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Mutations in the Human *UBR1* Gene and the Associated Phenotypic Spectrum

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ABSTRACT: Johanson–Blizzard syndrome (JBS) is a rare, autosomal recessive disorder characterized by exocrine pancreatic insufficiency, typical facial features, dental anomalies, hypothyroidism, sensorineural hearing loss, scalp defects, urogenital and anorectal anomalies, short stature, and cognitive impairment of variable degree. This syndrome is caused by a defect of the E3 ubiquitin ligase UBR1, which is part of the proteolytic N-end rule pathway. Herein, we review previously reported ($n = 29$) and a total of 31 novel *UBR1* mutations in relation to the associated phenotype in patients from 50 unrelated families. Mutation types include nonsense, frameshift, splice site, missense, and small in-frame deletions consistent with the hypothesis that loss of UBR1 protein function is the molecular basis of JBS. There is an association of missense mutations and small in-frame deletions with milder physical abnormalities and a normal intellectual capacity, thus suggesting that at least some of these may represent hypomorphic *UBR1* alleles. The review of clinical data of a large number of molecularly confirmed JBS cases allows us to define minimal clinical criteria for the diagnosis of

Additional Supporting Information may be found in the online version of this article.

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JBS. For all previously reported and novel *UBR1* mutations together with their clinical data, a mutation database has been established at LOVD.

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KEY WORDS: Johanson–Blizzard syndrome; *UBR1*; exocrine pancreatic insufficiency; aplasia of alae nasi; cognitive impairment

Background

Mutations of *UBR1* (MIM #605981) are known to cause Johanson–Blizzard syndrome (JBS; MIM #243800), a clinically distinct, autosomal recessively inherited congenital malformation syndrome. The clinical hallmarks of this multisystem disorder are nasal wing hypo-/aplasia and exocrine pancreatic insufficiency, which is typically present at birth or manifests in early infancy. Hearing impairment, aplasia cutis congenita of the scalp, dental defects, hypothyroidism, cognitive impairment of variable degree, short stature, and urogenital and anorectal malformations are additional common features of the syndrome [Zenker, 2008]. The eponymic name of this condition goes back to Ann Johanson and Robert Blizzard who in 1971 described three unrelated girls affected by congenital aplasia of the alae nasi, deafness, hypothyroidism, dwarfism, absent permanent teeth, and malabsorption [Johanson and Blizzard, 1971]. More than 60 patients have been reported in the literature since then. The birth prevalence of JBS in Europe has been estimated to be approximately 1:250,000 [Zenker et al., 2005].

The human *UBR1* gene is located on chromosome 15q15.2. It spans 163 kb and contains 47 exons that encode for a protein with a total length of 1749 amino acids. No protein isoform variants are known. The UBR1 protein contains two zinc finger motifs, a UBR-box and a RING-H2 domain (Fig. 1) [Kwon et al., 1998; Xie and Varshavsky, 1999; Kwak et al., 2004]. UBR1 represents one of at least four E3 ubiquitin ligases of the N-end rule pathway, an evolutionary conserved and ubiquitously expressed intracellular proteolytic pathway involved in ubiquitin-mediated degradation of many proteins. Specifically, this pathway relates the stability of a protein to the identity of its N-terminal amino acid [Varshavsky, 1996; Varshavsky, 1997]. The N-end rule pathway has been found to be involved in various basic biological functions, such as oxygen sensing, regulation of DNA repair, signaling by G proteins, metabolic regulation, apoptosis, meiosis, spermatogenesis, neurogenesis, and organ development and functioning [Varshavsky, 2011; Tasaki et al., 2012]. However, the full spectrum of its complex biological functions is still not well understood.

In 2005, Zenker et al. (2005) discovered mutations in *UBR1* to be responsible for JBS. The molecular pathogenesis and pathophysiology of JBS, however, still remain unclear. Although UBR1 has a ubiquitous expression pattern, the symptoms of JBS are limited to specific organs, thus giving rise to the hypothesis that the UBR1 defect may be partially rescued/compensated in some tissues by other E3 ubiquitin ligases [Zenker et al., 2005]. In the exocrine pancreas, which is invariably affected in JBS, the underlying pathogenetic mechanism appears to be an inflammatory damage of prenatal onset affecting acinar cells [Daentl et al., 1979; Moeschler et al., 1987; Vanlieferinghen et al., 2001; Zenker et al., 2005]. Other symptoms of the disease such as imperforate anus, scalp defects and renal anomalies are rather considered as primary defects of organ development and the causative mechanism of the UBR1 defect in their pathogenesis is unsolved. Mice that are deficient of Ubr1 do not show any obvious symptoms or malformations reminiscent of JBS except for being underweight [Kwon et al., 2001], which can be seen in patients that are not appropriately treated with pancreatic enzyme replacement. However, by reinvestigating these mice, it was shown that they do have some impairment in pancreatic enzyme production and increased susceptibility to experimentally induced pancreatitis [Zenker et al., 2005; Zenker et al., 2006].

The original patients included in the gene identification study showed the typical severe manifestations of JBS and exhibited predominantly truncating mutations [Zenker et al., 2005]. Subsequently, some patients with a milder and/or oligosymptomatic phenotype were described [Alkhouri et al., 2008; Fallahi et al., 2011], and a recent study provided evidence for the existence of hypomorphic alleles as the explanation of at least part of the phenotypic variability [Hwang et al., 2011]. Herein, we review a total of 60 mutations including 31 novel ones; we summarize clinical findings and discuss genotype–phenotype correlations.

Methods of Mutation Detection and *in Silico* Prediction

Patients with a suspected diagnosis of JBS were evaluated with a standardized clinical checklist submitted by the referring clinician. Moreover, clinical photographs were reviewed by an experienced clinical geneticist (M.Z.). The study was approved by the Ethics Board of the Medical Faculty of the University of Erlangen. Informed consent was obtained from all patients or parents. Molecular genetic studies included direct sequencing of the *UBR1* gene as described previously [Zenker et al., 2005]. Parental samples were examined as far as they were available to confirm the segregation of *UBR1* mutations.

Sequences generated by the ABI 3500xl Genetic analyzer (Applied Biosystems, Cheshire, UK) were evaluated and compared with the reference sequence (NM_174916.2) using the Sequence Pilot software package version 4.0.1 (JSI Medical Systems GmbH, Kippenheim, Germany). The nomenclature standards of Human Genome Variation Society (HGVS) [den Dunnen and Antonarakis, 2000; online update July 2013] were used for correct naming on nucleotide and protein level; additionally the denomination of mutations was verified using Mutalyzer 2.0.beta-21 (https://mutalyzer.nl/). Pathogenicity of all variants was checked with MutationTaster (http://www.mutationtaster.org/). Missense mutations were additionally rated using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (Sorting Intolerant From Tolerant, http://sift.jcvi.org/), and MutPred (http://mutpred.mutdb.org). For *in silico* prediction of hypothesized splice site mutations, NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/) and BDGP (Berkeley Drosophila Genome Project, http://www.fruitfly.org/seq_tools/splice.html) were used. Protein alignment was created using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and BLOSUM62-Matrix.

