（資料6） 遺伝医学セミナー・テキスト

染色体異常症（不均衡転座）

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[疾患名]　エマヌエル症候群　（OMIM#609029）

[病因]　核型　47,XX or 47,XY,+der(22)t(11;22)(q23;q11)

不均衡転座による 22q11 より近位、11q23 より遠位側の混合トリソミー

反復性 t(11;22)均衡転座を持つ親の第 1 減数分裂 3:1 分離配偶子による。

[頻度]　不明（新生転座発生頻度からの理論値では日本人に t(11;22)均衡転座保因者が約 10000 人、エマヌエル症候群患者が約 1000 人と推定される）。

[症状]　MCA/MR (multiple congenital anomalies/mental retardation)を呈する。特徴的顔貌（耳介、口蓋裂、小下顎など）、先天性心疾患 57%（ASD, VSD, PDA, 総肺静脈還流異常など）、泌尿・生殖器系の異常、鎖肛などあり。新生児期の筋緊張低下、呼吸障害、哺乳困難。その後、成長遅延、精神運動発達遅延、発語障害、聴覚障害、視力障害、けいれん、易感染性（繰り返す中耳炎、呼吸器感染）を伴う。

[診断]　染色体核型解析による。転座特異的 PCR も可能。

[遺伝]　過剰転座染色体の由来は、均衡転座保因者の母親 90%、父親 5%、不明 5%、de novo はまれ。t(11;22)均衡転座保因者は、表現型正常。不妊、習慣流産あり。均衡転座保因者の流産率は 23-37%程度。出生児は正常核型 38 (55) %、均衡転座 55.4 (41.2) %、エマヌエル症候群 5.7-6.1 (2.2-5) %（括弧内は男性保因者の場合）。

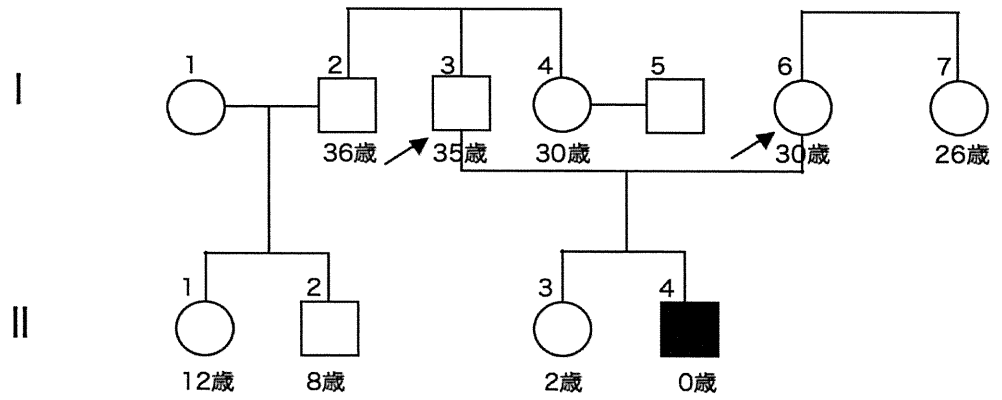
出生前の染色体検査で診断可能。t(11;22)均衡型新生転座は精子でのみ発生するが、その発生頻度が数万分の 1 と高頻度であるため、転座とは無関係な出生前診断などで偶然に均衡型新生転座が見つかるケースが増加している。

[関連サイトと参考文献]

