A. 研究目的

近年の画像診断技術の発展により、胎児の重篤な先天異常が出生前に診断されるケースが増加している。先天異常の一次予防は重要な課題であり、その関与因子の同定が望まれている。諸外国の疫学研究で明らかになった唯一の例は、葉酸投与が二分脊椎症や無脳症を減少させることであり、日本においても葉酸摂取の啓蒙が始まったが、我が国では、未だ何ら疫学研究が進行していない。葉酸の予防的投与は望ましいものと期待されるが、近年の日本人一般女性と先天異常児を出産した日本人女性の血中葉酸値や遺伝子多型に基づく薬剤有用性も比較検討した上で、その必要性を明確にしてゆく必要がある。

葉酸による先天異常（神経管閉鎖障害およびダウン症候群、自然流産・死産など）の発生予防効果に関する検討を目的として、これらの症例の収集と正確な臨床診断、葉酸代謝関連酵素遺伝子多型の解析、予防的葉酸投与の実施・現状調査・啓蒙を行う。

B. 研究方法

本研究は、「ヒトゲノム・遺伝子解析研究に関する指針」を遵守し、さらに日本産科婦人科学会、人類遺伝学会をはじめとする日本医学会分科会、及び関連組織の倫理規定の元に実施される。研究計画、インフォームドコンセント書式については所属施設の倫理委員会より承認を得た書式を用いて研究を実施する。

＊先天異常（神経管欠損症およびダウン症候群等）の症例収集

胎児の先天異常を主訴に当施設を受診した妊婦を対象に、超音波検査や胎児MRIを導入して、出生後の追跡調査や、死産や新生児死亡症例には病理解剖も可能な限り実施して正確な臨床診断を行う。神経管閉鎖障害患児（流・死産児も含む）を分娩した症例の検体を採取する。患者が解析結果の告知を希望する場合に備え、検体、臨床情報は検体採取機関で連結可能匿名化しID番号のみとする。さらに、葉酸代謝の関連が推定される先天異常児出産母体、および原因不明流産母体の検体も収集する。他施設からの症例の収集は、“遺伝性疾患の解析のための情報・検体の集積分配ネットワーク”（厚生科学研究ヒトゲノム・再生医療等研究事業「家族性遺伝性疾患の解析のための情報・検体の集積分配ネットワーク構築に関する研究」主任研究者：鈴森薫）にて構築したネットワークシステムを基盤として、産科婦人科拠点施設に症例情報と検体の集積を依頼する。

＊葉酸代謝関連遺伝子等の遺伝子多型及び染色体分析

葉酸代謝関連酵素の遺伝子多型分析は分担研究者の羽田、染色体分析は孫田に依頼する。

＊予防的葉酸投与の実施

当施設の産科婦人科では以前より遺伝カウンセリング専門外来を設け、平成16年からは臨床遺伝医療部も開設して、先天異常児の出生や遺伝カウンセリングに関する相談に対応している。これらの症例のうち、妊娠前より予防的葉酸投与を施行した症例の追跡調査を行う。

＊教育・啓蒙活動

分担研究者大橋と協力して、本研究への協力者への研究結果の還元や、一般の方々への教育・啓蒙を目的として、研修会、公開講座を実施した。

C. 研究結果

平成14年12月1日より平成15年11月30日までの一年間に、胎児の先天異常を主訴に受診した妊婦は53名で、そのうち頭部の形態異常（水頭症や小頭症疑いなど）を疑われたものが最も多く13例存在

した。超音波検査および胎児MRI等による診断の結果、水頭症3例、全前脳胞症3例、無頭蓋症2例、滑脳症1例、クモ膜嚢胞1例、脳ヘルニア1例、脳梁低形成1例、正常1例であった。全前脳胞症については水頭症あるいは小頭症として、無頭蓋症については小頭症として紹介されたケースが大半であった。

これらの症例のうち神経管閉鎖障害が原因と予想され、染色体異常や他の遺伝子異常が確認出来なかった症例と、他施設より遺伝子解析を目的として当施設へ送付された無頭蓋症と無脳症、あわせて20家系のうち、12名の母親より同意を得て検体よりDNAを採取し、羽田に依頼してMethylenetetrahydrofolate reductase (MTHFR)のC677T多型、Methionine synthase reductase (MTRR)のA66G多型、Thymidylate synthase (TS)のプロモーター領域にある28bpのリピート回数の多型(2回または3回)、Methionine synthase(MTR)の2756番目のAがGに置換する多型などの解析を実施した。しかし、まだ症例数が少ないこともあり、健常児を分娩したコントロール群の母親の結果との間には多型頻度の有意差は認めなかった。1例を除き、これらの母体の血中の葉酸値は正常範囲であった。神経管閉鎖障害に関しては発生頻度が低くさらなる症例収集が必要である。

自然流産症例については、孫田に染色体分析を依頼して、染色体異常群20例と染色体正常群20例に分け、羽田に送付してその母児の葉酸代謝関連酵素遺伝子多型について検討した。その結果、コントロール群と染色体異常を検出した流産胎児の母親との比較で、MTHFR C677T多型頻度の有意差が確認された。しかし、流産胎児の多型頻度には有意差は認められなかった。

当施設では、追跡調査継続中の症例のうち、神経管閉鎖障害分娩既往のある6例の妊婦が、次の妊娠前より葉酸(2.5～5mg)を内服して現在までに分娩に至り、全例で健児を得た。まだ妊娠に至っていない症例の母親も葉酸の予防的内服を希望している。

D. 考察　およびE. 結論

神経管閉鎖障害は稀な先天異常で、全国レベルでの症例収集が必要であるが、他院にて診断された胎児の頭部形態異常のうち、臨床診断が正確ではない症例が多いことがまず問題であった。わずかずつではあるが、症例が集まり始め、遺伝子多型解析を開始することができたが、葉酸代謝関連酵素遺伝子多型の有意差を確認するには至らず、さらなる症例収集と追跡調査が必須である。

しかしながら、以前から追跡調査を継続してきて本年度までに分娩となった神経管閉鎖障害の分娩既往母体のうち、予防的投与群の症例からは、再発例は認めなかったことは評価できる。まだ妊娠・分娩に至っていない症例についても、全例で次妊娠前からの葉酸の予防的内服を希望されている。

今回、染色体異常流産群でMTHFR C677T多型頻度の有意差が認められたことは新しい知見である。当院で以前に染色体異常の有無を分類せずに自然流産例での多型解析を試みた際には、明らかな有意差は確認されなかったため、この結果は、葉酸の不足により染色体異常流産が起きる可能性を示唆するものと思われる。

全国的には葉酸の予防的投与を行っている施設はまだ少ない。染色体異常による自然流産への関与も考慮して一般に啓蒙すれば、予防的な葉酸内服が広く普及して、その結果として、流産も神経管閉鎖障害の発生も抑制される可能性があると思われた。

F. 健康危険情報

特になし.

G. 研究発表

1. 論文発表

種村光代；胎児染色体異常の診断およびスクリーニング．小児科診療 67(2)，45-49，2004.

Yuko Kondo, Sami Tsukishiro, Mitsuyo Tanemura, Mayumi Sugiura-Ogasawara, Kaoru Suzumori, Sinichi Sonta ; Maternal uniparental disomy of chromosome 16 in a case of spontaneous abortion. J Hum Genet 49, 177-181, 2004

2. 学会発表

船水真紀子，蒔田芳男，大森博之，大橋博文，小橋　元，種村光代，鈴森　薫，羽田明；日本人集団におけるダウン症発生と葉酸代謝酵素遺伝子多型の関連．第 47 回日本人類遺伝学会（名古屋）2002 年 11 月

李　青英，月城沙美，中川千玲，野村紀子，種村光代，杉浦真弓，鈴森　薫，孫田信一；自然流産に見られたダブルトリソミーの起源と発生機序．中部出生前医療研究会（名古屋）2004 年 2 月 28 日

月城沙美、種村光代、杉浦真弓、松田義雄、鈴森薫、孫田信一；染色体の微細欠失を有する自然流産．第 56 回日本産科婦人科学会学術講演会（東京）2004 年 4 月 10 日-13 日

