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Spectral Karyotyping (SKY) permits the Characterisation of a de Novo unbalanced Translocation 46, XY, der(14)t(12;14)

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ABSTRACT A boy with a duplication 12q24 to qter shows the characteristic phenotype of this chromosome abnormality. The aberration could be analysed as an unbalanced translocation 12/14 de novo by combining different banding techniques with FISH and SKY. Heterologous euchromatin translocations on the heterochromatic short arm regions of the acrocentrics are an extremely rare type of rearrangement.

INTRODUCTION

The application of banding techniques is the conventional way to analyse human chromosomes. Unbalanced constitutional chromosomal aberrations often result in phenotypic changes. The probability of their detection depends on the quality of the metaphases, the size of the aberration and the frequency in which the aberration occurs in the investigated cell system. The limit of resolution is in a range of 5-10 Mb pairs. A problem for the analysis of subtle structural aberrations is the possibility of a similar banding pattern between the exchanged chromosome regions. Thus, the identification of unbalanced translocations, de novo complex rearrangements or additional marker chromosomes often remains questionable, incomplete or even impossible.

Molecular cytogenetic methods including fluorescence in situ hybridisation (FISH) (Cremer et al. 1988) and chromosome microdissection followed by reverse FISH (Viersbach et al. 1994; Müller-Navia et al. 1995) have been applied to clarify problematic cases. Various probe sets such as whole chromosome paints, alpha satellite probes or single copy probes are available to analyse chromosomal rearrangements. However, the application of conventional FISH requires some previous knowledge about the involved chromosomes in order to choose the DNA probes for hybridisation. Also, chromosome microdissection followed by PCR and reverse FISH might solve this problem but does not provide genome-wide screening capabilities.

In 1996, two new methods were described which combine the sensitivity and specificity of FISH with the possibility to analyse the whole genome simultaneously. For M-FISH, simultaneous colour differentiation of the 24 human chromosomes is achieved by combinatorial labelling, hybridisation of a probe cocktail followed by sequential acquisition of five to six images through fluorochrome specific narrow band pass filters and complex computational analysis (Speicher et al. 1996). The second method, a 24 colour FISH called spectral karyotyping (SKY) is based on the measurement of the entire emission spectrum through a single custom designed filter using a spectrophotometer and Fourier analysis to obtain a specific spectrum for each image point (Schröck et al. 1996).

We present the application of SKY for clinical diagnostics of a case with a derivative chromosome 14 showing unidentified additional material on the short arm. Fetal blood sampling and conventional banding analysis were performed prenatally. After birth of the child, SKY investigations of placental tissue revealed the chromosomal aberration that caused the abnormal phenotype. The SKY-results were confirmed using two-colour-FISH-experiments.

MATERIAL AND METHODS

Fetal blood sampling was performed in the 34th gestational week because of phenotypic abnormalities of the child that were detected by ultrasound.

Lymphocyte short-term cultures and

subsequent conventional cytogenetic analysis after QFQ and RBA banding were done according to standard protocols. FISH and SKY investigations were performed after the child was born using long-term cultures of placental tissue.

FISH with a whole chromosome painting probe for chromosome 12 was carried out as recommended by the manufacturers protocols.

For spectral karyotyping (SKY), chromosome painting probes were generated from flow sorted human chromosomes using sequence independent DNA amplification. In a secondary PCR reaction, labelling of the chromosomes was performed by directly incorporating haptenized or fluorochrome-conjugated nucleotides as described by Schröck et al. (1996). In the presence of an excess of human Cot-1 DNA (BRL) the differentially labelled chromosome painting probes were combined and precipitated. Slides were hybridised for 2 days at 37°C. The biotinylated probe sequences were visualised using avidin Cy5 (Amersham Life Science). The digoxigenin-labelled probe sequences were detected by incubation with an anti-mouse digoxin antibody followed by a goat anti-mouse-antibody conjugated to Cy5.5. Chromosomes were counterstained with DAPI and covered in paraphenylene-diamine.

