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Characteristics of nephroblastoma / nephroblastomatosis in children with a clinically reported underlying malformation or cancer predisposition syndrome

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Simple Summary:

It is well known that different cancer predisposition syndromes are associated with characteristic WT-features. The following findings from our retrospective analysis of patients with nephroblastoma treated according to the SIOP/GPOH trials between 1989 and 2017 are relevant:

- The outcome of patients with a cancer predisposition syndrome is not always favorable despite early diagnosis, small tumors and less metastatic disease. This finding is partly depending on complications related to the underlying syndrome.
- Predisposition syndromes seem to be underdiagnosed as several clinical and pathological features of Wilms tumor being clearly linked to a cancer predisposition syndrome did not lead to genetic counseling before and after WT diagnosis.

As a conclusion, in children with a nephroblastoma and specific clinical and pathological features that are in line with a nephroblastoma cancer predisposition syndrome such a syndrome should always be considered and ruled out if unknown at the time of tumor diagnosis.

Abstract:

Background: About 10% of Wilms Tumor (WT) patients have a malformation or cancer predisposition syndrome (CPS) with causative germline genetic or epigenetic variants. Knowledge on CPS is essential for genetic counselling. **Methods:** This retrospective analysis focused on 2927 consecutive patients with WTs registered between 1989 and 2017 in the SIOP/GPOH studies. **Results:** (GU, N=66, 2,3%), Beckwith-Wiedemann spectrum (BWS, N=32, 1,1%), isolated hemihypertrophy (IHH, N=29, 1,0%), Denys-Drash syndrome (DDS, N=24, 0,8%) and WAGR syndrome (N=20, 0,7%) were reported most frequently. Compared to others, these patients were younger at WT diagnosis (median age 24,5 months vs. 39,0 months), had smaller tumors (334,8mL vs. 496,9mL), less often metastasis (8,2% vs. 18%), but more often nephroblastomatosis (12,9% vs. 1,9%). WT with IHH was associated with blastemal WT and DDS with stromal subtype. Bilateral WTs were common in WAGR (30%), DDS (29%) and BWS (31%). Chemotherapy induced reduction in tumor volume was poor in DDS (7,7% increase) and favorable in BWS (84,6% reduction). The event-free survival (EFS) of patients with

BWS was significantly (p=0,002) worse than in others. **Conclusions:** CPS should be considered in WTs with specific clinical features resulting in referral to a geneticist. Their outcome was not always favorable.





Keywords: nephroblastoma; clinical malformations; cancer predisposition syndromes; tumor surveillance; outcome

1. Introduction

Nephroblastoma or Wilms tumor (WT), the most common kidney tumor in childhood (1), can be cured in more than 90% today (2),(3),(4). With 8 to 17% overall and up to 24% in bilateral WTs it has one of the highest association rates with congenital anomalies of all childhood cancers (5),(6). Such malformations and cancer predisposition syndromes (CPS) related to the development of WTs are characterized by genetic or epigenetic alterations. For example, the WAGR syndrome, is clinically defined by a variable occurrence of WT in combination with aniridia, genitourinary malformations and a range of developmental delays (7),(8),(9). It is caused by chromosome 11p13 deletions, including WT1 and neighboring genes, whereas Denys-Drash syndrome (DDS) is due to a dominant-negative WT1 mutation. DDS is characterized by the triad of WT, nephropathy and, if applicable, male pseudohermaphroditism (10),(11),(12). In addition, genitourinary malformations (GU) have been linked to WT1 mutations (13),(14). Isolated hemihypertrophy (IHH) (15),(16) and the Beckwith-Wiedemann spectrum (BWS) are overgrowth syndromes with elevated risk to develop WT. BWS shows a high variability of macroglossia, abdominal wall defects, visceromegaly, gigantism and hypoglycaemia caused by genetic and epigenetic alterations at 11p15.3 (12),(17),(18). Other WT associated syndromes like Perlman syndrome, or Simpson-Golabi-Behmel syndrome are much rarer and have a different genetic background. In this paper we focus on the five most frequent WT malformations or CPS, namely WAGR, DDS, GU, IHH and BWS, to compare their clinical, pathological and outcome data with data from WTs without a known CPS.