種村光代；市民公開セミナー1　妊婦さんに知って欲しい染色体や遺伝子の話．第 22 回日本染色体遺伝子検査学会総会（名古屋）2004 年 11 月 5～6 日

H. 知的財産権の出願・登録状況

特になし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル	発表誌名	巻名	ページ	出版年
鈴森薫	流産・死産・先天異常の原因検索と遺伝カウンセリングに有用な Spectral karyotyping（SKY）法について	産婦人科の世界	55	31-38	2003
Kondo, Y., Tsukishiro, S., Tanemura, M., Sugiura-Ogasawara, M., Suzu-mori, K., Sonta, S	Maternal uniparental disomy of chromosome 16 in a case of spontaneous abortion	J. Hum. Genet	49	177-181	2004
Li QY, Tsukishiro S, Nakagawa C, Tanemura M, Sugiura-Ogasawara M, Suzumori K, Sonta S	Parental origin and cell stage of nondis-junction of double trisomy in spontaneous abortion.	Congen. Anom.	45	27-32	2005
Tsukishiro S, Li QY, Tanemura M, Sugiura-Ogasawara M, Suzumori K, Sonta S	Paternal uniparental disomy of chromosome 14 and unique exchange of chromosome 7 in cases of spontaneous abortion.	J. Hum. Genet.			in press
Kondoh Y, Toma T, Ohashi H, Harada N, Yoshiura K, Ohta T, Kishino T, Niikawa N, Matsumoto N	Inv dup del(4)(:p14 --> p16.3::p16.3 --> qter) with manifestations of partial duplication 4p and Wolf-Hirschhorn syndrome	Am J Med Genet	A120(1)	123-6	2003
Akahoshi K, Spritz RA, Fukai K, Mitsui N, Matsushima K, Ohashi H	Mosaic supernumerary inv dup(15) chromosome with four copies of the P gene in a boy with pigmentary dysplasia	Am J Med Genet	A126(3)	290-2	2004
Miyake N, Harada N, Shimokawa O, Ohashi H, Kurosawa K, Matsumoto T, Fukushima Y, Nagai T, Shotelersuk V, Yoshiura K, Ohta T, Kishino T, Niikawa N, Matsumoto N	On the reported 8p22-p23.1 duplication in Kabuki make-up syndrome (KMS) and its absence in patients with typical KMS	Am J Med Genet	A128(2)	170-2	2004
Akahoshi K, Ohashi H, Hattori Y, Saitoh S, Fukushima Y, Wada T	A woman with 46,XX,dup(16)(p13.11 p13.3) and the ATR-X phenotype	Am J Med Genet	A132(4)	414-8	2005
1. Ohmori H, Makita Y, Funamizu M, Hirooka K, Hosoi T, Orimo H, Suzuki T, Ikari K, Nakajima T, Inoue I, Hata A	Linkage and association analyses of the osteoprotegerin gene locus with human osteoporosis	J Hum Genet	47	400-406	2002
2. Ohmori H, Makita Y, Funamizu M, Chiba S, Ohtani K, Suzuki Y, Wakamiya N, Hata A	Haplotype analysis of human collectin placenta 1 (hCH-P1) gene	J Hum Genet	48	82-85	2003
Ohmori H, Ando Y, Makita Y, Onouchi Y, Nakajima T, Saraiva MJM, Terazaki H, Suhr O, Sobue G, Nakamura M, Yamaizumi M, Munar-Ques M, Inoue I, Uchino M, Hata A	Common origin of the Val30Met mutation responsible for the amyloidogenic transthyretin type of familial amyloidotic polyneuropathy	J Med Genet	41	e51	2004
Kobashi G, Hata A, Ohta K, Yamada H, Hirayama E, Minakami H, Fujimoto S, Kondo K	A1166C variant of angiotensinogen II type 1 receptor gene is associated with severe hypertension in pregnancy independently of T235 variant of angiotensinogen gene	J Hum Genet	49	182-186	2004
Onouchi Y, Onoue S, Tamari M, Wakui K, Fukushima Y, Yashiro M, Nakamura Y, Yanagawa H, Kishi F, Ouchi K, Terai M, Hamamoto K, Kudo F, Aotsuka H, Sato Y, Nariai A, Kaburagi Y, Miura M, Saji T, Kawasaki T, Nakamura Y, Hata A	CD40 ligand gene and Kawasaki disease	Eur J Hum Genet	12	1062-1068	2004
Yamazaki K, Takazoe M, Tanaka T, Ichimori T, Saito S, Iida A, Onouchi Y, Hata A, Nakamura Y	Association analysis of SLC22A4, SLC22A5 and DLG5 in Japanese patients with Crohn disease	J Hum Genet	49	664-668	2004
種村光代	胎児染色体異常の診断およびスクリーニング	小児科診療	67	209-213	2004

流産・死産・先天異常の原因検索と遺伝カウンセリングに有用なSpectral Karyotyping（SKY）法について

鈴森 薫*

はじめに

ヒト遺伝子地図作製を目的に開発された染色体in situ hybridization法は，非放射性物質による核酸標識法の発展とともに，近年目覚ましい発展を遂げている．中でも蛍光標識を使ったFluorescent in situ hybridization（FISH）法は，染色体上のシグナルの位置を直接検出でき，古典的な染色体分染法では診断できなかったような疾患と関連性が示唆される責任領域をFISH法を用いて検出することにより，客観的な微細な異常の診断が可能になった．先天異常の診断にFISHが利用されたのは，各染色体に特異的なcentromere近傍のプローブを用いて13，18，21および性染色体（X，Y）の数的異常をスクリーニング的に迅速に検出する方法で，産科的には羊水の未培養細胞などによく使われている．この方法の有利な点は，数百の細胞をたちどころに解析することができるので，従来の培養羊水細胞の染色体分析よりは高い確率で低頻度のモザイクも検索することができる．先天性の疾患と関連性の染色体上の責任領域がわかればFISH法を用いて検出でき，客観的な微細な変化の判定も可能である．

このような疾患でよく目にするのは，Prader-Willi症候群（PWS），Angelman症候群（AS）とMiller-Dieker症候群（MDS）である．

PWSは乳児期の筋緊張低下と，その後顕著となる肥満，精神発育遅滞，性腺発育遅延を主徴とし，低身長，小肢端症，アーモンド様眼裂などの小奇形を呈する症候群である．典型例では第15番染色体長腕q11.2の染色体微細欠失やDNA欠失が認められる．一方，Angelman症候群は，重度精神遅滞，てんかん，発作的な笑い，筋緊張低下，小頭，下顎突出，あやつり人形様失調歩行などを特徴とし，患者はほぼ同一部位の欠失が報告されている．同一部位の欠失が認められるが，まったく臨床症状が異なることからgenomic imprintingの代表例として有名な一群の疾患で，欠失を持つ染色体が父由来ならPrader-Willi症候群に，母由来ならAngelman症候群となる．

Miller-Dieker症候群とは滑脳症と顔貌の異常

Suzumori Kaoru
* 名古屋市立大学大学院医学研究科 生殖・遺伝医学講座 生殖・発生医学（産科婦人科学）
（〒467-8601 愛知県名古屋市瑞穂区瑞穂町字川澄1）

を伴い，患者の大多数に第17番染色体の短腕p13.3を含む欠失が認められる（図1）．

以上述べたのは，代表的な遺伝子特定領域の欠失によって発症する先天異常であるが，微小な相互転座を保有する2家系の母親の転座部位近傍のcosmid DNAを用いて，FISH法で出生前診断に成功している[1]．

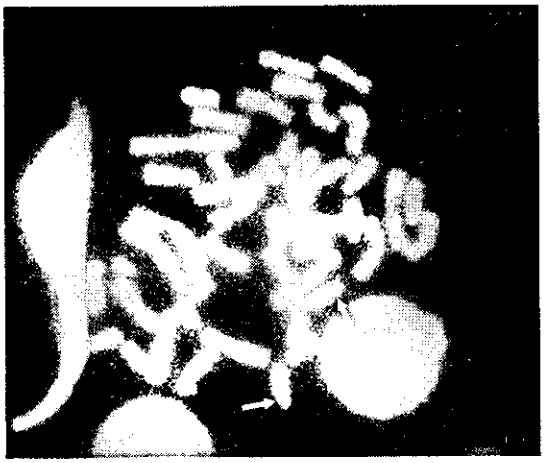

図1　Miller-Dieker症候群のFISH解析

このように分子細胞遺伝学の進歩，特にFISH法の登場は，染色体の構造や機能についてより微細な研究をもたらした．Centromeric satellite DNAプローブや染色体全体を染色するWhole-chromosome painting（WCP）は各染色体に特異的であり，従来のGバンド法などの分染法では識別できなかったような染色体特異領域を判別できるようになった[2]．しかしながら，通常のGバンド法やWCPによるFISH法をもってしても染色体の末端部（telomeric region）の変化を捉えることは不可能であった．近年，ヒト染色体末端部のtelomeric regionに特異的なプローブが開発され，cripticで確認できないような染色

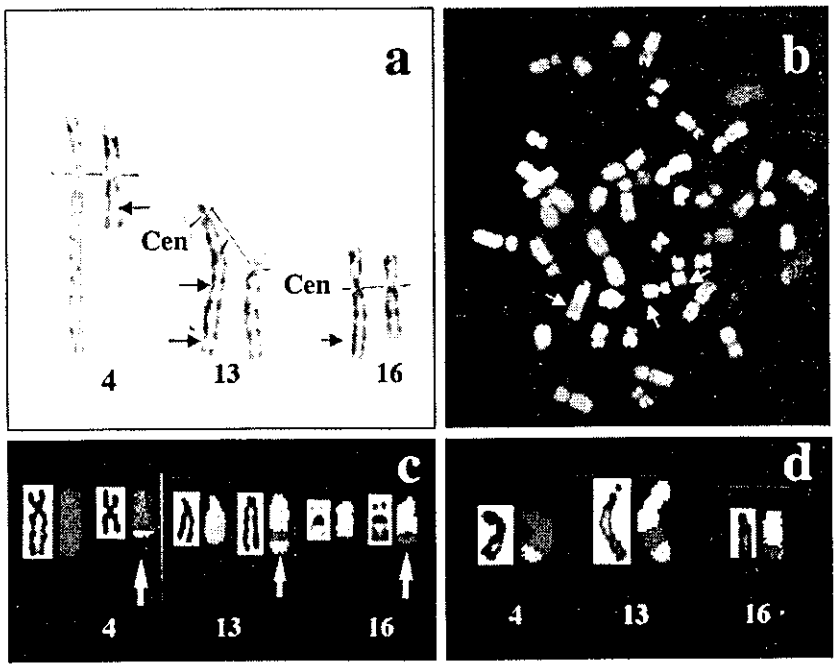

図2　症例1の染色体分析
a：Gバンド高精度分析法による母親の染色体．4，13と16番染色体との間で起こった複雑な転座．b：母親のSKY法による染色体像．矢印は転座，挿入染色体を示す．c：母親の部分核板，d：胎児の部分染色体核板で母親と同型．

