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Impact of clinical exomes in neurodevelopmental and neurometabolic disorders☆

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A B S T R A C T

Whole exome sequencing (WES) is well established in research and is now being introduced into clinically indicated diagnostics (so-called clinical exomes). We evaluated the diagnostic yield and clinical implications of WES in 72 patients from 60 families with undiagnosed neurodevelopmental disorders (NDD), neurometabolic disorders, and dystonias. Pathogenic or likely pathogenic variants leading to a molecular diagnosis could be identified in 21 of the 60 families (overall 35%, in 36% of patients with NDD, in 43% of patients with neurometabolic disorders, in 25% of patients with dystonias). In one family two coexisting autosomal recessive diseases caused by homozygous pathogenic variants in two different genes were diagnosed. In another family, a homozygous frameshift variant in *STRADA* was found to cause a severe NDD with early onset epilepsy, brain anomalies, hypotonia, heart defect, nephrocalcinosis, macrocephaly and distinctive facies so far designated as PMSE (polyhydramnios, megalencephaly, symptomatic epilepsy) syndrome. In 7 of the 21 families with a molecular diagnosis the pathogenic variants were only identified by clinical follow-up, manual reevaluation of the literature, a change of filter setting, and/or reconsideration of inheritance pattern. Most importantly, clinical implications included management changes in 8 cases and impact on family planning in 20 families with a molecular diagnosis. This study shows that reevaluation and follow-up can improve the diagnostic rate and that WES results have important implications on medical management and family planning. Furthermore, we could confirm *STRADA* as a gene associated with syndromic ID but find it questionable if the current designation as PMSE depicts the most important clinical features.

1. Introduction

Whole exome sequencing (WES) has proven to be a powerful tool to unravel the etiology of presumably monogenic diseases, in particular neurodevelopmental disorders (NDD), and to identify new disease or candidate genes. After having been tested in research, WES is now being introduced in clinically indicated diagnostics. For moderate to severe intellectual disability (ID) the identification of the underlying monogenic cause has been reported in 16% to 50% and most often consists of *de novo* heterozygous variants [1–3]; for patients with NDD or pediatric neurologic disorders, the yield of (likely) pathogenic findings ranges between 25 and 57%, settling at a level of 25 to 28% regarding large studies with broader inclusions (see Table 1). Recently, a high diagnostic rate of 68% (28/41) has been reported on investigation of 41 patients with ID and a broadly defined metabolic phenotype [4].

The benefit of a diagnosis for the assessment of the recurrence risk and for genetic counseling translating into informed decision making with regard to family planning, and possibly a prenatal diagnosis, has been known for a long time. Knowledge of a causal diagnosis may provide specific information on the disease course, facilitate the coping process, can give access to medical support and services, and helps to become involved in patient support groups [17]. Beyond these well-

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Table 1
Results of clinical exome studies in patients with neurodevelopmental disorders.

Reference	# subjects age	Phenotype	Diagnostic rate (%)	*Dn* mutation	Two or more diagnoses	Clinical implications by diagnosis	Clinical implications by incidental findings
De Ligt et al., 2012 [1]	100	Severe ID	16	81% (13/16)	0	n.d.	n.d.
Rauch et al., 2012 [2]	51	Severe non-syndromic ID	~50	~96%	0	n.d.	n.d.
Yang et al., 2013 [5]	250 89% ≤18 y	85% Neurol	25	53%	4 pts. (1.6%)	n.d.	12%
Hamdan et al., 2014 [3]	41	Moderate/ severe non-syndromic ID	29	100%	0	n.d.	n.d.
Srivastava et al., 2014 [6]	78 <18 y	NDD	41	59% (19/32)	1 pt. (1.3%)	All, management changes in 47%, impact on FP in 87%	n.d.
Lee et al., 2014 [7]	814 64% <18 y	Any, 37% DD,	26	50% (of trio-based diagnoses)	n.d.?	n.d.	Incidental findings in 5%
Yang et al., 2014 [8]	2.000 88% <18 y	Mostly NDD	25	51%	23 pts. (4.6%)	n.d.	4.6%
Wright et al., 2015 [9]	1.133 <18 y	87% DD/ID	27*	65%*	n.d.	n.d.	n.d.
Retterer et al., 2016 [10]	3.040 Any (mean 11.4 ± 13.2 y)	Any, 36% neurol/NDD	28.8	42.5%	28 pts. (± 1%)	n.d.	6%
Nolan & Carlson, 2016 [11]	50 <18 y	88% NDD	47	33%	0	In 78%, management changes in 52%, impact on FP in 48%	10%
Monroe et al., 2016 [12]	17	ID/NDD	29	n.d.	0	n.d.	n.d.
Tarailo-Graovac et al., 2016 [4]	41 90% <18 y	Neurometabolic disorders/ID + metabolic phenotype,	28/41	39% (11/28)	5/28 pts. (18%)	44% (18 pts)	2% (1/41)
Stark et al., 2016 [13]	80 ≤2 y	MCA + dysmorphism + other, 74% neurometabolic	57.5	36%	1 fam	Management changes in 32%, impact on FP in ≥62%	n.d.
Kuperberg et al., 2016 [14]	57 <18 y	NDD, Neurol	49	50%	0	All, changes in medic. in 5 pts	n.d.
Trujillano et al., 2017 [15]	1000 0–59 y 45% from cons. families	Any, 77% neurol/NDD	30.7	20%	3/307 pts. (1%)	n.d.	n.d.
Lisenka et al., 2017 [16]	150 <18 y	ID, Neurol	29.3	77% (34/44)	0	n.d.	n.d.

