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REVIEW

5th International ACC Symposium: Hereditary Predisposition to Childhood ACC and the Associated Molecular Phenotype

5th International ACC Symposium Session: Not Just for Kids!

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Abstract Adrenocortical carcinoma (ACC) affects children and adults. Roughly 50 % of very early onset ACCs occur in children with germline *TP53* mutations. Several recent studies have extended our understanding in basic, clinical, and translational genetics with regard to *TP53* germline predisposition in ACC patients. The recent description of the molecular landscape of pediatric ACCs provided insight into differences of tumors arising in patients with and without *TP53* germline mutation. Another recent important finding is that not all *TP53* mutations are equal in their tumor suppressing potential. It has now been shown that family histories as well as molecular characteristics of preserved TP53 functions vary greatly between mutations. It also has become clear that adult patients with ACC often harbor germline mutations causing hereditary syndromes, including Li-Fraumeni syndrome (LFS), Lynch syndrome, and multiple endocrine neoplasia type 1 (MEN1).

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The Molecular Landscape of Pediatric ACTs

Pinto et al. have recently provided a detailed insight into the molecular landscape of childhood adrenocortical carcinoma (ACC) [1]. Thirty-seven pediatric adrenocortical tumors (ACTs) (29 carcinomas, 3 adenomas, and 5 tumors of uncertain behavior) were studied by molecular profiling, including whole exome, whole genome, and transcriptome analysis. The cohort included a large number of ACCs from patients with hereditary tumor predisposition syndromes; 25 of 37 patients carried a germline *TP53* mutation and 2 patients had a clinical diagnosis of Beckwith-Wiedemann syndrome (BWS). In analogy to the studies in ACCs from adult patients, the findings confirm some of the hallmarks of genetic changes, such as maternal loss of heterozygosity (LOH) of the *IGF2* locus on chromosome 11p, resulting in IGF2 overexpression, and LOH of chromosome 17 at the *TP53* locus with selection against the maternal and wild-type TP53 allele [2]. In addition, *CTNNB1* (β-catenin) mutations and *ATRX* mutations were found in 8 % (and in 30 % of comparison cohort of 34 ACTs) and in one third of the cases, respectively. Importantly, somatic *ATRX* mutations always coexisted with germline *TP53* alterations, and somatic *CTNNB1* and germline *TP53* mutations were mutually exclusive. Although the telomerase reverse transcriptase locus (*TERT)* was amplified, no *TERT* expression was observed by RNA-sequencing analysis in those pediatric ACTs. Loss of *ATRX* is associated with alternative telomere lengthening (ALT), and in accordance, all samples with loss of *ATRX* displayed signs of ALT. ALT had not been described in pediatric ACCs but signs of ALT are present in up to ~20 % of adult ACCs often even in combination with detectable telomerase activity [3]. Most strikingly, loss of *TP53* together with *ATRX* mutations resulted in a significantly higher background mutational rate, higher number of single nucleotide and structural variants, and conferred a significantly worse prognosis,

making this a possibly suitable marker for future risk stratification and differentially aggressive therapeutic regimens. Tumors with *TP53* mutations, but without *ATRX* mutations, and tumors without *TP53* mutations, but with *CTNNB1* mutations, clustered in different groups with significantly better prognosis. Of note, this group contained eight of nine tumors from children with the Brazilian founder mutation *TP53* R337H [4]. As detailed below, the less aggressive phenotype might be due to some preserved TP53 function in these tumors [5]. Another interesting finding, which will need additional exploration in the future, was the observation of genomic integration of human herpesvirus-6 (HHV6) in the telomeric region of *11p* in two cases.

