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Two-year follow-up of Sanfilippo Disease patients treated with a genistein-rich isoflavone extract: Assessment of effects on cognitive functions and general status of patients

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Mucopolysaccharidoses (MPS) are inherited metabolic disorders caused by deficiencies in enzymes involved in degradation of glycosaminoglycans. MPS type III (Sanfilippo disease) is clinically characterized mainly by progressive and severe behavioral disturbances and cognitive dysfunction. Recent 1-year experimental treatment of 10 patients with a genistein (4', 5, 7-trihydroxyisoflavone)-rich extract resulted in improvement of tested parameters, including cognitive and behavioral functions.

Material/Methods: Eight pediatric patients with Sanfilippo disease were enrolled into the study. The modified version of the Brief Assessment Examination was used to assess cognitive functions. Moreover, 18 different parameters concerning changes in conditions of patients were assessed by their parents.

Results: During the first year of the treatment, an improvement of cognitive functions in 7 patients and stabilization in 1 patient were assessed, while after the third year (2-year follow-up) further improvement was observed in 2 patients, stabilization in 3 patients and some deterioration in 3 patients. Monitoring of general and behavioral symptoms revealed improvement in all patients after the first year of the treatment, further improvement in 5 patients, and deterioration in 3 patients during the next 2 years.

Conclusions: We conclude that the treatment of Sanfilippo patients with a genistein-rich soy isoflavone extract (called gene expression-targeted isoflavone therapy [GET IT]) may be effective in either inhibition (in some patients) or slowing down (in other patients) of behavioral and cognitive problems over a longer period. An increased dose of genistein may improve the efficacy of the treatment.

key words: **mucopolysaccharidosis • substrate reduction therapy • genistein • behavioral problems**

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Background

Mucopolysaccharidoses (MPS) are genetic disorders from the group of lysosomal storage diseases [1,2]. These disorders are caused by mutations in genes coding for enzymes involved in degradation of glycosaminoglycans (GAGs). Storage of GAGs in cells of patients results in progressive damage to the affected tissues, including the heart, respiratory system, bones, joints and, in most MPS types and subtypes, the central nervous system (CNS). MPS are usually fatal diseases, with average expected life span of 1 or 2 decades, although patients with milder forms can survive into adulthood [2]. There are 11 known types and subtypes of MPS, and each type is variable in severity of particular symptoms [2]. Prediction of severity and clinical progression of MPS is difficult, even when biochemical and genetic parameters are determined [3], but recent studies indicate that considering 2 or more parameters may give significantly better results than attempting to make conclusions based on a single biomarker [4].

MPS type III (MPS III, called also Sanfilippo disease) is a group of 4 conditions (MPS IIIA, B, C and D), revealing similar clinical symptoms and characterized by lysosomal storage of heparan sulfate (HS), one of GAGs [5]. Particular subtypes are classified according to gene coding for an enzyme involved in degradation of HS (heparan N-sulfatase in MPS IIIA, α-N-acetylglucosaminidase in MPS IIIB, acetyl-CoA: α-glycosaminide acetyltransferase in MPS IIIC and N-acetylglucosamine 6-sulfatase in MPS IIID) which is defective.

Although other MPS types are characterized by severe somatic symptoms and either some (sometimes also severe) or no neurological and behavioral problems, MPS III is specifically associated with severe learning difficulties and behavioral disturbances, accompanied with relatively mild somatic involvement. In most affected patients the progressive nature of the disease leads to death in the second (or rarely third) decade of life. As this disorder primarily affects the brain and nervous system, attempts to cure patients have been especially difficult, and aside from experimental treatments (see below), the best care that can be offered is palliative or symptomatic [5].

Enzyme replacement therapy (ERT), based on an intravenous infusion of an active, recombinant form of a deficient enzyme, is currently the only officially approved treatment of MPS, and is used in clinical practice for MPS types I, II and VI [6]. Unfortunately, neurological symptoms due to HS accumulation in CNS cannot be managed by ERT, due to an inefficient delivery of proteins, including recombinant enzymes, through the blood-brain barrier.

Substrate reduction therapy (SRT), called also substrate deprivation therapy (SDT), is an alternative approach for treatment of patients with lysosomal storage diseases [7,8]. This therapy is based on the action of small molecules that may, directly or indirectly, impair synthesis of compounds that cannot be efficiently degraded in cells of patients. Since such small molecules may likely cross the blood-brain-barrier, it is hoped that they could be used to manage neurological symptoms of these diseases.

