COMMENTARY ON *J. GENET*. CLASSIC

Parental-age effects in Down syndrome

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Introduction

Down syndrome is caused by a gene dosage-imbalance resulting from human chromosome-21 trisomy, and is the most commonly diagnosed congenital malformation/mental retardation syndrome (Jones 2006). In 1866, John Langdon Down made the first detailed description of all affected individuals (Down 1866). This pioneering study emphasized the unique clinical features of cases from heterogeneous ethnic backgrounds and distinguished them from all other cases with similar intellectual disabilities (Down 1866). While the syndrome was largely called 'mongolism' early on, for the diagnostic facial profile, there are three historical instances which have changed the context of Down syndrome. A letter published in *The Lancet* signed by illustrious biomedical scientists urged fellow researchers to abandon using the misleading connotation 'mongolism' for this mental deficiency syndrome and replace it with the designations 'Langdon-Down anomaly' or 'Down's syndrome/anomaly' or 'trisomy 21 anomaly' or 'congenital acromicria' (Allen *et al*. 1961). In 1965, a delegation from the Mongolian People's Republic approached the World Health Organization requesting them to avoid the terms mongol and mongolism (Patterson and Costa 2005). The possessive of the eponym was later discontinued and it is since widely called 'Down syndrome' (Nomenclature 1975).

The clinical features of Down syndrome are comprised of severe cognitive impairment, characteristic facial profile, short stature, speech and developmental delay, chronic ear infections and hearing loss, and hypotonia (Jones 2006). The diagnostic facial profile consists of epicanthal folds, flat nasal bridge, upslanting palpebral fissures, and protruding tongue (Jones 2006). Down syndrome patients can also develop congenital heart disease (40–50%), have an increased risk for Alzheimer disease (especially after the fourth decade) (Roper and Reeves 2006), acute megakaryocytic leukemia, Hirschsprung disease, and duodenal atresia (Jones 2006). Approximately 95% of diagnosed Down syndrome cases have a complete trisomy 21 and the remaining 5% either have somatic mosaicism (~1%) or chromosome-21 translocations (~4%) (Sherman *et al*. 2007). Overall, Down syndrome is estimated to affect 1 in 750 live births (Jones 2006); however, several reports have indicated variability in the estimates of Down syndrome among different ethnic groups. Moreover, variability of clinical presentation within a Down syndrome cohort provides evidence for gene dosage thresholds for specific phenotypes, and the action of genetic and environmental modifiers (Antonarakis *et al*. 2004). Prenatal testing for Down syndrome is performed by chorionic villus sampling (in first trimester), amniocentesis (in second trimester), biochemical analysis (Quad screen for maternal serum markers and alpha feto-protein analysis) and by ultrasound (for nuchal translucency) (Neilson and Alfirevic 2006; Saller and Canick 2008).

Penrose and parental-age effects in Down syndrome

Based on an analysis of 150 families (reproduced in pages 9–14 of this issue), each containing at least one affected Down syndrome child, Lionel Penrose (1933) suggested a partial correlation between advanced maternal age and the risk of having a child with Down syndrome (Penrose 1933). Using a regression analysis, described previously by Sewall Wright (Wright 1926), Penrose compared the paternal and the maternal ages of parents of Down syndrome offspring. After regressing out the paternal age effect, the difference between the observed and expected mean maternal ages at the time of birth of a Down syndrome child was significant. How-

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ever, after adjusting for maternal age, there was no significant difference in the observed and expected mean paternal age. Also, no effect of maternal parity or birth order of the affected child was observed (Penrose 1934). Thus, the association of increased paternal age and incidence of Down syndrome was merely due to a simultaneous association with maternal age. Over the next 75 years, several epidemiological and molecular genetic studies have examined Penrose's conclusions with significant implications for prenatal diagnosis and genetic counselling (Hassold and Jacobs 1984; Warburton 1989, 2005; Sherman *et al*. 2007; Hunt and Hassold 2008).

