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Molecular Basis of Neurodegeneration and Neurodevelopmental Defects in Menkes Disease

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Abstract

ATP7A mutations impair copper metabolism resulting in three distinct genetic disorders in humans. These diseases are characterized by neurological phenotypes ranging from intellectual disability to neurodegeneration. Severe ATP7A loss-of function alleles trigger Menkes disease, a copper deficiency condition where systemic and neurodegenerative phenotypes dominate clinical outcomes. The pathogenesis of these manifestations has been attributed to hypoactivity of a limited number of copper-dependent enzymes, a hypothesis that we refer as the oligoenzymatic pathogenic hypothesis. This hypothesis, which has dominated the field for 25 years, only explains some systemic Menkes phenotypes. However, we argue that this hypothesis does not fully account for the Menkes neurodegeneration or neurodevelopmental phenotypes. Here, we propose revisions of the oligoenzymatic hypothesis that could illuminate the pathogenesis of Menkes neurodegeneration and neurodevelopmental defects through unsuspected overlap with other neurological conditions including Parkinson's, intellectual disability, and schizophrenia.

Genetic defects in the trans-Golgi copper-transporter P-ATPase, ATP7A, cause three distinct X-linked recessive disorders: occipital horn syndrome (OMIM 304150), spinal muscular atrophy, distal, X-linked 3 (SMAX3, OMIM 300489), and Menkes disease (OMIM 309400)(Kaler, 2011). More than 350 different mutations affecting the *ATP7A* gene have been described (Moller et al., 2009; Tumer, 2013). These disease-associated mutations are quite heterogeneous in their genomic location and the type of DNA defect and, unlike other genetic disorders, there are not recurrent genetic defects that account for a significant number of cases (Tumer, 2013). Milder mutations in ATP7A result in occipital horn syndrome in which connective tissue and bone abnormalities predominate and patients lack the severe neurological phenotypes of Menkes disease (Das et al., 1995; Kaler et al., 1994). Yet another ATP7A-related disease is SMAX3, in which missense mutations not severe enough to perturbate systemic copper status cause a non-demyelinating spinomuscular

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Conflict of Interests

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atrophy (Kennerson et al., 2010; Takata et al., 2004). At the far end of the spectrum is Menkes disease in which the most severe loss-of-function mutations result in a multisystemic metabolic disorder of copper deficiency. Here we focus in Menkes disease, first described in 1962 in a single family that in two generations accumulated five male infants affected by intellectual disability, failure to thrive, prominent neurological manifestations, neurodegeneration, epilepsy, and ‘peculiar white hair’ (Menkes et al., 1962). Menkes disease is a rare affliction with an incidence of 1/140,000 to 1/300,000 (Gu et al., 2005; Tonnesen et al., 1991). Although this disease has been studied for more than 50 years and its metabolic foundations are known (Kaler, 2011; Menkes, 1988), we contend that the pathogenic mechanisms underlying neurodegeneration and neurodevelopmental defects remain poorly understood. In this review, we explore neuropathogenic hypotheses and argue that some of the classic ideas invoked to explain Menkes disease phenotypes, although logical, remain speculative and inadequate. We propose an updated modified hypothesis in light of newer findings to account for the neurological manifestations of ATP7A loss-of-function mutations.

Our interest in Menkes disease pathogenesis extends beyond this genetic disorder. Because the neurological symptoms associated with Menkes disease are common to other neuropsychiatric disorders of childhood and adulthood (Kaler, 2011; Menkes, 1988), it is increasingly recognized that Menkes disease studies may shed light into the mechanisms of other prevalent disorders. Menkes pathogenesis mechanisms can thus be a tool to understand: a) neuronal mechanisms where copper participates either as a micronutrient or a toxicant; b) pathways of neuronal cell death triggered by altered metabolic homeostasis; c) mechanisms that cells use to respond to neurotoxic anticancer agents such as platinum compounds, which bind to ATP7A (Gregg et al., 1992; Inesi et al., 2014; Liu et al., 2012; Rabik and Dolan, 2007); d) regulatory mechanisms of key receptors and channels involved in neurotransmission and neurodegeneration. These include N-methyl-D-aspartate (NMDA) receptors, voltagegated calcium channels, APP, and the prion protein to mention few (Gaier et al., 2013a; Hung et al., 2010; Kaler, 2011; Stys et al., 2012); and e) mechanisms of development that could account for defective cell positioning observed in Menkes gray matter (Mendelsohn et al., 2006).

