Title: Anticipation in familial hematological malignancies

Shortened running title: Familial hematological malignancies

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ABSTRACT
We describe a collection of eleven families with at least 2 generations of family members who have been diagnosed with a hematological malignancy (HM). In nine of these families there was a significant decrease in age at diagnosis in each subsequent generation (anticipation). The mean age at diagnosis in the first generation was 67.8 years, 57.1 years in the second and 41.8 years in the third (p < 0.0002). This was confirmed in both direct parent-offspring pairs with a mean reduction of 19 years in the age at diagnosis (p = 0.0087) and when the analysis was repeated only including cases of mature B cell neoplasm (p = 0.0007). We believe that these families provide further insight into the nature of the underlying genetic mechanism of predisposition in these families.

INTRODUCTION
Studies have established that a family history of hematological malignancy (HM) is a risk factor for the development of a HM\textsuperscript{1-5}. However in the literature collections of large families with multiple cases of varied types of HMs are relatively rare\textsuperscript{1} and that most studies of familial HM focus on one major subtype in each family such as chronic lymphocytic leukemia (CLL), myeloma, acute myeloid leukemia, and Hodgkin lymphoma\textsuperscript{4,6-8}. In a number of HM families, the phenomenon of ‘anticipation’ has been described\textsuperscript{4}. Anticipation is defined as the onset of the disease in question at an earlier age or disease severity in successive generations, and has implications for the nature of the genetic condition that underpins it.

We are studying 13 families, ascertained from a population-based study conducted between 1972-1980 in Tasmania\textsuperscript{9}. Herein we describe 11 of these families that have at least two generations affected with a HM. These families have been updated and the current and past generations included and cross-referenced with names from the Tasmanian Cancer Registry (TCR) and the genealogical databases of the Menzies Research Institute. These families have been recently published\textsuperscript{10}, but are continually being updated with new cases of HMs. We reviewed our families to determine whether anticipation could be shown. Here we describe nine HM families that exhibit anticipation. In contrast to most prior reports, we show that the phenomenon also occurs in families where the predominant HM is other than CLL. The demonstration of anticipation is shown both by whole generational analysis and by assessment of parent-offspring pairs.

MATERIAL AND METHODS
Families From The Original Study
The families from the original study were reviewed and prioritised for further study, based upon the number of cases affected, multiple generations affected, or sibling pairs affected. This study has received ethical approval from the Human Research Ethics Committee, Tasmania Network.

Confirmation Of Diagnosis
Medical and pathology records from the Royal Hobart Hospital (RHH), flow cytometry records from the University of Tasmania’s Oncology and Immunology Laboratory, files from the 1970’s study\textsuperscript{9} and records of the TCR were obtained and pathology samples located and reviewed by a hematologist (EMT) to confirm the type of HM.
Statistical Analysis
Student T-tests were generated with Microsoft excel. Pedigrees were drawn with Smart Draw. Anticipation was assessed with a logrank test for trend using GraphPad Prism software version 5.0.

RESULTS AND DISCUSSION
In these 11 families there were 123 people affected with a HM, and an overall mean of 3 generations (2-5 generations) affected. The male to female ratio was 91 to 32. Reconfirmation of diagnosis was sought in all cases. A total of 98 cases could be reconfirmed and classified according to the current World Health Organization classification of HMs. The 25 that could not be reconfirmed consisted of 4 diagnosed not in Tasmania and 21 who were in the original study, but whose pathology records are now no longer available. However for these 25 cases the age at diagnosis and the type of HM was recorded in the original study, and are thus these are referred to as confirmed cases.

The mean age at diagnosis was 56.5 years (2-88 years). Figure 1a shows the age at diagnosis for the first three generations for the 11 families. We have restricted our analysis of anticipation to the first 3 generations because of limited follow up time in the fourth and fifth generations. However, there is evidence to support anticipation in these generations (test for trend across all five generations p<0.0001), noting that the fifth generation consists of only 3 cases in one family. The mean age at diagnosis for the first generation in these families was 67.8 years compared to 57.1 years for the second generation (p=0.0437) compared to 41.8 years for the third generation (p=0.0094). There were 5 direct affected parent-offspring pairings, the pedigrees of these families are shown in Figure S1. The mean age of the parents was 63.8 years compared to 44.8 years in the offspring (p = 0.0087). This is a mean reduction in the age at diagnosis by 19 years. The types of HM found in each generation of these families are listed in Table 1. The analysis of the age at diagnosis was repeated for all cases of mature B cell neoplasms (including CLL) (Figure 1b) (therefore addressing the argument of young onset diseases only occurring in the younger generations) and there was still a significant decrease in the age at diagnosis for each subsequent generation (p=0.0007).