2. Materials and Methods

We conducted a retrospective investigation on data of 2927 patients with WT and/or nephroblastomatosis from Germany, Austria and Switzerland enrolled in the SIOP/GPOH 9, 93-01 and 2001 studies between 1989 and 2017. Details of their treatment protocols have been reviewed previously (19). Ethical approval was obtained from the Ärztekammer des Saarlandes (No: 136/01 from 20.09.2002 and, 248/13 from 13.01.2014). All parents or legal guardians of the affected children gave informed consent for study participation.

Pseudonymized data of all patients were stored in a central and encrypted SQL database. All patients identified in the database with a clinically documented malformation or CPS were reviewed by NW and NG, and details on these patients – including presentation, treatments, and outcome-were collected from the SIOP-RTSG/GPOH database and, retrospectively, from status report forms, radiology, pathology and surgery reports, progress letters, and telephone notes available at the data center. The identification of patients with malformations or CPS was based solely on clinical data provided by the registration CRF where associated congenital malformations or a syndrome were asked specifically for Aniridia, WAGR, genitourinary malformations, Denys Drash syndrome, BWS, IHH, Perlman syndrome. Free text could specify other malformations or syndromes that are not listed. This information is based on clinical characteristics. No information is provided if the syndrome was confirmed by genetic analysis. Patients with WAGR were also included in the paper by Hol et. al. (8). Tumor volume was calculated from imaging studies using the ellipsoid formula in those patients were CT or MRI of the tumor was available. Nephroblastomatosis was based on reference histology defined as multiple or diffuse nephrogenic rests but not further specified as perilobar, intralobar or both, as this information was not available for all patients with nephroblastomatosis. For statistical analysis all data were anonymized. IBM SPSS Statistics, version 25 and 27, was used for descriptive analyses (histograms, boxplots, pie charts, frequency charts and bar charts) and statistical comparisons (T-test for independent samples, Levene test, Chi-square test, multivariate analysis and Kaplan-Meier survival curves with Log Rank). P-values below 0.05 were considered as statistically significant. Overall survival (OS) included the time period between diagnosis and death of any reason, and event free survival (EFS) between diagnosis and any event, including recurrence of WT or nephroblastomatosis, death or loss to follow up.

3. Results

3.1. Characteristics of study population

An underlying malformation or syndrome was recorded in 198 (6,8%) patients (Table 1). Bilateral disease occurred in 253 (8.6%) of patients. In 137/2927 (4,6%) patients nephroblastomatosis was diagnosed, either isolated (73; 2,4%) or in conjunction with WT (64; 2,1%). This investigation focused on a sub-cohort of 171 patients, who presented with one of the five most frequent malformations or syndromes (**Table 1**), that is GU (N=66), BWS (N=32), IHH (N=29), DDS (N=24) and WAGR syndrome (N=20). 27 patients were diagnosed with a variety of other malformations or syndromes (**Suppl. Table 1**).

Table 1. Frequency of malformations and CPS in WT / nephroblastomatosis, *see **Suppl. Table 1**; **% related to the different malformations or CPS in bilateral WT, ***screening in 29 of 198 patients with malformations or CPS, ****% related to the different malformations or CPS in screened patients.

		WT / nej	WT / nephroblastomatosis						
	All WT		Only	y bilatera	l WT	diagnosis after screening			
All WT	2927	100%	253	8,6%	**	29 ***	****	**	
WAGR	20	0,7%	6	2,4%	30,0%	8	27,6%	40,0%	
GU	66	2,3%	8	3,2%	12,1%	1	3,4%	1,5%	
DDS	24	0,8%	7	2,8%	29,2%	3	10,3%	12,5%	
BWS	32	1,1%	10	4,0%	31,3%	10	34,5%	31,3%	
IHH	29	1,0%	4	1,6%	13,8%	4	13,8%	13,8%	
Other*	27	0,9%	7	2,8%	25,9%	3	10,3%	11,1%	
All	198	6,8%	42	16,6%	21,2%	29	100%	14,6%	