Clinical and Pathological Characteristics of Menkes Disease

Menkes disease manifests itself between two to twelve months after birth with hypotonia, failure to thrive, focal and generalized seizures, impaired cognitive development, and brain atrophy at the expense of the gray and white matter. Hypotonia at birth evolves into spastic paresis. Systemic features associated with the disease include the characteristic hypopigmented “kinky hair”, which at the microscopic level reveal pili torti (twisted hairs), monilethrix (beaded hairs) and thickening or weak nodes that cause hair fragility (trichorrhexis nodosa). In addition, affected Menkes infants exhibit sagging facial appearance, micrognathia and arched palate, laxity of the skin (cutis laxa) and joints, reduced bone density, bladder diverticula, aneurysms, vascular tortuosity, and bluish irises. This constellation of clinical features permits a high confidence of Menkes diagnosis when associated with serum copper deficiency and X-linked recessive transmission (Bankier,

1995; Gu et al., 2012; Gu et al., 2005; Kaler, 2011; Kodama et al., 2012; Menkes, 1988; Menkes et al., 1962; Prasad et al., 2011).

Menkes Disease Neuropathology

Menkes is characterized by widespread atrophy of the gray and white matter. At the light microscopic level there is focal degeneration that extends to all layers of the cerebral cortex. Neuronal cell loss is most pronounced in the cerebral cortex but affects hippocampus, striatum, hypothalamus and thalamus to a variable degree. In the cerebral cortex neuronal cell loss is commonly associated with astrogliosis (Barnard et al., 1978; Ghatak et al., 1972; Hirano et al., 1977; Menkes et al., 1962; Vagn-Hansen et al., 1973).

The cerebellum also shows astrogliosis, although this is more variable compared to the cerebrum. The cerebellum also exhibits marked atrophy in Menkes patients, a feature that is also observed in copper deficient animals giving rise to enzootic ataxia (Suttle, 2012). The Menkes cerebellum also shows scattered loss of Purkinje cells and pronounced reductions in neuronal numbers in the molecular and granular layers. The most prominent Purkinje cell phenotypes are defective cell positioning or heterotopia and abnormal cell architecture (Figure 1) (Ghatak et al., 1972; Hirano et al., 1977; Menkes et al., 1962; Vagn-Hansen et al., 1973). The heterotopia is characterized by irregular alignment of Purkinje cells and displacement within the molecular and granular layers of the cerebellar cortex (Barnard et al., 1978; Ghatak et al., 1972; Hirano et al., 1977; Menkes et al., 1962; Purpura et al., 1976; Vagn-Hansen et al., 1973). In addition, Purkinje cell dendrites are markedly swollen with an aberrant pattern of dendritic arborization (Figure 1) (Hirano et al., 1977; Purpura et al., 1976; Yamano and Suzuki, 1985). Sprouts directly extending from the Purkinje cell body, some of which resemble spines, appear less frequently with the age of the individual (Hirano et al., 1977). In addition to architectural phenotypes, the cell bodies of Purkinje cells contain clusters of mitochondria, which possess dense granules in their matrix and altered cristae (Iwane et al., 1990; Nagara et al., 1980; Onaga et al., 1987; Yoshimura and Kudo, 1983). This mitochondrial ultrastructure is important as it provides a morphological correlate to mitochondrial enzymatic defects, which has been postulated to underlie the neurodegeneration. However, despite their prominent mitochondrial ultrastructural pathology, the overall neurodegeneration of Purkinje cells is mild (Nagara et al., 1980; Yajima and Suzuki, 1979). This argues to factors in addition to mitochondria in the pathogenesis of Menkes disease neurodegeneration.

The emphasis on the neuropathology of Menkes disease has focused on the neurodegeneration associated with this disorder. However, it is important to emphasize that Menkes brains in humans or mice possess Purkinje cell heterotopy, abnormal neuroblast migration, and altered neuronal arborization (El Meskini et al., 2007; Hirano et al., 1977; Niciu et al., 2006; Purpura et al., 1976) (Figure 1). These defects precede neuronal cell death (El Meskini et al., 2007; Kodama et al., 2012; Niciu et al., 2006). The cellular basis of these structural abnormalities observed in Purkinje cells point to defective developmental mechanisms such as cell migration, polarity, and lamination of cortical layers in the cerebellum and possibly in the cortex (Mendelsohn et al., 2006).

