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CCSS ANALYSIS CONCEPT FORM

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STUDY TITLE: Genetic susceptibility genes and risk of obesity among survivors of childhood ALL

WORKING GROUP AND INVESTIGATORS:

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BACKGROUND AND RATIONALE:

Obesity has been identified as a potential late effect of therapy in survivors of childhood acute lymphoblastic leukemia (ALL) (1-3). Oeffinger et al (2002; submitted) explored whether adult survivors of childhood ALL were at an increased risk for obesity, and whether patient or treatment characteristics modified risk in the Childhood Cancer Survivor Study (CCSS). A total of 1,765 survivors of childhood ALL were compared with 2,565 adult siblings of childhood cancer survivors as well as participants in the National Health Interview Study. Using body mass index (BMI) to determine the prevalence of overweight individuals, the authors found that the age- and race adjusted odds ratio (OR) for obesity in survivors treated with cranial radiation therapy (CRT) ≥20 Gy in comparison with siblings was 2.59 for females (95% CI=1.88, 3.55) and 1.86 for males (95% CI=1.33, 2.57). Interestingly, the risk associated with CRT ≥20 Gy was modified by age at diagnosis and gender; females treated at ages 0-4 experienced the highest risk of BMI ≥ 30 and BMI 25.0-29.0 (OR 3.81; 95% CI=2.34, 5.99 and OR=3.19; 95% CI=2.07-4.82; respectively). There was no association found between obesity and ALL survivors who were treated with chemotherapy only or with chemotherapy and radiation less than 20 Gy. These data suggest that radiation therapy ≥20 Gy and female age at treatment are important effect modifiers in the association between childhood ALL and obesity, although the underlying biological relationships remain unknown.

Several possible candidate genes could be explored including polymorphisms in the leptin receptor gene. In mouse models, single nucleotide mutations (polymorphisms) of the leptin gene (results in a truncated protein) or in the leptin receptor (results in a termination of the signal) are linked with morbid obesity, and are also found in some humans (4). Interestingly, leptin levels increase with increasing fat mass in humans, which might suggest that obesity is a leptin-resistant state. Yiannakouris et al (5) explored the frequencies of 3 separate leptin receptor polymorphisms (K109R, Q223R, and K656N) in 118 genetically-homogenous individuals (including 89 normal weight, and 29 overweight/obese). Neither the

polymorphism at K109R or K656N was associated with obesity. However, for the Q223R polymorphism, there was a higher prevalence of the R223 alleles (homozygous form) among the overweight/obese subjects compared to the normal weight individuals (20.7% vs 4.5%, p=0.01). Further, the R223 allele was a significant predictor of both BMI (p=0.015) and percent fat mass (p=0.02) even after adjusting for age and gender. Since the Q223R polymorphism results in a change in charge (glutamine to arginine at codon 223), it is likely to have functional significance.

Another potential obesity candidate gene is the uncoupling protein 3 (UCP3) gene. A genetic polymorphism at codon 55 (C◊T) has been identified and is associated with BMI. In a study of 401 morbidly obese individuals and 231 control subjects, Otabe et al (6) reported that the homozygous T variant was a statistically signficiant risk factor for a higher BMI among obese subjects (p=0.004). Further, for normal weight subjects, individuals with the TT genotype also had a higher BMI (25.5) versus 22.6 and 22.7 for CC and CT, respectively, p=0.09. Finally, G-proteins comprise a family of ubiquitous signal-transducing proteins, and interest has focused on the β-3 subunit in a number of diseases as it preferentially links to the G-protein of inhibition (7). A common polymorphism in the gene (825C◊T) causes a truncated variant that is associated with an increased activity of specific G-proteins. Some studies have shown an increased body mass associated with the T-variant, as well as an increased risk of hypertension and blood pressure (reviewed in 7).

While genes associated with obesity may be important in understanding the risk of obesity among childhood ALL survivors, other potential candidate genes can also be explored. These include genes involved in DNA repair such as XRCC1, XPD, and XRCC3. For example, the XRCC1 gene is involved in the repair of DNA single strand breaks generated in response to either ionizing radiation or alkylating agents (8). The human XRCC1 gene is polymorphic in humans with single nucleotide polymorphisms occurring in at least 3 sites. Data suggest that this polymorphism is unlikely to be associated with complete loss of protein function, but that reduction in function may importantly influence ability to tolerate potentially carcinogenic DNA damage.