Database

We have established a collection of all mutations in the *UBR1* gene (http://databases.lovd.nl/shared/variants/UBR1), as well as all available phenotype data of patients that were clinically and molecularly ascertained to have JBS (http://databases.lovd.nl/shared/individuals/UBR1). The database was created on the Leiden Open Variation Database (LOVD) system, LOVD 3.0 build

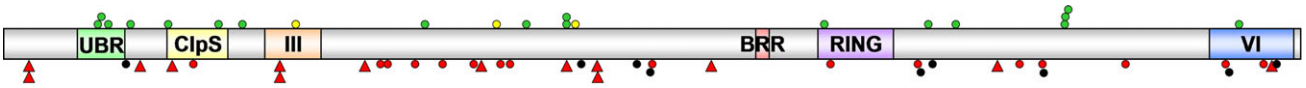

Figure 1. UBR1 protein with its conserved domains and distribution of mutations. The protein contains several distinct regions, such as the UBR box (green), a highly conserved substrate-binding domain. The ClpS region (yellow) shows sequence similarity to prokaryotic ClpS, which is an accessory subunit for recognition of degrons by the ATP-dependent protease ClpAP [Zeth et al., 2002]. Region III (orange) denotes a sequence that is highly conserved between UBR1 and UBR2 in different species but the function of which is unclear. The conserved region VI (blue) largely overlaps with a domain that is believed to function in regulation of protein activity by covering or exposing protein binding domains (autoinhibitory domain) [Tasaki et al., 2012]. The basic residue-rich region (BRR, pink) has been found in yeast ubr1 for binding to rad6 [Kwon et al., 1998; Xie and Varshavsky, 1999]. The RING domain (purple) is a cysteine- and histidine-rich region that is present in several E3 Ub ligases [Xie and Varshavsky, 1999; Kwon et al., 2001; Kwon et al., 2003]. Mutations are presented as circles and triangles. A total of 60 mutations are shown in this figure. Several of the missense mutations are clustered at the UBR box and in a region between RING and region VI that has not been described as a functional domain so far. Green circles: missense mutations (18). Yellow circles: small in-frame deletions (3). Red circles: nonsense mutations (16). Black circles: frameshift mutations (9). Red triangles: splice site mutations (14).

08 [Fokkema et al., 2005, 2011]. So far, the database contains 60 different *UBR1* mutations and phenotype data from 61 individuals.

Mutations in the *UBR1* Gene

Sequence changes that were classified as disease-causing mutations are listed in Table 1. They were found in patients with an unambiguous JBS phenotype as either homozygous or compound-heterozygous sequence changes with only two exceptions where only one disease-causing allele could be identified (see below). Pathogenic sequence changes comprise various types of mutations including nonsense and frameshift mutations that produce premature stop codons, splice site mutations that are predicted to interfere with correct splicing, as well as missense mutations and small in-frame deletions affecting conserved amino acid residues. 29 of the 60 mutations listed in Table 1 have been described previously [Zenker et al., 2005; Al-Dosari et al., 2008; Alkhouri et al., 2008; Elting et al., 2008; Almashraki et al., 2011; Fallahi et al., 2011; Hwang et al., 2011; Liu et al., 2011; Schoner et al., 2012; Godbole et al., 2013; Singh et al., 2014]. Thirty-one mutations are novel ones identified by conventional sequencing of all coding exons and flanking intronic regions in each patient. Sequence changes were generally classified as causative mutations, if they (1) produce a premature stop codon, (2) affect the highly conserved nucleotides −1, −2, +1, +2 near splice site junctions or if they (3) delete/substitute a conserved amino acid and were observed in combination with the presence of a mutation on the second allele (Table 2). As an exception to this rule we classified three novel intronic changes as pathogenic mutations that do not affect the invariant positions of the splice sites (c.1094-13A>G, c.1911+14C>G, c.5109-3A>G). All of these variants were predicted *in silico* to abrogate the authentic splice site or create an ectopic splice site that reaches higher scores than the authentic site. The predicted effect of these three mutations on RNA splicing could be confirmed by studies on the patients' mRNA in all cases. In two patients/families, a disease-causing mutation could be identified only on one allele (Families 24 and 26, Table 2). In one case the observed heterozygous change was a nonsense mutation (p.(Tyr1508*)), in the other a missense change (p.(Ala563Asp)) that was observed independently in a second unrelated family (22). We assume that these two individuals most likely harbor a mutation on the second allele that could not be identified because of the methodological limitations (e.g., intronic or promoter mutations, larger deletions or duplications). For patient 24.1, we demonstrated at the mRNA-level an overrepresentation of the allele carrying the p.(Ala563Asp) mutation (Supp. Fig. S1), thus suggesting instability of the mRNA produced from the allele assumed to harbor the unidentified mutation. Additionally, immunoblotting revealed clearly decreased expression of the UBR1 protein (data not shown). No other mutation detection technologies such as deep sequencing or MLPA were available to identify the presumed second *UBR1* mutation in these patients.

Of the 60 detected mutations, 25 (42%) are predicted to create a premature stop codon, including 16 nonsense and nine frameshift mutations. Fourteen (23%) of the disease-causing alleles represent splice site mutations, six of which could be analyzed at the mRNA level, revealing that the abnormal splicing leads to premature stop codons in five of them and an in-frame deletion of five amino acids in one (Table 1). Additionally, 18 mutations (30%) are predicted to be missense changes, and three are small in-frame deletions (5%). Their pathogenicity has been evaluated with the help of various online *in silico* prediction tools (MutationTaster, PolyPhen-2, SIFT, and MutPred). The evolutionary conservation of the affected amino acid residues and *in silico* prediction scores are shown in Supp. Figure S2 and Supp. Table S1. All missense alleles were observed in a single family only, except for the mutation p.(Ala563Asp). In nine cases, the missense change was in compound heterozygosity with a bona fide mutation on the second allele (6, 8, 15, 17, 18, 23, 27, 31, 40); in six families (22, 29, 39, 41, 46, 47), the missense mutation was homozygous in the patients; and three cases (21, 33, 37), had two different missense alleles or a small in frame deletion on the second allele (Table 2). All sequence changes listed in Table 1 were not found in over 200 individuals from a multiethnic cohort sequenced for *UBR1* in our laboratory, who did not have a clinical diagnosis of JBS. None of these changes is listed as a non-pathogenic variant at 1000 Genomes Project (TGP; http://www.1000genomes.org/home) and NHLBI Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS/). In families from which multiple affected individuals or unaffected siblings were available for testing, we could confirm segregation of the mutations with the phenotype.

Six mutations were recurrent (Table 1). The homozygous mutation c.2839+5G>A was previously reported in two unrelated families originating from Turkey and Iran, respectively (19, 25). The authors speculated that this might be a founder mutation in that region [Elting et al., 2008]. Another recurrent mutation, p.(Gln513*), was detected in three unrelated families from Costa Rica (1, 2, 44), thus establishing this mutation as a founder allele in that population. In contrast, the deletion c.660–2_660–1delAG was detected independently in two families with no obvious common background (8 German origin; 42 from Mexico). The same is true for the

