Complex chromosomal rearrangements: origin and meiotic behavior

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BACKGROUND: Complex chromosomal rearrangements (CCRs) describe structural rearrangements, essentially translocations, involving at least three breakpoints on two or more chromosomes. Although they are rare in humans, their clinical identification is important since CCR carriers can display various phenotypes which include phenotypically normal subjects, infertile males and patients with mental retardation and/or congenital abnormalities. The rearrangement can be de novo or familial. The use of fluorescent in situ hybridization assays and molecular techniques for the characterization of CCRs have indicated that the rearrangements could be more complex than initially assumed. Accumulating data have revealed that the mechanisms underlying the genesis of CCRs remain elusive.

METHODS: We performed a large PubMed search in order to summarize the current knowledge in this field and address important aspects of CCR formation and meiotic behavior, highlighting the complexity of these rearrangements at the chromosomal and genomic level.

RESULTS: The review of published data indicates that the complexity of CCRs is becoming increasingly known, thanks to the application of more and more efficient molecular techniques. These approaches have allowed the precise sequence analysis of breakpoints and the identification of insertions, deletions, inversions and recombination events. New models have been proposed for the formation of CCRs, based on replication-based mechanisms and specific sequence elements. Their meiotic behavior has been discussed in the light of these new molecular data.
Complex chromosomal rearrangements (CCRs) are structural abnormalities involving more than two chromosome breaks with exchanges of chromosomal segments. The great majority of CCRs are combinations of translocations. To date, <255 cases of CCRs involving three or more chromosomes have been reported. CCRs are rare events, which can be familial or de novo in origin (Pai et al., 1980). Various classifications of CCRs have been proposed according to their nature, the number of chromosome breaks, their distribution or the involvement of intra-chromosomal rearrangements. Heterozygous carriers of balanced CCRs are considered to have a high risk for spontaneous abortion or chromosomally abnormal offspring, since the meiotic segregation of a CCR can theoretically lead to numerous unbalanced chromosomal configurations. However, risk estimates remain highly empirical, ranging from 50 to 100% for spontaneous abortion (Batista et al., 1994), and from 20 to 90% for phenotypic abnormalities (Gorski et al., 1988). The difficulty of genetic and reproductive counseling of CCRs is linked to the unique and complex nature of each CCR, and the paucity of available data on their meiotic segregation. In addition, male carriers of CCRs are often subfertile or sterile due to spermatogenesis arrest.

The advent of molecular cytogenetic techniques has greatly improved the characterization of complex rearrangements. More recent studies have used microarray technologies to uncover cryptic rearrangements located near the breakpoints. They have indicated that many CCRs may harbor a more complex rearrangement not detectable by routine cytogenetics. In males, the application of fluorescent in situ hybridization (FISH) techniques on sperm nuclei allows the direct analysis of the meiotic chromosomal segregation of a few CCRs in gametes (Loup et al., 2010). The feasibility of preimplantation genetic diagnosis (PGD) of embryos in couples with CCRs by FISH strategies has also been demonstrated (Escudero et al., 2008).

The first part of this review presents the data related to the origin and formation of CCRs, and discusses their ascertainment, classification and pregnancy outcomes. The evolution of the techniques for CCR characterization is also discussed. The second part of this review focuses on the meiotic behavior of CCRs and implications for fertility, summarizing data on meiotic segregation and discussing recent results on direct sperm chromosome analysis in male carriers of CCRs and the introduction of PGD for CCR couples.

For the second part, we used combinations of the following search terms: meiosis, segregation, recombination, pachytene diagram, sperm FISH, fertility, prenatal diagnosis, PGD.

In addition, we searched references cited in the retrieved articles. The articles cited were selected on the basis of relevance and quality. We further included published and unpublished data from our own laboratory.

**Origins and features of CCRs**

**Ascertainment of CCRs**

In humans, CCRs are uncommon events, which have never been reported in any of the large cytogenetic surveys of newborns (Hamerton et al., 1975) or in couples experiencing spontaneous abortions (Creasy, 1989; De Braeckeleer and Lin, 2006).

To date, no more than 255 cases of CCRs have been described (Gouny et al., 2006; Ergul et al., 2009; Zhang et al., 2009a, b). According to the literature, about 70% of CCRs are detected in phenotypically normal subjects, 20–25% in patients with congenital abnormalities and/or mental retardation and 5–10% are detected during prenatal diagnosis (Fig. 1; Madan et al., 1997; Batanian and Eswara, 1998; De Gregori et al., 2007). Some rare cases have also been detected because of psychiatric troubles (Lespinasse et al., 2004; Gouny et al., 2006). In addition, it must be remembered that CCRs are also frequently observed in tumoral cells. In particular, CCRs are associated with hematological malignancies (Masuko et al., 2009; De Braeckeleer et al., 2010). The occurrence of CCRs in cancer cells constitutes a particular aspect of the tumoral genesis process which is beyond the scope of this review, and consequently this will not be discussed here.

When a CCR is detected in a phenotypically normal subject, the rearrangement is generally assumed to be balanced and it may represent a familial case. Familial transmission mainly occurs through female carriers (Batista et al., 1994). Thus, balanced familial CCRs are essentially identified in women referred for advanced maternal age, repeated spontaneous abortion or birth of a malformed child (Timar et al., 1991; Kotzot et al., 2001; Gardner et al., 2004; Karmous-Benailly et al., 2006; Park et al., 2007; Zhang et al., 2009a, b). Transmission through males has been documented in only a few families (Schwanitz et al., 1978; Meer et al., 1981; Röthlisberger et al., 1999; Gruchy et al., 2009). Nevertheless, the majority of CCRs, i.e. about 70–75%, are de novo in origin (Fig. 1). The de novo CCRs are found in equal proportion among phenotypically normal subjects (49%) and individuals with phenotypic abnormalities (51%) (Madan et al., 1997; Patsalis, 2007), due to submicroscopic imbalances or other genetic defects (Kumar et al., 1998). The de novo CCRs appear to be preferentially of paternal origin (Batista et al., 1994). Most of the male carriers of balanced de novo CCRs are diagnosed because of infertility troubles (Joseph and Thomas, 1982; Siffroi et al., 1997; Coco et al., 2004; Scott Sills et al., 2005; Bartels et al., 2007), although...
a few cases of fertile carriers have been reported (Bourrouillou et al., 1983; Walker et al., 1985; Cai et al., 2001; Karadeniz et al., 2008).

All these data have emphasized the difference in the parental origin between familial and de novo CCRs. This has led to the assumption that CCRs could arise preferentially during spermatogenesis and are mainly transmitted in families through oogenesis (Gorski et al., 1988; Batista et al., 1994). This hypothesis agrees with the epidemiological finding that most of the prenatally ascertained balanced CCRs are maternal in origin (70% maternal versus 30% paternal) (Giardino et al., 2005; Thomas et al., 2006). These observations are also in good concordance with the biological finding of a higher rate of structural chromosome aberrations in human male gametes (Templado et al., 2005). However, only a small proportion of CCRs has been detected at prenatal diagnosis (Bogart et al., 1986; Kim et al., 1986; Batista et al., 1993; Ruiz et al., 1996; Soler et al., 2005; Chen et al., 2006). In Giardino et al. (2006) reviewed 21 prenatally ascertained CCRs and noted that 15 (71%) were de novo in origin and apparently balanced (Sikkema-Raddatz et al., 1995; Mercier et al. 1996). Only five were maternally inherited (Bellec and de Perdigo, 1991; Delaroche et al., 1995; Lee et al., 2002; Bourthoumieu et al., 2004; Migliori et al., 2004) and one was inherited from the father (Soler et al., 2005). A significant proportion (45%) of CCRs detected prenatally represents a ‘re-building’ of the initial rearrangement through recombination, giving rise to simpler (Tihy et al., 2005) or more complicated karyotypes (Bass et al., 1985; Masuno et al., 1993; Zahed et al., 1998; Berend et al., 2002; Soler et al., 2005).