Mantel and Stark (1967) suggested that a high correlation between the paternal and maternal age masked the actual paternal age effect and reduced the power of correlation studies. While increased paternal age association was identified in some epidemiological studies (Erickson and Bjerkedal 1981; Stene *et al*. 1981, 1987), no significant effect was detected in several other studies on Down syndrome (Erickson 1978; Hook *et al*. 1981; Hook 1987; Fisch *et al*. 2003; Dzurova and Pikhart 2005). Discrepancies in these reports were mainly due to different methods of statistical analysis and, possibly, the small sample sizes analysed (Fisch *et al*. 2003). A more recent study suggested a significant paternal age effect on Down syndrome only with mothers aged 35 years or older (Fisch *et al*. 2003). Fisch *et al*. (2003) also showed that the paternal age effect was the greatest in couples older than 40 years with the risk for Down syndrome increasing to six times the rate in couples younger than 35 years (Fisch *et al*. 2003).

Interestingly, in familial cases of Down syndrome with more than one affected sibling, the mean maternal age for affected children was lower than the general maternal age estimates for Down syndrome (Penrose 1954). A similar reduction of mean maternal age was also observed in individuals when a maternal relative was also affected. No reduction of mean maternal age was observed when a paternal relative was affected. Further, a two to three times increased risk of Down syndrome in the siblings of affected individuals was observed, after accounting for selection bias (Penrose 1954). A recent study by Arbuzova and colleagues on familial cases of Down syndrome showed that while there is a greater recurrence risk for younger women, by the age of 40, the recurrence risk is not significantly different from non-familial cases (Arbuzova *et al*. 2001). Notably, a history of Down syndrome miscarriage also increases the risk of other fetal aneuploidies in subsequent pregnancies (Bianco *et al*. 2006).

Aetiology of Down syndrome

In his 1954 report, Penrose indicated several plausible aetiological factors for Down syndrome: advanced maternal age resulting in altered rate of crossing over between closely linked genes, chromosomal translocations and endocrine imbalances, a strong hereditary component in familial cases with reduced mean maternal age, and maternal-fetal genotype-specific susceptibility (Penrose 1954). Advances in cytogenetic analysis, including the discovery of the extra chromosome-21 independently by Jerome Lejeune and Patricia Jacobs in 1959 and isolation of chromosomes from amniotic fluid provided a molecular basis for Down syndrome (Lejeune *et al*. 1959; Steele and Breg 1966; Valenti *et al*. 1968). Additionally, in 1964 Penrose suggested that the Down syndrome cases influenced by advanced maternal age could be due to chromosomal nondisjunction, a failure of chromosomes to separate properly during meiosis (Penrose 1964; Sherman *et al*. 2007).

Analysis of chromosome heteromorphisms and highly informative DNA polymorphic markers from parents and Down syndrome offspring helped to determine the parental origin of the extra chromosome and stage of the meiotic error (Hassold and Jacobs 1984; Antonarakis 1991; Petersen *et al*. 1991). The maternal versus paternal basis for meiotic errors are well illustrated by their physiological timeline (figure 1) (Hassold and Hunt 2001). Female meiosis I (MI) is initiated during fetal development, but after homologous recombination, the oocytes undergo a period of arrest in prophase (reviewed by Hassold and Hunt 2001). MI is then resumed (10–50 years later) in the ovary just before ovulation (Hassold and Hunt 2001). After completion of MI, the oocytes are suspended in the metaphase of meiosis II (MII) and the second division is completed only after fertilization. The prolonged meiotic arrest phase likely allows accumulation of toxic effects including environmental insults, degradation of meiotic machinery causing MI and MII errors, and suboptimal ovarian functioning likely resulting in hormonal imbalance (Sherman *et al*. 2007). Male meiosis, however, begins at puberty and all events are sequentially completed without interruptions, in the adult testis (figure 1) (Hassold and Hunt 2001).

Research from the laboratories of Stephanie Sherman and Terry Hassold has provided insights into the origin of human aneuploidy, including those specific to trisomy 21. While maternal nondisjunction (majority being MI error) has accounted for >90% of trisomy 21 cases, 5%–10% were due to paternal nondisjunction (MI and MII errors), and <5% were due to mitotic errors (Hassold and Hunt 2001). However, only maternal meiotic nondisjunctions are associated with advanced maternal age (Lamb *et al*. 2005). Utilizing polymorphic markers, parent-to-trisomic offspring inheritance and recombination patterns of chromosome-21-specific segments have been studied (Savage *et al*. 1998; Lamb *et al*. 2005). MI errors are identified by the presence of a parental heterozygosity in the trisomic offspring and in MII error, the parental heterozygosity is converted to homozygosity in the offspring. However, if the parental heterozygosity is reduced to homozygosity in all informative loci (proximal, medial, and distal chromosome-21 regions) then a postzygotic, mitotic error is inferred (Savage *et al*. 1998) (figure 2,a–c).

