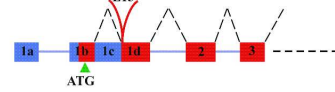
B Larin - variant



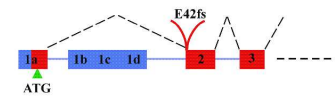
Reiss - variant



Gross-Hardt - variant



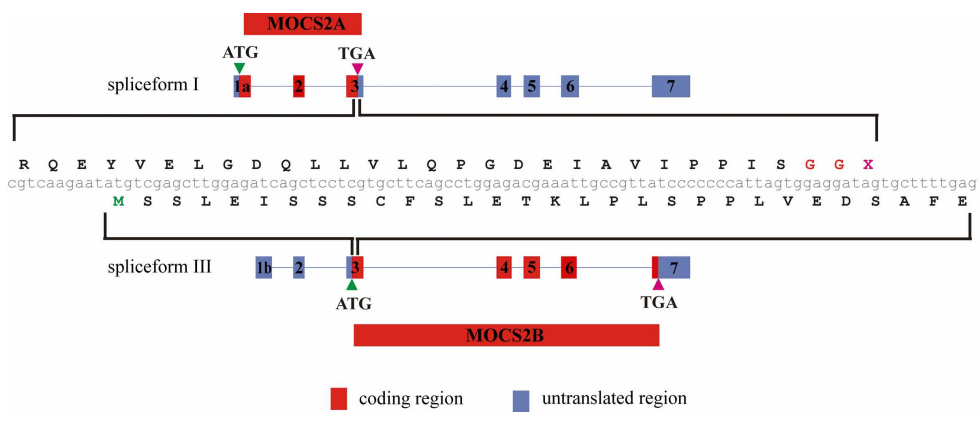
Arenas - variant



158x114mm (600 x 600 DPI)

