

On the origin of the maternal age effect in trisomy 21 Down syndrome: the Oocyte Mosaicism Selection model

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Abstract

We have recently documented that trisomy 21 mosaicism is common in human foetal ovaries. On the basis of this observation we propose that the maternal age effect in Down syndrome (DS) is caused by the differential behaviour of trisomy 21 in relation to disomy 21 oocytes during development from foetal life until ovulation in adulthood. In particular, we suggest that trisomy 21 oocytes, lagging behind those that are disomic, may escape the timed pruning of the seven million in foetal life to the 300–400 finally selected for ovulation. The net effect of this preferential elimination will be an accumulation of trisomy 21 oocytes in the ovarian reserve of older women. We here highlight the implications of this Oocyte Mosaicism Selection (OMS) model with respect to the prevalent view that the maternal age effect is complex, dependent on many different biological and environmental factors. We examine conclusions drawn from recent large-scale studies in families, tracing DNA markers along the length of chromosome 21q between parents and DS children, in comparison to the OMS model. We conclude that these family linkage data are equally compatible with the maternal age effect originating from the accumulation of trisomy 21 oocytes with advancing maternal age. One relatively straightforward way to get to grips with what is actually going on in this regard would be to compare incidence of trisomy 21 oocytes (and their pairing configurations) in foetal ovaries with that in oocytes at the meiosis I stage from adult women.

Reproduction (2010) **139** 1–9

Introduction

Down syndrome 50 years on: what do we know about the origin?

It is now more than 75 years since the maternal age effect in Down syndrome (DS) was first discovered (Penrose 1933, 1934) and 50 years since the genetic background in DS involving extra chromosome 21 material was identified (Book *et al.* 1959, Ford *et al.* 1959, Jacobs *et al.* 1959, Lejeune *et al.* 1959, Penrose *et al.* 1960, Polani *et al.* 1960). In the interim it has become abundantly clear that the origin of the most common type of DS associated with a free extra chromosome 21 (T21) differs from the more rare types that are dependent on extra chromosome 21 material caused by a structural chromosome rearrangement. In particular, it is exclusively this common type that is associated with an increase dependent on maternal age, a rise which is especially dramatic at later reproductive ages from around 35 years and onwards (Fig. 1).

What is the reason for the T21 maternal age effect?

Numerous hypotheses have over the years been put forward to explain the T21 maternal age effect (Table 1). Most authors assume that the main problem concerns meiotic segregation errors of a normal disomic oocyte, as reiterated by for example Hunt & Hassold (2008), Jones (2008), Mailhes (2008), Martin (2008), Oliver *et al.* (2008), Vogt *et al.* (2008), Allen *et al.* (2009), Coppédè (2009), Driscoll & Gross (2009), Garcia-Cruz *et al.* (2009) and Ghosh *et al.* (2009). We have recently performed a study (Hultén *et al.* 2008) relevant to the possibility that the maternal age effect is instead due to the accumulation of pre-existing T21 cells in ovaries of human females during their development from foetal life to adulthood, a hypothesis akin to the oocyte selection model by Vig (1984), Zheng & Byers (1992), Sensi & Ricci (1993), Zheng & Byers (1996a, 1996b), Zheng *et al.* (2000) and Zheng (2004). We found that in fact all eight normal fetuses analysed in this respect were T21 ovarian mosaics (average 0.54%, range 0.20–0.88;