Image acquisition was performed using a SD200 Spectracube system (Applied Spectral Imaging, Inc.) mounted on a Leica DMRBE microscope with a custom designed optical filter (SKY v3.0, Chroma Technology, Brattleboro, VT). Using a Sagnac interferometer in the optical head, an interferogram was generated at all image points that is deduced from the optical path difference of the light which in turn depends on the wavelength of the emitted fluorescence. The spectrum was recovered by Fourier transformation. The spectral information was displayed by assigning red, green and blue colours to certain ranges of the spectrum. This red, green, blue (RGB)-display renders chromosomes that were labelled with spectrally overlapping fluorochromes or fluorochrome combinations. Based on the measurement of the spectrum for each pixel, however, a spectral classification algorithm was applied that assigns a pseudocolour to all points in the image that show the same spectrum. This algorithm permits the unequivocal chromosome identification by spectral karyotyping (Garini et al. 1996). DAPI images were acquired from all metaphases analysed using a DAPI specific optical filter set.

RESULTS

Clinical Findings: The patient (Fig. 1) was born spontaneously in the 38th g.w. He showed dysmorphisms and malformations as detailed in Table 1. He was then reinvestigated at the age of 6. At that time, his length amounted to 102 cm (< 3rd P) and his weight to 12,5 kg (<3rd P). His IQ equaled about 50. His speach capacity was extremely retarded, he spoke only 2 words, while his hearing abilities were almost normal. He suffers from frequent infections of the middle ear. Because of an extreme muscular hypotonia, he can eat his meals in very small portions, only. Due to motoric redardation, he has been receiving physiotherapeutic treatment and is now able to walk and mount stairs.

Cytogenetic and Molecular-cytogenetic Findings: Conventional cytogenetic analysis revealed additional chromosomal material on the short arm of chromosome 14 derived de novo. After QFQ banding the additional material showed pale fluorescence (Fig. 2) and bright fluorescence after RBA banding (Fig. 3), respectively. However, its chromosomal origin could not be identified.

SKY analysis detected a chromosome 12 origin of the unknown material (Fig. 4). The pseudocolour (spectra based colour) of the additional segment on the short arm of chromosome 14 (Fig. 4B) unambiguously allowed

Table 1: Clinical symptoms of the patient in comparison to findings in the literature (Schinzel, 2001)

Findings in dup(12)(q24-qter)	*Patient*
low birth weight	2530g
asymmetric large head	+
frontal bossing	+
dolichocephaly	+
hypertelorism	+
heavy eyebrows	+
short nose with bride flat bridge	+
small, anteverted nostrils	+
macrostomia	+
small mandible	+
posterior rotated, large ears	+
small chin	+
very short neck	+
clino- and camptodactyly	+
cardiac defects (50%)	VSD
renal and anal malformations	anal atresia
moderate to severe developmentally retarded	+

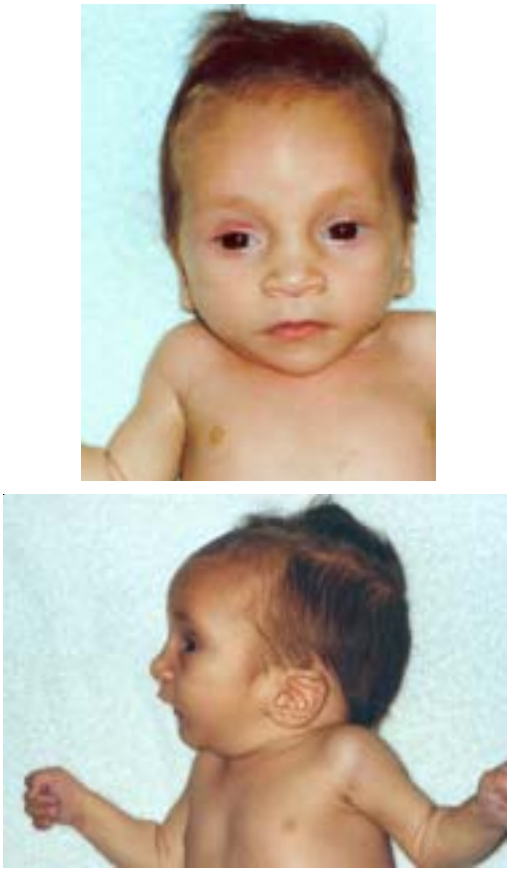

Fig. 1. patient with dup 12q24-qter (for description of clinical findings see Table 1), 3 months

