

**KARYOTYPES FOUND IN THE POPULATION DECLARED AT INCREASED
RISK OF DOWN SYNDROME FOLLOWING MATERNAL SERUM
SCREENING**

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ABSTRACT

Of the 65328 pregnancies of South Australian mothers screened by the South Australian Maternal Serum Antenatal Screening Programme between 1st January 1991 and 31st December 1997, 3431 (5.25%) were declared at increased risk of fetal Down syndrome. Fetal or neonatal karyotype was determined in 2737/3431 (79.8%) of these pregnancies, including 16 with early fetal loss. Interrogation of the database of the South Australian Neonatal Screening Service showed 643 liveborn infants whose phenotype was not subsequently questioned among the 694 pregnancies whose karyotype was not determined. Of the remaining 51/3431 pregnancies, 19 ended in early fetal loss without karyotyping and no newborn screening or other records could be found for 32. The 129 instances of abnormal karyotype found were Down syndrome (84), trisomy 18 (4), trisomy 13 (3), triploidy (2), female sex chromosome aneuploidy (6) and male sex chromosome aneuploidy (5), inherited balanced rearrangements (19), mosaic or *de novo* balanced abnormalities (4) and unbalanced karyotypes (2). In the pregnancies declared at increased risk of fetal Down syndrome, only the karyotype for Down syndrome occurred with a frequency greater than that expected for the general, pregnant population.

INTRODUCTION

While maternal serum screening is often thought of as screening for fetal Down syndrome, it has been clear from the outset that a number of other fetal chromosome anomalies also appear in the group found at increased risk of this condition. The summed prevalence of these other aneuploidies in this group may approximate that of Down syndrome (Haddow *et al.*, 1992; Brambati *et al.*, 1993; Benn *et al.*, 1995; Sheridan *et al.*, 1997; Chao *et al.*, 1999). Certain aneuploidies besides trisomy 21 are commonly reported, particularly trisomy 18 (Brambati *et al.*, 1993; Benn *et al.*, 1995; Hsu *et al.*, 1998) and Turner syndrome (McDuffie *et al.*, 1996; Wenstrom *et al.*, 1996). Other aneuploidies are also occasionally encountered (Harper *et al.*, 1994; Chen *et al.*, 1997; Hsu *et al.*, 1998; Zanini *et al.*, 1998). It is not certain, however, which of these aneuploidies are concentrated in the group declared at increased risk of Down syndrome, and which are there merely by chance. Distinguishing between these occurrences will permit an antenatal screening programme to make a clearer statement of its scope, i.e. the range of conditions for which it is truly screening.

When considering diagnostic karyotyping strategies to follow a result indicating an increased risk of fetal Down syndrome, the shorter turnaround time of 1-2 days for results from molecular cytogenetic techniques, primarily fluorescence *in situ* hybridisation (FISH) using labelled DNA probes to amniocytes, is attractive to obstetricians and patients alike. The specificity of molecular cytogenetic techniques is, however, a disadvantage in this setting (Isada *et al.*, 1994). Only standard numerical abnormalities are detected. Mosaic karyotypes also cause a problem using FISH.

Technical modifications are being published which may overcome these problems (Findlay *et al.*, 1998). Even so, the selection of probes for use in a FISH based diagnostic strategy requires the karyotypes selected by the screening procedure to be defined.

Determining the complete list of abnormal karyotypes present in the population of pregnancies screened at increased risk of fetal Down syndrome requires a comprehensive audit of the maternal serum screening programme, and presents the problem that not all mothers elect to undergo amniocentesis. Most reports of karyotype information focus only on those women who had amniocentesis (Benn *et al.*, 1995; Sheridan *et al.*, 1997; Chao *et al.*, 1999). Very few have attempted to identify fetal karyotypes in those who did not (Haddow *et al.*, 1992). In the present study, use was made of the database of the South Australian State Neonatal Screening Programme and other individual records as the avenues for addressing this problem.

MATERIALS AND METHODS

Seven years of maternal serum screening results, totalling 65328 singleton pregnancies from South Australian mothers screened between 1st January 1991 and 31st December 1997, were used as the test population. During this time the South Australian Maternal Serum Antenatal Screening Programme maintained constant test procedures using four analytes, alphafetoprotein, free α -glycoprotein subunit (α -hCG), free β -subunit of chorionic gonadotropin (free β -hCG), and unconjugated estriol (Ryall *et al.*, 1992).