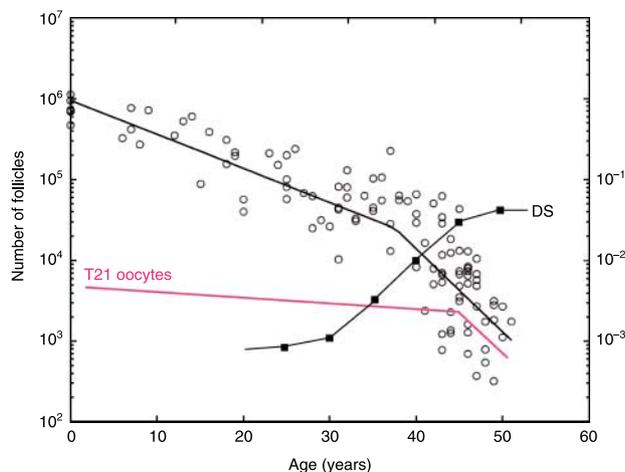


Figure 1 Increased proportion of T21 oocytes in the ageing ovary. The OMS hypothesis proposes that the T21 oocytes lag behind during development, resulting in higher proportions of the total oocyte pool over time. The figure illustrates the predicted number of T21 oocytes from birth until menopause (pink line) in comparison to the total (black circles) based on follicle counts (left hand Y axis) by Faddy (2000). The observed incidence (right hand Y axis) of T21 DS births (black squares) is represented by the data of Morris *et al.* (2002). The offset of the (pink) line showing the predicted number of T21 oocytes is based on the 0.54% mosaicism observed by Hultén *et al.* (2008). The slope is an approximation generating the expected DS birth rates with increasing maternal age.

s.d. 0.23), this foetal ovarian T21 mosaicism originating by pre-meiotic mitotic chromosome 21 segregation errors. Secondly, it is well known from investigation of meiotic behaviour in T21 fetuses that T21 oocytes, forming a bivalent plus a univalent or a trivalent (Fig. 2) may be retarded in their maturation in comparison to oocytes from fetuses with normal karyotypes (Jagiello *et al.* 1987, Cheng *et al.* 1998, Barlow *et al.* 2002). There is thus a distinct possibility that any T21 oocytes present in the normal ovary may accumulate by escaping apoptotic elimination during the drastic reduction from seven million in foetal life to the 300–400 selected for ovulation in adulthood (Fig. 1). The mechanism(s) underlying this developmental retardation is not known and requires further investigation, e.g. along the same lines as has recently been performed with respect to the premature aging in trisomy 21 DS children (Cairney *et al.* 2009).

Oocyte development takes decades to complete

It is in this context essential to remember the complexity in oocyte development and meiotic cell divisions, where the crucial crossover process and chiasma formation takes place during foetal development but the oocyte is not undergoing the final cell division until fertilisation in adulthood (see e.g. reviews in Hultén *et al.* (2005, 2009)). The precursor cells, the primordial germ cells, are differentiated among the endodermal cells of the yolk

sac already at around 4 weeks of foetal life. They then migrate to the gonadal ridge, where multiple pre-meiotic oogonial mitotic cell divisions lead to a peak of approximately seven million at 20 weeks gestation.

The meiotic prophase is initiated at around 9 weeks gestation and progresses in a semi-synchronous fashion until a few weeks after birth (Bendsen *et al.* 2006). At this time, further development is again arrested for several decades until maturation, following the FSH surge from puberty onwards. The first meiotic metaphase I (MI) to anaphase I (AI) division, normally halving the somatic chromosome number from 46 to 23 takes place just before ovulation followed by progression to metaphase II (MII). Oocytes again arrest at this stage with the second meiotic division at anaphase II (AII) not being completed until after fertilisation.

Quite remarkably, therefore, deviations in normal chromosome segregational behaviour from the very earliest mitotic cells divisions of the zygote until the completion of the second meiotic division taking place 15–50 years later following fertilisation may be of importance for the origin of T21 DS conceptions.