Cell Biology of Menkes Disease

Menkes disease is the product of either absent or impaired ATP7A copper pump activity and/or improper subcellular localization (Kaler, 2011; Kim and Petris, 2007; Kim et al., 2002; Kim et al., 2003). The consequence of such defects at the cellular level is an impaired intraluminal Golgi or cytoplasmic copper homeostasis. At low extracellular copper concentrations, wild type ATP7A resides in the trans-Golgi network (TGN) where it pumps copper into the lumen of the trans-Golgi network as a cofactor for copper-dependent apoenzymes (Polishchuk and Lutsenko, 2013). The most studied of these copper-dependent enzymes are tyrosinase, peptidylglycine α -amidating monooxygenase (PAM), dopamine beta hydroxylase (DBH), and lysyl oxidase (LOX), all of which are synthesized within the secretory pathway and loaded with catalytic copper by ATP7A at the trans Golgi network (Kaler, 2011; Lutsenko et al., 2007). Under increased extracellular copper concentration, the resulting increase in cytosolic copper induces ATP7A translocation from the trans-Golgi network to post-Golgi vesicles and the plasma membrane (Lutsenko et al., 2007; Petris et al., 1996; Polishchuk and Lutsenko, 2013). ATP7A facilitates copper extrusion from cells upon fusion of copper-laden vesicles with the plasma membrane and/or by direct pumping of this metal across the plasma membrane. Finally, restoration of the extracellular copper levels to low micromolar levels induces the retrieval of ATP7A from the plasma membrane back to the Golgi apparatus via endosomes (Lutsenko et al., 2007; Pascale et al., 2003; Petris and Mercer, 1999; Petris et al., 1996; Polishchuk and Lutsenko, 2013). This requirement for ATP7A in copper extrusion plays a critical role in supplying the body with copper via transport across the intestinal enterocytes, thus accounting for the severe systemic copper deficiency in Menkes patients. In the CNS, this copper deficiency is exacerbated due to the requirement for ATP7A in copper transport across the choroid plexus and/or blood brain barrier. Thus, the dual functions of ATP7A in copper transport into the secretory pathway and copper efflux provide a reasonable foundation to think that defects in these and other copper-dependent enzymes account, in part, for diverse aspects of the Menkes phenotype (Lutsenko et al., 2007; Pascale et al., 2003; Petris and Mercer, 1999; Petris et al., 1996; Polishchuk and Lutsenko, 2013).

The machinery required for anterograde trafficking of ATP7A from the Golgi is not fully understood. However, the retrieval of ATP7A via endosomes requires cytosolic trafficking complexes which include; clathrin, rab22, the clathrin adaptor complex AP-1, retromer, the WASH complex and possibly the BLOC-1 complex (Hirst et al., 2012; Holloway et al., 2013; Ryder et al., 2013; Steinberg et al., 2013). In particular, the WASH complex is part of a mechanism that also requires a complex of proteins that include COMMD1, CCDC22, CCDC93, and C16orf62 (the CCC complex) for endosome to Golgi retrieval of ATP7A (Phillips-Krawczak et al., 2014). Among these CCC complex components, evidence supports a role for COMMD1 controlling ATP7A levels in cultured cells and COMMD1 mutations cause autosomal recessive copper toxicosis in Bedlington terrier dogs (Materia et al., 2012; van de Sluis et al., 1999; Vonk et al., 2012). Mutations in AP-1 σ 1A subunit cause the MEDNIK syndrome in humans (Martinelli and Dionisi-Vici, 2014; Martinelli et al., 2013; Montpetit et al., 2008), a distinctive syndrome with abnormalities in copper homeostasis that can be attributed, in part, to aberrations in AP-1-dependent steps in ATP7A

trafficking. In contrast, copper content is normal in the brain of BLOC-1 deficient mice but at the expense of diverse molecular adaptations in copper homeostatic pathways (unpublished). Although the mechanisms by which these trafficking protein complexes are coordinated for the retrieval of ATP7A to the Golgi has not been elucidated, their connection with ATP7A trafficking offers new ways to understand mechanisms of Menkes neurodegeneration and neurodevelopmental abnormalities. For example, mutations that affect the retromer complex have been shown to cause certain cases of Parkinson's disease, a neurodegenerative disorder (McGough et al., 2014; Steinberg et al., 2013; Zavodszky et al., 2014). Moreover, mutations in AP-1 and the BLOC-1 complex are thought to contribute to neurodevelopmental disorders, such intellectual disability and schizophrenia (Borck et al., 2008; Montpetit et al., 2008; Mullin et al., 2011). Thus, because the proteins and mechanisms that control ATP7A trafficking appear to overlap with those of Parkinson's and other neurodevelopmental disorders, by extension these disorders could be caused in part by abnormal copper homeostasis.