Previous studies with rhodamine B ([9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene]-diethylammonium chloride) and genistein (5, 7-dihydroxy-3-(4-hydroxyphenyl) -4*H*-1-benzopyran-4-1) revealed that each of these compounds can reduce the efficiency of GAG synthesis in cells of various MPS types [9,10]. Importantly, these studies demonstrated not only a lack of further GAGs' accumulation, but also a reduction in lysosomal storage of these compounds. In fact, recently reported experiments indicated that RNA interference-mediated silencing of genes coding for various GAG synthetases causes a decrease in the amount of the storage material in cell lines of patients with Sanfilippo disease [11,12]. Tests on animal models of Sanfilippo disease demonstrated efficacy of rhodamine B and genistein *in vivo*, including behavior of affected and treated mice [9,13,14]. Although it is unlikely that rhodamine B can be used in clinical practice due to its potential toxicity to humans [9], genistein is a non-toxic and safe compound [15]. Moreover, unlike rhodamine B, the mechanism of genistein-mediated inhibition of GAG synthesis has been demonstrated. This isoflavone inhibits phsophorylation of the epidermal growth factor receptor (EGFR), which in turn causes an impairment of expression of genes coding for enzymes necessary for GAG synthesis [8]. Hence, this special kind of SRT has been called 'gene expression-targeted isoflavone therapy' (abbreviated as GET IT), and an open-label, pilot study in 10 pediatric MPS III patients, treated with a genistein-rich isoflavone extract for 12 months, was performed [15]. After 1 year of treatment, statistically significant improvement in all measured parameters (urinary GAG excretion, hair morphology and cognitive functions) was demonstrated [15].

Since longer-term effects of GET IT for MPS III are not known, we performed a follow-up study with genistein-treated MPS III patients. Considering learning difficulties and behavioral disturbances to be the most severe clinical problems of Sanfilippo disease, we focused on monitoring cognitive functions and general status of patients, including their behavior.

Material and Methods

Patients

The patients were diagnosed for Sanfilippo disease (either MPS IIIA, McKusick's OMIM no. 252900, or MPS IIIB, McKusick's OMIM no. 252920) by estimation of urinary GAG levels (highly elevated GAG levels, observed in hemi-quantitative electrophoretic analysis, gave a preliminary indication for MPS) and measurement of activities of particular lysosomal hydrolases in leukocytes. Deficiency in activity of heparan *N*-sulfatase (control value: 4.1±1.4 nmoles/mg of protein/18h) or α-N-aceltyl glucosaminidase (control value: 90±34 nmoles/mg of protein/42 h) was considered as a diagnosis for MPS IIIA or MPS IIIB, respectively. Mutations in genes coding for the above-mentioned enzymes were determined for 5 patients, and 1 mutation was determined for 1 patient. Eight patients (6 with MPS IIIA and 2 with MPS IIIB) were enrolled into the study. All patients were of Caucasian origin; 2 males and 6 females. Among the 8 patients, 5 (patients: IIIA-2, IIIA-4, IIIA-5, IIIB-2 and IIIB-5) were previously enrolled into the pilot study [15]. Information about all patients enrolled into this study is summarized in Table 1.

Treatment

The MPS III patients characterized in Table 1 were treated for 36 months with a genistein-rich soy isoflavone extract

Table 1. Patients' characteristics.

MPS type	Patient no.[a]	Gender	Age (years)[b]	Weight (kg)[b]	Mutation(s)[c]	Residual enzyme activity[d]
IIIA	IIIA-2	Female	5	18	R74C/ G90R	0.01
	IIIA-4	Female	6	28	nd/nd	0.12
	IIIA-5	Male	7	30	R74C / R74C	1.5
	IIIA-6	Female	3	22	S66W / S66W	0.15
	IIIA-7	Female	7	30	R74C / R74C	0.01
	IIIA-8	Female	18	30	R74C / R74C	0.01
IIIB	IIIB-2	Male	14	36	E120X/nd	3.0
	IIIB-5	Female	9	43	nd/nd	0.01

[a] Numbers of patients correspond to those described previously (15). Patients IIIA-2, IIIA-4, IIIA-5, IIIB-2 and IIIB-5 were enrolled also in that study; [b] the presented values were measured at baseline; [c] nd – not determined; [d] residual activities of enzymes deficient in MPS IIIA and MPS IIIB were measured in leukocytes, and were determined as heparan *N*-sulfatase (MPS IIIA) in nmoles/mg of protein/18 h (controls: 4.1±1.4) or as α-N-aceltyl glucosaminidase (MPS IIIB) in nmoles/mg of protein/42 h (controls: 90±34).