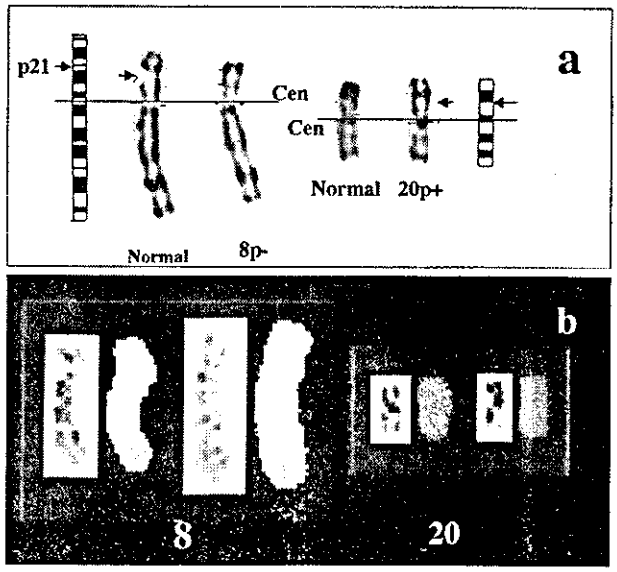

図3 a：46,XX,t(8；20)(p21；p11.2) を示した妻の染色体部分核板．矢印は切断部位を示すがバンドは類似しており判別は難しい．b：8回目の羊水検査におけるSKY法の結果で転座でないことはきわめて明瞭に識別できる．

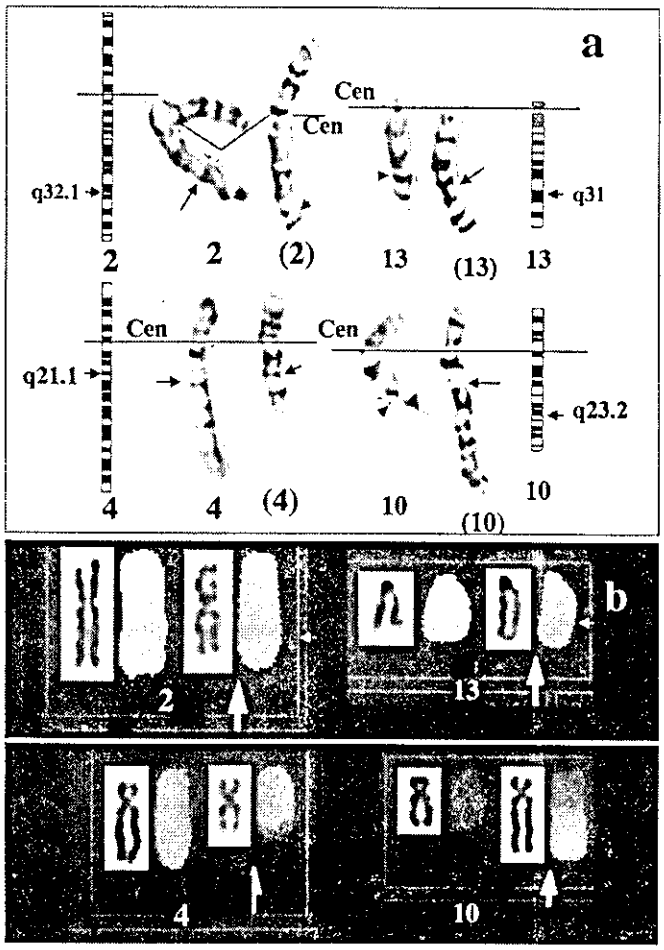

図4 2つの転座を保有する妻の染色体．a：Gバンド法と，b：SKY法による部分核板

体末端部の異常の有無も検出できるようになった．このtelomeric FISHは，染色体末端部に転座を有する家系の出生前診断にはきわめて有用で，いくつかの家系において臨床応用が試みられた[3]．

Spectral Karyotyping（SKY）法の臨床応用

特定の染色体の全体像を蛍光シグナルとして描出する染色体painting法と，複数のhybridization signalを異なった蛍光色素で描出するマルチカラー法は，FISH法の基本的な応用法の中でも特筆すべきものと言えよう．これら2つの技術を合わせて，マルチカラーFISH法の究極ともいうべきヒト染色体の24種類を染め分けるmultiplex-FISH法[4,5]が開発された．現在，筆者らはGバンド法，telomere FISH法に加え，SKY法を症例に応じて先天異常や出生前診断に応用しており，そのいくつかの症例を紹介する．

症例1

症例は27歳．先回出生児に異常を認め，遺伝カウンセリングのために紹介され来院した．先回出生児は女児で，心室中隔欠損，動脈管開存など先天心奇形を合併しており，染色体検査が施行され4qトリソミーが疑われたが，詳細は不明であった．そこで両親の染色体検査が行われた．Gバンド法にて分析し，父親には異常はなかったが，母親に複雑な4つの切断点を持つ，以下に述べるような複雑な均衡型転座であることが高精度分染法で判明した［der(4)t(4；16)(q22.2；q22.3)，der(13)ins(13；4)(q31.2；q22.2q31.3 or q31.3 q22.2)，der(16)t(4；16)(q31.3；q22.3)］（図2-a）．この複雑な変化をSKY法で見たのが図2-b，cである．両親は次の妊娠で出生前診断を強く希望した．CVSが妊娠9週で行われ，染色体分析はあまりにも複雑であるがゆえに，最初からSKY法を応用することとした．胎児の性染色体構成はXYで，母親とまったく同じ3 wayの転座を保有する健常児と診断した（図2-d）．その後の妊娠経過も何ら異常なく，妊娠38週に3100 gの男児を正常分娩した．

症例2

7回の連続習慣流産の精査目的で紹介された29歳の女性．夫婦の染色体検査の結果，本人が8pと20p[46,XX,t(8；20)(p21；p11.2)]であることが判明した（図3-a）．8回目の妊娠は問題なく推移し，夫婦は羊水検査を受けることを決心した．培養羊水細胞によるGバンド法による染色体分析では，妻と同じ均衡型であるように思われたが，分析できる良質の中期分裂像を得ることは困難であった．妊娠36週，CTG上distress patternが出現し，緊急帝王切開となった．児は2080 gの女児で異常は見られなかった．2年後，彼女は再度妊娠し来院した．しかし，今回は前の経験を生かし，SKY法で羊水細胞の染色体分析をすることとした．結果は女児で，図3-bのようにまったく正常の核型を示し，現在妊娠を続けている．

症例3

35歳の妊婦が3回の習慣流産の精査目的で当科を紹介された．彼女の1回目の妊娠では正常女児を出産，しかし2回目の妊娠は原因不明の初期流産であった．夫婦の染色体分析結果で，妻の46,XX,t(2；13)(q32.1；q31)，t(4；10)(q21.1；q23.2)（図4-a）であることがわかった．この2つの転座はSKY法で確認できた（図4-b）．図4-bでは，転座に関与する2番と13番染色体がほぼ類似の赤色で染色されたために判別は難しいが，切断点は白の矢印で提示した．その後の経過であるが，卵巣機能不全のためなかなか排卵が見られず，現在治療に専念している．

症例4

胎児水腫を疑って紹介された35歳の妊婦．最初の子は正常女児であったが，2回目の妊娠は原因不明の流産であった．最初に胎児水腫の原因検索の目的でTORCH検査を行ったが，いずれも

表1 Gバンド法で認められた異常をSKY法で詳細検討する際の目安

Gバンド法の結果	核型記号	SKY法適応の有用性	SKY法の捕捉
相互転座	t	○	Gバンド法と同程度の解析
過剰染色体	add	○○	過剰部の由来染色体を同定
派生染色体	der	○○	もととなる染色体を同定
挿入	ins	○○	挿入断片の由来染色体を同定
複雑転座 (3 way, 4 way 転座)		○○	関与する染色体を同定
遺伝子増幅	dmin/hsr	○○	増幅の由来染色体を同定
重複?	?dup	○○	重複であるか否かの同定 重複でない場合，関与する染色体の同定
逆位	inv	△	Gバンド法以上の解析は困難
欠失	del	△	Gバンド法以上の解析は困難
数的異常	±	△	Gバンド法以上の解析は困難
由来不明染色体	mar	△	感度限界内で由来染色体同定
隠れた転座 (cryptic 転座)		△	感度限界内で検出

○○：SKY法の有用性は高い，○：SKY法で解析可能であるがGバンド法以上の成果は期待できない，△：SKY法では検出できない場合もある

陰性であった．妊娠16週に染色体検査目的で羊水穿刺を行ったところ，胎児は18トリソミーであった．夫婦は3回のうち2回の妊娠に失敗したことを危惧し，精査を強く希望した．18トリソミーは突然変異で再発のリスクはきわめて低いことを十分に説明したが，できるだけの検査は受けたいと強く希望した．そこで夫婦の染色体分析を行うことから精査を開始したが，偶然にも夫が5番と8番の相互転座保因者［46,XY,t(5；8)(q21；q21.2)］であることがわかった．我々はこの所見をSKY法で確認した（**図5**），彼らは，次の妊娠ではぜひ出生前診断を受けたいという希望を伝え帰宅した．

症例5

28歳の妊婦が，姪にJackson痙攣と小頭症が認められることを心配し，遺伝カウンセリングのために来院した．姪の染色体検査はすでに済んでいたが，正常女性核型46,XXであった．しかし，夫婦は強く染色体異常だけでも妊娠中の胎児から除外したいと主張したので，出生前診断の前に両者の検査を行うこととした．ところが驚くことに，妻の側に染色体異常が検出された．その核型は複雑で，高精度分析で46,XX,der(7)(7pter→7q21.2::8p21.1→8qter)，der(8)(7qter→7q31.2::7q22→8p21.1→8qter）であった（**図6-a**，青色の矢印が逆位を示す）．この分析にはSKY法も併用した（**図6-b**）が，腕間逆位を検出することはSKY法ではできない．妊娠16週に羊水検査を行ったが，複雑さゆえにSKY法を用いた結果，母親と同じ核型であることが判明した（**図6-c**）．