3.2. Ultrasound surveillance every 3 months

Altogether 29 (14,6%) patients had been screened by ultrasound every 3 months after the diagnosis of a CPS. The highest screening frequency resulting in the diagnosis of WT/nephroblastomatosis was reported in patients with WAGR (40%) and BWS (31,3%). (**Table 1**). No data were available on why not all children with an underlying syndrome were included in a screening program

3.3. Gender distribution and age at diagnosis of WT / nephroblastomatosis

Gender distribution in the whole cohort of patients with syndrome-associated WT is similar to the total group of patients with WT / nephroblastomatosis with a predominance of females (51,4% females vs. 48,1% males, 0,5% gender not known) with the exception of GU cases. 43 males (3,2% of 1353) and 23 females (1,5% of 1562) were affected by GU malformations. In DDS a slight male predominance has also been observed, but without statistical significance.



Figure 1. Age of WT / nephroblastomatosis at diagnosis of syndrome-associated WTs displayed as a boxplot (The line in the bar shows the median, the left end of the bar the lower quartile and the upper end of the bar the upper quartile, the horizontal line ranges from the minimum to the maximum of data with dots and stars as outliers). DDS and WAGR show a significant lower age at diagnosis compared to all other syndromes (p<=0,001). In 3 patients with CPS WT was diagnosed beyond 10 years of age (IHH (173 months), GU (146 months, 325 months)) and in 143 WT without a syndrome beyond 120 months, the last one at 628 months (data not shown).

With a median age of 24,5 months (mean: 40,3 +/- 36,7 months), patients with associated malformations or syndromes were significantly (t-test: p<0,001) younger at diagnosis of WT / nephroblastomatosis than patients without a malformation or syndrome (median age: 39,0 months; mean age: 50,0 +/- 51,6 months;). Patients with WAGR (median age: 21 months; mean age: 23,8 +/- 9,0 months) and DDS (median age: 16,0 months; mean age: 16,7 +/- 12,2 months) were even significantly younger at diagnosis than patients with other syndromes (**Figure 1**).

3.4. Bilaterality

There was a statistically significant higher incidence of bilaterality in patients with (21,2%) than without a syndrome (7,4%) (p<0,05), especially in patients with BWS (31,3%), WAGR (30,0%) and DDS (29,2%) compared to other patients with WT and/or nephroblastomatosis (**Table 1**).

3.5. Metastatic disease in patients with CPS or malformations

In the whole group of patients 18% (529/2927) had a primary metastatic disease (stage IV) while in the cohort of patients with the five most common WT associated syndromes, primary metastatic disease was observed in less than 8,0% (14/171) (p< 0,001) with the exception of IHH (17,2%, p=0,014).

3.6. Histology

Mixed type is, with the exception of WAGR syndrome, the most common histological subtype for all WT with or without syndromes. Patients with CPS are significantly more likely to have isolated nephroblastomatosis. In particular, a significantly increased proportion of isolated nephroblastomatosis is observed in WAGR, BWS, and IHH (**Table 2**).

	Isolate	Isolated NBL		WT + NBL		WT only		Total	
Total	73	2,5%	64	2,2%	2790	95,3%	2927	100%	
Patients with CPS or GU	22*	12,9%*	11	12,9%	138	80,7%	171	100%	
Patients without CPS or GU	51	1,9%	53	1,9%	2652	96,2%	2756	100%	
WAGR	7*	35,0%*	2	10,0%	11	55,0%	20	100%	
BWS	7*	21,9%*	3	9,4%	22	68,8%	32	100%	
ІНН	5*	17,2%*	0	0,0%	24	82,8%	29	100%	
DDS	1	4,2%	2	8,3%	21	87,5%	24	100%	
GU	2	3,0%	4	6,1%	60	90,9%	66	100%	

Table 2. Association between nephroblastomatosis (NBL) and CPS or malformation; *chi-square: $p \le 0,001$

There was also a statistically significant association of IHH with the blastemal subtype after preoperative chemotherapy (p=0,040) and of DDS with stromal subtype (p<0,001) (**Table 3**).

