Significance

Descriptive epidemiology of rhabdomyosarcoma (RMS)

Incidence: RMS is the most common type of soft tissue sarcoma in children. The incidence of RMS is 4.5 cases/million children per year. There are two major RMS subtypes, embryonal RMS (ERMS) and alveolar RMS (ARMS). Together they comprise 80% of all RMS diagnoses, of which the vast majority are ERMS (75%). ERMS is characterized by an early age of onset (Fig.1) with approximately half of diagnoses established before the age of 5 years[1]. Although survival of ERMS has improved substantially over the last three decades, now reaching 75%[2], patients with advanced disease fair poorly. Likewise, ERMS patients with anaplastic morphology (approximately 17% of ERMS cases) have significantly worse prognosis (63%, 95%CI=50%-76% for 5-year failure-free survival (FFS)), compared to non-anaplastic morphology (77% FFS, 95%CI=72%-82%), after adjustment for competing risks[3]. In addition, ERMS treatment requires multimodality therapy that can be associated with significant toxicities and long-term effects, particularly when administered to young children[4]. Therefore improved understanding of disease etiology is needed to prevent ERMS in the future, but progress is hampered as causes of ERMS remain largely unknown.

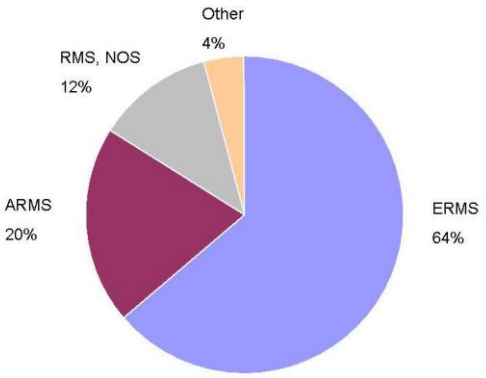

Figure 1 *Prevalence of RMS diagnoses by subtype in children <5 years of age*.

Epidemiology: *Environmental risk factors*: Few studies of RMS have examined potential environmental etiologic factors. The largest case-control study of childhood RMS thus far was conducted in the US in the mid-1980s and included 351 RMS cases[5]; the majority of reported associations between environmental exposures and risk of RMS result from this study[6-8]. Environmental exposures suggested by the available literature to be associated with increased RMS risk include paternal cigarette smoking[7], advanced maternal age, and x-ray exposure in utero[8], maternal[7] and child's[9] antibiotic use, stillbirths[10] and maternal recreational drug use[6].

Genetic risk factors: As with most types of childhood cancer inherited syndromes markedly raise risk of RMS but account for a small proportion of cases. These syndromes include neurofibromatosis, Li Fraumeni syndrome (LFS), involving germline mutations in NF1 and p53 genes, respectively, Costello syndrome, Beckwith-Wiedemann Syndrome (BWS), and Gorlin's nevoid basal cell carcinoma syndrome. Studies of family history of cancer and RMS mostly reflect known familial syndromes. Congenital malformations are more frequently (32%) observed in children and adolescents with RMS[11] compared to the general population, where the frequency of such malformations is approximately 3%[12]. This evidence, together with the lack of overt environmental risk factor for ERMS, suggests that ***genetic factors are important for ERMS development.***

Genetics: Clinical and pathological differences between ERMS and ARMS support the largely accepted view that these RMS types develop by different genetic mechanisms of tumorigenesis[13]. Due to considerable differences in underlying biology of the two major RMS subtypes, and the predominance of ERMS subtype, we decided to focus our proposal on unraveling the genetic predisposition to ERMS.

For some of the *syndromes predisposing to ERMS*, underlying genetic changes have been widely reported. For example, in BWS, 11p15 locus is frequently dysregulated[14]. In this region, both epigenetic alterations (H19 inactivation, biallelic expression of normally imprinted IGF2)[15], and genetic alterations (e.i, point mutations in CDKN1C, rearrangement of KCNQ1) have been described[16, 17]. However, specific genetic alterations of genes in this region have not been identified in sporadic ERMS, and whether BWS and ERMS share common genetic mutations is yet to be proven[18].

While genetic changes underlying rare familial syndromes that predispose to ERMS may play a role in ERMS predisposition, this concept remains to be proven. In addition, only a small proportion of ERMS are thought to arise through this mechanism, and it is not clear if the same variants would also play a role in ERMS that develops outside such familial context.