The Oligoenzymatic Pathogenic Hypothesis of Menkes Disease

Is the Menkes neuropathology due to nutritional copper depletion or an intrinsic lack of ATP7A in neurons? Menkes disease neuropathology is recapitulated by conditional deletion of ATP7A in the gut (Wang et al., 2012). This powerful evidence argues that copper depletion in the brain leads to Menkes neuropathology. Menkes neurological manifestations have been ascribed to five enzymes expressed in brain that require copper for their function. Presently, these enzymes include mitochondrial cytochrome oxidase C, and four enzymes that acquire copper in the Golgi apparatus: PAM, DBH, LOX, and tyrosinase (Bankier, 1995; Kaler, 2011; Prasad et al., 2011). We refer to this hypothesis as the 'oligoenzymatic hypothesis'.

The oligoenzymatic hypothesis was born in 1988 and its appeal was immediate due to its explanatory power for some of the disease features (Menkes, 1988). Defective tyrosinase activity explains the hypopigmentation observed in Menkes patients but not the neurological phenotypes (Table 1). Similarly, hypoactivity of LOX family members provides an explanation for the vascular defects, cutis laxa, and diverticula in Menkes patients (Kaler, 2011; Lutsenko et al., 2007; Polishchuk and Lutsenko, 2013). LOX initiates the crosslinking of collagens and elastin catalyzing oxidative modification of lysine residues present collagens and elastin. Some of the systemic Menkes phenotypes are recapitulated by mouse *Lox* null alleles. However, none of these *Lox* mutant mice are known to present with neuroanatomical alteration (Table 1) (Hornstra et al., 2003; Maki et al., 2002; Maki et al., 2005). In contrast, LOX propeptide excess regulates Purkinje cell development, thus a role for LOX in Purkinje dysfunction in the Menkes brain cannot be excluded (Li et al., 2010).

PAM, DBH, and mitochondrial cytochrome oxidase C are frequently invoked to explain the neurological manifestation in Menkes (Bankier, 1995; Lutsenko et al., 2007; Polishchuk and Lutsenko, 2013; Prasad et al., 2011). PAM and DBH participate in the synthesis of neurotransmitter and neuropeptides (Bousquet-Moore et al., 2010; Stewart and Klinman, 1988), yet there is little evidence that they alone account for neurodegeneration and neurodevelopmental abnormalities in Menkes patients or mouse models. Mice homozygous

for null alleles of PAM and DBH enzymes neither recapitulate the neurodevelopmental nor the neurodegenerative phenotypes observed in Menkes though the mice die in utero (Czyzyk et al., 2005; Thomas et al., 1995) (Table 1). Consistent with these observations, embryonic lethality caused by DBH deficiency in mice can be rescued by systemic administration of L-threo-dihydroxyphenylserine, and while this treatment corrects brain neurochemical abnormalities in Menkes mice, it does not correct neurodegeneration (Donsante et al., 2013). PAM haploinsufficiency impairs long-term potentiation without neurodegeneration or abnormalities in cortical development (Gaier et al., 2010). PAM haploinsufficiency selectively alters copper content in the amygdala (Gaier et al., 2014; Gaier et al., 2013b). This suggests that PAM could contribute to Menkes disease in an anatomically restricted manner.

