

SHORT REPORT

Coexisting variants in *OSTM1* and *MANEAL* cause a complex neurodegenerative disorder with NBIA-like brain abnormalities

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Coexistence of different hereditary diseases is a known phenomenon in populations with a high consanguinity rate. The resulting clinical phenotypes are extremely challenging for physicians involved in the care of these patients. Here we describe a 6-year-old boy with co-occurrence of a homozygous splice defect in *OSTM1*, causing infantile malignant osteopetrosis, and a loss-of-function variant in *MANEAL*, which has not been associated with human disease so far. The child suffered from severe infantile-onset neurodegeneration that could not be stopped by bone marrow transplantation. Magnetic resonance imaging demonstrated global brain atrophy and showed hypointensities of globus pallidus, corpora mamillaria, and cerebral peduncles, which were comparable to findings in neurodegeneration with brain iron accumulation disorders. LC-MS/MS analysis of urine and cerebrospinal fluid samples revealed a distinct metabolic profile with accumulation of mannose tetrasaccharide molecules, suggestive of an oligosaccharide storage disease. Our results demonstrate that exome sequencing is a very effective tool in dissecting complex neurological diseases. Moreover, we suggest that *MANEAL* is an interesting candidate gene that should be considered in the context of neurological disorders with brain iron accumulation and/or indications of an oligosaccharide storage disease.

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INTRODUCTION

The underlying causes of neurodegenerative disorders in childhood are manifold and straight-forward candidate gene prioritization is challenging. The era of next-generation sequencing has clearly advanced the diagnostic process for affected individuals. Apart from known disease entities, also many novel genetic disorders have been identified and characterized during the last years. In addition, the relevance of clustering of multiple hereditary diseases in consanguineous families has come to attention.^{1,2} Coexistence of multiple, functionally relevant gene variants may cause unique clinical syndromes that are extremely difficult to address via routine diagnostics.

Here, we report on an individual of a consanguineous family that suffered from a complex infantile-onset neurodegenerative disorder, characterized by developmental delay, optic nerve atrophy, dyskinetic movement disorder, and a neurodegeneration with brain iron accumulation (NBIA)-like pattern on brain magnetic resonance imaging (MRI).

Case report

The boy is the third child of healthy, consanguineous parents from Saudi Arabia. Two older brothers and one younger sister are

healthy. Pregnancy and birth were reportedly uneventful. First abnormalities were noticed by the parents around the age of 2 months. The infant had feeding difficulties, was irritable and constantly unsettled. Moreover, he did not make eye contact. Further investigations performed in Saudi Arabia led to the identification of a splice defect in *OSTM1* consistent with a diagnosis of osteopetrosis. Accordingly, allogeneic stem cell transplantation was successfully performed at the age of 7 months. In the following, the clinical condition of the child stabilized. However, starting between the age of 2 and 3 years, the parents noted regression with loss of previously acquired skills and development of a dyskinetic movement disorder. Further diagnostics performed at different university hospitals including extensive metabolic work-up (including lysosomal enzyme analysis in skin fibroblasts, screening for congenital disorders of glycosylation, etc) and genetic testing (NGS panels for leukodystrophies, peroxisomal disorders, lysosomal storage disorders, etc) did not identify the cause of his neurological deterioration. Brain MRI demonstrated massive brain atrophy (Figure 1a and b) and symmetrical iron accumulation in pallidum (Figure 1c), corpora mamillaria, and cerebral peduncles. Therefore, a disorder within the NBIA spectrum was suspected. At the current age of 6 years, the child

