Constitutional and somatic rearrangement of chromosome 21 in acute lymphoblastic leukaemia

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Changes in gene dosage are a major driver of cancer, known to be caused by a finite, but increasingly well annotated, repertoire of mutational mechanisms. This can potentially generate correlated copy-number alterations across hundreds of linked genes, as exemplified by the 2% of childhood acute lymphoblastic leukaemia (ALL) with recurrent amplification of megabase regions of chromosome 21 (iAMP21). We used genomic, cytogenetic and transcriptional analysis, coupled with novel bioinformatic approaches, to reconstruct the evolution of iAMP21 ALL. Here we show that individuals born with the rare constitutional Robertsonian translocation between chromosomes 15 and 21, rob(15;21)(q10;q10), have approximately 2,700-fold increased risk of developing iAMP21 ALL compared to the general population. In such cases, amplification is initiated by a chromothripsis event involving both sister chromatids of the Robertsonian chromosome, a novel mechanism for cancer predisposition. In sporadic iAMP21, breakage-fusion-bridge cycles are typically the initiating event, often followed by chromothripsis. In both sporadic and rob(15;21)c-associated iAMP21, the final stages frequently involve duplications of the entire abnormal chromosome. The end-product is a derivative of chromosome 21 or the rob(15;21)c chromosome with gene dosage optimized for leukaemic potential, showing constrained copy-number levels over multiple linked genes. Thus, dicentric chromosomes may be an important precipitant of chromothripsis, as we show rob(15;21)c to be constitutionally dicentric and breakage-fusion-bridge cycles generate dicentric chromosomes somatically. Furthermore, our data illustrate that several cancer-specific mutational processes, acting sequentially, can coordinate to fashion copy-number profiles over large genomic scales, incrementally refining the fitness benefits of aggregated gene dosage changes.

Acute lymphoblastic leukaemia (ALL) is the most common childhood cancer, with an annual incidence of 35/50,000 million children aged 0–14 years. Approximately 2% of these cases show intrachromosomal amplification of one copy of chromosome 21, IAMP21, which defines a distinct ALL subgroup13–15, 21–22. They are found in about 1 in 1,000 newborns8,9, but rob(15;21)c accounts for only 0.5–1% of cases. To confirm this, we interrogated cytogenetics databases. Only three patients among 93,000 referrals for haematological malignancies to the Munich Leukaemia Laboratory and West Midlands Regional Genetics Laboratory carried rob(15;21)c. Similarly, only 16 cases were found among approximately 300,000 referrals to UK regional cytogenetics laboratories for investigation of infertility or previous Down syndrome birth.

From these data, we estimate the risk of IAMP21 ALL in carriers of rob(15;21)c to be increased approximately 2,700–fold over the general population (Supplementary Table 1). This association is remarkably specific. All patients in this study with rob(15;21)c had IAMP21 ALL, implying that they are not predisposed to other forms of ALL, nor other cancers, as far as we can ascertain. Furthermore, the only Robertsonian translocation associated with IAMP21 ALL was rob(15;21)c. For clarity, we use ‘rob(15;21)c’ to denote the germline configuration and ‘der(15;21)c’ to describe the rearranged and amplified chromosome in these cases.

Using cytogenetics, fluorescence in situ hybridization (FISH) and copy-number profiling, we studied 21 patients with sporadic IAMP21 ALL and 12 patients with ALL associated with rob(15;21)c. Five sporadic IAMP21 and four cases associated with rob(15;21)c were sequenced to identify genomic rearrangements16 (Supplementary Table 2; Extended Data Fig. 1, Supplementary Fig. 1). We applied deductive approaches, supported by confirmatory simulations, to reconstruct principles underlying the temporal evolution of iAMP21 ALL. This reasoning is explored in considerable detail, together with a sample-by-sample analysis, in Supplementary Results, Extended Data Figs 3–8, Supplementary Tables 3–6 and Supplementary Figs 4–23.

