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ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Lysosomal storage disease in the brain: mutations of the β-mannosidase gene identified in autosomal dominant nystagmus

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Purpose: Genetic etiology of congenital/infantile nystagmus remains largely unknown. This study aimed to identify genomic mutations in patients with infantile nystagmus and an associated disease network.

Methods: Patients with inherited and sporadic infantile nystagmus were recruited for whole-exome and Sanger sequencing. β-Mannosidase activities were measured. Gene expression, protein–protein interaction, and nystagmus-associated lysosomal storage disease (LSD) genes were analyzed.

Results: A novel heterozygous mutation (c.2013G>A; p.R638H) of *MANBA*, which encodes lysosomal β-mannosidase, was identified in patients with autosomal-dominant nystagmus. An additional mutation (c.2346T>A; p.L749H) in *MANBA* was found by screening patients with sporadic nystagmus. *MANBA* was expressed in the pretectal nucleus of the developing midbrain, known to be involved in oculomotor and optokinetic nystagmus. Functional validation of these mutations demonstrated a significant decrease of β-mannosidase activities in the patients as well as in mutant-transfected HEK293T cells. Further analysis revealed that nystagmus is present in at least 24 different LSDs involving the brain.

Conclusion: This is the first identification of *MANBA* mutations in patients with autosomal-dominant nystagmus, suggesting a new clinical entity. Because β-mannosidase activities are required for development of the oculomotor nervous system, our findings shed new light on the role of LSD-associated genes in the pathogenesis of infantile nystagmus.

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Key Words: lysosomal storage diseases; MANBA; neuronal ceroid lipofuscinoses; nystagmus; whole-exome sequencing

INTRODUCTION

Congenital/infantile nystagmus is an oculomotor disorder characterized by uncontrollable bilateral ocular oscillations. This disorder is also referred to as “idiopathic,” given that no known nervous or ocular diseases have yet to be found.[1] In fact, approximately half of cases of infantile nystagmus are accompanied by structural or electrophysiological derangements involving central foveal vision in both eyes.[2] For example, foveal hypoplasia and presenile cataract syndrome (OMIM 136520) or oculocutaneous albinism (OMIM 203100), which are caused by mutations in the *PAX6* or the *SLC38A8* gene, respectively, may be accompanied by decreased visual acuity and secondary nystagmus.

In a survey regarding genetic eye diseases among 27,193 Chinese, the prevalence of infantile nystagmus was estimated to be 0.025%,[3] whereas it is 0.24% in Western countries.[2] The most common cause of congenital nystagmus seems to be mutations of the X-linked *FRMD7* gene (NYS1), which also have been detected by us and others in China.[4] Mutations in the *GPR143* gene have been found to be responsible for another X-linked ocular albinism and nystagmus (NYS6). Moreover, at least six susceptibility loci have been mapped from 1996 to 2014, including NYS2 (6p12; OMIM 164100), NYS3 (7p11; OMIM 608345), NYS4 (13q; OMIM 193003), NYS5 (Xp11.4; OMIM 300589), NYS7 (1q31-q32; OMIM 614826), and one additional locus in the X chromosome identified by a recent study.[5] However, the responsible genes in the mapped loci have not been identified.

The genetic etiology and pathogenesis of infantile nystagmus remain largely unknown. Several recent hypotheses have been proposed to explain how sensory deficits could lead to nystagmus generation. One key area of controversy, however, is whether infantile nystagmus subtypes share a common mechanism. Identification of new genes and determination of their cellular functions will therefore advance our understanding of infantile nystagmus.[1]

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Exome sequencing is a transformative technology that produces unbiased genetic data on a whole-genome scale.[6] Identification of disease-causing mutations will provide a basis for determining the molecular pathogenesis of the disease and for the development of effective therapies. To identify potential genetic etiology for these ocular disorders in our hereditary disease program,[4] we applied whole-exome sequencing (WES) and bioinformatics to recently recruited patients with nystagmus. In this study we report both the identification of heterozygous mutations in the *MANBA* gene in patients with infantile nystagmus and the molecular association of nystagmus with lysosomal storage disease (LSDs).

MATERIALS AND METHODS

Patient ethics statement

Informed consent was obtained from each of the patients and, in the case of minors, from their parents. This study and associated research protocols were approved by the ethics committees of the Changzhi Medical College as well as Wenzhou Medical University.