Table 3. Association between histological subtypes and CPS or malformation; *chi-square: p≤0,040

	Stroma	Stromal subtype		subtype after erative therapy	Other histological subtypes		
All WT	270 9,2%		215	7,3%	2442	83,4%	
WAGR	0 0,0%		1	5,0%	19	95,0%	
GU	4	6,1%	5	7,6%	57	86,3%	
DDS	9*	37,5%*	0	0,0%	15	62,5%	
BWS	0 0,0%		3	9,4%	29	90,6%	
ІНН	0	0,0%	5*	17,2%*	24	82,8%	

3.7. Tumor volume

Tumor volume (TV) was available in 2068 of 2927 (70,7%) patients at the time of diagnosis (1950 in patients without and 118 with CPS or GU) and in 1802 (61,6%) patients after preoperative chemotherapy (1702 in patients without and 100 with CPS or GU) (**Table 4**). In children with WT and CPS or GU TV was significantly lower than in patients without (334,8ml vs 496,9ml; p<0,001) (**Table 4**). Furthermore, with the exception of DDS a significant TV reduction can be achieved by preoperative chemotherapy in WTs with CPS or GU with the largest effect of 84,6% in patients with BWS showing an average TV after preoperative chemotherapy of only 38,3ml (Error! Reference source not found.). In contrast, in DDS even an increase in TV under preoperative chemotherapy of 7,7% (27,3ml) was observed (**Table 4**).

Table 4. Tumor volume (TV) at diagnosis and volume reduction achieved by preoperative (preop.) chemotherapy, *t-test: p<0,001 for lower initial TV in patients with CPS. TV is not available for all patients.

	Mean tumor volume (TV)									
	at diagnosis	after preop. chemo	volume ree	duction						
Patients without CPS or GU	496,9 ml * (N=1950)	236,1 ml (N=1702)	260,8 ml	52,5 %						
Patients with CPS or GU	334,8 ml* (N=118)	196,0 ml (N=100)	138,8 ml	41,5 %						
WAGR	99,7 ml (N=14)	76,9 ml (N=11)	22,8 ml	22,9 %						
GU	448,2 ml (N=43)	245,3 ml (N=36)	202,9 ml	45,3 %						
DDS	352,5 ml (N=17)	379,8 ml (N=18)	-27,3 ml	-7,7 %						
BWS	249,4 ml (N=22)	38,3 ml (N=17)	211,1 ml	84,6 %						
ІНН	334,3 ml (N=22)	135,5 ml (N=18)	198,8 ml	59,5 %						
Patients with CPS or GU undergoing surveillance	53,6 ml (N=17)	105,4 ml (N=13)	-51,8 ml	-96,6 %						

Tumor volume at diagnosis in the 17 patients with CPS undergoing surveillance is significantly smaller (TV in CPS patients with surveillance: mean 53,6 ml, median: 21,1 ml. TV in CPS patients without surveillance: mean: 382,1 ml, median: 320,0 ml) (**Figure 2**)



Figure 2. TV at diagnosis of CPS patients as a function of CPS surveillance displayed as a boxplot. TV at diagnosis of the 17 patients with CPS surveillance and of 101 patients without CPS surveillance. 2 outliers in patients with CPS surveillance at 160,0 ml and 396,0 ml and 3 outliers in patients without CPS surveillance at 1631,0 ml, 1632,0 ml and 2051,0 ml. CPS patients with CPS surveillance show significantly smaller TV at diagnosis (p<0,001).

3.8. Outcome

There was no statistically significant influence on EFS for the whole group of patients with a CPS (**Figure 3A**). But patients with BWS showed a significantly worse EFS (**Figure 3B**) and a higher relapse rate (34,4%) compared to other patients with WT and/or nephroblastomatosis (13,7%). Out of 22 patients with BWS and only unilateral disease at diagnosis 5 patients relapsed of whom 3 showed metachronic disease (3, 4.5 and 6 years after initial diagnosis). One of these 3 patients developed also lung and liver metastasis and died 6 years after diagnosis. Of the other two relapsed patients one patient developed a local relapse in the same kidney and the other one devloped lung metastasis without local or metachronic relapse and both survived. Further analysis suggested that EFS tends to be worse in patients with nephroblastomatosis and a syndrome than in patients without nephroblastomatosis (**Figure 3C**), particularly if they had developed WT in addition (**Figure 3D**). Supplemental Table 2 gives an overview of outcome data. The only significant difference was seen in BWS for 5- and 10y EFS.