The participation of mitochondrial mechanisms in Menkes disease neurodegeneration is appealing as Menkes patients and Atp7a mutant mouse models have ultrastructural alterations in mitochondria (Iwane et al., 1990; Nagara et al., 1980; Onaga et al., 1987; Yoshimura and Kudo, 1983). These observations have been linked to impaired function of the mitochondrial cytochrome oxidase C complex (complex IV). Cytochrome oxidase C is a 13-subunit copper-dependent complex necessary for oxidative phosphorylation (Saraste, 1999). Decreased content of cytochrome oxidase C subunits have been frequently reported in human cells in culture and mouse tissues affected by Menkes (Hunt, 1977; Kodama et al., 1989; Kumode et al., 1994; Kunz et al., 1999; Kuznetsov et al., 1996; Maehara et al., 1983; Meguro et al., 1991; Rezek and Moore, 1986; Rossi et al., 2001; Seki et al., 1989; Sparaco et al., 1993; Yoshimura et al., 1990). This is in contrast with our quantitative mass spectrometry studies performed in cultured Menkes fibroblasts. We have found that there is no appreciable difference in the levels of nearly 80 mitochondrial proteins including complex IV subunits in the Menkes mitochondrial proteome (unpublished data). The most consistent finding concerning complex IV is a reduction in the cytochrome oxidase C enzymatic activity (Hunt, 1977; Kodama et al., 1989; Kunz et al., 1999; Kuznetsov et al., 1996; Maehara et al., 1983; Meguro et al., 1991; Seki et al., 1989). Despite this decreased activity, the metabolism of high-energy phosphate compounds, such as ATP, does not seem to be affected in mouse cells lacking Atp7a (Kunz et al., 1999; Kuznetsov et al., 1996). Thus, it is possible that redox alterations rather than triphosphate nucleotide depletion may account for part of the neurological phenotypes. Do mutations in complex IV phenocopy aspects of Menkes disease? Genetic defects affecting the assembly or function of the cytochrome oxidase C complex subunits generate diverse disorders. These disorders span from isolated myopathy to severe multisystem disease such as infantile cardioencephalomyopathy and Leigh syndrome. This spectrum of genetic disorders encompasses defects in mitochondria-rich tissues such as heart and skeletal muscle, severe neurodegeneration, and lactic acidosis (OMIM: 256000, 220110, 604377) (DiMauro et al., 2012; Shoubridge, 2001). While mitochondrial disorders (e.g. Leigh's or Alper's) result in similar neurological defects to Menkes disease, and underscore the likely contribution of mitochondrial dysfunction to some aspects of Menkes neurodegeneration phenotypes, the constellation of neurological Menkes manifestations points to the contribution of non-mitochondrial defects in the neuropathology of this disorder.

These observations challenge the oligoenzymatic pathogenic hypothesis, which has remained unchanged for nearly 25 years (Bankier, 1995; Kaler, 2011; Prasad et al., 2011).

Proposed Revisions to the Oligoenzymatic Hypothesis

The oligoenzymatic hypothesis seeks to link ATP7A copper-sensitive targets to disease manifestations. However, the oligoenzymatic hypothesis alone may not adequately explain neurodegeneration and neurodevelopmental phenotypes due to the paucity of copper-sensitive targets that it considers. We propose that simply considering ontological categories to which these few enzymes belong can enhance the oligoenzymatic hypothesis. Cytochrome C oxidase, PAM, SOD3, DBH, LOX, and tyrosinase are part of the GO term GO:0005507, which defines gene products capable of copper ion binding (<http://amigo.geneontology.org/amigo/landing>). This ontological category encompasses 56 gene products in humans and mice (Table 1). This category includes many proteins documented to require copper for their activity and accordingly, their hypoactivity could directly contribute to disease pathogenesis. Analysis of these 56 copper binding proteins using the mouse genomic informatics server (<http://www.informatics.jax.org/>) shows that fundamental Menkes disease phenotypes can be identified using a bottom-up approach. For example, these 56 proteins predict as phenotypes hypopigmentation (MP0005408), abnormal blood vessel (MP0001614), abnormal skin tensile (MP0005275), and neurodegeneration (MP0002229) (Table 2). Among the neurodegeneration group MP0002229, there are several interesting gene products to consider in the pathogenesis of neurodegeneration and neurodevelopmental phenotypes: PRNP which encodes the prion protein; SOD1; and SNCA, encoding alpha synuclein (Table 2). Searching the OMIM database with the 56 copper binding proteins offers additional insight (Table 3, <http://www.ncbi.nlm.nih.gov/omim/>). This search identifies Parkinson's disease and other dementias with significant association and commonalities to Menkes disease. However, data mining misses some suggestive molecules. Take for example Apoa4, Gpc1, Rnf7, and Sco2. The evidence supporting copper-binding by some of these molecules is either incipient or indirect, yet when mutated in mice they produce diverse neurological manifestations (Table 1) (Chen and Chan, 2012; Cheng et al., 2006; Leary, 2010).

The bioinformatics analyses presented here rest on the assumption of a copper requirement for all downstream targets of ATP7A. In addition to these copper sensitive targets, there may be ATP7A downstream effectors that are copper independent that contribute to disease. For example, proteins whose stability requires ATP7A polypeptides instead of their pump activity or molecules indirectly connected to ATP7A whose expression/activity is dependent on a copper-sensitive intermediary. Unbiased studies of the proteomes and transcriptomes of diverse Menkes mutations will provide insight into the existence of these copper independent effectors.