WES and Sanger sequencing

The steps of WES and pathogenesis analysis are shown in **Figure 1**. Genomic DNAs were isolated from peripheral blood leukocytes. Whole-exome capture using the SureSelect Human All Exon Kit (Agilent, Santa Clara, CA) and high-throughput sequencing by the HiSeq2000 sequencer (Illumina, San Diego, CA) were conducted in house, as previously described.[7] The reads were aligned to hg19 and variants identified through the GATK pipeline. All variants were prioritized by allele frequency, conservation, and predicted effect on protein function, and they were tested for segregation with disease. Detected variants—if present in the dbSNP (www.ncbi.nlm.nih.gov/projects/SNP/), HapMap, 1000 Genomes Project (http://www.1000genomes.org/home), and ESP6500 (evs.gs.washington.edu/EVS/), and our in-house Chinese Exome Database (1,300 individuals of Chinese Han nationality)—were all removed. Deleterious single-nucleotide variants (SNVs) were predicted by SIFT (http://sift.bii.a-star.edu.sg/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and MutationTaster (http://www.mutationtaster.org/) programs. Candidate SNVs were validated by the ABI3730 sequencer. Thirty patients with sporadic, idiopathic infantile nystagmus were screened for potential mutation evaluation of the *MANBA* gene by polymerase chain reaction and Sanger sequencing. Exons 1 through 17 of *MANBA*, including intron–exon junctions, were individually amplified by primers (**Supplementary Table S2** online) and bidirectionally sequenced. The 5,499 SNVs and 172 insertions/deletions shared by three patients and normal family members (**Supplementary Tables S3** and **S4** online), 96 SNVs and 3 insertions/deletions presented in only three patients (**Supplementary Tables S5** and **S6** online), and 12 novel SNVs and 1 novel insertion/deletion in three patients (**Supplementary Tables S7 and S8** online) are supplemented.

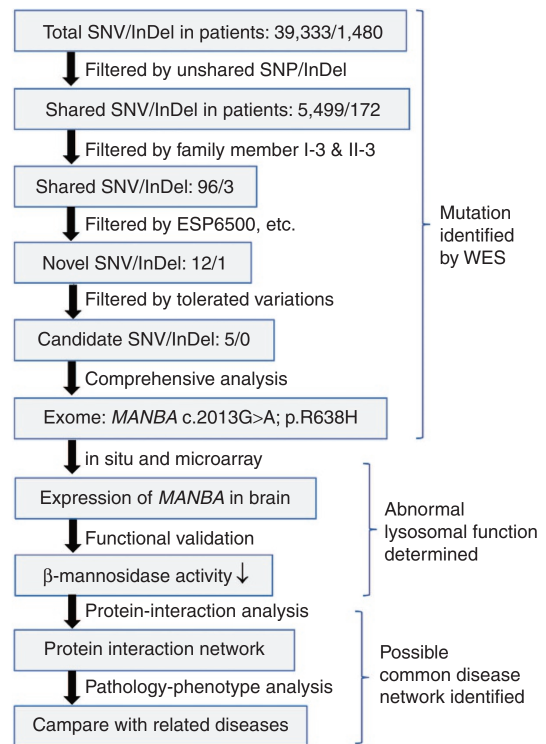

Figure 1 Whole-exome sequencing (WES) and pathogenesis analysis. The schematic illustrates the main steps of WES analysis and gene expression/function studies. Known or nondeleterious single-nucleotide polymorphisms and insertions/deletions were excluded. Causative genes for nystagmus and their potential interactive network are explored by Ingenuity Pathway Analysis. Detailed information of the shared single-nucleotide variants/insertions and deletions detected in the patients is provided in **Supplementary Tables S3–S8** online.

Expression of *MANBA*

The expression data of *MANBA* in prenatal and adult human brains were extracted from the Allen Brain Atlas (http://www.brain-map.org/). Images of in situ hybridization for *Manba* transcriptional expression in mouse embryonic tissues were analyzed and selected from EURExpress and GenePaint (http://www.genepaint.org/Frameset.html). Expression levels of *MANBA* in the optic nerve were assessed in Gene Expression Omnibus profiles (http://www.ncbi.nlm.nih.gov/geoprofiles).

Mutagenesis and western blots

Wide-type *MANBA* complementary DNA (Open Biosystems, Pittsburgh, PA) was mutagenized (Mutagenesis kit; Promega, Madison, WI) to create the c.2013G>A or c.2346T>A mutation in the pcDNA3.1 vector using specific primers (**Supplementary Table S2** online). HEK293T cells were then transfected with 2 μg of wild-type *MANBA* or R638H-*MANBA* or L749H-*MANBA* construct DNA by Lipofectamine 2000 (Invitrogen, Grand Island, NY). Cells were harvested after 48 h of culturing for enzyme activity analyses. Western blots were performed using the ECL Western Blotting System (GE Healthcare Life Sciences, Pittsburgh, PA) and MANBA mouse polyclonal antibody (1:1,000; Abnova, Taipei, Taiwan).

Enzyme assays

β-Mannosidase activity in peripheral blood leukocytes and HEK293T cells was assessed using the human β-mannosidase enzyme-linked immunosorbent assay kit (Yanji Biotech, Shanghai, China). Briefly, leukocytes or HEK293T cells were homogenized by sonication, and protein concentrations were measured by bicinchoninic acid protein assay (Pierce, Rockford, IL). Following modifications of a previous protocol,[8] 10 µl of the cell lysates (2 µg protein) were incubated with 20 µl of 2.0 mmol/l substrate 4-methylumbelliferyl-β-D-mannopyranoside (4MU-β-mannoside; Sigma, St. Louis, MO) in a reaction buffer (0.2 M sodium phosphate/0.1 M citric acid buffer; pH 4.2) at 37 °C for 1 h. After the reaction was stopped with 200 µl of the buffer (0.5 M Na_2CO_3/$NaHCO_3$; pH 10.7), the fluorescence of 4-MU was measured (excitation, 362 nm; emission, 448 nm) using a Multi-label Counter 1420 Microplate Reader (Perkin Elmer, Waltham, MA). All experiments were performed independently at least three times, and each experiment consisted of triplicate samples.

