**KLHDC8B** in Hodgkin lymphoma and possibly twinning

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A key feature of Hodgkin lymphoma is that the malignant cells are binucleated, as a consequence of failed cytokinesis. We recently ascertained a family in which multiple cases of Hodgkin lymphoma had occurred among individuals who inherited a balanced chromosomal translocation. We cloned the translocation breakpoints and found that it disrupted a previously uncharacterized gene, **KLHDC8B**, encoding a Kelch family protein whose deficiency impairs cytokinesis and leads to binucleated cells. In other families we found a rare single nucleotide polymorphism affecting mitotic translation of **KLHDC8B** that was associated with and linked to Hodgkin lymphoma. Interestingly, the index family demonstrated an unusual frequency of twins, and there is a previously reported association between Hodgkin lymphoma and twins. Here we review the unusual genetic features of Hodgkin lymphoma, including gender concordance among siblings, and genetically test the hypothesis that **KLHDC8B** may participate in twinning by disrupting cytokinesis through impediment of polar body separation from oocytes.

Hodgkin lymphoma is a lymph node cancer of B-cell origin.\(^1\) An important feature is that in classical Hodgkin lymphoma, the malignant cells, known as "Reed-Sternberg" cells, are typically binucleated. In contrast to other cancers, where the majority of the cells comprising the tumor appear to be malignant, Reed-Sternberg cells are few in number and are surrounded by a sea of reactive, yet benign, inflammatory cells.

Of all types of malignancy, Hodgkin lymphoma demonstrates particularly strong heritability, in that relatives of cases are at an especially high risk for also developing the disease, compared to many other types of cancer.\(^2\) However, except for HLA associations\(^3\) and rare autoimmune lymphoproliferative disorders\(^4\) whose clinical features include other problems, no genes predisposing to development of Hodgkin lymphoma in the general population had been recognized.

**Gender Concordance in Hodgkin Lymphoma**

We had previously proposed a pseudoautosomal locus for Hodgkin lymphoma.\(^5,6\) A curious phenomenon is that when Hodgkin lymphoma runs within families, affected individuals tend to be of the same sex.\(^2,7\) Among sibling pairs with Hodgkin lymphoma, male-male or female-female pairs predominate; male-female pairs are fewer. The most striking example (no pun intended), occurs in what is perhaps the largest Hodgkin disease kindred ever described, that of New York Yankee baseball legend, Mickey Mantle\(^8\) (Fig. 1A). In his family (Fig. 1B), six people developed Hodgkin lymphoma, and a seventh developed non-Hodgkin lymphoma. (Mickey himself succumbed to hepatocellular carcinoma). All were male. Pseudoautosomal genetic transmission could explain gender concordance in a pedigree such as the Mantle family, because the pseudoautosomal regions contain identical genes on both sex chromosomes. Should a pseudoautosomal mutation derive from the father, then it could reside on either the X or Y chromosome and, recombination notwithstanding, would necessarily follow gender.

A supportive observation is that Hodgkin lymphoma has been observed to co-segregate in a pedigree\(^9,10\) transmitting the only...
translocation involving chromosomes 2
and 3. In the hopes that the translocation could point to a potential tumor suppressor gene, we molecularly cloned the translocation and determined that it indeed disrupted a previously uncharacterized gene, \textit{KLHDC8B}, located at a site of recurrent cytogenetic abnormalities and loss of heterozygosity (LOH) in B-cell lymphomas\textsuperscript{12} and other malignancies\textsuperscript{13,14}. Nevertheless, our laboratory has maintained interest in Hodgkin lymphoma and has continued to seek rare families with multiple occurrences in the hope that studying them might illuminate the intriguing genetic principles underlying this form of cancer. Recently, a family presented to us with multiple cases of the nodular sclerosis type of classical Hodgkin lymphoma and other malignancies.\textsuperscript{11} Interestingly, all individuals with cancer for whom genetic material was available carried a constitutional, balanced translocation involving chromosomes 2 and 3.