症例6

27歳の妊婦が，妊娠22週になって頭蓋内にcystic echoが存在するという理由で紹介されてきた（**図7**）．妊娠の経過とともにcystic echoは増大し，脳梁の低形成と脳室の拡大が顕著となってきた．これらの脳内変化を家族に説明し，18トリソミーを疑っていることを話したところ，確認のために羊水検査を希望した．胎児染色体分析結果では18トリソミーではなく，8番染色体短腕部に過剰部分を認めた（**図8**）．**図9**はGバンドとSKY法による8番染色体であるが，Gバン

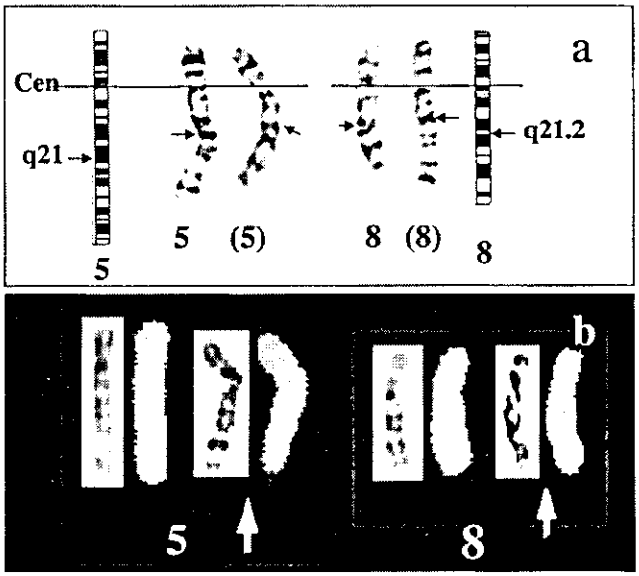

図5　a：5番と8番の染色体と切断部位（Gバンド法）．b：同染色体のSKY法による所見

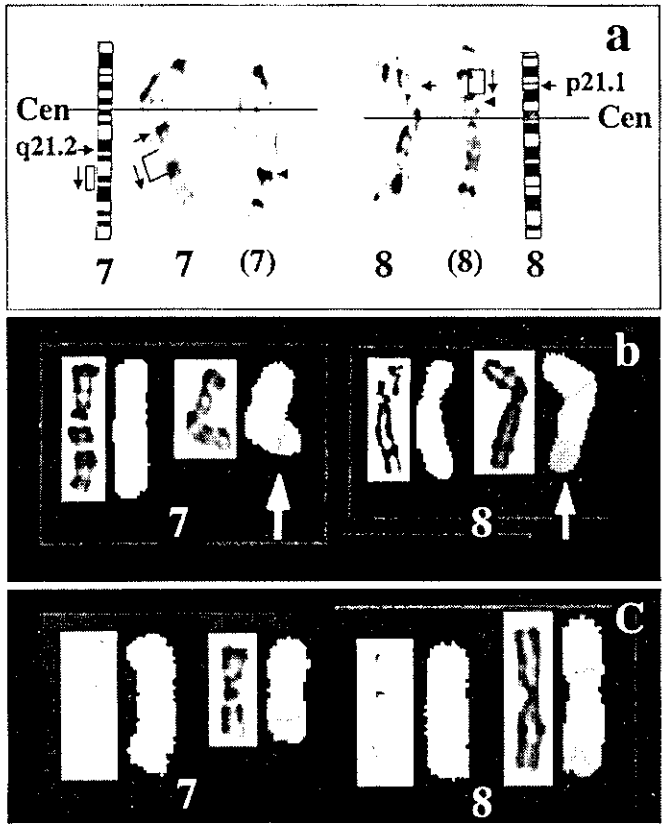

図6　a：母親の高精度分染法，b：そのSKY法による部分核板．c：羊水細胞のSKY法による分析で母親と同じ異常を保有している．

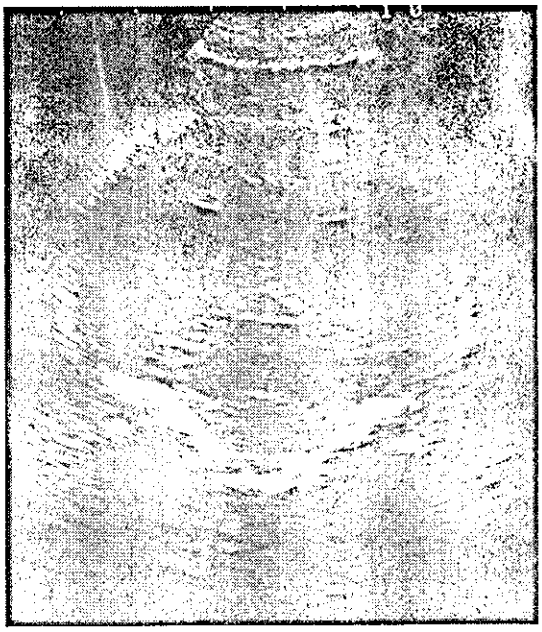

図7 症例6の胎児の脳超音波所見

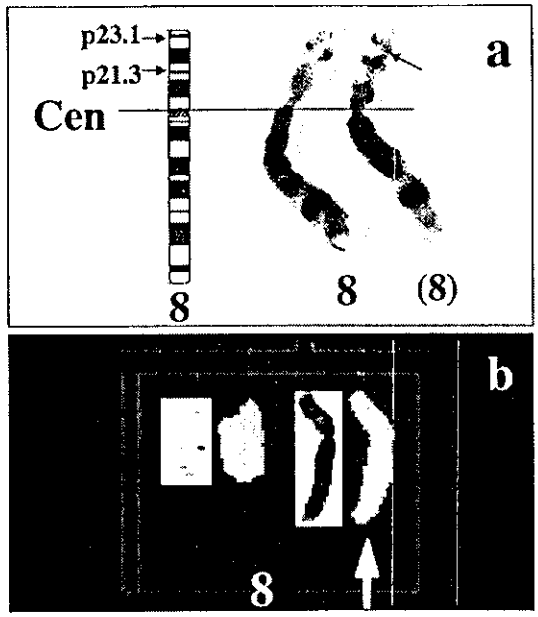

図9 a：8番染色体のGバンド分析．矢印より上が過剰部分（重複？）．b：SKY法による分析ですべて同色で8番染色体由来であることを示している．

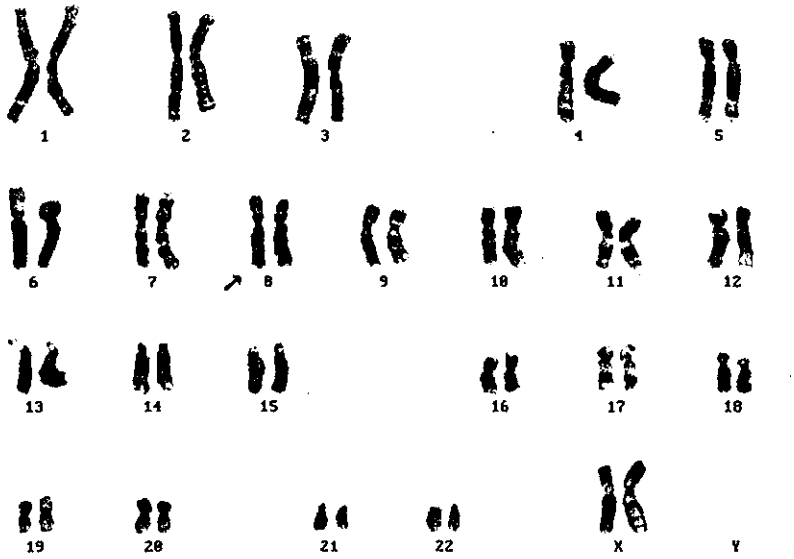

図8 症例6の羊水細胞染色体，8番染色体の短腕に過剰部分が疑われる．

ドではp21.3-23.1が逆転して短腕末端部に存在するように見られた．SKY法では詳細は不明であったが，過剰部分は8番染色体に由来することが，染色に変化のないことから確認された．妊娠

37週に水頭症でfloppyな女児が出産された．出生後の超音波検査で脳の異常のみではなく，動脈管開存，心室中隔欠損などの心奇形の合併があることが判明した．

おわりに

今回紹介した6症例はいずれも，通常のGバンド法では明らかにできなかった染色体上の微細な，また複雑な変化をSKY法を用いることにより一層明らかにすることができた．SKY法は周産期における胎児異常や両親の複雑な転座に威力を発揮するのみならず，腫瘍でよく観察されるマーカー染色体の由来を明らかにするなど多様な使用が試みられており，将来は染色体異常のデータをもとに疾患遺伝子座が決定されたり，疾患遺伝子の候補となる特定の染色体領域にある遺伝子を見つけるのにも貢献することが期待されている．

文　献

1) Suzumori K, Tanemura M, Oya N, Suzumori N, Kim KC, Ohashi H, Fukushima Y : Minute chromosomal rearrangement detected prenatally by fluorescence in situ hybridization. Prenat Diagn 18 : 725-730, 1998.
2) Trask BJ : Fluorescence in situ hybridization : applications in cytogenetics and gene mapping. Trends Genet 7 : 149-154, 1991.
3) Wakui K, Tanemura M, Suzumori K, Hidaka E, Ishikawa M, Kubota T, Fukushima Y : Clinical applications of two-color telomeric fluorescence in situ hybridization for prenatal diagnosis : identification of chromosomal translocation in five families with recurrent miscarriages or a child with multiple congenital anomalies. J Hum Genet 44 : 85-90, 1999.
4) Schöck E, du Monoir S, Verdman T, et al : Multicolor spectral karyotyping of human chromosomes. Science 273 : 494-497, 1996.
5) Verdman T, Vigon C, Schöck E, Rowley JD, Ried T : Hidden chromosomal abnormalities in haematological malignancies detected by multicolour spectral karyotyping. Nature Genet 15 : 406-410, 1997.