Table 1. Mutations in the *UBR1* Gene Causing Johanson–Blizzard Syndrome

Location	Nucleotide alteration	Predicted effect[a]	Family ID	Previous publications
Intron 01	c.81+2dupT	r.spl.? p.?	34	
Intron 01	c.81+5G>C	r.spl.? p.?	23	Alkhouri et al. (2008)
Exon 03	c.364G>C	p.(Val122Leu)	18	Hwang et al. (2011)
Exon 03	c.380G>T	p.(Cys127Phe)	41	
Exon 03	c.407A>G	p.(His136Arg)	6	Zenker et al. (2005); Hwang et al. (2011)
Exon 04	c.477delT	p.(Gly160Alafs*5)[b]	10	Zenker et al. (2005)
Exon 04	c.497A>G	p.(His166Arg)	31	
Intron 04	c.529-13G>A	*p.(Asn177Leufs*10)*	20, 48	Godbole et al. (2013)
Exon 05	c.650T>G	p.(Leu217Arg)	40	
Intron 05	c.660-2_660-1delAG	r.spl.? p.?	8, 42	Zenker et al. (2005)
Exon 06	c.753_754delTG	p.(Cys251*)	11	Zenker et al. (2005)
Exon 07	c.857T>G	p.(Ile286Arg)	17	
Exon 08	c.950T>C	p.(Leu317Pro)	33	Liu et al. (2011)
Intron 09	c.1094-13A>G	*p.(Val365Glufs*2)*	14	
Intron 09	c.1094-12A>G	r.spl.? p.?	13	Zenker et al. (2005)
Exon 10	c.1166_1177del12	p.(Ala389_Phe392del)	37	
Intron 12	c.1440-1G>A	r.spl.? p.?	36	Al-Dosari et al. (2008)
Exon 13	c.1507C>T	p.(Arg503*)	15, 32	Hwang et al. (2011)
Exon 13	c.1537C>T	p.(Gln513*)	1, 2, 44	Zenker et al. (2005)
Exon 14	c.1648C>T	p.(Gln550*)	3	Zenker et al. (2005)
Exon 15	c.1688C>A	p.(Ala563Asp)	22, 24	
Exon 15	c.1759C>T	p.(Gln587*)	4	Zenker et al. (2005); Schoner et al. (2012)
Exon 16	c.1886C>G	p.(Ser629*)	28	
Intron 16	c.1911+14C>G	*p.(Glu638Valfs*29)*	9	
Exon 17	c.1979_1981delTTG	p.(Val660del)	23	Alkhouri et al. (2008)
Exon 17	c.1993C>T	p.(Arg665*)	17	
Exon 18	c.2034C>A	p.(Tyr678*)	29	
Exon 19	c.2098T>C	p.(Ser700Pro)	39	Almashraki et al. (2011)
Intron 20	c.2254+2T>C	r.spl.? p.?	6	Zenker et al. (2005); Hwang et al. (2011)
Exon 21	c.2260C>T	p.(Arg754Cys)	37	
Exon 21	c.2261G>A	p.(Arg754His)	46	
Exon 21	c.2294_2296delAAG	p.(Glu766del)	21	
Exon 21	c.2319dupT	p.(His774Serfs*6)	18	Hwang et al. (2011)
Intron 21	c.2379+1G>C	r.spl.? p.?	5	Zenker et al. (2005)
Intron 21	c.2380-1G>A	r.spl.? p.?	45	
Exon 24	c.2546_2547insA	p.(Met849Ilefs*13)[c]	9	Zenker et al. (2005)
Exon 25	c.2598delA	p.(Pro867Hisfs*12)[d]	12	Zenker et al. (2005)
Exon 25	c.2608G>T	p.(Glu870*)	42	
Intron 26	c.2839+5G>A	*p.(Arg947Aspfs*7)*	19, 25	Elting et al. (2008)
Exon 30	c.3304C>G	p.(Gln1102Glu)	15	Hwang et al. (2011)
Exon 30	c.3328G>T	p.(Glu1110*)	50	
Exon 33	c.3682C>T	p.(Gln1228*)	30	
Exon 33	c.3694delC	p.(Leu1232Trpfs*17)	27	
Exon 33	c.3724A>G	p.(Arg1242Gly)	21	
Exon 33	c.3745dupA	p.(Arg1249Lysfs*4)	49	
Exon 34	c.3835G>A	p.(Gly1279Ser)	8	Zenker et al. (2005)
Intron 35	c.3998-1G>C	*p.(Glu1333_Gly1337del)*	16	
Exon 37	c.4093C>T	p.(Gln1365*)	40	Almashraki et al. (2011)
Exon 38	c.4188C>A	p.(Cys1396*)	35	Fallahi et al. (2011)
Exon 38	c.4193delT	p.(Leu1398Argfs*3)	43	
Exon 39	c.4277C>T	p.(Pro1426Leu)	33	Liu et al. (2011)
Exon 39	c.4280C>T	p.(Ser1427Phe)	27	
Exon 39	c.4291T>C	p.(Ser1431Pro)	29	
Exon 41	c.4524T>A	p.(Tyr1508*)	26	
Exon 45	c.4927G>T	p.(Glu1643*)	7	Zenker et al. (2005)
Exon 45	c.4942delG	p.(Glu1648Lysfs*21)	16	
Exon 45	c.4981G>A	p.(Gly1661Arg)	47	Singh et al. (2014)
Exon 46	c.5080G>T	p.(Glu1694*)	34	
Intron 46	c.5109-3A>G	*p.(Arg1704Glyfs*26)*	28	
Exon 47	c.5135_5144del10	p.(Arg1712Leufs*14)	38	

[a]Italic letters indicate that the effect of splicing mutations was demonstrated on mRNA level.
[b–d]Denominations of these previously published mutations were corrected according to current guidelines [den Dunnen and Antonarakis, 2000; online update July 2013]: [b]previously published as T159fsX164; [c]previously published as M849fsX861; [d]previously published as P866fsX878.
Mutation nomenclature refers to GenBank reference sequence NM_174916.2. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Table 2. Overview of All Patients Who were Included in this Study

Family	Patient	Genotype	Gender	Age	Exocrine pancreatic insufficiency	Nasal wings gestalt	Oligodontia of permanent teeth	Hearing impaiment	Scalp defect	Cognitive impair-ment	Short stature (P<3)	Hypothy-roidism	Micro-cephaly (P<3)	Intra uterine growth restriction	Congenital heart defect	Imper-forate anus	Genital malfor-mations	Renal anomalies	Diabetes (age of onset/ diagnosis)	Reference
1	1.1	[p.Q513*] + [p.Q513*]	M	11.8y	+	A, C	NA	+	–	NS	+	NA	–	–	–	–	–	–	NA	1
2	2.1	[p.Q513*] + [p.Q513*]	F	8y	+	A, C	NA	+	+	ID	+	–	–	–	–	–	–	–	NA	1, 2
	2.2	[p.Q513*] + [p.Q513*]	M	9m†	+	A, C	NA	NA	+	NA	+	–	+	–	–	–	–	–	NA	1, 2
3	3.1	[p.Q550*] + [p.Q550*]	F	10y	+	A	+	+	+	ID	–	+	–	–	–	+	–	–	–	1
4	4.1	[p.Q587*] + [p.Q587*]	M	7y	+	A	+	+	+	ID	+	+	+	–	–	+	HY, CR	–	NA	1, 3
	4.2	[p.Q587*] + [p.Q587*]	M	1d†	+	A	NA	NA	+	NA	NA	NA	–	+	HCM	–	MI, O	+	NA	1, 3
5	5.1	[c.2379+1G>C] + [c.2379+1G>C]	M	14.8y	+	A	+	+	+	ID	+	+	+	–	–	–	O	–	NA	1, 4
	5.2	[c.2379+1G>C] + [c.2379+1G>C]	M	3w†	+	A	NA	NA	+	NA	NA	NA	NA	NA	TOF	+	–	–	NA	1, 4
	5.3	[c.2379+1G>C] + [c.2379+1G>C]	F	4m	+	H	NA	+	+	NA	+	NA	+	+	–	–	CL	–	NA	–
6	6.1	[p.H136R] + [c.2254+2T>C]	F	13.5y	+	A	+	+	+	MID	+	+	–	–	–	+	NS	+	–	1, 5
7	7.1	[p.E1643*] + [p.E1643*]	M	14d†	+	A	NA	+	+	NA	NA	–	–	+	VSD, O	+	MI	–	NA	1, 6
	7.2	[p.E1643*] + [p.E1643*]	M	TOP	NA	A	NA	NA	+	NA	NA	NA	–	–	–	+	–	+	NA	7
8	8.1	[c.660-2_660-1delAG] + [p.G1279S]	F	11y	+	H	+	–	–	–	–	–	NA	–	–	–	–	–	NA	1, 8
9	9.1	[c.1911+14C>G] + [p.M849Ifs*13]	M	25y	+	H	+	+	–	–	+	–	–	+	–	–	–	–	–	1, 4, 9
10	10.1	[p.G160Afs*5] + [p.G160Afs*5]	F	12y	+	A, C	+	+	+	ID	–	+	–	–	ASD	+	–	+	(12)	1, 10–12
11	11.1	[p.C251*] + [p.C251*]	M	13y	+	A	+	+	+	NS	+	+	+	+	VSD	–	–	–	–	1, 10–12
12	12.1	[p.P867Hfs*12] + [p.P867Hfs*12]	M	4.5y	+	NS	+	+	–	NS	+	–	–	–	–	–	–	–	NA	1
13	13.1	[c.1094-12A>G] + [c.1094-12A>G]	F	1.1y	+	A	NA	+	+	NA	–	–	–	–	–	–	–	–	NA	1, 13
14	14.1	[c.1094-13A>G] + [c.1094-13A>G]	M	3y	+	A	NA	+	+	NS	+	+	NA	–	VSD	–	HY	–	NA	–
15	15.1	[p.R503*] + [p.Q1102E]	F	15.3y	+	H	+	+	+	–	–	–	NA	–	–	–	–	–	–	5
16	16.1	[c.3998-1G>C] + [p.E1648Kfs*21]	M	11.9y	+	H	+	–	+	MID	+	–	–	–	–	+	–	–	–	14
17	17.1	[p.I286R] + [p.R665*]	M	19y	+	H	+	+	–	BL	+	+	–	–	–	–	–	–	–	–
18	18.1	[p.V122L] + [p.H774Sfs*6]	F	20y	+	H	+	–	+	–	–	+	–	–	–	–	–	–	–	5
19	19.1	[c.2839+5G>A] + [c.2839+5G>A]	F	7.1y	+	H	+	+	+	–	–	+	–	–	DCM	–	–	–	–	15
	19.2	[c.2839+5G>A] + [c.2839+5G>A]	F	22y	+	H	+	–	–	–	–	–	–	+	–	–	–	–	–	15
20	20.1	[c.529-13G>A] + [c.529-13G>A]	F	5m	+	A	NA	+	+	NS	–	–	+	–	PDA	–	–	–	–	–
21	21.1	[p.E766del] + [p.R1242G]	M	12.1y	+	H	+	–	–	–	–	–	NA	–	–	–	–	–	–	–
22	22.1	[p.A563D] + [p.A563D]	F	12y	+	H	+	–	+	–	–	–	NA	NA	–	–	–	–	–	16
	22.2	[p.A563D] + [p.A563D]	F	7y	+	H	+	+	+	BL	–	–	–	NA	–	–	–	–	–	16
23	23.1	[c.81+5G>C] + [p.V660del]	F	12.3y	+	H	+	+	–	–	–	–	–	–	–	–	–	–	–	17
24	24.1	[p.A563D] + [?]	F	37y	+	H	+	+	–	BL	+	–	NA	+	–	–	–	–	(18)	–
25	25.1	[c.2839+5G>A] + [c.2839+5G>A]	F	5.5y	+	H	NA	+	+	NS	+	+	+	+	ASD	–	–	–	–	15
	25.2	[c.2839+5G>A] + [c.2839+5G>A]	F	3m†	+	H	NA	NA	+	NA	NA	NA	+	+	–	–	–	–	NA	15
26	26.1	[p.Y1508*] + [?]	F	5.6y	+	A	+	+	+	NS	–	+	–	–	–	–	–	–	–	–
27	27.1	[p.L1232Wfs*17] + [p.S1427F]	M	8.9y	+	H	+	NA	–	–	+	–	NA	NA	–	–	NA	–	NA	–
28	28.1	[p.S629*] + [c.5109-3A>G]	F	18y	+	H	+	+	–	–	+	–	–	–	–	–	–	+	(11)	–

