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Liver findings in patients with Carney complex, germline *PRKAR1A* pathogenic variants, and link to cardiac myxomas

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Abstract

This study aimed to evaluate liver involvement in patients with Carney Complex (CNC) based on a large cohort and to analyze any germline *PRKAR1A* genotype - phenotype association of liver

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Declaration of interest

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disease. The study included 83 patients with CNC, followed between 1995 to 2018 at a tertiary research center. We reviewed liver images, recorded types and number of lesions and analyzed per genotype: all patients were sequenced for the *PRKAR1A* gene.

A total of 29/83 patients (24.0%) had liver radiological findings. Patients with liver lesion had a significantly higher rate of pathogenic variants detected in the *PRKAR1A* gene (72.4% vs. 38.9%, p=0.005, respectively). Patients with a pathogenic variant detected on germline *PRKAR1A* analysis had a higher risk for having a liver lesion compared with patients with wild-type (WT) *PRKAR1A* alleles (21/42 [50.0%] vs. 8/41 [19.5%], respectively, p=0.004]. Among patients with liver lesions, those with a nonsense *PRKAR1A* pathogenic-variant had more liver lesions (7/7) than among those with other pathogenic-variant types (8/22, p=0.001). In multivariable analysis, detection of liver lesion(s) was associated with an odds ratio of 5.2 for cardiac myxomas (95% confidence interval 1.55–17.49, p=0.008). In conclusion, patients with CNC, particularly with a *PRKAR1A* pathogenic-variant, have a higher rate of liver lesions. Additionally, liver lesions are associated with a high-risk for cardiac myxomas in this population.

Keywords

Carney Complex; genotype-phenotype; PRKAR1A; Liver

Introduction

Carney Complex (CNC) is a rare multiple neoplasia syndrome, that is either inherited as an autosomal dominant trait, or occurs sporadically as a result of a de-novo defect (Correa et al. 2015). CNC is mostly caused by a germline pathogenic variant in the *PRKAR1A* gene (OMIM 188830), which encodes the regulatory subunit type I alpha of protein kinase A (Stratakis et al. 2000).

CNC is a multi-organ syndrome and it most commonly manifests with spotty skin pigmentation, cutaneous, cardiac and breast myxomas, and primary pigmented nodular adrenocortical disease (PPNAD) associated with an atypical form of Cushing syndrome (CS) (Stratakis 2016). However, patients with CNC may also have other endocrine neoplasms including growth hormone- and/or prolactin-secreting pituitary adenoma(s), thyroid neoplasms, large-cell calcifying Sertoli cell tumors in the testis and ovarian cysts and carcinomas (Courcoutsakis et al. 2013).

We have recently reported a higher frequency of liver manifestations in pediatric patients with CNC, in association with failure to thrive (Tirosh et al. 2017). Liver involvement in CNC has been reported previously in case reports and small case series (Terracciano et al. 2004; Veugelers et al. 2004; Gennari et al. 2008), with some support from animal models, which developed liver lesions in association with abnormal protein kinase A activity (Veugelers et al. 2004).

However, until today liver involvement has not been validated in a large cohort of patients with CNC. Hence, in the current study we performed an imaging-based screening for liver manifestations in patients with CNC. We then analyzed our data that were collected without

prior knowledge of the genetics, as per the genotype. Clearly, patients with CNC due to *PRKAR1A* pathogenic variant had more liver lesions.

Patients and Methods

This is a retrospective study of 83 patients with CNC, followed between 1995 to 2018 at the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). One patient was diagnosed, treated and followed at Hadassah-Hebrew University Medical Center and genetic diagnosis was confirmed at the NIH. The NICHD institutional review board has approved the study of patients with CNC (protocol 95-CH-0059); the parents of the patients signed the proper consent forms for clinical and genetic investigations related to the diagnosis of their child.

The diagnosis of CNC was made by using previously published diagnostic criteria (Correa et al. 2015). Genetic sequencing for *PRKAR1A* defects was obtained in all patients as was previously described (Stratakis et al. 2000). The patients' medical files were reviewed, and data were retrieved on demographics, medical and family history, physical findings, biochemical evaluation results, adrenal and liver imaging and treatment. Analysis of the imaging files and diagnoses were recorded without knowledge of the genetic testing results.