Conclusions

Menkes neurological and neurodevelopmental manifestations have been attributed to defects in a select group of enzymes that require copper. However, knowledge gained from genetic experiments affecting these enzymes indicates that in isolation they are insufficient to

account for the neurological manifestations in Menkes. We propose a revised version of the oligoenzymatic hypothesis that includes all molecules in the copper-binding ontological category and molecules that may be sensitive to ATP7A content rather than cellular copper content. This revised hypothesis suggests intriguing connections between Menkes and Parkinson's disease (SCNA), risk factors for Alzheimer's (APOE4), and neurodevelopmental disorders. We postulate that regional differences in the neuropathology of Menkes, as those observed between the cerebral and cerebellar cortex, emerge from varying degrees of impairment in molecular networks downstream of ATP7A, including cell migration, polarity, and survival.

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Figure 1. Branching abnormalities of cerebellar cortex Purkinje cells

Human tissue was processed with Golgi stain. Panels depict camera lucida drawings of Purkinje cells. Note the dendritic systems of Purkinje cells in Menkes' disease are atrophic and lack organized tertiary branches. Modified from Purpura et al. 1976 (Purpura et al., 1976).

Table 1

Copper Binding Gene Products Define by Gene Ontology

NRP is the acronym for no reported phenotype.

Mouse Gene	MGI Entry Number	MGI Phenotypes	OMIM Entry Number	OMIM Phenotypes
Aoc1	MGI:1923757	NRF		
Adnp	MGI:1338758	Developmental defects. Failure of the cranial neural tube to close. Embryonic death between E8.5 and E9		
Alcy	MGI:87968	NRF	613752	failure to thrive, mental and motor retardation, facial dysmorphism with abnormal hair and teeth, and myocardiopathy
Ang	MGI:88022	NRF	611895	ALS9 Parkinsonism
Aoc1				
Aoc2	MGI:2668431	NRF		
Aoc3	MGI:1306797	Homozygous null display decreased lymphocyte migration and homing in response to inflammation		
Apoa4	MGI:88051	Homozygous null have neurodegeneration, lower HDL cholesterol levels but normal lipid absorption, growth, and feeding behavior.		
Atox1	MGI:1333855	Homozygote null mutation have impaired intracellular copper trafficking and exhibit high postnatal mortality, retarded growth, hypoactivity, loose skin, hypopigmentation, and seizures		
Atp7a	MGI:99400		309400	
Atp7b	MGI:103297	Copper accumulation in various organs, including brain. Liver cirrhosis that resembles Wilson disease in humans and the 'toxic milk' phenotype in mice	277900	Wilson disease
Commd1	MGI:109474	Embryonic lethal with growth retardation, failure to turn, increased apoptosis in brain mesenchyme and defects in extraembryonic tissue development		
Cox11	MGI:1917052	NRF		
Cox17	MGI:1333806	Homozygous null are growth retarded and die between E8.5 and E10, with severe reductions in cytochrome c oxidase activity at E6.5		
Cp	MGI:88476	Accumulation of iron in the liver, spleen, cerebellum, and brainstem, mild iron deficiency anemia, and impaired motor coordination associated with loss of brainstem dopaminergic neurons.	604290	dementia and diabetes mellitus, chorea, and ataxia
Cr11	GI:88513	die by E16.5 with abnormal C3 deposition		
Cutc	MGI:1913638	NRF		
Dct	MGI:102563	Pigmentation defect	191275	