(*Continued*)

Table 2. Continued

Family	Patient	Genotype	Gender	Age	Exocrine pancreatic insufficiency	Nasal wings gestalt	Oligodontia of permanent teeth	Hearing impaiment	Scalp defect	Cognitive impair-ment	Short stature (P<3)	Hypothy-roidism	Micro-cephaly (P<3)	Intra uterine growth restriction	Congenital heart defect	Imper-forate anus	Genital malfor-mations	Renal anomalies	Diabetes (age of onset/ diagnosis)	Reference
29	29.1	[p.S1431P] + [p.S1431P]	M	12.2y	+	H	+	–	–	–	–	–	–	–	–	–	–	–	–	–
	29.2	[p.S1431P] + [p.S1431P]	F	9.8y	+	H	+	–	–	–	–	–	–	–	–	–	–	–	–	–
30	30.1	[p.Q1228*] + [p.Q1228*]	M	15.2y	+	H	+	+	+	ID	+	–	+	+	–	–	CR	–	–	–
31	31.1	[p.H166R] + [p.Y678*]	F	15y	+	H	+	–	+	–	–	–	–	–	–	–	–	–	–	–
32	32.1	[p.R503*] + [p.R503*]	F	23y	+	H	NA	+	–	NA	+	–	NA	NA	–	–	–	–	–	–
	32.2	[p.R503*] + [p.R503*]	M	20y	+	H	NA	+	–	NA	+	–	NA	NA	–	–	–	–	–	–
	32.3	[p.R503*] + [p.R503*]	F	17y	+	H	NA	+	–	NA	+	–	NA	NA	–	–	–	–	(16)	–
33	33.1	[p.L317P] + [p.P1426L]	F	1.8y	+	H	+	–	+	ID	+	–	NA	NA	–	+	–	–	–	18
34	34.1	[c.81+2dupT] + [p.E1694*]	M	7.7y	+	H	+	+	–	NA	–	–	+	–	–	–	–	+	–	–
35	35.1	[p.C1396*] + [p.C1396*]	M	3.5y	+	H	+	+	+	BL	+	+	–	–	PDA, O	–	HY	–	–	19
36	36.1	[c.1440-1G>A] + [c.1440-1G>A]	M	3m	–	A	NA	+	+	NA	+	+	+	+	–	+	–	+	–	20
37	37.1	[p.A389_F392del] + [p.R754C]	M	13y	+	A, C	+	+	+	–	–	–	–	NA	–	–	–	–	–	–
38	38.1	[p.R1712Lfs*14] + [p.R1712Lfs*14]	M	8m	+	A	NA	+	–	NA	+	–	+	NA	ASD	+	–	–	NA	–
39	39.1	[p.S700P] + [p.S700P]	M	3y	+	A	NA	–	+	NA	+	+	+	+	ASD	–	HY	–	–	21
40	40.1	[p.L217R] + [p.Q1365*]	M	22y	+	H	+	+	+	ID	+	+	NA	–	–	–	–	+	–	–
41	41.1	[p.C127F] + [p.C127F]	F	2.3y	+	A	+	+	+	NS	+	+	–	+	–	–	–	–	–	–
42	42.1	[c.660-2_660-1delAG] + [p.E870*]	F	13.6y	+	A	+	+	+	NA	–	+	–	–	–	+	CL, O	–	–	–
43	43.1	[p.L1398Rfs*3] + [p.L1398Rfs*3]	F	1.7y	+	H	+	+	–	NS	+	–	–	–	–	–	–	–	–	–
44	44.1	[p.Q513*] + [p.Q513*]	F	4m†	+	A, C	+	NA	–	NA	NA	–	NA	+	–	–	–	–	–	–
45	45.1	[c.2380-1G>A] + [c.2380-1G>A]	M	1.3y	+	A	NA	+	+	NS	–	+	–	–	–	–	HY	–	–	–
46	46.1	[p.R754H] + [p.R754H]	F	4m†	+	A	NA	NA	+	NA	+	–	+	+	ASD	–	–	–	NA	–
47	47.1	[p.G1661R] + [p.G1661R]	M	2.5m	+	H	NA	NA	+	NA	+	NA	+	–	–	–	–	–	–	22
48	48.1	[c.529-13G>A] + [c.529-13G>A]	F	10m	+	H	NA	+	–	NA	+	+	–	–	ASD, PDA	–	–	–	NA	23
49	49.1	[p.R1249Kfs*4] + [p.R1249Kfs*4]	F	1.2y	+	A	NA	+	+	NA	+	–	+	–	–	–	–	–	–	–
50	50.1	[p.E1110*] + [p.E1110*]	F	2.5m†	+	A	NA	NA	–	NA	NA	+	–	–	ASD	–	–	–	–	–
% of all patients presenting with a symptom					98	100	100	78	64	61	61	39	34	29	25	20	20	13	10	

M, male; F, female; y, year(s); m, month(s); w, week(s); d, day(s); †, deceased; TOP, termination of pregnancy; NA, no data available; H, hypoplasia; A, aplasia; C, facial clefting; NS, present but not specified; ID, moderate to severe intellectual disability; MID, mild intellectual disability; BL, borderline intellectual disability; ASD, atrial septal defect; VSD, ventricular septal defect; HCM, hypertrophic cardiomyopathy; TOF, tetralogy of fallot; PDA, patent ductus arteriosus; O, other anomalies; HY, hypospadias; CR, cryptorchidism; MI, micropenis; CL, clitoral hypertrophy/clitoromegaly. References: (1) Zenker et al. (2005); (2) Guzman and Carranza (1997); (3) Schoner et al. (2012); (4) Rudnik-Schoneborn et al. (1991); (5) Hwang et al. (2011); (6) Vanlieferinghen et al. (2001); (7) Vanlieferinghen et al. (2003); (8) Zerres and Holtgrave (1986); (9) Swanenburg de Veye et al. (1991); (10) Jones et al. (1994); (11) Timoney et al. (2004); (12) Cheung et al. (2009); (13) Vieira et al. (2002); (14) Mcheik et al. (2002); (15) Elting et al. (2008); (16) Reichart et al. (1979); (17) Alkhouri et al. (2008); (18) Liu et al. (2011); (19) Fallahi et al. (2011); (20) Al-Dosari et al. (2008); (21) Almashraki et al. (2011); (22) Singh et al. (2014); (23) Godbole et al. (2013).

recurrent mutation p.(Arg503*) that was identified in a family of Arab ancestry (32) and additionally in a patient originating from Portugal (15). We could not study in detail the allelic background of these mutations to prove that they have emerged independently. The missense mutation p.(Ala563Asp) was detected in unrelated families from Germany (22) and England (24) and may have a common origin in the Middle European population.