Insights into the Role of TP53 Mutations as Germline Predisposing Variants for ACC

As mentioned, *TP53* germline mutations are identified in a substantial proportion of childhood ACCs. Although the association of ACC and LFS was known for a long time, it was only recently substantiated in a larger study by testing 88 consecutive unselected children with the diagnosis of ACC [5–7]. However, *TP53* mutations in the cohort analyzed by Wasserman et al. were not observed in the usual hotspots of the gene. In addition, children with these germline mutations often lacked a history of classical LFS. Only 11 of 19 children with a germline *TP53* mutation had a diagnosis of classic LFS or even Li-Fraumeni-like syndrome (LFLS) by Birch or the somewhat looser Eeles criteria, respectively. Of the remaining eight patients, four had proven de novo mutations, underscoring the importance to test children regardless of their family history. Two of the remaining four patients had an inherited mutation with a parent unaffected by age 42 and 51, and for the additional two patients, parental DNA was not available. Further investigation of the mutant proteins observed in this study showed that TP53 activity of de novo and classical LFS/LFSL alleles was significantly lower than for the variants found in patients without a family history of LFS. Interestingly, the well-described Brazilian hotspot mutation R337H showed 69 % preserved wild-type TP53 activity in an assay for TP53 activation of known target genes. The variability in preserved TP53 function was also confirmed in colony-forming assays using a TP53 deficient cell line. Together, these data suggest that the degree of disturbed TP53 function might explain the lower penetrance observed in some families and provide invaluable insight into the predisposition to cancer development in patients with germline *TP53* mutation in a translational research perspective. In the future, these data will hopefully be the basis of risk assessment for families with different *TP53* mutations and might lead to a tailored screening protocol.

Germline Predisposition to ACC as an Important Clinical Consideration

Two hereditary syndromes are contributing to childhood ACC predisposition: Li-Fraumeni syndrome and Beckwith-Wiedemann syndrome. Genetic predisposition to ACC in the adult population has been researched over the last years through efforts of systematic genetic counseling and testing of patient populations with the diagnosis of ACC or at risk individuals carrying a genetic diagnosis.

The increased risk for ACC development in BWS appears to be restricted to childhood. Although the risk for ACC in LFS patients seems to decline with age, it has recently been shown that 4–6 % of adult patients with the diagnosis of ACC harbor a germline mutation in *TP53* [5, 8, 9]. It is important to mention that just like in children with ACC, germline *TP53* mutations occurring in adult ACC patients may represent either low penetrance alleles or de novo mutations (up to 25 % in LFS), and therefore, the family history might appear negative.

Over the last decade, there had been accumulating evidence of Lynch syndrome-associated ACC. Indeed, one of Lynch's initial families (family N) was ascertained through an index patient with ACC. In a study by Raymond et al., 3.2 % of patients with ACC were found to have a mutation in one of the genes causing Lynch syndrome [10]. Mutations in *MSH2*, *MSH6*, and *MLH1* have been described. Not all families had a definitive family history of Lynch syndrome, but the personal and family history was often suggestive of Lynch syndrome. Remarkably, microsatellite instability was less obvious than in colorectal malignancies associated with the syndrome. Absence of the respective MMR proteins was found in all but one patient with a *MSH6* mutation, which is in accordance with data for other core malignancies associated with Lynch syndrome [11, 12].

A recent retrospective analysis of patients with MEN1 showed that 1.4 % of MEN1 patients developed an ACC, and a total of 20 % had adrenal nodules or enlargement [13]. At the University of Michigan, we find MEN1 in ~1–2 % of all ACC patients, and all of these patients had criteria for a clinical or molecular diagnosis of MEN1 prior to ACC development.

Familial adenomatous polyposis (FAP) has long been mentioned as a predisposing syndrome for ACC. Particularly the fact that activating *CTNNB1* mutations are present in ~30 % of benign adenomas, and ACCs further underscore the importance of this pathway in the pathogenesis of ACC. However, the actual contribution of FAP patients to the number of ACC patients is rather small. One reason might be that FAP patients only recently started to receive care that will prevent early morbidity and mortality from the numerous colon adenomas and colorectal cancers. The chance of progression of any of the often very numerous colon adenomas (100–1000 s) might

likely be the same as for the adrenal adenomas present in 10–15 % of FAP patients, but only nowadays, FAP patient might survive long enough to be at risk for these adrenal lesions to progress to cancer.

