02-05

CHILDHOOD CANCER SURVIVOR STUDY

Analysis Concept Proposal

1. **Title: Cardiac Events among Survivors of Childhood and Adolescent Cancer – Role of gene-environment interactions**

2. **Working Group and Investigators:** This proposed study will be within the Chronic Disease Working Group. Proposed investigators (with email address and fax no.)will include:

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3. Background and Rationale:

3.1 *Congestive heart failure:* Anthracyclines have gained widespread use in the treatment of childhood leukemia and solid tumors. A rapidly growing number of survivors of childhood cancer are at risk of developing substantial morbidity and mortality because of anthracycline-related cardiac disease.[1-3] Clinically, the most important side effect of anthracycline chemotherapy is a dose-dependent cardiotoxicity. The incidence of congestive heart failure secondary to doxorubicin-induced cardiomyopathy depends on the cumulative dose of the drug. At total doses of less than 400 mg/m2 body surface area, the incidence of congestive heart failure is 0.14%; this increases to 7% at a dose of 550 mg/m2 and to 18% at a dose of 700 mg/m2. The rapid increase in clinical toxicity at doses greater than 550 mg/m^2 has made the 550-mg dose the popular empiric limiting dose for doxorubicin-induced cardiotoxicity. Mortality directly related to doxorubicin-induced cardiac failure is substantial; large series have reported rates of more than 20%. However, recent reports have suggested a better prognosis with up to 59% clinical recovery among patients with anthracycline-induced CHF. Although reports conflict, proposed risk factors for chronic anthracycline cardiotoxicity include higher rates of drug administration, higher cumulative doses, mediastinal irradiation, younger age, female sex, black race, pre-existing heart disease and hypertension.[4-7] An additional confounding factor in the identification of patients at high risk is the wide variation in individual sensitivity to anthracyclines. Doses in excess of 1000 mg/m2 can be well tolerated by some patients. In contrast, appreciable decreases in left ventricular ejection fraction have been documented at doses as low as 300 mg/m2. Progressive ventricular dysfunction after an initial myocardial insult probably underlies late-onset decompensation. Reductions in left ventricular mass, mass index, and compliance have been reported in anthracycline-treated survivors of childhood cancer for more than 7 years after completion of chemotherapy.[8-9] Dexrazoxane is the only cardioprotectant clinically approved for use against anthracyclines.

3.2 *Coronary artery disease (CAD):* Mediastinal irradiation for Hodgkin's disease at a dose of 40 to 45 Gy has been shown to increase the risk of subsequent death from coronary artery disease. The risk increases with high mediastinal doses, minimal protective cardiac blocking, young age at irradiation, and increasing duration of follow-up.[10-12] Therefore, the possibility of radiation-induced myocardial infarction (MI) needs to be taken into account both in treatment planning and follow-up of patients with Hodgkin's disease. However,

In the total group, the risk of fatal cardiac ischemic events and/or sudden unexpected death was significantly elevated with a relative risk of 4.2 for myocardial infarction and 6.7 for myocardial infarction or sudden death. However, among females, and among those without other cardiovascular risk factors, the risk of serious cardiac events after conventionally fractionated irradiation of the mediastinum with 30 to 40 Gy was low. However, this study is different from the previous reports that do report a higher incidence in two respects – I) the cohort size is relatively small, and ii) the patient population consists of adults at the time of treatment of their Hodgkin's disease, (young age having been shown to be risk factor for the development of CAD). However, this study does emphasize the need for examining other cardiovascular risk factors when describing the risk of CAD among survivors of Hodgkin's disease.

3.3 *Genetic Susceptibility*: Anthracycline-induced cardiotoxicity limits effective cancer chemotherapy by causing early cardiomyopathy, and it can produce late-onset ventricular dysfunction years after treatment has ceased. Conventional doses of anthracycline often lead to permanent myocardial damage and reduced functional reserve. Underlying pathogenic mechanisms may include free-radical-mediated myocyte damage, adrenergic dysfunction, intracellular calcium overload, and the release of cardiotoxic cytokines. Several genes have been putatively implicated in anthracycline-induced cardiotoxicity, including Carbonil reductases, metallothioneins, intracellular calcium-independent phospholipase A2 and IPLA2, cyclooxygenase 2 (PTGS2), endothelin converting enzyme (ECE1), hepcidin (HAMP), p-glycoprotein, etc. (See Table 1) The most interesting candidates for anthracycline-related cardiotoxicity are those related with some aspects of iron metabolism and oxidative damage to the heart, since the anthracycline-induced damage resembles that seen in iron overload syndromes or oxidative damage. We will therefore focus on metallothioneins, the carbonyl reductases and the hepcidin genes.