Legend: cons.: consanguineous, DD: developmental delay, *Dn*: *de novo*, fam: family, FP: family planning, ID: intellectual disability, n.d.: not documented, NDD: neurodevelopmental disorder, MCA: multiple congenital anomalies, neurol: neurologic disease, pts.: patients, y: years, * incl. CNVs.

known aspects of a causal diagnosis, there is growing evidence that a diagnosis influences the management of the disease, including surveillance for directly or indirectly related morbidities, and can pave the way to a targeted disease-modifying treatment [4,6,11,14].

We evaluated the diagnostic clues, the yield and the clinical implications of trio-based WES in a group of patients with presumably monogenic diseases, predominantly children with NDD, neurometabolic or movement disorders, following a phenotype-first approach and using a semi-automated bioinformatics pipeline. An illustrative case report of siblings with a *STRADA* associated NDD is included.

2. Patients and methods

2.1. Patients

WES was performed in a cohort of 72 patients from 60 families with undiagnosed, suspected genetic conditions. The patients were included between 1/2013 and 12/2015 after a phenotype-first approach, meaning that patients were thoroughly phenotypically characterized prior to WES and that the patient's phenotype was taken into consideration for the interpretation of WES data. The study cohort comprised 45 index patients with developmental delay (DD)/ ID and/or congenital malformations (NDD group) seen at the Institute of Human Genetics, eight patients with infantile dystonia (dystonia group), and seven patients with a neurometabolic disorder (neurometabolic group), seen at the Department of General Pediatrics, Division of Neuropediatrics and Metabolic Medicine, of Heidelberg University Hospital. The neurometabolic group includes one patient with clearly reduced concentration of 5-methyltetrahydrofolate in CSF (ZKJM003-014), four patients with abnormal glycosylation of alpha-1 antitrypsin and two patients with a general glycosylation deficiency (therefore presenting a presumed congenital disorder of glycosylation, CDG). Apart from continuously reduced serotonergic metabolites in CSF without clear pathophysiological significance in one of the patients with dystonia, there were no metabolic abnormalities observed among the eight patients of the dystonia group. For that reason, these patients have not been included in the neurometabolic group, although infantile dystonia can be caused by neurometabolic diseases. All patients were evaluated and phenotypically characterized by an experienced clinical geneticist and/or neuropediatrician. The evaluation included the medical history and family history of at least three generations and a physical, dysmorphological, and neurologic examination. Brain MRI was performed in patients with abnormal findings on neurologic examination in addition to DD/ID (*e.g.* focal neurologic deficits), or micro- or macrocephaly and in patients with a suspected genetic condition with expected brain phenotype. If clinically indicated examinations by other medical specialist (*e.g.* ophthalmologic evaluation) were performed. Clinical characteristics of the index patients are given in Table 2.

The patients had a mean age at diagnosis of 8.5 years compared to a mean age of 6.4 years at WES analysis regarding the patients in whom no diagnosis could be achieved. 50% of patients were males, and most were of German or Turkish origin (55% and 28%, respectively). 25% of families, mainly those of Turkish origin, reported consanguinity. 70% of index patients with known family history were sporadic cases, 30% had at least one affected sibling. Patients displayed a wide range of symptoms (Table 2), with 77% having DD/ID. Other common phenotypes were micro- or macrocephaly (53% and 12%, respectively), dysmorphic signs (40%), short stature (32%), epilepsy (28%), and

Table 2
Clinical characteristics of the 60 index patients.

Characteristics	Patient no. *(%)*		
	NDD ($n = 45$)	Dystonia ($n = 8$)	NM ($n = 7$)
Sex			
Male	22 (49%)	5 (62%)	3 (43%)
Female	23 (51%)	3 (38%)	4 (57%)
Age of molecular diagnosis (solved cases)			
<10 years	11 (69%)	0 (0%)	2 (67%)
≥10 years	5 (31%)	2 (100%)	1 (33%)
Age at whole exome sequencing (unsolved cases)			
<10 years	14 (61%)	0 (0%)	4 (100%)
≥10 years	9 (39%)	6 (100%)	0 (0%)
Parental consanguinity			
Yes	15 (33%)	0 (0%)	0 (0%)
No	30 (66%)	1 (13%)	6 (86%)
N/A	0 (0%)	7 (87%)	1 (14%)
Number of affected family members			
One	30 (66%)	1 (13%)	4 (57%)
Two	12 (27%)	0 (0%)	0 (0%)
≥Two	3 (7%)	0 (0%)	0 (0%)
N/A	0 (0%)	7 (87%)	3 (43%)
Region of origin			
Middle Europe (Germany)	25 (56%)	3 (38%)	5 (71%)
Eastern Europe (Turkey)	16 (33%)	0 (0%)	1 (14%)
Eastern Europe (other than Turkey)	1 (<1%)	0 (0%)	1 (14%)
Southern Europe[a]	2 (<1%)	0 (0%)	0 (0%)
Israel	1 (<1%)	0 (0%)	0 (0%)
N/A	0 (0%)	5 (63%)	0 (0%)
Phenotype			
ID/DD	43 (96%)	2 (25%)	6 (86%)
Learning disability	1 (<1%)	0 (0%)	0 (0%)
Mild	9 (20%)	1 (13%)	1 (14%)
Moderate	6 (13%)	0 (0%)	0 (0%)
Severe	18 (40%)	1 (13%)	5 (71%)
Unspecified, N/A	9 (20%)	0 (0%)	0 (0%)
Epilepsy	11 (25%)	0 (0%)	6 (86%)
Behavioral abnormalities including ASD	11 (25%)	0 (0%)	0 (0%)
Additional neurologic symptoms[b]	21 (47%)	8 (100%)[c]	3 (43%)
Abnormal neuroimaging	19 (42%)	4 (50%)	3 (43%)
Mikro−/Makrocephalie	24 (53%)/6 (13%)	3 (38%)/0 (0%)	5 (71%)/1 (14%)
Short stature/overgrowth	17 (38%)/1 (<1%%)	1 (13%)/0 (0%)	1 (14%)/0 (0%)
Failure to thrive	3 (7%)	2 (25%)	0 (0%)
Dysmorphic features	22 (49%)	0 (0%)	2 (29%)
Congenital malformations	17 (38%)	0 (0%)	2 (29%)
Other organ manifestations	17 (18%)	1 (13%)	5 (71%)