In a multivariate analysis of all patients, nephroblastomatosis and bilaterality had a significant influence on the risk of relapse and death. If only CPS and GU WT patients were considered, such risk was only found for relapse but not for death (**Table 5**).



Figure 3. Event-free survival in different subgroups: A: Influence of a syndrome on EFS in patients with WT and/or nephroblastomatosis; Log Rank: p=0,890; B: Influence of BWS on EFS in patients with WT and/or nephroblastomatosis; Log Rank: p=0,002: C: Influence of nephroblastomatosis on EFS in patients with a CPS; Log Rank: p=0,086; D: Influence of WT on EFS in patients with nephroblastomatosis; Log Rank: p=0,315.

	WT and/or nephroblastomatosis						CPS patients					
Factor	relapse			death			relapse			death		
values	p- value	hazard ratio	EFS (%)	p- value	hazard ratio	OS (%)	p- value	hazard ratio	EFS (%)	p- value	hazard ratio	OS (%)
CPS patients	0,594	0,931	83,2	0,139	1,422	88,1						
bilaterality	0,000	1,579*	73,4	0,030	1,976*	88,2	0,003	3,013*	65,4	0,639	1,861	85,6
nephro- blastomatosis	0,005	1,220	72,1	0,074	0,266*	96,2	0,032	1,264	73,3	0,167	0,225	96,7

Table 5. Multivariate analysis in patients with a WT and/or nephroblastomatosis and only in patients with a syndrome; *: p<0,05

4. Discussion

In our retrospective analysis we found that 5,8% of patients with WT and/or nephroblastomatosis are associated with the top five syndromes (WAGR, BWS, DDS, IHH and GU) in agreement with previous literature. With the exception of patients with GU and DDS, female patients are more frequently affected. Patients with syndromes show smaller TVs both at diagnosis and after preoperative chemotherapy, which might be due to the inclusion in a screening program (20). The statistically significant lower frequency of metastatic disease at diagnosis in patients with a syndrome does not translates into a better EFS. Therefore, other factors such as nephroblastomatosis and comorbidities must be considered to explain their EFS, especially in patients with BWS. (**Table 6**).

Table 6. Summary of results in the top 5 syndromes associated with WT. * significant results (p<0.05); ** isolated nephroblastomatosis

	Prevalence (%)	Age at diagnosis (month)	Gender	Characteristic Histology	Bilaterality (%)	Volume reduction by preoperative chemotherapy	5y-EFS (%)	Confirmed by
WAGR	0,7	23,8*	m <f< td=""><td>nephroblastomatosis**</td><td>30,0*</td><td>22,8 ml</td><td>77,8</td><td>(21), (9),(22)</td></f<>	nephroblastomatosis**	30,0*	22,8 ml	77,8	(21), (9),(22)
GU	2,3	42,9	m>f*	-	12,1	202,9 ml	87,6	(23)
DDS	0,8	16,7*	m>f	stromal subtype	29,2*	-27,3 ml	94,7	(24),(25), (26), (27)
BWS	1,1	35,3	m <f< td=""><td>nephroblastomatosis**</td><td>31,3*</td><td>211,1 ml</td><td>60,6*</td><td>(28), (29)</td></f<>	nephroblastomatosis**	31,3*	211,1 ml	60,6*	(28), (29)
ІНН	1,0	45,3	m <f< td=""><td>nephroblastomatosis** / blastemal subtype after preop. chemo</td><td>13,8</td><td>198,8 ml</td><td>84,6</td><td>(6), (15), (30), (31)</td></f<>	nephroblastomatosis** / blastemal subtype after preop. chemo	13,8	198,8 ml	84,6	(6), (15), (30), (31)