Table 1. Genes implicated in anthracycline-induced cardiotoxicity

Gene And official gene symbol	***Brief Description***	***Locus Link ID http://www.ncbi.nlm.nih.gov/LocusLink/***	***SNP's linked from Locus Link***	***PUB MED link (from Locus Link)***
Carbonil reductase Isoforms 1 and 3	Overexpression in mice enhances doxorubicin toxicity. 21q22.13 (isoform 1) 21.q22.2 (isoform 3) Down syndrome (interesting) ?	http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=873 (isoform 1) http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=874 (isoform 3)	http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=874 (isoform 3) http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=873 (isoform 1)	isoform 3 isoform 1
P-glycoprotein (MDR1)	Higher accumulation of dox and dox-ol in cardiac tissue of mdr1a -/- mice	http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=5243	http://www.pharmgkb.org/PharmGKB/query/search.jsp?CMD=Search&frame=PharmGKB_Production_v7_01819	http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=10716719
Metallothioneins Different functional genes and pseudogenes (same chromosomal band)	Storage of heavy metals. Antioxidant activity. Inhibited by doxo. Chromosomal band: 16q13	http://www.ncbi.nlm.nih.gov/LocusLink/list.i		metallothioneins

Hepcidin HAMP	Iron storage (involved in a specific signaling pathway?). Iron overload (cardiac) during doxorubicin ? Apoptosis (cardiomyocites)?	HAMP	locusId=57817	HAMP

Multiple polymorphisms have been associated with the risk of MI, with most associations more prominent in certain subsets of patients. None have been studied in cancer survivors. We will focus on polymorphisms that are relatively common and have been associated with the risk of early onset MI (Table 2).

Table 2. Genetic polymorphisms associated with risk of MI

Gene and official gene symbol	***Brief Description***	***Locus Link ID http://www.ncbi.nlm.nih.gov/LocusLink/***	***SNP's associated with MI risk***	***Reference***
MTHFR	Methylenetetrahydrofolate reductase	http://www.ncbi.nlm.nih.gov/locuslink/LocRpt.cgi?l=4524	C677T A1298C	{11753,11761,11799,11886}
ITGB3	Platelet glycoprotein IIIa—PIA2 allele	http://www.ncbi.nlm.nih.gov/locuslink/LocRpt.cgi?l=3690	PIA2 allele	{11759}
ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor) or glycoprotien Ia	http://www.ncbi.nlm.nih.gov/locuslink/LocRpt.cgi?l=3673	C807T	{11760, 11775,11796}
NOS3	nitric oxide synthase 3 (endothelial cell)	http://www.ncbi.nlm.nih.gov/locuslink/LocRpt.cgi?l=4846	393 allele	{11768}
F7	Factor VII F7: coagulation factor VII (serum prothrombin conversion accelerator)	http://www.ncbi.nlm.nih.gov/locuslink/LocRpt.cgi?l=2155	Promoter A1 and A2 alleles	{11780,11782}
THPO	Thrombopoietin	http://www.ncbi.nlm.nih.gov/locuslink/LocRpt.cgi?l=7066	4830 A and 5713A	{11839}
CCR5	Chemokine receptor	http://www.ncbi.nlm.nih.gov/locuslink/LocRpt.cgi?l=1234	CCR5 and early onset	{11912}

3.4 *Present Study:* In the proposed study, we plan on utilizing the banked DNA from patients with congestive heart failure, or myocardial infarction and from matched controls with no evidence of CHF or MI. The aim is to compare the frequencies of polymorphisms in candidate gene(s) among patients who develop clinically overt heart failure or myocardial infarction (cases) and those who don't (controls). The clinical

characteristics of the 91 patients with banked DNA samples (75 with CHF and 16 with MI) are shown in Table 3.

4. Specific Aims/Objectives/Research Hypothesis

4.1 Hypothesis:

1. Patients who develop anthracycline-associated congestive heart failure or radiation-associated myocardial infarction may have a genetic susceptibility to do so.
2. An interaction between genetic susceptibility and environment (anthracycline or radiation exposure) plays a role in the development of these cardiac events.

4.2 *Specific Aims of Research:* We propose to determine the frequencies of polymorphisms in candidate genes among patients with anthracycline-associated CHF or radiation-associated myocardial infarction and among survivors of childhood cancer with no evidence of CHF or MI, in order to explore gene-environment interactions.

The specific aims are:

Aim 1: To characterize each patient with CHF or MI with respect to the nature of the primary malignancy (pathology, stage), demographic characteristics (race, age, gender), therapeutic exposures (including modality and dose, with particular emphasis on agents known or suspected to be cardiotoxic).

Aim 2: To use the above information to determine the associated risk factors in survivors of childhood cancer who are registered and treated on specific COG protocols.

Aim 3: To utilize the banked constitutional DNA to support directed molecular studies of these patients. These samples will be valuable for studies aimed at the identification of mutations in candidate genes or investigation of new susceptibility genes, and gene-environment interactions.

Aim 4: To explore the nature of gene-environment interaction in the development of CHF or MI.