Abbreviations: ASD, autism spectrum disorder; DD, developmental delay; ID, intellectual disability; n, total number; N/A, not available; NDD, neurodevelopmental disorders; NM, neurometabolic disorders.

[a] Italy/Croatia (father/mother) and Portugal (both parents).

[b] Including ataxia, nystagmus, tremor, hyper- or hypertonia, dystonia, hemi- or tetraparesis, spasticity, hyperekplexia, eye movement disorder/opsoclonus, facial nerve palsy, congenital miosis, peripheral neuropathy, hyperventilation.

[c] All dystonia.

behavioral abnormalities including autism spectrum disorders (18%). 32% of patients had congenital malformations, most often congenital heart disease (CHD). Before enrollment and in addition to deep phenotyping, all patients had undergone an extensive diagnostic workup. For the NDD group this included selective metabolic screening, standard G-banded chromosome analysis at 550-band resolution or more, molecular karyotyping (using Affymetrix® Cytogenetics Whole-Genome 2.7 M Array or Affymetrix® CytoScan HD Oligo/SNP-Array), and targeted gene tests whenever indicated. The patients with neurometabolic disorders received an extensive metabolic testing, including organic acids in urine; amino acids in plasma, urine and CSF; homocysteine in plasma; oligosaccharides and mucopolysaccharide electrophoresis in urine; biogenic amines, pterins and 5MTHF in CSF, acylcarnitines in dried blood spots and plasma; transferrin electrophoresis for CDG syndromes; and very long chain fatty acids. In all patients of the dystonia group CSF neurotransmitter monoamine metabolite analysis was performed and resulted normal or with unspecific changes. For each patient written consent for participation had been given by the patient or his/her legal representative. The study adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee of the Faculty of Medicine at the University of Heidelberg.

2.1.1. Case report of family 5

A girl of 16 years (individual A0170701) from consanguineous Turkish parents (fam. 5) presented with severe ID, cortical malformation and anomalies of the midbrain and fossa posterior on cranial MRI, symptomatic epilepsy with onset at the age of three months, and significant hypotonia. In addition, she showed an atrial septal defect (ASD), nephrocalcinosis, and relative macrocephaly. She was evaluated many times at the Division of Neuropediatrics and Metabolic Medicine of the Children's Hospital and at the Outpatient Clinic of the Institute of Human Genetics at Heidelberg, Germany. When last seen at 16 years of age, she was constrained to a wheelchair, had no speech, showed a neurogenic kyphoscoliosis and increasing behavioral aggressiveness. Epilepsy was well controlled by therapy with oxcarbacepine and valproate. Her height was according to the mean whereas her occipitofrontal circumference (OFC) was plotted to the 88th centile. Facial features comprised a long narrow face, broad eyebrows, synophris, mild hypertelorism, broad nasal bridge, smooth philtrum, full lower lip, and ear dysplasia.

An older brother had been affected by the same disorder and died at 10 years of age in status epilepticus. His cranial MRI showed only mild unspecific findings. There were two healthy sisters.

2.2. Methods

Genomic DNA was isolated from leukocytes from the proband, both parents, affected (if appropriate) and in selected cases also unaffected siblings by standard procedures. Trio-based WES and analysis of the sequence data was performed at the German Cancer Research Center (DKFZ) at Heidelberg, Germany. Table S2 provides details on the index patients with a molecular diagnosis. For data analysis we used the previously described Heidelberg exome data analysis bioinformatics pipeline; in case A0100401, the sequencing data were analyzed in parallel at the Institute of Medical Genetics and Applied Genomics, Tübingen, using the Tübingen bioinformatics pipeline [18]. Variants with a minor allele frequency (MAF) >1% in the Exome Aggregation Consortium (ExAC) and 1000 genome phase III database were considered common alleles and removed from the candidate list. Local control samples were used to remove the recurrent technical artifacts and common alleles that were not seen in the above databases. Gene-based annotations from Gencode V19 were added using ANNOVAR [19,20]. All SNVs and indels that affect protein sequences and variants within ±2 bases around the intron-exon junction, classified as splice-site variants, were considered as functional. Variants were further assessed by 7 different variant effect prediction tools (SIFT [21], PolyPhen2 [22], LRT [23], MutationTaster [24], MutationAssessor [25], FATHMM [26], and PROVEAN [27]) from dbNSFP v2.0 [28] and CADD scores [29]. Variants were classified and described using the recommended terminology 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign', and 'benign' according to standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) [30]. Genes harboring variants were described as 'candidate','newly identified' ('novel') or 'known to cause disease' according to previous publications with slight modifications [1,4]: genes which had not been previously implicated in human disease were defined as 'candidates'if variants were found in one family, were predicted to be pathogenic by the majority of *in silico* prediction tools, were linked to brain or embryonic development in the literature, and met at least two of the following criteria:

Table 3
Clinical data, results of WES, and clinical implications of identified variants for the patients with pathogenic or likely pathogenic variants.a*, b**

Fam. no.	Case ID	Group	Sex	Gene(s)	Variant(s) Reference transcript	*VS*	IP	ExAC-MAF	Reported	Class	No. of affected/seq. patients	Diagnosis/MIM	Phenotype	Expanding phenotype/ novel features	Age at dx (y; mo)	Impact on clinical management (e.g surveillance, therapy)	Impact on family planning	Ref.
4	A0100401	NDD	m	*PGAP1*	c.1090-2A>G; p.? ENST00000354764	hom	AR	–	No	Likely path	3/3	Mental retardation, autosomal recessive 42/ #615802	severe DD, hypotonia, microcephaly,	yes/retinal dystrophy	7;7	No	Yes	Granzow et al., 2015 [18]
	A1420405[a]		f	*IFT140*	c.3577G>T; p.(E1193X) ENST00000426508.2	hom	AR	–	No	Likely path		Short-rib thoracic dysplasia 9 with or without polydactyly/#266920	retinal dystrophy hydrops, shortened tubular bones, omphalocele, cystic kidney	Yes/omphalocele	pre-natal			
5	A0120501	NDD	f	*STRADA*	c.891dupC; p.(C298Lfs*11/ ENST 00000336174.6	hom	AR	–	No	Likely path	2/1	Polyhydramnios, megalencephaly, and symptomatic epilepsy/#611087	severe DD, epilepsy, hypotonia, cortical malformation, rel. Macrocephaly, facial dysmorphism, ASD, nephrocalcinosis	No	16;0	No	Yes	
7	A0170701	NDD	m	*CWF19L1*	c.467delC; p.(P156fs) ENST00000354105.4	hom	AR	–	No	Likely path	2/1	Spinocerebellar ataxia, autosomal recessive 17/#616127	mild ID, ataxia, dysmetria, intention tremor, dysarthria, cerebellar atrophy, mild microcephaly	Yes[b]/hexadactyly, vertebral malformation	13;8	No	Yes	Evers et al., 2016 [35]
9	A0210901	NDD	m	*PTCH1*	c.2978dupA; p.(K993 fs) ENST00000331920.6	het (dn)	AD	–	No	Likely path	1/1	Basal cell nevus syndrome/ #109400	severe ID, autism, hydrocephalus, chiari I malformation, macrocephaly, facial dysmorphism, craniosynostosis	Yes/craniosynostosis	8;9	Surveillance: regularly developmental assessment, physical and skin examinations because of increased risk for brain and skin tumors, orthopantogram to identify jaw keratocysts [36]	No	
10	A0241001	NDD	m	*DNMT3A*	c.1156delG; p.(V386 fs) ENST00000264709.3	het (dn)	AD	–	No	Likely path	1/1	Tatton-Brown--Rahman syndrome/#615879	mild ID, macrocephaly, overgrowth, facial dysmorphism, cryptorchism	No	9;5	no	Yes	
16	A0451701	NDD	m	*COASY*	c.728C>T; p.(A243V)	het	AR	–	No	Likely path	2/2	Neurodegeneration with brain iron accumulation 6/#615643	mild ID, obsessive--compulsive behavior, ataxia, spasticity, MRI abnormalities	Yes/novel brain (MRI) and metabolic abnormalities	6;10	Surveillance: annual neurologic examination for progressive cognitive impairment, spastic-dystonic paraparesis and	Yes	Evers et al. 2017 [37]
					c.1582C>T; p.(R528C) ENST00000590958.1	het		0,0000094	Yes[p]	Likely path								

17	A0551801	NDD	m	*USP9X*	c.6360A>G; p.(I2120M) ENST00000324545.8	hemi (mat)	XL	0,0001036	No	Likely path*	1/1	Mental retardation, X-linked 99/ #300919	DD, autism, macrocephaly, malformations toe and fingers	No	9;5	axonal neuropathy No	Yes	
18	A0581901	NDD	m	*MYT1L*	c.1990C>T; p.(Q664X) ENST00000428368.2	het (dn)	AD	–	No	Path	1/1	Mental retardation, autosomal dominant 39/#616521	severe ID, behavioral problems, microcephaly	No	8;5	No	Yes	
23	A0752401	NDD	f	*SYNGAP1*	c.403C>T; p.(R135X) ENST00000418600.2	het (dn)	AD	–	No	Likely path	1/1	Mental retardation, autosomal dominant 5/ #612621	severe global DD, severe speech delay, epilepsy, hyperventilation, movement disorder, microcephaly, low weight, PEG tube	No	12;4	No	Yes	
24	A0782501	NDD	f	*SIPA1L3*	c.4489C>T; p.(R1497X) ENST00000222345.6	hom	AR	0,0000094	No	Likely path	2/2	Cataract 45/#616851	bilateral congenital cataract	No	4;5	No	Yes	Evers et al., 2015 [32]
26	A1003101	NDD	f	*GNAO1*	c.607G>A; p.(G203R) ENST00000262493.6	het (dn)	AD	–	Yes[P]	Path	1/1	Epileptic encephalopathy, early infantile, 17/ #615473	severe ID, epilepsy, nystagmus, dystonia, hypotonia; microcephaly, dystrophy, strabismus convergens	Yes/severe dystrophy	4;7	Surveillance for movement disorder which might respond to specific treatment (deep brain stimulation [38,39]).	Yes	
27	A1033201	NDD	m	*PIGV*	c.607C>T; p.(R203C) c.1022C>A; p.(A341E) ENST00000374145.1	het het	AR	0,0000094 0,0000942	No	Likely path Likely path	1/1	Hyperphosphatasia with mental retardation syndrome 1/#239300	severe ID, epilepsy, MRI abnormalities, microcephaly, facial dysmorphism, brachydactyly, hypoplastic nails, intestinal malrotation, Hirschsprung disease, cryptorchism, conductive hearing loss	No	1;6	No	Yes	
28	A1063301	NDD	f	*PIBF1*	c.1453C>T; p.(Q485X) c.1508A>G; p.(Y503C) ENST00000326291.6	het het	AR	0,0000094 0,0000565	No	Path path	1/1	Joubert-Syndrome/-none (Stand: 18.12.2016)	severe DD, spastic tetraparesis, polymicrogyria, hypoplasia of vermis cerebelli, molar tooth sign, microcephaly, failure to thrive, cleft palate, hepatopathy	Yes/cleft palate, hepatopathy	3;2	Surveillance: Annual evaluations of growth, vision, and liver and kidney function; periodic neuropsychologic and developmental testing according to [40]	Yes	
35	A1324101	NDD	f	*DDX3X*	c.1703C>T; p.(P568L) ENST00000399959.2	hemi (dn)	XL	–	No	Path	1/1	Mental retardation, X-linked 102/#300958	severe DD, epilepsy, severe hypotonia, hypoplastic	Yes/CHD, anal anomalies	10;8	No	Yes	Dikow et al., 2017 [34]