Finally, it is important to note the impact of new technologies on the ascertainment of CCRs, and the fact that what is found is only what is investigated. A good illustration of this point is the example of insertional translocations (ITs). The intercalation of a segment of one chromosome into another non-homologous chromosome requires at least three chromosomal breaks and is thus qualified as a CCR (Gray et al., 1972). ITs are rarely observed in routine chromosomal analysis. Most ITs are inherited and discovered because of congenital abnormalities and mental retardation. In a review on ITs, Van Hemel and Eussen (2000) estimated the incidence of ITs to be 1:80 000, which is similar to the previous finding of Fryns et al. (1984) with only four ITs among 40 000 karyotyped patients. A recent re-evaluation of IT frequency by applying array-comparative genomic hybridization (CGH) led to an estimate of 1:500, i.e. an incidence 160 times higher than previously recognized (Kang et al., 2010). In 2005, de Ravel et al. (2005) reported a de novo IT of chromosome 16p13.3 into the short arm of chromosome 22, in a dysmorphic boy with severe mental retardation. This small interstitial duplication could not be detected without array-CGH. This observation, like several recent case reports (Fauth et al., 2006; Carvalho et al., 2009; Spreiz et al., 2010), reinforces the power of array-CGH procedures for detecting and clarifying CCRs. Next to high-resolution array-CGH analysis, FISH assays remain essential for delineating CCRs in situ. One can predict that the combined application of array-CGH technologies and FISH will lead to the identification of an increased number of CCRs, indicating that CCRs have an unexpected level of complexity and are probably much more common than considered, particularly in patients with apparently balanced rearrangements.

Classification of CCRs

Faced with the large variety of CCRs and their complex nature, various classifications have been proposed in order to define and compare both the significance and the outcome of CCRs of similar complexity. A categorization based on the mode of transmission was initially proposed by Kleczkowska et al. (1982). They reviewed 38 cases of CCRs and divided them into familial and de novo rearrangements. According to this classification, the authors found a significant prevalence of maternal origin in familial CCRs, and more CCRs in de novo CCRs. In 1993, Kousseff et al. (1993) proposed a classification based on the number of chromosome breaks (Table I). This classification divided CCRs into two large groups: those with four or fewer breaks, and those with more than four breaks. About 150 CCRs were thus classified. The authors observed that most familial CCRs belonged to group I and have been transmitted through a balanced...
female carrier, whereas most de novo CCRs belonged to group II (Joyce et al., 1999; Lee et al., 2002; Grasshoff et al., 2003; Kuechler et al., 2005). An alternative categorization was based on both the location and the distribution of breakpoints (Lurie et al., 1994), distinguishing CCRs with intrachromosomal rearrangements (insertions, inversions, duplications) from those without.

Finally, Kausch et al. (1988) categorized CCRs according to their structure, dividing them into three classes (Fig. 2): (i) three-way rearrangements, (ii) exceptional CCRs and (iii) double two-way translocations. The first type constitutes the most common class of CCRs, including cases where three chromosomes break and exchange chromosomal segments. These CCRs are principally familial and can be transmitted from generation to generation (Meer et al., 1981; Farrell et al., 1994; Zahed et al., 1998). The group of exceptional CCRs involves rearrangements in which more than one breakpoint per chromosome is found (Bass et al., 1985; Batanian and Eswara, 1998). These rearrangements can reach an extremely high degree of complexity with as many as seven derivative chromosomes (Kousseff et al., 1998) as in 7 balanced CCRs (5 females; 2 males) identified by prenatal diagnosis among 246 balanced chromosomal rearrangements (Giardino et al., 2009). Such a sex ratio bias in the occurrence of CCRs has no direct biological explanation. This might not reflect a real difference at conception, but could be a bias linked to the small number of reported cases of CCRs in the studies mentioned. These sex ratio observations must also be clearly distinguished from the well-documented preferential maternal transmission of both classical chromosomal translocations and CCRs (Page and Shaffer, 1997; Patsalis et al., 2009). Consequently, heterozygous females can be fertile and have pregnancies that produce phenotypically normal children. Men appear to be more susceptible to disturbances of gametogenesis.

In 1997, Madan et al. (1997) refined the above classification by proposing an improved classification of exceptional CCRs, which integrates the presence of additional inversions, insertions or deletions in the translocated chromosomes. Thus, intrachromosomal and interchromosomal ITs are also classified (qualified) as CCRs (de Ravel et al., 2001; Juchniuk de Vozzi et al., 2009).

**Pregnancy outcomes in CCR carriers**

Useful information regarding the pregnancy outcome of individuals carrying CCRs has been provided only by a few studies. In 1988, Gorski et al. (1988) collected data on 67 pregnancies in 25 families where CCRs were identified. They found an overall incidence of 48.3% spontaneous abortions, then 53.7% abnormal pregnancy outcomes, including 18.4% with malformation in live born children. In a review of 35 familial CCRs, Batista et al. (1994) compiled 63 karyotyped individuals born to the index CCR carriers. Among them, 27% had normal karyotypes, 31.7% were balanced carriers and 41.3% displayed an unbalanced karyotype. In accordance with Gorski's data, the authors reported a 50% risk of miscarriage. Madan et al. (1997) reviewed 60 familial and de novo cases of balanced CCRs, and estimated that carriers have a 50% risk of spontaneous abortion and a 20% risk of having a child with an unbalanced karyotype. Among these 60 cases, 27 with post-natal data showed 48% normal individuals and 52% phenotypically abnormal subjects (Madan et al., 1997).

Batista et al. (1994) also observed a noticeable excess of balanced female offspring versus male offspring (16 females; 4 males) and a slight excess of unbalanced females (15 females; 11 males), whereas the sex ratio in normal offspring remained equitable (9 males; 8 females). A similar excess of female offspring was previously reported by Walker et al. (1985) among the offspring of 16 CCR families, as well as in 7 balanced CCRs (5 females; 2 males) identified by prenatal diagnosis among 246 balanced chromosomal rearrangements (Giardino et al., 2009). Such a sex ratio bias in the occurrence of CCRs has no direct biological explanation. This might not reflect a real difference at conception, but could be a bias linked to the small number of reported cases of CCRs in the studies mentioned. These sex ratio observations must also be clearly distinguished from the well-documented preferential maternal transmission of both classical chromosomal translocations and CCRs (Page and Shaffer, 1997; Patsalis et al., 2009). Consequently, heterozygous females can be fertile and have pregnancies that produce phenotypically normal children. Men appear to be more susceptible to disturbances of gametogenesis. The presence of a chromosomal rearrangement can alter chromosomally, because of the absence of a sex vesicle as contact element, and the less stringent female meiotic checkpoints (LeMaire-Adkins et al., 1997; Steuerwald, 2005; Kurahashi et al., 2009).