Mouse Gene	MGI Entry Number	MGI Phenotypes	OMIM Entry Number	OMIM Phenotypes
Dbh	MGI:94864	Embryonic lethal probably due to cardiovascular failure	223360	orthostatic hypotension, ptosis, nasal stuffiness, and a neonatal history of delayed eye opening, skeletal muscle hypotonia
F5	MGI:88382	50% of homozygous null allele die at E9-E10 with defects in yolk-sac vasculature and somite formation; the remaining half develop to term but die of massive hemorrhage within hours of birth.		
F8	MGI:88383	Prolonged, exsanguinating bleeding following tail-clipping	306700	hemophilia A
Gpc1	MGI:1194891	Reduced brain size with mild cerebellar patterning defects. Otherwise viable and fertile		
Heph	MGI:1332240	Small and pale at birth, exhibit a hypochromic anemia which tends to disappear with age. Mutants have impaired iron transport in the placenta and in the gut.		
Heph11	MGI:2685355	NRF		
Il1a	MGI:96542	Development of Th2 helper cell responses and some antibody responses are compromised		
Lox	MGI:96817	Altered arterial wall structure, aortic aneurysms, cardiovascular dysfunction, diaphragmatic hernia, and perinatal death. Abnormal development of the respiratory system, and elastic and collagen fiber abnormalities in the lung and skin are also observed		
Lox11	MGI:106096	Elastic fiber homeostasis is disrupted, loose skin, abnormal lung morphology, intestinal defects, and post partum uterine prolapse		
Lox12	MGI:2137913	NRF		
Lox13	MGI:1337004	NRF		
Lox14	MGI:1914823	NRF		
Mett11d1	MGI:1098577	NRF		
Mett117	MGI:1098577	NRF		
Moxd1	MGI:1921582	NRF		
Moxd2	MGI:2388042	NRF		
mt-Co2	MGI:102503	NRF	540000	mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, is a genetically heterogeneous mitochondrial disorder with a variable clinical phenotype. The disorder is accompanied by features of central nervous system involvement, including seizures, hemiparesis, hemianopsia, cortical blindness, and episodic vomiting
Mt1	MGI:97171	Abnormal zinc absorption and abnormal circadian rhythm response to melatonin. Mice homozygous for null alleles of Mt1 and Mt2 exhibit increased sensitivity to xenobiotics and injury with decreased wound healing and abnormal mineral absorption.		

Mouse Gene	MGI Entry Number	MGI Phenotypes	OMIM Entry Number	OMIM Phenotypes
Mt3	MGI:97173	Zinc deficiency in several brain regions, abnormal astrocyte morphology, increased susceptibility to kainic acid induced seizures, and altered zinc accumulation and neuronal death in certain brain areas following seizure-induced or acute brain injury		
P2rx2	MGI:2665170	Viable and show no gross pathology. Mice show abnormal ventilatory and electrophysiological responses to hypoxia	608224	autosomal dominant deafness-41
P2rx4	MGI:1338859	Hypertension, abnormal artery morphology, abnormal nitric oxide homeostasis, and impaired flow induced vascular remodeling and vasodilation		
P2rx7	MGI:1339957	Fertile and viable with no obvious phenotypic abnormality. Cellular responses of macrophages to extracellular ATP are frequently normal however. In addition, long bones are thinner than normal in adult mice		
Pam	MGI:97475	Embryonic lethality during fetal growth and development, edema, abnormal yolk sac vasculature, thin arterial walls, and abnormal bronchial epithelial morphology		
Prnd	MGI:1346999	Homozygous null mice display male infertility		
Prnp	MGI:97769	Homozygous mutants also show impaired locomotor coordination and reduced mitochondria numbers with unusual morphology	123400 137440	memory loss, dementia, ataxia, and pathologic deposition of amyloid-like plaques in the brain
Rnf7			600072	insomnia with or without a diurnal dreaming state, hallucinations, delirium, and dysautonomia preceding motor and cognitive deterioration
Rnpep	MGI:2384902	NRF		
S100a13	MGI:109581	NRF		
S100a5	MGI:1338915	NRF		
Sco1	MGI:106362	NRF		
Sco2	MGI:3818630	Null allele exhibit embryonic lethality. Mice heterozygous for a knock-out allele and a knock-in allele exhibit muscle weakness and reduced exercise endurance.	220110	Leigh syndrome, heterogeneous, ranging from isolated myopathy to severe multisystem disease, with onset from infancy to adulthood
Slc11a2	MGI:1345279	Microcytic, hypochromic anemia associated with impaired intestinal iron absorption and erythroblast iron uptake. Mutants have reduced viability and fertility.	206100	hypochromic microcytic anemia with iron overload-1
Snai3				
Sncg	MGI:1277151	Disruptions in this gene display resistance to the effects of MPTP on dopamine levels. Mice expressing a knock-in allele exhibit impaired coordination, long stride length, abnormal response to reserpine and reduced brain dopamine levels.	127750 168601	Parkinson
Sod1	MGI:98351	Homozygous mutants exhibit increased motor neuron loss after axonal injury and enhanced susceptibility to ischemic reperfusion injury. Homozygous females have irregular and small litters, and for some alleles exhibit immature ovarian follicles with few corpora lutea.	105400	ALS1

Mouse Gene	MGI Entry Number	MGI Phenotypes	OMIM Entry Number	OMIM Phenotypes
Sod3	MGI:103181	Mice homozygous for a knock-out allele exhibit increased sensitivity to hyperoxia, increased LPS-stimulated spleen production of TNF, and enhanced severity of collagen-induced arthritis.		
Trp53	MGI:98834	Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.	191170	Multiple neoplasms
Tyr	MGI:98880	Albinism or hypopigmentation. Albinism is associated with reduced number of optic nerve fibers and mutants can have impaired vision	606933	Occulo cutaneous albinism

Table 2
Enrichment Analysis of Mouse Phenotypes Associated to Genetic Defects in the Ontological Category Copper Binding.

