Original Article

Molecular Syndromology

Mol Syndromol 2015;6:58–62
DOI: 10.1159/000371399

Accepted: October 21, 2014
by M. Schmid
Published online: March 4, 2015

MAN1B1 Mutation Leads to a Recognizable Phenotype: A Case Report and Future Prospects

Sabine Hoffjan[a] Jörg T. Epplen[a] André Reis[b] Rami Abou Jamra[b]

[a]Department of Human Genetics, Ruhr-University, Bochum, and [b]Institute of Human Genetics, Friedrich-Alexander-University, Erlangen-Nuremberg, Germany

Key Words
CDG syndrome · Intellectual disability · *MAN1B1* · Mental retardation

Abstract
Intellectual disability (ID) is one of the most common reasons for referral to genetic counseling. Nevertheless, in over 50% of the cases no diagnosis can be made. Here, we present how exome sequencing in combination with medical genetics evaluation led to the identification of a known pathogenic homozygous mutation in *MAN1B1* in a consanguineous Turkish family. The phenotype comprised mild ID, truncal obesity and facial dysmorphism, comparable to that of the patients in the 3 recent publications on mutations in this gene. Clinically, the majority of patients in the literature showed congenital disorder of glycosylation syndrome type 2. In this study, we summarize the current knowledge about *MAN1B1* mutations from the literature as well as databases and suggest that exome sequencing should be implemented in a larger scale in routine diagnostics, since autosomal recessive ID has proven to be extremely heterogeneous. Even syndromic patterns may only become recognizable retrospectively.

© 2015 S. Karger AG, Basel

Early-onset cognitive impairment, also referred to as intellectual disability (ID), has a prevalence of about 3% in the general population and represents a major socioeconomic burden worldwide [Leonard and Wen, 2002; Musante and Ropers, 2014]. In fact, ID is one of the most common reasons for referral to genetic counseling [Ropers, 2010; own unpubl. observations]; yet, in over 50% of the cases, routine examination does not reveal the cause or clinical entity. Whole-exome sequencing has enabled a rapid progress in this field, and recent studies show that undiagnosed cases are probably due to de novo dominant or inherited recessive point mutations in autosomal genes [Najmabadi et al., 2011; de Ligt et al., 2012; Rauch et al., 2012; Gilissen et al., 2014]. Here, we report on our clinical and scientific experience in this field, leading to the suggestion that whole-exome sequencing should be implemented in a larger scale in clinical genetic practice. A family sought a diagnosis for their 3 affected children with mild ID. We identified a mutation in the *MAN1B1* gene and thereby, retrospectively, diagnosed a recently described syndromic form of ID. Further, we discuss the phenotypic aspects in our family in comparison to reported cases in the literature.

KARGER 125

© 2015 S. Karger AG, Basel
1661–8769/15/0062–0058$39.50/0

E-Mail karger@karger.com
www.karger.com/msy

Sabine Hoffjan, MD
Department of Human Genetics, Ruhr-University
Universitätsstrasse 150
DE–44801 Bochum (Germany)
E-Mail sabine.hoffjan@ruhr-uni-bochum.de

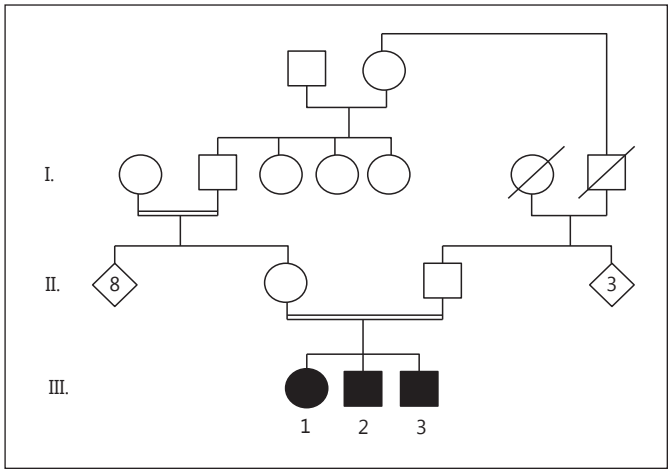


Fig. 1. Pedigree of the Turkish family with 3 children showing ID. Filled symbols represent affected children.