**Table 1** Incidence of breakpoints detected in 251 CCRs reported in the literature to date.

<table>
<thead>
<tr>
<th>Number of breakpoints per CCR</th>
<th>Number and % of detected cases</th>
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<tbody>
<tr>
<td>3</td>
<td>75 (29.9%)</td>
</tr>
<tr>
<td>4</td>
<td>73 (29.1%)</td>
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<tr>
<td>5</td>
<td>38 (15.1%)</td>
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<tr>
<td>6</td>
<td>22 (8.76%)</td>
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<tr>
<td>7</td>
<td>17 (6.77%)</td>
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<tr>
<td>8</td>
<td>11 (4.38%)</td>
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<tr>
<td>9</td>
<td>9 (3.58%)</td>
</tr>
<tr>
<td>10</td>
<td>2 (0.79%)</td>
</tr>
<tr>
<td>11</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>12</td>
<td>2 (0.79%)</td>
</tr>
<tr>
<td>13</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>14</td>
<td>1 (0.04%)</td>
</tr>
<tr>
<td>15</td>
<td>1 (0.04%)</td>
</tr>
<tr>
<td>Total</td>
<td>251</td>
</tr>
</tbody>
</table>

Source: Madan et al. (1997), Batanian and Eswara (1998), Gorski et al. (1988), Vermeulen et al. (2004), Soler et al. (2005), De Gregori et al. (2007), Zhang et al. (2009a, b), Schluth-Bolard et al. (2009), Giardino et al. (2009), Ballarini et al. (2009).
or CCRs (Mau-Holzmann, 2005; Oliver-Bonet et al., 2005). In rare cases, probably because of less robust checkpoints, male carriers of CCRs are able to complete spermatogenesis (Ferguson et al., 2007). A few exceptional cases of male inheritance of CCRs have been documented (Röthlisberger et al., 1999; Grasshoff et al., 2003).

Concerning the pregnancy outcome of individuals carrying a double two-way translocation, information is limited to a few cases. In 1970, Jacobs et al. (1970) documented the case of two women carrying a t(1q;Cq) and a t(Dq;Dq), featuring 10 miscarriages, two children with only the balanced t(1q;Cq) and one child with both balanced translocations. In 1986, Burns et al. (1986) analyzed the meiotic segregation in sperm of a man heterozygous for both a t(5;11) and a t(7;14) reciprocal translocations. The proband was ascertained through prenatal diagnosis after his wife had experienced four spontaneous abortions and given birth to a balanced t(7;14) male infant and to an unbalanced child with the cri-du-chat syndrome. It is clear from the published reports that the risk of phenotype alterations increases with the number of chromosomes involved in CCRs and the number of breakpoints (Ruiz et al., 1996; Madan et al., 1997; Giardino et al., 2006).

**Evolution of technical approaches for CCR investigation**

Both the identification of CCRs and the accurate delineation of their breakpoints are dependent on the quality of the chromosomal analysis. The initial identification of CCRs was made using conventional cytogenetics based on banding techniques and high-resolution chromosome analysis (Fitzgerald, 1974; Seabright et al., 1978; Stoll et al., 1979; Smith et al., 1985; Köhler et al., 1986). In numerous cases, these approaches did not allow resolution the complexity of CCRs. Indeed, only large structural rearrangements, >5 Mb, can be identified by routine cytogenetic techniques, due to their limited level of resolution. In addition, the interpretation of banding may be complicated by chromosomal condensation and imperfect banding.

The characterization of CCRs has been greatly improved by the introduction of molecular cytogenetic techniques, essentially FISH. Various FISH methods have been tested for investigating CCRs, illustrated by a growing number of reports, demonstrating that CCRs

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**Figure 2** Classification of CCRs according to their structure. Three classes can be distinguished: (A) Three-way CCR in which three segments from three chromosomes (A, B, C) break off and translocate, leading to the formation of three derivative chromosomes der(A), der(B) and der(C). (B) Exceptional CCR, encompassing a wide range of complicated structural rearrangements (i.e. translocation, inversion, insertion, duplication, deletion) and involving more breakpoints than chromosomes. Derivative chromosomes are produced and recombination may provide supplementary complexity in the meiotic segregation of these CCRs. (C) Double two-way CCR, which includes two or more simultaneous independent simple reciprocal translocations between pairs of chromosomes, which independently segregate through meiosis, producing normal chromosomes (A, B, C, D) and derivative chromosomes [der (A), der(B), der(C), der(D)].
could be more complex and more common than initially thought (Zhang et al., 2009a, b). The simplest FISH technique used has been whole chromosome painting (WCP) to identify chromosome fragments involved in complex rearrangements (Van der Burgt et al., 1992; Friling et al., 1995; Ruiz et al., 1996; Wallerstein et al., 1996; Fuster et al., 1997; Kim et al., 2001). Analyses using subtelomeric- and/or locus-specific probes have also been performed (Wang et al., 1993; Curott et al., 1999; Lee et al., 2002; Astbury et al., 2004; Patsalis et al., 2004; Park et al., 2007). Anecdotally, the Primed IN Situ (PRINS) technique and Fiber-FISH procedure have been combined with standard FISH for *in situ* investigations of CCRs (Brandt et al., 1997; Gajecka et al., 2006a). The FISH approach has often allowed the re-interpretation of CCRs by detecting additional complexity. Thus, Batista et al. (1994) reported the case of a familial CCR initially interpreted as a three-way translocation, which was re-evaluated by FISH to be a translocation and an insertion. In the same way, Gibson et al. (1997) reported the case of a woman carrying an apparently balanced translocation between chromosomes 1 and 5 for which FISH analysis detected an additional insertion, not revealed by G-banding. Unusual CCRs exhibiting recombination and subsequent change in derivative chromosomes are another good example of the usefulness of molecular cytogenetic techniques in order to delineate the exact nature of CCRs. Soler et al. (2005) documented the study of a family with a double two-way translocation for which FISH assays were essential for characterizing the rearrangement since one of the two reciprocal translocations was cryptic. However, even results of routine FISH analyses may be difficult to interpret because of highly rearranged karyotypes. Alternative approaches were provided by multicolor FISH techniques, based on the *in situ* hybridization of 24 fluorescent-labeled chromosome painting probes, such as in M-FISH, spectral karyotyping (SKY) or COBRA-FISH (Speicher et al., 1996; Schröck et al., 1996; Tanke et al., 1999), which are relevant for more accurate characterization of CCRs (Tanemura et al., 2001; Vermeulen et al., 2004; Rosenberg et al., 2005). However, these techniques also present limits for the detection of intra-chromosomal rearrangements (Lee et al., 2002), and they have given rise to misclassifications. Thus, Houge et al. (2003) reported a discrepancy between M-FISH and SKY results in the study of a 10-year-old boy with a constitutional CCR involving 5 chromosomes and 15 breakpoints. A further increase in resolution in the *in situ* analysis of CCRs was obtained with the multicolor-banding (MCB) technique, introduced by Liehr et al. (2002) and improved by Weise et al. (2003). This sophisticated technique gives to each individual chromosome a color banding pattern based on 138 overlapping chromosomal region-specific microdissection libraries (Fig. 3). This procedure allows a more refined delineation of chromosomal breakpoints at the GTG sub-band level, with a resolution of a few megabase pairs (Weise et al., 2008). The efficiency of the MCB technique has been demonstrated in several recent cytogenetic studies of CCRs (Kuechler et al., 2005; Bartels et al., 2007; Karadeniz et al., 2008; Ergul et al., 2009). In the report of Houge et al. (2003) mentioned above, the clarification of the 16 fragments in the CCR was obtained thanks to the MCB technique.

All these data demonstrate that FISH and its derivative techniques have considerably facilitated the characterization of CCRs and become essential for the precise delineation of chromosomal breakpoints. The application of CGH and array-CCG techniques has represented supplementary advancements, essential for elucidating the molecular nature and the origin of CCRs.

A particularly disturbing aspect of CCRs, is the observation of subjects carrying apparently balanced CCR, who present phenotypic abnormalities and/or psychiatric disorders (Batanian and Eswara, 1993; Curotti et al., 1993; Weise et al., 2006a). The FISH approach has considerably facilitated the characterization of CCRs and represented supplementary advancements, essential for elucidating the molecular nature and the origin of CCRs.