1．*t(11;22)*　http://www.fujita-hu.ac.jp/~genome/11&22/

2．Emanuel syndrome.org (Chromosome 22 Central)
http://emanuelsyndrome.org/

3．大江瑞恵、倉橋浩樹　エマヌエル症候群の臨床像と遺伝　小児科 51(4), 443-450, 2010



＜症例＞

クライエントは一郎（I-3;35 歳）と明子（I-6;30 歳）夫婦。結婚後、３年の不妊期間の後に第１子を自然妊娠し、健常女児が出生。その２年半後に、第２子の男児を自然妊娠で得たが、児には心房中隔欠損、鎖肛、小顎、口蓋裂があり、哺乳力や体重増加が悪く、生後３ヶ月の時に小児科で染色体検査をおこなった。その結果、47,XY,+der(22)t(11;22)(q23;q11)と判明した。小児科の主治医はこの疾患名を聞いたことがなかったという。夫婦は児の今後について多くの情報を得る事が難しいと判断し、遺伝外来で詳しい話をきくことにした。夫は３人兄弟で、兄(I-2)には 2 人の子どもがいて、妹(I-4)は先日結婚をしたばかり。妻には、未婚の妹（I-7）がいる。

＜ロールプレイ＞

場面 1；染色体検査の結果、第 2 子が 47,XY,+der(22)t(11;22)(q23;q11)と判明した後の初回の GC（疾患について。子供の将来。なぜ？）

場面 2；次子の再発率と出生前診断についての GC（保因者診断、着床前診断について）

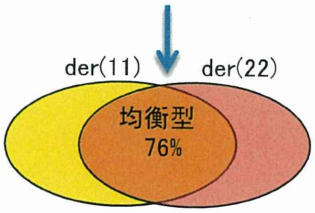
＜ポイント＞

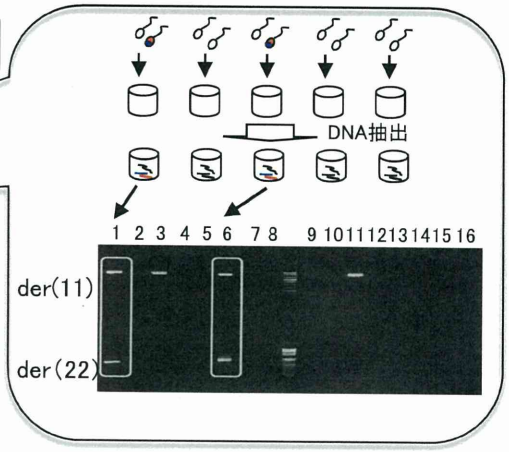
- クライエントのほとんどは、疾患に関する情報不足で来談する。クライエントのかかえる不安に対して、情報提供、心理的サポートをどうできるか。
- 保因者診断がもたらす意義や、結果の家系内への開示（どこまでの範囲の親族が可能性を知っていた方がよいのか？）。
- 流産をしていない均衡転座保因者の着床前診断について。