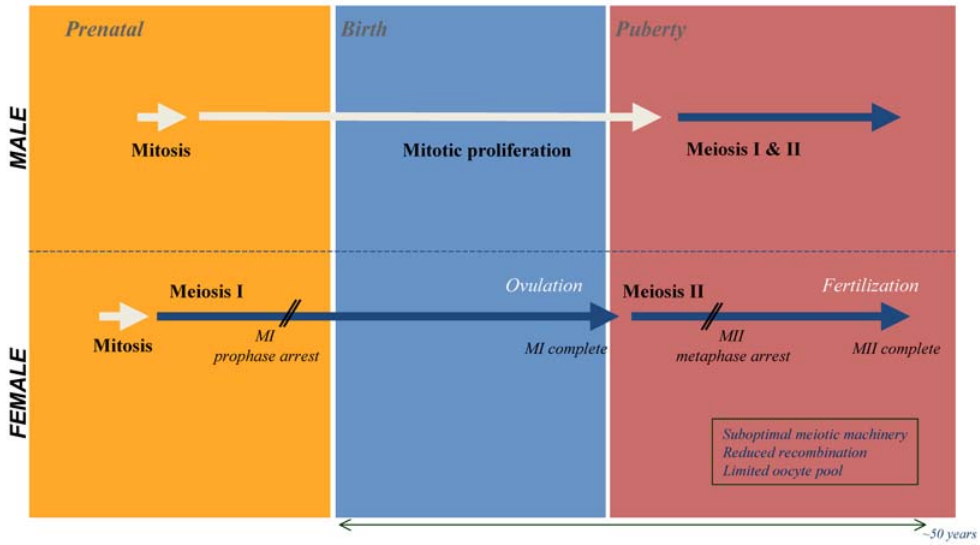

Figure 1. Male and female physiological timeline. Male and female germ cells have different timelines for mitotic and meiotic events in the developing testis and ovary. In male fetal testis, after initial mitotic arrest, a longer mitotic proliferation is resumed (from prenatal period to puberty) until the onset of puberty when meiotic events are initiated and completed. Sperm production is continued throughout the lifetime. In contrast, in the female ovary, after a smaller mitotic proliferation event, the germ cells enter into protracted meiosis I and II periods. After homologous recombination, MI is arrested at prophase, and is completed only after ovulation, at the onset of puberty. MII is then initiated only to be arrested again, in metaphase II; MII is completed after fertilization. Possible advanced maternal age-associated events are also shown (box). The figure is modified from Hassold and Hunt (2001).