Patients and Methods

Case Report

A consanguineous Turkish family with 3 children showing mild to moderate ID presented at the Department of Human Genetics of the Ruhr-University Bochum (fig. 1). At the time of the first physical examination, the siblings were 17, 14 and 10 years old. For all 3 children, pregnancy and delivery were uneventful. They concordantly showed muscular hypotonia from the beginning, and the motor milestones were rather late, though in the normal range (e.g. unassisted walking was achieved at ~18 months of age). All 3 children were rather small (but in the range of the parents' height) and displayed truncal obesity that started at ~5 years of age. They showed foot deformities (flat feet) and tapering fingers and had common facial features including a prominent nose, short philtrum, a thin upper lip and broad eyebrows; however, these features were considered unspecific at the beginning.

ID was diagnosed in the daughter (III.1) when integration into a regular primary school was not possible. At the age of 17, she was able to read and write a few words as well as understand and follow simple assignments. She attended a special school for intellectually disabled children and did not show any other malformations, medical or behavioral symptoms. Karyotyping as well as analysis of fragile X syndrome yielded normal results. Her brother (III.2) showed comparable psychomotor delay. In addition, he developed pectus excavatum and underwent surgery at the age of 5 years. The youngest brother (III.3) showed comparable psychomotor development and additionally had strabismus convergens. Ultrasounds of muscle and brain in infancy, ophthalmologic evaluation and orienting metabolic tests (lactic acid and ammonia in serum, amino acid analysis in plasma and urine, and organic acid analysis in urine) were inconspicuous in the youngest brother. An MRI at the age of almost 3 years showed slight widening of the outer ventricles. At ~3 years of age, he had one short episode of non-ketotic hyperglycemia (blood sugar level >350 mg/dl). An autoimmune-mediated diabetes mellitus type 1 was excluded and subsequently blood sugar levels remained within the normal range without further therapy.

Due to the lack of obvious malformations or clearly dysmorphic features, the affected children were classified as having unspecific ID, most probably of autosomal recessive inheritance.

Mutation Detection

As described by Abou Jamra et al. [2011], autozygosity mapping in the family with DNAs from the 2 affected brothers restricted the candidate regions to 12 with a total length of 170 Mb. Subsequently, exome sequencing using DNA from individual III.2 was performed as described in former studies [Hansen et al., 2013; Murakami et al., 2014], resulting in an average coverage of 58×. 65% of the target sequences were covered with a depth of at least 20×, and 79% were covered with a depth of at least 5×. 466 SNVs and indels were rare (i.e. minor allele frequency of ≤0.01 in the databases of 1000 Genomes and Exome Variant Server (EVS) as well as in 700 in-house exomes) and predicted to affect the protein sequence (nonsynonymous or splicing). Under the hypothesis of a homozygous identical by a descent founder mutation, we considered homozygous variants followed by filtering based on in silico parameters (conservation and prediction programs, see online suppl. table 1; filtering strategy, see online suppl. fig. 1 ; see www.karger.com/doi/10.1159/000371399 for all online suppl. material). This revealed only one candidate mutation: c.1000C>T, p.R334C in exon 7 of *MAN1B1* (NM_016219). We validated the mutation and confirmed segregation in the family using Sanger sequencing.

