A novel deletion cluster at 13q14.2-q21.33 in an 80-year man with late onset leukemia: Clinical and molecular findings

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Introduction

Genome-wide, high-resolution arrays are rapidly becoming a reliable method of molecular investigation across individuals, families, and populations. Developed just a few years ago, they have now become a primary method for assessment of the quantitative and qualitative genomic variations involving single nucleotide polymorphisms, copy number variations (CNVs), and insertions and deletions. They have been instrumental in establishing genome-wide associations in a variety of disorders including cancers, and congenital anomalies. Further, the results have helped identify the involvement of specific genes and genomic alterations in a number of cancers. This case report represents one such example and deals with identification of a major genomic rearrangement by Affymetrix Human Array 6.0 screening.

MATERIALS AND METHODS: The breakpoints for individual deletions in this cluster was identified by Affymetrix Human Array 6.0 screening.

RESULTS: The deleted segments harbours a number of genes, most associated with cancer as well as a high concentration of LINEs, SINEs and related repeats. The derived chromosome represents an intra-chromosomal re-arrangement that quickly overtook blood progenitor cells probably before age 69 as a cause of CLL.

CONCLUSIONS: The study highlights the role of ongoing de novo changes at susceptible sites, such as repeat rich regions, in the human genome. Also, it argues for the involvement of genes/deletions in the 13q(14.2-21.33) region in the development of CLL.

Key words: Copy number variations, deletions, de novo mutations, LINES, leukemia, SINES

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The subject was an 80-year-old at the time of assessment. He had a high school diploma and had worked in sales until he retired. The subject had no major illness until he was diagnosed with leukemia at age 69. At age 70 he was noted to be hypertensive and 3 years later had a quadruple coronary bypass. Immediately following his coronary artery bypass the subject developed low mood and anxiety. And then after discharge from hospital...
he started to experience panic attacks and was prescribed lorazepam for 2 months. At age 80 the subject’s gallbladder ruptured and he developed septicemia. He had emergency surgery following which he lost weight, became debilitated and experienced further symptoms of depression. The subject received the last course of nine chemotherapy treatments for leukemia 6 months before the blood was drawn for genetic assessment. At the time when the blood was drawn the subject was taking atorvastin sodium daily to treat hypercholesterolemia.

**Results and Discussion**

The subject’s leucocyte genomic DNA was hybridized to the Affymetrix Human Array 6.0 following the manufacturer’s protocol at the London Regional Genomics Center, The University of Western Ontario. Briefly, 5 µg of genomic DNA was labeled and hybridized to the array. Calls for CNVs were made using the Affymetrix Genotyping Console 4.0 as well as Partek H Genotyping Suite™ software suites. In both cases, the CNVs were identified by continuity of markers on a segment. Two CNVs that overlapped by 50% in the two methods of data analysis were given the same identity. Every measure was undertaken to avoid inclusion of false positives including correction for segmental duplications. The CNVs identified were further assessed by comparison to the Database of Genomic Variants (http://projects.tcag.ca/variation/) and annotated with gene symbols by importing the annotation file from the UCSC genome browser (NCBI36/hg 18). This analysis identified an unusual cluster of gains and losses on 13q that forms the focus of this report.

Table 1 shows the cytogenetic and molecular (nt) breakpoints as well as the size of deleted fragments and gene affected. The genomic features of this abnormality as shown in Figure 1, suggests that it harbors a number of genes, some labeled in this figure. More important the genes affected are implicated in a number of pathways [Table 2]. It is apparent from this table that a large number of genes are known to play a role in cancers, cell cycle regulation. Further, the region harbors several MicroRNA coding genes, which function in cell survival, proliferation, differentiation, and angiogenesis and is the primary target of genomic amplification that occurs in several lymphomas and solid tumors. A recent study by Parker et al. has assessed 13q deletions in chronic lymphocytic leukemia (CLL) using genomic profiling. They identified 205 copy number alterations on chromosome 13 in 132 cases. These deletions were highly heterogeneous (845 Kb to 96.2 Mb) in size. They also identified two breakpoint cluster regions within short interspersed nuclear elements proximal to DLEU5 (TRIM13) and within long interspersed nuclear elements/L1 repeats distal to GUCY1B2.

Forty breakpoints, almost all associated with the distribution of LINES and SINES [Figure 1]. Parker et al. also suggested that the larger deletions (Class II), as seen in our subject have an increased risk of disease progression (odds ration = 12.3; P = 0.005). Interestingly, deletions on the long arm of chromosome-13 have also been reported with mental and motor retardation, craniofacial dysmorphic facial appearance and various congenital malformations. Furthermore, del13q is known to manifest with a range of abnormalities, including, retinoblastoma, mental and growth retardation, brain malformations, heart defects, distal limb deformities, and digestive, and urogenital abnormalities (www.diseasesdatabase.com). Interestingly, none of these symptoms are apparent in this case.