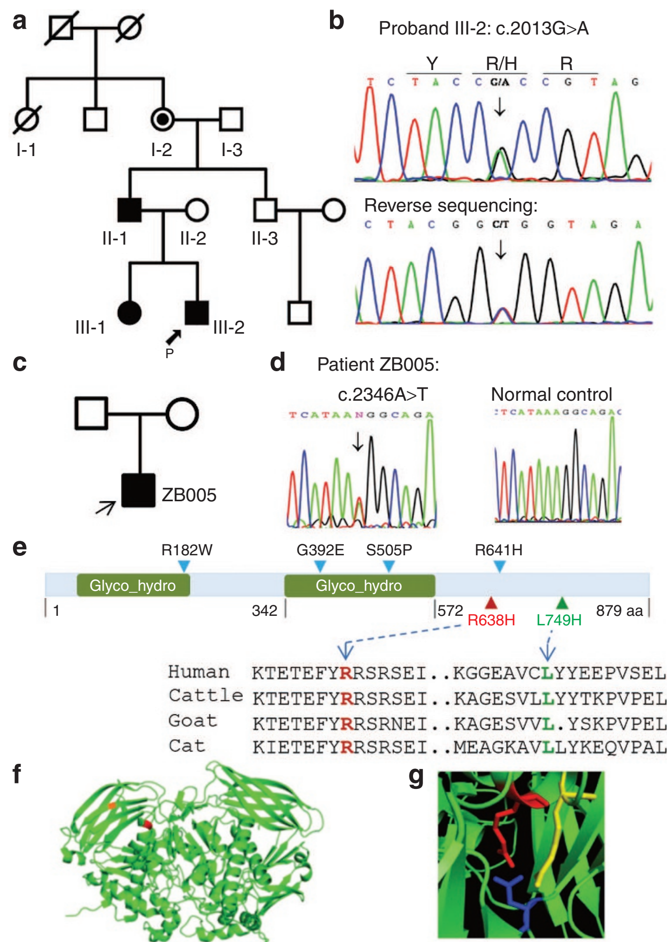

Figure 2 ***MANBA* mutations and β-mannosidase structure.** (**a**) Pedigree of the family. (**b**) The c.2013G>A mutation of *MANBA* is confirmed in all patients and the carrier (I-2) by Sanger sequencing. (**c**) An additional patient (ZB005) with infantile nystagmus. (**d**) The c.2346A>T mutation of *MANBA* is identified. Filled squares or circles represent subjects with nystagmus. The arrows denote the probands. (**e**) The encoded protein with two enzymatic active domains is a member of the glycosyl hydrolase 2 family. Multiple sequence alignment shows the evolutionary conservation of the indicated residues. The β-mannosidosis disorder caused by *MANBA* mutations has been identified in the four species listed. (**f**) The residue R638 (in red) and L749 (in orange) were positioned in the three-dimensional structural model. (**g**) The residue R638 (in red) and R641 (in yellow) are predicted to be associated with D709 (in blue).

Nystagmus in other inherited LSDs

A list of genes that share biological similarity to *MANBA* was generated through careful curation of OMIM (http://www.ncbi.nlm.nih.gov/omim/) gene entries implicated as causative in LSDs involving the brain. Convergent pathogenic pathways were predicted and assessed by protein–protein interaction network (Ingenuity Pathway Analysis; http://www.ingenuity.com/).

RESULTS

Clinical presentations

Three patients with idiopathic infantile nystagmus and reduced visual acuity (i.e., bilateral correctable low vision) in a nonconsanguineous family (HH02) were recruited at Heping Hospital in Northwestern China (**Figure 2a**). The diagnosis was made based on the 10 general features of infantile nystagmus.[9] The patients showed horizontal, conjugate, and pendular nystagmus predominant in the primary position (**Table 1**) since 2–3 months of age. No colorblindness, oscillopsia, vertigo, or imbalance were found. No angiokeratomas, organomegaly, coarse facial features, or dysostosis multiplex were found. No other abnormalities were found during a general physical examination. No other neurologic abnormalities outside of the visual system were identified. Optical coherence tomography, flash electroretinography, visual evoked potential examinations, and brain magnetic resonance imaging did not show any noticeable abnormality in the patients (**Supplementary Figure S3** online).