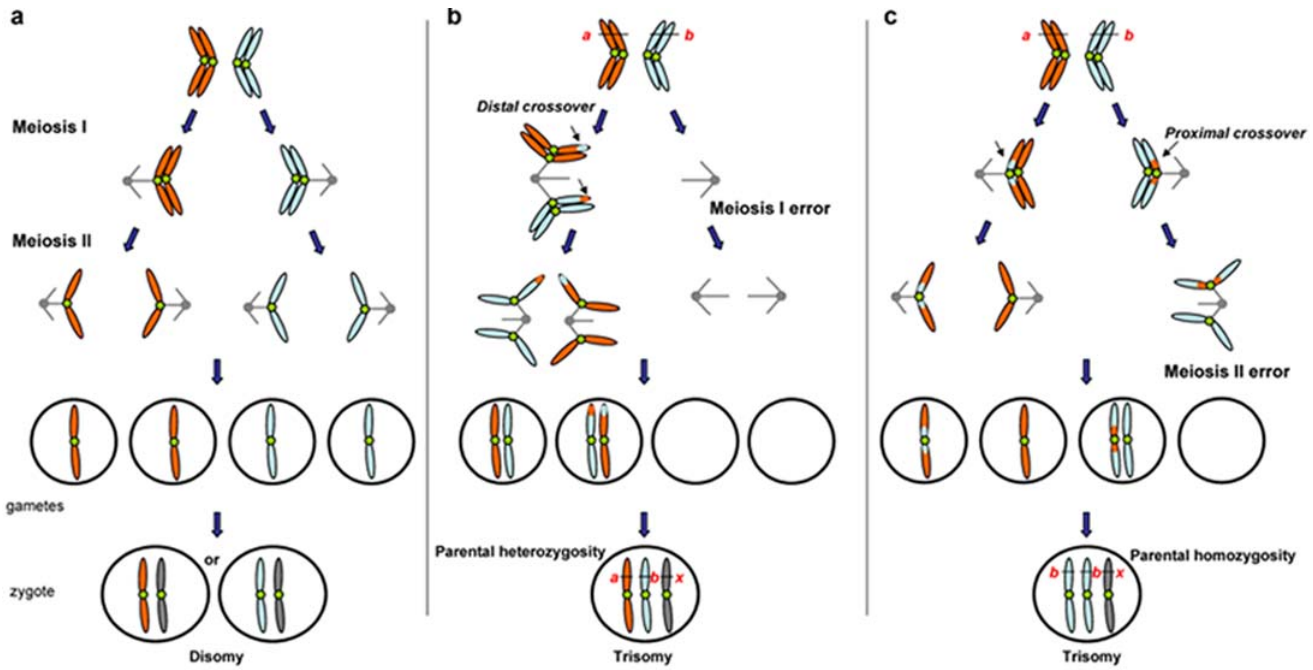

Figure 2. Normal meiosis and maternal meiotic errors. (a) The schematic shows the normal series of meiotic events. Briefly, MI ends in the separation of chromosomal homologs and MII results in the separation of sister chromatids. (b & c) Nondisjunction due to MI and MII errors is shown. Genotyping using informative microsatellite markers (a, b and x) in an individual with Down syndrome with MI error will show parental heterozygosity, while MII errors are characterized by marker homozygosity for one of the parental chromosome. Note that an excess of distal crossovers lead to MI errors and increased proximal crossover causes MII errors (modified after Lamb *et al.* 2005).

For maternal meiosis, location and number of crossovers in trisomic offspring compared to controls have shown significant differences and have indicated that: (a) increased distal (telomeric) crossovers (with shorter linkage maps) contribute to MI errors in younger women (aged <29 yr) but not so in older women (aged >34 yr), (b) increased proximal (pericentromeric) exchanges (with longer linkage maps) associated with MII errors are more common in older women and not in younger women, and finally, (c) absence of recombination (with no chiasmata formation) is associated with MI error in both age groups (Lamb *et al*. 2005). As recombination occurs during the prophase of MI, at least some MII errors are probably initiated early during MI (figure 3). It is therefore apparent that, apart from advanced maternal age, the only other factor that is consistent with maternal meiotic nondisjunction is altered recombination pattern (Sherman *et al*. 2007). Evaluation of paternal non-disjunction cases revealed a reduction in recombination in MI cases and an excess of MII errors associated with a slight increase in the amount of proximal-medial exchange compared to exchange at telomeres (Savage *et al*. 1998). Interestingly, there was an altered sex-ratio among the paternally derived MII cases with an overall increase in male probands (Savage *et al*. 1998; Petersen *et al*. 1993). The skewed sex-ratio was consistent with proximal-medial exchange in MII and was hypothesized to result from imbalanced non-disjunction products segregating with the Y chromosome during anaphase (Savage *et al*. 1998).

It has been suggested that increased biological ageing of the ovaries is a major factor for aneuploidy conditions in females -a 'limited oocytes pool' hypothesis (Warburton 1989). According to this hypothesis, the ageing of the ovary is associated with availability of limited and less optimal oocytes for fertilization. While several correlations with biological ageing of the ovary, including cigarette smoking, history of unilateral ovariectomy, and hormonal levels are still being evaluated, the median age for menopause was consistently found to be 0.9–1 year earlier in women with trisomic pregnancies compared to controls (Kline and Levin 1992; Kline *et al*. 2000). Kline *et al*. (2004) suggested that changes in follicular development, unrelated to the size of the oocyte pool, likely influence abnormal chromosome segregation (Kline *et al*. 2004). A preferential survival in older mothers of fetuses with Down syndrome prompted a 'relaxed selection' hypothesis (Erickson 1978; Ayme and Lippman-Hand 1982). Accordingly, a significant component of maternal-age association in Down syndrome live births was suggested to be caused by relaxed selection against aneuploid fetuses

