The human Y chromosome: a sole survivor
Noordam, M.J.
Chapter 7

Intrachromosomal homologous recombination between inverted amplicons on opposing Y chromosome arms generates pseudoisochromosomes and pericentric inversions

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Abstract

Amplicons – large, nearly identical repeats in direct or inverted orientation – are abundant in the male-specific region of the human Y chromosome (MSY) and provide numerous targets for intrachromosomal homologous recombination. Thus far, ectopic recombination resulting in interstitial deletions, duplications, inversions, or isodicentric chromosomes, has been reported only for amplicon pairs located exclusively on either the short arm (Yp) or the long arm (Yq). Here we report our finding of four men with Y chromosomes that evidently formed by intrachromosomal homologous recombination between inverted repeat pairs composed of one amplicon on Yp and one amplicon on Yq. In two men with spermatogenic failure, sister chromatid crossing over between inverted amplicons resulted in pseudoisochromosome formation and loss of distal Yq including, in one case, the azoospermia factor c (AZFc) region. In two other cases, intrachromatid crossing over between inverted amplicons generated pericentric inversions. These findings highlight the recombinogenic nature of the MSY, as intrachromosomal homologous recombination occurs for nearly all Y-chromosome amplicon pairs, even those located on opposing chromosome arms.
Introduction

The male-specific region of the human Y chromosome (MSY) contains many amplicons – large, nearly identical repeats – whose sequence similarity is maintained by gene conversion (Skaletsky et al., 2003; Rozen et al., 2003). These long segments of high sequence identity render the Y chromosome susceptible to intrachromosomal homologous recombination that can result in interstitial deletions, duplications, inversions, or isodicentric chromosomes (Kamp et al., 2000; Sun et al., 2000; Blanco et al., 2000; Kuroda-Kawaguchi et al., 2001; Repping et al., 2002; Repping et al., 2003; Blanco et al., 2004; Repping et al., 2004; Repping et al., 2006; Lange et al., 2009). Interstitial Y deletions and isodicentric Y chromosomes are associated with a wide range of sex disorders including male infertility, Turner syndrome, and sex reversal.

Whereas each of the intrachromosomal homologous recombination events reported to date involved amplicons located on the same Y-chromosome arm, the Y chromosome also contains two sets of inverted repeats (IRs) that are composed of one amplicon on the short arm (Yp) and one amplicon on the long arm (Yq), namely IR1 and IR4 (Skaletsky et al., 2003). IR1 is composed of two 65-kb amplicons that share 99.66% sequence identity whereas IR4 is composed of two 275-kb amplicons that share 93.76% sequence identity. Of note, the IR1 repeat on Yq is located within the azoospermia factor c (AZFc) region that contains genes essential for spermatogenesis and is almost entirely ampliconic.

We hypothesized that intrachromosomal homologous recombination between amplicons of IR1 or of IR4 can generate two types of rearrangements: pseudoisochromosomes and pericentric inversions (Figure 1). For example, resolution of a double-strand break (DSB) in the Yq copy of IR1 by interchromatid crossing over with the Yp copy would produce a pseudoisoYp chromosome, which carries a partial duplication of Yp and a partial deletion of Yq, and a pseudoisoYq chromosome, which carries a partial duplication of Yq and partial deletion of Yp. Transmission of the former would likely result in male offspring with impaired spermatogenesis due to the removal of multiple genes from the AZFc region.

![Figure 1](image.png)

**Figure 1.** Mechanisms of pericentric inversion and pseudoisochromosome formation: homologous recombination between inverted repeats composed of one amplicon on the short arm (Yp) and one amplicon on the long arm (Yq): Intrachromatid crossing over produces a pericentric inversion (top of figure), while crossing over between sister chromatids generates pseudoisochromosomes.
Alternatively, resolution of a DSB in the Yq copy of IR1 by intrachromatid crossing over with the Yp copy would lead to a pericentric inversion. Although pseudoisoY chromosomes and Y-chromosome pericentric inversions have been described previously (Jacobs and Ross, 1966; Bernstein et al., 1986; Spurdle and Jenkins, 1992), it is unknown if these rearrangements indeed are generated via homologous recombination between inverted amplicons.