Table 1 Clinical characteristics of the patients with congenital nystagmus

Subject	Age	Pendular nystagmus	Visual acuity (OD/OS)	Corrected visual acuity (OD/OS)	Fundal examination	Other examination	β-Mannosidase activity (nmol/h/mg)
II-1	40	+	4.2/4.2	−6.00 DS→4.9/−4.50 DS→4.9	–	–	24.62
III-1	9	+	4.3/4.3	−3.50 DS→4.8/−3.50 DS→4.8	–	–	N.A.
III-2	5	+	4.1/4.1	+7.00 DS→4.7/+7.00 DS→4.7	–	–	15.49

No detectable abnormality was found during fundal examination, which included the optic disc, macula, and retina. No strabismus accompanied the nystagmus. Other examinations included optical coherence tomography, flash electroretinography, visual evoked potential, and brain magnetic resonance imaging. β-Mannosidase activity (normal value is ~35 nmol/h/mg protein) was measured in peripheral blood leukocytes.

DS, diopter sphere; N.A., serum sample was not available; OD, right eye; OS, left eye; +, present; –, absent.

To identify potential causative mutations, we performed WES in seven members of the family, including three patients with autosomal-dominant nystagmus and four unaffected subjects. An average of 57.82 Mb of aligned base reads per sample were generated, of which 96.93% of reads reached ≥10× coverage (**Supplementary Table S1** online). The pathogenic cause was assumed to be the same heterozygous mutation in a single gene. A total of 39,333 SNVs (missense, nonsense, and splice-site mutations) and 1,480 insertions/deletions (short coding insertions or deletions) were identified in the three patients (**Figure 1**). After the known variants (present in the normal family members or in the databases as mentioned in the Materials and Methods) and nondeleterious SNVs were removed, the candidate genes were reduced to five. After bioinformatic analyses, we excluded four unrelated genes (*DOK3*, *ASB16*, *FGD6*, and *PRDM2*) because variants of these genes were nonpathogenic based on SIFT/PolyPhen-2/MutationTaster analyses and because the functions of these genes are unrelated to the visual system. Finally, the *MANBA* gene was prioritized because mutations in the gene have caused a wide spectrum of neurological disorders in both humans and animals. More convincingly, nystagmus also has been observed in ruminants such as goats and cattle with β-mannosidosis[10] caused by disruptive homozygous mutations of *MANBA*.[11]

Identification of mutations

Using bidirectional Sanger sequencing with specific primers (**Supplementary Table S2** online), we confirmed the novel inherited heterozygous mutation (c.2013G>A) in exon 14 of the *MANBA* gene at chromosome 4q25 (GenBank accession no. NM_005908.3) in all three patients (Sanger sequencing data of proband III-2 are shown in **Figure 2b**), but not in the unaffected family members I-3, II-2, and II-3 (data not shown). This mutation changed the codon 638 CGC for arginine into histidine (p.R638H), resulting in a missense mutation. This mutation is segregated with the disease phenotype and predicted to be deleterious or to cause disease by three different programs: SIFT (affecting protein function, with a score of 0.01), PolyPhen-2 (probably damaging, with a score of 0.977), and MutationTaster (disease causing, with a score of 0.983). Also, this mutation was not found in the ESP6500, 1000 Genomes Project, or the in-house exome databases, suggesting that it is a private SNV cosegregated with the phenotype nystagmus in the family. Subject I-2 was the obligate carrier because of reduced penetrance. Reduced penetrance also was observed in the *FRMD7*-associated nystagmus, probably as a result of the type of mutation in *FRMD7* and random inactivation of the X chromosome.[12] Furthermore, haplotype analysis with single-nucleotide polymorphisms flanking the MANBA locus confirmed that the c.2013G>A mutation-containing region segregates with the patients' phenotypes and also matches the haplotype of the obligate carrier (**Supplementary Table S10** online). In addition, with the exception of decreased vision, no other medical history was documented, and whether her sister (I-1) had nystagmus or not is unknown.

To examine whether additional mutations in *MANBA* could be identified in unrelated patients, we used polymerase chain reaction and Sanger sequencing to screen the whole coding region of the gene, including 17 exons and intron–exon junctions (**Supplementary Table S2** online), in a cohort of 30 patients with sporadic, idiopathic infantile nystagmus. As a result, we found one more heterozygous missense mutation (c.2346T>A, p.L749H) in patient ZB005 (**Figure 2c,d**). This substitution was not shown in the in-house Chinese exome database (see Methods), and it was predicted to be harmful by the three programs mentioned above. Because the parent DNAs were unavailable, whether c.2346T>A is an inherited or de novo mutation was not determined.

To illustrate the potential effects of the mutations on the protein's function, the locations of p.R638H and p.L749H in β-mannosidase were compared with four known missense mutations that cause recessive β-mannosidosis, which is one LSD (**Figure 2e**). Multiple sequence alignment of a region of human MANBA harboring the mutations with that of goats, cattle, and cats showed that both p.R638 and p.L749 are evolutionarily conserved in the four species, and β-mannosidosis has been previously reported in these species (**Figure 2e**, lower panel; also see **Supplementary Figure S4** online).