SPECIFIC AIMS:

- Determine whether polymorphisms in genetic susceptibility genes previously associated with obesity, including the leptin receptor gene (Q223 (R-allele)), the UCP3 gene (T allele), and the G Protein β-3 subunit (T-allele), are associated with an increased risk of obesity in children with ALL.

- Determine whether polymorphisms in DNA repair genes including XRCC1, XPD, and XRCC3 are associated with an increased risk of obesity in children with ALL.

- Explore whether the dose of radiation treatment modifies the association between the above genetic polymorphisms and risk of obesity in children with ALL.

ANALYSIS FRAMEWORK/METHODS:

Buccal cell collection. Buccal cells represent a unique source of genetic material and are being utilized in a number of epidemiologic studies. Buccal cell collection from CCSS

participants began in May 1999 using the Molecular Genetics Bank at the University of Minnesota. To date, viable DNA samples have been collected on 4,412 CCSS cases (including 711 ALL survivors) and 666 sibling controls. The following buccal cell samples for ALL patients (by treatment and BMI status) in the CCSS are available:

	BMI STATUS (kg/m^2)					
Treatment	**< 18.5**	**18.5-24.9**	**25.0-29.9**	**30.0-34.9**	**35.0-39.9**	**>= 40.0**
Chemo only	9	106	36	18	6	3
10-19.9 Gy	9	86	54	17	5	3
> 20 Gy	7	166	102	58	17	9
Total	**25** 27	**358** 376	**192** 196	**93** 98	**28** 29	**15** 16

B) Genotyping. We have considerable experience in genotyping for the selected polymorphisms of interest. Below we demonstrate genotypes obtained from population control samples for the each of the three obesity genes of interest (UCP3, Q233R, and G protein β-3 subunit). Dr. Davies laboratory has previously demonstrated the capacity to examine polymorphisms in the DNA repair genes (9).

UCP-3

W M M W W/M W/M M

Q233R

W/M W M M M W/M W

G-Protein

W/M W/M W W W M

For all, W indicates the more frequently found (wild-type) variant, while M represents the less commonly found (mutant) variant.

	Odds Ratio				
Prevalence of Polymorphism in controls	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**
.05	0.12	0.35	0.60	0.78	0.90
.10	0.21	0.58	0.85	0.96	0.99
.15	0.28	0.72	0.93	0.99	1.00
.20	0.34	0.80	0.97		
.25	0.38	0.84	0.98		
.30	0.41	0.87	0.99		
.35	0.43	0.88	0.99		
.40	0.44	0.89	0.99		
.45	0.44	0.89	0.99		
.50	0.44	0.88	0.99		

OUTCOMES OF INTEREST. With obesity defined as BMI ≥30, we will initially compare the genotype frequencies of patients with BMI ≥ 30 to two patient controls with BMI < 25.0 (frequency-matched by age and gender). Our power to evaluate statistically significant differences between 136 obese patients and 272 non-obese patients is shown above. For example, if the frequency of the R variant allele (in Q233R polymorphism) is 5% in non-

obese patients, we will have sufficient statistical power to detect odds ratios of approximately 3.0 or greater using a two-sided test (α=0.05). Moreover, if the frequency of the T variant allele in the UCP3 gene in patient controls is 21%, we will have sufficient statistical power to detect odds ratios of approximately 2.0 or greater. We will also explore whether the dose of radiation treatment modifies the association between the above genetic polymorphisms and risk of obesity in children with ALL, recognizing that our power will be diminished for these stratified analyses.

SUBJECT POPULATION TO BE INCLUDED: ALL patients with an available buccal cell sample (matched sets only). The laboratory analyses will require approximately 5μg of DNA from each patient.

EXPLORATORY VARIABLES: Sex, age, radiation exposure

TIMELINE: We expect that the laboratory aspect of this project will take approximately 6 months to complete. Analysis, write-up of results, and submission of a manuscript would follow.

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