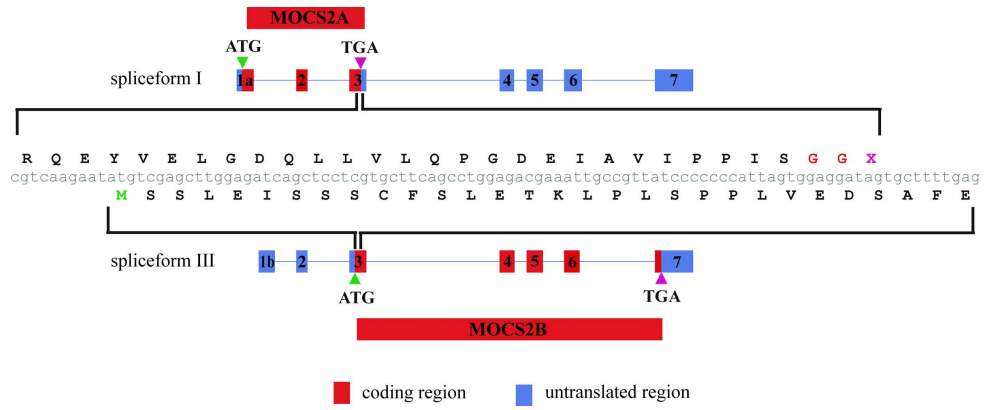
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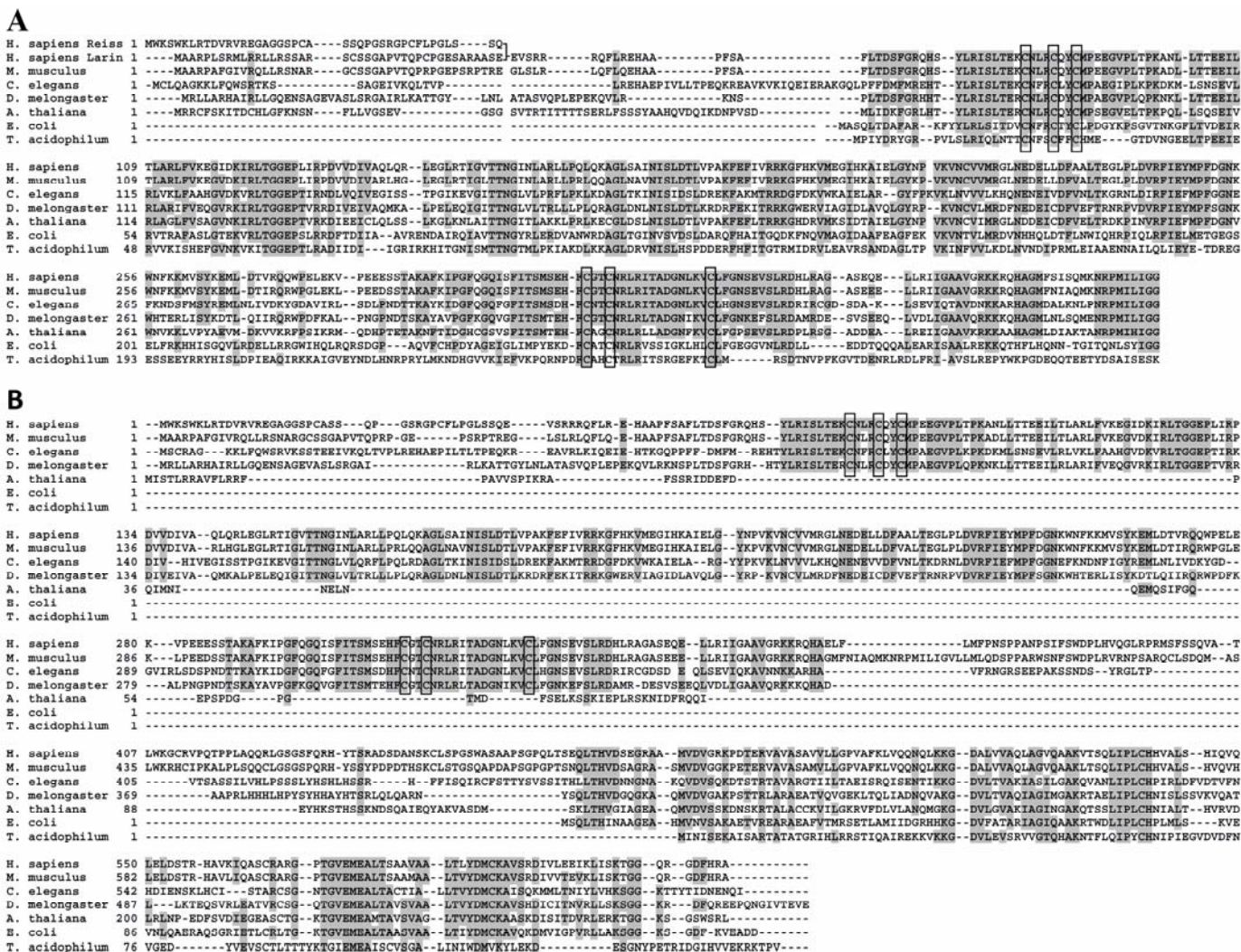
Peer Review



104x41mm (600 x 600 DPI)

Peer Review

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Supp. Figure S1. Alignment of the proteins MOCS1A (A) and MOCS1AB (B). Conserved amino acid positions are shaded. The essential Cys clusters (Menendez, et al., 1996) are boxed.

