EPIDEMIOLOGY OF BURKITT LYMPHOMA IN EAST AFRICA CHILDREN OR MINORS

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Outline
- Definition of BL
- Etiology of BL
- EMBLEM scientific objectives
- Achievements to date
- Opportunities for collaboration

Burkitt lymphoma

- B cell lymphoma
- Clinically aggressive
- Affects mostly children in Africa
  - Jaw
  - Orbit
  - abdomen

Etiology of Burkitt lymphoma

- Plasmodium falciparum malaria
- Genetic translocations and mutations
  - MYC/IgH
  - TP53, BIM, BCL2
- Viruses
  - EBV
  - HIV
- EBV & Malaria not shown to cause translocation

Pathogenesis of BL involves
- Genetic instability, infection & geographic factors
- BL valuable model to study relationship between (EBV, malaria, HIV)

Etiology of BL

- MYC/IgH translocations & variants
- Epstein-Barr virus (EBV) discovered in BL cells
IARC Monograph 104

P. falciparum malaria is "probably carcinogenic to humans" (Group 2A)

- Ecological studies individual level data lacking, subject to ecological fallacy
- Direct evidence from individual level studies difficult to adduce
- Difficult to measure life time exposure to malaria individuals
  - Carpenter et al (2008), OR 5, antibodies in cases than controls
  - Mulatima et al (2008), OR 5, , antibodies in cases than controls
- Both studies suffered from
  - Lack of hostopathological confirmation, cases not geographically matched, reverse causality bias

**GENETIC RESISTANCE TO MALARIA & RISK FOR BL**

- Genetic studies using sickle cell trait
  - Williams et al, 1966 [OR 0.52, p<0.05], based on 95 cases & 313 controls
    - Hospital based, clinical malaria may be over represented
  - Pike et al, 1969, [OR 0.8, NS], based 36 cases, 36 controls
  - Nkrumah et al., 1978, [OR 1.3, NS], 110 cases, 112 controls

**BL and Malaria in endemic BL**

<table>
<thead>
<tr>
<th>EBV</th>
<th>Malaria</th>
<th>Cases/Controls</th>
<th>aOR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low</td>
<td>12/17</td>
<td>1.0</td>
<td>0.5-2.4</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>15/16</td>
<td>1.1</td>
<td>0.5-2.4</td>
</tr>
<tr>
<td>Medium/high</td>
<td>Low</td>
<td>22/19</td>
<td>1.0</td>
<td>0.5-2.2</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>77/18</td>
<td>5.0</td>
<td>2.8-8.9</td>
</tr>
</tbody>
</table>

*P interaction=0.04*

Carpenter et al. JIC 2008

**Antibodies to Whole schizont extract**

- Marker of exposure but not malaria immunity
  - Provides information about importance of exposure
  - Provides no information about importance of immunity
- Subject to reverse causation

**Malaria exposure, immunity and BL**

- BL risk may arise from
  - Intense exposure leading to more malaria
  - Abnormal immunity leading to more chronic infection
- Exposure may be measured by
  - Questionnaire data
  - Antibody markers of exposure
- Abnormal immunity may be measured using
  - Genetic markers of malaria resistance
  - Markers of humoral immunity

Specific objectives for BL

- Role of:
  - Humoral immunity in BL
    - Multi-clonal infections
    - Abnormal humoral responses
  - Malaria resistance genes (25 genes; 42 SNPs)
  - EBV genetic variants
  - Host genetic variants

Study countries

Study Design

<table>
<thead>
<tr>
<th>1500 Hospital-based cases</th>
<th>3000 Matched Population Controls</th>
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<tbody>
<tr>
<td>Specimens</td>
<td></td>
</tr>
<tr>
<td>1. Blood</td>
<td></td>
</tr>
<tr>
<td>2. Saliva</td>
<td></td>
</tr>
<tr>
<td>3. Stool</td>
<td></td>
</tr>
<tr>
<td>4. Tissue</td>
<td></td>
</tr>
<tr>
<td>Malaria resistance genotypes</td>
<td></td>
</tr>
<tr>
<td>EBV Variants</td>
<td></td>
</tr>
<tr>
<td>Genome-wide association studies</td>
<td></td>
</tr>
<tr>
<td>Questionnaire data</td>
<td></td>
</tr>
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</table>