Comprehensive annual audits of programme performance were maintained. Throughout this period, and introduced at various times, the data management software included algorithms for identification of pregnancies at risk of fetal neural tube defects, Down syndrome, non-viability, unsuspected twins, trisomy 18 and significant (greater than 3 weeks) misdating. No separate algorithms for the identification of triploidy, Turner syndrome or any of the other aneuploidies which are associated with known, abnormal patterns of maternal serum analyte results, were applied prospectively to these data.

The databases and sources of information accessed to ascertain the outcome of those pregnancies screened at increased risk of fetal Down syndrome from this population were: (a) the database of the South Australian Maternal Serum Antenatal Screening Programme; (b) the database of the South Australian State Neonatal Screening Programme; (c) the databases of the Department of Cytogenetics and Molecular Genetics of the Women's and Children's Hospital and of GCAT Pty Ltd, which between them provide all karyotype analyses in South Australia; (d) the Annual Reports of the South Australian Birth Defects Register.

In determining the probability that the given karyotype was detected by the screening algorithm, a simple χ^2 test was applied to the uncorrected, observed frequency of that karyotype in the group screened at increased risk, and the birth or second trimester estimates of the prevalence of the condition in general taken from Fergusson-Smith and Yates (1984), and Warburton (1991).

RESULTS

From the 65328 singleton pregnancies screened, 3431 (5.25%) were declared to be at increased risk of fetal Down syndrome following confirmation of gestational and maternal age information. Of these 3431 pregnancies, fetal karyotype was determined on 2737 (79.8%), either from chorion villus, amniocyte, blood or *post mortem* tissue specimens (Table 1). The majority of the results (2608/2737, 95.3%) showed a karyotypically normal fetus, including 2 following elective termination of pregnancy because of severe fetal structural abnormalities, 10 following fetal death *in utero*, and 4 stillborn.

In 129 pregnancies there was an abnormal fetal karyotype (Table 2). There were 95 abnormal karyotypes associated with significant clinical abnormality, 11 sex chromosome abnormalities, and 3 *de novo* balanced translocations which have a 3.1-10.3% risk of being phenotypically abnormal (Warburton, 1991). In addition there was one level 2 mosaicism for trisomy 8 which may be associated with phenotypic abnormality. Nineteen other instances of abnormal karyotype were inherited balanced rearrangements which were not expected to be associated with clinical abnormalities.

Of the 694 pregnancies (20.2%) screened at increased risk of fetal Down syndrome and for whom karyotyping studies were not performed, newborn screening records were found on 643. These were the infants liveborn to mothers who declined amniocentesis upon receipt of an increased risk result from maternal serum screening, and whose appearance at 18 weeks gestation on morphology ultrasound, and at birth, raised no

suspicion of abnormal karyotype. They were consequently not karyotyped from cord blood specimens at birth or *post mortem*. The continued absence of these infants from the cytogenetics databases of South Australia, given that they are now between 3 and 9 years old, permits the assumption that each has a normal karyotype or, if abnormal, presents no clinical problems. This assumption is further supported by the coincidence between the annual numbers of pregnancies identified in this study as being affected by fetal Down syndrome and the numbers given independently in the Annual Reports of The South Australian Birth Defects Register 1991-1997.

Reference to case records showed 16 of the remaining 51 pregnancies to have suffered early fetal loss, two were electively terminated because of fetal neural tube defects, and one was stillborn. Karyotyping studies were not requested on any of these. No outcome records could be located for the remaining 32/3431 (0.9%) pregnancies.

Fetal karyotype was therefore known in 2737/3431 (79.8%) of pregnancies screened at increased risk of fetal Down syndrome, and could be assumed normal or clinically insignificant if abnormal in a further 643/3431 (18.7%). Total ascertainment of fetal and neonatal karyotypes in the study group can thus be considered 3380/3431 (98.5%).