Here, we first catalog all variations of IR1 and of IR4 that could potentially be targets for such homologous recombination events, as well as the putative resultant pseudoisoY chromosomes and pericentric inversions. We then report four Y chromosomes – two pseudoisoYp chromosomes and two with pericentric inversions – that evidently have been formed by homologous recombination between amplicons of IR1 or of IR4. Finally, we describe the spermatogenic phenotypes of the two men with pseudoisoYp chromosomes.
Materials and Methods

Samples

The men studied originated from sample collections of the Onze Lieve Vrouwe Gasthuis, Amsterdam, the Maastricht University Medical Center, the Academic Medical Center in Amsterdam and the Whitehead Institute, Cambridge, USA. They were selected based on their karyotype data that indicated a pericentric inversion or a pseudoisoYp chromosome. Karyotyping of these men was performed as part of either fertility workup or prenatal screening.

For each patient, at least two semen analyses were performed according to WHO guidelines. From each patient genomic DNA was extracted from a venous blood sample.

Low resolution STS deletion screening

All included men were screened for deletions in the AZFc region using plus/minus PCR for the following low-resolution STSs: sY142, sY1191, sY1197, sY1201, sY1206 and sY1291 as described previously (Kuroda-Kawaguchi et al., 2001; Repping et al., 2004).

High resolution STS deletion screening

WHT5557 was screened for deletions using STSs listed in Table 2.

Fluorescence in situ hybridization

Metaphase and interphase nuclei were hybridized with probes that target the red (18E8), green (RP11-0363G06), yellow (79J10) amplicons in the AZFc region, a region proximal to IR1 on Yp (17224/17225), a region distal to IR1 on Yp (17228/17229), AMELY (RP11-0199M02), SRY (PDP1335), TSPY (RP11-0516H), and the centromere (PDP97DYZ), as previously described (Lange et al., 2009) For counting signals in interphase FISH, at least 200 nuclei were scored for each sample and set of probes.
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Results

A catalog of inverted repeat pairs composed of one Yp amplicon and one Yq amplicon

We closely examined the MSY reference sequence to determine the precise structure of the IR1 and IR4 repeats on Yp and on Yq (Skaletsky et al., 2003). On Yp, the IR1 and IR4 amplicons are each present in one complete copy. Similarly, on Yq, the IR4 amplicon is present in only one contiguous copy. By contrast, the IR1 amplicon on Yq is located within the 3.5-Mb AZFc region, which is comprised of five amplicons (blue [four copies b1-b4], turquoise [two copies t1 and t2], green [three copies g1-g3], red [four copies r1-r4], gray [two copies g1 and g2], yellow [two copies y1 and y2]) arrayed in direct and inverted orientations (Figure 2) (Kuroda-Kawaguchi et al., 2001). Since IR1 is composed of part of the blue amplicon b2 and part of the green amplicon g1, there are four additional loci on Yq that contain segments of IR1: in amplicons b3, b4, g2, and g3. (The portion of IR1 found in the blue amplicons b2, b3, and b4 is deleted in b1.) We termed these new loci IR1-like-1 (IR1L1) through IR1L4 based upon their position in the MSY reference sequence (Figure 2 and Table 1).

Table 1. Catalog of possible homologous recombination events involving IR4 or IR1/IR1Ls on Yp and Yq.

<table>
<thead>
<tr>
<th>Parental structure of IR3</th>
<th>Parental structure of AZFc Yp target Yq target</th>
<th>Size (kb)</th>
<th>Sequence identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Reference</td>
<td>IR4</td>
<td>IR4</td>
<td>275</td>
</tr>
<tr>
<td>Reference gr/rg inversion</td>
<td>IR4</td>
<td>IR1</td>
<td>65</td>
</tr>
<tr>
<td>Reference gr/rg inversion</td>
<td>IR1 IR1L2</td>
<td>35</td>
<td>99.61</td>
</tr>
<tr>
<td>Reference gr/rg inversion</td>
<td>IR1 IR1L4</td>
<td>30</td>
<td>99.73</td>
</tr>
<tr>
<td>Reference gr/rg inversion</td>
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<td>Reference gr/rg inversion</td>
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<td>99.73</td>
</tr>
<tr>
<td>Reference b2/b3 inversion</td>
<td>IR4</td>
<td>IR4</td>
<td>275</td>
</tr>
<tr>
<td>Reference b2/b3 inversion</td>
<td>IR1 IR1L1</td>
<td>30</td>
<td>99.73</td>
</tr>
<tr>
<td>Reference b2/b3 inversion</td>
<td>IR1 IR1L2</td>
<td>35</td>
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<td>IR1 IR1L4</td>
<td>30</td>
<td>99.73</td>
</tr>
<tr>
<td>IR3/IR3 inversion Reference</td>
<td>IR1 IR1L1</td>
<td>30</td>
<td>99.73</td>
</tr>
<tr>
<td>IR3/IR3 inversion gr/rg inversion</td>
<td>IR1 IR1L3</td>
<td>35</td>
<td>99.61</td>
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<tr>
<td>IR3/IR3 inversion b2/b3 inversion</td>
<td>IR1 IR1L3</td>
<td>35</td>
<td>99.61</td>
</tr>
</tbody>
</table>