It is assumed that the effect of JBS-associated *UBR1* mutations is a loss of function or diminished expression of the gene product. The lack of UBR1 protein has been proven by immunoblotting in some patients with biallelic truncating mutations [Zenker et al., 2005]. In contrast, it has been shown for a few missense mutations that the mutant protein may be expressed but functionally impaired [Hwang et al., 2011]. The missense mutations p.(Val122Leu) and p.(His136Arg) that were investigated in detail affect the extremely conserved UBR box, allowing study of these changes in the yeast Ubr1 homologue. Aside from those mutations affecting the UBR box, there is no strict clustering of missense mutations and small in-frame deletions to certain protein regions of known function (Fig. 1). An apparent clustering of missense mutations (p.(Pro1426Leu), p.(Ser1427Phe), p.(Ser1431Pro)) was observed in a protein domain of unknown function and may indicate that this region is critically involved in intra- or intermolecular interactions.

Genotype–Phenotype Correlations

All individuals carrying homozygous or compound heterozygous *UBR1* mutations as well as the two individuals in which the mutation could only be identified on one allele had a clear clinical diagnosis of JBS. However, the clinical expression of the syndrome and of individual manifestations showed wide variation. This variability was more pronounced between than within families. All patients reviewed in this article were affected by clinically apparent exocrine pancreatic insufficiency leading to failure to thrive, except for one patient (36.1) reported by Al-Dosari et al. (2008). This patient was seen at the age of three months and could not be followed up (Fowzan Alkuraya, personal communication). He may have developed this symptom later. In all other cases manifestation of exocrine pancreatic insufficiency was within the first year of life, in most of them shortly after birth. All patients who were followed up for long term had a need of permanent pancreatic enzyme supplementation. Moreover, all patients displayed anomalies of the nasal wings that ranged from complete aplasia sometimes associated with more severe lateral facial clefting, to quite subtle hypoplasia (Fig. 2). Anomalies of secondary teeth (oligodontia, hypodontia) were also present in all patients on whom appropriate information of the dental status was available.

In order to determine to what extent the *UBR1* genotype may contribute to the variability of the clinical expression, we compared the group of patients with biallelic "truncating" (nonsense, frameshift) mutations (= cohort 1; $n = 21$) to those with a "nontruncating" mutation (missense or small in-frame deletion) on at least one allele (= cohort 2; $n = 21$), assuming that truncating mutations most likely lead to complete lack of a functional UBR1 protein, whereas nontruncating mutations may in some cases retain some residual protein function. In both cohorts, 100% of the individuals presented with pancreatic insufficiency, dental defects and total/partial absence of alae nasi (Fig. 3). However, regarding the facial phenotype, we observed that milder expression of nasal wing hypoplasia was significantly associated with the presence of at least one nontruncating allele, whereas facial clefting and complete aplasia of the alae nasi were typically found in patients with biallelic truncating

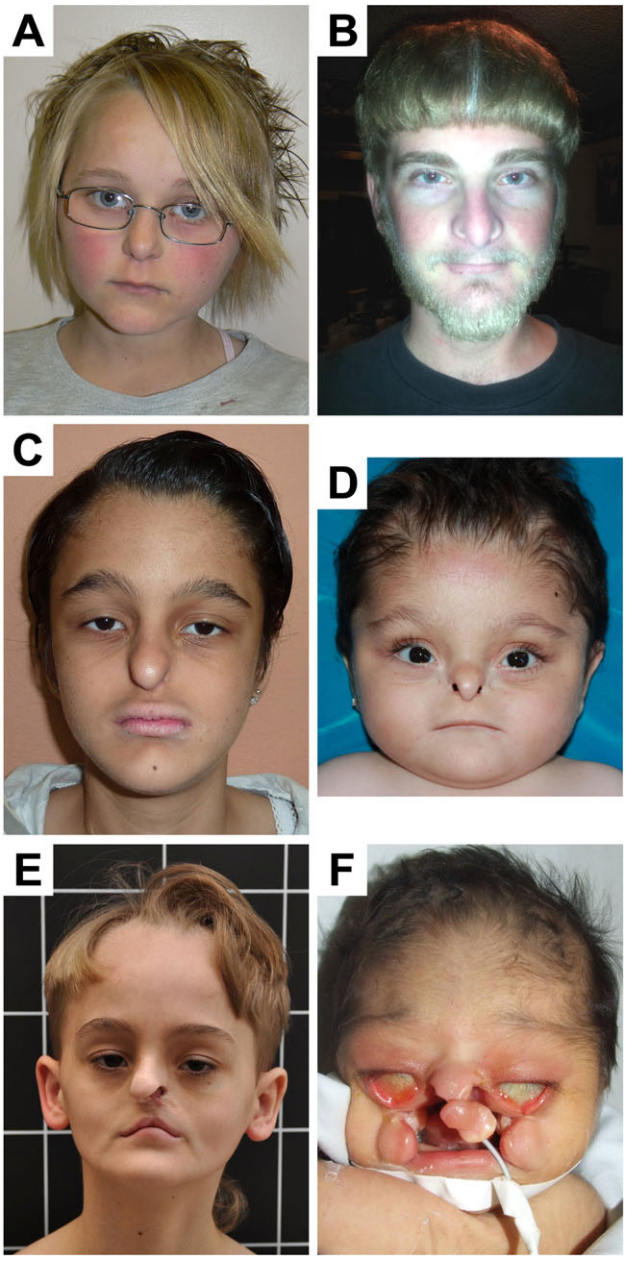

Figure 2. Variability in the expression of facial anomalies in JBS. Mild hypoplasia of nasal wings and frontal hair upsweep in patient 28.1 (A). Nasal wing hypoplasia in patient 40.1 (B). Complete aplasia of nasal wings in patient 41.1 (C) and patient 42.1 (D). More extensive defects of the lateral portion of the nose together with zygomatic hypoplasia and a surgically repaired cleft lip and palate in patient 37.1 (E), and severe facial malformations with a bilateral cleft lip extending into lateral facial clefts and lower eyelid coloboma in patient 44.1 (F).

alleles. On the severe end of the spectrum of facial malformations, lateral facial clefting was observed in several individuals, notably in four patients from three Costa Rican families (1, 2, and 44) with the same homozygous nonsense mutation p.(Arg503*). We speculate that this may rather be an effect of genetic background than directly correlated with this specific *UBR1* genotype. Patients with two biallelic truncating mutations frequently presented with hearing impairment (100%), cognitive impairment (100%) and short stature (88%), whereas individuals of the cohort with nontruncating