Of the abnormal karyotypes found (Table 2), only that of Down syndrome was significantly greater in frequency in the group declared at increased risk of Down syndrome than in the general population. The expected prevalence in second trimester is 1:585, calculated from an expected birth prevalence in the screened population on the basis of its age profile of 1:734 (Staples *et al.*, 1991), and a spontaneous loss rate of

25.5% from second trimester to term (Hook *et al.*, 1983). The observed prevalence of 1:41 (84/3431) in the increased risk group is greater than this ($0.01 < p < 0.05$).

The four-fold increase in frequency of trisomy 13 found in the increased risk group, based on an observed prevalence of 1:1144 compared to an expected prevalence of 1:5080 calculated from a birth prevalence of 1:8000 and a fetal loss rate of 36.5% (Fergusson-Smith and Yates, 1984), failed to reach significance ($0.2 < p < 0.3$). Any adjustment of the expected prevalence applied on the grounds that the study population was older than that published by Fergusson-Smith and Yates (1984) will only make this figure less significant.

No other karyotypes were found at greater frequency in the increased risk group than in the general pregnant population. An observed prevalence of trisomy 18 of 1:858 in the increased risk group was indistinguishable from an expected prevalence of 1:2389 in the general population based on a birth prevalence of 1:6600 and a subsequent loss rate of 63.8% ($0.4 < p < 0.5$) (Fergusson-Smith and Yates, 1984). This was also true for the frequency of total female sex chromosome aneuploidies. Assuming that half the fetuses in the increased risk group were female gave an observed frequency of 1:286 female fetuses, compared to an expected frequency at birth of 1:526 girls ($0.6 < p < 0.7$). Correcting for any spontaneous loss of these fetuses after second trimester would only reduce the likelihood of these frequencies being different. Similar figures follow for the male sex chromosome aneuploidies (found 1:343 male fetus' vs expected 1:384 boys at birth, $0.9 < p < 0.95$), balanced translocations (found 1:181 vs expected 1:526 at birth,

0.4<p<0.5) and those unbalanced translocation and *de novo* abnormalities listed in Table 1 which may be associated with an abnormal phenotype.

DISCUSSION

A screening test functions to concentrate individuals affected by the screened condition into a small group. Typically, maternal serum screening for Down syndrome in second trimester concentrates about 70% of pregnancies affected by fetal Down syndrome in an 'increased risk' group of around 5% of the total screened. It is in the nature of prenatal screening that other abnormal fetal karyotypes are also found in this increased risk group. These may be obligatory detections if the alternative karyotype generates screening variables which mimic the parameters set for fetal Down syndrome, or they may be there by chance. The distinction is important since it defines the scope of the screening programme, and thereby defines the content of pre- and post-test counselling information (Sheridan *et al.*, 1997) and determines the diagnostic strategy which can follow an increased risk screening result.

The accumulated results of seven years operation of the South Australian Maternal Serum Antenatal Screening (SAMSAS) Programme show one karyotypically abnormal fetus was found for every 26 pregnancies declared at increased risk of fetal Down syndrome, a figure consistent with other reports (Haddow *et al.*, 1992; McDuffie *et al.*, 1996). One fetus affected by Down syndrome was detected for every 41 reports issued stating increased risk of fetal Down syndrome. The screening programme thus

concentrated pregnancies affected by fetal Down syndrome more than 14-fold over their expected prevalence in the general population in the second trimester.

In contrast, none of the other abnormal fetal karyotypes found in the group declared at increased risk of fetal Down syndrome could be shown to have a greater frequency in that group than in the general pregnant population. This is not surprising for trisomy 18, which is characterised by a pattern of analytes quite different from that for fetal Down syndrome. Turner syndrome, however, could have been expected to be an obligatory screened condition using an algorithm targeted to Down syndrome, since the serum biochemical pattern for this aneuploidy also features lowered alphafetoprotein and unconjugated estriol values and raised free β -hCG. However, this was not so, and the presence of this and all the other abnormal karyotypes found in the group declared at increased risk of fetal Down syndrome is no more than chance.