Furthermore, structural bioinformatics analysis showed that the last domain from residue 572 to 879 (**Figure 2e,f**) stabilizes the β-mannosidase active sites (residues 342–571).[13] Because the positively charged arginine 638 was substituted by a slightly positive histidine, p.R638H may destabilize β-mannosidase by affecting the salt bridge D709 or the interaction between R641 and D709, as shown in a three-dimensional structural model (**Figure 2f,g**) that was established using PyMOL (L. Schrödinger, version 1.3r1; PDB ID code 2JE8). This assumption is supported by previous analysis of the p.R641H mutation, which caused β-mannosidosis deficiency.[14]

Analysis of *MANBA* localization in brain tissues

To examine whether *MANBA* is expressed in the brain region involving regulation of eye movement, we compared publicly accessible transcriptional profiles. Expression analysis of the Allen Brain Atlas showed that *MANBA* was localized in various brain regions, primarily restricted to the cerebellar cortex, midbrain, pons, thalamus, and corpus callosum (**Supplementary Table S9** online). *MANBA* also was expressed at a low level across all periods of human brain development, from 8 weeks after conception to adult stages (**Supplementary Figure S1** online). Furthermore, an in situ hybridization study of whole-mount mouse embryos at embryonic day 14.5 showed that *MANBA* was expressed in the central nervous system (CNS), particularly in the pretectum of the midbrain (**Figure 3a–c**), which primarily involves oculomotor and optokinetic nystagmus.[15] *MANBA* also was abundantly expressed in the optic nerve of humans (National Center for Biotechnology Information, Gene Expression Omnibus profile ID no. 4031643) and mouse (Gene Expression Omnibus profile ID no. 71898221). Notably, light and electron microscopic analyses showed a substantial

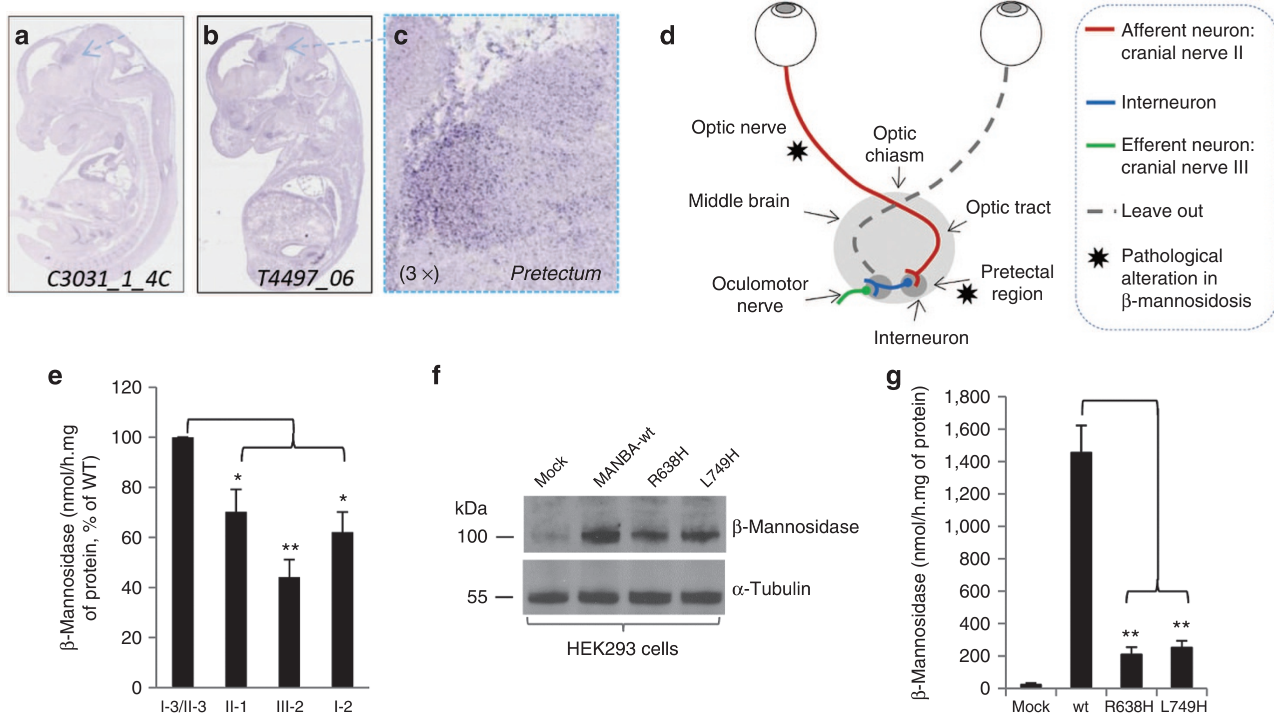

Figure 3 ***MANBA*** **expression and β-mannosidase activity.** (**a**) Sagittal in situ hybridization image in a mouse embryo at embryonic day 14.5 (GenePaint) shows *Manba* expression in the pretectum of the midbrain. (**b**) A similar pattern is also shown using EURExpress. (**c**) Threefold amplification of the midbrain. (**d**) Dorsal view of the afferent and efferent pathways. The impulse starts in the retina and crosses over the optic chiasm to synapse in the pretectal nucleus, and then crosses over to synapse in the nucleus of cranial nerve III, which is involved in ocular movement. Regions with pathological alterations in animals with β-mannosidosis are indicated. (**e**) β-Mannosidase activity in patients and controls. (**f**) MANBA protein level is shown by western blots. A mock, nontransfected control was used. (**g**) β-Mannosidase activity in HEK293 cells (mean ± SEM from three independent transfections conducted in triplicate). $*P < 0.05$; $**P < 0.01$.

decrease in the number of myelinated axons in the fetal optic nerve of β-mannosidosis-affected goats.[16] Overall, the expression of *MANBA* was relatively restricted to the mid- and hindbrain (i.e., the cerebellum, pons, and medulla), regions known to be involved in the optokinetic nystagmus neural pathway. These findings also revealed that in β-mannosidosis, pathological alterations in the optic nerve and pretectal region are involved in the oculomotor pathway of the brain (**Figure 3d**).