Case definition

<table>
<thead>
<tr>
<th></th>
<th>Eligible</th>
<th>Non-Eligible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0–16 years</td>
<td>Greater than 16 years</td>
</tr>
<tr>
<td>Geography</td>
<td>Pre-defined geographic area for at least 4 months</td>
<td>Outside pre-defined geographic area for 4 months (except for children &lt; 4 months)</td>
</tr>
<tr>
<td>Previous BL Treatment</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinically stable</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>BL diagnosis</td>
<td>New diagnosis</td>
<td>Previous diagnosis of BL more than 1 month</td>
</tr>
</tbody>
</table>
Control definition

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
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<td>0 - &lt; 16 years</td>
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<td>Geography</td>
<td>Pre-defined geographic area for at least 4 months</td>
<td>Outside pre-defined geographic area for 4 months (except for children &lt; 4 months)</td>
</tr>
<tr>
<td>BL diagnosis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Illness</td>
<td>No</td>
<td>Yes</td>
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</tbody>
</table>

Study hypothesis

- CARRIAGE OF ONE OR MORE MALARIA RESISTANCE GENES IS PROTECTIVE TO BL
- This will be tested by studying:
  - 1500 children with BL matched to 3000 age, sex, district HC II controls in EA
  - Using malaria resistance genes as markers of exposure to malaria

Public health significance of the study

- BL is the most common childhood cancer in equatorial Africa
- Malaria is the most spread childhood treatable and preventable exposure
- Confirming the association will:
  - Suggest novel ways to prevent and treat BL
- The use of multiplex genomic methods offers rare opportunity to:
  - Gain new insights in gene-environmental interactions that influence the geographic distribution of BL

Choice of controls

- We enroll three types of controls
  - Matched 2 per case enrolled from 100 randomly selected villages
  - Unmatched Pilot population enrolled from 12 selected villages
  - HC II pilot controls enrolled from children coming from survey villages
- The matched controls enrolled from randomly selected villages were cases arise
- Controls will be frequency matched to sex, age of cases from the general area
- Randomly select 100 parishes, stratify wet/dry, rural/urban
- List all villages in that parish, select one village
- Construct village household list, select households, screen children for eligibility
- Enroll eligible children according to pre-determined criteria

Analysis & use of unmatched population vs HC pilot controls

- 180 HC II controls without clinical malaria vs 600 Population control
- Assuming that the prevalence of mild clinical malaria is 50% in HC II and 10 in population
- This comparison will have the power to detect the postulated minimum deviations of key exposures between the two groups
- If distribution of key exposures among HC II controls without mild clinical malaria is similar, controls will be enrolled from HC II as planned
- If distribution of key exposures among all HC II controls and population controls are similar we will drop mild clinical malaria as selection criteria for controls
- Correlation of children from the same households will be controlled for

Choice of controls

- Unmatched pilot population controls
  - Will be selected from 12 (of the 100) villages
  - They will be compared to children from the survey villages enrolled from the HC II
    - The 12 villages will be stratified into 4/urban, 8/rural, 6/wet, 6/dry
- HC II pilot controls
  - These will be enrolled from children coming from the survey villages
  - 30 children will be enrolled from each village
  - The distribution of age, sex, will be matched to that of BL cases seen in the region
  - The enrollment will follow a pre-determined criteria
Primary outcome analysis

- Test association of BL & malaria resistance genes among 600 cases & 1500 controls
- Genotypes will be coded using and additive models
- In additive models genotypes will be coded as the number of variant alleles (0, 1 or 2)
- First fit single SNP models
- Second test for gene level effects that account for linkage equilibrium
- Fit haplotypes using single window approach, window size of 3-5 SNPs
- Finally use pathway-based approach for specific malaria biologic mechanism of malaria association with BL
- Pearson Ch-square to test for associations
- Unconditional logistic regression to estimate odds ration & 95% CI

Modeling of malaria-resistance genes, questionnaires & antibody variables

- We will fit multivariable logistic models to determine relative contribution of each variable to the risk of BL
- Compare full vs nested models
- If the malaria questionnaire, or antibody variables capture the full risk by malaria
  - There will be no residual effects of malaria resistance genotypes

Study power

- The study has > 80% power to detect a 50% heterozygosis in malaria resistance genes among 600 vs 1500 controls
- Power calculations assumes:
  - That children are uniformly exposed to malaria year round
  - Malaria risk is modified by carriage of SNPs
- The study can detect a minimum odds ratio of 1.4 of association with other malaria exposures assuming 25% prevalence of exposure
- Calculation based on additive models 600 cases 1500 controls, two sided