![Figure 3](image_url) Example of the use of MCB probes for investigating a CCR. In a female with fertility problems banding cytogenetics revealed a karyotype 46,XX,der(4),der(5),der(6); derivative chromosomes are marked by arrowheads. Using MCB the final karyotype was: 46,XX,der(4)t(4;5:6)(4pter→4q24::5q14→5q32.2::5q32–33.1→5q23.2→6q12→6qter),der(5)t(4;5)(4qter→4q24::5q14→5pter),der(6)t(5;6)(6pter→6q12::5q32–33.1→5qter).
1998; Goumy et al., 2006; Borg et al., 2007). Such features, observed in about 30–40% of apparently balanced CCRs, have suggested the occurrence of genomic alterations in the vicinity of breakpoints or elsewhere in the genome. A somewhat different troubling scenario is the occurrence of de novo cryptic gains or losses on the background of a parental balanced rearrangement (Aboura et al., 2003; Lespinasse et al., 2004; Sismani et al., 2008). With respect to these questions, CGH and array-CGH have provided new essential tools for mapping genomic aberrations such as cryptic imbalances or gene disruption, since they allow a rapid genome-wide survey with a resolution ranging from 5 Mb for CGH to 35 kb for array-CGH (Ness et al., 2002; Vissers et al., 2007; Higgins et al., 2008; Ballarati et al., 2009; Haj et al., 2009; Lee et al., 2010). In the case of small imbalances of a few nucleotides, an additional STS marker analysis and/or sequence analysis have been sometimes performed (Gajecka et al., 2006b). In the study of a de novo balanced t(2;10;11), Rosenberg et al. (2005) found a 0.15–1.5 Mb cryptic deletion associated with one of the breakpoints. More recently, Zhang et al. (2009a, b) identified two microdeletions and one microduplication in a fetus carrying an apparently balanced CCR, t(4;5;15), on the basis of G-banding and FISH assays. Among seven unrelated mental retardation patients carrying an apparently balanced de novo (5 patients) or inherited (2 patients) rearrangement CCRs, Schluth-Bolard et al. (2009) reported that five of these CCRs were imbalanced, with deletions ranging from 690 kb to 4.9 Mb. These findings were consistent with results of previous studies (Gribble et al., 2005; De Gregori et al., 2007) and led the authors to suggest that about 30–40% of apparently balanced de novo rearrangements with abnormal phenotype could be associated with a cryptic genomic imbalance, not systematically related to the breakpoints. Similar conclusions have been expressed in other recent studies of patients with apparently balanced CCRs (Sismani et al., 2008; de Vree et al., 2009).

The significances of these data are still unclear but they emphasize that more detailed molecular analyses are required to elucidate the complexity of rearrangements and their genesis.

**The formation of CCRs**

The mechanism(s) underlying the formation of CCRs are complex as testified by the level of complexity of CCRs in karyotypes, evidenced by recent studies using FISH and molecular methodologies.

In order to explain the formation of CCRs through the smallest possible number of chromosomal events, the simplest proposition could be that CCRs simply originate from two coincidental classical translocations. Nevertheless, even if the occurrence of three or more simultaneous but independent breakage-reunion events is consistent with the fact that most CCRs are de novo in origin, this hypothesis constitutes a simplistic explanation. The assumption of such a ‘catastrophic’ event, producing multiple chromosome breakages and derivative chromosomes within the span of a single cell division, has been evoked in several studies (Gribble et al., 2005; Seller et al., 2006).

Another hypothesis is based on a possible chain of chromosomal events. A plausible scenario could be chromosome ‘jumping’. Jumping translocations (JTs) are complex chromosomal events in which a donor chromosomal segment is translocated to various recipient chromosome sites. JTs are rarely observed in constitutional karyotypes, with <50 reported cases (Reddy, 2010), and they usually describe mosaicism with chromosome jumping occurring over the course of several cell divisions. JTs may be associated with various phenotypic abnormalities (Rivera et al., 1990; Lefort et al., 2001; Edwards and Waters, 2008) or malignancy (Berger and Bernard, 2007). Little is known about the origin of JTs, but their breakpoints frequently involve areas of repetitive DNA sequences such as telomeric or centromeric domains (Farrell et al., 1993; Bouffer et al., 1996). This has suggested that telomere shortening, pericentromeric demethylation and architecture of repeat sequences might contribute to the multistep process of JT formation (Sawyer et al., 1998; Reddy, 2010).

In a sequential formation, the occurrence of an initial chromosome break should lead to the formation of an unstable chromosomal constitution, followed by sequential secondary breaks that will bring final genomic stabilization (Rosenberg et al., 2005). In agreement with this postulate, numerous complex rearrangements observed in cancers are presumed to result from structural aberrations accumulated through several cell divisions. In constitutional CCRs, karyotypes might appear to be ‘chaotic’ with no evidence for a specific order of events. A ‘chaotic’ multi-way rearrangement might thus reflect the cellular efforts to repair meiotic chromosomal knots. Houge et al. (2003) suggested such a faulty meiotic repair process to explain the origin of the most complex constitutional CCR reported so far, involving 5 chromosomes and 15 breakpoints. In accordance with the predominant paternal origin of de novo CCRs, a chaotic sequential procedure might occur preferentially during spermatogenesis rather than oogenesis. Both the higher number of mitoses in sperm versus oocyte formation, and the lack of efficient DNA repair system in late spermatogenesis could contribute to generation of CCRs.

However, a post-zygotic origin cannot be ruled out, since chromosomal instability appears to be a hallmark of early development (Boué, 1992; Pellestor, 1995). Whole genome-wide array screening of human preimplantation embryos has revealed the high frequency (90%) and the complexity of chromosomal aberrations in early human embryos (Vanneste et al., 2009a). Not only aneuploidies, mosaicsisms or uniparental disomies, but also segmental deletions, amplifications and duplications were observed in early human embryos (Vanneste et al., 2009b), suggesting that a particular vulnerability applies to these very early mitoses. This high prevalence of chromosome instability might favor the genesis of numerous secondary rearrangements and subsequent CCRs during early embryological development, as observed in some tumor cells (Smith et al., 2001). It has been established that impaired mitotic assembly checkpoints and deregulated microtubule dynamics cause enhanced chromosome instabilities (Rao et al., 2009; Thompson et al., 2010). Recently, gene expression profiling performed on human oocytes and preimplantation embryos has indicated that genes implicated in DNA repair processes as well as in chromosome segregation and cell division checkpoints, are detected and potentially functional in these cells (Kocabas et al., 2006; Li et al., 2006; Jones et al., 2008; Jaroudi et al., 2009). However, there are significant changes in the human transcriptome following fertilization through Day 3 of development, with the degradation of maternal transcripts and the activation of zygote genes (Dobson et al., 2004; Jaroudi et al., 2009). This confirms the assumption that the activation of embryonic genes in humans occurs between the 4- and 8-cell stages of preimplantation development (Braude et al., 1988; Schultz, 2002). In rapidly dividing blastomeres, characterized by
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brief G1 and G2 phases, the recruitment and/or the activation of the cellular machinery for DNA repair and cell cycle checkpoints might be less efficient. This could explain why during this early stage of development, the human embryo is more vulnerable to DNA damage and to the genesis of chromosome rearrangements.