MAN1B1 Mutations in the Literature and in Databases

The *MAN1B1* gene is located on chromosome 9q34.3 and comprises 13 exons (fig. 2). It encodes the protein endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase (ERManI) that is believed to play an important role in the disposal of misfolded glycoproteins [Karaveg et al., 2005]. Rafiq et al. [2011] first described mutations in this gene, identified by homozygosity mapping and next-generation sequencing, as causative for nonsyndromic ID in 4 consanguineous families from Pakistan and Iran. The affected children in the Iranian family carried the same missense mutation (c.1000C>T, p.R334C) that was found in our Turkish family (fig. 2; table 1). Shortly thereafter, additional *MAN1B1* mutations were identified in a group of patients with congenital disorder of glycosylation type 2 (CDG2) syndrome [Rymen et al., 2013], which was confirmed in a second cohort [Van Scherpenzeel et al., 2014]. Despite individual variability in clinical phenotype, most described patients shared truncal obesity, a broad nasal bridge, a prominent nose, a thin upper lip, down-slanting palpebral fissures, broad eyebrows with lateral thinning, and muscular hypotonia, suggesting that *MAN1B1* deficiency may rather be a syndromic form of ID [Rymen et al., 2013; Van Scherpenzeel et al., 2014].

Mol Syndromol 2015;6:58–62
DOI: 10.1159/000371399

Table 1. *MAN1B1* mutations and associated phenotypes

Mutations	No. of patients	Ethnicity	Intellectual disability (IQ)	Dysmorphic features	Additional features	Truncal obesity	Hypotonia	CDG2	Reference
c.1000C>T, p.R334C homozygous	10 (5 families)	Turkish, Iranian	mild-moderate (55–69)	hypertelorism, broad eyebrows (laterally thin), flat philtrum, thin upper lip, prominent nose, macrocephaly	joint hypermobility, skoliosis (1), autism (1), strabismus (1), pectus excavatum (1)	+	+	+/n.s.	Rafiq et al., 2011; Rymen et al., 2013; Van Scherpenzeel et al., 2014; present study
c.1189G>A, p.E397K homozygous	6 (3 families)[a]	Pakistani	mild-moderate (45–60)	down-slanting palpebral fissures, thin sparse eyebrows, broad nose, mild dolichocephaly, short neck	seizures	+	?	n.s.	Rafiq et al., 2011
c.1418G>A, p.Trp473* homozygous	3 (1 family)	Pakistani	mild (55–65)	long face, down-slanting palpebral fissures, hypertelorism, short philtrum, broad nasal root, small chin, clinodactyly, syndactyly (toes 2/3)	seizures	–	n.s.	n.s.	Rafiq et al., 2011
c.1863G>A, p.W621X homozygous	2 (1 family)	n.s.	moderate (n.s.)	thin lateral eyebrows (1), inverted nipples, abnormal fat distribution (1)	strabismus	–	+/–	+	Van Scherpenzeel et al., 2014
c.530_542del, p.L177Pfs*32/ c.621-2A>G	2 (1 family)	n.s.	mild-moderate (35–55)	macrocephaly, dolichocephaly, bulbous nose tip, thin upper lip	seizures	+	+/–	+	Van Scherpenzeel et al., 2014
c.1789C>T, p.R597W/ c.2065G>A, p. E689K	2 (1 family)	n.s.	moderate-severe (40)	macrocephaly, inverted nipples, abnormal fat distribution	strabismus	+	+	+	Van Scherpenzeel et al., 2014
c.224G>A, p.W75X homozygous	1	n.s.	severe (33)	bulbous nose tip	autism, ataxia	+	–	+	Van Scherpenzeel et al., 2014
c.1225T>C, p.S409P homozygous	1	Portuguese	psychomotor retardation (n.s.)	macrocephaly, slight facial dysmorphism	MRI: cerebellar hypoplasia with vermian atrophy	+	+	+	Rymen et al., 2013
c.1225T>C, p.S409P/ c.172G>T, p.E58X	1	Portuguese	moderate (43)	slight facial dysmorphism, long thin fingers	autoaggressive behavior, repetitive movements	+	+	+	Rymen et al., 2013
c.1225T>C, p.S409P/ c.1282delA, p.I428fs*43	1	n.s.	moderate (50)	macrocephaly, thin lateral eyebrows, bulbous nose tip, inverted nipples, abnormal fat distribution	strabismus	+	transient	+	Van Scherpenzeel et al., 2014
c.465+1460_620+527del/ c.1445+2delTGAG	1	Belgian	mild (52)	slight facial dysmorphism	joint hypermobility, skin laxity, autism spectrum disorder, VSD; MRI: multiple small white matter lesions	–?	+	+	Rymen et al., 2013
c.1001G>C, p.R334P/ c.1849C>T, p.Q617X	1	n.s.	severe (35)	thin lateral eyebrows, thin upper lip, inverted nipples, macrocephaly	strabismus	+	+	+	Van Scherpenzeel et al., 2014
c. 1833_1834delAG homozygous	1	Belgian	mild (n.s.)	slightly dysmorphic features, long thin fingers	joint hypermobility, skin laxity, pectus excavatum	(+)[b]	+	+	Rymen et al., 2013
c.1976T>G, p.F659C homozygous	1	n.s.	moderate (50)	thin lateral eyebrows, bulbous nose tip, thin upper lip	seizures, ataxia	+	transient	+	Van Scherpenzeel et al., 2014
c.2065G>T, p.E689X	1	n.s.	developmental delay (n.s.)	macrocephaly, thin lateral eyebrows, thin upper lip, inverted nipples, abnormal fat distribution	joint laxity	–	+	+	Van Scherpenzeel et al., 2014