(continued on next page)

Table 3 (*continued*)

Fam. no.	Case ID	Group	Sex	Gene(s)	Variant(s) Reference transcript	*VS*	IP	ExAC-MAF	Reported	Class	No. of affected/seq. patients	Diagnosis/MIM	Phenotype	Expanding phenotype/ novel features	Age at dx (y; mo)	Impact on clinical management (e.g surveillance, therapy)	Impact on family planning	Ref.
													corpus callosum, enlarged CSF spaces, delayed myelinisation, microcephaly, short stature, dysmorphism, CHD, anal atresia, spine anomalies					
39	A1494401	NDD	m	*ZBTB18*	c.1382A>G; p.(N461S) ENST00000358704	het (dn)	AD	–	Yes	Path	1/1	Mental retardation, autosomal dominant 22/#612337	severe ID, ataxia, microcephaly, short stature	No	5;11	No	Yes	
41	ZKJM001–001	NDD	m	*SCN2A*	c.5798A>T;p.(K1933M) ENST00000357398.3	het (dn)	AD	0,0000094	No	Likely path	1/1	Epileptic encephalopathy, early infantile, 11/#613721	severe ID, behavior abnormalities, epilepsy, ataxia, hypertonia, tremor	No	16;2	Specific antiepileptic treatment	Yes	
46	ZKJM003–014	NM	f	*DHFR*	c.335T>G;p.(M112R) ENST00000439211.2	hom	AR	–	No	Likely path*	NA/1	Megaloblastic anemia due to dihydrofolate reductase deficiency/#613839	severe DD, epilepsy, hypoplastic corpus callosum, brain atrophy, intracerebral calcifications, microcephaly, megaloblastic anemia, metabolic abnormalities: 5-MTHF in CSF not detectable	No	4;2	Surveillance: frequent EEG, evaluation of anticonvulsant medication, annual evaluation of blood count, vision, orthopedic complications, feeding difficulties, neuropsychologic and developmental testing	Yes	
47	ZKJM003–008	NM	f	*SLC35A2*	c.831C>G, p.(N277K) ENST00000376521.1.	hemi (mat)	XL	–	No	Likely path	NA/1	Congenital disorder of glycosylation, type IIm/#300896	severe DD, epilepsy, spastic movement disorder, progressive brain atrophy, macrocephaly, sensorineural hearing loss, congenital cataract, short	Yes/HCM, hearing problems, short stature	2;0	treatment trial with galactose [41], surveillance: periodic evaluation of movement disorder, neuropsychologic and developmental	Yes	

												stature, VSD, AV anomaly, HCM, CDG metabolic abnormalities: abnormal glycosylation of serum transferrin (CDG type II pattern)			testing; evaluation of blood coagulation, immune and renal function [41]:	
48	ZKJM003–009	NM	m	*ATP6AP1*	c.542T>G; p.(L181R) ENST00000369762.2	hemi (mat)	XL	–	No	Likely path	NA/1	Immunodeficieny and hepatopathy with or without neurologic features/#300972	normal cognitive development, hepatopathy, exocrine pancreas insufficiency, glomerulo tubulopathy, immune deficiency, cutis laxa, metabolic abnormalities: abnormal glycosylation of serum transferrin (CDG type II pattern)	Yes/cutis laxa	8;0	surveillance: periodic evaluations of vision, hearing, liver and renal function; annual neuropsycholo-gic and developmental testing	Yes
49	ZKJM003–001	Dys	m	*GNAO1*	c.736G>A; p.(E246K) ENST00000262493.6	het (dn)	AD	–	Yes	Path	1/1	Epileptic encephalopathy, early infantile, 17/#615473	severe ID, muscular hypotonia, action induced dystonic movement disorder and myoclonus, choreoathetosis, failure to thrive	No	9;1	No	Yes
50	ZKJM003–010	Dys	f	*GNAO1*	c.625C>T; p.(R209C) ENST00000262494.7	het (dn)	AD	–	Yes[P]	Path	1/1	Epileptic encephalopathy, early infantile, 17/#615473	mild to moderate ID, severe muscular hypotonia, dystonic movement disorder with very severe hyperkinetic crises triggered by infections or stress, anarthria, microcephaly, abnormal MRI	No	14; 8	No	Yes