Concern about population stratification

- Few studies have examined genetic associations in populations in East Africa
- The extent of population stratification for this study is unknown
- The study will assess potential effects of population stratification
  - By Examining the prevalence of malaria –resistance-alleles in sub-groups age, tribe, region
  - Assess genetic relatedness & population stratification using markers of ancestry haplotype markers from the Yoruba
- These markers of heredity are not linked to malaria resistance genes therefore:
  - Will enable the study to assess for false positive associations due to stratifications

LIMITATION OF THE STUDY

- Biases: Recall and reverse causality
  - To minimize biases;
    - Measure malaria-resistance-genetic polymorphism
  - Supplement genetic measurements with;
    - Questionnaire and antibodies measurements
    - Focus on EBV diversity instead of EBV load
  - Second limitation is selection of controls;
    - Select population controls from 5% of villages
    - Use their distribution to correct, for differences in risk distribution in HC LL controls

Benefits of the study

- Individual
  - Timely high quality CBC, Malaria BS, stool microscopy, treat Malaria and parasites
  - One time donation of ITN, health education on malaria prevention
  - Indirect knowledge about diseases that are common in Uganda, BL, malaria
  - HIV testing in line with local laws & policy
- Research community
  - Training of research community, training East African and International collaborating scientists in virology, epidemiology and Biostatistics
  - Results might yield discoveries – diagnostics and
**ETHICAL CONSIDERATIONS**

- Formal ethical review & approval from IRB National Cancer Institute – USA
- Reviewed & approved by IRBs participating hospitals, Uganda, Kenya, TZ
- IRBs in Uganda, Kenya, Tanzania & USA revisit the study annually
- Study procedures are reviewed with all participants before enrollment
- Participants provide informed signed consent indicating understanding of procedures, risks & benefits
- Participants free to withdraw from the study
- All study procedures conducted using (SOPs) to maximize safety ensure scientific integrity of results
- Data stored without patients identifiers

**Case accrual**

<table>
<thead>
<tr>
<th>Country</th>
<th>Uganda</th>
<th>Kenya</th>
<th>Tanzania</th>
<th>Total</th>
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<tbody>
<tr>
<td>Male</td>
<td>157</td>
<td>50</td>
<td>92</td>
<td>483</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>36</td>
<td>70</td>
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<table>
<thead>
<tr>
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<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Screened</td>
<td>73</td>
<td>37</td>
<td>13</td>
<td>5</td>
<td>12</td>
<td>7</td>
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<th>Female</th>
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<th>Female</th>
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<tbody>
<tr>
<td>Eligible</td>
<td>69</td>
<td>32</td>
<td>10</td>
<td>4</td>
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<table>
<thead>
<tr>
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<th>Female</th>
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<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Enrolled</td>
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<td>32</td>
<td>10</td>
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<th>Female</th>
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<tbody>
<tr>
<td>Total</td>
<td>299</td>
<td>147</td>
<td>73</td>
<td>45</td>
<td>108</td>
<td>81</td>
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**Control Accrual**

<table>
<thead>
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**Samples**

<table>
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<tr>
<th>Country</th>
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<tbody>
<tr>
<td>Plasma</td>
<td>2275</td>
<td>56</td>
<td>32</td>
<td>2362</td>
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<tr>
<td>Buffy coat</td>
<td>1479</td>
<td>28</td>
<td>16</td>
<td>1523</td>
</tr>
<tr>
<td>RBCs</td>
<td>1374</td>
<td>28</td>
<td>13</td>
<td>1415</td>
</tr>
<tr>
<td>Saliva</td>
<td>2424</td>
<td>44</td>
<td>31</td>
<td>2499</td>
</tr>
<tr>
<td>Tissue</td>
<td>66</td>
<td>14</td>
<td>14</td>
<td>94</td>
</tr>
<tr>
<td>Slides</td>
<td>57</td>
<td>13</td>
<td>8</td>
<td>78</td>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>Uganda</th>
<th>Kenya</th>
<th>Tanzania</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>7675</td>
<td>183</td>
<td>114</td>
<td>7972</td>
</tr>
</tbody>
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**OPPORTUNITIES FOR COLLABORATION**

- Sharing of GWAS data

**APPRECIATION TO**

- AFENET
- St. Mary’s Hospital Lacor
- Kuluva Hospital
- Webuye District Hospital
- SHED Foundation/Bugando Medical centre
- INCTR
- OHIO University/AIDS cancer specimen resource