J Hum Genet (2004) 49:177–181
DOI 10.1007/s10038-004-0128-5

ORIGINAL ARTICLE

Yuko Kondo · Sami Tsukishiro · Mitsuyo Tanemura
Mayumi Sugiura-Ogasawara · Kaoru Suzumori
Shin-ichi Sonta

Maternal uniparental disomy of chromosome 16 in a case of spontaneous abortion

Received: 4 November 2003 / Accepted: 7 January 2004 / Published online: 2 March 2004

Abstract To investigate the involvement of uniparental disomies (UPDs) in spontaneous abortions, we analyzed in detail the polymorphism of microsatellites on each chromosome in cases of abortion. Of the 52 spontaneous abortions investigated, 25 had a normal karyotype. The polymorphic analysis of these cases revealed that, in the villi from 24 of the 25 cases, biparental patterns were present in informative microsatellites in all autosomes. In the remaining case with a 46, XX karyotype (case 18), however, the informative patterns of the microsatellites of chromosome 16 appeared to be both of maternal origin. The results also showed that the region from the distal end of the short arm to near the middle point of the long arm of chromosome 16 (pter to D16S3107) were heterozygous, and those of the remaining region of the long arm (D16S3018 to qter) were homozygous. That is, this fetus had maternal isodisomy and heterodisomy of chromosome 16, originating from a maternal, meiosis I non-disjunction of dyad 16 that accompanied a cross-over at near the middle point of the long arm. The present finding suggests that some UPDs may become a cause for spontaneous abortions.

Y. Kondo · S. Tsukishiro · M. Tanemura ·
M. Sugiura-Ogasawara · K. Suzumori
Department of Obstetrics and Gynecology,
Nagoya City University Medical School,
Nagoya 467-8601, Japan

Present address: Y. Kondo
Department of Obstetrics and Gynecology,
Moriyama Municipal Hospital,
Nagoya 463-8567, Japan

S. Sonta (✉)
Department of Genetics,
Institute for Developmental Research,
Aichi Human Service Center, 713-8 Kamiya-cho,
Kasugai, Aichi 480-0392, Japan
E-mail: ssonta@inst-hsc.jp
Tel.: +81-568880811
Fax: +81-568880829

Keywords Uniparental disomy (UPD) · Chromosome 16 · Spontaneous abortion · Microsatellite polymorphism · Maternal origin · Normal karyotype

Introduction

Among all recognized pregnancies, about 10–15% end in spontaneous abortion. About half of these abortions are caused by chromosomal abnormalities, including many kinds of aneuploidy, polyploidy, monosomy of the X-chromosome, and so on (Lauritsen 1976; Hassold et al. 1980; Kajii et al. 1980; Warburton et al. 1980). Some of the chromosomal abnormalities detected in these abortions are also seen in liveborns, and the frequency of pregnancies with each of these chromosomal abnormalities that end in abortion is far higher than the frequency of those which produce liveborns (Epstein 1986; Gardner and Sutherland 1996). The findings have indicated that chromosomally unbalanced embryos are mostly eliminated as spontaneous abortion during the developmental stages.

On the other hand, the causes of other cases of abortions of fetuses with a normal karyotype are mostly unknown, although immunological and other defects (Gill 1986; Chiu et al. 1996; Kaider et al. 1999) have been detected in some cases. However, the fact that typical chromosomal abnormalities account for a large portion of spontaneous abortions suggests that functional, structural, and constitutional abnormalities, which are undetectable by usual chromosomal analysis, may contribute to these abortions. These abnormalities could be, for example, a deletion of fine chromosomal segments including genes essential to fetal development, abnormal inactivation of the X-chromosome, or abnormal imprinting. Uniparental disomy (UPD) of chromosomes having an imprinting region is also included in this category. UPD cases cannot usually be detected by chromosome banding, except for a few cases with remarkable heteromorphism of the

chromosomes. To-date, only a few UPD cases have been found among spontaneous abortions (Shaffer et al. 1998; Smith et al. 1998; Fritz et al. 2001). In the present study, we analyzed the chromosomal origin of spontaneous abortions with a normal karyotype, using microsatellite polymorphic markers to investigate the involvement of UPDs, and found one case of UPD for chromosome 16 in 52 abortions.

Materials and methods

Cases of abortions analyzed

We obtained 143 cases of spontaneous abortion from the patients admitted to the Department of Obstetrics and Gynecology, Nagoya City University School of Medicine, Nagoya and from the Cell Bank, constructed by a Health Sciences Research Grant for Research on Human Genome (H10-Genome-008) from the Ministry of Health and Welfare of Japan. Of the 143 cases, the 52 that were miscarriages of first-time pregnancies at weeks 7–9 were analyzed in the present study. After the women and their spouses in these cases had been given understandable and detailed information on this study and its purposes, all agreed to allow the use of parental and fetal materials for analysis. Peripheral blood of the woman and her spouse and chorionic villi from the abortion were obtained in each case.

Chromosomal analysis

Part of the villi from the abortions was cultured and prepared for chromosomal analysis after 7–23 days. Peripheral blood lymphocytes from the patient and her spouse were also cultured and harvested conventionally for chromosomal analysis.

DNA extraction and polymorphic analysis of microsatellites

Genomic DNA was extracted from the villi of the aborted fetuses and the blood of the women and their spouses by the standard methods. Two hundred polymorphic microsatellite markers on about every 2 Mb in all autosomes and the X-chromosome were selected from the Genethon collections (Dib et al. 1996). Most of the primer sets used in this study were gratefully accepted gifts from Prof. Y. Nakamura of the Medical Institute of Tokyo University, and other primers were synthesized. Genotypes of the fetus and the parents for each marker locus were determined using the DNA-sequencer-assisted method with fluorescent microsatellite marker DNAs (Fujimoto et al. 1998; Alf Express Fragment Manager, Pharmacia Biotech). When the results suggestive of UPD for certain chromosomes were obtained, further analyses using other microsatellite markers on the same chromosome were carried out to clearly identify the parental chromosome origin. To examine the exact stage occurred, a non-disjunction and the existence of recombination on chromosome 16 during maternal meiosis, we detected the exact arrangement of allelic microsatellite patterns of each chromosome 16 in the mother of the aborted fetus (case 18) by analysis of the microsatellite patterns of the grandparents on the mother's side.

Results

Of the 52 spontaneous abortions investigated, 27 (51.9%) had chromosomal abnormalities: 47,XX or XY,+16 and 45,X in five cases; 47,XX or XY,+22 in three cases; 47,XX or XY,+9 and 69,XXY in two cases; and 47,XX,+2, 47,XY,+10, 47,XX,+13, 47,XX,+15, 47,XY,+18, 47,XY,+21, 48,XX,+16,+22, 69,XXX, 70,XXY,+22, 92,XXYY in one case. Polymorphic analysis of microsatellites was carried out in the remaining 25 cases with normal karyotypes: 46,XX in 13 cases and 46,XY in 12 cases. Polymorphic analysis of the villi from 24 of the 25 cases revealed biparental patterns, one paternal and the other maternal, in informative microsatellites in all autosomes and in the X-chromosome of the 46,XX cases. The results thus indicated that every paired chromosome originated from one paternal and one maternal chromosome.

In the remaining case of 46,XX (case 18), the microsatellite polymorphic patterns of all paired autosomes and the X-chromosome, except chromosome 16, indicated to be one paternal and one maternal chromosome; however, the informative patterns of three microsatellites of chromosome 16 were both of maternal origin. Further polymorphic analysis of this case was carried out using other primer sets for chromosome 16 (Fig. 1). The exact arrangement of microsatellite patterns in each chromosome 16 in the mother was detected by analysis of the grandparents on the mother's side (data not shown). Eight microsatellites showed informative patterns clearly indicating both maternal origin, and all patterns of the other 17 microsatellites were consistent with maternal origin. The results also showed that the microsatellite patterns in the region from the end of the short arm to about the middle part of the long arm of chromosome 16 (D16S423 to D16S3107) were heterozygous, while those in the remaining region from that point to the distal end of the long arm (D16S3018 to D16S413) were homozygous. That is, the microsatellite analyses indicated that this fetus had maternal isodisomy and heterodisomy of chromosome 16. The results also revealed that both chromosomes 16 originated from a maternal, meiosis I non-disjunction of dyad 16; one accompanied one meiotic recombination at about the middle of the long arm, and the other had no recombination. In this case, all the 100 cells analyzed had a normal karyotype of 46,XX, and there was no evidence of mosaicism including trisomy 16.

Case 18 was the first pregnancy of a 32-year-old woman. Her spouse was 33 years old. The karyotypes of the woman and her spouse were normal. There was nothing remarkable either before or during the early period of pregnancy. When the pregnancy was confirmed at 6 weeks and 2 days, the gestational sac was about 16 mm and heartbeat was confirmed. The fetus was diagnosed as having a stopped heartbeat in the eighth week of pregnancy, and aborted finally at 8 weeks and 4 days of pregnancy. In the aborted materials, the fetus could not be found. Abnormal figures indicating signs of hydatidiform, and cystic features were not found in the chorionic villi obtained from the abortion in this case.