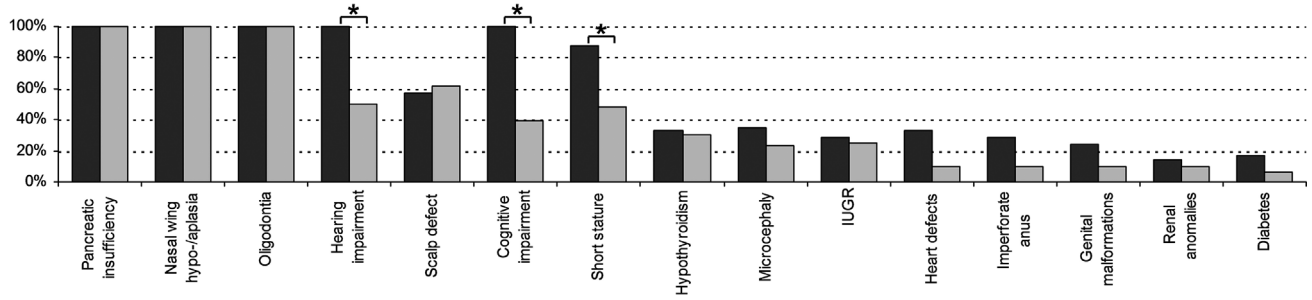

Figure 3. Genotype–phenotype correlation in JBS patients. Frequency of different clinical signs and symptoms in JBS patients with biallelic truncating mutations (black bars) and patients with at least one nontruncating mutation (gray bars). Symptoms showing statistically significant differences ($P < 0.05$/Fisher's exact test) between those two cohorts are marked with asterisks. IUGR: intrauterine growth restriction.

mutations showed these features in a significantly lower frequency, namely 50%, 39%, and 48%, respectively (Fig. 3). Several other symptoms were also seen at a higher frequency in cohort 1, although the differences did not reach statistical significance because of a lower overall prevalence. Those included microcephaly (35% in cohort 1 vs. 23% in cohort 2), heart defects (33% vs. 10%), imperforate anus (29% vs. 10%), genital malformations (24% vs. 10%), and diabetes (17% vs. 6%). The clinical data collected for the study do not allow to exclude that some of the observed differences (e.g., differences in stature, head growth) might at least in part be secondary to nutritional aspects related to the severity of pancreatic dysfunction. Scalp defects, hypothyroidism, intrauterine growth restriction, and renal anomalies had an almost equal distribution regarding our two cohorts. The most evident difference was related to the mental status of the patients. In cohort 1, all patients had some degree of cognitive impairment, with half of them classified as having moderate to severe intellectual disability, whereas in cohort 2 more than half of the individuals were reported to have intellectual abilities in the normal range (Fig. 4). Together, these findings suggest that at least some of the nontruncating mutations represent hypomorphic alleles. Different levels of residual function have indeed been demonstrated for three missense mutations in a yeast model [Hwang et al., 2011]. However, there is currently no method available to easily assess the function of mutant UBR1 proteins. Our data also suggest that the minimal requirements for UBR1 function vary between different cells/tissues. Although some residual UBR1 protein function seems to be sufficient for rescuing the brain function, it is insufficient to prevent pancreatic insufficiency, oligodontia, and nasal wing hypo-/aplasia.

Although we can provide evidence for a significant impact of the genotype on phenotypic expression, we also observed intrafamilial clinical variability between siblings carrying the same mutations. For example, patient 19.1 was reported to have diabetes, hearing impairment and a serious congestive cardiomyopathy, whereas her older sister (19.2) did not exhibit any of these symptoms [Elting et al., 2008]. Patients 22.1 and 22.2 [Reichart et al., 1979] were sisters with the same homozygous missense mutation. Both exhibited a relatively mild phenotype, but there were differences in mental and hearing abilities. In another family, one affected child had severe lethal urogenital malformations, whereas the older brother had no

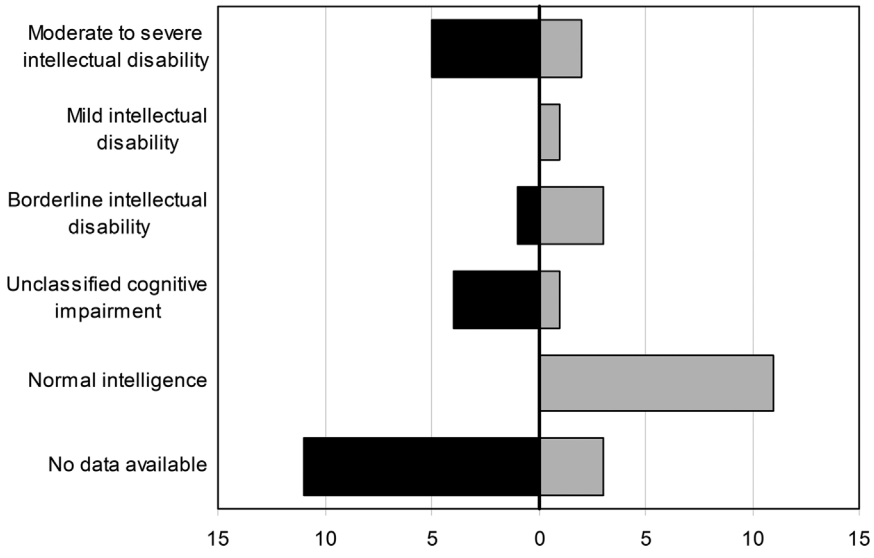

Figure 4. Intellectual functioning and degree of intellectual impairment in cohort 1 (biallelic truncating mutations, black bars) versus cohort 2 (at least one nontruncating mutation, gray bars). The level of intellectual functioning was classified according to the results of formal IQ testing, if available, or estimated by the referring clinicians based on the classification proposed by Zhang et al. (2005). The majority of patients without information about the intellectual status were too young for proper classification. This was particularly true for cohort 1 (median age 7 years 6 months) compared with cohort 2 (median age 11 years 9 months).

such anomalies [Schoner et al., 2012] (Table 2). Taking this into account, it is obvious that the *UBR1* genotype alone cannot explain all the phenotypic variability. The existence of unlinked genetic or nongenetic modifiers or stochastic factors has to be assumed, but their nature as well as their possible impact on the phenotype is currently unknown.

In the original report describing *UBR1* mutations in JBS, the majority of mutations were truncating ones (eight of 14 = 57%) or were predicted to cause abnormal splicing (29%), whereas only two (14%) were missense mutations [Zenker et al., 2005]. In this review, 21 out of 60 mutations represent missense alleles or small in-frame deletions, equaling 35% of all detected mutations (Table 1). The excess of truncating mutations in the first report may be because of the fact that patients with the full phenotype were selected for the gene identification study, while later molecular testing was also available for more mildly affected or oligosymptomatic patients in whom nontruncating mutations are more common.