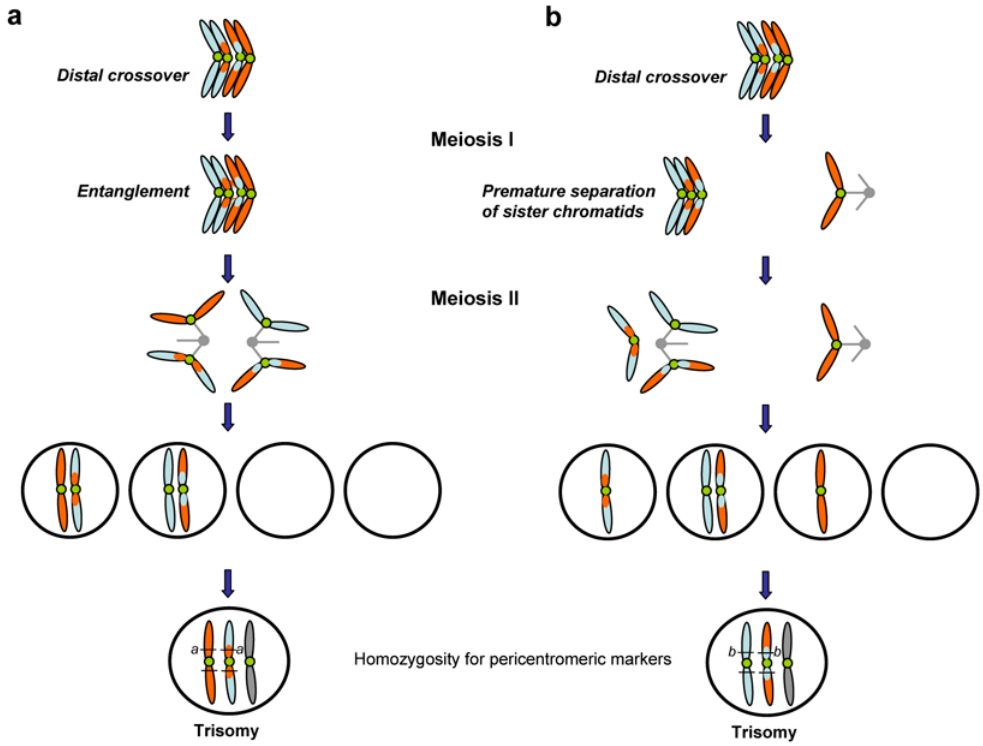

Figure 3. MI non-disjunctional errors manifesting in MII. Neil Lamb and colleagues suggested that the consequence of increased proximal crossovers contributing to MII errors may be due to (a) chromosomal entanglement at MI, wherein the bivalents are not separated until MII, or (b) premature separation of sister chromatids at MI, due to loss of sister chromatid cohesion. Here, there is separation of the whole chromosome and a single chromatid to each pole of the meiotic spindle (Lamb *et al*. 1997). Notably, there are several other possible patterns of MI and MII errors (refer to Hassold and Hunt 2001).

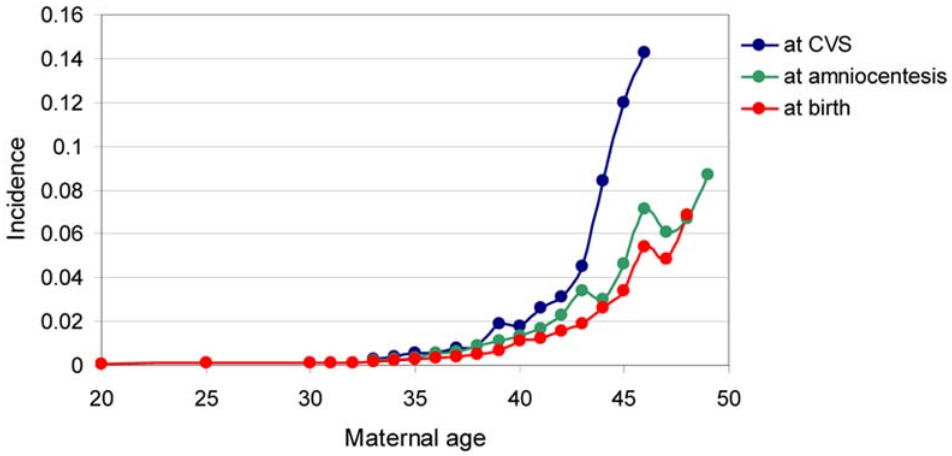

Figure 4. Incidence of Down syndrome at increasing maternal ages as identified by chorionic villus biopsy (first trimester) or amniocentesis (mid-trimester) leading to miscarriage and at birth is depicted. Note that the incidence of Down syndrome-related miscarriage increases with age. The graph is derived from published data in Savva *et al*. (2006) and Nussbaum *et al*. (2007).