In the hopes that the translocation could point to a potential tumor suppressor gene, we molecularly cloned the translocation and determined that it indeed disrupted a previously uncharacterized gene, \textit{KLHDC8B}, located at a site of recurrent cytogenetic abnormalities and loss of heterozygosity (LOH) in B-cell lymphomas\textsuperscript{12} and other malignancies\textsuperscript{13,14}. The figure is Figure 1. Gender concordance in familial Hodgkin lymphoma. (A) Mickey Mantle and his four sons. (Photo courtesy of Danny Mantle). (B) Pedigree of the Mantle family. Hodgkin (HL, black) or non-Hodgkin lymphoma (NHL, grey). HCC, hepatocellular carcinoma.
on chromosome 3. This region has also been implicated in nasopharyngeal carcinoma,\(^{15,27}\) which, like Hodgkin lymphoma,\(^1\) shares an association with Epstein-Barr virus.\(^{18}\) We additionally found that a rare 5‘-UTR SNP, which appears to alter mitotic-specific expression of KLHDC8B, was both associated with and linked to Hodgkin lymphoma in three more families. Further, in one of three sporadically occurring cases of Hodgkin lymphoma, we detected somatic LOH for KLHDC8B in Reed-Sternberg cells, but not in reactive T-lymphocytes purified from the tumor.

We developed an antibody to KLHDC8B and determined that the protein is expressed only during cytokinesis and locates to the midbody, which is a structure that connects dividing cells just prior to their separation. RNAi knockdown of KLHDC8B resulted in an increase in binucleated cells. When cytokinesis cannot be completed, the cleavage furrow regresses, culminating in a binucleated cell. Thus, deficiency of KLHDC8B recapitulates the signature Reed-Sternberg cell of Hodgkin lymphoma.

**KLHDC8B in Twinning**

KLHDC8B encodes a protein predicted to contain seven repeated “Kelch” domains.\(^{19}\) The Kelch domain was discovered in a Drosophila protein component of ring canals, which form syncytia interconnecting oocytes and which derive from incompletely cytokinetic of primordial germ cells.\(^{20}\) Other mammalian homologs of Kelch, including KLHL9 and KLHL13, also locate to the midbody and produce binucleated cells when knocked-down by RNAi.\(^{21}\) In mammals, oocytes similarly develop from “oocyte nests”—clusters of primordial germ cells joined through ring canals that break down to release individual eggs.\(^{22}\) Gametogenesis is therefore coordinated with cytokinesis.

Interestingly, the index family\(^{21}\) segregating Hodgkin lymphoma with the chromosomal translocation disrupting KLHDC8B had three sets of apparent dizygotic twins occurring among eight births. We therefore entertained the hypothesis that KLHDC8B may contribute to twinning, as well as Hodgkin lymphoma. It is conceivable that disruption of KLHDC8B may lead to production of pairs of gametes tethered to one another through a persistent cytoplasmic bridge, akin to the physiologic role of Kelch in Drosophila. Along these lines, it is worth noting that the chromosomal region containing KLHDC8B was among top-scoring loci in a genome-wide linkage scan for dizygotic twinning.\(^{23}\) Moreover, rather uniquely among different forms of cancer, being born as a dizygotic twin is associated with an increased risk for Hodgkin lymphoma,\(^{24}\) suggesting that there may be a common genetic basis for both.

We specifically weighed the possibility that a deficiency of cytokinesis may impair dissociation of an oocyte from a polar body, during either first or second meiosis, thus resulting in the phenomenon of “polar body twins”. (Polar bodies are byproducts of female gametogenesis not maturing to oocytes but that are nonetheless genetically equivalent to oocytes). While there is only scant evidence that polar body twinning has ever actually occurred,\(^{25}\) twins derived from an ovum and its first polar body would be expected to be less genetically similar to one another than ordinary siblings or dizygotic twins, while twins derived from an ovum and its second polar body are postulated to be intermediate in genetic identity between dizygotic twins (or ordinary siblings) and monozygotic twins.\(^{26,27}\)

The genetic relationship between polar body twins, if they do really occur, would be determined by two major factors: which polar body undergoes fertilization and the number and distribution of chiasmata during oogenesis. The first polar body is created after the initial stage of meiosis and therefore is necessarily genetically distinct from the secondary oocyte; however, the second polar body is derived from the secondary oocyte so is genetically identical (Fig. 2).