A

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H. sapiens      1 -----MVPLCQVEVLYFAKSAEITGVRSETISVPQEIKALQLWKE---IETRHPLGLADVRNQIIFAVRQEYVELGDQLLVLQPGDEIAVIPPISGG
M. musculus    1 -----MVPRCQIDVLYFAKSAEIAGRVSETISVPQEIKASELWKE---LESHPGLADVRNQVIFAVRQEYVELGDQLLVLQPGDEVAVIIPPISGG
C. elegans     1 -----MISIKVLFFGEACQLVVGKREAIIDFPEETDYEEIRKT---ILENYPALQKIEKVMMLAVDQYANPGDRFELVR-FTEIAVIPPISGG
D. melongaster 1 -----MNADGCVVNVHVLFFAKSRELANTPRSTVEVPTETATELLDH---LVSKF-GLTSIRDNLILAHNESYIDNLSDRILFKGDELAIIIPPISGG
A. thaliana    1 MDKEVTKIESDDTSSVEIKVLLFARARELTGVDPDLTKMPSGSTTQKCLDE---LVLFKPSLEEVRSVVLALNEEY---TTDSAVIQHRDELAIIIPPISGG
E. coli        1 -----MIKVLFFAQVRELVG--TDATEVAADPTVEALRQHMAAQSDRWALALEDEGKLLAAVNQTLVSPDHP---LTDGDEVAFFPVPVTGG
T. acidophilum 1 -----MRCVQMDVTVKYFAYRERVGKDSKIQLDQGKRISDLI---DMLMARYPGIFNEKN-LFVARNYRYADRGE---ALSDGDVVAIMPHVSSGG
    
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B

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H. sapiens      1 -----MSSLEISSSCFSLETKLPLSPPLVEDSAFEP
M. musculus    1 -----MSSLEISNSCFSPEMRLPSSRQSVEDNASEP
C. elegans     1 MTSTKAYVLALAGCTNSGKSTLAKEFORFFGAGETTIINQDEFYKTENEVEKIYHPDAEHLTDEGYYSFDEKEAINIDQFRQRI LNESKVKYKNVIIDGN
D. melongaster 1 -----
A. thaliana    1 -----
E. coli        1 -----
T. acidophilum 1 -----

H. sapiens     32 SRKDM-----EVEE---KSKDVINF-----
M. musculus    32 SGKDVD-----DVQE---KPKDIIQF-----
C. elegans     101 MITEMDEIVDLCDRIVVMTLDFKTKRRREARTDYVPADTPGYFVNVAFPAYVRHLERARQRSRTDSRLTFIDVSESKFDDKNSIVNFRQIILDDHIKL
D. melongaster 1 ---MD-----HVKL---VNDPIDIA-----
A. thaliana    1 ---M-----SAEE---KNLIEILE-----
E. coli        1 -----
T. acidophilum 1 ---MM-----YV-----DII-----

H. sapiens     50 TAEKLSVDEVSQLVIS---PL---CGAISLVFGTTRNNFEGKKVISLEYEAYLPMANEVRK-ICSDIRQKWPV-KHIAVFHRLGLVVPVSEASIIIAV
M. musculus    50 TAEKLSVGEVSQLVVS---PL---CGAVSLVFGTTRNNFEGKKVISLEYEAYVPMANEVRK-ICNDRIRQKWPV-RHIAVFHRLGLVVPVSEASTVIAV
C. elegans     201 TDEVIDVKVVDQLV---SHFS---CGAISTFNGVTRDNHGGRDVSNLSYDCHDLMAKCLR-GICAEIRAEYDPVKKIAIFHRLGKVDIGESSVVIIST
D. melongaster 15 HIHQLLADE-----G---CGASSVFGTTRDNFQGGKVLSLAYEAYDSMALKEMNK-ICSDLRSKWLDLKHIVYHRLGTVFVCEASVVIAS
A. thaliana    14 EGHKVDVVKYIDYVSA---PQ---AGAIATFSGTTRDMFEGKTVLELRYEAYVPMATRLSS-ICTARSTWDLHK-IAVAHRLGVPVPGVCEASVLIAS
E. coli        2 AETKIVVGPQFFSVGEEYPLAERDEGAVVTFQKVRNHNLDGSDVNALTLEHYPCMTKALAE-IVDEARNRWFPLGRVT-VIHRIGELWPGDEIVFVGV
T. acidophilum 8 ---ESEIPVMDLIDRIRNPK---AGAIVTFEGTVREDSGKVDALFYESYREMAIREIRDMIEYCIHQF--GITDAAVVHRIGRNVLTEDSVVIAV