Aneuploidy mosaicism is common in human embryos

One particular aspect of this notion concerns recent information obtained by chromosome analysis of embryos at the 8-cell stage, indicating that chromosome malsegregation of one or a few chromosomes is common, leading to embryonic mosaicism including a cell line with an aberrant chromosome number (see e.g. Hultén *et al.* 2009, Vanneste *et al.* 2009). The relevance of this observation in relation to the origin of ovarian T21 mosaicism that we have documented previously (Hultén *et al.* 2008) is, however, not yet known. First of all, the number of embryos so far investigated in this respect at the 8–9-cell stage is still small, and the involvement of T21 mosaicism *per se* has not been clarified. Secondly, in the absence of knowledge as regards the origin of any such T21 embryonic mosaicism, the relevance of the indication by Katz-Jaffe *et al.* (2004) that it is only those that are 'meiotic' that survive until the time of amniocentesis (around 16 weeks gestational age) remains unknown. Thirdly, we do not know what the relation might be between any T21 mosaicism in somatic and germ line cells of the embryo. Fourth, bearing in mind the tiny founding germ cell population (2–3 cells) suggested by Zheng *et al.* (2005) it seems highly unlikely that earlier malsegregation underlies the foetal ovarian T21 mosaicism at an average of 0.54% that we detected in the eight cases at gestational age 14–22 weeks. Tentatively, we propose that this has been caused by oogonial malsegregation at around 5-week gestational age, i.e. when the migrating germ cells have reached their final destination in the mesenchyme of the urogenital ridge (Bendsen *et al.* 2006, Pereda *et al.* 2006).

Table 1 Examples of hypotheses aimed at explaining the maternal age effect in T21 Down syndrome.

Hypothesis	References
Altered meiotic recombination laid down in foetal oocytes/mechanical instability at MI – AI transition/AI chromosome malsegregation	Penrose (1954), Henderson & Edwards (1968), Warren <i>et al.</i> (1987), Sherman <i>et al.</i> (1991), Lamb <i>et al.</i> (2005), Hunt & Hassold (2008), Mailhes (2008), Oliver <i>et al.</i> (2008) and Allen <i>et al.</i> (2009)
Variation in centromeric alphoid DNA	Maratou <i>et al.</i> (2000)
Impaired meiotic sister chromatid cohesion at MI – AI	Watanabe & Nurse (1999), Schon <i>et al.</i> (2000), de Bruin <i>et al.</i> (2004), Eichenlaub-Ritter <i>et al.</i> (2004), Hodges <i>et al.</i> (2005), Steuerwald <i>et al.</i> (2005), Mailhes (2008), Subramanian & Bickel (2008) and Vogt <i>et al.</i> (2008)
Impaired chromatin condensation/mechanical instability at MI – AI transition	Hultén (1990)
Impaired spindles at AI and AII	Hawley <i>et al.</i> (1994), Battaglia <i>et al.</i> (1996), Van Blerkom <i>et al.</i> (1997), LeMaire-Adkins <i>et al.</i> (1997), Eichenlaub-Ritter (1998), Volarcik <i>et al.</i> (1998), Steuerwald <i>et al.</i> (2001), Homer <i>et al.</i> (2005), Ma <i>et al.</i> (2005), Mailhes (2008) and Vogt <i>et al.</i> (2008)
Accumulation of toxic effects/environmental insults/virus/mitochondrial mutations/cytoplasmic agents/energy deficit/protein deficit in oocytes during foetal and postnatal development	Penrose (1954), Stoller & Collmann (1965), Evans (1967), Beermann & Hansmann (1986), Van Blerkom <i>et al.</i> (1998), Jansen & de Boer (1998), Schon <i>et al.</i> (2000) and Sherman <i>et al.</i> (2007)
Foetal ovarian T21 mosaicism, the OMS model	Vig (1984), Zheng & Byers (1992), Sensi & Ricci (1993), Zheng & Byers (1996a, 1996b) and Hultén <i>et al.</i> (2008)
Grandmaternal age effect implying foetal ovarian T21 mosaicism	Penrose (1954), Greenberg (1963), Stoller & Collmann (1969), Richards (1970), Papp <i>et al.</i> (1977), Aagesen <i>et al.</i> (1984) and Malini & Ramachandra (2006)
Limited oocyte pool/reduced ovarian reserve/premature menopause	Eichenlaub-Ritter & Boll (1989), Warburton (1989), Gaulden (1992), Kline & Levin (1992), Freeman <i>et al.</i> (2000), Kline <i>et al.</i> (2000), van Montfrans <i>et al.</i> (2002), Kline <i>et al.</i> (2004) and Warburton (2005)
Defective oocytes through hormonal imbalance, e.g. raised FSH/E2, serum AMH changes	van Montfrans <i>et al.</i> (1999), Nasserri <i>et al.</i> (1999), Seifer <i>et al.</i> (2007) and Sherman <i>et al.</i> (2007)
Decreased time to ovulation/delayed fertilisation due to reduced sexual activity	German (1968) and Klein <i>et al.</i> (1996)
Increased miscarriage rate/relaxed selection against T21 conceptions	Erickson (1978), Aymé & Lippman-Hand (1982) and Neuhäuser & Krackow (2007)