A measure of caution accompanies this statement. Statistical analysis was limited to performing a simple χ^2 test on the observed *versus* expected frequencies of the disorders. Even though the data cover seven full years of screening results, the rarity of the conditions encountered means that the numbers of affected pregnancies found remains low. Working from a larger database may produce different conclusions. It is noted, however, that adjustments to the expected prevalences of the conditions in mid-trimester, applied on the grounds that the study population was older or that fetal loss rates may be higher than that used as reference (Fergusson-Smith and Yates, 1984), will only reduce the significance of the differences found between observed and expected prevalences in those conditions already found insignificant by the present analysis.

Several conclusions follow from this analysis. Firstly, the algorithm used for second trimester screening for fetal Down syndrome properly detects only Down syndrome. Secondly, broadening the scope of the screening programme to include other abnormal karyotypes such as trisomy 18 and Turner syndrome, requires separate algorithms specific to those conditions to be built into the screening software. Thirdly, the distinction between those conditions deliberately screened and those found by chance is not always obvious, and must be determined so that accurate pre-test counselling information (Sheridan *et al.*, 1997) and post-test diagnostic strategies can be properly prepared.

The presented data show that a post-screening diagnostic strategy based on rapid, molecular cytogenetic techniques must certainly include testing for Down syndrome, since this condition is specifically targeted by the screening algorithm. Given that a woman will have undergone an invasive procedure to acquire the specimen for testing, including trisomy 18, trisomy 13 and sex chromosome abnormalities in the rapid diagnostic strategy can be justified on the grounds of the likelihood of such pregnancies surviving to term and the severity of clinical symptoms in any infant which may be born. There would seem little justification, however, for including other conditions in a post-screening, rapid diagnostic strategy.

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Table 1. Detection of chromosome abnormalities in 3431 pregnancies screened at increased risk of fetal Down syndrome

	Amniotic fluid	Chorion villus	Blood	Post mortem tissue	Not studied
Normal karyotype	2579	22	1	6	-
Down syndrome	69	0	12	3	-
Other abnormal karyotype	40	0	1	4	-
Liveborn - no evidence of abnormality	-	-	-	-	643
Fetal loss - no karyotype	-	-	-	-	19
Lost to study	-	-	-	-	32

Table 2. Abnormal karyotypes found in the population declared “at increased risk of Down syndrome”

Autosomal aneuploidies (associated with significant clinical abnormalities)

Down syndrome	84 cases
Trisomy 18	4 cases
Trisomy 13	3 cases
Triploidy	2 cases

Sex chromosome abnormalities (variable clinical significance)

<i>Female</i>	
45,X	3 cases
46,X,idic(X)(p11.2)	1 case
47,XXX	2 cases
<i>Male</i>	
47,XXY	3 cases
47,XXY/46,XY	1 case
47,XYY	1 case

Unbalanced karyotypes (associated with significant clinical abnormality)

46,XX,der(6)t(6;?)(q21;?)	1 case
47,XX,+mar.ish der(2)(p11.2q11.2)(3647+)/46,XXde novo	1 case

Mosaic or de novo cytogenetically balanced karyotypes (may be associated with a phenotypically abnormal individual)

46,XX,t(2;21)(q35;q22.1)de novo	1 case
46,XX,t(3;15)(p21;q13)de novo	1 case
46,XX,t(4;14)(q21;q22)de novo	1 case
47,XX,+8/46,XX	1 case

Balanced rearrangements (expected to be associated with a phenotypically normal individual)

46,XX,inv(1)(p13q21)mat	1 case
46,XX,inv(2)(p11.2q13)pat	1 case
46,XY,inv(9)(p13q12)inv(9)(p24q32)pat	1 case
46,XY,inv(11)(p13q23)pat	1 case
46,X,inv(Y)(p11.2q11.2)pat	4 cases
45,XY,dic(13;14)(p11.2;p11.2)pat	1 case
45,XX,dic(14;22)(p11.2;p11.2)pat	1 case
46,XX,t(1;9)(q12;q12)mat	1 case
46,XY,t(2;16)(q21;q22)pat	1 case
46,XX,t(4;10)(q23;p15)pat	1 case
46,XY,t(5;19)(q11.1;q12)pat	1 case
46,XY,t(9;12)(q13;q13)pat	2 cases
46,XY,t(9;16)(q32;p13.1)mat	1 case
46,XX,t(11;15)(q25;q13)pat	1 case
46,XY,t(12;17)(q22;21.1)mat	1 case