The IR1 and IR4 amplicons on Yp and all IR1 amplicons on Yq are located in regions known to be subject to large-scale inversion. On Yp, a 3.6-Mb region containing the IR1 and IR4 amplicons and bounded by the IR3 inverted repeats has been inverted repeatedly during human history (Page, 1986; Affara et al., 1986; Jobling et al., 1998; Tilford et al., 2001; Skaletsky et al., 2003; Repping et al., 2006). On Yq, the orientations of segments that contain IR1, or any of the IR1Ls, can be polymorphic due to the frequently occurring gr/rg and b2/b3 inversions, and the palindrome 1 (P1) inversion (Repping et al., 2003; Fernandes et al., 2004; Repping et al., 2004) (Figure 2B, C). Thus, additional inverted repeat pairs potentially exist among extant human Y chromosomes. Taking these relatively common inversions into account, we find eighteen inverted repeat pairs as putative
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substrates for the proposed model of inter-arm homologous recombination (Figure 1, Table 1). For each of these eighteen pairs, sister chromatid crossing over would generate either a pseudoisoYp chromosome or a pseudoisoYq chromosome while intrachromatid crossing over would produce a pericentric inversion. Thus, homologous recombination between IR1 or IR4 copies could produce a total of 50 MSY structures that are distinct from the reference sequence (see Suppl. Note 1).

Figure 2. Possible location and orientation of inverted repeats IR1 and IR4 in the human Y chromosome. (A) Inverted repeats IR1-4, each composed of one amplicon on Yp and one amplicon on Yq, are indicated as well as IR3 from which both copies are located on Yp. Furthermore the probe positions used for FISH are indicated. Blocks of heterochromatin are shown in orange. (B, C) IR combinations that can result in pericentric inversions or pseudoisochromosomes are indicated in black whereas the ones that do not are indicated in red. B1 represents reference sequence Yp and Yq, B2 represents reference sequence Yp and Yq with gr/rg inversion, B3 represents reference sequence Yp and Yq with b2/b3 inversion. C1 represents Yp with IR3 inversion and reference sequence Yq, C2 represents Yp with IR3 inversion and Yp with gr/rg inversion, C3 represents Yp with IR3 inversion and Yq with b2/b3 inversion. See table 1 for sizes and sequence similarity between the various targets.
Patients

We identified from our sample collections two men, namely WHT5557 and AMC1574 that on the basis of karyotyping had pseudoiso Yp chromosomes and two men that had pericentric inverted Y chromosomes, namely AMC0972 and AMC0973. We performed plus/minus PCR assays and FISH to determine which homologues recombination event generated these chromosomes.

PseudoisoYp chromosomes

We had previously localized, in case WHT5557, a single breakpoint in the IR4 amplicon on Yq bounded by STSs sY1279 and sY1278 (Lange et al., 2009) (Figure 3). We used high-resolution breakpoint mapping to further delineate the breakpoint region in this case. Making use of the sequence divergence between IR4 on Yq and IR4 on Yp we designed plus/minus STS assays specific to the Yq repeat unit (Lange et al., 2008), and we precisely localized the breakpoint to an 800-bp interval (Table 2).

We then designed a primer pair for junction amplification by PCR, with one primer immediately proximal to the Yq breakpoint interval and a second primer immediately distal to the homologous interval on Yp. This primer pair amplified a product in WHT5557 but not in control individuals. Sequencing of the junction product confirmed the expected sequences to each side of a 155-bp segment of perfect homology to be those of Yp and Yq (Figure 4).

Figure 3. Location of the breakpoints in the two men with pseudoisochromosomes. (A) Schematic representation of the human Y chromosome, with the STS positions used. (B) STS results for WHT5557 and AMC1574. Black indicates presence of region whereas grey indicates the region where the deletion occurred. See Table 2 for high-resolution STS markers used to detect the breakpoint in WHT5557.