The Oocyte Mosaicism Selection model

As regards the origin of T21 emphasis has previously been placed on maternal T21 oocyte selection taking place postnatally during oocyte development from puberty until menopause (Vig 1984, Zheng & Byers 1992, Sensi & Ricci 1993, Zheng & Byers 1996a, 1996b) and a mathematical model to this effect has been produced by Zheng *et al.* (2000). The implication of our version of this model, which we have here termed OMS for Oocyte Mosaicism Selection, is twofold. First, we hypothesise that the majority of T21 conceptions may originate by obligate non-disjunction of a maternal T21 oocyte at the first meiotic division (T21-ND), a type of non-disjunction called secondary non-disjunction in classical genetics (Cooper 1948). This proposal stands in contrast to the current dogma implying that the most common reason for T21 DS conceptions is non-disjunction of the two chromosomes 21 in a normal disomy 21 maternal oocyte (D21-ND). The differentiation between these two alternatives is illustrated in Figs 3 and 4. Secondly, and most importantly we explore the view that the maternal age effect in trisomy 21 DS is most readily explained by accumulation of pre-existing T21 oocytes during maternal oogenesis.

Data analysis and discussion

The OMS model changes the way we may interpret observations using family linkage analysis, performed with a view to obtaining information on the mode of chromosome 21 parental chromosome segregation errors. We here exemplify this notion by a revised analytical approach to the data presented in recent large-scale family linkage studies, where the authors emphasise the likelihood of a complex association between advanced maternal age and non-disjunction of chromosome 21 during oogenesis (Lamb *et al.* 2005, Oliver *et al.* 2008, Allen *et al.* 2009).

This type of study first of all demonstrates that, in the outstanding majority of DS families, the extra chromosome 21 in the DS child is of maternal origin. It is further concluded that maternal chromosome 21 non-disjunction, taking place just before ovulation (maternal meiosis I non-disjunction, MMI ND), is three times as common as chromosome 21 non-disjunction, taking place after fertilisation (maternal meiosis II non-disjunction, MMII ND). Thirdly, the data are taken to indicate that there are three types of aberrant ‘vulnerable’ maternal recombination patterns, i.e. 1) lack of a maternal crossover on 21q, 2) a more distal than normal single maternal crossover on 21q, and 3) a more proximal than normal maternal

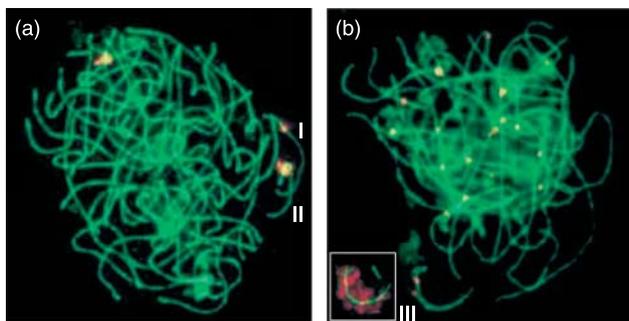


Figure 2 Trisomy 21 oocytes at the full pairing (pachytene) stage, following immunofluorescence staining for identification of the Synaptonemal Complexes (the proteinaceous structure holding the homologues together). (a) Foetal T21 oocyte containing a bivalent 21 (II) and a univalent 21 (I) identified by FISH, using a 13/21 centromeric probe (I); the univalent 21 lies close to and is much longer than the bivalent 21. (b) Foetal T21 oocyte containing a trivalent 21 (III) that shows lengthly triple synapsis. The centromeres identified by the CREST antibody (in yellow and red) are asymmetrically located, suggesting non-homologous association within the region of triple synapsis. Distally, one axis protrudes from the configuration. The insert shows the T21 highlighted by FISH (in yellow) using a whole chromosome 21 paint. Reproduced from Barlow *et al.* (2002) with permission.

single crossover on 21q. Fourthly, it is only the more proximal than normal maternal single crossover on 21q that is maternal age dependent (Fig. 5).