（資料7）

エマヌエル症候群患者数の推定

PCRによる精子中の派生染色体(der(11)、der(22))の検出頻度　1/13,681人





精子中の新生均衡型転座 = 新生転座保因者数　;1/18,241人
よって、百万人中なら54.8人の保因者

女性保因者は27.4人

男性保因者は27.4人

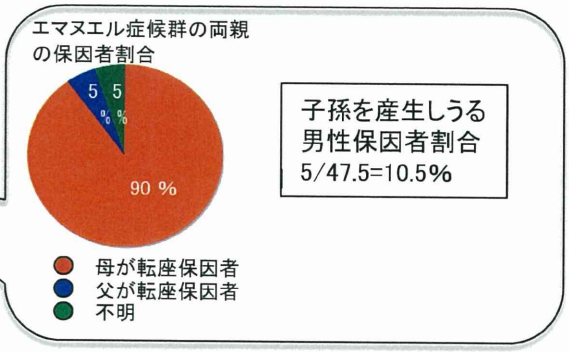
27.4人

子孫をえられる

Yes　No

2.9人

24.5人
（不妊）



子孫を産生しうる男性保因者割合
5/47.5=10.5%

1カップル平均2人の子どもを持つと(昭和22年から平成20年までの統計)、転座保因者から転座保因者が産まれる数

22.6人

3.2人

25.8人

t(11;22)均衡型転座保因者から産まれる生児の頻度

	男性保因者	女性保因者
t(11;22)保因者	41.2%	55.4%

その子孫が保因者を出産していくと、ある世代100万人中103人の保因者が存在する。

日本人の人口　1億1,800万人中には12,184人

女性保因者は6092人

男性保因者は6092人

1カップル平均2人の子どもを持つと、転座保因者からエマヌエル症候群の患者が産まれる数

女性保因者は719人

男性保因者は25人

t(11;22)均衡型転座保因者から産まれる生児の頻度

	男性保因者	女性保因者
エマヌエル症候群	3.6%	5.9%

日本でのエマヌエル症候群の患者数　743人

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エマヌエル症候群患者 11 例の口蓋裂を伴った浸出性中耳炎患者では、Hirschsprung 病、難聴などの合併症のほか、急性上気道炎、感染性胃腸炎、結膜炎、急性鼻咽頭炎等に反復罹患していた。報告ではエマヌエル症候群合併症の中で横隔膜ヘルニア（CDH）の発症率は 8%と、一般頻度（1 人/3500 から 15000 出生）に比べて高率である。妊娠 ICR マウス（妊娠 8 日）に除草剤ニトロフェン 25mg 経口投与し、マウス胎仔の CDH の有無を観察した結果、マウス胎仔における CDH の発生率は 15%（92 匹中 14 匹）、肺低形成は 80%（92 匹中 74 匹(80%)と高率であった。母獣への酸素投与治療によるマウス胎仔の CDH 発生率は 18%（56 匹中 10 匹）であったが、肺低形成は 39%（56 匹中 22 匹）と減少し改善した。当センター倫理委員会の承認を経て、胎児診断された CDH57 例のうち、2 例に対し、母体酸素投与を施行した。その結果、母体酸素投与は重症 CDH においても左室の発育が得られ、本治療法はエマヌエル症候群の致死的な合併症でもある CDH の補助治療となる可能性が示された。

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重度の知的および感覚・運動障害を伴う一人のエマヌエル症候群児に対して,その感覚活用を促し，コミュニケーション行動の形成・促進を図りながら、生活上の障害状況からの立ち直りをめざす療育支援を行った．初年度は行動観察を中心に、コミュニケーション行動の様相について実態把握を行い、感覚活用の可能性と具体物や半具体物をコミュニケーションの手がかりにできるかどうか、AACの利用可能性について検証した。次年度は、主に低年齢児用に開発された感覚機能評価法を活用して、自覚的・他覚的感覚機能評価を行い、環境づくりやコミュニケーション行動に生かす方法を探った。研究実施期間中、一貫して外界探索とコミュニケーション行動の促進を目標に、教育的対処を行った．最終年度には，該当児童の3年間にわたる外界探索およびコミュニケーション行動について、その教育的対処の結果と、対処にあたっての基本方針の意義を考察した。本事例においては、ゆっくりとしてではあるが、外界探索、対象物の操作、コミュニケーションいずれもが発達し、機能向上が見られた。これまでに見いだされている対症療法に加えて、相互作用を重視した療育支援と、特に感覚機能評価に基づく代替コミュニケーション（AAC）支援を継続していくことの重要性が示唆された．

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エマヌエル症候群は染色体異常を原因とする先天異常症候群であり、患児の母親が転座保因者であることが多い。平成２２年度は、保因者診断を受けた者がどのような心理社会的影響を受けるかを知るために染色体構造異常保因者11名の半構造化面接を行った。保因者診断は診断された者に様々な心理社会的な影響を及ぼしていた．配偶者や両親など周囲の者の理解と支援が保因者の心理的安定に鍵となっていた．このような患者・家族の心理支援として様々な患者家族会が活動しているが、本症候群では家族会がまだ設立されていない。平成２３年度は、埼玉県立小児医療センターの疾患集団外来の活動実績を検討した。本年度開催した集団外来は６疾患（コステロ、4p モノソミー、ベックウィズウィーデマン、コフィンシリス、片側肥大、プラダーウィリーの各症候群）で計７回だった。平均参加患者数は12家族（参加率52%）で総人数は27人だった。まだ家族会のない稀少疾患では、医療機関による疾患の集団外来は有意義と考える。そのために、都市圏では小児医療専門施設が、また地域圏では一定の規模の医療圏にまたがる先天異常症候群の情報センター機能をもつ医療機関の存在が重要と考えた。