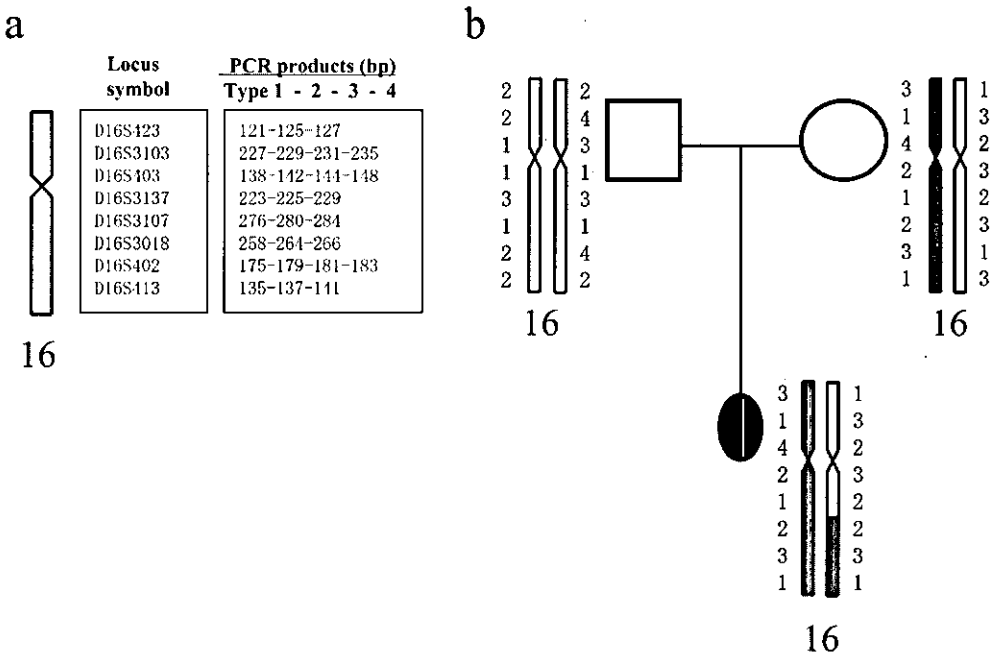

Fig. 1a, b Polymorphic patterns of microsatellites of chromosome 16 seen in the aborted fetus and the parents in case 18. **a** List of primers that showed informative patterns of microsatellite polymorphism and the size of PCR products (bp). The arrangement of markers and the locus of the centromere are roughly shown. **b** Polymorphic patterns of microsatellites in the fetus (case 18) and the parents. The arrangement of microsatellite patterns in each chromosome 16 of the mother was detected by analysis of the grandparents on the mother's side (data not shown). The polymorphic analysis indicates that this is a case of maternal iso/heterodisomy of chromosome 16

Discussion

The frequency and distribution of chromosome abnormalities in the present study of spontaneous abortions were similar to the data in the previous studies (Carr and Gedeon 1977; Hassold et al. 1980; Kajii et al. 1980). Whereas the frequency of polyploidies was comparatively low, and the respective frequency of some abnormalities differed as compared with the previous studies, these discrepancies may be due to a small number of cases in the present analysis.

Full UPDs for various chromosomes have been detected by DNA polymorphic analysis and other methods in human individuals with chromosomal abnormalities, imprinting disturbances, and non-Mendelian inheritance of recessive genes. In a previous review (Engel 1998), maternal (mat) UPDs of chromosomes 1, 2, 4, 6, 7, 9, 10, 13–16, 21, 22, and X, and paternal (pat) UPDs of chromosomes 1, 5–8, 11, 13–16, 20–22, and X have been reported. Among these UPDs, some cases, such as of UPDs 1, 13, 21, and 22, which do not relate to anomalous transmission of recessive genes, had almost no clinical features (Ledbetter and Engel 1995; Morison and Reeve 1998). In contrast, abnormal clinical features have been distinctly shown in cases of both pat and mat UPDs 14 and 15 (Nicholls et al. 1989; Antonarakis et al. 1993; Bottani et al. 1994; Cotter et al. 1997). Abnormal clinical features were also shown in cases of pat UPDs 6 and 11 (Henry et al. 1991; Temple et al. 1995) and in cases of mat UPDs 2, 7, and 16 (Vaughan et al. 1994; Kotzot et al. 1995; Johnston et al. 1996). In particular, serious clinical features have been described in some cases of mat UPD 2 showing phenotypes of severe growth retardation, renal failure, and pulmonary dysplasia (Webb et al. 1996; Shaffer et al. 1997) of pat UPD 14 showing skeletal dysplasia and thoracic narrowing (Cotter et al. 1997), and of mat UPD 16 showing congenital heart and digestive-tract anomalies and stunted growth (Vaughan et al. 1994). Furthermore, no UPDs of chromosomes 3, 12, and 17–19 have been found in any case to-date. These findings, therefore, suggest the possibility that some UPD cases may also exhibit serious abnormalities before birth, including during the periods of embryogenesis and early fetal development. Previous mouse studies have clearly indicated that some UPDs affect the development of embryos and placentas (Ferguson-Smith et al. 1991). In humans, however, it has not been sufficiently ascertained whether UPDs affect development before birth, including early embryogenesis, implantation, organogenesis, and differentiation, through the involvement of some imprinting genes. In the present study, we documented the first case of mat UPD 16 in a human spontaneous abortion. This finding indicates the possibility that UPDs may affect the development of the fetus.

To-date, many UPD 16 cases in fetuses and liveborns have been reported in the literature. Whereas one was a child of pat UPD 16 without any clinical abnormality (Kohlhase et al. 2000), mat UPD 16 cases showed clinical abnormalities such as body stalk anomaly, intrauterine growth retardation, imperforate anus, and congenital heart disease (Kalousek et al. 1993; Vaughan et al. 1994; O'Riordan et al. 1996; Abu-Amero et al. 1999; Chan et al. 2000). Mat UPD 16 has frequently been associated with trisomy 16-confined placental mosaicism (CPM) (Kalousek et al. 1993; Yong et al. 2003). In the present study, the analysis of a large number of cells from cultured chorionic villi excluded the possibility of CPM and, thus, suggests that the case of UPD 16 was probably derived from a trisomy rescue

event at the first mitotic stage or from fertilization between disomic and nullisomic gametes. The result also suggests the possibility that the clinical or pathological effects of full mat UPD 16 on pregnancies may differ from cases of mat UPD 16 associated with CPM of trisomy 16.

The mechanism of UPDs is first considered to be derived from the trisomy rescue event (Cassidy et al. 1992; Purvis-Smith et al. 1992). Full trisomy 16 is the major cause of spontaneous abortions, and the frequency of trisomy 16 seen in spontaneous abortions may be higher than that of any other trisomy. Therefore, UPD 16 may be due to the process of trisomy rescue. One can also consider UPD by fertilization between nullisomic and disomic gametes for the same chromosome, but the frequency of UPDs produced by this mechanism may be low. Though the results confirmed a low incidence of UPD in spontaneous abortion (Fritz et al. 2001), the possibility of UPD 16 causing spontaneous abortion has not been excluded.

Most trisomies seen in liveborns, including trisomies 18 and 21, are also found in spontaneous abortions at several times the rate in liveborns; for example, trisomy 21 is found at about 3.3 times the rate in liveborns and trisomy 18 at more than 15 times, according to the calculations of the data of Carr and Gedeon (1977) and Hook and Hamerton (1977) with the assumption that spontaneous abortions occur at the rate of about 15% of conceptions. These facts also suggest that UPDs seen in liveborns with congenital abnormalities may become a cause for spontaneous abortions, in the same way as trisomies. However, the relationship between UPDs and abortion is still not well understood. In the literature to-date, there have been only two reported UPD cases (UPDs 9 and 21) among spontaneous abortions (Fritz et al. 2001), and the present case is the first report of UPD 16 in spontaneous abortions. So far, there have been four studies searching for UPD in consecutively corrected spontaneous abortions of a normal karyotype (Shaffer et al. 1998; Smith et al. 1998; Fritz et al. 2001; present study). The combined frequency of UPD cases from these studies is 1.65% (3/182), which confirms a low incidence of UPDs in spontaneous abortions (Fritz et al. 2001). The data indicated that while the frequency of UPDs in spontaneous abortions may be low, the possibility of UPDs causing spontaneous abortion has not been excluded.

However, there is still an insufficient number of detailed studies on the origin of whole chromosomes in spontaneous abortions, using DNA polymorphic analyses and other means. In order to clarify the relationship between UPDs and spontaneous abortions, or the effects of UPDs during the developmental stages in humans, further investigations of abortions using DNA polymorphic markers and other means are needed. Through these studies, one may discover unknown imprinting regions in human chromosomes and genes only activated during limited periods such as embryogenesis and fetal development.

Acknowledgements The authors would like to thank Professor Yusuke Nakamura of the Medical Institute of Tokyo University, Tokyo, for proffering oligonucleotide primers capable of detecting many polymorphic microsatellite markers. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 13470356) and a Health Sciences Research Grant for Research on Human Genome (H10-Genome-008) from the Ministry of Health and Welfare of Japan.