Imaging techniques

Examinations of the liver were done using commercially available high magnetic resonance imaging (MRI) and/or computerized tomography (CT) scanners for all subjects. The routine liver imaging examinations were evaluated by two radiologists in consensus who were blinded to genetic results, as above. Both radiologists had at least 15 years of experience in body and liver imaging. Standard diagnostic criteria and methods were used (Sahani & Kalva 2004; Jang et al. 2009). Briefly, cysts are defined as hypodense well-defined lesion(s) on CT with no enhancement in any of the phases of enhancement. On MRI, cysts are T1 hypointense (dark) and T2 hyperintense (bright) with no mural enhancement noted after IV contrast administration. Adenoma is initially hypodense in CT with intense enhancement in arterial phase and wash-out in delayed phases. On MRI the lesion is T1 hypointense and T2 iso- to mildly hyperintense with enhancement pattern similar to that in CT. Hemangioma is hypodense in CT with initial nodular enhancement in early phases and gradual fill-in in porto-venous and delayed phases. On MRI, hemangioma is T2 hyperintense and remain hyperintense in heavy T2WI with enhancement pattern parallel to that described in CT.

Genetic analysis

DNA was extracted from peripheral blood leukocytes using the Quick-DNA™ Universal Kit, according to manufacturer protocols (Zymo). The complete *PRKAR1A* coding sequence of the blood samples was analyzed by bidirectional Sanger sequencing, as we have previously described.

Statistical analysis

The analysis was performed on R statistics (version 3.4.4) via R studio (version 1.0.153). Data were described with simple descriptive statistics. Continuous variables are presented as

mean ± standard deviation (SD) and were compared using the Student's t-test, while parametric variables were compared using the chi-square test, unless otherwise indicated. Multivariable analysis was performed using logistic regression, including age (categorized to <=40 years and >40 years), gender, diagnosis with PPNAD and detection of liver lesion. Odds ratio (OR), 95% confidence interval (CI) and adjusted p-value are presented for each variable in the model. Statistical significance was set at two tailed p-value < 0.05.

Results

The current analysis included 83 patients with CNC, with an age at last follow-up of 39.3±17.3 years (range 9–75), including 59 females (71.1%). Of 83 patients included in the current analysis, 42 had germline pathogenic variant detected in the *PRKAR1A* gene, including 15 frameshift mutations, 14 nonsense mutations, 8 splice site variants, 3 missense mutations and two unknown types of pathogenic variants.

A total of 29 patients (24.0%) had liver radiological findings: 17 cysts, 3 cysts with other enhancing lesions, 2 hemangiomas (Fig. 1) and 6 undefined hepatic lesions. Patients with liver lesions had a significantly higher rate of pathogenic variants detected in the *PRKAR1A* gene (72.4% vs. 38.9%, p=0.005, respectively), and were older at last evaluation (43.6±6.0 vs. 34.9±17.5 years, respectively, p=0.02). The patients' characteristics, compared by presence/absence of liver lesion, are detailed in Table 1.

Patients with pathogenic variants detected on germline *PRKAR1A* analysis had a higher risk for having a liver lesion compared with patients with wild-type (WT) *PRKAR1A* alleles (21/42 [50.0%] vs. 8/41 [19.5%], respectively, p=0.004]. Among patients with any liver lesion, patients harboring pathogenic variants in the *PRKAR1A* gene had higher rate of multiple liver lesions compared with patients with WT *PRKAR1A* allele, with borderline statistical significance (13/21 [61.9%] vs. 2/8 [25.0%], respectively, p=0.08).

No difference was found in the risk to develop liver lesions between patients with different pathogenic variant types (p = 1.0); however, among patients with a liver lesion, all patients with nonsense *PRKAR1A* pathogenic variant had multiple liver lesions (7/7, 100%) vs. 8/22 (36.4%) for patients with any other pathogenic variant types (p = 0.001).

To better assess the clinical implication of the liver lesion detection in CNC, we reviewed all the pathology reports of patients with reported liver lesions in our cohort. We found three liver tissue pathology reports: one patient underwent biopsy for a liver mass that was diagnosed as focal nodular hyperplasia, second patient underwent adrenalectomy, with resection of normal liver tissue and the last had metastatic breast cancer with liver metastases, with normal background liver parenchyma.

Since cortisol and growth hormone over-secretion have systematic manifestations, we screened for liver lesions among patients with non-CNC related Acromegaly and Cushing syndrome. Among 95 patients with hypercortisolemia, 15 (23.5%) had focal liver lesion (7 liver cysts, 3 hemangiomas, one adenoma and four lesions too small to characterize). The rate of liver lesions was significantly higher among patients with CNC in general vs. non-CNC hypercortisolemia (34.9% vs. 15.8%, p=0.003), and especially compared to patients

with CNC with pathogenic variant in PRKAR1A (50.0% vs. 15.8%, p<0.001). Among ten patients with Acromegaly, none had focal liver abnormality, lower than patients with CNC (15.8% 0%, Fisher's exact, p=0.003).