The phenomenon of ‘anticipation’ has been described in relation to a number of familial diseases including HM. In the literature anticipation has been reported in CLL families with a reduction in the age at diagnosis by 15 years. It has also been observed in AML families with the mean age at diagnosis in the first generation being 57 years, second generation 32 years and 13 years for the third generation. Anticipation has also been observed in lymphoma families, families with plasma cell dyscrasia and for Hodgkin lymphoma. In fact over 140 families with HMs have been documented in the literature that show anticipation, although this phenomenon is not universally observed. It is often argued that this phenomenon is due to selection bias, however if this was the case, then it would be present in all families. It is also argued that the youngest generation is not old enough to have developed a HM in their 6th decade, but in the 11 families no one in the first generation developed a HM before the age of 35 years. When this analysis was repeated with just one type of HM, mature B cell neoplasm (n=60), anticipation was still present. It was interesting to note that the two families that did not show a consistent decrease in the age at diagnosis for each generation were families that were studied because of a cluster of affected siblings whose parents were first cousins. It is well recognised that children of consanguineous relationships are at an increased risk of recessive disorders.
In the other nine families there was a reduction in the age at diagnosis for each subsequent generation. This was confirmed in direct parent-offspring affected cases with a mean reduction of 19 years in the age at diagnosis between the generations. Previous reports have shown that anticipation in familial HM has been seen across 3 generations, we now show affected individuals with anticipation across five generations in one family and across four generations in an additional 2 families. This is consistent with a dominantly acting mutation in nine families and a recessively acting mutation in two families.

Anticipation is reported to be consistent with a dominantly acting mutation, however the causative mutation in HMs has not been identified. In other disorders where anticipation has been reported, it has been found to be due to an unstable dynamic mutation that expands with each generation. This type of mutation has been implicated in over 40 diseases. Triplet repeats have been studied in familial CLL, however no causative repeat was identified.

It is clearly recognised in the literature that there is a genetic basis for the familial risk of HMs, however genome wide association studies of CLL, childhood ALL and NHL have identified genetic variants accounting for only a small percentage of the heritable risk. We believe that further study of these families will provide valuable insights into the genetic predisposition underlying familial HMs.

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Authorship contribution:
EMT wrote the manuscript, confirmed the pathology and reviewed all medical records. RJT and JMS provided statistical support. AB updated the pedigrees. KAM provided haematology input and assisted with the manuscript preparation. RML provided clinical input and assisted with the manuscript preparation. SJF and assisted with the manuscript preparation and JLD assisted with the manuscript preparation.

Conflict of interest:
The authors declare they have no conflict of interest.
REFERENCES:


**Table 1: Distribution of HMs in each generation.**

<table>
<thead>
<tr>
<th>Neoplasm Type</th>
<th>Generation 1</th>
<th>Generation 2</th>
<th>Generation 3</th>
<th>Generation 4</th>
<th>Generation 5</th>
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<td>2</td>
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<td>0</td>
<td>0</td>
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<td>1</td>
<td>0</td>
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</tr>
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</tr>
<tr>
<td>Acute Lymphoid Leukemia</td>
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<td>2</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Mature B cell Neoplasms*</td>
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<td>28</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Mature T and NK cell Neoplasms</td>
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<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hodgkin Lymphoma</td>
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<td>0</td>
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<td>1</td>
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<tr>
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<td><strong>34</strong></td>
<td><strong>54</strong></td>
<td><strong>23</strong></td>
<td><strong>9</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

* Excluding CLL cases
Figure 1a: Age at diagnosis by generation for all subjects in the first three generations

Figure 1b: Age at diagnosis by generation for subjects with a mature B cell neoplasm
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