H. sapiens     140 SSAHRAASLEAVSYAIDTLKAKVPIWKKEIYEES-ST---WKGKNECFWASNS-----
M. musculus    140 SSAHRAASLEAVSYAIDSLKAKVPIWKKEIYEESTSS---WKRKNECFWAAGD-----
C. elegans     292 SSPHRKTAIQATEKCIDLLKEHAPIFYE--EYNGEVDGVWKSNIEDSK-----Q
D. melongaster 98 SSPHRSEALESVSFAIDQLKTRVPIWKKEIYDGDNDSE---WKENKESIRPKKSKGPNYAACPCKVEESHVPRTLVQIRVNDALTKRLECFVNRKRD
A. thaliana    104 SSVHRADGLDACKFLIDELKASVPIWKKEVY--TNGEI---WKENSEFMKRELEAKRDSIVKKTVEEHRRCGCGSKVRVEEEDHKDITGDNKSSS + 146 AAs
E. coli        100 TSAHRSSAFEAGQFIMDYLKRTRAPFWKREATPEGDR---WVEARESDQQAARW---
T. acidophilum 97 SAPHRGQAFDACYIIDQIKERAPIWKRDIVQGSFASW---H-----
    
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Supp. Figure S2. Alignment of the proteins MOCS2A (A) and MOCS2B (B). Conserved amino acid positions are shaded.

Reiss et al., *Human Mutation*

Larin-variant (exon 1a)

1 atggcggcgcggccactgtcccggatgctgcggcggcttctgaggtccagcgcgccggagc
 2 M A A R P L S R M L R R L L R S S A R S 20
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 9 61 tgcagctcaggggctccggtagaccagccctgccccggggagtcgcgcgagctgcctcg
 10 C S S G A P V T Q P C P G E S A R A A S 40
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 13 121 gag..
 14 E 41
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Reiss-variant (exon 1b and c)

1 atgtggaagagttggaagctccgcacagatgtcagagtaagggagggggcagggcggttct
 2 M W K S W K L R T D V R V R E G A G G S 20
 3
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 5
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 7 61 ccttgtgcctcttcccagcccggtagcaggggcccattgcttccctcctgggtctgtcctcg
 8 P C A S S Q P G S R G P C F L P G L S S 40
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 13 121 cag..
 14 Q 41
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Both variants (exons 1d - 10)

124 ...gaggtgtccagggcggaggcagttcctgcgggagcatgcggcccccttctccgccttc
 1 E V S R R R Q F L R E H A A P F S A F 60
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 3
 4 181 ctcacagacagcttcggccggcagcacagctacctgcggatctccctcacagagaagtgc
 5 L T D S F G R Q H S Y L R I S L T E K C 80
 6
 7
 8 241 aacctcagatgtcagtactgcatgccccgaggaggggtcccgctgacccccaaagccaac
 9 N L R C Q Y C M P E E G V P L T P K A N 100
 10
 11
 12 301 ctgctgaccacagaggagatcctgaccctcgcccggctctttgtgaaggaaggcatcgac
 13 L L T T E E I L T L A R L F V K E G I D 120
 14
 15
 16 361 aagatccggctcacaggtggagagccgcttatccggccggacgtggtggacattgtggcc
 17 K I R L T G G E P L I R P D V V D I V A 140
 18
 19
 20 421 cagctccagcggctggaagggctgagaaccataggtggtaccaccaatggcatcaacctg
 21 Q L Q R L E G L R T I G V T T N G I N L 160
 22