Maternal age dependent T21-ND cannot be distinguished by family linkage analysis

In the most recent study, Allen *et al.* (2009) compared polymorphic DNA markers along the length of the long arm of chromosome 21 in a large cohort of parents and live born DS children, selected from more than 1000 DS families and controls respectively. At least one 21q marker had to be informative when DNA from both parents could be analysed and eight markers when only maternal DNA was available. In a total population of 930 such cases they classified those where parental heterozygosity had been retained as originating from MMI ND, implying failure of the two homologs 21 in a normal disomy 21 oocyte to separate properly (Fig. 3b and c). On the alternative OMS model we note that the very same result is a likely outcome of obligate MMI ND in a T21 oocyte, containing a univalent and a bivalent (Fig. 2a), i.e. when the chromatids of the univalent undergo precocious separation at AI (Fig. 4a). We conclude that 1) any maternal age effect caused by accumulation of pre-existing T21 oocytes under the OMS model will not have been detected by the approach used by Allen *et al.* (2009), and 2) their data are not inconsistent with a maternal age effect caused by accumulation of pre-existing T21 oocytes with advancing maternal age.

The maternal age dependent 21q proximal recombination is the typical in T21 trivalents

In the study by Allen *et al.* (2009), the MMI ND type of segregation error, presumed to involve the two chromosomes 21 in a normal disomy 21 oocyte (D21-ND; Fig. 3b and c) was recorded as the most common, occurring in 76% of cases (730/960). A subset of these 730 cases has previously been investigated by Lamb *et al.* (2005) and Oliver *et al.* (2008). In these earlier investigations, the majority of cases interpreted to be due to MMI ND were found to either lack a parental chromosome 21 crossover or showing a crossover near the end of 21q. Both these types of MMI ND were considered to be equally common among the different age groups, these patterns thus interpreted to influence the risk for ND irrespective of maternal age. Under the OMS model, we again note that these results are those expected in respectively a T21 oocyte forming a bivalent plus a univalent (Fig. 4a) and a T21 oocyte forming a trivalent (Fig. 4b in Hultén *et al.* (2008)).