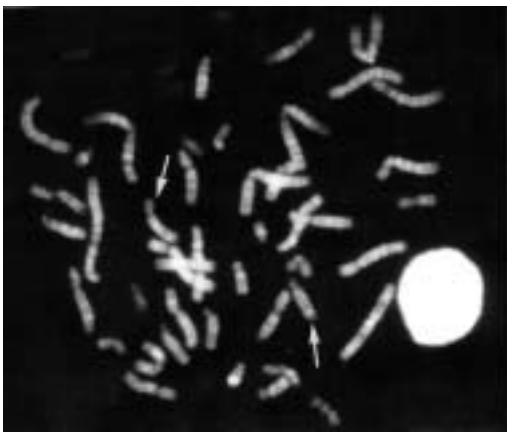

Fig. 2. Metaphase after QFQ banding (arrows denote normal and aberrant chromosome 14).

the classification as chromosome 12 material. After comparison with the chromosome banding pattern obtained by conventional cytogenetics it was concluded that the additional material originated from 12(q24-qter).

FISH with a whole chromosome paint for chromosome 12 (AGS) confirmed the SKY-result (Fig. 5):

Karyotype: 46,XY, der(14). ish t(12;14)(q24; p11.2)(wcp12+; SKY).

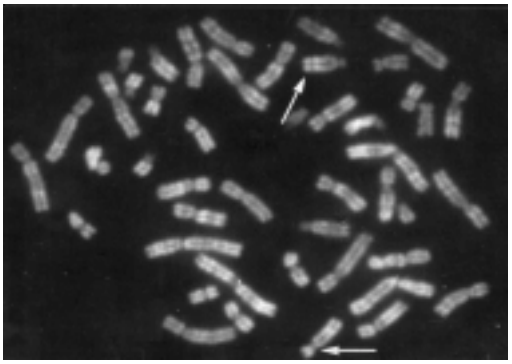

Fig. 3. Metaphase after RBA banding (arrows denote normal and aberrant chromosome 14).

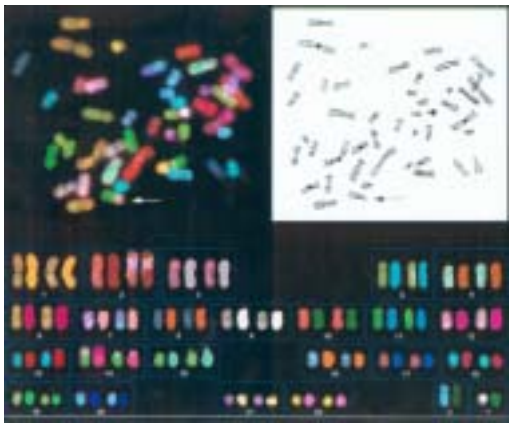

Fig. 4. Metaphase spread after simultaneous SKY-hybridisation of 24 combinatorially labelled chromosome-painting probes. Chromosomes are shown in RGB display colours (A); chromosome spectra are visualised by applying an RGB look up table to different regions of the spectrum, for each homologue chromosome it is shown on the left side the display colour and of the identical chromosome on the right side the spectra based colour (B); electronically inverted DAPI image (C); the aberrant chromosome 14 is denoted by arrow.

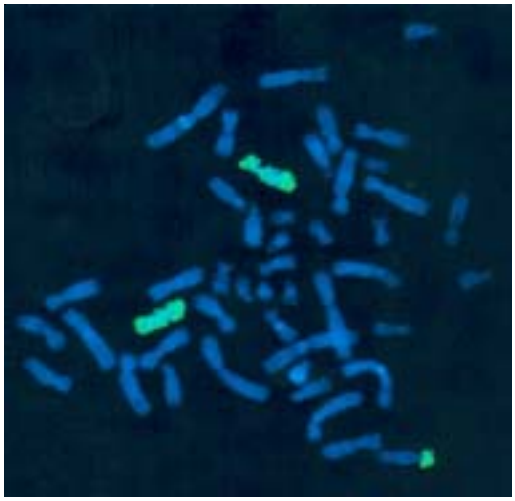

Fig.5. Metaphase after FISH with wcp 12, both normal chromosomes 12 and the additional segment on the short arm of chromosome 14 show hybridisation signals.