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ARTICLE

Paternal origin of the *de novo* constitutional t(11;22)(q23;q11)

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The constitutional t(11;22)(q23;q11) is a well-known recurrent non-Robertsonian translocation in humans. Although translocations generally occur in a random fashion, the break points of t(11;22)s are concentrated within several hundred base pairs on 11q23 and 22q11. These regions are characterized by palindromic AT-rich repeats (PATRRs), which appear to be responsible for the genomic instability. Translocation-specific PCR detects *de novo* t(11;22)s in sperm from healthy males at a frequency of $1/10^4$–10^5, but never in lymphoblasts, fibroblasts or other human somatic cell lines. This suggests that the generation of t(11;22) rearrangement is linked to gametogenesis, although female germ cells have not been tested. Here, we have studied eight cases of *de novo* t(11;22) to determine the parental origin of the translocation using the polymorphisms on the relevant PATRRs. All of the eight translocations were found to be of paternal origin. This result implicates a possible novel mechanism of sperm-specific generation of palindrome-mediated chromosomal translocations.
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Keywords: recurrent non-Robertsonian translocation; parental origin; germ cells

INTRODUCTION

The constitutional t(11;22)(q23;q11) is a well-known recurrent non-Robertsonian translocation in humans. Carriers of this balanced translocation usually have no clinical symptoms and are often identified after chromosomal malsegregation resulting in the birth of offspring with an unbalanced form of the translocation. The unbalanced individuals have a distinctive phenotype called Emanuel syndrome [MIM 609029], that consists of severe mental retardation, preauricular tag or sinus, ear anomaly, cleft or high-arched palate, micrognathia, heart defects and genital abnormalities in the male.[1,2] Most constitutional translocations generally occur in a random fashion. However, the break points of t(11;22)s are concentrated within several hundred base pairs on 11q23 and 22q11, which are characterized by palindromic AT-rich repeats (PATRRs).[3–7] Recently, molecular cloning of various translocation break points has shown similar palindromic sequences on other chromosomes, such as 17q11, 4q35.1, 1p21.2 and 8q24.1.[8–11] It has been acknowledged that palindrome-mediated genomic instability contributes to a diversity of genomic rearrangements including not only translocations, but also deletions and gene amplification.[12,13] Palindromic sequences have the potential to form stem-loop (hairpin or cruciform) structures by intrastrand base pairing in the single-stranded DNA. Thus, we proposed that such unusual DNA secondary structures give rise to genomic instability that leads to the recurrent translocation.[14,15]

As all of the t(11;22) break points are located in a small region within both the PATRR11 (~450 bp) and the PATRR22 (~595 bp), we established a PCR detection system for translocation-derivative chromosomes.[16] Using this PCR system, *de novo* t(11;22)s are detected in sperm from healthy males at a frequency of $1/10^4$–10^5, but never in lymphoblasts, fibroblasts or other human somatic cell lines[17,18], suggesting that the generation of a t(11;22) is linked to meiosis. However, female germ cells have not been tested because the number of human oocytes that can be examined is limited. To investigate whether the translocation is meiosis-specific or male germ cell-specific, we attempted to determine the parental origin of *de novo* t(11;22) cases.