Reduced β-mannosidase activity

To examine whether heterozygous mutations affect the function of *MANBA*-encoded protein, we measured β-mannosidase activity in peripheral blood leukocytes collected from 5 available family members as well as 20 unrelated normal controls. Using a human β-mannosidase enzyme-linked immunosorbent assay kit, we found a 40–60% reduction of β-mannosidase activity in the patients (II-1 and III-2) and carrier (I-2) compared with the normal family members (I-3, II-3; **Figure 3e**) and the 20 unrelated normal controls. This result indicates that the subjects with heterozygous mutations only have intermediate levels of normal β-mannosidase activity in peripheral blood leukocytes (~35 nmol/h.mg of protein). To test whether the c.2013G>A and c.2346T>A mutations are responsible for the reduced β-mannosidase activity in the patients, we expressed the wild-type and mutated *MANBA* genes in HEK293T cells. As shown in the western blots, the levels of mutant β-mannosidase proteins (R638H and L749H) were slightly less abundant than the wild-type MANBA (**Figure 3f**), suggesting possible instability of the mutants. As expected, the β-mannosidase activity in the mutant-transfected cells was much lower than that in the wild-type *MANBA*-transfected cells (**Figure 3g**), indicating that the mutant β-mannosidase produced by either R638H- or L749H-containing construct exhibited only very low enzymatic activity. In addition, by using a lysosomal enrichment kit and western blot analysis, we confirmed that these β-mannosidase proteins were still located in the lysosome (data not shown).

Nystagmus and LSDs

Because nystagmus is genetically heterogeneous, protein–protein interaction and associated disease network analyses may provide the landscape of nystagmus etiology. The association between the lysosomal gene *MANBA* and nystagmus prompted us to examine whether nystagmus is also present in other LSDs. After an extensive evaluation of OMIM entries of LSDs involving the brain, we curated a total of 27 LSDs involving brain, which can be divided into five categories (**Table 2**). Surprisingly, by genotype–phenotype analysis of each of the diseases, we found that nystagmus was shown in most, if not all, LSDs involving the CNS.[17] In particular, patients with ceroid lipofuscinosis CLN4B caused by heterozygous mutations of *DNAJC5* have also been reported with nystagmus in the autosomal-dominant form, like our case.[18]

Table 2 Nystagmus is associated with lysosomal storage disease involving brain

Disease	OMIM	Gene	Protein function	Nystagmus	References
Sphingolipidoses					
Gaucher disease (type 2, 3, perinatal)	230900, 231000, 608013	*GBA*	Acid β-glucosidase, lysosomal membrane protein, regulate glycolipid metabolism	+	1–3
Niemann–Pick disease					
Type A	257200	*SMPD1*	Sphingomyelin phosphodiesterase in lysosome	+	4
Type C1	257220	*NPC1*	Intracellular cholesterol transporter in lysosome	+, (+)	5, 6
Type C2	607625	*NPC2*	Intracellular cholesterol transporter in lysosome	±	7
Krabbe disease	245200	*GALC*	Galactosylceramidase in sphingolipid metabolism	+	8, 9
Tay-Sachs disease	272800	*HEXA*	Degradation of GM2 gangliosides	+	10, 11
Sandhoff disease	268800	*HEXB*	Degradation of GM2 gangliosides	+	12
GM1-gangliosidosis, type 1	230500	*GLB1*	Hydrolyzing β-galactose	+, (+)	13, 14
Mucopolysaccharidoses	—	—	—	+	15
Scheie syndrome	607016, 607015	*IDUA*	An iduronidase for lysosomal degradation of glycosaminoglycans	N.D.	—
Mucopolysaccharidosis					
Type 2	309900	*IDS*	Iduronate 2-sulfatase for lysosomal degradation	(+)	16
Type 3A	252900	*SGSH*	Required for lysosomal degradation of heparan sulfate	+	17
Type 4A	253000	*GALNS*	Required for the degradation of the glycosaminoglycans in lysosome	+	18
Glycoproteinoses					
α-Mannosidosis	248500	*MAN2B1*	α-Mannosidase for catabolism of N-linked carbohydrates	+, (+)	19–21
β-Mannosidosis	609489, 248510	*MANBA*	β-Mannosidase for lysosomal oligosaccharide catabolism	+, (+)	22–25 and this study
Fucosidosis	230000	*FUCA1*	Lysosomal a-fucosidase	N.D.	—
Aspartylglucosaminuria	208400	*AGA*	Aspartylglucosaminidase	N.D.	—
Neuraminidase deficiency	256550	*NEU1*	Lysosomal sialidase	+	26–28
Glycogen storage disease II	232300	*GAA*	α-Glucosidase for degradation of glycogen to glucose in lysosomes	+	29
Neuronal ceroid lipofuscinoses					
Ceroid lipofuscinosis, neuronal, 1	256730	*PPT1*	For catabolism of lipid-modified proteins in lysosomal degradation	+	30
Ceroid lipofuscinosis, neuronal, 2	204500	*TPP1*	Lysosomal serine protease with tripeptidyl-peptidase I activity	+	31
Neuronal ceroid lipofuscinosis	204200	*CLN3*	Lysosomal membrane protein in microtubule-dependent transport	+	32
Ceroid lipofuscinosis, neuronal, autosomal dominant	162350	*DNAJC5*	Membrane trafficking and protein folding; presynaptic role	+	33
Other genetic diseases involving lysosomes					
Hermansky-Pudlak syndrome 1	203300	*HPS1*	Organelle biogenesis, including lysosome	+	34
Chediak-Higashi syndrome	214500	*LYST*	Lysosomal trafficking regulator	+	35, 36
Salla disease	604369	*SLC17A5*	Lysosomal membrane transporter	+	37
Farber lipogranulomatosis	228000	*ASAH1*	Degradation of ceramide into sphingosine in lysosome	+	38
Fabry disease	301500	*GLA*	Hydrolyses α-galactosyl moieties in lysosome	+	39