Thus, an unbiased, comprehensive genome-wide examination is a good approach to study ERMS predisposition and therefore improve understanding of genetic causes of ERMS.

Genome-wide association (GWA) studies and childhood cancer susceptibility. The proof of concept that genetic predisposition likely underlies a childhood malignancy comes from recent studies in leukemia[19, 20] and neuroblastoma[21, 22]. These authors used a GWA approach and identified germline genomic variants that are associated with these pediatric malignancies. These studies demonstrate that common genetic variants in specific genes, predominantly those associated with transcriptional regulation and differentiation, can increase cancer risk. These studies used previous generation of GWA chips, while we propose to use the new generation of chips with 10-fold increased coverage (5 million compared to previously available 500,000 SNPs). This latest technology assures reasonable coverage of genome-wide variation down to 1% of minor allele frequency (MAF). Thus, compared to these earlier studies examining childhood cancer susceptibility, our approach will have an opportunity to also examine rare variants (MAF ranging between 1% and 5%) and their association with ERMS risk. ***We therefore anticipate that our proposed project will fill in the gap in knowledge on genetic causes of ERMS.***

Early onset of ERMS suggests possible role of maternal genotype that may affect in utero environment. It has been well established that for certain phenotypes (e.g., birth defects, perinatal outcomes, pediatric cancers) the maternal genotype can directly contribute to risk, by affecting the *in utero* environment [23, 24]. Although the importance of maternal genetic effects has been largely recognized in genetic epidemiology studies [25-34], nearly all GWA studies (GWAS) to date have not studied maternal genetic effects[35]. It has been suggested that this failure to account for maternal genetic effects in previous GWAS studies could partially explain why GWAS have not identified the majority of genetic contribution to he diseases under study[23]. ***Given the high prevalence of birth defects among pediatric RMS patients and several perinatal outcomes associated with ERMS risk[36, 37], the maternal genotype is likely to play a role in susceptibility to ERMS.***

Our hypotheses that there may be genetic predisposition to a specific ERMS morphology associated with prognosis will likely lead to a number of specific benefits. We anticipate that we will identify susceptibility to a high risk disease, e.g., anaplastic ERMS, thereby identifying patients who should be treated more aggressively. Conversely, we anticipate to identify patients susceptible to less aggressive forms of ERMS (such as botryoid ERMS) who would benefit from a less aggressive treatment associated with fewer short and long-term treatment-related adverse effects. Finally, it is plausible that some of the variants we discover will be associated with treatment outcome, and, if proven, such knowledge would inform consideration of treatment options. For example, patients carrying germline *TP53* mutations (underlying LFS) are extremely sensitive to radiation treatment and current guideline recommend avoiding such treatment, if possible. Thus, we anticipate to identify variants associated with responsiveness to a particular therapeutic agent (a specific type of chemotherapy) or to radiation therapy. In addition, discovery of variants predisposing to ERMS overall, will open the way for future development of prevention strategies.

Innovation

Due to its rarity and diagnostic diversity, genetic predisposition to ERMS has not been studied to date. We are using the Childhood Cancer Research Network, a Children's Oncology Group pediatric cancer registry to identify a substantial number of ERMS cases, and controls selected among friends of cases, required to address ERMS etiology. The following features of our proposal are novel:

1) Our approach to use ERMS cases and controls available from public databases to discover variants associated with EMS predisposition, followed by case-parent triad design to account for population stratification by examining the identified candidate variants is novel. This approach will also determine which variants appear de novo, and which are inherited.
2) The use of case-parent triad design will enable us to evaluate maternal genetic effects. We are not aware of other GWA studies that evaluated these effects, thus this is highly innovative.
3) We are using the latest technology, Illumina Omnibead arrays, to discover genes underlying ERMS susceptibility. This technology for the first time allows studying of relatively rare variants (e.g., MAF = 1-5%) which will be further strengthened by deep sequencing, allowing us assess the contribution of rare variants to ERMS susceptibility.
4) Statistical methods for analyses of such complex design are not readily available. The methods used in GWA studies are designed to analyze common variants and are not suited for analysis of rare variants. Thus, our approach requires development of additional statistical methods.

In summary, our innovative approach will unravel genetic predisposition to ERMS and open the way to reduce the burden of this devastating malignancy affecting young children. The analysis methods developed for this proposal can be applied to other studies of genetic predisposition to cancer.