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 2
 3 481 gcccggctactgccccagcttcagaaggctgggtctcagtgccatcaacatcagcctggac
 4 A R L L P Q L Q K A G L S A I N I S L D 180
 5
 6 541 accctgggtgcctgccaagtttgagttcattgtccgcaggaaaggcttccacaagggtcatg
 7 T L V P A K F E F I V R R K G F H K V M 200
 8
 9 601 gagggcatccacaaggccatcgagctgggctacaaccctgtgaagggtgaactgtgtgggtg
 10 E G I H K A I E L G Y N P V K V N C V V 220
 11
 12 661 atgcgaggccttaacgaggatgaactcctggactttgcggccttgactgagggcctcccc
 13 M R G L N E D E L L D F A A L T E G L P 240
 14
 15 721 ctggatgtgcgcttcatagagtatatgccctttgatggcaacaagtggaacttcaagaag
 16 L D V R F I E Y M P F D G N K W N F K K 260
 17
 18 781 atggctcagctataaggagatgctagacactgtccggcagcagtgggccagagctggagaag
 19 M V S Y K E M L D T V R Q Q W P E L E K 280
 20
 21 841 gtgccagaggaggaatccagcacagccaaggcctttaaataccctggcttccaaggccag
 22 V P E E E S S T A K A F K I P G F Q G Q 300
 23
 24 901 atcagcttcatcacatccatgtctgagcatttctgtgggacctgcaaccgctgccaatc
 25 I S F I T S M S E H F C G T C N R L R I 320
 26
 27 961 acagctgatgggaacctcaaggtctgcctctttggaaactctgaggtatccctgcgggat
 28 T A D G N L K V C L F G N S E V S L R D 340
 29
 30 1021 cacctgagctggggcctctgagcaggagctgctgagaatcattggggctgctgtgggc
 31 H L R A G A S E Q E L L R I I G A A V G 360
 32
 33 1081 aggaagaagcggcagcatgcaggcatgttcagatatttcccagatgaagaaccggcccatg
 34 R K K R Q H A G M F S I S Q M K N R P M 380
 35
 36 1141 atcctcatcgggtgggtgacccatcaagttatTTTTGATGTTCCCAATTCCCCACCAGCC
 37 I L I G G - 385 (end of MOCS1A in spliceform I)
 38
 39 / / (spliced out in spliceform II for MOCS1AB)
 40
 41 384 E L F L M F P N S P P A 395
 42
 43 1201 aatccaagcattttctcctgggacccgctccatgttcagggtctaagaccagaatgagt
 44 N P S I F S W D P L H V Q G L R P R M S 415
 45
 46 1261 ttctccagccaggtggccactttatggaaaggatgcagggtccccagaccctcctcta
 47 F S S Q V A T L W K G C R V P Q T P P L 435
 48