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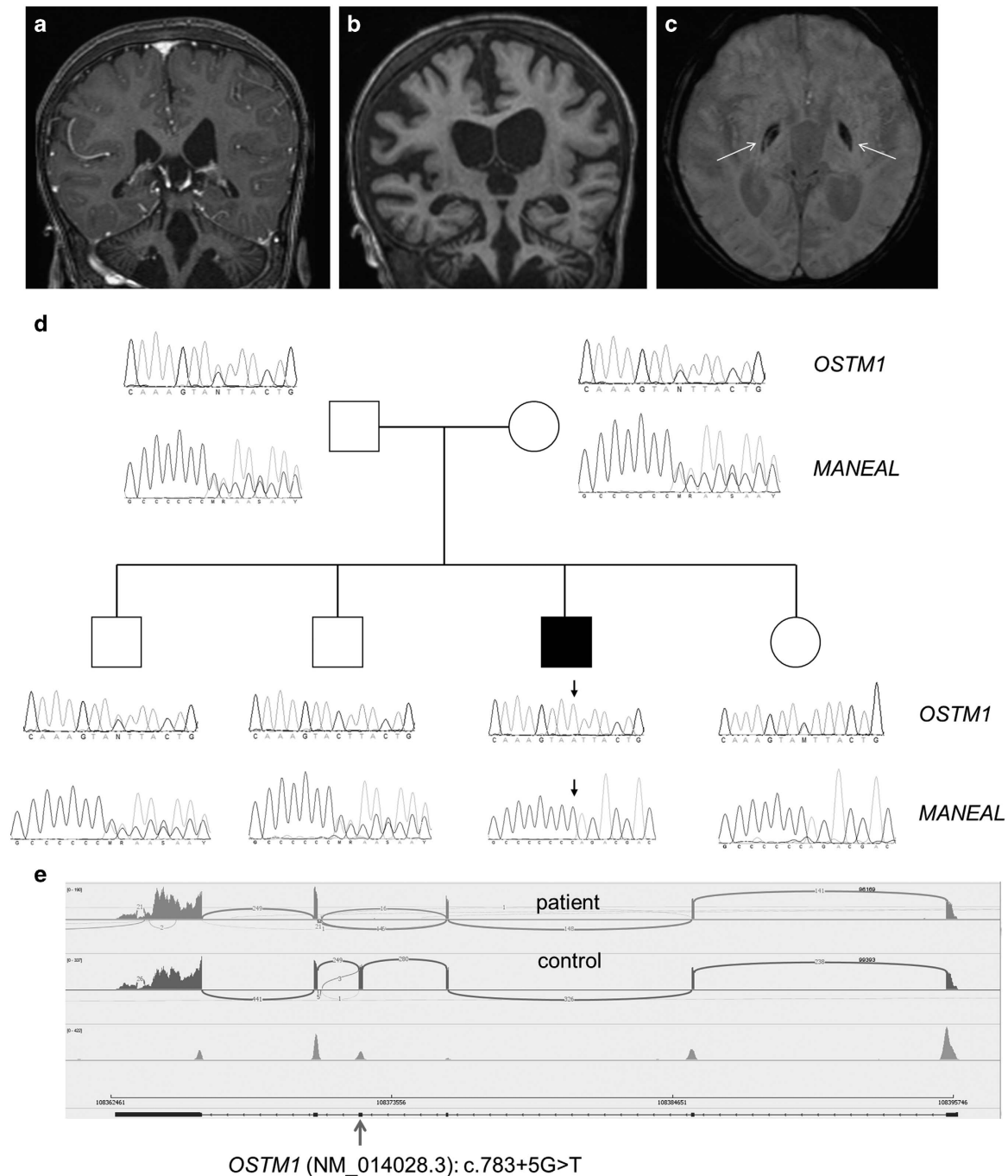


Figure 1 (a) Brain MRI (T1 weighted, coronal view) at the age of 2 years. (b) Brain MRI (T1 weighted, coronal view) at the age of 6 years. Images demonstrate severe brain atrophy during the course of the disease. (c) Susceptibility weighted MRI images (axial view) at the age of 6 years showing bilateral signal alterations within the globus pallidum (white arrows), which are reminiscent of those reported in NBIA disorders. (d) Pedigree of the investigated family and electropherograms showing identified variants in *OSTM1* (NM_014028.3, NG_007262.1) and *MANEAL* (NM_001031740.2). Mutation status of affected (closed symbols) and unaffected (open symbols) family members is depicted. (e) Sashimi plot of RNA sequences produced from patient's (red, upper panel) and control (blue, lower panel) fibroblast cell lines. These data are in line with the change c.783+5G>T, resulting in a skipping of exon four of the *OSTM1* transcript. A full color version of this figure is available at the *European Journal of Human Genetics* journal online.

is wheelchair-bound and shows severe intellectual disability. Swallowing difficulties required placement of a PEG tube and due to episodic vomiting fundoplication was performed. The boy is unable to communicate and does not make visual contact. Because of his dyskinetic movement disorder various drug regimens were tried; however, without convincing therapeutic effect. Only recently, he

suffered from generalized seizures, which were controlled by levetiracetam treatment.