At a cytogenetic level of resolution, and according to the structure of human chromosomes, the length of individual chromosomes could be a determining factor for the occurrence of breakages. The observation of several breaks in small chromosomes like chromosomes 18, 21 and 22 (Batanian and Eswara, 1998; Ergul et al., 2009), has contradicted the belief that large chromosomes are more prone to breakage. This assumption has been confirmed by Giardino et al. (2009) in prenatally diagnosed de novo balanced rearrangements. They reported that the number of breakpoints was not directly proportional to the length of chromosomes. With regard to the ratio between the haploid genome length and the number of breakpoints, some short chromosomes like chromosomes 16, 20, 21 and 22 display a higher density of breakpoints than some long chromosomes (chromosomes 1, 6, 12, X) (Giardino et al., 2009). Also, statistical analyses of both the involvement and re-involvement of chromosomes in CCRs have pointed out that some chromosomes, such as chromosomes 2, 3, 4, 7, 11 are more frequently implicated in CCRs than would be expected (Lurie et al., 1994; Zhang et al., 2009a, b). The possibility that some chromosome bands could be ‘hot spots’ for breakage and CCR formation has thus been postulated. In a study of four cases of de novo CCRs and a review of the literature, Vermeulen et al. (2004) found that 30% of CCRs, had breakpoints on chromosome 7, especially on region q21.1 which contains genes involved in neuronal development. Batanian and Eswara (1998) noted that band 18q21 was the assigned breakpoint in 11 of 14 reported CCR cases involving chromosome 18. Battisti et al. (2003) confirmed the hotspot on 18q21, leading to the speculation of the existence of clusters of breakage within specific bands. A non-random distribution of breakpoints in R-bands for structural chromosome rearrangements was postulated by Nakagome and Chiyo (1976). In the breakpoint FISH mapping of 152 apparently de novo balanced chromosomal rearrangements from patients with or without associated phenotype abnormalities, Fantes et al. (2008) observed the preferential location of breakpoints in R-bands, but they also indicated that the frequency of breakpoints disrupting genes was similar in the two study groups. This finding was supported by the results of a similar study performed by Baptista et al. (2008), on 31 normal and 14 abnormal carriers of balanced translocations. The authors also indicated that the great majority of the breakpoints were located in the vicinity of genes, within <200 kb (Baptista et al., 2008). Since the R-bands contain most of the genes (Saitoh and Laemmli, 1994), the involvement of genes in breakpoints could be completely fortuitous. Also, it is important to note that even if some chromosomes or specific chromosome bands appear to be preferentially involved in CCRs, most of the CCRs do not share specific breakpoints or partner chromosomes. Consequently, speculation about the possibility of chromosome or chromosomal region predisposition remains subject to caution.

With regards to molecular mechanism(s), the occurrence of chromosomal structural rearrangement is essentially dependent on two distinct events: double-strand breaks (DSBs) and DSB repair (Shaw and Lupski, 2004). Various endogenous and exogenous factors (ionizing radiations, chemotherapeutic drugs, free radical oxidative damage, viral infection) can damage chromosomes and lead to DSB (Lucas et al., 1992; Tsai and Lieber, 2010). Unrepaired DNA DSBs are powerful initiators for generating structural rearrangements (Richardson and Jasin, 2000). Accumulating data have indicated that both the recombination-based mechanisms, i.e. non-allelic homologous recombination (NAHR) and non-homologous end joining (NHEJ), might underlie the formation of genomic rearrangements (Lupski and Stankiewicz, 2005). Molecular analyses of chromosome breakpoints have revealed NAHR as the major mechanism for recurrent rearrangement whereas NHEJ could be responsible for many of the non-recurrent rearrangements (Lieber, 2008). The apparent randomness of CCRs and the lack of homology in their breakpoint regions suggest the preferential involvement of NHEJ DNA repair processes. Lee et al. (2010) have postulated that autosomal dominant traits could predispose to complex recombination between non-homologous chromosomes. However, due to the multiple breakpoints involved and the often large distance between them, the CCRs observed in humans cannot be simply generated by single recombination events, such as NAHR or NHEJ. Recently, alternate processes, based on errors of DNA replication, have been proposed. These are called ‘Fork Stalling and Template Switching’ (FoSTeS) (Lee et al., 2007) and the ‘micro-homology-mediated break-induced replication’ (MMBIR) model (Hastings, 2009). Briefly, in the FoSTeS model, it is assumed that the DNA replication fork could stall and that the lagging strand disengages from the original template, thus invading another replication fork, and restarting DNA synthesis via the sequence micro-homology between the switched template and the initial fork. This procedure of disengaging, invading and re-synthesizing can occur multiple times in series, with the new template strand being not necessarily adjacent but probably in 3D physical proximity (Zhang et al., 2009a, b). The MMBIR process requires a short homology at the breakpoints and collapsed DNA replication forks as substrates for initiating the replication restarting. MMBIR proposes that a collapsed replication fork occurring as the fork proceeds through a nicked dsDNA, results in a one-ended, double-stranded DNA that is processed to expose a 3’ end which is then used to prime DNA synthesis after a template switch (Hastings, 2009). In the initial model proposed for amplification in Escherichia coli, it was suggested that the switches occur between different replication forks, because the genomic distances involved exceeded the lengths expected to be exposed as single-stranded, at a single replication fork (Slack et al., 2006). The relatively long micro-homology (20–40 bp) shared by Alu sequences could facilitate template switching (Zhang et al., 2009b). However, the sequence analyses of breakpoints in some complex rearrangements have shown the absence of micro-homology at the junctions. This suggests that implication of NHEJ or NHAR mechanisms can be potentially involved in one step of CCR formation, in association with the replication-based processes. All these long-distance template-switch models have also been proposed to explain the origin of gross non-recurrent chromosomal rearrangements seen in the PLP1 gene regions of patients with Pelizaeus–Merzbacher disease, as well as in a low proportion of patients with cystic fibrosis or hemophilia A (Férec et al., 2006; Branzei and Foiani, 2007; Lee et al., 2007).

A variant form of strand break, called a ‘one-ended, DSB’ might also occur, resulting from a collapsed or broken replication fork, which the cell would manage differently from the way it handles a ‘two-ended,
DSB'. Homology of only a few nucleotides might then mediate an alternative mechanism of homologous recombination repair, known as Break-Induced Replication (BIR) (Ricchetti et al., 2003). This one-ended recombination procedure can occur when the single 3’ end of a DSB invades a DNA duplex at any chromosome location presenting homology, primes DNA synthesis and establishes a replication fork, thus replicating the sequence till the end (Smith et al., 2007). The BIR has been proposed to be the most probable mechanism for telomere healing or capture in mammalian cells (Ballif et al., 2004; Riha et al., 2006; Lydeard et al., 2007).

These data stress the extreme complexity of CCR genesis. These various mechanisms are known to play a crucial role in the maintenance of chromosomal stability in the face of spontaneous or induced DNA breaks (Fergusson et al., 2000; Aguilera and Gomez-Gonzalez, 2008), but they are also repair pathways, which can induce additional structural alterations such as cryptic insertions, deletions or duplications, detected at breakpoint junctions in some reciprocal translocations and CCRs (Carvalho et al., 2009; Schluth-Bolard et al., 2009). In this context, the analysis of sequence elements at the junction but also around breakpoints could help to clarify the molecular mechanism of CCR formation. Sequence analyses have shown that breakpoints are mostly located within unusual DNA sequences such as low-copy repeats (LCRs), Alu repetitive sequences, fragile sites or palindromic sequences (Gajecka et al., 2006b; Kato et al., 2008; Lukusa and Fryns, 2008). Molecular cloning of translocation breakpoints has evidenced palindromic, often AT-rich sequences, on partner chromosomes. Thus, in constitutional translocations involving the recurrent site on 22q11.2, such as the t(11;22)(q23;q11.2) or the t(8;22)(24.13;q11.2) translocations, the breakpoints occur at the center of AT-rich palindromic sequences (Gotter et al., 2007). Palindromic DNA sequences induce genomic instability and they have the potential to form various secondary structures in the form of hairpin loops or cruciform’s, which are capable of impeding replication (Leach, 1994; Inagaki et al., 2009; Kurahashi et al., 2009). The formation of these particular DNA conformations stimulates DSBs and underlies the genesis of CCRs (Bacolla et al., 2004; Kurahashi et al., 2009). Replication stalling at different times during S phase might occur at sites having a similar propensity for secondary structures, making them substrates for CCR genesis (Gotter et al., 2004). An interesting observation is that the de novo occurrence of palindromic-mediated translocation has never been detected in any tissues other than sperm (Kurahashi and Emanuel, 2001). This is in good agreement with the predominant paternal origin of de novo CCR. Such an apparent sperm-specificity implies that there might exist a spermatogenesis-specific mechanism for the development of these rearrangements. An explanation could be that during the transition of the histones to protamines, their release from nucleosomes could favor the subsequent release of free-negative supercoiling and thus facilitate extrusion of stem-loop secondary structures at palindromic sites (Kurahashi et al., 2009). In addition, the higher number of cell divisions during spermatogenesis compared with oogenesis, probably increases the probability of DSB formation in male gametes, and replication-based mechanisms such as MMBIR, acting in the germline through cell divisions. This gives rise to more copy number variations and accompanying chromosomal rearrangements in male than in female gametes.