In the absence of chiasmata it should be relatively straightforward to discriminate between first and second polar body twins. If a derivative of the first polar body were subsequently fertilized, then all maternally derived genes would be necessarily non-identical. However, if the second polar body is fertilized then all maternally derived genes will be identical. This is complicated significantly by crossing over during meiosis. At an individual locus, if no chiasmata have occurred, then the assumption outlined in the previous paragraph is correct. A single chiasma, however, would result in all-maternally derived alleles telemeric of the breakpoint being non-identical in second polar body twins. In contrast, for first polar body twins, a single chiasma would result in a 50% chance of maternally derived alleles telmeric of the breakpoint being identical (and a 50% chance of being non-identical) (Fig. 2). This is complicated further when two or more chiasmata occur.

The overall expectation for sharing in first and second polar body twins was examined by Goldgar and Kimberling.\(^{26}\) In summary, the correlation between first and second polar body twins is expected to be 38 and 51%, respectively. Using a set of markers spread throughout the genome and calculating overall allele sharing would permit identification of first polar body twins, but would not be sufficient to distinguish second polar twins from dizygotic twins. These limitations could be overcome in two ways: either by mapping all chiasmata or by using markers adjacent to the centromere to reconstruct allele sharing in the absence of chiasmata.

We selected one pair of twins from the pedigree, whose mother had the translocation disrupting KLHDC8B and who developed Hodgkin lymphoma. Each of the twins was male, neither inherited the translocation nor has had Hodgkin lymphoma, and they were considered by their family to be non-identical. To examine their relationship we undertook a whole genome screen using the Genechip Mapping 10K Array (Affymetrix), containing approximately 10,200 SNPs.

We initially determined overall correlation using the program PLINK.\(^{28}\) This demonstrates an overall sharing of 54%, with sharing of 0, 1 and 2 alleles being 21, 49 and 30%, respectively (compared to an expected distribution of 25, 50 and 25%). This indicates that it is unlikely that the twins were first polar body twins. However, by analyzing overall sharing statistics we can not rule out the twins being second polar body twins.

To explore the relationship between the twins further, we analyzed identity by...
Nevertheless, the results are not definitive, because, in the absence of parental genotypes, allele sharing will be overestimated due to fortuitous sharing of common descent (IBD) sharing using the programs MERLIN\(^2\) and RELATE.\(^3\) Probabilities of sharing 0, 1 and 2 alleles were graphed, and the most probable pattern of sharing at centromeric regions was established (Table 1). The two programs gave similar results, with both indicating that the twins examined in this study are most likely dizygotic.

Overall, this analysis does not suggest that there is anything genetically special about this particular pair of twins. Nevertheless, the results are not definitive, because, in the absence of parental genotypes, allele sharing will be overestimated due to fortuitous sharing of common
markers between both parents. Nor can we exclude that a disruption of cytokinesis might contribute to twinning through a mechanism other than involving ejection of polar bodies. For example, one could imagine that primordial germ cells remain connected, such that two primary oocytes may be deposited into a follicle and then ovulated simultaneously. The possibility that twinning and gender concordance could somehow be coupled (noting that there is a pair of twins in the Mantle family) through a mechanism other than that involving pseudoautosomal inheritance should also not be overlooked. We therefore plan to further explore the link between KLHDC8B, failed cytokinesis, and the curious genetic phenomena of twinning and gender concordance that have been associated with Hodgkin lymphoma.

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Table 1. Expected and observed allele sharing at centromeric regions throughout the genome indicative of twin type

|          | Sharing 0 alleles | Sharing 1 allele | Sharing 2 alleles |
|----------|------------------|-----------------|------------------|
| Expected |                  |                 |                  |
| MZ Twin  | 5.5 (25%)        | 11 (50%)        | 5.5 (25%)        |
| DZ Twin  | 11 (50%)         | 11 (50%)        | 11 (50%)         |
| 1st Polar Body Twin | 11 (50%) | 11 (50%) | 11 (50%) |
| 2nd Polar Body Twin   | 11 (50%)        | 11 (50%)        |                  |

| Observed |                  |                 |                  |
|----------|------------------|-----------------|------------------|
| Merlin   | 4 (18%)          | 12 (55%)        | 6 (27%)          |
| Relate   | 5 (23%)          | 9 (41%)         | 8 (36%)          |

Numbers indicate number of centromeric regions and numbers in parentheses indicate percentage of all chromosomes. 22