(Erickson 1978; Ayme and Lippman-Hand 1982). This hypothesis was not supported by maternal age-specific prevalence of trisomic spontaneous abortions and live births, which showed that the chance for miscarriage is, in fact, much higher in older women (figure 4) (Hook 1983; Hassold *et al*. 1984; Hook *et al*. 1989).

In general, the incidence of aneuploidy in humans varies during development; for example, ~ 1% – 2% in sperms and ~ 20% in oocytes during gametogenesis, ~ 20% incidence at pre-implantation stage, ~ 35% in spontaneous abortions, ~ 4% in stillbirths, and ~ 0.3% incidence in live births, suggesting a strong *in utero* negative selection (Hassold and Hunt 2001). Snijders *et al*. (1999) estimated that approximately one-half of Down syndrome pregnancies are lost during the first trimester (diagnosed by chorionic villus sampling at 9–11 weeks) and approximately one-fourth are lost after mid-trimester amniocentesis (Snijders 1999; Snijders *et al*. 1999).

Several common themes emerge from available Down syndrome epidemiological studies: first, the mean maternal age for Down syndrome live births is about five years greater than that of control unaffected births (Cuckle *et al*. 1987). Second, the incidence of Down syndrome among pregnancies ending in miscarriage increases with maternal age (figure 4). Third, a paternal-age effect exists, but is very small in comparison to maternal-age effect in Down syndrome prevalence (Fisch *et al*. 2003).

Conclusions

Penrose's studies helped understand the hereditary basis, prenatal diagnosis and risk analysis of Down syndrome. Validation of Penrose's analysis on parental-age-related risk utilizing modern molecular diagnostic tools is interesting in several aspects. Given the phenotypic variability of Down syndrome and clinical overlap with several other congenital anomalies/mental retardation disorders or isolated disorders such as Alzheimer disease or hypothyroidism, Penrose's ascertainment of a large cohort was consistent. Penrose's clinical acumen is notable as most genetic diagnosis of mental retardation disorders, in the present day, are routinely performed by karyotyping or higher resolution DNA-based approaches and biochemical analysis — which was non-existent during his time. Finally, despite the fact that the mean age of mothers was lower 75 years ago (Irving *et al*. 2008), it is remarkable that the subtle maternal-age correlation was expanded qualitatively by Penrose's study.

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References

Allen G., Benda C. E., Book J. A., Carter C. O., Ford C. E., Chu E. H. *et al*. 1961 "Mongolism". *Lancet* **1**, 775.

Antonarakis S. E. 1991 Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. *N. Engl. J. Med*. **324**, 872–876.

Antonarakis S. E., Lyle R., Dermitzakis E. T., Reymond A. and Deutsch S. 2004 Chromosome 21 and Down syndrome: from genomics to pathophysiology. *Nat. Rev. Genet*. **5**, 725–738.

Arbuzova S., Cuckle H., Mueller R. and Sehmi I. 2001 Familial Down syndrome: evidence supporting cytoplasmic inheritance. *Clin. Genet*. **60**, 456–462.

Ayme S. and Lippman-Hand A. 1982 Maternal-age effect in aneuploidy: does altered embryonic selection play a role? *Am. J. Hum. Genet*. **34**, 558–565.

Bianco K., Caughey A. B., Shaffer B. L., Davis R. and Norton M. E. 2006 History of miscarriage and increased incidence of fetal aneuploidy in subsequent pregnancy. *Obstet. Gynecol*. **107**, 1098–1102.

Cuckle H. S., Wald N. J. and Thompson S. G. 1987 Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br. J. Obstet. Gynaecol*. **94**, 387–402.

Down J. L. H. 1866 Observations on an ethnic classification of idiots. In *Clinical lecture reports*, **3**, 259–262. London Hospital.

Dzurova D. and Pikhart H. 2005 Down syndrome, paternal age and education: comparison of California and the Czech Republic. *BMC Public Health* **5**, 69–79.