4.1. Prevalence and surveillance

The prevalence of syndromes in patients with WT is lower in our series compared to the 8-17% in the literature (5),(6). This may be due to an underreporting in our retrospective multicenter study where standardized reporting was carried out at diagnosis, hence early in life with a probably incompletely symptomatology. BWS, for example, a syndrome with variable features, is described with a prevalence of 1 up to 8% in other studies (12),(28),(29). Therefore, not all patients with a WT CPS are included in ongoing ultrasound screening programs. In our data the screening rate is depending on the clinical symptomatology and highest in WAGR with 40% (**Table 1**). MacFarland et al. reported 12 patients diagnosed with BWS after a WT was already known (32). This may explain our low prevalence of 1,1% and also undiagnosed BWS in other studies. Clinicians need to recognize subtle manifestations of syndromes in WT patients to not overlook them. For clinical diagnosis of BWS a new consensus statement has been published in this respect (33). Knowledge about specific associations between different syndromes and WT will allow an earlier diagnosis of such WT, with CPSs demanding genetic testing, counselling and subsequently screening programs.

4.2. Age at diagnosis of WT / nephroblastomatosis

Of all patients with syndromes, those with DDS are diagnosed the earliest followed by WAGR (9). Patients with GU and BWS also tend to be diagnosed earlier than those without syndromes. The late median diagnosis at 45 months in IHH compared to other syndromes suggests that IHH manifests itself clinically rather subtly (12),(30) which is why no surveillance for WT was done. Therefore, our results suggest that early age at diagnosis of a WT without a syndrome should always raise awareness of a CPS. In addition, in patients with the diagnosis of such a syndrome screening for WT needs to start early and regularly up to the age when the manifestation of a WT gets more unlikely (20). According to our data, for patients with DDS and WAGR such a screening may stop already at the age of 4 or 5 years as also recommended by Hol et al. (8), whereas in the other syndromic patients screening should continue at least up to the age of 7 years as also consented for BWS (20) (**Figure 1**). In the work of Diller et al. the average age of diagnosis of patients with GU was 13 months and thus significantly earlier compared to our data (42.9 months) underscoring the need for a regular screening in patients with GU (34).

4.3. Tumor volume and response to preoperative chemotherapy

Tumor response to preoperative chemotherapy varies significantly between the different syndromes and depends on the presence of a stromal subtype or WT1 mutation. Thus, we confirm a poor response in patients with DDS (26),(27). These patients have comparably larger initial TVs that can even increase after preoperative chemotherapy (Error! Reference source not found.). A WT1 mutation/deletion path driven propensity for stromal components or even stromal predominance is a likely reason (35), (36). The TV reduction after preoperative chemotherapy is also poor in WAGR patients with WT1 deletion despite of missing stromal type WT (Error! Reference source not found.). In contrast, an excellent response on preoperative chemotherapy is achieved in patients with BWS. As a consequence of a poor TV reduction, a stromal subtype or WT1 aberrations with an underlying syndrome may be possible. This is especially true for patients with bilateral disease.

4.4. Bilaterality

The significantly increased frequency of bilaterality in patients with WAGR, GU, DDS is consistent with previous work (9),(23),(24). In patients with BWS we found a higher percentage of bilaterality (31,3%) than Breslow et al. (21%) (9). Consequently, patients with unilateral disease and WAGR, DDS or BWS should always be regarded as predisposed for bilaterality (4) and in cases of bilateral disease these three syndromes need to be kept in mind, if not diagnosed yet.

4.5. Metastatic disease

Metastases are a significantly less frequent event in patients with syndromes as compared to non-syndromic WT. This may be due to an early diagnosis of WT or nephroblastomatosis in patients with syndromes and underscores the importance of a screening program (20).

4.6. Histology

The majority of patients with syndromes have intermediate-risk WT. As described earlier, patients with DDS are significantly more often affected by WT with a stromal subtype and never with high risk tumors (24),(25). There is a significant association with blastemal subtype after preoperative chemotherapy in patients with IHH and a trend in BWS due to the frequently IGF2 driven biology. In contrast to the work of Green et al., focal and diffuse anaplasia do not occur as first histology in our data (30). However after nephroblastomatosis the development of diffuse anaplasia in case of a secondary WT is a relatively frequent event (37). We confirm the association between WAGR, BWS and IHH and nephroblastomatosis found by others (9),(21),(31),(33) in contrast to patients with DDS and GU (23). As nephroblastomatosis is found more often in patients with the above mentioned three syndromes this raises the question whether all patients with nephroblastomatosis need to be examined for CPS if not yet known (33).