The broad themes are illustrated by two representative cases (Figs 1, 2). In PD9020a, a patient with sporadic IAMP21, the boundaries of the amplified region are demarcated by fold-back inversion rearrangements (Fig. 1a). These indicate breakage-fusion-bridge (BFB) repair11, previously proposed to trigger IAMP2112,13. Breakage-fusion-bridge repair is a mutational process initiated by a telomeric double strand (ds) DNA break that is replicated in S phase. In G2, the two copies of the dDNA break are fused by non-homologous end-joining (marked (1) in Fig. 1a). This creates a dicentric chromosome in which the two centromeres are pulled to opposite poles during mitosis, forming an anaphase bridge. With cytokinesis, the bridge breaks, and the process can repeat in the next cell cycle (marked (2) in Fig. 1a). In the region

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LETTER

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98 | NATURE | VOL 508 | 3 APRIL 2014
between the fold-back inversions in PD9020a, we also found a cluster of back-and-forth rearrangements (marked (3)) of all four possible orientations, associated with copy-number profiles that oscillate among three states (Fig. 1a, zoomed-in panel). These clusters bear the hallmarks of back-and-forth rearrangements (marked (3)) of all four possible orientations between the fold-back inversions in PD9020a, we also found a cluster (Supplementary Results), a mutational process in which a one-off catastrophic event shatters one or a few chromosomal regions leading to large numbers of localized genomic rearrangements.

Two features of this genomic architecture allow reconstruction of the temporal evolution of the iAMP21 chromosome. First, the rearrangements frequently link together genomic segments of different copy number (off-diagonal histograms, Fig. 1b). Second, as we traverse chromosome 21 from first to last base-pair, the copy-number segments on either side of each breakpoint position typically differ in copy number by one (Fig. 1c). FISH confirms widespread RUNX1 signals along the iAMP21 chromosome (Fig. 1d). On the basis of reasoning outlined in detail in Supplementary Results, these features indicate that chromothripsis occurred after two BFB cycles and was likely the final major event, stabilizing the chromosome (Fig. 1e).

In PD7170a, a der(15;21) iAMP21 derived from rob(15;21)c, the picture is dominated by a series of back-and-forth rearrangements spanning chromosomes 15 and 21 (Fig. 2a–c). Cytogenetic and FISH studies confirmed that it was the Robertsonian chromosome undergoing rearrangement (Fig. 2d–e). A sizable number of rearrangements link together segments of different copy number (off-diagonal histograms, Fig. 2b), and copy number oscillates among three, rather than two, states. Together with occasional inverted rearrangements with no breakpoints between the two joined ends, this pattern indicates that chromothripsis was the initiating event, and that the chromothripsis process involved both sister chromatids of the Robertsonian chromosome (Extended Data Figs 7, 8; Supplementary results; Supplementary Table 4). Importantly, the shattered sister chromatids are repaired into one derivative chromosome, thereby amplifying the copy number of some chromosomal regions (Fig. 2f). The amplification was completed by whole-chromosome duplication of the der(15;21) chromosome through isochromosome formation (Fig. 2d).

These broad temporal sequences of events are reflected in the other samples (Fig. 3; Extended Data Fig. 6; Supplementary Results). In the other four sequenced cases of sporadic iAMP21, a telomeric fold-back inversion suggests at least one BFB cycle. In each, this was a critical early event, defining the break between the most amplified region of chromosome 21 and subtelomeric loss. Chromothripsis occurred after the BFB cycles in three cases. Finally, partial or whole-chromosome duplications usually completed the evolution. In the other three sequenced
Figure 2 | Rearrangements of der(15;21) in patient PD7170a. a. Rearrangement and copy-number pattern. The temporal order of the two major rearrangement events are marked (1) and (2). Rearrangements are separated based on their orientation: D, deletion-type; TD, tandem duplication-type; HH, head-to-head inverted; TT, tail-to-tail inverted. b. Copy-number jump distribution, showing the copy number at each end of each rearrangement. c. Copy-number step distribution, showing the distribution in magnitude of copy-number change at copy-number segmentation breakthroughs. 

Figure 3 | Rearrangement processes of the iAMP21 chromosome in the remaining patients. a, b. Rearrangement and copy-number patterns for chromosome 21 of sporadic iAMP21 ALL patients (a) and der(15;21) rearrangements in der(15;21) iAMP21 ALL patients (b). The inferred temporal orders of the major rearrangement events are shown with symbols (1), (2) and (3). In patients PD4117a and PD9021a, the fold-back rearrangement demarcating the second BFB repair breakpoint have probably been lost or obscured due to subsequent rearrangement events, and a '?' symbol is used to denote the uncertainty of their location. Inferred evolution of the derivative iAMP21 chromosomes are shown in the bottom panel. WC, whole chromosome; WCD, whole-chromosome duplication. Events with incomplete understanding are labelled '??'.