MATERIALS AND METHODS

A detailed description of materials and methods used for this paper can be found in the supplementary part of this article.

Results and discussion

On the basis of the complex clinical picture and unusual brain abnormalities whole-exome sequencing was performed. Analysis yielded an average 207-fold coverage of the target region with >98% being covered >20-fold. A search for autosomal homozygous rare (MAF>0.1% in 9000 in-house control exomes) loss of function, splice, and near splice variants prioritized two genes (*MANEAL* and *OSTM1*). The splice variant c.783+5G>T, p.(?) in *OSTM1* (NM_014028.3, NG_007262.1) was found in a 62 Mb run of homozygosity. In *MANEAL* (NM_001031740.2), exome sequencing identified the frameshift variant c.446dup, p.(Asp150Argfs*48) located within a 38 Mb homozygous region. Sanger sequencing confirmed the two variants in a homozygous state in the patient with his parents being heterozygous carriers of both variants (Figure 1d). Sequencing of the healthy siblings showed heterozygous changes or wild-type sequences at the respective genomic positions. We next performed whole-transcriptome sequencing on patient-derived fibroblast cell lines to further investigate the functional consequences of the above-mentioned variants. This analysis confirmed that the *OSTM1* variant c.783+5G>T results in a complete skipping of exon four of the canonical *OSTM1* transcript (Figure 1e). This finding is in line with a loss-of-function character of the c.783+5G>T *OSTM1* variant.

The information regarding both variants was submitted to the Leiden Open Variation Database (LOVD v.3.0) (<http://databases.lovd.nl/shared/individuals/00100446>).

Expression of *MANEAL* was found to be generally very low in both, patient's and control fibroblasts (data not shown). Therefore, albeit overall *MANEAL* expression levels in patient's RNA were within the lower range of controls this finding is not clearly supportive of nonsense-mediated decay of the mutant transcript. Data from The Human Protein Atlas suggest that *MANEAL* is mainly expressed in brain tissue (<http://www.proteinatlas.org>).

OSTM1 encodes osteopetrosis-associated transmembrane protein-1 and functionally relevant variants within this gene may cause infantile malignant osteopetrosis (OMIM #259720), which is a very severe sub-form of this disease.

The clinical phenotype includes generalized osteosclerosis, anemia, hepatosplenomegaly, and progressive neurodegeneration. The clinical course is usually fatal during the first years of life. Bone marrow transplantation (BMT) was shown to be ineffective in stopping neurodegeneration and according to current guidelines (00_OP_GUIDELINES_V3.1108015), BMT is contraindicated in patients with *OSTM1* defects. Clinical features of the child here include severe optic atrophy and global brain atrophy, which can be attributed to this defect.

However, the MRI abnormalities possibly indicating brain iron accumulation demonstrated in Figure 1c appeared unusual in this context and might be associated with the homozygous frameshift variant in *MANEAL*. This gene encodes mannosidase endo- α -like protein (also known as glycoprotein endo- α -1,2-mannosidase-like), which is as a putative membrane protein of the Golgi apparatus, existing as three alternatively spliced isoforms.^{3,4} So far, no functional studies regarding *MANEAL* have been performed. Nevertheless, it was suggested that *MANEAL* might be involved in glycoprotein metabolism. Interestingly, in several oligosaccharide/glycoprotein storage diseases brain pathology on MRI includes iron accumulation patterns similar to those observed in NBIA disorders.^{5,6} The exact pathomechanism behind this phenomenon is unclear. However, lysosomes are known to play a critical role in cellular iron homeostasis.^{7,8}

In view of these findings, we further investigated a potential role of *MANEAL* in the disease reported here. Analysis of urine and cerebrospinal fluid samples of the index patient revealed almost identical patterns of diagnostic ions as depicted in Figure 2. We observed a specific elevation of the hexose tetrasaccharides (Hex4 peak at m/z 997). This 'mass spectrum fingerprint' was distinctly different when compared to urine samples of healthy controls or of patients with other lysosomal storage diseases (eg, α -mannosidosis, Pompe disease, and GM1-gangliosidosis; for examples see Supplementary Figure 1) as well as a patient with osteopetrosis caused by disease-causing variants in *TCIRG1* (Supplementary Figure 2). Of note, mannose is an epimer of glucose and has the same molecular weight. Therefore, it is not possible to distinguish between them by measuring their mass spectra. To answer the question