Other interesting sequences identified in the vicinity of translocation breakpoints are the consensus translin-binding motifs (Aoki et al., 1995; Abeysinghe et al., 2003; Gajecka et al., 2006b, 2008). Translins are DNA-binding proteins that specifically recognize consensus sequences at the breakpoint junctions in chromosomal translocations and form a ring around the DNA. The precise role of translin proteins in joining the broken chromosome ends has not yet been elucidated. However, these proteins appear to be implicated in the control of recombination and DNA repair (Ishida et al., 2002; Jacob et al., 2004).

All these data tend to indicate that certain sequence features within LCRs may predispose to, facilitate, even induce CCR formation. However, as stressed by Lupski and Stankiewicz (2005), LCRs may stimulate but do not appear to mediate non-recurrent rearrangement, and probably CCRs arise as unique, complex events. Several lines of evidence seem to indicate that not all DNA breaks are mechanically equivalent, and there is no common mechanism underlying their formation. There could be diverse mechanisms. One could speculate that it is the genomic architectural feature rather than point mutations or sequence predisposed ‘hot spots’ which is the determining factor for CCR formation. First of all, the intranuclear localization of the associated breakpoints may affect the nature of CCRs. The general consensus is that chromosomes are compartmentalized into discrete territories (Cremer and Cremer, 2001; Mateos-Langerak et al., 2007). As evidenced for the t(11;22) translocation, the spatial proximity of breakpoint loci might facilitate illegitimate repair processes leading to more complex rearrangements (Roix et al., 2003; Ashley et al., 2006). Secondly, the unusual stable secondary DNA structures formed between the DNA ends at translocation breakpoints may exclude histones and other architectural proteins, making the DNA molecule more vulnerable to illegitimate recombination.

Consequently, the study of the 3D organization of nuclei, the plasticity of the genome as well as the identification of genome architectural features conferring susceptibility to chromosome rearrangements, make important contributions for elucidating the mechanisms involved in the formation of CCRs. The heterogeneity of CCRs illustrates the difficult challenge associated with identifying these mechanisms.

**The behavior of CCRs throughout meiosis**

### The meiotic segregation of CCRs

The nature of CCRs and the number of chromosomes involved make the analysis of the meiotic behavior and consequences of CCRs, particularly difficult, and consequently there are relatively sparse data concerning the meiotic segregation of CCRs in humans. As in the usual reciprocal translocations, the carrier of a CCR has a risk for abnormal conception due to either malsegregation of derivative chromosomes or the meiotic generation of a recombinant chromosome. Different modes of meiotic segregation can be defined according to the structure of CCRs, with reference to the classification introduced by Kausch et al. (1988). The most common type of CCR, i.e. the three-way exchange, will form during meiosis I a hexavalent configuration, allowing the full synopsis of homologous segments (Fig. 4). The in situ pachytene analysis of a few CCR has confirmed the occurrence of such hexavalent structures in spermatocytes. Saadallah and Hulten (1985) analyzed spermatocytes from a subfertile man carrying...
Complex chromosomal rearrangements

Figure 4 Hexavalent pachytene configuration adopted at meiosis I by three-way CCRs. This configuration allowed the efficient synopsis of the six chromosomes involved in the complex rearrangement. In this example, corresponding to the complex three-way translocation t(5;13,14) investigated in our laboratory, the color and the specific location of each MCB probe are indicated on the schema.

A three-way translocation t(2;4;9). They reported that all spread pachytene spermatocytes analyzed displayed a chromosomal hexavalent configuration. A similar analysis was conducted on a three-way translocation t(9;12;13) in three heterozygous male carriers from two generations. Pachytene analysis in all three patients showed the formation of a hexavalent structure in all spermatocytes (Johannsson et al., 1988). Synaptonemal complex analysis of spermatocytes was also reported for a subfertile bull with a three-way translocation t(1;8;9), and revealed hexavalent structures in 52 of 53 pachytene cells analyzed (Kovacs et al., 1992). In all these studies, the hexavalent configuration showed efficient synopsis with the six chromosomal arms fully paired, except in the small central segments around the breakpoints.

For such three-way CCRs, the modes of meiotic segregation are theoretically 3:3, 4:2, 5:1 and 6:0, leading to the production of a large variety of imbalances. If the disjunction is symmetric (i.e. 3:3 mode), 20 different genic chromosomal combinations can occur, including the normal and balanced arising from alternate segregation, and 18 unbalanced combinations. If the disjunction is asymmetric (modes 4:2, 5:1 or 6:0), 44 extremely unbalanced different gametes can be produced (30 types for 4:2, 12 for 5:1 and 2 for 6:0, respectively). Meiotic studies of classical two-way reciprocal translocations have stressed the relationship between the formation of chain tetravalents and the preferential segregation toward the 3:1 unbalanced segregation, as well as the correlation between the formation of a ring tetravalent and the 2:2 segregation (Templado et al., 1990; Oliver-Bonet et al., 2006). The application of this concept to the setting of CCRs suggests the opening of the hexavalent structure and the formation of a chain hexavalent structure favoring both 4:2 and 5:1 segregations. Nevertheless, several reports on families with unbalanced three-way translocations have concluded that abnormal live births are most commonly due to 3:3 adjacent 1 segregation, as in classical reciprocal translocations, followed by 4:2 segregation (Batista et al., 1994; Lespinasse et al., 2003). The 4:2 segregation mode appears to be characteristic of the segregation of CCRs in which an acrocentric chromosome is involved. A few cases of extra derivative chromosomes resulting from 4:2 malsegregation have been described and most of these cases were maternally transmitted (Schwinger et al., 1975; Fuster et al., 1997; Migliori et al., 2004).

The type of segregation that a translocation configuration can undergo through meiosis depends on various genomic and genetic factors, including the number and the nature of chromosomes involved, the location of breakpoints and the size of the translocated segments. Non-genetic factors such as age, sex and environment also affect segregation patterns (Jalbert et al., 1980). Some computer programs and interactive web sites (i.e. https://www.hc-forum.net) integrating all these parameters are available, which calculate a prediction of imbalances at birth with high accuracy (Cans et al., 1993; Cohen et al., 2001). Unfortunately, these tools are not available for complex three-way translocations. Also, the occurrence of double interstitial chiasma may affect the disjunctional process and lead to the formation of additional rearranged chromosomes (Cifuentes et al., 1998). In contrast, as set forth for classical reciprocal translocations, the occurrence of single interstitial recombination between homologous chromosomal regions, will not generate new recombinant chromosomes, but lead to similar meiotic products from alternate and adjacent segregations. Contrary to the increase in chiasma frequency observed in the interstitial segments of balanced reciprocal translocations, the hexavalent configuration shows a reduced number of chiasma, probably because of the restricted size of unpaired segments (Saadallah and Hulten, 1985). Consequently, in the cases of three-way CCRs, recombination is rare. Only a few examples of recombinant three-way CCRs have been reported (Masuno et al., 1993; Berend et al., 2002).