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der(15;21) iAMP21 cases, amplification was initiated by chromothripsis involving both sister chromatids of the rob(15;21)c chromosome. This seemed to be followed by further rearrangements in two cases and was completed by whole-chromosome duplications. These data provide insight into why there is such specific enrichment of iAMP21 ALL in carriers of rob(15;21)c. Universally, the amplification is initiated by chromothripsis that affects both sister chromatids of the Robertsonian chromosome. This suggests that rob(15;21)c has a structural abnormality that specifically predisposes it, after replication, to the catastrophic shattering of chromothripsis. Using FISH, we demonstrated that the rob(15;21)c chromosome has centromeres from both chromosomes 15 and 21, and is thus dicentric (Fig. 2e and Extended Data Fig. 1). Our hypothesis, therefore, is that the two centromeres of the Robertsonian chromosome can occasionally confound attachment of mitotic spindles to the sister kinetochores, such that each chromatid connects to spindles emanating from opposite poles (Fig. 2f). During anaphase, this merotelic attachment would lead to lagging of both sister chromatids, rendering them jointly prone to chromothripsis. The der(15;21) iAMP21 chromosomes consistently lose the chromosome 15 centromere, shown by FISH (Fig. 2e) and sequencing (Figs 2a and 3b), potentially enhancing stability of the derivative chromosome. In sporadic iAMP21 cases, chromothripsis frequently follows BFB cycles. Although we cannot know whether chromothripsis is an immediate consequence, it is plausible that the dicentric chromosome created by BFB repair could trigger chromothripsis, analogous to that seen with the dicentric rob(15;21)c17,18.

The preceding analysis provides insight into the mutational processes shaping chromosome 21, but unless the resulting chromosome profile confers a selective advantage on the clone, it will not expand. We combined copy-number profiles and gene expression data from additional patients with iAMP21 ALL (Fig. 4). A consensus copy-number profile emerged in which regions from 35.9–36.4 Mb and 38.0–40.0 Mb of chromosome 21 were consistently the most highly amplified and over-expressed, including genes important in haematological malignancies such as RUNX1, DYRK1A and ETS2.2

The final stage of iAMP21 generation usually involves duplication of the whole derivative chromosome, through whole-chromosome duplication,

Figure 4 | Chromothripsis alters the copy-number landscape of chromosome 21 in a non-random fashion. a, b, Chromosome arm level (a) and zoomed-in view (b) of chromosome 21, showing gene expression, copy-number (CN) distribution, chromothripsis effect and distribution of rearrangement breakpoints. In the gene expression panels, positive-strand genes are shown in blue and negative-strand genes are shown in red. c, Correlation between average rate of deletion in the ref. 19 data set and chromothripsis effect for chromosome 21. IQR, interquartile range.
isochromosome or ring formation. All duplications occurred after chromothripsis, suggesting that chromothripsis might be remodelling chromosome 21 in a non-random fashion. We used the inferred temporal evolution of somatic rearrangements to extract and average copy-number changes resulting from chromothripsis (Fig. 4). As expected, chromothripsis spared the most amplified regions, whereas on average one to two copies were deleted from other parts of the chromosome. Analysis of der(15;21) iAMP21 indicated that regions of chromosome 15 were also consistently lost or retained, although sample numbers are small (Extended Data Fig. 9).

Remarkably, the consensus chromothripsis landscape in iAMP21 closely mirrored the copy-number profile of chromosome 21 averaged over thousands of cancer samples across different cancer types\(^{9,20}\) (\(P = 0.0003\); Fig. 4c; Supplementary Figs 27–30). This indicates that chromothripsis has a critical role in optimising the copy-number landscape of chromosome 21 to maximise the net selective advantage gained from subsequent rounds of whole-chromosome duplication.