References

Abu-Amero SN, Ali Z, Abu-Amero KK, Stanier P, Moore GE (1999) An analysis of common isodisomic regions in five mUPD 16 probands. J Med Genet 36:204–207

Antonarakis SE, Blouin J-L, Maher J, Avramopoulos D, Thomas G, Talbot CC Jr (1993) Maternal uniparental disomy for human chromosome 14, due to loss of a chromosome 14 from somatic cells with t(13;14) trisomy 14. Am J Hum Genet 52:1145–1152

Bottani A, Robinson WP, DeLozier Blanchet CD, Engel E, Morris MA, Schmitt B, Thun-Hohenstein L, Schinzel A (1994) Angelman syndrome due to paternal uniparental disomy of chromosome 15: a milder phenotype? Am J Med Genet 51:35–40

Carr DH, Gedeon M (1977) Population cytogenetics of human abortuses. In: Hook EB, Porter IH (eds) Population cytogenetics: studies in humans. Academic, New York, pp 1–9

Cassidy SB, Lai L-W, Erickson RP, Magnuson L, Eappen T, Gendron R, Herrmann J (1992) Trisomy 15 with loss of the paternal 15 as a cause of Prader-Willi syndrome due to maternal disomy. Am J Hum Genet 51:701–708

Chan Y, Silverman N, Jackson L, Wapner R, Wallerstein R (2000) Maternal uniparental disomy of chromosome 16 and body stalk anomaly. Am J Med Genet 94:284–286

Chiu L, Nishimura M, Ishii Y, Nieda M, Maeshima M, Takedani Y, Shibata Y, Tadokoro K, Juji T (1996) Enhancement of the expression of progesterone receptor on progesterone-treated lymphocytes after immunotherapy in unexplained recurrent spontaneous abortion. Am J Reprod Immunol 35:552–557

Cotter PD, Kaffe S, McCurdy LD, Jhaverzi M, Willner JP, Hirschhorn K (1997) Paternal uniparental disomy for chromosome 14: a case report and review. Am J Med Genet 70:74–79

Dib C, Faure S, Fizames C, Marc S, Vignal A, Heilig R, Lathrop M, Morrissette J, Gyapay G, Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Engel E (1998) Uniparental disomies in unselected populations. Am J Hum Genet 63:962–966

Epstein CJ (1986) The consequences of chromosome imbalance, principles, mechanism and models. Cambridge University Press, Cambridge

Ferguson-Smith AC, Cattanach BM, Barton SC, Beechey CV, Surani MA (1991) Embryological and molecular investigations of parental imprinting on mouse chromosome 7. Nature 351:667–670

Fritz B, Aslan M, Kalscheuer V, Ramsing M, Saar K, Fuchs B, Rehder H (2001) Low incidence of UPD in spontaneous abortions beyond the 5th gestational week. Eur J Hum Genet 9:910–916

Fujimoto M, Kantaputra PN, Ikegawa S, Fukushima Y, Sonta S, Matsuo M, Ishida T, Matsumoto T, Kondo S, Tomita H, Deng HX, D'urso M, Rinaldi MM, Ventruto V, Takagi T, Nakamura Y, Niikawa N (1998) The gene for mesomelic dysplasia Kantaputra type is mapped to chromosome 2q24-q32. J Hum Genet 43:32–36

Gardner RJM, Sutherland GR (1996) Chromosome abnormalities and genetic counseling, 2nd edn. Oxford University Press, New York

Gill TJ III (1986) Immunological and genetic factors influencing pregnancy and development. Am J Reprod Immunol Microbiol 10:116–120

Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, Matsuyama A, Wilson C, Yamane JA, Jacobs PA (1980) A cytogenetic study of 1,000 spontaneous abortions. Ann Hum Genet 44:151–178

Henry I, Bonaiti-Pellie C, Chehensse V, Beldjord C, Schwartz C, Utermann G, Junien C (1991) Uniparental paternal disomy in a genetic cancer-predisposing syndrome. Nature 351:665-667

Hook EB, Hamerton JL (1977) The frequency of chromosomal abnormalities detected in consecutive newborn studies-differences between studies-results by sex and by severity of phenotypic involvement. In: Hook EB, Porter IH (eds) Population cytogenetics: studies in humans. Academic, New York, pp 79–92

Johnston KM, Baker JC, Egli CA, McCaskill C, Shaffer LG (1996) Maternal uniparental isodisomy of chromosome 2 in a child with growth retardation, hypospadias and a cytogenetic abnormality. Am J Hum Genet Suppl 59:A95

Kaider AS, Kaider BD, Janowicz PB, Roussev RG (1999) Immunodiagnostic evaluation in women with reproductive failure. Am J Reprod Immunol 42:335-246

Kajii T, Ferrier A, Niikawa N, Takahara H, Ohama K, Avirachan S (1980) Anatomic and chromosomal anomalies in 639 spontaneous abortuses. Hum Genet 55:87–98

Kalousek DK, Langlois S, Barrett I, Yam I, Wilson DR, Howard-Peebles PN, Johnson MP, Giorgiutti E (1993) Uniparental disomy for chromosome 16 in humans. Am J Hum Genet 52:8–16

Kohlhase J, Janssen B, Weidenauer K, Harms K, Bartels I (2000) First confirmed case with paternal uniparental disomy of chromosome 16. Am J Med Genet 91:190–191

Kotzot D, Schmitt S, Bernasconi F, Robinson WP, Lurie IW, Ilyina H, Mehes K, Hamel BC, Otten BJ, Hergersberg M, Hamel BC, Otten BJ, Hergersberg M (1995) Uniparental disomy 7 in Silver-Russell syndrome and primordial growth retardation. Hum Mol Genet 4:583-587

Lauritsen JG (1976) Aetiology of spontaneous abortion. A cytogenetic and epidemiological study of 288 abortuses and their parents. Acta Obstet Gynecol Scand Suppl 52:1–29

Ledbetter DH, Engel E (1995) Uniparental disomy in humans: development of an imprinting map and its implications for prenatal diagnosis. Hum Mol Genet 4:1757–1764

Morison IM, Reeve AE (1998) A catalogue of imprinted genes and parent-of-origin effects in humans and animals. Hum Mol Genet 7:1599–1609

Nicholls RD, Knoll JHM, Butler MG, Karani S, Lalande M (1989) Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. Nature 342:281–285

O'Riordan S, Greenough A, Moore GE, Bennett P, Nicolaides KH (1996) Case report: uniparental disomy 16 in association with congenital heart disease. Prenat Diagn 16:963–965

Purvis-Smith SG, Saville T, Manass S, Yip M-Y, Lam-Po-Tang PRL, Duffy B, Johnston H, Leigh D, McDonald B (1992) Uniparental disomy 15 resulting from "correction" of an initial trisomy 15. Am J Hum Genet 50:1348-1350

Shaffer LG, McCaskill C, Egli CA, Baker JC, Johnston KM (1997) Is there phenotype associated with maternal isodisomy for chromosome 2 in the presence of two isochromosomes? Am J Hum Genet 61:461–462

Shaffer LG, McCaskill C, Adkins K, Hassold TJ (1998) Systematic search for uniparental disomy in early fetal losses: the results and a review of the literature. Am J Med Genet 79:366–372

Smith MJ, Creasy MR, Clarke A, Upadhyaya M (1998) Sex ratio and absence of uniparental disomy in spontaneous abortions with a normal karyotype. Clin Genet 53:258–261

Temple IK, James RS, Crolla JA, Sitch FL, Jacobs PA, Howell WM, Betts P, Baum JD, Shield JP (1995) An imprinted gene(s) for diabetes? Nat Genet 9:110–112

Vaughan J, Ali Z, Bower S, Bennett P, Chard T, Moore G (1994) Human maternal uniparental disomy for chromosome 16 and fetal development. Prenat Diagn 14:751-756

Warburton D, Stein Z, Kline S, Susser M (1980) Chromosome abnormalities in spontaneous abortions: data from the New York City study. In: Porter IH, Hook EB (eds) Human embryonic and fatal death. Academic, New York, pp 261–268

Webb AL, Sturgiss S, Warwicker P, Robson SC, Goodship JA, Wolstenholme J (1996) Maternal uniparental disomy for chromosome 2 in association with confined placental mosaicism for trisomy 2 and severe intrauterine growth retardation. Prenat Diagn 16:958-962

Yong PJ, Barrett IJ, Kalousek DK, Robinson WP (2003) Clinical aspects, prenatal diagnosis, and pathogenesis of trisomy 16 mosaicism. J Med Genet 40:175–182

ORIGINAL ARTICLE

Parental origin and cell stage of non-disjunction of double trisomy in spontaneous abortion

Qing Ying Li[1], Sami Tsukishiro[1], Chiaki Nakagawa[2], Mitsuyo Tanemura[1], Mayumi Sugiura[1], Kaoru Suzumori[1], and Shin-ichi Sonta[2]

[1]Department of Obstetrics and Gynecology, Nagoya City University Medical School, Nagoya and [2]Department of Genetics, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Japan

ABSTRACT **Using polymorphic analysis of microsatellites, we investigated the parental origin and mechanism of double trisomies seen in cases of spontaneous abortion. We obtained chorionic villi from spontaneous abortions, and peripheral blood from females who experienced abortion and their spouses. Chromosomal analysis of 170 cases revealed four cases with double trisomy. The karyotypes of these cases are 48,XX,+16,+22, 48,XXY,+18, 48,XX,+15,+21 and 48,XX,+2,+5. In the present study, the incidence of double trisomy was 2.4% of spontaneous abortions. Polymorphic analysis of microsatellites indicated that extra chromosomes were all of maternal origin in the four cases of double trisomy. The predominance of maternal origin in cases of double trisomy is similar to cases of single trisomy. The result also indicated that both extra chromosomes in two cases occurred by non-disjunction at the first meiotic division, and extra chromosomes in the other two cases occurred by non-disjunction at the first mitotic division. The mean maternal age in cases of double trisomy was significantly higher than that in cases of single trisomy. These findings suggest the possibility that abnormal separation of two or more chromosomes may occur simultaneously in oogonia, and that this phenomenon may increase in relation to the increase in age of women.**

Key Words: double trisomy, microsatellite, non-disjunction, origin, spontaneous abortion

INTRODUCTION

Chromosomal aneuploidy is one of the major causes of pregnancy wastage. About a half of spontaneous abortions before 15 weeks of gestation have chromosomal abnormalities. Around 50% of these abnormalities are aneuploidies (Hassold *et al.* 1980). Most of them are single trisomy for various chromosomes; double trisomies are rarely observed in cases of spontaneous abortion (Creasy *et al.* 1976; Hassold *et al.* 1980; Kajii *et al.* 1980; Eiben *et al.* 1990; Reddy 1997).

Following the development of reproductive diagnosis in humans and methods for tissue culture, the detectable probability of double trisomy among successfully karyotyped cases of spontaneous abortion may increase (Reddy 1997). Recently, polymorphic DNA markers have been used to investigate the parental origin of extra chromosomes and the stages at which the extra chromosomes occurred in cases of spontaneous abortion with trisomy. These studies have indicated that the majority of extra chromosomes in cases of single trisomy are maternal in origin. Advanced maternal age remains the only well-documented risk factor in non-disjunction (Epstein 1986). On the other hand, few data are available to investigate the genetic mechanisms of double trisomy (Zaragoza *et al.* 1994; Park *et al.* 1995; Devriendt *et al.* 1998; Chen *et al.* 2000). In the present study, polymorphic analysis of microsatellites was performed to ascertain the parental origin and genetic mechanisms of the extra chromosomes of double trisomies found in four cases of spontaneous abortions.