Abbreviations: AD, autosomal dominant; ASD, atrial septal defect; AR, autosomal recessive; AV, aortic valve; CHD, congenital heart disease; Class, variant classification according to [30]; CSF, cerebrospinal fluid; DD, developmental delay; dn, *de novo*; Dys, dystonia; dx, diagnosis; ENST, ensemble transcript reference; ExAC-MAF, minor allel frequency of variant in ExCAC database; fam, family; f, female; HCM, hypertrophic cardiomyopathy; incl., including; IP, inheritance pattern; IS pred, *in silico* prediction of variant; hemi, hemizygous; het, heterozygous; hom, homozygous; IP, inheritance pattern; m, male; mat, maternal inherited; NDD, neurodevelopmental disorder; NM, neurometabolic disorder; mo, month; NA, not available, No, number; path/likely path, pathogenic/likely pathogenic; path*, see comment on classification in section 3.1 Molecular Diagnoses by WES; Ref; Reference (if published); rel., relative; Reported; variant reported as pathogenic in patient with similar phenotype ([P] if already reported before identification of the variant in the present study); seq., whole exome sequenced; *VS*, variant status; VSD, ventricular septal defect; XL; x-linked; y, years.

[a] fetal sibling of case A0100401 (induced abortion).

[b] in fetal sibling of case A0170701 (induced abortion) with the same homozygous *CWF191* variant.

evolutionary conservation, an expression pattern consistent with the phenotype (*e.g.* brain expression), or implication on the basis of animal models. If such variants were found in at least two affected patients with striking phenotypic overlap from unrelated families the gene was defined as 'newly identified'('novel'). Genes described as 'known to cause disease'have been described previously as a cause of an overlapping phenotype.

3. Results

3.1. Molecular diagnoses by WES

Phenotypic data, results of WES, and clinical implications of identified variants are summarized in Table 3 for the index patients with a molecular diagnosis. Results of *in silico* predictions are provided in Table S1. Overall, pathogenic or likely pathogenic variants could be identified in 21 out of 60 families (21/60, 35%), including variants in two patients (A0551801 and ZKJM003–014) who did not fully meet the ACMG criteria for (likely) pathogenic variants but exhibited additional aspects in favor of pathogenicity. Individual ZKJM003–014 showed a clearly reduced concentration of 5-methyltetrahydrofolate in CSF, and normal homocysteine and methionine. Biochemical and clinical characteristics where therefore compatible with DHFR deficiency described in 2011 [31]; the biochemically possible differential diagnosis of a folate receptor 1 defect had been ruled out previously, and no other variants explaining the phenotype were identified. In individual A0551801 a maternally inherited variant in the X-linked *USP9X* was found which was not present in the healthy brother, a situation insufficiently represented in ACMG guidelines, classified as pathogenic by most *in silico* prediction tools, with a convincing overlap between the patient's and reported phenotypes. The diagnostic rate was highest for the neurometabolic group with 3/7 index patients (43%) receiving a diagnosis, followed by the NDD group with 16/45 solved index cases (36%), and the dystonia group with 2/8 diagnosed index patients (25%). In 9 of the 21 families with a molecular diagnosis (43%) the causative variant was autosomal heterozygous *de novo*, in further 8 families (38%) an autosomal recessive disorder was diagnosed, and 4 (19%) had an X-linked disease. In the 13 families with solved 'sporadic cases'(only one affected individuum) autosomal heterozygous *de novo* variants were found in 9 cases (69%). In three families with sporadic cases (fam. 26, 49 and 50) different pathogenic variants in the same gene (*GNAO1)* were found. Of the 5 consanguineous families with a molecular diagnosis, 4 (80%) had an autosomal recessive disease. We observed one consanguineous family with two coexisting autosomal recessive diseases caused by pathogenic homozygous variants in two single genes (fam. 4). The index patient (individual A0100401) and his sister were both found to have a homozygous variant in *PGAP1* (for details see Granzow et al. 2015 [18]). In a later pregnancy, the fetus (A1420405) was also affected by the same alteration in *PGAP1* but showed sonographic features which could not be explained by it. Subsequent WES on fetal DNA in addition revealed a homozygous variant in *IFT140*, responsible for most of the sonographic anomalies.

Taken together the results of the 21 families with a molecular diagnosis 25 different pathogenic or likely pathogenic variants in 20 distinct genes were identified. At the time of diagnosis 19 of these 20 genes were known to cause disease, most of them had been described as disease causing since 2013. One gene (*SIPA1L3*) was a candidate gene that had not been described in human disease before (for details see [32]). Meanwhile a pathogenic missense variant and a chromosomal translocation affecting this gene have been described in two other patients with phenotypic overlap [33].

Distinctly novel phenotypes could not be identified in association with known genes, but in 9 cases, variants were associated with features expanding a known phenotypic spectrum (see Table 3 for details). For example, case A1324101 with variant in *DDX3X* had a phenotype overlapping Toriello-Carey syndrome with congenital heart disease and anal anomalies, unknown to be associated with *DDX3X* variants so far, thus widening the clinical spectrum [34]. In family 7 with two affected children and the molecular diagnosis of autosomal recessive spinocerebellar ataxia type 17 (SCAR17, *CWF19L1* gene, MIM #616127), the sibling of the index patient showed additional features that may be unrelated to SCAR17. Due to the lack of DNA, WES could not be performed on this child to detect possible further pathogenic variants [35].