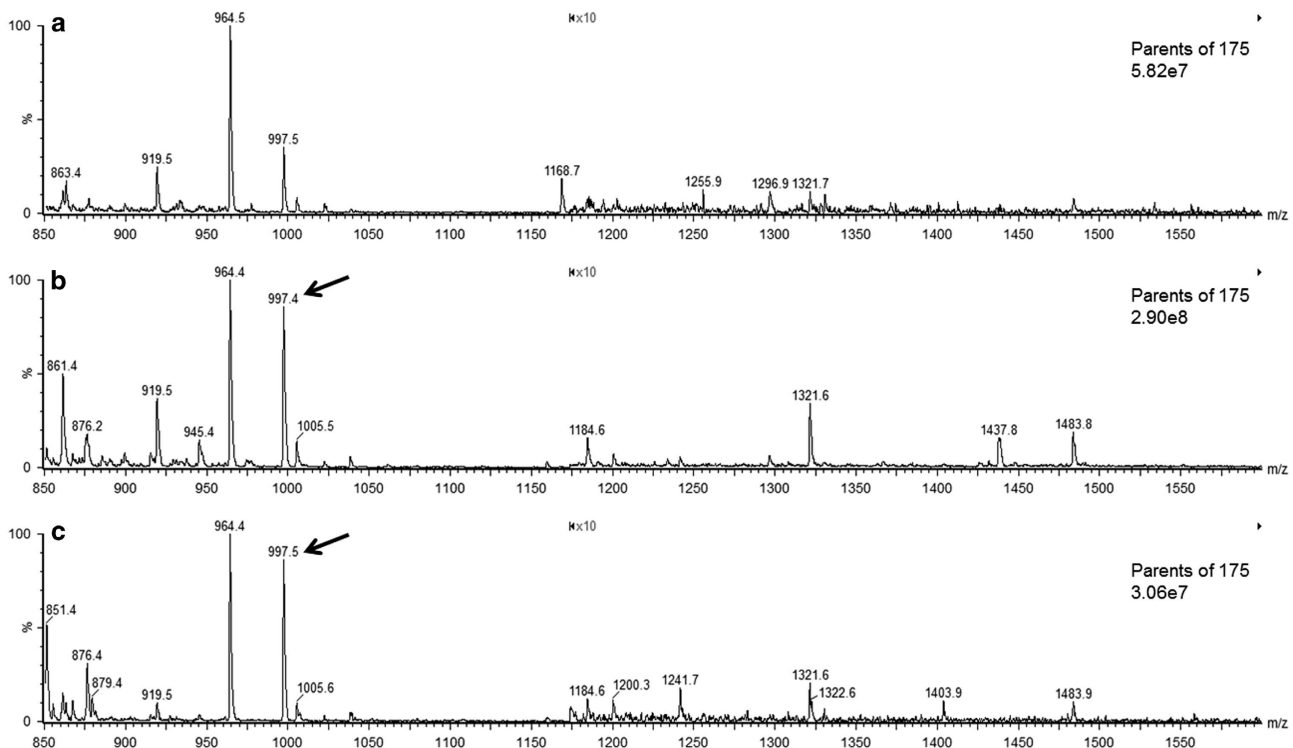


Figure 2 Tandem mass spectra as parents scan of m/z 175 of the PMP-derivatized control urine sample (a) as well as patient's urine (b) and cerebrospinal fluid samples (c). We observed a specific elevation of the hexose tetrasaccharides (Hex4 peak at m/z 997; black arrows) in the patient samples. Images (a, b) depict representative MS/MS spectra of several independent measurements ($n=3$). The mass ranges between m/z (mass-to-charge) 1160 and 1600 are magnified ten-fold. For additional details regarding the assignments of characteristic diagnostic PMP-derivatized ions to their molecular fragments see Supplementary Table 1.