In 2012, two patients with Carney complex (CC) and ACC have been described [14, 15]. CC is a very rare hereditary syndrome predisposing to PPNAD-associated Cushing syndrome as well as other manifestations, such as cardiac myxomas and growth hormone-producing pituitary tumors. The true prevalence of CC is unknown, and the incidence and risk increase for ACC remain to be determined, but the co-occurrence of a very rare cancer (ACC) in a very rare syndrome with predisposition to an unusual benign proliferative adrenal phenotype makes this likely a true association.

In terms of practical clinical care, these recent findings translate into two areas: surveillance of patients with a hereditary syndrome that provides a risk increase for ACC and the evaluation of patients with ACC for an underlying hereditary syndrome.

Roughly 10 % of all patients with ACC will have an identifiable hereditary syndrome that can be diagnosed by genetic diagnosis [16, 17]. With regard to general screening, physicians should pay particular attention to any of the common elements warranting a further work-up, and every patient should be offered genetic counseling and genetic evaluation. The patient's personal and family history should be thoroughly evaluated for any suggestive features. Genetic counselors should obtain at least a four-generation pedigree. An additional general screening approach is the analysis of paraffin-embedded tissue for microsatellite instability (MSI) and immunohistochemistry (IHC) for the MMR proteins. The proportion of patients with ACC with underlying Lynch syndrome is in the same order as the proportion of patients with colorectal cancer, where general screening has long been instituted. All patients with ACC fulfill the Chompret testing criteria for *TP53* mutations regardless of family history; therefore, all ACC patients should be offered *TP53* germline testing following genetic counseling and informed consent. All other genetic testing should depend on clinical suspicion based on personal and family history as well as patient preferences.

While surveillance is well established for the core cancers of common hereditary syndromes, such as colon cancer with Lynch syndrome or breast cancer for hereditary breast and ovarian cancer (HBOC), ACC is not part of any screening surveillance for any of the predisposing hereditary syndromes. A general recommendation for all screening procedures is to avoid radiation-containing procedures to minimize cancer risk due to surveillance, which is a specific concern with LFS. With regard to LFS, recent studies suggest the feasibility of screening for ACC [18, 19]. The highest risk is in early childhood, and most of the tumors are hormone secreting. LFS surveillance guidelines are not well established, and several experimental protocols have been proposed. In this young population, treating physicians should consider ultrasound and hormone evaluation (dehydroepiandrosterone sulfate [DHEAS] and testosterone) every 6 months. Even more importantly, parent counseling should include the fact that most ACC patients present with symptoms and signs of precocious puberty, and that any of these clinical findings need further evaluation. For MEN1, expert opinion-based guidelines are in place and cover the adrenal glands by imaging in a yearly fashion [20]. Attention should be paid specifically to those patients with a prior adrenal mass. While there is no adrenal imaging in the guidelines for FAP and Lynch syndrome surveillance, treating physicians should be aware of the increased risk of ACC and the need to evaluate any adrenal mass in these patients. However, the absolute risk is relatively small (likely <1 %), and general surveillance might not be justified.

In summary, every patient with a diagnosis of ACC should be offered genetic counseling, baseline genetic testing for *TP53* by germline DNA sequencing, and deletion/duplication analysis and IHC for the MMR proteins. All other genetic testing should be guided by clinical suspicion. It is important to keep in mind that a genetic diagnosis of a patient very well influences further treatment decisions (e.g., avoidance of adjuvant radiation therapy for LFS patients) and impacts future surveillance for the patient and their family with regard to screening for other associated tumors (e.g., yearly colonoscopies with Lynch syndrome) in at risk gene mutation carriers.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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