Node Name	Corrected P-value Benjamini-Hochberg	Total Genes in Gene Set	Total Genes Intersected	Intersecting Genes
MP0005636_ABNORMAL_MINERAL_HOMEOSTASIS_	1.96E-10	195	10	MT3;ATP7B;MT1;CP;SLC11A2;HEPH;ATOX1;PRNP;ATP7A;SCO2
MP0003632_ABNORMAL_NERVOUS_SYSTEM_	2.01E-05	111	5	ATOX1;CP;PRNP;SNCA;ATP7B
MP0003631_NERVOUS_SYSTEM_PHENOTYPE_	7.06E-05	241	6	DBH;CP;ATOX1;PRNP;SNCA;ATP7B
MP0005408_HYPOPIGMENTATION_	1.30E-04	31	3	ATOX1;ATP7B;TYR
MP0003718_MATERNAL_EFFECT_	4.64E-04	49	3	ATOX1;SLC11A2;ATP7B
MP0002295_ABNORMAL_PULMONARY_CIRCULATION_	6.39E-04	55	3	LOX;ATP7A;ATOX1
MP0001186_PIGMENTATION_PHENOTYPE_	9.35E-04	258	5	ATOX1;ATP7B;TYR;ATP7A;TRP53
MP0005171_ABSENT_COAT_PIGMENTATION_	9.82E-04	14	2	ATP7A;TYR
MP0003186_ABNORMAL_REDOX_ACTIVITY_	8.53E-04	61	3	SOD1;ATP7A;MT1
MP0004147_INCREASED_PORPHYRIN_LEVEL_	1.11E-03	15	2	HEPH;SLC11A2
MP0008438_ABNORMAL_CUTANEOUS_COLLAGEN_	1.39E-03	17	2	LOX;ATP7A
MP0000609_ABNORMAL_LIVER_PHYSIOLOGY_	1.71E-03	442	6	ATP7B;ATP7A;ATOX1;SCO2;HEPH;SLC11A2
MP0001614_ABNORMAL_BLOOD_VESSEL_	1.58E-03	779	8	LOX;TRP53;CP;PAM;P2RX4;ATP7A;IL1A;APOA4
MP0002128_ABNORMAL_BLOOD_CIRCULATION_	1.87E-03	450	6	F5;LOX;TRP53;ATP7A;MT1;ATOX1
MP0005275_ABNORMAL_SKIN_TENSILE_	2.41E-03	23	2	LOX;ATP7A
MP0002229_NEURODEGENERATION_	2.32E-03	318	5	PRNP;ATP7B;CP;SOD1;SNCA
MP00008770_DECREASED_SURVIVOR_RATE_	2.66E-03	196	4	P2RX2;F5;TRP53;TYR
MP0003279_ANEURYSM_	3.24E-03	27	2	ATP7A;LOX
MP0003329_AMYLOID_BETA_DEPOSITS_	3.24E-03	27	2	ATP7B;PRNP
MP0005395_OTHER_PHENOTYPE_	4.46E-03	111	3	ATOX1;SLC11A2;ATP7B

Table 3

Enrichment Analysis of Human Phenotypes Associated to Genetic Defects in the Ontological Category Copper Binding.

Node Name	Corrected P-value Benjamini-Hochberg	Total Genes in Gene Set	Total Genes Intersected	Intersecting Genes
PARKINSON_DISEASE	5.68E-02	100	2	DBH;SNCA
LATERAL_SCLEROSIS	5.39E-02	97	2	ANG;SOD1
CUTIS_LAXA	4.26E-02	85	2	ATP7A;LOX
SKIN/HAIR/EYE_PIGMENTATION	3.04E-01	90	1	TYR
DEMENTIA	3.04E-01	90	1	SNCA
THROMBOPHILIA	3.01E-01	89	1	F5
ALBINISM	2.96E-01	87	1	TYR
WAARDENBURG_SYNDROME	2.93E-01	86	1	TYR
ATAXIA	2.15E-01	60	1	CP