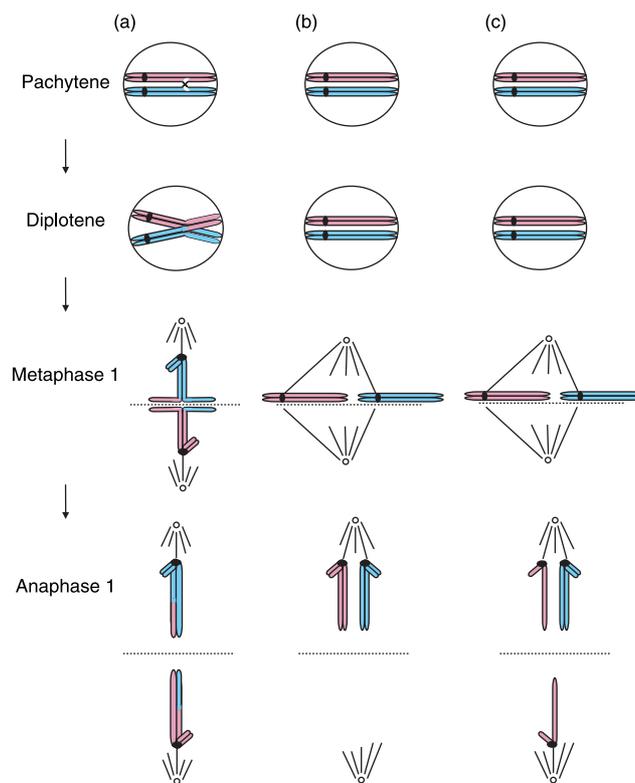


Figure 3 Cartoon illustrating the different types of meiosis I segregation that may take place in a normal disomy 21 oocyte. (a) Normal chromosome pairing and crossing-over, attachment of the movement centres (kinetochores) at metaphase I and separation at anaphase I. (b) Lack of crossing-over and chiasma formation may lead to primary non-disjunction at anaphase I. (c) Lack of a chiasma can also lead to the same type of segregation at anaphase I as during mitosis (precocious meiotic disjunction).

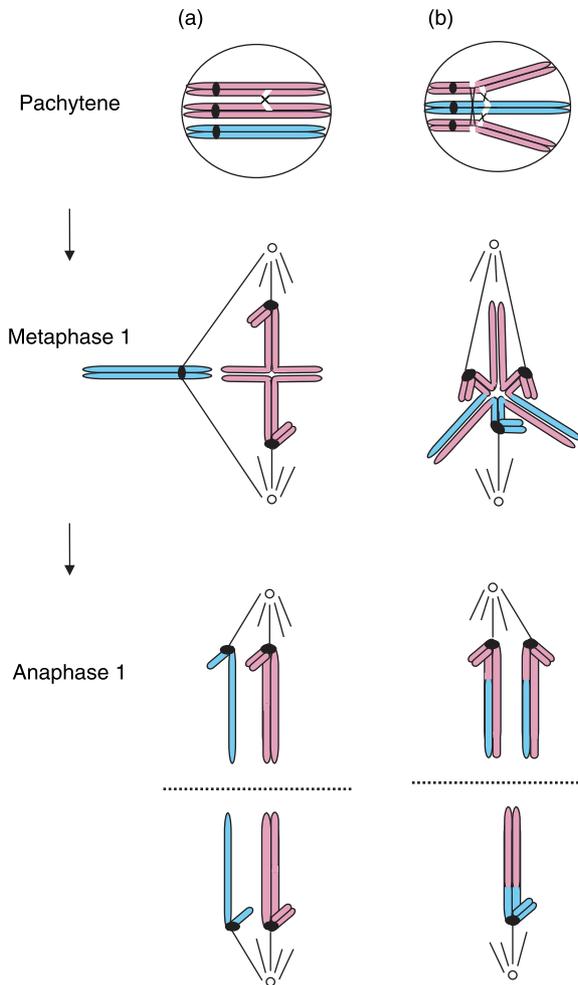


Figure 4 Cartoon illustrating the different types of secondary non-disjunction that may take place in a T21 oocyte. (a) Formation of a bivalent with a chiasma formed in the middle of the long arm plus a univalent, where the univalent is undergoing precocious disjunction leading to a chromosome 21 plus a chromatid in each of the daughter cells. (b) Formation of a trivalent with chiasmata in aberrant positions, i.e. in this example proximally within the long arm. Obligate secondary non-disjunction at anaphase I will lead to two chromosomes 21 travelling into one of the daughter cells and one chromosome 21 into the other.

In contrast, cases interpreted by Lamb *et al.* (2005) and Oliver *et al.* (2008) to be due to MMII ND on the basis of reduction of parental heterozygosity (including near-centromeric markers) with a near-centromeric crossover, were different in as much as they were considered to be maternal age dependent. On the basis of the OMS model, we suggest that these cases could equally well be the result of obligate non-disjunction (T21-ND), i.e. in an oocyte carrying a trivalent 21 (Figs 2b and 4b).

We conclude 1) that these earlier family linkage data are compatible with the OMS model explaining the maternal age effect in T21 DS. We further suggest that 2) the net result as regards those oocytes finally selected for ovulation is likely to be dependent on three main factors,

i.e. i) the incidence of T21 oocytes in the original foetal pool, ii) the specific pairing configurations (bivalent plus univalent or trivalent) and iii) their respective fate due to differential oocyte selection during oogenesis (Fig. 1).