In AMC1574, STS mapping revealed a single breakpoint in the distal arm of palindrome P1 distal to sY1206 (Table 2, Figure 3). Given the multi-copy nature and high sequence similarity of IR1 on Yq, high-resolution breakpoint mapping was not feasible for this case. Instead, we performed single-color and two-color FISH experiments on metaphase spreads to assay the copy number and arrangement of several loci on Yp and Yq. Single-color FISH demonstrated that the green, yellow, and red amplicons from the AZFc region were located on Yq only, whereas FISH probe 17228/17229, located distal to the Yp copy of IR1, and RP11-0516H08 targeting TSPY, were present only on Yp (Figure 5). However, FISH probes 17224/17225, located proximal to the Yp copy of IR1, and RP11-
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Table 2. STSs used to detect breakpoints in the cases with pseudoisoY chromosomes. STSs are ordered from centromere to telomere on Yq. For the locations of sY1279 and sY1278, see Figure 4.

<table>
<thead>
<tr>
<th>STS</th>
<th>GenBank Accession Number</th>
<th>Results for WHT5557</th>
<th>Results for AMC1574</th>
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<td>sY1279</td>
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<td>+</td>
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<td>sY3014</td>
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<tr>
<td>sY1278</td>
<td>G75504</td>
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Figure 4. Junction sequence of WHT5557. (A, B) Sequence of the junction product of WHT5557 of a 153-bp segment of perfect homology marked by unique sequences from Yq on one side and from Yp on the other side.
0199M02 targeting AMELY, were present on both Yp and Yq. Two-color FISH showed that SRY was present on Yp and on Yq and that a single centromere was located between these two copies of SRY (Figure 5). In sum, these results indicate that the pseudoYp isochromosome in AMC1574 was formed due to a recombination event between the IR1 amplicon on Yp and the IR1L4 amplicon on Yq.

### Y-chromosome pericentric inversions

For AMC0972, single-color FISH on metaphase spreads demonstrated that copies of the green, yellow, and red amplicons of the AZFc region were present on Yp and on Yq. Further, whereas RP11-0199M02 and 17224/17225 were present on Yp, RP11-0516H08 and 17228/9 were located on Yq (Figure 5). Two-color FISH showed a green-red-green amplicon organization on Yp and a green-red amplicon organization on Yq (Figure 5). These results indicate that the pericentric inversion in AMC0972 is the result of two homologous recombination events: a gr/rg inversion on Yq which was followed by an inversion for which IR1 on Yp and IR1L1 on Yq were the targets.

For AMC0973, single-color FISH showed that green, yellow and red amplicons were present on Yp and Yq and two color FISH showed a green-red amplicon organization on Yp and a green-red-green amplicon organization on Yq (Figure 5). Unfortunately, insufficient cells were available for additional FISH experiments for this patient. These results indicate that the pericentric inversion in AMC0973 is most likely the result of homologous recombination between IR1 on Yp and IR1L2 on Yq.

### Spermatogenic phenotype

Whereas pericentric inversions do not result in loss of genomic content and thus should not influence spermatogenesis, recombination events that result in pseudoisoYp chromosomes can lead to loss of genes that are involved in spermatogenesis or to loss of the second pseudoautosomal region (PAR2), which might affect chromosome pairing during meiosis and thereby also impair spermatogenesis. Indeed, both men with pseudoisoYp chromosomes were phenotypically normal but had reduced semen quality: WHT5557 and AMC1574 were diagnosed with azoospermia and severe oligozoospermia, respectively. In contrast, both men with pericentric inversions, i.e. AMC0972 and AMC0973, were both phenotypically normal men with normozoospermia, as expected.
Figure 5. FISH results. N.D. stands for not done.
Discussion

We have demonstrated that intrachromosomal homologous recombination between two sets of inverted amplicons with copies on both the short and long arm of the Y chromosome, namely IR1, IR1Ls and IR4, can generate pericentric inversions and pseudoisoY chromosomes.

Both men with pericentric inversions had normal spermatogenesis according to WHO criteria which was to be expected as no genomic content is removed by the pericentric inversion. The two men with pseudoisoYp chromosomes on the other hand had severe oligozoospermia and azoospermaia and lacked a significant part of their Y chromosomes, namely the PAR2 region, a region that recombines with the X-chromosome during meiosis. The absence of this region could hamper proper chromosome segregation during meiosis and thereby spermatogenesis. In addition, one man also lacked the entire \textit{AZFc} region, a region essential for normal sperm production (Kuroda-Kawaguchi et al., 2001).