[a] Three families from the same village in Pakistan, probably with shared inheritance. [b] Growth and weight are in the normal range, but truncal fat accumulation is apparent. n.s. = Not stated; VSD = ventricular septal defect.

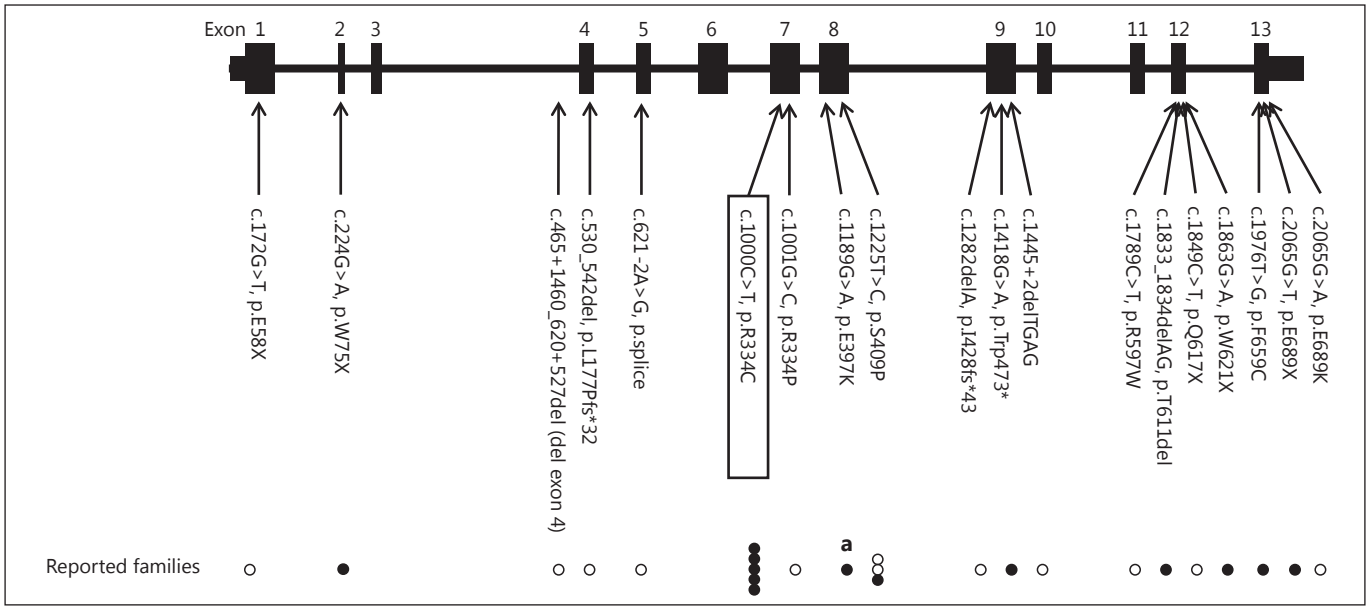


Fig. 2. Structure of the *MAN1B1* gene, located on chromosome 9q34.3, and localization of the mutations described in the literature [Rafiq et al., 2011; Rymen et al., 2013; Van Scherpenzeel et al., 2014] and the present study. Filled circles: homozygous mutations; empty circles: heterozygous mutations; box: the most frequently reported *MAN1B1* mutation to date (c.1000C>T, p.R334C). **a** Three families from the same village in Pakistan, probably with shared inheritance.