MATERIALS AND METHODS

Samples

Samples were collected from cases with *de novo* constitutional t(11;22)s and their parents after obtaining written informed consent. Genomic DNA was extracted from peripheral blood samples, chorionic villus or amniotic fluid samples and saliva samples. The study was approved by the Ethical Review Board for Human Genome Studies at Fujita Health University.

DNA analysis

Translocation-specific PCR was performed as previously described.[16] The primer sets were designed on both sides of the PATRR on chromosomes 11 and 22 (Figure 1a). We directly sequenced the PCR products on the ABI Prism 3100 Genetic Analyzer.

Amplification of PATRR11 and PATRR22 was also performed as previously described.[7,16] The PCR primers were as follows; 199F 5′-GAGAGTAAAGAA ATAGTTCAGAAAGG-3′ and 190R 5′- CCACAGACTCATTCATGGAACC-3′ for PATRR11, -469F 5′-CCATATGCAGTTATAAATATGTTTCATGATTAT-3′ and +440R 5′-ACAAGTAAACAGGTTTTCAAAGCT-3′ for PATRR22. The PCR condition was to heat at 94°C 2 min, 30 cycles of 10 s of 98°C and 10 min of 60°C. Each PCR product containing the PATRR was cloned into the plasmid vector, pT7Blue (Novagen, Madison, WI, USA), and then sequenced. We used the SURE strain (Stratagene, La Jolla, CA, USA) to maintain the highly unstable PATRR insert.

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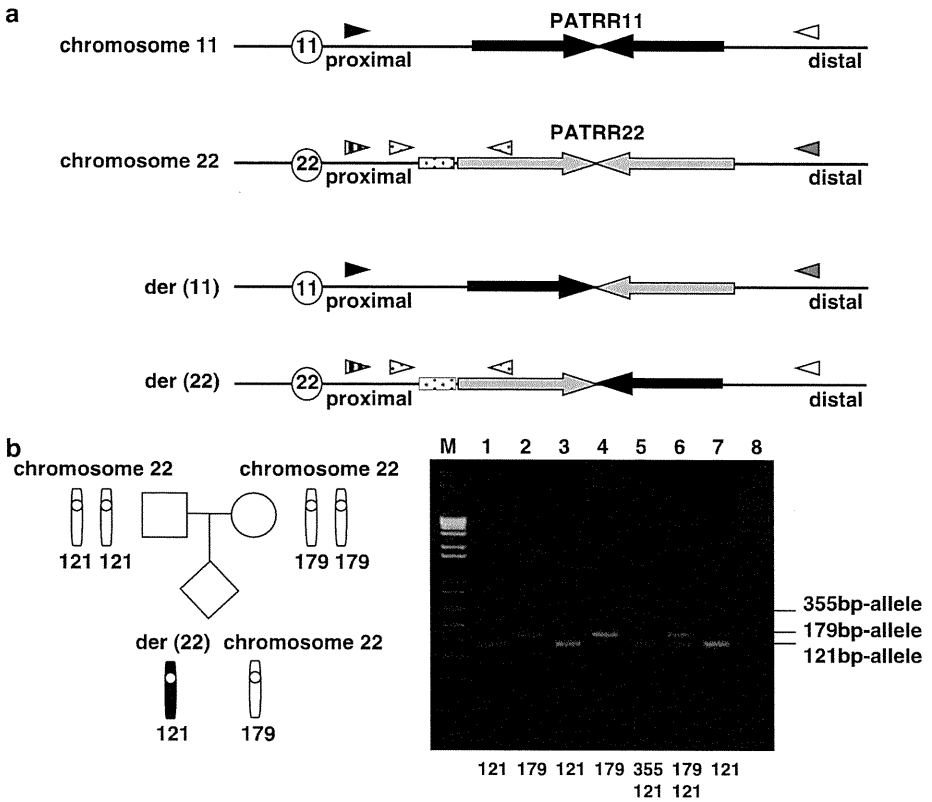