Chromosomal deletions are among the most common genetic events observed in hematologic malignancies. It includes differential loss of genetic material from 13q in lymphoid neoplasias, in non-Hodgkin's lymphoma and in chronic lymphoproliferative diseases. The presence the complex deletion involving 13q14.2-13q21.33 in our subject is best explained by the origin of this abnormality later in life, probably before age 69 when he was diagnosed with CLL. Given the genes involved, it is argued that this abnormality must have provided proliferative advantage that led to the development of CLL at age 69. Given that, the observed deletion is large and contains ~50 genes including genes implicated in CLL, this feature may have accounted for the fast progression of CLL in this patient. Such a speculation is compatible with the concentration and specific location of the LINE1, SINE, and related repeats [Figure 1] in 13q14.2-21.33. This feature will make this genomic region prone to intra-chromosomal recombination during mitotic DNA replication. This would support recent
### Table 1: Genomic (cytogenetic and nucleotide numbers) breakpoints, corresponding size of deletions and genes affected in the 13q (14.2-21.33) region in the subject

| Chromosome | Cytogenetic band start | Cytogenetic band end | Nucleotide position start | DNA sequence position end | Size (kb) | Gain or loss | Gene affected |
|------------|------------------------|----------------------|---------------------------|---------------------------|-----------|-------------|---------------|
| 13         | q12.3                  | q12.3                | 29253785                  | 29357162                  | 103       | Gain        | SLC7A1        |
| 13         | q12.1                  | q12.1                | 47237551                  | 47666738                  | 429       | Loss        | MED4          |
| 13         | q14.2                  | q14.2                | 48148998                  | 48696505                  | 548       | Loss        | SUVCL2        |
| 13         | q14.3                  | q14.3                | 48751953                  | 49328768                  | 561       | Loss        | NUPT1         |
| 13         | q14.3                  | q14.3                | 49343727                  | 51639572                  | 2296      | Loss        | LOPL1         |
| 13         | q14.2                  | q14.2                | 52437155                  | 52763266                  | 326       | Loss        | PCDH8         |
| 13         | q14.1                  | q14.1                | 52826196                  | 52932732                  | 107       | Loss        | SUCLA2        |
| 13         | q14.2                  | q14.2                | 53057893                  | 53192116                  | 134       | Loss        | SUCLA2        |
| 13         | q14.3                  | q14.3                | 54680266                  | 54790556                  | 110       | Loss        | SETDB2        |
| 13         | q14.3                  | q14.3                | 54935168                  | 55098944                  | 164       | Loss        | PHF11         |
| 13         | q14.3                  | q14.3                | 55750401                  | 55874339                  | 124       | Loss        | TPTE2P3       |
| 13         | q14.3                  | q14.3                | 56398440                  | 56585146                  | 187       | Loss        | TPTE2P3       |
| 13         | q21.1                  | q21.1                | 52437155                  | 52763266                  | 326       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 52826196                  | 52932732                  | 107       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 53057893                  | 53192116                  | 134       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 53550461                  | 53590407                  | 334       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 54680266                  | 54790556                  | 110       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 54935168                  | 55098944                  | 164       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 55750401                  | 55874339                  | 124       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 55974036                  | 56272375                  | 298       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 56398440                  | 56585146                  | 187       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 56934307                  | 57038858                  | 105       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 57282053                  | 57390283                  | 698       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 58340087                  | 58443546                  | 103       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 58676802                  | 58970079                  | 293       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 59126155                  | 59350332                  | 224       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 59494917                  | 59781724                  | 363       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 60075937                  | 60201120                  | 125       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 60685046                  | 61083734                  | 399       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 61379792                  | 61483739                  | 104       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 61650921                  | 61897775                  | 247       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 62086051                  | 62225287                  | 139       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 62279951                  | 62396589                  | 107       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 63059363                  | 63205520                  | 146       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 63386740                  | 63794997                  | 408       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 63956144                  | 64073049                  | 117       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 64177520                  | 64483758                  | 306       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 64835495                  | 65128686                  | 293       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 65196691                  | 65485151                  | 288       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 65671222                  | 66142551                  | 471       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 66444238                  | 66561990                  | 118       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 66767856                  | 67064534                  | 297       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 67105010                  | 67226610                  | 122       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 67389818                  | 67536058                  | 146       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 67647031                  | 67858955                  | 212       | Loss        | PCDH17        |
| 13         | q21.1                  | q21.1                | 69163283                  | 69315829                  | 152       | Gain        | MIR548H4,     |
| 13         | q21.1                  | q21.1                | 69386740                  | 69754997                  | 408       | Loss        | KLHL1         |

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reports\(^6\) that \textit{de novo} mutations that are operational during the life-time\(^9\) can occur both randomly and in response to external challenges. The phenotypic description of deletion 13q syndrome is dependent on the location and size of the deleted segment. At present, the syndrome is divided into three groups based on the deletion’s location relative to chromosomal band 13q32. Groups 1 (proximal to q32) and 2 (including q32) have shown distinctive phenotypes including mental retardation and growth deficiency.\(^{10}\) However, del13q manifests with a range of abnormalities, including, retinoblastoma, mental and growth retardation, brain malformations, heart defects, distal limb deformities, and digestive, and urogenital abnormalities have been reported previously (www.diseasesdatabase.com). This case adds yet another example of large deletions on 13q that account for the initiation and rapid progression of chronic lymphocytic leukemia.

**Conclusion**

The human genome continues to undergo genomic changes randomly and in response to a variety of internal and external factors. We report a cluster of somatic deletions covering 13q14.2-13q21.33, along with individual breakpoints and genes affected in a male that the most likely has caused progressing CLL. Such results support the dynamic nature of the human genome and the role of specific genomic aberrations in leukemia.

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