Clinical and Diagnostic Relevance

The clinical diagnosis of JBS is principally based on the recognition of the association of exocrine pancreatic insufficiency and the characteristic facial anomalies. In typical cases with pancreatic involvement, complete or partial absence of the nasal wings, oligodontia of permanent teeth, hearing impairment, scalp defects, short stature, and cognitive impairment, the diagnosis is obvious. Molecular testing of *UBR1* will very likely be able to confirm the diagnosis in those patients. In our cohort of patients with a clinically well-defined JBS phenotype, we were able to detect the likely disease-causing mutation on 67 out of 69 alleles (mutation detection rate: 97%). Mutational screening by sequencing of exonic and flanking intronic regions should especially be attempted in families who are seeking prenatal testing for JBS in future pregnancies. Although fetuses affected by JBS may present with dilated sigmoid colon as a sign of anal atresia and with aplastic alae nasi on high-resolution ultrasound scanning [Auslander et al., 1999; Vanlieferinghen et al., 2003], only molecular testing of the *UBR1* gene allows an early and reliable prenatal diagnosis, whereas all of the physical anomalies that may be detected by prenatal ultrasound are variable. Molecular testing may also be indicated to identify a familial mutation in order to offer subsequent carrier testing in healthy family members if requested, although the risk of having a child with JBS in relatives of a patient is very small (at least in the absence of parental consanguinity), given a presumably low carrier frequency in the general population. In cases with relatively mild or oligosymptomatic JBS, molecular analysis of *UBR1* may help to establish the diagnosis. In patients with some features of the JBS-associated malformation spectrum but with a lack of pancreatic involvement, detection of a mutation in *UBR1* seems very unlikely. The authors of this review have performed *UBR1* testing in more than 100 individuals with various phenotypes partially overlapping JBS but without exocrine pancreatic insufficiency and could not identify any disease-causing *UBR1* mutations in this cohort (data not shown). The same is true for a number of patients with pancreatic insufficiency whose phenotypes did otherwise not fit with JBS. We could also not confirm the diagnosis in two cases reported in the literature as having JBS, in which the clinical diagnosis appeared doubtful to us [Dumic et al., 1998; Steinbach and Hintz, 2000]. Generally, exocrine pancreatic insufficiency manifesting in early infancy is a rare symptom. Besides JBS, cystic fibrosis (MIM #219700), as well as Shwachman–Bodian–Diamond syndrome (MIM #260400), have to be considered in the differential diagnosis of early onset exocrine pancreatic insufficiency. Among these entities, JBS can usually be distinguished without difficulties on the basis of accompanying anomalies. However, nasal wing hypoplasia may sometimes be quite subtle considering that the degree of nasal wing development is quite variable in the normal population as well. Other manifestations, such as cognitive impairment, deafness and oligodontia may not be noticeable at early age. Our current experience with 61 individuals with molecularly confirmed JBS, and *UBR1* testing in multiple patients with phenotypes partially overlapping with JBS, suggests that besides exocrine pancreatic insufficiency, nasal wing hypo-/aplasia and oligodontia of permanent teeth are invariant features of JBS. Hearing impairment of variable degree occurs in 78% of the JBS patients. The following symptoms have been observed in more than half of the molecularly proven cases: scalp defects (64%), cognitive impairment (61%) ranging from learning difficulties to severe intellectual disability, and short stature (61%). Further common features include hypothyroidism (39%), microcephaly (34%), intrauterine growth restriction (29%), and congenital heart defects (25%). Genital (20%) and anorectal (20%) malformations, renal anomalies (13%) and diabetes (10%) were seen occasionally. Notably, diabetes had an age of onset ranging from 11 to 18 years. This suggests that diabetes might represent the consequence of an ongoing destructive process of the pancreas and/or the lack of trophical factors derived from exocrine cells as in diabetes type 3c of other etiologies. We also noticed some rare malformations in patients with a molecularly confirmed diagnosis of JBS, some of these might be coincidental associations and not a result of *UBR1* mutations. The rarer abnormalities are: cleft lip and palate (1.1, 37.1, 44.1), tethered spinal cord (11.1, 35.1), neural tube defect (44.1), poly-/syndactyly of the feet (31.1, 42.1), brain malformations such as arhinencephaly (4.2) and Arnold-Chiari type II malformation (44.1), respiratory problems due to hypoplasia of the lung (4.2) or pneumopathy of unknown cause (4.1), osseous malformations including left hip dislocation (2.1, 2.2) and vertebral anomalies (11.1), situs inversus (7.1), prostate aplasia (4.1), gastroesophageal reflux (35.1), cholestatic liver disease (36.1), and natal teeth (44.1). Although we have demonstrated a correlation between nontruncating mutations and less severe expressions of the disease, predictions on the individual phenotypic expression on the basis of the genotype should be made with much caution. This is particularly true for novel (private) mutations, which still constitute the majority of identified alleles.

When JBS is diagnosed in an individual, a panel of baseline investigations is recommended to detect all medical issues that may be related with the disease. These include assessment of the pancreatic exocrine function, abdominal ultrasound or MRI, echocardiography, audiometry, and screening for hypothyroidism [Sukalo et al., 2014]. During followup, it is of particular importance to regularly check for hearing loss, diabetes, and hypothyroidism because these symptoms may develop later in life [Trellis and Clouse, 1991; Nagashima et al., 1993].

For patients affected by JBS, there is thus far no causal treatment available. Symptoms have to be treated according to general guidelines: These include enzyme supplementation for pancreatic insufficiency, insulin for diabetes mellitus, surgery for anorectal and genitourinary malformations, hearing aids for deafness, as well as early intervention programs and specific education for individuals with intellectual disabilities. Dental implantation is usually required during the teenage years [Zerres and Holtgrave, 1986]. Facial malformations may be corrected by plastic surgery [Kobayashi et al., 1995; Fichter et al., 2003; Timoney et al., 2004].

On the basis of current experience, there is no convincing evidence of genetic heterogeneity of JBS. Undetected *UBR1* mutations in a small number of patients with an unequivocal clinical

diagnosis of JBS may rather be explained by methodological limitations. Likewise there is no evidence that *UBR1* mutations might cause other phenotypes than JBS, and any specific clinical manifestations or disease predispositions in heterozygous *UBR1* mutation carriers are not yet established.

Future Prospects

Future research needs to be directed toward the identification of possible modifiers of the phenotype caused by UBR1 defects. Understanding the pathophysiology caused by the UBR1 deficiency is a particular challenge. Since UBR1 acts in targeted protein degradation, it is reasonable to assume that inappropriate levels of certain intracellular proteins are mainly involved. The exocrine pancreas, which is most severely and invariably affected in JBS, is in the focus of ongoing research. Identification of proteins and mechanisms involved in the pathophysiology of pancreatic involvement in JBS may be the clue to a more general insight into pancreatic damage and pancreatitis. Treatment prospects might eventually arise from a more detailed knowledge of molecular pathophysiology and modifying factors. Moreover, genetic research on disorders partially overlapping with JBS may reveal other genes encoding components of the N-end rule pathway as disease-causing genes and may lead to a more complete picture of human N-end rule pathway disorders. It is of great interest to continue deciphering genotype–phenotype correlations in order to enable more precise predictions regarding the clinical course and severity of this syndrome. Furthermore, functional analysis of naturally occurring disease-associated mutations has the potential to lead to the discovery of new domains and functions of the UBR1 protein.

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References

Al-Dosari MS, Al-Muhsen S, Al-Jazaeri A, Mayerle J, Zenker M, Alkuraya FS. 2008. Johanson–Blizzard syndrome: report of a novel mutation and severe liver involvement. Am J Med Genet A 146A:1875–1879.

Alkhouri N, Kaplan B, Kay M, Shealy A, Crowe C, Bauhuber S, Zenker M. 2008. Johanson–Blizzard syndrome with mild phenotypic features confirmed by UBR1 gene testing. World J Gastroenterol 14:6863–6866.

Almashraki N, Abdulnabee MZ, Sukalo M, Alrajoudi A, Sharafadeen I, Zenker M. 2011. Johanson–Blizzard syndrome. World J Gastroenterol 17:4247–4250.

Auslander R, Nevo O, Diukman R, Morrad E, Bardicef M, Abramovici H. 1999. Johanson–Blizzard syndrome: a prenatal ultrasonographic diagnosis. Ultrasound Obstet Gynecol 13:450–452.

Cheung JC, Thomson H, Buncic JR, Heon E, Levin AV. 2009. Ocular manifestations of the Johanson–Blizzard syndrome. J AAPOS 13:512–514.

Daentl DL, Frias JL, Gilbert EF, Opitz JM. 1979. The Johanson–Blizzard syndrome: case report and autopsy findings. Am J Med Genet 3:129–135.

den Dunnen JT, Antonarakis SE. 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat 15:7–12.

Dumic M, Ille J, Bobonj G, Kordic R, Batinica S. 1998. The Johanson–Blizzard syndrome. Lijec Vjesn 120:114–116.

Elting M, Kariminejad A, de Sonnaville ML, Ottenkamp J, Bauhuber S, Bozorgmehr B, Zenker M, Cobben JM. 2008. Johanson–Blizzard syndrome caused by identical UBR1 mutations in two unrelated girls, one with a cardiomyopathy. Am J Med Genet A 146A:3058–3061.

Fallahi GH, Sabbaghian M, Khalili M, Parvaneh N, Zenker M, Rezaei N. 2011. Novel UBR1 gene mutation in a patient with typical phenotype of Johanson–Blizzard syndrome. Eur J Pediatr 170:233–235.

Fichter CR, Johnson GA, Braddock SR, Tobias JD. 2003. Perioperative care of the child with the Johanson–Blizzard syndrome. Paediatr Anaesth 13:72–75.

Fokkema IF, den Dunnen JT, Taschner PE. 2005. LOVD: easy creation of a locus-specific sequence variation database using an "LSDB-in-a-box" approach. Hum Mutat 26:63–68.

Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. 2011. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat 32:557–563.

Godbole K, Sukalo M, Hiremath L, Zenker M. 2013. Johanson–Blizzard syndrome. Indian Pediatr 50:510–512.