The meiotic segregation of exceptional CCRs (with one or more chromosomes with more than one breakpoint) gives rise to a greater range of abnormal gametes. In the study of a woman with a five breakpoint CCR involving a translocation and an inversion, Kausch et al. (1988) distinguished up to 70 different unbalanced gametes, according to the octavalent configuration theoretically adopted by the translocated chromosomes at pachytene stage and the possible 4:4, 5:3, 6:2, 7:1 and 8:0 resulting segregations. Several studies of de novo balanced CCRs with similar complexity have been reported to date (Joyce et al., 1999; Röthlisberger et al., 1999; Houge et al., 2003; Bartels et al., 2007), confirming that rearrangements of greater complexity confer a greater potential range of gametes abnormalities. In addition, the occurrence of interstitial crossing-over will lead to the formation of additional recombinant gametes. In a review of 17 cases of balanced CCR involving more breaks than the number of chromosomes and additional insertions, Madan et al. (1997) observed that 12 CCRs could potentially produce a simpler translocation in the next generation, by the occurrence of a crossing-over in the inserted segment. The authors concluded that in exceptional CCRs, recombination events might increase the proportion of normal or balanced offspring. This idea has been documented by several cases of familial CCR in which the rebuilding of an involved chromosome through recombination, lead
to more complicated or more simple karyotypes in offspring (Tuck et al., 1996; Tihy et al., 2005).

The third type of CCR, the double or triple two-way translocation, must undergo two (or three) separate and independently operating quadrivalent configurations. Several examples of such CCRs have been described in the literature (Tabor et al., 1981; Miller and Flatz, 1984; Zahed et al., 1998; Soler et al., 2005). Bowser-Riley et al. (1988) proposed an empirical risk calculation to assess the segregation mode of both double and triple two-way translocation, based on the principle that each separate translocation risk can be estimated and the extrapolated total risk should be additive. Nevertheless, the proposed method clearly has limitations since it assumed that there was no interference between the assortments of the separate exchanges and it did not take into consideration the occurrence of recombination as an additional parameter. Thus, Walker et al. (1985) reported the case of a complex three-way translocation t(7;8;9) that arose in the index patient from two independent translocations, t(7;8) and t(7;9) in the grandparent. More recently, Soler et al. (2005) documented a case of recombination in a male carrier of two reciprocal translocations, leading to balanced and unbalanced rearrangements in offspring.

**Sperm analysis of CCR carriers**

Chromosomal analysis, directly on spermatozoa of CCR carriers, may provide an accurate method for analyzing meiotic segregation of CCRs. However, only a few sperm studies have been performed because male infertility is a common problem in male CCR carriers. To date, all the sperm analyses discussed in this paper, have been carried out on CCR carriers displaying oligozoospermia, with sperm counts <10 x 10^6/ml. The pioneer segregation analysis on sperm samples from a CCR concerned a man heterozygous for a double two-way translocation t(5;11), t(7;14), analyzed using the human sperm-hamster oocyte fusion technique, also called ‘Humster’ test (Burns et al., 1986). Only 23 sperm karyotypes were obtained, among them 19 (87%) displayed unbalanced complements resulting from adjacent 1, 3:1 and even 4:0 segregation modes. In 1994, Lu et al. (1994) reported the first analysis of a three-way translocation t(2;4;8) using FISH, but this assay was only focused on the incidence of aneuploidy for chromosomes 4 and 8, which appeared to be significantly higher (3.3 and 4.4%, respectively) than the normal range. The first sperm segregation analysis on a three-way CCR was performed by combining the ‘Humster’ test and WCP probes for the carrier of a complex translocation t(2;11;22) (Cifuentes et al., 1998). The use of the ‘Humster’ test limited the number of scored sperm complements, but also introduced a potential bias linked to the success of the in vitro fertilization of hamster oocytes by human spermatozoa. A total of 208 spermatozoa were analyzed. The frequency of sperm carrying a normal or balanced complement was 13.5%. The authors found a 86.5% frequency of unbalanced sperm, including a large majority of 3:3 unbalanced products (64.9%), lower rates of 4:2 and 5:1 imbalances (20.7 and 0.96%, respectively) and no imbalance resulting from the 6:0 segregation mode. Interestingly, 18 complements displayed disomies for the chromosomes involved in the CCR, originating from either the extra non-disjunction at meiosis II or the occurrence of chiasma in the interstitial segments of the pachytene hexavalent (Cifuentes et al., 1998).

The use of FISH allows one to analyze a large sample of sperm nuclei and to eliminate the risk of a selection of sperm through the *in vitro* heterogeneous fertilization process. By using FISH probes derived from region-specific microdissected libraries (MCB probes) (Liehr et al., 2002), we have analyzed the meiotic segregation of a balanced CCR t(5;13;14)(q23;q21;q31) on 667 sperm nuclei (Fig. 5). Only 27% of spermatozoa displayed a normal or balanced chromosome complement. The rate of unbalanced sperm was 69.4%, including different types of 3:3, 4:2 and 5:1 segregations. No case of 6:0 segregation was observed, and 21 sperm nuclei (3.15%) displayed unexpected fluorescent patterns, due to non-disjunctions at the second meiotic division. An alternative

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**Figure 5** Examples of *in situ* labeling obtained in sperm nuclei of carriers of CCRs. (A) A sperm nuclei from a t(5;13;14) carrier, labeled by using the MCB specific probes for chromosome 5 (in blue), chromosome 13 (in red) and chromosome 14 (in green), according to the pachytene configuration presented in Fig. 4. This sperm nucleus shows a fluorescent profile corresponding to an unbalanced 3:3 segregation pattern (13 + 14 + der14). (B) A sperm nuclei from a patient carrying a t(1;19;13) CCR, labeled by combining PRINS and FISH techniques. Chromosome 1 was labeled in red (1pter) and Alexa-blue (centromere). Chromosome 13 was labeled in yellow (13q34) and green (centromere). Chromosome 19 was labeled in Spectrum-blue (19qter). This sperm nucleus displays a fluorescent profile corresponding to an unbalanced 3:3 segregation pattern (der 1 + 13 + 19).
approach was used by Loup et al. (2010) by combining FISH and PRINS techniques, in order to increase the number of fluorochromes simultaneously used on the same sperm preparation (Pellestor et al., 1996). This method was applied for the segregation analysis of a t(1;19;13) on a total of 1882 sperm (Fig. 5). The main frequency of unbalanced sperm was 75.9% including mainly 4:2 segregations (38.2%) and 3:3 segregations (34.1%). Only 14.8% of sperm patterns were consistent with a normal or balanced chromosome complement. In addition, it is relevant to note that 9.3% of spermatozoa showed fluorescent patterns that did not correspond to any of the established segregation profiles. This observation emphasizes the difficulty encountered when developing an in situ segregation analysis of CCR on sperm, according to the number of chromosomes implicated and the location of breakpoints. The occurrence of chiasma may also affect the segregation pattern by leading to the formation of additional recombinant chromosomes. In the FISH studies reported, this phenomenon did not change the panel of fluorescent signals and thus was not taken into account. Most of the unassigned signal combinations observed was consistent with the occurrence of non-disjunction during the second meiotic division. This reflects the complexity of the meiotic behavior of a hexavalent, but also indicates that unbalanced complements well proceed through spermatogenesis and spermiogenesis.

All these data drawn from sperm analysis confirm the high production of unbalanced gametes during meiotic segregation of CCRs. As in reciprocal translocations, the meiotic behavior of each CCR seems to be unique and consequently, the in situ study constitutes an interesting way for obtaining an accurate estimation of gametic output in CCRs and, by extrapolation, for assessing the risk of producing abnormal offspring. Nevertheless, such a sperm FISH study is not always possible because of the frequent alteration of spermatogenesis in male CCR carriers.