DISCUSSION

Although chromosome-banding techniques are well established and allow recognition of most karyotypic changes, they are limited with regards to the identification of specific aberrations. Even under optimal conditions the detection and/or analysis of certain aberrations may not be possible, for example in cases of marker chromosomes, some de novo unbalanced translocations with unspecific banding patterns in the additional material, complex rearrangements or subtle translocations. For these cases, fluorescence in situ hybridisation (FISH) is a suitable additional method. If a suggestion can be made about the involved chromosomes, FISH permits to identify the origin of marker chromosomes (Eggermann et al. 2002), to characterise complex chromosome rearrangements (Mercier et al. 1996; Phelan et al. 1998; Peschka et al. 1999) and subtle translocations (Engels et al. 2003). Numerical aberrations in interphase cells are analysable with centromere specific probes (Raff et al. 2001). Also deletions, amplifications, insertions and translocations have been visualised using locus specific probes on metaphase chromosomes (Bosse et al. 2004) or fibre FISH preparations (Florijn et al. 1995). However, for each in situ hybridisation the DNA probes must be carefully selected. The FISH-analysis of completely unclear cases can become rather complex, expensive and time consuming until the adequate DNA probes are found.

In addition, microdissection in combination with probe amplification and reverse hybridisation has been developed especially for the analyses of marker chromosomes (Viersbach et al. 1998). The problem of this method is that one must be able to unambiguously detect the chromosomes or regions in question under the microscope that they can be removed with a fine glass needle. Cross hybridisation because of similarities between short DNA sequence motifs between two or more chromosome regions is possible and may cause difficulties for the interpretation of the results. Microdissection of cases showing unbalanced translocations is problematic as only additional material can be characterised but not deleted regions.

Comparative genome hybridisation (CGH) offers a global approach as a genome-wide screening technique for detection of chromosome imbalances in a single experiment (Du Manoir et al. 1993). The CGH technique has mostly been used in research laboratories for the identification of copy number changes in tumour genomes. More recently, CGH has been successfully applied in clinical cytogenetics as an additional method to conventional banding techniques (Lapierre et al. 1998; Breen et al. 1999). However, balanced chromosomal rearrangements or inversions, ploidy changes and weak mosaicism (<40%) cannot be detected by CGH (Kallioniemi et al. 1994) . The limit of resolution for copy number increases or decreases is estimated to be in the range of 10 Mb pairs. Gains of smaller chromosomal regions are identified if they are present in high copy numbers. A great advantage of the CGH-technique is the possibility to use DNA for hybridisation from uncultivated tissue.

Since its introduction in 1996 by Schröck et al. SKY continues to be a powerful tool for the investigation of chromosomal aberrations that are difficult to clarify by conventional cytogenetic or molecular cytogenetic methods. SKY has been applied for in clinical cytogenetics (Schröck et al. 1997), in cancer cytogenetics of haematological malignancies (Veldmann et al. 1997; Rao et al. 1998; Sawyer et al. 1998) or solid tumors (Macville et al. 1999) and in comparative cytogenetics (Liyanage et al. 1996; Coleman et al. 1997). SKY revealed subtle translocations with a size of approximately 1-2 Mb pairs depending

on the chromosome resolution (Schröck et al. 1996). Limitations of this method are the detection of small intrachromosomal aberrations and the identification of marker chromosomes consisting of repetitive DNA sequences.

In the present case of a de novo derived aberrant chromosome 14 with unidentified material on the short arm, SKY proved to be a rapid and reliable method for identification of the additional material as being derived from chromosome 12. The phenotype of the patient resembled the dysmorphisms known for dup (12) (q24-qter) (Schinzel 2001).