5. Analysis Framework:

5.1 Study design: Case-control study design will be used. Polymorphisms of the two groups of CCSS subjects will be compared.

5.2 Outcome of interest: All CCSS subjects with documented congestive heart failure or myocardial infarction *AND* banked DNA samples. (See Table 3)

5.3 Subject Population: all CCSS cases (whose cardiac status and vital status is known)

5.4 Control selection: A case-control study design will be used. For each case with CHF, several controls (maximum 4) will be randomly sampled without replacement from the "risk set" of the case. The risk set of each case will consist of all CCSS subjects who do not report CHF and have been followed for the same length of time as the cases' "failure time" (time from first administration of anthracyclines/ radiation/ cyclophosphamide/ ifosfamide to congestive heart failure) of the case. Controls will be matched by race, primary diagnosis, cumulative anthracycline exposure, radiation to the mediastinum, and time at risk from administration of cardiotoxic agent(s).

Table 3. Clinical characteristics of patients with cardiac events in the CCSS cohort		
Variable	**Congestive Heart Failure (n=75)**	**Myocardial Infarction (n=16)**
Gender		
Males (n=51)	43 (57%)	11 (69%)
Females (n=40)	32 (43%)	5 (31%)
Diagnosis		
Acute lymphoblastic leukemia (n=12)	10 (13%)	2 (13%)
Acute myeloid leukemia (n=4)	3 (4%)	1 (6%)
Brain tumor (n=3)	1 (1%)	0 (0%)
Osteosarcoma (n=9)	9 (12%)	0 (0%)
Rhabdomyosarcoma (n=6)	5 (7%)	0 (0%)
Ewing's sarcoma (n=5)	4 (5%)	1 (6%)
sarcoma (n=5)	5 (7%)	1 (6%)
Wilms (n=3)	3 (4%)	2 (13%)
Neuroblastoma (n=6)	4 (5%)	3 (19%)
Hodgkin's disease (n=30)	24 (32%)	5 (31%)
Non-Hodgkin's lymphoma (n=8)	7 (7%)	1 (6%)
Radiation		
No (n=26)	20 (27%)	3 (19%)
Yes (n=59)	50 (67%)	11 (69%)
Missing (n=6)	5 (6%)	2 (13%)
Radiation to the heart		
Don't know (n=6)	6 (8%)	0 (0%)
Maybe (n=3)	2 (3%)	1 (6%)
Missing (n=6)	5 (7%)	2 (13%)
No (n=47)	38 (51%)	6 (38%)
Yes (n=29)	24 (32%)	7 (44%)
Anthracycline exposure (39 to 1395 mg/m^2)		
Yes (n=51)	48	6
Cumulative dose of anthracyclines (mg/m^2)	358 (53-1395)	230 (71-480)

5.5 Explanatory variables: Sex, age at diagnosis, age at follow-up, recurrence, type of treatment (other than anthracycline exposures), dose of radiation to the heart, smoking, educational attainment, family history of heart disease, body mass index, and presence of mutations in the candidate genes.

5.6 Specific tables (Table 4, 5):

Table 4. Description of patients with CHF and controls

Variable	CHF cases	Controls	p-value
Total			
Males			
Age at dx Mean (range)			
Age at study Mean (range)			
Primary Diagnosis			
Race			
Smoking status			
Educational attainment			
Recurrence			
Type of treatment Chemotherapy only Radiation only Surgery only Chemotherapy + surgery Chemotherapy + radiation Radiation + chemotherapy Chemotherapy + radiation + surgery			
Dose of radiation to the Mantle region 1-999 cGy 1000-2499 cGy 2500-3499 cGy 3500-4499 cGy > 4500 cGy			
Use of each chemotherapy drug (yes/no)			
Dose of each chemotherapy agent			

Table 5. Genotype of candidate gene(s) and risk of developing CHF or MI: Multivariate analysis

Variable	Controls n (%)	CHF Cases		
		n (%)	OR	95% CI
Age at Dx				
Sex				
Radiation dose to Mantle region				
Treatment Modality				
Genotype of candidate gene				
Smoking				
Family History of Heart Disease				
Recurrence				

E.2 *Statistical analysis:* Odds Ratio (OR) and 95% confidence intervals (CIs), will be estimated by using conditional logistic regression. The candidate gene(s) will be analyzed as dichotamous or trichotamous variables. The multivariate analysis will include age at diagnosis, sex, treatment modality, radiation dose,

dose of chemotherapy agents (other than anthracyclines), smoking history, Family history of heart disease, recurrence of primary disease, and the genotype of the candidate gene in the model.

Sample Size: Banked DNA is available for 91 CCSS subjects with CHF (n=75) or MI (n=16). We will match each case with as many as 4 controls (matching criteria described in section 5.4). Thus there will be DNA available from approximately 455 CCSS subjects for this study.

6. Literature Cited

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