Table 2. Assessment of cognitive functions of patients by using the modified BAE test, and estimation of a generalized score for estimation of general and behavioral symptoms in patients.

Patient	BAE score[a]			Questionnaires (general score)[b]		
	Baseline	1 year	3 years	Baseline	1 year	3 years
IIIA-2	14	19	21	–1	+16	+19
IIIA-4	0	4	4	+4	+21	+5
IIIA-5	11	17	19	–10	+22	+23
IIIA-6	25	29	25	0	+13	+8
IIIA-7	38	38	38	–1	+8	+7
IIIA-8	2	4	2	–3	+14	+15
IIIB-2	33	36	32	–4	+6	+13
IIIB-5	27	32	32	–16	+26	+29

[a] For information about calculation of the BAE score, see Appendix; [b] General score was calculate by summing all answers given by parents to 18 questions included in the questionnaire (for details see Table 3), giving following weights to particular types of answers: (–2) considerably deteriorated, (–1) somewhat deteriorated, (0) no changes, (+1) somewhat improved, (+2) considerably improved.

(called SE-2000), provided by the manufacturer (Biofarm, Poznań, Poland) in the form of tablets (product name Soyfem). The extract consisted of genistin and genistein (26.90%), daidzin and daidzein (13.37%), glycitin and glycitein (1.98%), and soy proteins, carbohydrates and lipids (remaining amount). This extract was administered orally (in the form of whole tablets or tablets crushed into powder) at the dose corresponding to 5 mg of genistin and genistein (genistin is a glycan that can be converted to genistien by either acid environment or intestinal bacterial flora) per 1 kg of body weight daily. The extract was administered twice a day, with equal amounts at morning and evening. Two types of evaluation (the modified BAE test and parents' questionnaire, both described in the next subsections) were performed at baseline and after 12 and 36 months of treatment. The monitoring of adverse effects was based on reports of parents, who were appropriately instructed to signal any such effects immediately (by either phone or email, with confirmation of receipt of the information), and who provided an assessment of such effects in writing every 3 months (even if no adverse effects were observed). This experimental treatment was approved by the Independent Bioethics Committee of the Medical University of Gdańsk, Poland (approval no. NKEBN/398/2005). Parents of the children involved in this study signed the informed consent form.

The modified BAE test

To assess cognitive functions and behavioral changes, a modified version of the Brief Assessment Examination [15] was used. This test estimated the following parameters: (i) alertness/activity, (ii) obeying commands, (iii) pointing at objects, (iv) pointing at colors, (v) matching shapes, (vi) speech, (vii) auditory digit span, and (viii) mathematics. The maximum possible score was 52 points. Details of this test are shown in the Appendix.

Tabele 3. Parents' assessment of patients' condition.