We also identified 9 variants with potential pathogenicity, 7 of them in the NDD and 2 in the dystonia group, and functional studies are pending in 4 of them. Neither in the 72 individuals subjected to this study, nor in their parents, any medically actionable incidental finding could be made.

3.2. Clinical implications

All (likely) pathogenic variants summarized in Table 3 were reported to the respective family in a personal consultation followed by a written report including information about the diagnosed disease, inheritance pattern, and recommended changes of management, if appropriate. During this consultation the impact on family planning and clinical management was documented in each individual case. A clinical implication was defined as an influence on family planning (*e.g.* parents said they would opt for prenatal diagnosis or abstain from further pregnancies) and/or change of clinical management (*e.g.* beginning of regular screening for complications or comorbidities, or adaptation of medication).

According to 20 of the 21 families who received reports, the result was important for family planning, either with regard to a further pregnancy of the parents or for the future family planning of their non-affected children. So far, the results of WES of this study have been used for a prenatal diagnostic procedure in 4 cases.

Clinical implications included management changes in 8 cases. These changes consisted of a specific treatment in 2 cases (change of antiepileptic medication in ZKJM001–001 and treatment with galactose in ZKJM003–008), and cancer surveillance according to established guidelines because of a tumor disposition in 1 case (A0210901 with basal cell nevus syndrome, MIM #109400). In 6 index cases (A0451701, A1003101, A1063301, ZKJM003–014, ZKJM003–008, and ZKJM003–009) surveillance for other disease associated complications was initiated (for details see Table 3). For example, patient A0451701 and his affected sister with variants in *COASY* were affected by a neurodegenerative disease associated with progressive cognitive impairment, spastic-dystonic paraparesis and axonal neuropathy so that annual neurologic examinations were initiated. Patient A1003101 has been diagnosed with mutations in *GNAO1* that can lead to a phenotype with severe dystonia, which might respond to deep brain stimulation [38,39]. As dystonia was mild in this patient, deep brain stimulation was currently not considered a treatment option, but regular follow-up visits in a specialized center were recommended. In patient A1033201 a rare subtype of Joubert-Syndrome was diagnosed. According to Parisi et al. [40] annual evaluations of growth, vision, and liver and kidney function; periodic neuropsychologic and developmental testing were initiated.

3.3. Results in family 5

In family 5, a homozygous frameshift variant c.891dupC / p.(C298Lfs*11) in *STRADA* (*LYK5*) was identified in the index with both parents being heterozygous carriers. DNA of her deceased brother was unavailable. One of her healthy sisters tested negative for the variant, the other sister was still very young and not tested. *STRADA* encodes a pseudokinase acting as an upstream inhibitor of the mTOR complex 1. A homozygous intragenic deletion (exons 9–13) has been reported in 16 members of a Mennonite family who showed polyhydramnios, a severe NDD, syndromic epilepsy with early onset, macrocephaly,

ventriculomegaly and subependymal dysplasia on cMRI (if available), distinctive facial features comparable to those of individual A0170701, ASD in 4/16 and nephrocalcinosis in 2/16 individuals [42]. This so-called PMSE (polyhydramnios, megalencephaly, symptomatic epilepsy) syndrome (MIM #611087) is associated with high mortality due to medical complications. In 2013, Rapamycin was shown to ameliorate seizure frequency in 5 affected members of the original family [43]. Very recently, a further patient with a loss of function alteration of *STRADA* has been reported [44].

Based on the so far known patients with homozygous loss of function mutations in *STRADA*, the *STRADA* associated phenotype consists of severe DD/ ID, early onset epilepsy, cMRI anomalies, severe hypotonia, relative macrocephaly and facial dysmorphism as described above. MRI findings are available of 8 patients including individual A0170701 and her brother, and so far inconsistent. Polyhydramnios is frequent and nephrocalcinosis or ASD may be associated.

4. Discussion

The overall diagnostic yield of 35% in this study is in line with previous findings (see Table 1). It was highest (43%) for the small neurometabolic subgroup with 3/7 solved cases. A diagnostic rate of 68% has been reported previously for a larger group of 41 patients with neurometabolic disorders [4]. It is conceivable that the possibility of correlating the function of a candidate gene with biochemical markers is of advantage for the prioritization of variants. Also the largest subgroup of patients with NDD had a relatively high diagnostic yield of 36% which was aided by a careful phenotyping and serial reevaluation of data (see below). In 2 of the 8 patients with dystonia (25%) a diagnosis could be made: both patients carried previously described pathogenic *de novo GNAO1* variants. A previous study has reported clinically relevant mutations in 6 out of 16 cases with early-onset generalized dystonia analyzed by exome sequencing, resulting in a diagnostic rate of 37.5% [45]. Dystonia defines a class of hyperkinetic movement disorders, which are clinically and genetically highly variable. Etiological categorization based on clinical grounds is not always easy, given the fact that primary genetic or secondary (after neurologic damage) dystonic symptoms might overlap. Taking together WES results of our 8 and the previously published 16 dystonia cases [45] a substantial portion of dystonias (33%) is caused by single gene mutations. Furthermore, our study confirms that mutations in *GNAO1*, which were initially described as causative for early onset epilepsy in individuals with Ohtahara syndrome [46] cause a much broader neurologic phenotype with severe dystonia as the main feature [38,39,47,48].