From a detailed dissection of the mutational forces causing one particular subtype of one particular cancer, findings with general significance have emerged. Carriers of constitutional rob(15;21)c chromosomes are specifically but highly predisposed to iAMP21 ALL. Usually, constitutional risk of cancer is mediated by variation in coding sequence or gene regulation, but here it seems to be transmitted through a propensity for the Robertsonian chromosome to undergo chromothripsis after replication. This may be because it is dicerentric and prone to anaphase bridging, which would dovetail with the frequent occurrence of chromothripsis following BFB cycles in sporadic iAMP21 ALL. This hypothesis is consistent with the finding that lagging chromosomes during anaphase can become sequestered in micronuclei and subjected to chromosomal pulverisation before rejoining the main nucleus\(^{7,18}\). More generally, the study of iAMP21 ALL has illustrated how large-scale copy-number changes can be optimised by spatially and temporally coordinated genomic instability taking several complementary forms. BFB cycles can generate rapid-fire, focal amplification; chromothripsis causes loss of multiple, non-contiguous chromosomal regions; and whole-chromosome duplication gives expansive, low-amplitude amplification. Their combined activity, therefore, gives considerably more flexibility to shaping large-scale chromosomal copy-number profiles than any one process alone. Of course, clones sample these mutational processes randomly, so only when the aggregate fitness of such changes is positive will the clone have the selective advantage to expand.

**METHODS SUMMARY**

Information was available from 21 iAMP21 patients and 12 with iAMP21 and rob(15;21)(q10q10)c (Supplementary Table 2). Paired-end sequencing data were generated as 37–75 bp paired reads from 400–500 bp fragments as previously described\(^{10}\). The ddcutative reasoning for reconstructing temporal evolution of complex rearrangements followed principles formulated previously\(^{21}\). Confirmatory PCR across the breakpoints was performed for the vast majority of identified rearrangements (Supplementary Results).

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**Author Contributions** C.J.H. and P.J.C. designed the study; Y.L. carried out and interpreted the sequencing and associated analyses, assisted by E.P. and P.J.C.; S.C. and S.R. coordinated the study; C.S. carried out the FISH analyses and interpreted the FISH and SNP6.0 results; S.R. carried out the initial sequence analysis and associated validation; B.D.Y. assisted with the analysis of SNP6.0 data; C.S. and H.M.R. interpreted the cytogenetic findings; O.J., B.R. and M.M. performed laboratory analyses; P.J., M.G., P.T., N.B., N.T., C.H. and L.G. provided data on incidence of rob(15;21)c: cases; P.J., F.M.R., N.A.H., A.J.C., N.B., N.T., M.R.T., S.D., J.B. and N.D. and P.V. provided rob(15;21)c: cases associated and clinical and genetic data to be included in the study; A.V.M. and R.J.Q.M. provided the incidence data and calculated the relative risk values; P.S. and V.R. provided data interpretation; J.C. and P.V.L. ran copy-number analyses and coordinated analysis of publicly available solid tumour cancer data; M.R.S. contributed to the analysis and interpretation of the sequencing studies. P.J.C. and C.J.H. assimilated the data and wrote the manuscript, with support from all authors.

**Author Information** Genome sequence data have been deposited at the European Genome-Phenome Archive (http://www.ebi.ac.uk/ega/, hosted by the EBI) with accession number EGA0000010000658 (https://www.ebi.ac.uk/ega/datasets/EGA0000010000658). Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.J.H. (christine.harrison@newcastle.ac.uk) or P.J.C. (pjc@dameng.ac.uk).

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Extended Data Figure 1 | FISH studies of patient PD10009a indicating the role of the centromeres in the formation of the iAMP21 chromosome from rob(15;21)c patients. a, A representative normal metaphase from a non-leukaemic cell hybridized with centromere specific probes for chromosomes 15 (CEP15, Cytocell) labelled green and chromosomes 13 and 21 (this probe cross hybridizes to both chromosomes 13 and 21, CEP13/21, Cytocell) labelled red. The chromosomes are counterstained with DAPI (blue). The discrete green signal indicates one copy of the normal chromosome 15, the two discrete red signals at the bottom of the cell indicate the two normal copies of chromosomes 13 and 21 and the discrete red signal at the top indicates the normal chromosome 21. The closely apposed red green signals at the bottom of the cell are hybridized to the rob(15;21)c confirming that this chromosome is dicentric with intact copies of both centromeres. In order to maintain stability and normal segregation at mitosis, one centromere of a dicentric chromosome needs to be inactive. In Robertsonian translocations, chromosome 15 is most frequently inactivated. This is often depicted by decondensation of the chromatin, in contrast to tightly condensed chromatin of an active centromere. In some cells the chromosome 21 centromere of the rob(15;21)c appeared smaller (condensed) than the chromosome 15 centromere (decondensed).