Parameter	Scores given to patients by parents[a]							
	Patient no.							
	IIIA-2	IIIA-4	IIIA-5	IIIA-6	IIIA-7	IIIB-8	IIIB-2	IIIB-5
Speech performance	0/+1/0	0/+1/0	–1/0/0	0/+1/–1	–1/0/0	0/0/0	0/0/0	–1/+2/+2
Speech understanding	0/+1/0	0/+1/0	–1/+1/+1	0/+2/0	0/+1/+1	0/+1/+1	0/+1/+1	–1/+2/+1
Activity	0/+1/+1	0/+2/+2	–1/+2/+2	0/+2/+1	0/+1/0	–1/0/+1	0/0/+1	0/+1/0
General behavior	0/+1/+1	0/+2/0	–1/+2/+2	0/+1/+1	0/+1/+1	0/+1/+2	0/+1/+1	–1/+2/+2
Sleeping problems	0/+1/+1	0/+1/+2	–1/+2/+2	0/+1/+1	0/0/0	0/+2/+2	–1/+1/+1	–2/+2/+2
Pain	0/0/+1	0/0/0	0/0/0	0/0/+1	0/0/–1	+1/+2/+2	0/0/0	–2/+2/+2
Walking	0/+2/–1	0/+1/–2	0/+2/0	0/+2/+2	0/0/0	–1/0/0	–1/0/+1	–2/+1/0
Hand mobility	0/0/+1	0/+1/–1	–1/+2/+2	0/0/+2	0/0/0	0/0/+1	0/0/+1	0/+1/+2
Joint mobility	0/0/+1	0/+2/–1	–1/+2/0	0/0/+1	0/0/0	0/0/0	0/0/0	–1/+1/+2
Breathing problems (day)	0/+1/+2	+1/+2/0	0/0/0	0/–1/0	0/+2/0	0/0/0	0/0/0	0/0/+2
Breathing problems (night)	0/+1/+2	+1/+2/+1	0/+2/+2	0/–1/+1	0/+2/0	0/0/0	0/0/0	–1/+2/+2
Infections	–1/+2/+2	+2/+2/+2	0/+1/+2	0/–1/0	0/0/+2	0/0/0	0/+2/+2	–1/+2/+2
Vision	0/0/0	0/0/0	0/0/+2	0/0/0	0/0/+1	0/0/0	–1/0/+2	0/0/0
Hearing	0/0/0	0/+2/0	0/0/+2	0/+2/–1	0/0/0	0/0/0	0/0/0	0/0/+2
Skin	0/+2/+2	0/+1/+1	0/0/0	0/0/0	0/0/+2	0/+2/+2	0/0/0	–1/+2/+2
Hair	0/+2/+2	0/+1/+1	–1/+2/+2	0/+2/–1	0/+1/+1	0/+2/+2	0/0/+1	–1/+2/+2
Dyspeptic symptoms	0/+1/+2	0/+1/+1	–1/+2/+2	0/+2/–1	0/0/0	–1/+2/+1	–1/0/0	–1/+2/+2
Stools	0/0/+2	0/–1/–1	–1/+2/+2	0/+1/+2	0/0/0	–1/+2/+1	0/+1/+2	–1/+2/+2

[a] The first number (before the first slash sign) is a score for the comparison of changes in a particular child from a year before the onset of the treatment to the start of the therapy, the number between slashes is a score for the comparison of changes in a particular child from the onset of the treatment to a year after the start of the therapy, and the number after the second slash is a score for the comparison of changes in a particular child from the onset of the treatment to 3 years after the start of the therapy. The following score scale was used: considerably deteriorated (–2), somewhat deteriorated (–1), no changes (0), somewhat improved (+1), considerably improved (+2).

Parents' observations

Parents of patients were asked to evaluate 18 different parameters concerning changes in conditions of their children from a year before the onset of the treatment to the start of the therapy, from the onset of the treatment to a year after the start of the therapy, and from the onset of the treatment to 3 years after the start of therapy. Parents were instructed to be objective. Some parents reported they depended also on the opinion of other, uninitiated, people (eg, teachers).

Results

Eight patients suffering from Sanfilippo disease (MPS IIIA and IIIB) were monitored during an experimental therapy consisting of a genistein-rich soy isoflavone extract (Soyfem) at the dose of 5 mg/kg/day for 24 months. Five of these patients were enrolled in a 1-year pilot study (patients IIIA-2, IIIA-4, IIIA-5, IIIB-2, IIIB-5), described previously [15], and the treatment (called GET IT) was continued for the next 2 years (parents of these patients collaborated with researchers for the next 2 years and sent back their questionnaires on time; although parents of the other 5 patients also agreed to collaborate, they failed to return completed questionnaires on time). Three patients were selected after the onset of the previous trial. Urinary GAG levels and hair morphology are relatively simple assays that can be used for monitoring of MPS treatment; however, they do not reflect the behavioral and cognitive status of examined patients. Moreover, a statistically significant improvement in GAG excretion and normalization of hair morphology were reported after 1 year of GET IT [15]; hence, one would not expect further improvement in these parameters during the next 24 months. Since cognitive and behavioral dysfunctions are the most severe symptoms in Sanfilippo disease, we decided to monitor cognitive ability and behavior of patients by using a specific psychological test and a questionnaire for patients' parents, as described previously [15].