 49 1321 gccagcagcggctggggctctggctcctttcagagacactacacttcccgtgcagactca
 50 A Q Q R L G S G S F Q R H Y T S R A D S 455
 51
 52 1381 gatgccaactcaaagtgcttagcccagggttctgggcttctgctgccccctcaggacc
 53 D A N S K C L S P G S W A S A A P S G P 475
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 3 1441 cagctaacctcagaacaactaactcatgtggactcgggaaggacgggcagctatggtagat
 4 Q L T S E Q L T H V D S E G R A A M V D 495
 5
 6 1501 gtgggcaggaagccagacacagagcgggtggctgtggcttcagccgtggctcctcctggga
 7 V G R K P D T E R V A V A S A V V L L G 515
 8
 9 1561 ccggtagccttcaagcttgtccagcagaaccagctcaagaaaggagatgccctagtgggtg
 10 P V A F K L V Q Q N Q L K K G D A L V V 535
 11
 12 1621 gccagctggctggagtccaggcagccaaggtgaccagccagctgatccctctgtgccac
 13 A Q L A G V Q A A K V T S Q L I P L C H 555
 14
 15 1681 cacgtggccctgagccacatccaggtgcagctggagctggacagcacacgccatgccgtg
 16 H V A L S H I Q V Q L E L D S T R H A V 575
 17
 18 1741 aagatccaggcatcttgccgggctcggggccccaccggggtggagatggaggccctgacc
 19 K I Q A S C R A R G P T G V E M E A L T 595
 20
 21 1801 tctgctgcagtgccgcctcaccctgtatgacatgtgcaaggctgtcagcagggacatc
 22 S A A V A A L T L Y D M C K A V S R D I 615
 23
 24 1861 gtggtggaggagatcaagctcattagcaagactgggtggtcagcggggggacttccatcgg
 25 V L E E I K L I S K T G G Q R G D F H R 635
 26
 27 1921 gcttag
 28 A - 636 (end of MOCS1AB)
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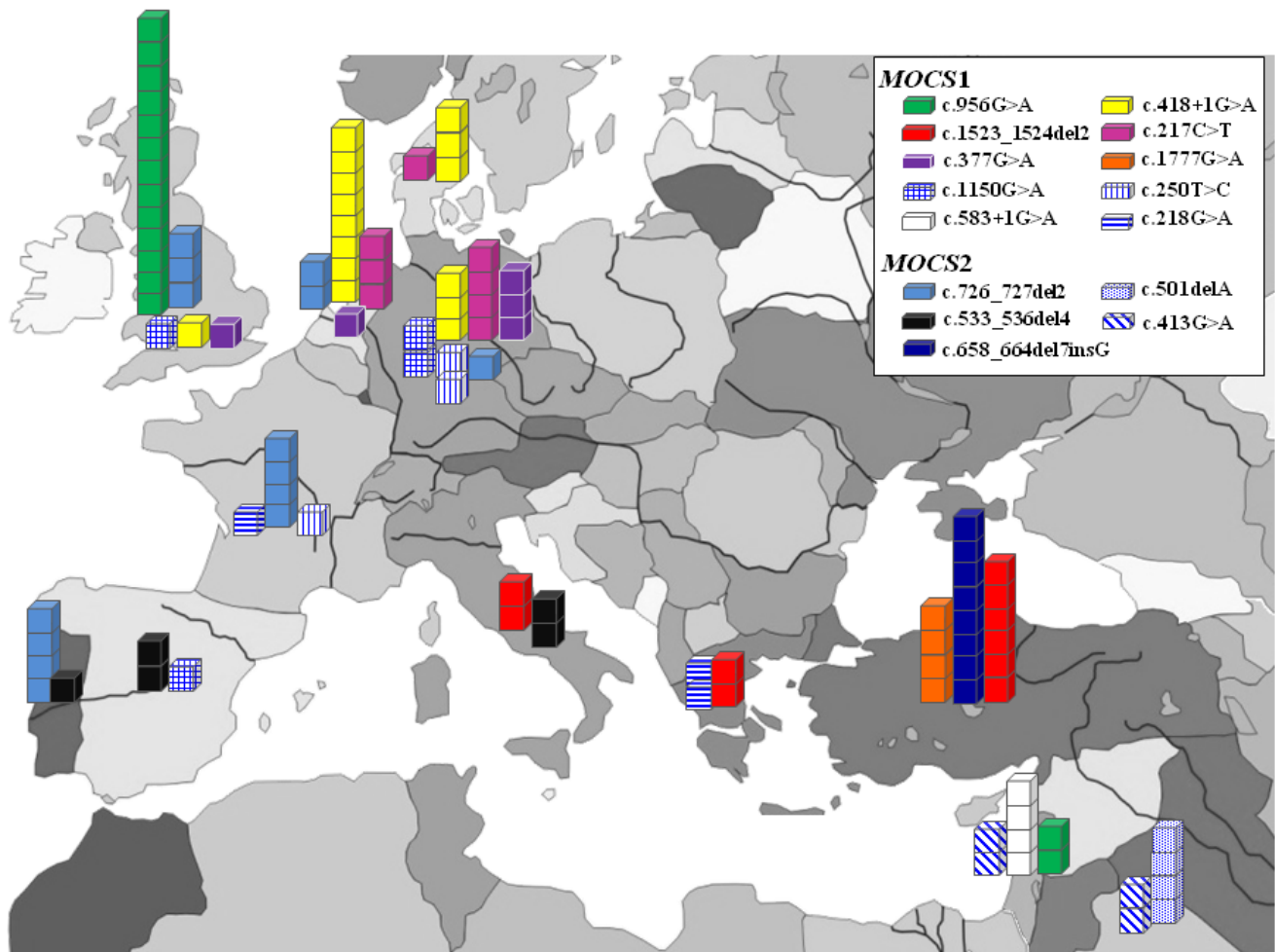
Supp. Figure S3. Sequences of the *MOCS1* cDNA and the proteins MOCS1A and MOCS1AB. Nucleotide numbering refers to the full length transcript including the complete exon 9 (spliceform I). Mutations in exon 1a should be explicitly marked “Larin variant” since they are not included in the reference sequence (GenBank AJ224328.1). Mutations in exon 10 affect the MOCS1AB fusion protein, which should be specified in the nomenclature for the predicted effect.