Erickson J. D. 1978 Down syndrome, paternal age, maternal age and birth order. *Ann. Hum. Genet*. **41**, 289–298.

Erickson J. D. and Bjerkedal T. O. 1981 Down syndrome associated with father's age in Norway. *J. Med. Genet*. **18**, 22–28.

Fisch H., Hyun G., Golden R., Hensle T. W., Olsson C. A. and Liberson G. L. 2003 The influence of paternal age on Down syndrome. *J. Urol*. **169**, 2275–2278.

Hassold T. J. and Jacobs P. A. 1984 Trisomy in man. *Annu. Rev. Genet*. **18**, 69–97.

Hassold T. and Hunt P. 2001 To err (meiotically) is human: the genesis of human aneuploidy. *Nat. Rev. Genet*. **2**, 280–291.

Hassold T., Warburton D., Kline J. and Stein Z. 1984 The relationship of maternal age and trisomy among trisomic spontaneous abortions. *Am. J. Hum. Genet*. **36**, 1349–1356.

Hook E. B. 1983 Down syndrome rates and relaxed selection at older maternal ages. *Am. J. Hum. Genet*. **35**, 1307–1313.

Hook E. B. 1987 Issues in analysis of data on paternal age and 47,+21: implications for genetic counseling for Down syndrome. *Hum. Genet*. **77**, 303–306.

Hook E. B., Cross P. K., Lamson S. H., Regal R. R., Baird P. A. and Uh S. H. 1981 Paternal age and Down syndrome in British Columbia. *Am. J. Hum. Genet*. **33**, 123–128.

Hook E. B., Topol B. B. and Cross P. K. 1989 The natural history of cytogenetically abnormal fetuses detected at midtrimester amniocentesis which are not terminated electively: new data and estimates of the excess and relative risk of late fetal death associated with 47,+21 and some other abnormal karyotypes. *Am. J. Hum. Genet*. **45**, 855–861.

Hunt P. A. and Hassold T. J. 2008 Human female meiosis: what makes a good egg go bad? *Trends Genet*. **24**, 86–93.

Irving C., Basu A., Richmond S., Burn J. and Wren C. 2008 Twenty-year trends in prevalence and survival of Down syndrome. *Eur. J. Hum. Genet*. **16**, 1336–1340.

Jones K. L. 2006 *Recognizable patterns of human malformation*. Elsevier Saunders, Philadelphia.

Kline J. and Levin B. 1992 Trisomy and age at menopause: predicted associations given a link with rate of oocyte atresia. *Paediatr. Perinat. Epidemiol*. **6**, 225–239.

Kline J., Kinney A., Levin B. and Warburton D. 2000 Trisomic pregnancy and earlier age at menopause. *Am. J. Hum. Genet*. **67**, 395–404.

Kline J., Kinney A., Reuss M. L., Kelly A., Levin B., Ferin M. and Warburton D. 2004 Trisomic pregnancy and the oocyte pool. *Hum. Reprod*. **19**, 1633–1643.

Lamb N. E., Feingold E., Savage A., Avramopoulos D., Freeman S., Gu Y. *et al*. 1997 Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. *Hum. Mol. Genet*. **6**, 1391–1399.

Lamb N. E., Yu K., Shaffer J., Feingold E. and Sherman S. L. 2005 Association between maternal age and meiotic recombination for trisomy 21. *Am. J. Hum. Genet*. **76**, 91–99.

Laxova R. 1998 Lionel Sharples Penrose, 1898–1972: a personal memoir in celebration of the centenary of his birth. *Genetics* **150**, 1333–1340.

Lejeune J., Gautier M. and Turpin R. 1959 Etude des chromosomes somatiques de neuf enfants mongoliens. *C. R. Acad. Sci. Paris* **248**, 1724–1722.

Mantel N. and C. R. Stark 1967 Paternal age in Down's syndrome. *Am. J. Ment. Defic*. **71**, 1025–1027.

Neilson J. P. and Alfirevic Z. 2006 Optimising prenatal diagnosis of Down's syndrome. *BMJ* **332**, 433–434.

Nomenclature 1975 Classification and nomenclature of morphological defects. *Lancet* **1**, 513.

Nussbaum R. L., McInnes R. R. and Willard H. F. 2007 Prenatal diagnosis. In *Thompson and Thompson Genetics in Medicine*. Saunders, Philadelphia.