References cited here are provided in the **Supplementary References** online for Table 2.

N.D., not determined; +, present in patients; (+), present in animals.

To find potentially shared pathophysiological mechanisms at the molecular and cellular levels, we performed Ingenuity Pathway Analysis for the causative genes of these 27 LSDs. As a result, these genes are implicated in multiple lysosomal functions, such as neuronal development, lipid metabolism, molecular transport, small-molecule biochemistry, cellular assembly and organization, and cellular function and maintenance. As an example of these complex interactions, a network of protein–protein interactions that depict an interconnection of the 16 lysosome-associated genes involving neuronal development and nystagmus is illustrated in **Supplementary Figure S2** online.

DISCUSSION

Using WES analysis, we identified a novel *MANBA* mutation in patients with inherited nystagmus. To the best of our knowledge, this is the first identification of infantile nystagmus in an autosomal-dominant form linked to a causative gene of inherited disorders. The following provide evidence for a pathogenic role of *MANBA* mutations in nystagmus: (i) *MANBA* is the only candidate deleterious gene linked to the nystagmus phenotype of all affected subjects. (ii) An additional rare deleterious mutation was identified by screening a cohort of patients with the same disorder. (iii) *MANBA* is expressed in brain regions involving eye movements and nystagmus; the expression pattern of *MANBA* in the brain is also similar to the pattern of the X-linked nystagmus gene *FRMD7* in the developing brain.[19] (iv) Functional assays demonstrated that β-mannosidase activity in the samples from affected subjects is significantly decreased. (v) In particular, nystagmus and substantially reduced myelinated axons in the fetal optic nerve have been observed in ruminant animals with *MANBA* mutations. (vi) Gene–disease pathway analysis has revealed that a majority of LSDs involving the brain are accompanied by the nystagmus phenotype.

Normal functioning of the lysosome is important for degradation of macromolecules and cell homeostasis. The lysosome-endosomal system is intimately involved in the regulation of autophagy, apoptosis, and cell death involving in neurological diseases and many other conditions.[20] LSDs are a group of rare, hereditary metabolic disorders that result from mutations in genes encoding catabolic enzymes for the degradation of macromolecules. The accumulated substrate(s) of the defective enzyme(s) lead to dysfunction in the nervous system, eye, and other systems and ultimately lead to cell death and organ-specific clinical manifestations. Most LSDs are inherited in an autosomal-recessive manner,[21] but some, like patients with neuronal ceroid lipofuscinosis 4B and nystagmus[18,22] and the case presented here, present autosomal-dominant inheritance.[23,24]

Mutations in *MANBA* have been previously linked to β-mannosidosis (OMIM 248510), an autosomal-recessive LSD resulting in demyelination of the CNS. The encoded β-mannosidase subcellularly localizes to the lysosome, where it is the final exoglycosidase for *N*-linked glycoprotein oligosaccharide catabolism. Patients with β-mannosidosis have a wide spectrum of neurological involvement, including intellectual disability, hearing loss, ataxia, and seizures.[13] Phenotype–genotype correlation analysis revealed that truncation mutations often cause severe phenotypes, whereas substitution mutations may show mild phenotypes (**Supplementary Table S11** online).[13]

This study extends the scope of β-mannosidosis to the autosomal-dominant inheritance of infantile nystagmus. The heterozygous mutations in *MANBA* identified in this study may represent the least severe β-mannosidosis that is limited to the eye regulatory regions in brain. Because of significantly reduced β-mannosidase activity in these patients (**Figure 3**), the substrate *N*-linked glycoprotein oligosaccharide is expected to build up over time, leading to excess cellular storage of this material and potentially leading to neuronal cell death and hypomyelination.