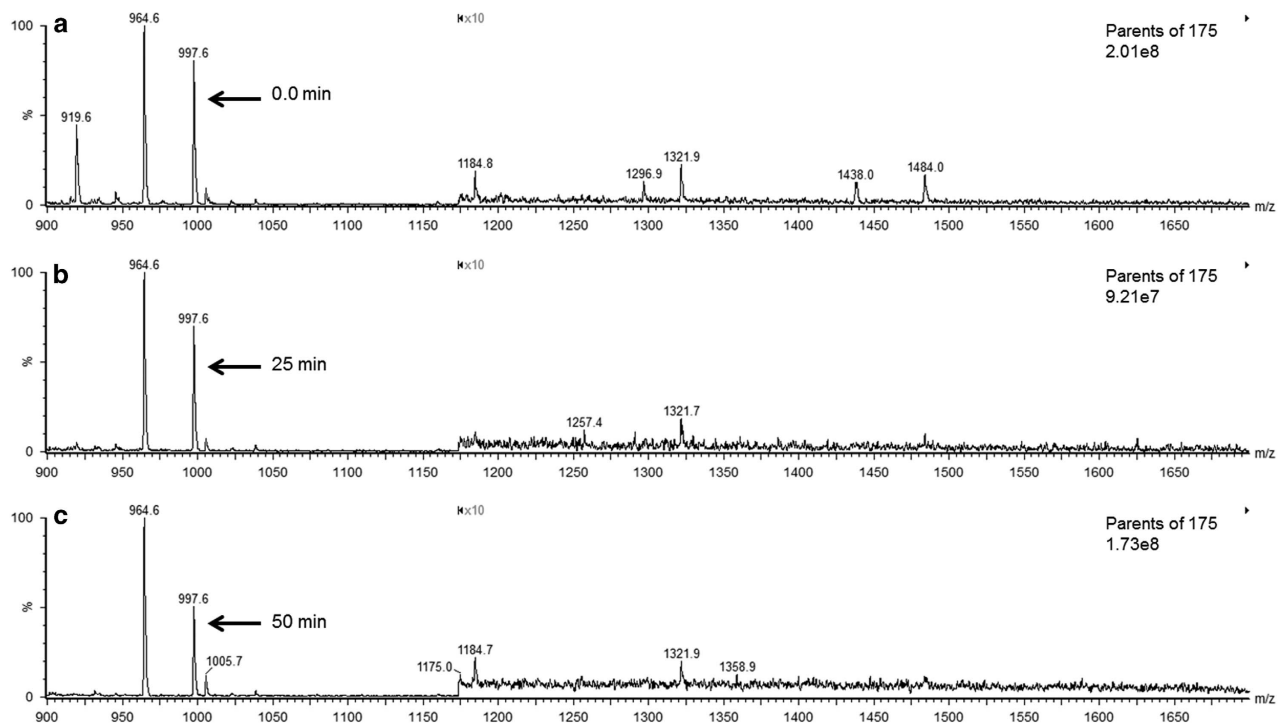


Figure 3 MS/MS spectra of time-dependent enzymatic digestion experiments using α -mannosidase in patient's urinary oligosaccharide samples. (a–c) As depicted, α -mannosidase treatment induced a time-dependent decrease of the Hex4 peak at m/z 997 (black arrows) indicating that the Hex4 peak represents mannose moieties. The mass ranges between m/z (mass-to-charge) 1160 and 1600 are magnified ten-fold.

whether *MANEAL* deficiency really affects mannose metabolism, we performed enzymatic experiments using recombinant α -mannosidase on patient samples in a time-dependent manner. As depicted in Figure 3, only treatment with α -mannosidase induced a significant decrease of the Hex4 peak up to 50% after 3 h of incubation at 37 °C (Figure 3). In addition, we also performed similar experiments using recombinant α -glucosidase, which revealed no effect on the Hex4 peak (data not shown). Hence we conclude that the Hex4 peak in the *MANEAL* patient's body fluids most likely represents mannose moieties.

These findings suggest disturbed mannose metabolism in the affected patient. However, the contribution of this metabolic abnormality to the clinical phenotype is extremely difficult to address. The severe neurological phenotype of *OSTM1* deficiency and the effects/side effects of BMT that the patient received at the age of 7 months certainly have a major influence on current clinical problems. Moreover, *OSTM1* plays an important role in subcellular endosome/lysosome dispersion and autophagy processes, which could be associated with lysosomal dysfunction.⁹ Nevertheless, the unusual NBIA pattern on brain MRI, which has not been described for *OSTM1* patients, and the above-mentioned findings in body fluids suggest a potential impact of the frameshift variant in *MANEAL*. Therefore, *MANEAL* represents certainly an interesting candidate gene that should be considered in the context of neurological disorders with neuroimaging findings suggestive of brain iron accumulation and/or indications of a glycoprotein storage disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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