Table 2. Features of *MAN1B1* deficiency syndrome as revealed from the most consistent clinical phenotypes reported in the literature and the present study

Features
Intellectual disability
Mild to moderate
General built
Truncal obesity
Muscular hypotonia
Facial features[a]
Broad nasal bridge/hypertelorism
Broad eyebrows (laterally thin)
Prominent nose with bulbous tip
Thin upper lip
Additional features[b]
Macrocephaly
Joint hypermobility
Skin laxity
Long, thin fingers
Strabismus
Autism spectrum disorder
Inverted nipples
Abnormal fat distribution
Seizures
Potential features[c]
Scoliosis
Pectus excavatum
Ataxia
Biochemical findings
Glycosylation profile according to CDG2

[a] Reported in the majority of patients. [b] Reported in some, but not all patients. [c] Reported in a few or single patients.

The mutations described so far include nonsense, missense and splice site mutations as well as deletions of single exons and are located throughout the gene (fig. 2). The p.R334C mutation has been found most frequently so far, being in homozygous state in an Iranian family with 3 children [Rafiq et al., 2011], 2 Turkish families with CDG2 [Rymen et al., 2013], a third family with a CDG2 patient of unknown origin [Van Scherpenzeel et al., 2014] as well as in the 3 affected children in our Turkish family, summing up to 10 individuals from 5 unrelated families, mostly of Turkish origin (table 1). This mutation is located at the base of the active-site pocket and thus is believed to influence enzymatic function and substrate binding [Van Scherpenzeel et al., 2014].

Screening the EVS database (http://evs.gs.washington.edu/EVS/) revealed 30 reliable (coverage of >20×) heterozygous variants in the *MAN1B1* gene in 51 individuals that are predicted 'damaging' by PolyPhen2 and highly conserved (online suppl. table 2), theoretically giving a carrier frequency of 51/6500 (0.0078).

Discussion

In the present study, we further confirm p.R334C in the *MAN1B1* gene as a causative mutation in autosomal recessive ID. Taking into account data from the literature, it appears that p.R334C is a founder mutation in the Turkish population. Autosomal recessive ID is highly heterogeneous, and private familial mutations are usually identified [Musante and Ropers, 2014]. Nevertheless, further examples such as a recently reported mutation in *CLP1* in 4 independent Turkish pedigrees with neuropediatric disease [Schaffer et al., 2014] show that one should be aware of such founder mutations which may be reported as variants in public databases. Based on EVS data, potentially damaging mutations in *MAN1B1* have a carrier prevalence of 0.0078 (online suppl. table 2), and the disease prevalence may be as high as 0.000015, comparable to several well-known metabolic disorders (http://www.orpha.net/).

The phenotype associated with *MAN1B1* mutations appears comparatively uniform for the patients described so far (summarized in table 2). This includes mild to moderate ID, truncal obesity, muscular hypotonia and modest dysmorphic features (broad nasal bridge, prominent nose, thin upper lip, curved eyebrows) as well as a glycosylation type typical for CDG2 [Rymen et al., 2013; Van Scherpenzeel et al., 2014]. Although mutations in *MAN1B1* lead to a syndromic disorder, we have to admit that this was only achieved retrospectively in our patients. This is probably a result of the growing number of genes and mutations that make it a difficult task to establish differential diagnoses. Although for some distinct phenotypes clinical examination will still draw attention to a specific disorder or syndrome, it seems that this is getting more and more difficult. We, therefore, suggest that exome sequencing should be implemented on a larger scale in routine diagnostics for ID, especially in cases suspected of autosomal recessive inheritance. We acknowledge that interpretation of the results may not always be as forthcoming as in our case and especially distinguishing disease-causing mutations from benign sequence variants has proven a difficult task [MacArthur et al., 2014]. However, large-scale application of whole-exome sequencing, and eventually also whole-genome sequencing which can additionally detect noncoding regulatory variants, appears warranted in order to get a more comprehensive picture of autosomal recessive ID in both consanguineous and non-consanguineous families in the future.