Especially in prenatal diagnosis, a comprehensive karyotype analysis must be performed as fast as possible to provide the parents with the necessary information about the prognosis for the development of the fetus. In unbalanced cases decisions for the possible interruption of the pregnancy or for the adapted birth management are to be made.

In the future, SKY shall be widely used to complement clinical cytogenetics. The combined application of multicolour karyotyping and high resolution banding analysis might become the standard of care in prenatal and postnatal diagnostics.

SUMMARY

SKY has proven to be a powerful and rapid tool for the characterisation of an unbalanced translocation that could not be identified by conventional cytogenetics. Fetal blood sampling was performed in a fetus of the 34th gestational week because of abnormalities detected by ultrasound. The cytogenetic analysis after QFQ and RBA banding revealed a derivative chromosome 14 with unidentified additional material on the short arm. The chromosomes of the parents were normal.

SKY was performed to determine the chromosomal origin of the additional material. This method combines the sensitivity and specificity of FISH with the possibility to analyse the whole genome simultaneously. SKY revealed a chromosome 12 origin of the additional region. Comparing the SKY-data with the conventional banding pattern we concluded that the additional material originated from chromosome 12q24-qter. The phenotype of the child was in concordance with reports from the literature involving aberrations of this chromosomal region.

The advantages and disadvantages of different cytogenetic and molecular-cytogenetic methods are discussed.

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REFERENCES

Bosse K, Eggermann T, Van der Ven K, Raff R, Engels H, Schwanitz G 2004. Unbalanced translocation 8;Y (45,X,dic(Y;8)(q11.23;p23.1)): case report and review of terminal 8p deletions. *Ann Génét*, **00**: 000-000(DOI:10.1016)

Breen CJ, Barton L, Carey A, Dunlop A, Glancy M, Hall K, Hegarty AM, Khokhar MT, Power M. Ryan K, Green AJ, Stallings RL 1999. Application of comparative genomic hybridization in constitutional chromosome studies. *J Med Genet*, **36**: 511-517.

Coleman AE, Schröck E, du Manoir S, Weaver Z, Wienberg J, Ferguson-Smith M, Potter M, Ried T, Janz S 1997. Multicolor spectral karyotyping (SKY) in T(12;15)-positive BALB/c plasmacytomas. *Cancer Res*, **57**: 4585-4592.

Cremer T, Lichter P, Borden J, Ward DC, Manuelidis L 1988. Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome-specific library probes. *Hum Genet*, **80**: 235-246.

Du Manoir S, Speicher MR, Joos S, Schröck E, Popp S, Dohner H. Kovacs G, Robert-Nicoud M, Lichter P, Cremer T 1993. Detection of complete and partial chromosome gains and losses by comparative genomic in situ hybridization. *Hum Genet*, **90**: 590-610.

Eggermann K, Mau UA, Bujdosó G, Koltai E, Engels H, Schubert R, Eggermann T, Raff R, Schwanitz G 2002. Supernumerary marker chromosomes derived from chromosome 15: analysis of 32 new cases. *Clin Genet*, **62**: 89-93.

Engels H, Ehrbrecht A, Zahn S, Bosse K, Vrolijk H, White S, Kalscheuer V, Hoovers JMN, Schwanitz G, Proppinh P, Tanke HJ, Wiegant J, Raap AK 2003. Comprehensive analysis of human subtelomeres with combined binary ratio labbeling fluerescence *in situ* hybridisation. *EJHG*, **11**: 643-657.

Florijn RJ, Bonden LAJ, Vrolijk , Wiegant J, Vaandrager JW, Baas F, Den Dunnen JT, Tanke HJ, Van Ommen GJB, Raap AK 1995. High-resolution DNA fibre-FISH for genomic DNA mapping and colour barcoding of large genes. *Hum Mol Genet*, **4**: 831-836.

Garini Y, Macville M, du Manoire S, Buckwald RA, Lavi M, Katzir N, Wine D, Bar-Am I, Schröck E, Cabib D, Ried T 1996. Spectral karyotyping. *Bioimaging*, **4**: 65-72.

Kallioniemi O-P, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D 1994. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. *Genes Chromosomes Cancer*, **19**: 231-243.