In order to assess the potential prognostic utility of liver lesion detection on other manifestation of CNC, we performed logistic regression, controlling for demographic and clinical covariates. In the multivariable analysis, detection of any liver lesion(s) in patients with CNC was associated high risk for cardiac myxomas (odds ratio [OR] 5.21, 95% confidence interval [CI] 1.55–17.49, p=0.008), in contrast to female gender (OR 1.84, 95% CI 0.52–6.58, p=0.35), older age (>40 years, OR 1.1, 0.3–3.3, p=0.93) and diagnosis with PPNAD (OR 0.32, 95% CI 0.08–1.35, p=0.12).

Discussion

In the current study we report for the first time a high prevalence of liver lesions in a large cohort of patients with CNC, based on a prospective natural history study with genetic analysis. We found a high rate of cystic lesions, together with liver nodules and hemangiomas, which were most common among patients with a pathogenic variant detected in the *PRKAR1A* gene. Moreover, patients with a detected pathogenic variant in the germline DNA, had increased risk for multiple liver lesions. Specifically, patients with nonsense *PRKAR1A* pathogenic variant had higher risk for multiple liver lesions compared with any other pathogenic variant type.

The prevalence of hepatic cysts and hemangiomas in the general population is debatable due to the high discrepancy in the published literature. Nevertheless, both cysts and other hepatic lesions are increasingly found as a mere coincidence on abdominal imaging techniques, such as ultrasonography computed tomography and magnetic resonance imaging (Lantinga et al. 2013). The prevalence of simple hepatic cysts is estimated by 15–18% in the United States (Lantinga et al. 2013) and the prevalence of hepatic hemangiomas is estimated as 0.4% to 10% based on radiological findings and autopsy series (Choi & Nguyen; Brouwers et al. 1997; Mocchegiani et al. 2016).

When compared to the general population, the rates of liver cysts and hemangiomas is not exceeding 20%, whereas in our analysis, the rate of liver lesions (mainly cysts and hemangiomas) in patients with a detected *PRKAR1A* pathogenic variant was 50% and most of these patients (61%) had multiple lesions. Furthermore, multiple liver lesions are rare in the general population and are usually associated with either polycystic kidney disease, affecting up to 0.2% of the general population (Lavö et al. 1990), or isolated polycystic liver disease (has prevalence of less than 0.01%) (Qian 2010).

Patients with multiple liver lesions in non-CNC context are usually asymptomatic. However, a small fraction of these patients develop acute liver cyst-related complications and/or massive cystic liver enlargement, causing morbidity and mortality (Qian 2010). Currently, the management of symptomatic patients is centered on palliating symptoms and treating complications, however, new therapies are under investigation (Masyuk et al. 2017). The absence of pathological finding in liver pathology reports in patients with CNC in our

cohort, suggests low clinical implication for this manifestation *per se.* However, with the high risk found for cardiac myxomas among patients with liver lesions in the multivariable analysis, detecting such lesion should alert the clinician to survey for cardiac myxomas more closely.

Our findings raise a question regarding screening strategies for liver lesions in patients with CNC and in particular in patients with a detected *PRKAR1A* pathogenic variant. With that being said, the clinical significance of multiple liver cysts in CNC is not known and further investigation is needed.

This analysis is based on the largest CNC-patient cohort worldwide. Nevertheless, it has its limitations, including the retrospective methodology and the cross-sectional analysis, with their known drawbacks. In addition, long term follow-up on the complications of liver disease is required to establish the benefit from screening.

In conclusion, patients with CNC have a high-rate of liver lesions, with higher rates in the patients' subgroups with a *PRKAR1A* pathogenic variant detected, and specifically in those harboring a nonsense pathogenic variant. Although liver lesions by themselves might not indicate follow-up or intervention based on our data, a closer surveillance for cardiac myxomas development may be indicated among patients in which liver lesions are detected.