Acknowledgements

We thank the patients and their family for participation in the study. We thank Katharina Batzke and Farah Radwan for technical assistance and Arif Ekici, Mandy Krumbiegel and Steffen Uebe for support in mapping and NGS. This study was supported by a DFG grant AB393/2-2 to Rami Abou Jamra.

References

Abou Jamra R, Wohlfart S, Zweier M, Uebe S, Priebe L, et al: Homozygosity mapping in 64 Syrian consanguineous families with non-specific intellectual disability reveals 11 novel loci and high heterogeneity. Eur J Hum Genet 19: 1161–1166 (2011).

de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, et al: Diagnostic exome sequencing in persons with severe intellectual disability. N Engl J Med 367:1921–1929 (2012).

Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, et al: Genome sequencing identifies major causes of severe intellectual disability. Nature 511:344–347 (2014).

Hansen L, Tawamie H, Murakami Y, Mang Y, ur Rehman S, et al: Hypomorphic mutations in *PGAP2*, encoding a GPI-anchor-remodeling protein, cause autosomal-recessive intellectual disability. Am J Hum Genet 92:575–583 (2013).

Karaveg K, Siriwardena A, Tempel W, Liu ZJ, Glushka J, et al: Mechanism of class 1 (glycosylhydrolase family 47) {alpha}-mannosidases involved in N-glycan processing and endoplasmic reticulum quality control. J Biol Chem 280: 16197–16207 (2005).

Leonard H, Wen X: The epidemiology of mental retardation: challenges and opportunities in the new millennium. Ment Retard Dev Disabil Res Rev 8:117–134 (2002).

MacArthur DG, Manolio TA, Dimmock DP, Rehm HL, Shendure J, et al: Guidelines for investigating causality of sequence variants in human disease. Nature 508:469–476 (2014).

Murakami Y, Tawamie H, Maeda Y, Büttner C, Buchert R, et al: Null mutation in *PGAP1* impairing Gpi-anchor maturation in patients with intellectual disability and encephalopathy. PLoS Genet 10:e1004320 (2014).

Musante L, Ropers HH: Genetics of recessive cognitive disorders. Trends Genet 30:32–39 (2014).

Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, et al: Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature 478:57–63 (2011).

Rafiq MA, Kuss AW, Puettmann L, Noor A, Ramiah A, et al: Mutations in the alpha 1,2-mannosidase gene, *MAN1B1*, cause autosomal-recessive intellectual disability. Am J Hum Genet 89:176–182 (2011).

Rauch A, Wieczorek D, Graf E, Wieland T, Endele S, et al: Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. Lancet 380:1674–1682 (2012).

Ropers HH: Genetics of early onset cognitive impairment. Annu Rev Genomics Hum Genet 11: 161–187 (2010).

Rymen D, Peanne R, Millón MB, Race V, Sturiale L, et al: MAN1B1 deficiency: an unexpected CDG-II. PLoS Genet 9:e1003989 (2013).

Schaffer AE, Eggens VR, Caglayan AO, Reuter MS, Scott E, et al: *CLP1* founder mutation links tRNA splicing and maturation to cerebellar development and neurodegeneration. Cell 157: 651–663 (2014).

Van Scherpenzeel M, Timal S, Rymen D, Hoischen A, Wuhrer M, et al: Diagnostic serum glycosylation profile in patients with intellectual disability as a result of MAN1B1 deficiency. Brain 137:1030–1038 (2014).