4.7. Outcome

With the exception of patients with BWS showing a significantly worse EFS and increased risk of relapse, CPS or GU in general have no impact on EFS. Breslow et al. found no difference in WT with BWS neither in OS nor in EFS (9). However, if nephroblastomatosis is present in our data, EFS tends to be worse, especially if these patients develop a WT as already shown by Furtwängler et al. (37),(38). Therefore, patients

with nephroblastomatosis independent of a predisposition syndrome must be followed in regular intervals after the end of treatment for longer periods of time to diagnose a relapse early in order to keep their overall survival as high as for other patients (39).

5. Conclusions

Diagnosis of WT at an early age, bilateral tumors or nephroblastomatosis in patients without a known CPS should always raise suspicion of an underlying CPS and genetic testing and counselling should be offered to these patients and families. Screening for WT in patients with a syndrome may stop earlier after the age of 4 to 5 years in patients with DDS and WAGR as also recommended by Hol et al. (8), whereas for the other syndromes this should last up to the age of 7 years and needs to continue in cases of nephroblastomatosis even in CR after the end of first line treatment.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Further WT associated syndromes and malformations (number of involved patients in brackets), Table S2: Data on outcome of different patient groups. (NBL: Nephroblastomatosis); *p<0,05.

Supplemental Table 1. Further WT associated syndromes and malformations (number of involved patients in brackets).

Other syndromes	Gigantism (4) Townes-Brook Fanconi synd syndrome, Mu	sm (4), Trisomy 21 (2), VACTERL (2), Perlman-syndrome (2), Neurofibromatosis type I (2), -Brooks, Prader type III, Bloom-syndrome, Klinefelter syndrome, Gorlin-Goltz syndrome, syndrome, Conradi-Hünermann syndrome, Rubinstein–Taybi syndrome, Oral-facial-digital ne, Mulibrey nanism, Trisomy 8, Trisomy 18, Trisomy 1, Crigler–Najjar syndrome, 11P syndrome							
Malformations	cardiac	rdiac VSD (4), ASD (2), Fallot, Pulmonary valve stenosis, PDA (3), other cardiac defects (2)							
	renal	Horseshoe kidney (5)							
	facial Auricular dysplasia (2), cleft lip and cleft palate (3), Microphthalmia, Anop coloboma, Hereditary ptosis								
	visceral	anal atresia, omphalocele, lateral thyroglossal cyst, tracheal stenosis							
	limb	syndactyly (2), hexadactyly, Amelia, pigeon toe, congenital hip malformation							
	cerebral	isolated cerebellar atrophy							
	neoplastic	lipoma (3), lymphangioma, stromal granuloma							

Supplemental Table 2: Data on outcome of different patient groups. (NBL: Nephroblastomatosis); *p<0,05

	Event	Last event	Death	Last death	EFS	EFS	EFS	OS	OS	OS
	n	(months)	n	(months)	2y	5у	10y	2y	5y	10y
No syndrome	65	115	54	143	88,2	85,3	84,1	95,0	93,0	92,2
With syndrome	16	83	15	86	87,1	84,2	83,2	96,3	92,0	88,1
WAGR	2	83	2	19	87,5	87,5	77,8	88,4	88,4	88,4
GU	7	21	7	86	87,6	87,6	87,6	96,9	92,0	88,3
DDS	1	5	2	6	94,7	94,7	94,7	90,6	90,6	90,6
BWS	8	59	3	85	83,0	60,6*	60 <i>,</i> 6*	100	95,5	80,4
ІНН	4	22	2	40	84,6	84,6	84,6	100	91,8	91,8

WT without NBL	62	111	60	143	87,7	85,6	84,6	94,9	92,8	91,7
Isolated NBL	10	115	0	-	85,0	83,3	76,1	100	100	100
WT with NBL	14	61	4	38	80,5	71,8	69,1	94,7	92,4	92,4

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