b, A representative normal metaphase indicating the chromosome 15 centromere (CEP 15, green) and a probe set designed to cover the common region of amplification of chromosome 21 (CRA); probes RP11-777J19, RP11-383L18 and RP11-773I18 (as previously described) were hybridized together and labelled red. The discrete green signal indicates the centromere on the normal chromosome 15, the discrete red signal indicates the CRA on the normal chromosome 21, whereas the red and green signals close together show the centromere of chromosome 15 and the CRA on the rob(15;21)c.  c, e, The same representative abnormal metaphase hybridized with CEP15 (green) and CEP13/21 (red) as above. The discrete green signal indicates the intact chromosome 15 centromere on the normal chromosome 15. The three discrete red signals show the centromeres on the two normal chromosomes 13 located either side of the red signal on the normal chromosome 21. The iAMP21 chromosome, which we know to be composed of both chromosomes 15 and 21, as patient PD7170a (Fig. 2), and to be in the formation of a ring chromosome has one red signal indicating the presence of an intact chromosome 21 centromere, but absence of the green signal indicating loss of the chromosome 15 centromere, which is present in the normal cells as shown in a. This loss of chromosome 15 centromere from the der(15;21) is also confirmed by sequencing (Fig. 3b). In e, the metaphase shown in panel c has had colours inverted to confirm the origin of the chromosomes and indicate the ring formation of the iAMP21 chromosome (arrow). d, f, The same representative abnormal metaphase hybridized with CEP15 (green) and the probe set specific for the CRA (red) as above. The discrete green signal at the bottom indicates the intact chromosome 15 centromere on the normal chromosome 15. The discrete red signal (bottom left) shows one copy of the CRA on the normal chromosome 21. The iAMP21 chromosome has multiple red signals indicating multiple copies of the CRA interspersed throughout this abnormal ring chromosome. In f, the image shown in d is inverted to confirm the origin of the chromosomes and indicate the ring formation of the iAMP21 chromosome (arrow).
Extended Data Figure 2 | Rearrangement orientations are distributed equally on chromosome 21 in iAMP21 patients. Comparison of rearrangement orientations for rearrangements on chromosome 21 (leftmost 4 bars for each panel) against the rest of the genome (rightmost 4 bars for each panel) for the 9 patients sequenced. These show that the distribution was not statistically different from uniform for the chromosome 21 rearrangements (P values under x axis legend). For rearrangements in the rest of the genome, deletion-type rearrangements (TH, coloured red) predominated. Multinomial distribution statistical significances (raw P values, rounded to 4 decimals) for the null hypothesis of equal distribution between all orientation types are shown in the x axis labels.
Extended Data Figure 3 | Rearrangement metrics used to describe rearranged sections of a genome. a, b, Illustration of the process of compiling rearrangement metrics in a hypothetically rearranged chromosome (a) and summarization of these values into the rearrangement metrics representation used in this study (b). CN, copy number.
Extended Data Figure 4 | Rearrangement metrics of 50 simulated chromosomes under two alternative sequences of two rearrangement events, two BFB cycles and chromothripsis. In copy-number step-size distribution plot, every simulated instance is represented by a point at each of the 5 copy-number change-size value, showing the frequency of copy-number steps with each copy-number step size. In copy-number jump-size distribution, rearrangements from all 50 simulated chromosomes are aggregated. In copy-number trajectory, every connected line represents a single simulated chromosome.
Extended Data Figure 5 | Comparison of rearrangement metrics of two alternative sequences of rearrangements that generate a chromothripsis-like copy-number pattern involving three copy-number states. In copy-number step-size distribution plot, every simulated instance is represented by a point at each of the five copy-number change-size value, showing the frequency of copy-number steps with each copy-number step size. In copy-number jump-size distribution, rearrangements from all 50 simulated chromosomes are aggregated. In copy-number trajectory, every connected line represents a single simulated chromosome.
Extended Data Figure 6 | Copy-number and rearrangement pattern in the iAMP21 chromosome of patient PD9022a. a, Copy-number and rearrangement pattern of PD9022a chromosome 21. D, deletion type rearrangement link; TD, tandem duplication type; TT, tail-to-tail rearrangement; HH, head-to-head rearrangement. b, the deletion type (tail-to-head) rearrangement resolved by five unmapped split reads whose mates mapped to the vicinity of the associated copy-number breakpoint. The mapped mates and unmapped split reads are joined together by dashed lines. The 5 split reads are aligned to the two ends of the rearrangement.
Extended Data Figure 7 | Fold-back-like rearrangements of patient PD7170a.  
a–c, Three fold-back-like rearrangement of the der(15;21) chromosome of patient PD7170a are shown (arrows). In a, the rearrangement is tail-to-tail type. In b and c, the rearrangement is head-to-head type.
Extended Data Figure 8 | Formation of fold-back-like rearrangements. If two copies of the same chromosome are rearranged in the same event, fold-back-like rearrangements may form (left). If two copies of the same chromosome undergo separate rearrangements, fold-back-like rearrangements cannot form.
Extended Data Figure 9 | Chromothripsis effect based on the four sequenced rob(15;21)c iAMP21 ALLs patients on chromosome 15.
Extended Data Table 1 | Application of published criteria for chromothripsis\textsuperscript{14} to the chromosome 21 amplifications analysed in this manuscript