**Impact of CCRs on fertility**

The majority of familial CCRs are transmitted through female carriers, indicating that human oogenesis can handle the complexity of CCRs and produce phenotypically normal children. The scarcity of transmitting males is mainly due to the impairment or the arrest of spermatogenesis, frequently associated with CCRs and resulting in sterility or subfertility (Rodriguez et al., 1985; Lee et al., 2006; Bartels et al., 2007; Salashourifar et al., 2009). Nevertheless, male transmission of CCRs has been reported in a few rare cases (Meer et al., 1981; Grasshoff et al., 2003). To date, <20 cases of CCRs in fertile male carriers have been documented, and when fertility is maintained, there is a high risk of abnormal pregnancy outcome (Cai et al., 2001).

The great vulnerability of male gametogenesis with CCR finds its origin in the disturbance of homologous pairing during male meiosis. Failure of synapsis, heterosynapsis and subsequent association of asynaptic segments with the X–Y body causing interference with the X inactivation are considered to be the major reasons for the breakdown of spermatogenesis. Also, the transcriptional silencing of the X–Y body may spread to close autosomal chromatin and disturb the transcription of genes necessary for the completion of meiosis (Solari, 1999). The complex pachytene configurations of CCRs may have different degrees of terminal asynapsis, according to the number of chromosomes and breakpoints involved in the rearrangement. Consequently, various levels of association with the sex body

<table>
<thead>
<tr>
<th>Table II</th>
<th>Summary of PGD procedures performed in patients carrying CCRs.</th>
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<tr>
<td><strong>Rearrangement, reference</strong></td>
<td><strong>Number and types of probes (r round of hybridization)</strong></td>
</tr>
<tr>
<td>45,XX,t(8;12)(q24.1;q22),der(13;14)(q10;q10),</td>
<td>Three telomeric and one centromeric (r = 3)</td>
</tr>
<tr>
<td>46,XX,t(13;16)(q35.1;q32.1;q11.1),</td>
<td>Three telomeric and one centromeric (r = 3)</td>
</tr>
<tr>
<td>Escudero et al. (2008)</td>
<td>Four telomeric, one centromeric and one LSI (r = 3)</td>
</tr>
<tr>
<td>46,X,del(X)(p22.3),t(2;18)(q14.1;q21)/46,XY,t(5;13;8)(q24.3;q14.3), Lim et al. (2008)</td>
<td>Three telomeric, one centromeric, one LSI (r = 1)</td>
</tr>
<tr>
<td>46,XX,t(2;18)(q14.1;q22), Escudero et al. (2008)</td>
<td>Two telomeric, one centromeric, one LSI (r = 1)</td>
</tr>
<tr>
<td>46,XX,t(5;13;16)(q35.1;q32.1;q11.1), Lim et al. (2008)</td>
<td>Two telomeric, one centromeric, one LSI (r = 1)</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
</tr>
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</table>

**Number of cycles: 22**

**Diagnosed embryos: 136**

**Biopsied embryos: 129**

**Transfer: 9**

**Pregnancy: 4**

**Delivery: 2**
may be observed (Coco et al., 2004). It has been suggested that the involvement of an acrocentric chromosome in the CCR may favor the contact with the sex body (Lespinasse et al., 2003). In addition, pachytene checkpoints can detect failure in chromosome asynapsis and recombination and lead to pachytene arrest and apoptosis (Roeder and Bailis, 2000; Sanderson et al., 2008). The disturbance of spermatogenesis occurring at the pachytene stage leads to the death of the majority of spermatocytes and the lack of spermiogenesis (Coco et al., 2004; Scott Sills et al., 2005). In a recent cytogenetic and histological investigation of 10 CCR carriers with infertility problems, Kim et al. (2011) described several cases of maturation arrest, and ‘Sertoli cells only’ in one patient.

Other factors could also affect spermatogenesis. Thus, the hypothesis of the dysregulation of autosomal genes involved in male gametogenesis by chromosome breakpoints has been proposed by several authors (Luciani et al., 1984; Bache et al., 2001; Guo et al., 2002).

The deleterious influence of ITs on carrier reproduction appears less pronounced than for other types of CCRs. In contrast with balanced complex translocations, most ITs are inherited and transmission of ITs over two, three or even four generations has been reported several times (Moller et al., 1984; Shaffer et al., 1993; Gruchy et al., 2009). Both the small size of the inserted segments and their less complex meiotic behavior might explain this tolerance.

The defect in spermatogenesis in CCR carriers can be bypassed by the use of the ICSI procedure. A successful result was obtained by chance in the case of a three-way CCR initially diagnosed as a cryptic rearrangement. The use of ICSI clearly raises the question of the propagation of a paternal CCR into the next generation.

**CCR and PGD**

Sperm segregation studies using multicolor FISH technologies have demonstrated the possibility of developing efficient and accurate procedures for in situ analysis of CCRs on isolated interphase nuclei. This has led several laboratories to propose PGD as a strategy for couples with CCRs in order to select embryos with no genetic imbalance and hence increase their chances of a successful pregnancy. The literature has reported PGD for six carriers of CCRs (Escudero et al., 2008). Results are summarized in Table II. Lim et al. (2008) reported a healthy live birth after four cycles of PGD were performed in three patients. The pregnancy was achieved by a patient carrying a CCR combining a reciprocal translocation t(2;18) and an X deletion. Escudero et al. (2008) reported PGD results for three couples with a CCR carrier including two cases of double two-way translocations, and one three-way CCR. In total, blastomeres were biopsied from 136 embryos and 120 embryos among the 129 diagnosed were unbalanced (93%). The cumulative pregnancy rate per transfer was 2/5 (40%). Each of these couples had experienced a history of repeated pregnancy loss. After PGD, no spontaneous abortion occurred. These two studies point out the relevance and the feasibility of PGD in case of CCRs. However, one must emphasize the high incidence of chromosomally abnormal embryos, making it necessary to obtain a sufficient number of oocytes to provide additional chances of pregnancies.

**Concluding remarks for genetic counseling of CCRs**

Genetic counseling for families with CCR is not easy because the risk of imbalances probably differs with the nature of the rearrangement, and both the number of chromosomes involved and the number of underlying breakpoints. Thus, the general figures determined by Gorski et al. (1988) and Madan et al. (1997), for the overall risk of spontaneous abortion in phenotypically normal carriers of CCRs (50%) and the risk of an unbalanced live born child if a pregnancy develops to term (20%), are probably not suitable for the genetic counseling of all CCRs because of the private nature of each CCR.

The reproductive risks seem to be very specific for each carrier and its precise prevalence is impossible to establish.

According to Madan et al. (1997), the risk of phenotypic abnormalities in balanced de novo CCRs detected prenatally, should be greater than the 4–6% risk associated with balanced reciprocal translocations and will increase with the number of breakpoints. However, balanced CCRs must be extensively investigated to rule out the occurrence of cryptic rearrangements and ensure that the fetal balanced CCR is truly balanced. In a recent survey published by Giardino et al. (2009) on de novo apparently balanced chromosome rearrangements identified prenatally, about 35% of couples with a balanced CCR diagnosed in their fetus decided to electively terminate the pregnancy. This rate is higher than that for reciprocal translocation (24%) and Robertsonian translocation (3%). This well illustrates the lack of information regarding the frequency and type of clinical features associated with the prenatal diagnosis of apparently balanced complex rearrangements. The complexity of CCRs reinforces the necessity for molecular investigations as adjuncts to conventional cytogenetics and FISH methods in the characterization of CCRs.

The genetic counseling in prenatal cases of balanced CCRs can greatly benefit from the use of microarray technologies for the potential detection of most possible imbalances.

**Author’s roles**

F.P. was responsible for the main writing of the study, reference search and picture and schema selection. J.P. collected the data and provided diagrams. All authors drafted the paper and revised the paper critically.

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