Lapierre JM, Cacheux V, Da Silva F, Collot N, Hervy N, Wiss J, Tachdjian G 1998. Comparative genomic

hybridization: technical development and cytogenetic aspects for routine use in clinical laboratories. *Ann Génét,* **41**: 56-62.

Liyanage M, Coleman A, du Manoir S, Veldman T, McCormack S, Dickson RB, Barlow C, Wynshaw-Boris A, Janz S, Wienberg J, Ferguson-Smith MA, Schröck E, Ried T 1996. Multicolor spectral karyotyping of mouse chromosomes. *Nature Genet,* **14**: 312-315.

Macville M, Schröck E, Padilla-Nash H, Ghadimi BM, Keck C, Zimonjic D, Popescu N, Ried T 1999. Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping. *Cancer Res,* **59**: 141-50.

Mercier S, Fellmann F, Cattin J, Bresson JL 1996. Molecular analysis by fluorescence in situ hybridization of a prenatally detected de novo complex chromosomal rearrangement t(2q;3p;4q;13q). *Prenat Diagn,***16**: 1046-1050.

Müller-Navia J, Nebel A, Schleiermacher E 1995. Complete and precise characterization of marker chromosomes by application of microdissection in prenatal diagnosis. *Hum Genet,* **96**: 661-667.

Peschka B, Leygraaf J, Hansmann D, Hansmann H, Schröck E, Ried T, Schwanitz G, Schubert R 1999. Analysis of a de novo complex chromosome rearrangement involving chromosomes 4, 11, 12 and 13 and eight breakpoints by conventional cytogenetic, fluorescence in situ hybridization and spectral karyotyping. *Prenat Diagn,* **19**: 1143-1149.

Phelan MC, Blackburn W, Rogers RC, Crawford EC, Cooley Jr NR, Schröck E, Ning Y, Ried T 1998. FISH analysis of a complex chromosome rearrangement involving nine breakpoints on chromosomes 6, 12, 14 and 16. *Prenat Diagn,* **18**: 1174-1180.

Raff R, Schwanitz G, 2001. Fluorescence *in situ* hybridization. General principles and clinical application with special emphasis to interphase diagnostics. *Int J Hum Genet,* **1**: 65-75.

Rao PH, Cigudosa JC, Ning Y, Calasanz MJ, Iida S, Tagawa S, Michaeli J, Klein B, Dalla-Fevara R, Jhanwar SC, Ried T, Chaganti RSK 1998. Multicolor spectral karyotyping identifies new recurring breakpoints and translocations in multiple myeloma. *Blood,* **92**: 1743-1748.

Sawyer JR, Lukacs JL, Munshi N, Desikan KR, Singhal S, Metha J, Siegel D, Shaughnessy J, Barlogie B 1998. Identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. *Blood,* **92**: 4269-4278.

Schinzel A 2001. *Catalogue of Unbalanced Chromosome Aberrations in Man.* 2nd Ed. Berlin, New York: Walter de Gryter.

Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T 1996. Multicolor spectral karyotyping of human chromosomes. *Science,* **273:** 494-497.

Schröck E, Veldman T, Padilla-Nash H, Ning Y, Spurbeck J, Jalal S, Shaffer LG, Papenhausen P, Kozma C, Phelan MC, Kjeldsen E, Schonberg SA, O´Brien P, Biesecker L, du Manoir S. Ried T 1997. Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities. *Hum Genet,* **101**: 255-262.

Speicher M, Ballard SG, Ward Dc 1996. Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nature Genet,* **12**: 368-375.

Veldmann T, Vignon C, Schröck E, Rowley JD, Ried T 1997. Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. *Nature Genet,* **15**: 406-410.

Viersbach R, Schwanitz G, Nöthen M 1994. Delineation of marker chromosomes by reverse chromosome painting using only a small number of DOP-PCR amlified microdissected chromosomes. *Hum Genet,* **93:** 663-667.

Viersbach R, Engels H, Gamerdinger U, Hansmann M 1998. Delineation of supernumerary marker chromosomes in 38 patients. *Am J Med Genet,* **76**: 351-358.