| Criterion                                                                 | Applicability/Assessment in data                                                                                                                                                                                                 |
|---------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Clustering of breakpoints                                                | **Applicable.** Statistical evidence for breakpoint clustering in 5/9 of chromosomes 21 and 2/4 of chromosomes 15 of rob(15;21) iAMP21 samples (Supplementary Figure 2).                                                            |
| Regularity of oscillating copy-number states                             | **Applicable.** As opposed to ‘conventional chromothripsis’, copy numbers oscillate over multiple copy number states since chromothripsis takes place on amplified chromosomes (by BFBs in sporadic iAMP21 and by whole-chromosome duplication in rob(15;21) iAMP21). Copy number profiles are shown in Figure 1A, Figure 2A, Figure 3 and Extended Data Figure 6. |
| Interspersed loss and retention of heterozygosity                        | **Applicable.** Sporadic iAMP21 and der(15;21) show interspersed loss and retention of LOH in multiple samples (Supplementary Figure 3). Segments of LOH are expected to occur less frequently as chromothripsis takes place on amplified chromosomes (by BFBs in sporadic iAMP21 and by whole-chromosome duplication in rob(15;21) iAMP21). |
| Prevalence of rearrangements affecting a specific haplotype             | **Applicable.** FISH and cytogenetic analysis demonstrated that gross chromosomal rearrangements only affected one of the two chromosomes 21 in sporadic cases, and only the rob(15;21) chromosome in the Robertsonian cases (Figure 1D, Figure 2D-E, Supplementary Figures 12E, 16E, 18E, 20D-E, 21D-E, 23D-E). |
| Randomness of DNA segment order and fragment joins                      | **Applicable.** Rearrangement join orientations in iAMP21 and der(15;21) chromosomes are consistent with random draws from a uniform multinomial distribution. However aggregated data of all other chromosomes show a significant departure from this null distribution, where deletion-type rearrangements seem to dominate the rearrangement landscape (Extended Data Figure 2). We also evaluated randomness of DNA segment orders, but found that as noted earlier\textsuperscript{14}, DNA segment orders were non-random even in chromosomes presumed to have undergone chromothripsis (data not shown). |
| Ability to walk the derivative chromosome                                | **Not applicable,** as in both sporadic and rob(15;21) associated iAMP21, chromothripsis takes place on chromosomes with preceding or synchronous duplication (by BFBs in sporadic iAMP21 and by whole-chromosome duplication in rob(15;21) iAMP21). In this scenario, it would be impossible to walk the derivative chromosome as outlined in the criteria. |