Additional complexity in interpreting results and further studies

In this context, it is essential to recognise the complexity in extrapolation of data obtained by family linkage to 1) original patterns of grandparental recombination taking place during maternal foetal development (Hultén & Tease 2003a, 2003b) and 2) segregation of grandparental chromosomes during maternal AI taking place just before ovulation. It is also important to recognise that the variant synapsis in any T21 oocyte (Jagiello *et al.* 1987, Cheng *et al.* 1998, Barlow *et al.* 2002) may lead to a number of different patterns of recombination, where those illustrated here (Fig. 4) and in Hultén *et al.* (2008) (Fig. 4) only represent some possibilities.

Further information on patterns of 21q recombination would be valuable, using analysis of MLH1 recombination foci along the length of the meiosis-specific chromosome pairing structure, the synaptonemal complex at the pachytene stage (Baker *et al.* 1996, Barlow & Hultén 1998, Tease *et al.* 2002, 2006, Tease & Hultén 2004, Lenzi *et al.* 2005, Robles *et al.* 2007). The normal positioning of a single MLH1 recombination focus in the middle of 21q is illustrated in Fig. 6. Our own studies using this approach in T21 fetuses have so far been hampered by the maturation delay of T21 oocytes in comparison to fetuses with normal karyotypes. Thus, analysis of foetal T21 ovaries, where termination of pregnancy had been performed at around 16 weeks gestation, has not allowed the relevant information on MLH1 recombination foci to be obtained, as only oocytes at the earlier leptotene and zygotene stages have been identified (M Hultén, S Patel & E Iwarsson 2008, unpublished observations).

Until detailed information on the recombination along 21q in a number of T21 fetuses and a large population of oocytes per case becomes available, it is difficult to state what the reason might be for the reduced rate of MMI- type D21 non-disjunction detected by Allen *et al.*

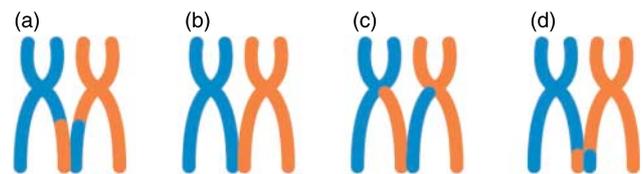


Figure 5 Cartoon illustrating normal positioning, absence and aberrant crossover positions on 21q; revised from Fig. 1 in Hassold & Hunt (2007). (a) Optimal location of a single crossover, in the middle of the long arm. (b) Absence of crossover. (c) Single crossover located too near to the centromere. (d) Single crossover located too far from the centromere.

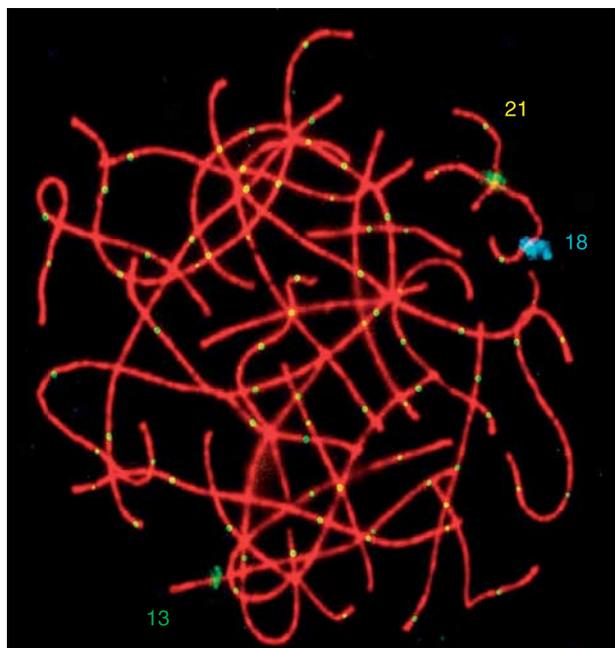


Figure 6 Normal 21 oocyte at the full pairing (pachytene) stage following dual immunofluorescence staining for identification of the synaptonemal complexes and the MLH1 recombination foci (in yellow) and subsequent fluorescence *in situ* hybridisation (FISH) with probes, highlighting the centromeres of chromosomes 21 and 13 (in green) and chromosome 18 (in blue). Revised from Fig. 1 in Tease *et al.* (2002).