No adverse effects of the therapy were reported during this study. This corroborates the conclusion that GET IT is a safe treatment, as described previously [15].

To assess cognitive functions of the patients, a modified BAE psychological test (see Appendix for details) was performed at baseline, and at 12 and 36 months after the start of treatment. During the first year of the treatment, an improvement in the score in 7 patients and stabilization in 1 patient (IIIA-7) were observed (Table 2), which corroborates previous results [15]. After the third year (2-year follow-up) further improvement (relative to results after the first year) was observed in 2 patients (IIIA-2, IIIA-5), stabilization was observed in 3 patients (IIIA-4, IIIA-7, IIIB-5), and deterioration was observed in 3 patients (IIIA-6, IIIA-8, IIIB-2). In this last-mentioned group of patients, the scores after 36 months of treatment were equal or very similar to those achieved at baseline (Table 2).

To estimate the efficacy of the treatment, patients' parents were asked to evaluate 18 different parameters concerning changes in conditions of their children from a year before the start of the treatment to the start of the therapy, from the onset of the treatment to a year after the start of the therapy, and from the start of the treatment to 3 years after the start of the therapy. As expected, parents reported deterioration of patients in many parameters in the period from 1 year before the treatment to the start of the trial, which confirmed that the children were in the clinically progressive stage of the disease (Table 3).

To estimate status of the patients, we calculated a general score for each patient on the basis of answers to the questionnaires. This general score was calculated by summing all the answers and giving the following weights to particular types of answers: (–2) considerably deteriorated, (–1) somewhat deteriorated, (0) no changes, (+1) somewhat improved, and (+2) considerably improved.The results of questionnaires revealed improvement in all patients after the first year of treatment, further improvement in 5 patients (IIIA-2, IIIA-5, IIIA-8, IIIB-2, IIIB-5), and deterioration in 3 patients (IIIA-4, IIIA-6, IIIA-7), during the next 2 years (Table 2).

Discussion

After unsuccessful trials with bone marrow transplantation and with treatments employing small molecules, either miglustat or glucosamine, to our knowledge, this pilot study on a specific substrate deprivation therapy, called GET IT, demonstrates the first treatment of patients with Sanfilippo disease that demonstrates a statistically significant improvement in biochemical and morphological parameters, as well as improvement in clinical status of patients [1,15,16]. Since GET IT appeared to be a promising treatment, in the absence of information on clinical effects of longer treatment, a follow-up of treated patients was necessary. In this report we present a 2-year follow-up of Sanfilippo disease patients treated with a genistein-rich soy isoflavone extract (Soyfem) at the dose corresponding to the amount of genistein equal to 5 mg/kg/day.

When monitoring cognitive abilities and general status of the patients after the third year of the treatment, we observed either further improvement/stabilization of the patients, or their slow deterioration. This is in contrast to rapid deterioration of untreated MPS III patients presented in the literature [5] and evident from questionnaire responses in this report (Table 3). Interestingly, such a picture of the clinical manifestation of symptoms in treated MPS III patients is similar to that observed during ERT of MPS I, although only effects on somatic tissues could be considered in the latter case. Namely, MPS I patients treated with laronidase improve relatively rapidly during the first several months of the treatment, and then slow improvement, stabilization or slow deterioration occurs [6,17]. It is plausible that during the first period of both ERT and GET IT, the reversible effects of diseases are corrected, and thereafter it is only possible to either prevent further progression or to slow the irreversible changes.

The question remains whether it is possible to make GET IT more efficient. The main mechanism of genistein-mediated impairment of GAG synthesis is inhibition of phosphorylation of EGFR [8]. Thus, one might predict that when using higher doses of genistein it should be possible to slow the GAG production even more effectively. The dose of the SE-2000 extract used in both the previous trial [15] and this study corresponded to the amount of genistein equal to 5 mg/kg/day. Since it was recently reported that a dose as high as 160 mg/kg/day is safe (with no adverse effects) for MPS IIIB mice [14], it is likely that significantly (at least 200%) higher doses than that used in this study may be safe for humans. Therefore, there is great need for a clinical trial evaluating GET IT for Sanfilippo disease.