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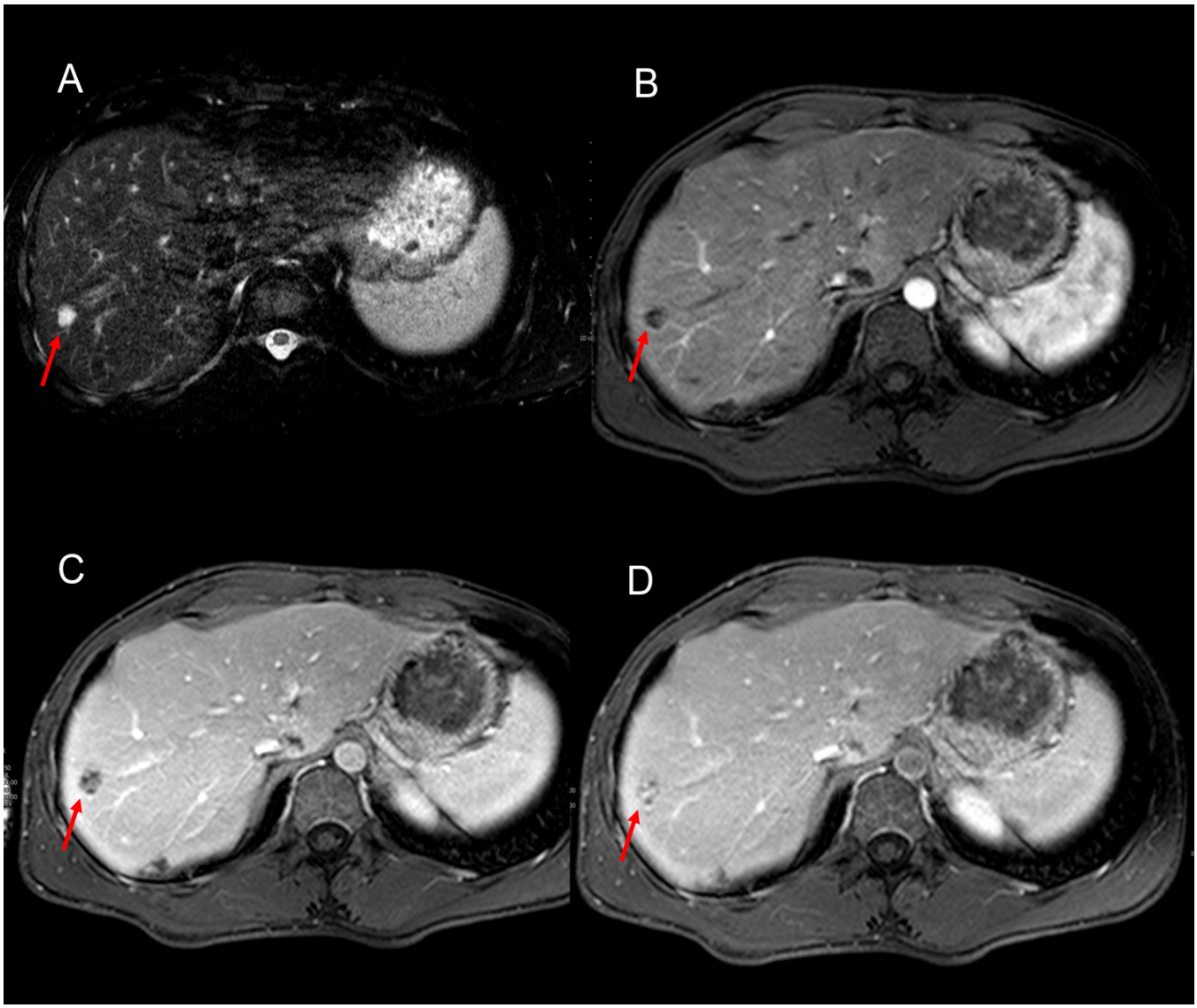

Figure 1.

Magnetic Resonance Imaging of the liver of a 28 year old male with Carney Complex showing hemangioma. T2 weighted image showing bright lesion in right lobe of the liver (A). T1 weighted image with contrast in arterial phase showing no enhancement (B) with subsequent filling-in on porto-venous phase (C) and delayed venous phase (D). This pattern is consistent with hemangioma.

Table 1

Patients characteristics, compared by presence/absence of liver lesion on abdominal imaging

	Liver lesion on imaging		p value
	Not Detected (n=54)	Detected (n=29)	
Female n(%)	41/54 (75.9)	18/29 (62.1)	0.2
Age (years)	34.9±17.5	43.6±6.0	0.02
Imaging type [CT/MRI/US, n(%)]	9/42/3 (16.7/77.8/5.6)	4/25/0 (13.8/86.2)	0.4
Number of lesion n(%)			
Single lesion	NA	14/29 (48.3%)	NA
Multiple lesions		15/29 (51.7%)	
Lesion characteristics Cyst/adenoma/hemangioma	NA	19/5/5 (65.52/17.24/17.24)	NA
PPNAD [yes/no/unknown, n(%)]	30/8/16 (55.6/14.8/29.6)	16/7/6 (55.2/24.1/20.7)	0.5
Cardiac myxoma [yes/no/unknown, n(%)]	14/24/16 (25.9/44.4/29.6)	9/13/7 (31.0/44.8/24.1)	0.8
Germline pathogenic variant in *PRKAR1A* detected n(%)	21/54 (38.9)	21/29 (72.4)	0.005
Serum alkaline phosphatase level (IU/L)	95.2±70.3	81.9±55.2	0.4
Serum ALT level (IU/L)	25.4±18.6	34.7±31.6	0.2
Serum AST level (IU/L)	21.1±7.8	24.0±15.4	0.3
Serum total bilirubin level (mg/dl)	0.51±0.30	0.51±0.26	1.0

CT, computerized tomography; MRI, magnetic resonance imaging; US, ultrasound; PPNAD, primary pigmented nodular adrenocortical disease; IU/L, international units/liter; NA, not applicable