From a total of 50 predicted potential intrachromosomal homologous recombination events, we identified four: two pericentric inversions and two pseudoiso Yp chromosomes. FISH indicated that one pericentric inversion occurred on a Y chromosome with the reference organization on Yp and a \textit{gr/rg} inversion on Yq. The other pericentric inversion most likely occurred on a Y chromosome with the reference organization on Yp and Yq. It is currently unknown which factors determine the occurrence rate for such intrachromosomal recombinations although it has been speculated that the size of the amplicons, their overall sequence identity and their distance affect the frequency of occurrence (Repping et al., 2002).

Most likely all the Y chromosomes with pericentric inversions and the pseudoisoY chromosomes we have predicted exist in the human population. Since Y-chromosome pericentric inversions do not influence spermatogenesis or other phenotypes, identifying Y chromosomes with such inversions is rather a process of chance. PseudoisoYp chromosomes are expected to result in impaired spermatogenesis and thus these chromosomes should be identified during karyotype analysis as part of fertility work-up. The fact that our sample collection did not contain additional men with pseudoisoYp chromosomes is most likely due to the low prevalence of pseudoisoYp chromosomes in the population. This low prevalence is in turn caused by the poor reproductive capacity of men carrying a pseudoisoYp chromosome. An individual carrying a pseudoisoYq chromosome is expected to be phenotypically a female since \textit{SRY} is absent, and may present with Turner syndrome stigmata. Since our sample collection only contained males, we did not identify any pseudoisoYq chromosomes.

In conclusion, we have found that in addition to generating Y chromosomes carrying deletions, duplications, inversions and isodicentric Y chromosomes, intrachromosomal homologous recombination can generate Y chromosomes with pericentric inversions and pseudoisoY chromosomes. The catalogue of all possible MSY structures resulting from homologous recombination between IR1 or IR4 copies as well as the methods used to detect them presented in this paper should facilitate the molecular classification of all Y chromosomes with pericentric inversions and pseudoiso Y chromosomes.
Acknowledgements

The authors thank Lia Knegt for karyotype analysis.
References


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Supplementary Note 1. The 50 possible structures resulting from homologous recombination between IR1 or IR4 repeats on opposing arms of the Y chromosome:

Reference Y chromosome structure (A1) resulting in pericentric inversions (A1.1-A1.4)
Reference Y chromosome structure (A2) resulting in pseudoisoYp chromosomes (A2.1-A2.4)
Reference Y chromosome structure (A3) resulting in pseudoisoYq chromosomes (A3.1-A3.4)
Y chromosome with gr/rg inversion (B1) resulting in pericentric inversions (B1.1-B1.4)
Y chromosome with gr/rg inversion (B2) resulting in pseudoisoYp chromosomes (B2.1-B2.4)
Y chromosome with gr/rg inversion (B3) resulting in pseudoisoYq chromosomes (B3.1-B3.4)
Y chromosome with b2/b3 inversion (C1) resulting in pericentric inversions (C1.1-C1.4)
Y chromosome with b2/b3 inversion (C2) resulting in pseudoisoYp chromosomes (C2.1-C2.4)
Y chromosome with b2/b3 inversion (C3) resulting in pseudoisoYq chromosomes (C3.1-C3.4)
Y chromosome with IR3/IR3 inversion (D1) resulting in pericentric inversions (D1.1-D1.2)
Y chromosome with IR3/IR3 inversion (D2) resulting in pseudoisoYp chromosomes (D2.1-D2.2)
Y chromosome with IR3/IR3 inversion (D3) resulting in pseudoisoYq chromosomes (D3.1-D3.2)
Y chromosome with IR3/IR3 and gr/rg inversion (E1) resulting in pericentric inversions (E1.1-E1.2)
Y chromosome with IR3/IR3 and gr/rg inversion (E2) resulting in pseudoisoYp chromosomes (E2.1-E2.2)
Y chromosome with IR3/IR3 and gr/rg inversion (E3) resulting in pseudoisoYq chromosomes (E3.1-E3.2)
Y chromosome with IR3/IR3 and b2/b3 inversion (F1) resulting in pericentric inversions (F1.1-F1.2)
Y chromosome with IR3/IR3 and b2/b3 inversion (F2) resulting in pseudoisoYp chromosomes (F2.1-F2.2)
Y chromosome with IR3/IR3 and b2/b3 inversion (F3) resulting in pseudoisoYq chromosomes (F3.1-F3.2)
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Intrachromosomal homologous recombination between inverted amplicons