Guzman C, Carranza A. 1997. Two siblings with exocrine pancreatic hypoplasia and orofacial malformations (Donlan syndrome and Johanson–Blizzard syndrome). J Pediatr Gastroenterol Nutr 25:350–353.

Hwang CS, Sukalo M, Batygin O, Addor MC, Brunner H, Aytes AP, Mayerle J, Song HK, Varshavsky A, Zenker M. 2011. Ubiquitin ligases of the N-end rule pathway: assessment of mutations in UBR1 that cause the Johanson–Blizzard syndrome. PLoS One 6:e24925.

Johanson A, Blizzard R. 1971. A syndrome of congenital aplasia of the alae nasi, deafness, hypothyroidism, dwarfism, absent permanent teeth, and malabsorption. J Pediatr 79:982–987.

Jones NL, Hofley PM, Durie PR. 1994. Pathophysiology of the pancreatic defect in Johanson–Blizzard syndrome: a disorder of acinar development. J Pediatr 125:406–408.

Kobayashi S, Ohmori K, Sekiguchi J. 1995. Johanson–Blizzard syndrome facial anomaly and its correction using a microsurgical bone graft and tripartite osteotomy. J Craniofac Surg 6:382–385.

Kwak KS, Zhou X, Solomon V, Baracos VE, Davis J, Bannon AW, Boyle WJ, Lacey DL, Han HQ. 2004. Regulation of protein catabolism by muscle-specific and cytokine-inducible ubiquitin ligase E3alpha-II during cancer cachexia. Cancer Res 64:8193–8198.

Kwon YT, Reiss Y, Fried VA, Hershko A, Yoon JK, Gonda DK, Sangan P, Copeland NG, Jenkins NA, Varshavsky A. 1998. The mouse and human genes encoding the recognition component of the N-end rule pathway. Proc Natl Acad Sci USA 95:7898–7903.

Kwon YT, Xia Z, An JY, Tasaki T, Davydov IV, Seo JW, Sheng J, Xie Y, Varshavsky A. 2003. Female lethality and apoptosis of spermatocytes in mice lacking the UBR2 ubiquitin ligase of the N-end rule pathway. Mol Cell Biol 23:8255–8271.

Kwon YT, Xia Z, Davydov IV, Lecker SH, Varshavsky A. 2001. Construction and analysis of mouse strains lacking the ubiquitin ligase UBR1 (E3alpha) of the N-end rule pathway. Mol Cell Biol 21:8007–8021.

Liu ZF, Zhang ZH, Li M, Jin Y, Lian M, Tang WW. 2011. Report of a case with Johanson–Blizzard syndrome and literatures review. Zhonghua Er Ke Za Zhi 49:66–69.

Mcheik JN, Hendiri L, Vabres P, Berthier M, Cardona J, Bonneau D, Levard G. 2002. Johanson–Blizzard syndrome: a case report. Arch Pediatr 9:1163–1165.

Moeschler JB, Polak MJ, Jenkins JJ, 3rd, Amato RS. 1987. The Johanson–Blizzard syndrome: a second report of full autopsy findings. Am J Med Genet 26:133–138.

Nagashima K, Yagi H, Kuroume T. 1993. A case of Johanson–Blizzard syndrome complicated by diabetes mellitus. Clin Genet 43:98–100.

Reichart P, Flatz S, Burdelski M. 1979. [Ectodermal dysplasia and exocrine pancreatic insufficiency—a familial syndrome]. Dtsch Zahnarztl Z 34:263–265.

Rudnik-Schoneborn S, Keller B, Beemer FA, Pistor K, Swanenburg de Veye HF, Zerres K. 1991. Johanson–Blizzard syndrome. Klin Padiatr 203:33–38.

Schoner K, Fritz B, Huelskamp G, Louwen F, Zenker M, Moll R, Rehder H. 2012. Recurrent Johanson–Blizzard syndrome in a triplet pregnancy complicated by urethral obstruction sequence: a clinical, molecular, and immunohistochemical approach. Pediatr Dev Pathol 15:50–57.

Singh A, Chaudhary N, Dhingra D, Sukalo M, Zenker M, Kapoor S. 2014. Johanson–Blizzard syndrome: Hepatic and hematological features with novel genotype. Indian J Gastroenterol 33:82–84.

Steinbach WJ, Hintz RL. 2000. Diabetes mellitus and profound insulin resistance in Johanson–Blizzard syndrome. J Pediatr Endocrinol Metab 13:1633–1636.

Sukalo M, Mayerle J, Zenker M. 2014. Clinical utility gene card for: Johanson–Blizzard syndrome. Eur J Hum Genet 22:e1–3.

Swanenburg de Veye HF, Heineman-de-Boer JA, Beemer FA. 1991. A child of high intelligence with the Johanson–Blizzard syndrome. Genet Couns 2:21–25.

Tasaki T, Sriram SM, Park KS, Kwon YT. 2012. The N-end rule pathway. Annu Rev Biochem 81:261–289.

Timoney N, Weinberg MJ, Ross DA, Thomson HG. 2004. Johanson–Blizzard syndrome: a challenge in nasal reconstruction. Can J Plast Surg 12:81–85.

Trellis DR, Clouse RE. 1991. Johanson–Blizzard syndrome. Progression of pancreatic involvement in adulthood. Dig Dis Sci 36:365–369.

Vanlieferinghen P, Gallot D, Francannet C, Meyer F, Dechelotte P. 2003. Prenatal ultrasonographic diagnosis of a recurrent case of Johanson–Blizzard syndrome. Genet Couns 14:105–107.

Vanlieferinghen PH, Borderon C, Francannet CH, Gembara P, Dechelotte P. 2001. Johanson–Blizzard syndrome. a new case with autopsy findings. Genet Couns 12:245–250.

Varshavsky A. 1996. The N-end rule: functions, mysteries, uses. Proc Natl Acad Sci USA 93:12142–12149.

Varshavsky A. 1997. The N-end rule pathway of protein degradation. Genes Cells 2:13–28.

Varshavsky A. 2011. The N-end rule pathway and regulation by proteolysis. Protein Sci 20:1298–1345.

Vieira MW, Lopes VL, Teruya H, Guimaraes-Lamonato L, Oliveira LC, Costa CD. 2002. Johanson–Blizzard syndrome: the importance of differential diagnostic in pediatrics. J Pediatr (Rio J) 78:433–436.

Xie Y, Varshavsky A. 1999. The E2–E3 interaction in the N-end rule pathway: the RING-H2 finger of E3 is required for the synthesis of multiubiquitin chain. Embo J 18:6832–6844.

Zenker M. 2008. *UBR1* and the N-end Rule Pathway and the Johanson-Blizzard Syndrome. In: Epstein CJ, Erickson RP, Wynshaw-Boris A, editors. *Inborn Errors of Development.* Oxford, U.K.: Oxford University Press. p 1190–1194.

Zenker M, Mayerle J, Lerch MM, Tagariello A, Zerres K, Durie PR, Beier M, Hulskamp G, Guzman C, Rehder H, Beemer FA, Hamel B, et al. 2005. Deficiency of UBR1, a ubiquitin ligase of the N-end rule pathway, causes pancreatic dysfunction, malformations and mental retardation (Johanson–Blizzard syndrome). Nat Genet 37:1345–1350.

Zenker M, Mayerle J, Reis A, Lerch MM. 2006. Genetic basis and pancreatic biology of Johanson–Blizzard syndrome. Endocrinol Metab Clin North Am 35:243–253, vii–viii.

Zerres K, Holtgrave EA. 1986. The Johanson–Blizzard syndrome: report of a new case with special reference to the dentition and review of the literature. Clin Genet 30:177–183.

Zeth K, Ravelli RB, Paal K, Cusack S, Bukau B, Dougan DA. 2002. Structural analysis of the adaptor protein ClpS in complex with the N-terminal domain of ClpA. Nat Struct Biol 9:906–911.

Zhang X, Snijders A, Segraves R, Niebuhr A, Albertson D, Yang H, Gray J, Niebuhr E, Bolund L, Pinkel D. 2005. High-resolution mapping of genotype-phenotype relationships in cri du chat syndrome using array comparative genomic hybridization. Am J Hum Genet 76:312–326.