The identification of two monogenic disorders in one out of 21 families with a genetic diagnosis is in accordance with the literature where double diagnoses have been reported in 0–4.6% (see Table 1). An exception is the study by Tarailo-Graovac et al., 2016 [4], who found pathogenic variants at two distinct disease loci in 12% of patients with broadly defined neurometabolic disorders. Generally, the possibility of more than one independent single gene disorder in one family has to be born in mind when interpreting WES data, in particular those of consanguineous families, and re-evaluation in case of additional clinical symptoms within a family should be considered.

Also limitations of WES should be taken into account. WES is not able to detect all causal mutation types, for example pathogenic repeat expansions (as found in fragile X syndrome or spinocerebellar ataxia), larger insertions/deletions and many copy number variants will be missed. Clinically relevant genomic sequences outside the coding exons, *e.g.* deep intronic splice variants, can not be detected by WES. In addition, WES has limitations in sufficiently covering the whole exome, especially GC-rich regions. In our study, also the mitochondrial genome is not covered.

Worthy of note is that 13 of the 20 genes harboring disease causing variants were identified shortly before an inclusion of the patient in the study or during the study. This implies that the respective diagnosis probably would not have been made by targeted panel diagnostics. In several cases, *e.g.* in individual A0241001 with a *DNMT3A* variant (MIM #615879), the causative gene was only identified reconsidering the data and the current literature. It was only after the publication on *de novo* heterozygous variants in the X linked gene *DDX3X* as a cause of syndromic and non-syndromic ID in females ([49], MIM #300958) that heterozygous *de novo* variants in this and in so far unknown X linked genes were considered in female patients. This led to the detection of a *DDX3X* variant in a girl with a preliminary clinical diagnosis of Toriello-Carey syndrome (individual A1324101), and subsequently to the identification of another *DDX3X* variant in a second patient with a similar phenotype not within this study [34].

Other variants could only be identified as (probably) pathogenic because additional clinical data, follow-up data or information on a further pregnancy in the family were available. In individuals A1063301 and A0451701, a thorough neuroradiological characterization was crucial for considering *PIBF1* and *COASY* (MIM #615643) as the causative genes, respectively. In individual A1033201, additional information on high serum alkaline phosphatase activity (hyperphosphatemia) provided support for considering a diagnosis of Mabry syndrome (hyperphosphatasia with mental retardation syndrome 1, MIM #239300). In family 4, persistent contact with the family and following the course of the next pregnancy led to the identification of a second single gene disorder in this consanguineous family. In total, 7 variants could only be identified due to manual reevaluation of the literature, a change of filter settings, reconsideration of inheritance pattern and/or clinical follow-up.

Currently, WES can be carried out with good coverage at reasonable costs. However, the analysis of WES data can be challenging and time-consuming. For genetically heterogeneous diseases, such as neurodevelopmental and neurometabolic disorders, the following 2-tier approach may be applied: After enrichment and sequencing of all exonic sequences, a targeted evaluation for variants in genes known to be associated with the particular phenotype (virtual gene panel) is performed. In a second step, if no diagnosis could be made, the evaluation is expanded to the whole exome. In contrast to gene panels based on enrichment of selected exons, newly identified disease genes can easily be incorporated in the virtual gene panel.

Analysis of the clinical implications of the established diagnoses showed that in almost every case, knowledge of a genetically identifiable diagnosis and the corresponding recurrence risk was important for the family planning of the parents or the siblings. However, there might have been a bias in the selection of families because in view of the restricted numbers of available exomes, parents with an urgent demand of assessing a diagnosis, mainly because of implications for family planning, were given priority in some cases. At the time of writing this manuscript, prenatal diagnosis based on the results of this study has been performed 4 times.

In 8 families, the diagnosis led to alterations in the clinical management of the disease which most often meant that a surveillance program was started or had to be changed because of possible complications directly or indirectly related to the diagnosis. In two cases, a specific treatment could be initiated. With regard to family 5 in whom a homozygous *STRADA* nonsense mutation had been identified, it cannot be excluded that the demise of the brother of individual A0170701 in status epilepticus could have been prevented if a *STRADA* defect would have been detected earlier and Rapamycin treatment initiated. No changes in medication or management had become necessary because of incidental findings.

Significant implications of a genetic diagnosis established by WES on family planning, medication selection and systemic investigations have also been reported by other groups [4,6,11,13,14] (see Table 1). Changes in medication or medical management have been reported in 32–78% [6,11,13]. The literature concerning the subgroup of patients with neurometabolic disorders is still scarce reporting on treatment changes in 44% [4]. In the present study, three out of seven individuals with a

neurometabolic disease, where a molecular diagnosis was performed, underwent a surveillance program for disease associated complications; one individual (ZKJM003–008) in addition received a specific treatment.

In conclusion, the present study showed a high diagnostic rate on exome sequencing of patients with NDD (36%). Several monogenic disorders could also be diagnosed in the patients with neurometabolic diseases and dystonia, although these groups of patients appear too small to draw reliable conclusions with regard to the diagnostic yield. *STRADA* could be confirmed as a gene for severe autosomal recessive syndromic NDD and its phenotypic spectrum could be further delineated. WES in a diagnostic setting may have a significant impact on both the medical management of the disease and on family planning. Although the cohort is too small for statistical conclusions, it is noteworthy that a significant proportion of diagnoses could only be made due to reconsideration of sequencing data in relation to the current literature, alteration of filter settings and/or the availability of careful phenotypic data and follow-up information. WES is superior to targeted panel diagnostics due to rapidly growing number of recently discovered genes that would be missed by panel diagnostics. Reevaluation of phenotypic and genotypic data and follow-up can significantly improve the diagnostic rate.

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