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2
3 1 atggtgccgctgtgccaggttgaagtattgtatTTTTGcaaaaagtgctgaaataacagga
4 M V P L C Q V E V L Y F A K S A E I T G 20
5
6 61 gttcgttcagagaccatttctgtgcctcaagaaataaaagcgttgcagctgtggaaggag
7 V R S E T I S V P Q E I K A L Q L W K E 40
8
9
10 121 atagaaactcgacatcctggattggctgatgtagaaatcagataatatttgctgttctgt
11 I E T R H P G L A D V R N Q I I F A V R 60
12
13 181 caagaatatgtcgagcttggagatcagctcctcgtgcttcagcctggagacgaaattgcc
14 Q E Y V E L G D Q L L V L Q P G D E I A 80
15 (MOCS2B) M S S L E I S S S C F S L E T K L P 18
16 241 gttatccccccattagtgaggatagtgcttttgagccatctaggaaagatatggatga
17 V I P P I S G G - 88 (end of MOCS2A)
18 L S P P L V E D S A F E P S R K D M D E 38
19
20 301 agttgaagagaaatctaaagatgttataaactttactgccgagaaactttcagtagatga
21
22 V E E K S K D V I N F T A E K L S V D E 58
23
24 361 agtctcacagttggtgatttctccgctctgtggtgcaatatccctattttgtagggactac
25
26 V S Q L V I S P L C G A I S L F V G T T 78
27
28 421 aagaaataactttgaagggaaaaaagtcattagcttagaataatgaagcatatctacccat
29
30 R N N F E G K K V I S L E Y E A Y L P M 98
31
32 481 ggcgaaaatgaagtcagaaagatttgtagtgacattaggcagaaatggccagtcaaaca
33
34 A E N E V R K I C S D I R Q K W P V K H 118
35
36 541 catagcagtgttccatagacttggcttggttccagtgctcagaagcaagcataatcattgc
37
38 I A V F H R L G L V P V S E A S I I I A 138
39
40 601 tgtgtcctcagcccacagagctgcatctcttgaagctgtgagctatgccattgatacttt
41
42 V S S A H R A A S L E A V S Y A I D T L 158
43
44 661 aaaagccaaggtgcccatatggaaaaaggaaatatacgaagagtcactcaacttggaaagg
45
46 K A K V P I W K K E I Y E E S S T W K G 178
47
48 721 aaacaaagagtgcTTTTGGGCATCCAACAGTTAA
49
50 N K E C F W A S N S - 188
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Supp. Figure S4. Sequences of the *MOCS2* cDNA (spliceform I) and the proteins MOCS2A (upper one letter code) and MOCS2B (lower one letter code).



Supp. Figure S5. Geographical distribution of MOCS1 and MOCS2 mutations found in more than one family within Europe. Each box represents one allele. For each family only one index patient was counted.