Oxidative stress, inflammation, cytotoxicity and apoptosis may be involved in the mechanism of neurodegeneration in Sanfilippo disease [18–20]. Moreover, accumulation of hyperphosphorylated tau protein (P-tau), which forms aggregates characteristic of Alzheimer's disease, has been detected in brains of MPS IIIB mice [21]. Thus, it is intriguing that genistein has been described as a factor attenuating oxidative stress in the brain [22], having a neuroprotective effect against beta amyloid-induced neurotoxicity [23,24], inhibiting apoptosis in primary neuronal cell cultures [25], preventing Alzheimer's disease-associated inflammation [26], and revealing a general neuroprotective effect [27]. Genistein, apart from its action as an indirect inhibitor of GAG synthesis [8], may also be beneficial for MPS III patients due to its neuroprotective functions. Although some of these functions were observed *in vitro* at genistein concentrations as low as 100 nM, in most *in vivo* studies some effects were evident only at 10–15 mg/kg [22–27]. These facts strengthen the need for results of clinical studies in which patients with Sanfilippo disease are treated with genistein at doses of 10–15 mg/kg/day or higher.

Results of recent studies on animals suggest that GET IT may be effective in treatment of other types of MPS [28]. Since improvement in CNS was observed in genistein-treated mice with MPS II [28], an X chromosome-linked disorder [2], GET IT could be considered as a potential treatment for MPS II-associated mental retardation [29].

Conclusions

The treatment of Sanfilippo patients with a genistein-rich soy isoflavone extract (GET IT), at the dose corresponding to 5 mg/kg/day, may be effective in either inhibition (in some

patients) or slowing (in other patients) of the cognitive dysfunction, behavioral problems and general deterioration caused by the disease, over a period of 3 years. An increase in the dose of genistein is suggested to improve the efficacy of the treatment.

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APPENDIX

The modified Brief Assessment Examination (for the original test see refs. 30 and 31)

A. The point scale

1. Activity
General excitement 0 points
Short-term goal directed behaviour 2 points
Short-term goal directed activity 4 points
Repeated goal directed activity 6 points
Full adequate activity 8 points

2. Fulfilling simple commands
None fulfilled 0 points
1 fulfilled 3 points
2 fulfilled 5 points

3. Pointing at objects
None pointed 0 points
1 pointed 3 points
2 pointed 5 points

4. Pointing at colours
None pointed 0 points
1 pointed 3 points
2 pointed 5 points

5. Matching shapes
None matched 0 points
1 matched 3 points
2 matched 5 points

6. Speech
Absent 0 points
Individual echoic words 2 points
Individual words 4 points
Simple sentences 6 points
Expanded statements 8 points

7. Aural digit span
None reproduced 0 points
1 reproduced 4 points
2 reproduced 6 points
3 reproduced 8 points

8. Counting
Does not count 0 points
Counts to 5 4 points
Counts to10 8 points

B. Description of the scale

1. Activity
General excitement – kinetic excitement, no goal-oriented activity, lack of even short-term concentration on a single stimulus. Also dynamic lack of any activity.
Short-term goal directed behaviour – temporary concentration, e.g. watching somebody or something.
Short-term goal directed activity – conscious, purposeful manipulation of an item, visual examination, intentional attempts to socialize.
Repeated goal directed activity – likewise, repeated or long-term conduct.
Full adequate activity – goal directed activity dominates in child's behaviour.

2. Fulfilling simple commands
Commands like "give mammy the toy", „put the book on the shelve", „put the ball into the bag" may be supported by the gesture but without presentation.
3. Pointing at objects
Pointing as a response to a verbal request, e.g. „show me, where the flower is", etc.

4. Pointing at colours
Pointing items in a named colour, e.g. show the blue block (among several basic colours: red, yellow, blue, green or white).

5. Matching shapes
Matching the shapes in a big puzzle – into the holes in a desk: ● ▲ ■

6. Speech
Absent, beside no goal-directed vocalization, babbling, prattling.
Individual echoic words: any attempts to direct towards anybody vocal communication, any repeated voice reactions to the stimuli, etc.
Individual words: can be mispronounced or „own expressions" can be used consistently.

7. Aural digit span
Repeating by heart digits in a line of two or three elements (e.g. 7–4, 5–8–2).

8. Couning
Does not count, has no idea of numbers
Counts to 5: counts items in the limit of five
Counts to 10: counts items in the limit of 10.

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