MATERIALS AND METHODS

Cases of abortions analyzed

We obtained information from 174 cases of spontaneous abortion from patients admitted to the Department of Obstetrics and Gynecology, Nagoya City University School of Medicine, Nagoya, Japan, and from Cell Bank, constructed

Correspondence: Shin-ichi Sonta, PhD, Department of Genetics, Institute for Developmental Research, Aichi Human Service Center, 713-8 Kamiya-cho, Kasugai, Aichi 480-0392, Japan. Email: ssonta@inst-hsc.jp

Received September 10, 2004; revised and accepted November 22, 2004.

by a Health Sciences Research Grant for Research on Human Genome (H10-Genome-008) from the Ministry of Health, Labour and Welfare of Japan. After the women and their spouses in these cases had been given easy to understand, yet detailed information on this study and its purposes, all parties agreed to allow the use of parental and fetal materials for analysis. Peripheral blood from the woman and her spouse, and chorionic villi from the abortion were obtained in each case. The study was approved by the Institutional Review Board (IRB) of Institute for Developmental Research, Aichi Human Service Center, and the IRB of Nagoya City University Medical School.

Chromosomal analysis

Part of the chorionic villi from 174 cases of spontaneous abortion were cultured, and cells were harvested at 6–22 days of cultivation to analyze the chromosomes. Chromosomal analysis was performed with Q- and G-banding.

DNA preparation

Genomic DNAs for molecular studies were extracted from chorionic villi of spontaneous abortions and from peripheral blood of the parents using a DNA extraction kit (ISOTISSUE, Nippon Gene, and Dr GenTLE, Takara Bio).

Microsatellite analysis

Microsatellite markers were used to determine the parental origin of extra chromosomes and the stage at which the extra chromosomes occurred. Six to 18 microsatellite markers from the Genethon collections (Dib *et al.* 1996), which spanned the whole length of extra chromosomes in cases with double trisomy, were analyzed. Most of the primer sets used in this study were gratefully accepted gifts from Professor Y. Nakamura of the Institute of Medical Sciences, University of Tokyo, Tokyo. The remaining primers were synthesized. Genotypes of the fetus and the parents for each marker locus were determined using the DNA-sequencer-assisted method with fluorescent microsatellite marker DNAs (Fujimoto *et al.* 1998; Alf Express Fragment Manager, Amersham Pharmacia Biotech, USA).

RESULTS

Of 174 cases of spontaneous abortion that we obtained and cultured, 170 (97.7%) were successfully karyotyped. In these cases, 83 (48.8%) were euploidies and 87 (51.2%) had chromosomal abnormalities, of which 47 (27.7%) were trisomies, four (2.4%) were double trisomies, 15 (8.8%) were polyploidies, seven (4.1%) were X monosomies, six (3.5%) were translocations, and eight (4.7%) were mosaics. The karyotypes of the four cases with double trisomy were 48,XX,+16,+22 (Case 1), 48,XXY,+18 (Case 2), 48,XX,+15,+21 (Case 3) and 48,XX,+2,+5 (Case 4).

The results of polymorphic analysis of microsatellites are summarized in Table 1. More than two informative patterns were demonstrated on each extra chromosome. Examples of microsatellite polymorphic pattern of abortions and parents are given in Figs 1 and 2.

Polymorphic analysis of microsatellites on extra chromosomes 16 and 22 in Case 1 and extra chromosomes X and 18 in Case 2 were all revealed to be maternal in origin. In addition, polymorphic patterns of microsatellites on loci near

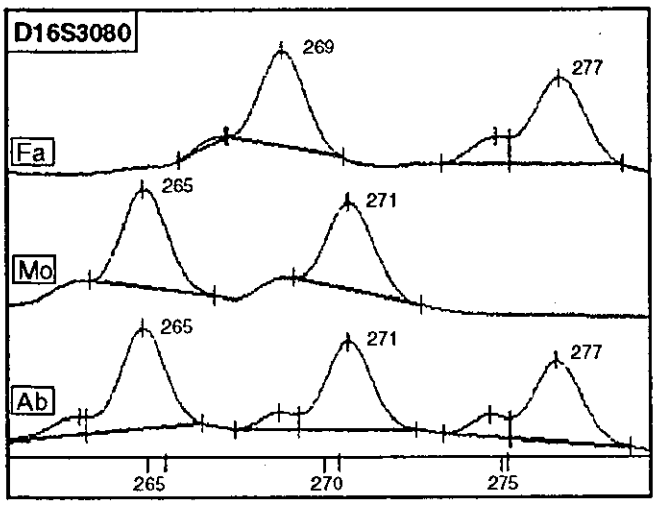

Fig. 1 Sample of microsatellite polymorphic patterns analyzed by ALF DNA sequencer (see text). The figure was obtained by polymerase chain reaction (PCR) using the primer D16S3080. Three distinct patterns are seen in the abortus (Ab) of Case 1. One pattern was derived from the father (Fa) and the other two were from the mother (Mo).

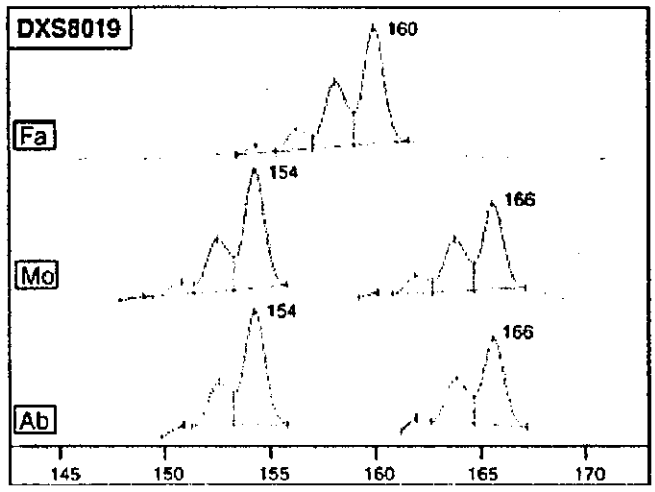

Fig. 2 Microsatellite polymorphic patterns obtained by PCR using the primer DXS8019. The figure indicates that the two X chromosomes of the abortus originated from the different X chromosomes of the mother. Ab, abortus; Fa, father; Mo, mother.

Table 1 Polymorphic analysis of microsatellites in abotuses and their parents

Chromosome constitution	Chromosome no.	Symbol	PCR products (base pair) Father	Mother	Abortus†	Origin of an extra chromosome
48XX,+16,+22	16	D16S423	135, 139	**137, 141**	135, **137, 141**	MI
		D16S407	140, 154	**152, 156**	**152**, 154, **156**	
		D16S3080	269, 277	**265, 271**	**265, 271**, 277	
		D16S3140	135, 157	**149, 155**	135, **149, 155**	
		D16S511	186, 204	**194, 196**	186, **194, 196**	
	22	D22S446	196, 200	**194, 194**	**194, 194**, 196	MI
		D22S275	160, 166	**156, 162**	**156, 162**, 166	
		D22S1158	220, 234	**220, 238**	**220**, 234, **238**	
		D22S283	132, 134	**128, 132**	**128, 132**, 134	
		D22S1171	134, 138	**142, 144**	134, **142, 144**	
48XXY,+18	18	D18S59	142, 152	**140, 148**	**140, 148**, 152	MI
		D18S453	141, 143	**139, 141**	**139, 141**, 143	
		D18S57	88, 92	**88, 96**	**88**, 92, **96**	
		D18S1147	202, 218	**206, 216**	**206, 216**, 218	
		D18S1141	233, 235	**237, 243**	233, **237, 243**	
	X	DXS7108	252	**244, 250**	**244, 250**	MI
		DXS8019	160	**154, 166**	**154, 166**	
		DXS8020	197	**195, 199**	**195, 199**	
		DXS8094	233	**221, 221**	**221, 221**	
		DXS1073	203	**205, 207**	**205, 207**	
48XX,+15,+21	15	D15S1035	172, 178	**172, 176**	**176, 176**, 178	Mi
		D15S978	213, 217	**211, 213**	**211, 211**, 213	
		D15S155	265, 265	**253, 263**	**263, 263**, 265	
		D15S979	157, 165	**147, 157**	**147, 147**, 157	
		D15S1014	178, 180	**180, 182**	178, **182, 182**	
	21	D21S1899	161, 161	**161, 163**	161, **163, 163**	Mi
		D21S270	197, 199	**203, 207**	199, **207, 207**	
		D21S1899	268, 272	**258, 272**	**258, 258**, 268	
		D21S1890	159, 161	**153, 159**	**153, 153**, 161	
48XX,+2,+5	2	D2S281	243, 245	**245, 247**	245, **247, 247**	Mi
		D2S2153	154, 164	**146, 154**	**146, 146**, 154	
		D2S114	218, 220	**224, 228**	218, **228,228**	
		D2S163	213, 227	**221, 223**	**223, 223**, 227	
		D2S206	119, 125	**125, 133**	125, **133, 133**	
	5	D5S1987	193, 205	**201, 203**	**201, 201**, 205	Mi
		D5S646	285, 285	**277, 285**	**277, 277, 285**	
		D5S495	223, 237	**221, 223**	**221, 221**, 223	
		D5S404	186, 190	**188, 188**	186, **188, 188**	
		D5S498	177, 183	**177, 177**	**177, 177**, 183	

†PCR, products of probable maternal origin are shown in bold; MI, meiosis I; Mi, first mitotic division.