Patterson D. and Costa A. C. 2005 Down syndrome and genetics - a case of linked histories. *Nat. Rev. Genet*. **6**, 137–147.

Penrose L. S. 1933 The relative effects of paternal and maternal age in mongolism. *J. Genet*. **27**, 219–224.

Penrose L. S. 1934 The relative aetiological importance of birth order and maternal age in Mongolism. *Proc. R. Soc. London. B* **115**, 431–450.

Penrose L. S. 1954 Mongolian idiocy (mongolism) and maternal age. *Ann. N. Y. Acad. Sci*. **57**, 494–502.

Penrose L. S. 1964 Genetic aspects of mental deficiency. In *Proceedings of the international Copenhagen congress on the scientific study of mental retardation*, Vol. 2 (ed. J. Öster), pp. 165–172, Det Berlingske Bogtrykkeri, Copenhagen.

Petersen M. B., Schinzel A. A., Binkert F., Tranebjaerg L., Mikkelsen M., Collins F. A. *et al*. 1991 Use of short sequence repeat DNA polymorphisms after PCR amplification to detect the parental origin of the additional chromosome 21 in Down syndrome. *Am. J. Hum. Genet*. **48**, 65–71.

Petersen M. B., Antonarakis S. E., Hassold T. J., Freeman S. B., Sherman S. L., Avramopoulos D. and Mikkelsen M. 1993 Paternal nondisjunction in trisomy 21: excess of male patients. *Hum. Mol. Genet*. **2**, 1691–1695.

Roper R. J. and Reeves R. H. 2006 Understanding the basis for Down syndrome phenotypes. *P LoS Genet*. **2**, e50.

Saller Jr D. N. and Canick J. A. 2008 Current methods of prenatal screening for Down syndrome and other fetal abnormalities. *Clin. Obstet. Gynecol*. **51**, 24–36.

Savage A. R., Petersen M. B., Pettay D., Taft L., Allran K., Freeman S. B. *et al*. 1998 Elucidating the mechanisms of paternal nondisjunction of chromosome 21 in humans. *Hum. Mol. Genet*. **7**, 1221–1227.

Savva G. M., Morris J. M., Mutton D. E. and Alberman E. 2006 Maternal age-specific fetal loss rates in Down syndrome pregnancies. *Prenat. Diagn*. **26**, 499–504.

Sherman S. L., Lamb N. E., Feingold E. 2006 Relationship of recombination patterns and maternal age among non-disjoined chromosomes 21. *Biochem. Soc. Trans*. **54**, 578–580.

Sherman S. L., Allen E. G., Bean L. H. and Freeman S. B. 2007 Epidemiology of Down syndrome. *Ment. Retard. Dev. Disabil. Res. Rev*. **13**, 221–227.

Snijders R. 1999 Fetal loss in Down syndrome pregnancies. *Prenat. Diagn*. **19**, 1180.

Snijders R. J., Sundberg K., Holzgreve W., Henry G. and Nicolaides K. H. 1999 Maternal age and gestation-specific risk for trisomy 21. *Ultrasound Obstet. Gynecol*. **13**, 167–170.

Steele M. W. and Breg Jr W. R. 1966 Chromosome analysis of human amniotic-fluid cells. *Lancet* **1**, 383–385.

Stene J., Stene E., Stengel-Rutkowski S. and Murken J. D. 1981 Paternal age and Down's syndrome: data from prenatal diagnoses (DFG). *Hum. Genet*. **59**, 119–124.

Stene E., Stene J. and Stengel-Rutkowski S. 1987 A reanalysis of the New York State prenatal diagnosis data on Down's syndrome and paternal age effects. *Hum. Genet*. **77**, 299–302.

Valenti C., Schutta E. J. and Kehaty T. 1968 Prenatal diagnosis of Down's syndrome. *Lancet* **2**, 220.

Warburton D. 1989 The effect of maternal age on the frequency of trisomy: change in meiosis or in utero selection? *Prog. Clin. Biol. Res*. **311**, 165–181.

Warburton D. 1997 Human female meiosis: new insights into an error-prone process. *Am. J. Hum. Genet*. **61**, 1–4.

Warburton D. 2005 Biological aging and the etiology of aneuploidy. *Cytogenet. Genome Res*. **111**, 266–272.

Wright S. 1926 Effect of age of parents upon characteristics of the Guinea-pig. *Am. Nat*. **60**, 552–559.

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