Nystagmus has been identified as one of the main signs of demyelinating or hypomyelinating leukoencephalopathies in the CNS of ruminants with β-mannosidosis.[10,25–27] In particular, microscopic analysis observed a substantial decrease in the number of myelinated axons and a decreased proportion of oligodendrocytes in the fetal optic nerve of β-mannosidosis-affected goats,[16] indicating that the pathogenetic process was initiated during an early stage of myelination in the optic nerve (**Figure 3d**). Another study confirmed that oligosaccharide accumulates in the regional CNS in caprine β-mannosidosis.[28] These findings clearly link the prenatal lesions of the optic nerve and regional CNS in β-mannosidosis to ocular motor phenotypes such as infantile nystagmus in goats. Genetic inactivation of the *MANBA* gene in mice, however, did not show signs of lysosomal storage in neuronal tissues or β-mannosidosis-related phenotypes,[29] suggesting a compensatory mechanism that might be specific to the mouse.

As a matter of fact, demyelination occurs in many other LSDs in brain. Because each neuronal cell or glial cell subtype can synthesize different sphingolipids, LSD pathologies can be cell specific, and different disorders can manifest with diverse neurological syndromes.[30] Abnormal eye movements can present as initial clinic signs in type 2 Gaucher disease, as a major sign in type 3 Gaucher disease, and as an early sign in many other LSDs[17] (**Table 2**). Also, distinct eyemovement disorders occurred in late-onset GM2-gangliosidosis disease (i.e., Tay-Sachs disease), Gaucher disease type 3, and Niemann–Pick type C. For instance, limitation of the upward gaze is a common finding in Niemann–Pick type C and serves as a valuable clinical sign of this disorder. Optic nerve atrophy and retinopathy are common in neuronal ceroid lipofuscinoses and some classic LSDs that profoundly affect the brain and optic nerves, causing blindness and eyemovement disorders.[30] The shared clinical features suggest that the various proteins operate in similar functional pathways in these disorders.

Interestingly, the most common cause of acquired nystagmus probably is multiple sclerosis,[31] in which the nerves are demyelinated in the brain and spinal cord. Notably, *MANBA* (the molecular marker rs228614) has been recently identified as a genetic risk locus for multiple sclerosis by genome-wide

association study analyses of a large population,[32] suggesting that this polymorphic allele of *MANBA* might be associated with nystagmus in multiple sclerosis.

Nystagmus may also be observed in non-LSDs in which myelin synthesis is impaired. As an example, nystagmus frequently occurs in microcephaly disorder resulting from mutations of *CASK*.[33,34] Yet, the G-protein-coupled receptor 143 encoded by *GPR143*, which is responsible for X-linked congenital nystagmus 6, also targets the lysosome[35] and delays delivery of multivesicular bodies, not the *GPR143* gene product itself, to the lysosome.[36] Many G-protein-coupled receptors regulate myelin-associated glycoprotein in neuronal cells. Moreover, it is worth noting that nystagmus has been proposed as a key clinical symptom for the diagnosis of CNS hypomyelination.[37] Nystagmus as an early ocular alteration was recently present in 88% of patients with autosomal-dominant Machado–Joseph disease and 17% of carriers who were nonpenetrant for the disease, but it did not occur in those who did not carry the mutation.[38] These observations are in agreement with the hypothesis that impairment of neuronal connections between subcortical and cortical optokinetic pathways may be associated with pathogenesis of congenital/infantile nystagmus.[9]

Nevertheless, how the mutations in *MANBA* and other lysosomal disease–associated genes are attributed to the pathogenesis of nystagmus is still not clear. Variable phenotypes and reduced penetrance, as well as potential genetic modifiers, may also contribute to the underlying complex mechanisms. Theoretically, generation and control of eye movements require the participation of the cortex, cerebellum, and brain stem.[39] Accordingly, the bifurcation model has proposed that the waveform variability in the network between the brain stem and cerebellum is associated with hereditary eye-movement disorders.[40] Our findings presented here seem to support this hypothesis in terms of the association of this network with abnormal connectivity or impaired plasticity of neurons in these tissues.

Given the fact that nystagmus is often described as an accompanying symptom in many different disorders, including LSDs, our findings instead indicate that nystagmus may present as an isolated or early clinical manifestation in these disorders as a result of subtle mutations or compensatory mechanisms. Therefore, developing gene-specific enrichment methods for molecular examination of undiagnosed nystagmus is conceivable. In the case of LSDs, earlier identification of mutations may raise questions as to whether patients should receive enzyme replacement therapy; this will require further study.

Taken together, our results provide compelling evidence that the heterozygous mutations of *MANBA* identified here are responsible for infantile nystagmus. This is the first identification of an autosomal-dominant nystagmus, suggesting a new clinical entity in LSDs involving the brain. Furthermore, gene–disease network analyses suggest that mutations in LSD-related genes, as well as other causative genes, which affect myelination and development of oculomotor nervous system, may play an important role in the pathogenesis of eye-movement disorders.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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