(2009) in mothers within the youngest and oldest age groups. Tentatively, we suggest that under the OMS model this may be due to the combination of differences in 1) degree of foetal T21 ovarian mosaicism, where young mothers may more often be high-grade T21 ovarian mosaics, and 2) the behaviour of the different types of T21 synaptic configurations during development, where oocytes forming a trivalent 21 may show some specificity in apoptotic selection/developmental delay in comparison to those carrying a bivalent plus a univalent.

The OMS model may apply to other constitutional aneuploidies

Finally, although we have here focused attention on the maternal age effect in trisomy 21 DS, this condition being the most common genetic disorder in the human population, it is important to note that the same model may apply to other common constitutional aneuploidies, such as trisomy 18 Edwards and trisomy 13 Patau syndrome as well as to some of the sex chromosome aberrations. Thus, we have previously suggested that under the OMS model trisomy 21 foetal mosaicism might represent only the tip of the iceberg (Hultén *et al.* 2008). Further studies will obviously be required to sort out the potential role of gonadal mosaicism for the origin

of constitutional aneuploidy in the human population. It will also be of special interest to find out to what extent foetal testicular T21 mosaicism is underlying the paternal origin of T21 offspring, constituting only around 5–10% of the total, and where there is by comparison only a very weak age effect (De Souza *et al.* 2009).

Conclusion

In conclusion, we suggest that the new information that we have recently reported on as regards the common occurrence of ovarian T21 mosaicism (Hultén *et al.* 2008), and the support thereby provided for the OMS model underlying the maternal age effect in trisomy 21 DS, imply that the time is now ripe for a paradigm shift. Thus, we propose that the OMS model should be seriously considered as a relevant alternative to other proposals on the origin of the maternal age effect in trisomy 21 DS, including in particular those concluding that this is a highly complex situation, where many different biological and environmental factors may be at play (Mailhes 2008, Martin 2008, Oliver *et al.* 2008, Vogt *et al.* 2008, Allen *et al.* 2009, Coppedè 2009, Driscoll & Gross 2009, Garcia-Cruz *et al.* 2009, Ghosh *et al.* 2009). In contrast, we suggest that, by comparison, the OMS model provides a straightforward explanation. Thus, we propose that 1) obligate first meiotic T21-ND may be the prevailing mechanism for T21 conceptions of maternal origin, 2) the maternal age effect is due to accumulation of T21 oocytes with advancing age, and 3) this model is compatible with results obtained on maternal segregation and recombination patterns recently recorded by DS family linkage analysis (Lamb *et al.* 2005, Hunt & Hassold 2008, Oliver *et al.* 2008, Allen *et al.* 2009).

As we have commented on in our previous report (Hultén *et al.* 2008) one relatively straightforward way to test the OMS model would be to compare the incidence of T21 oocytes in a pool of foetal oocytes in relation to that in oocytes from adult women at different biological ages, obtained from for example oophorectomies and during IVF treatment due to male factor fertility problems. In view of the statement by Allen *et al.* (2009) that the basis of the maternal age effect in aneuploidy remains one of the most important questions in medical genetics, it is hoped that such a study can be realised in the not too distant future. We would welcome notification from any colleagues who would be interested in taking part in this type of collaborative study.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work has been supported by grants from the Wellcome Trust (Grant No 061202/ZOOZ), BBSRC (Grant No BB/C003500/1), The Swedish Research Council and Stockholm County Council.

Acknowledgements

This paper is dedicated to Marthe Gautier and Patricia Jacobs for their pioneering work on Down syndrome.

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Received 5 March 2009

First decision 1 May 2009

Revised manuscript received 30 August 2009

Accepted 15 September 2009