Figure 1 The PCR system to determine the allele type of the translocated or normal chromosomes. (**a**) Translocation-specific PCR. Black arrows indicate each proximal and distal unit of the PATRR11, whereas light gray arrows depict the PATRR22. The PCR primers are indicated as arrowheads. The PATRRs on the normal chromosomes were amplified with a PATRR11-specific primer set (black and white arrowheads) or that for PATRR22 (vertical-striped and gray arrowheads). Translocations can be detected using one primer flanking the PATRR11 (black or white arrowheads) and one primer flanking the PATRR22 (vertical-striped or gray arrowheads). Stippled boxes indicate the AT-rich regions flanking the PATRR22. To determine the size of the AT-rich region, we performed nested PCR. The first PCR amplified the PATRR22 or der(22) breakpoint region using specific-primer sets, respectively. Next, internal primers (stippled arrowheads) were used to amplify the AT-rich regions flanking the PATRR22. (**b**) Determination of the parental origin in family 6. PCR was performed to amplify the AT-rich region adjacent to the PATRR22. The figure shows the ideograms for the normal chromosome 22, and the der(22). M: 1 kb Plus size marker, lane 1: the der(22) of the translocation carrier, lane 2: chromosome 22 of the carrier, lane 3: father, lane 4: mother, lane 5: heterozygote for the 355 bp- and 121 bp-alleles 6: heterozygote for the 179 bp- and 121 bp-alleles, 7: homozygote for the 121 bp-alleles, 8: no DNA. The additional band observed in lane 5 originates from the heteroduplex. Allele type of the der(22) of the carrier corresponds to the paternal type, and that of the PATRR22 of the normal homolog to the maternal type.

We determined the genotype of the size polymorphism at the AT-rich region adjacent to the PATRR22 by PCR.[7] The PCR primer sets were as follows; 22a 5′-CCCAGTGTGAATTGGGATTCAG-3′ and Rev-22c 5′-CAGTAGTATGGATC CGTTGGAGG-3′. Three different length PCR (355 bp-, 179 bp- and 121 bp-allele) products can be identified. We determined the allele type of the der(22) and PATRR22 separately by means of a nested PCR (Figure 1a).

RESULTS

We studied eight carriers of *de novo* t(11;22)s and their parents. All t(11;22) carriers were diagnosed by standard karyotyping followed by fluorescence *in situ* hybridization with appropriate probes. The testing of their parents provided the information that the translocation was *de novo* in origin, which was also confirmed by translocation-specific PCR.

To examine parental origin of the translocated chromosomes, the PATRRs and flanking regions on chromosome 11, 22, der(11) and der(22) were amplified by PCR and the nucleotide sequences were determined[16] (Figure 1a). The highly polymorphic nature of the PATRRs allows one to distinguish between alleles of the translocated and normal chromosomes in the t(11;22) carrier and permitted us to analyze the segregation of the rearranged parental homologs.

In family 1, the allele type of both the proximal part of the PATRR11 on the der(11) and the distal part of the PATRR11 on the der(22) of the translocation carrier corresponded with either of the normal PATRR11s of the father but with neither of the mother (Figure 2 and Supplementary Table 1). On the other hand, the PATRR11 on the normal chromosome 11 of the carrier corresponded with that of the mother but not with that of the father. These results clearly indicate that the translocated chromosomes are of paternal origin. Similarly, the PATRR11 on the der(11) and the der(22) of the translocation carrier in family 2 corresponded with one of the PATRR11s of the father but with neither of the mother. The PATRR11 on the normal chromosome 11 of the carrier was entirely deleted. The mother, but not the father, was found to carry this deleted allele, indicating that the translocated chromosomes are also of paternal origin.

In family 3, the PATRR11 on the der(11) and the der(22) of the translocation carrier corresponded with one of the PATRR11s of the father but with neither of the mother. Although the PATRR11 on the normal chromosome 11 of the carrier was not determined, the results indicate that the translocated chromosomes are also of paternal

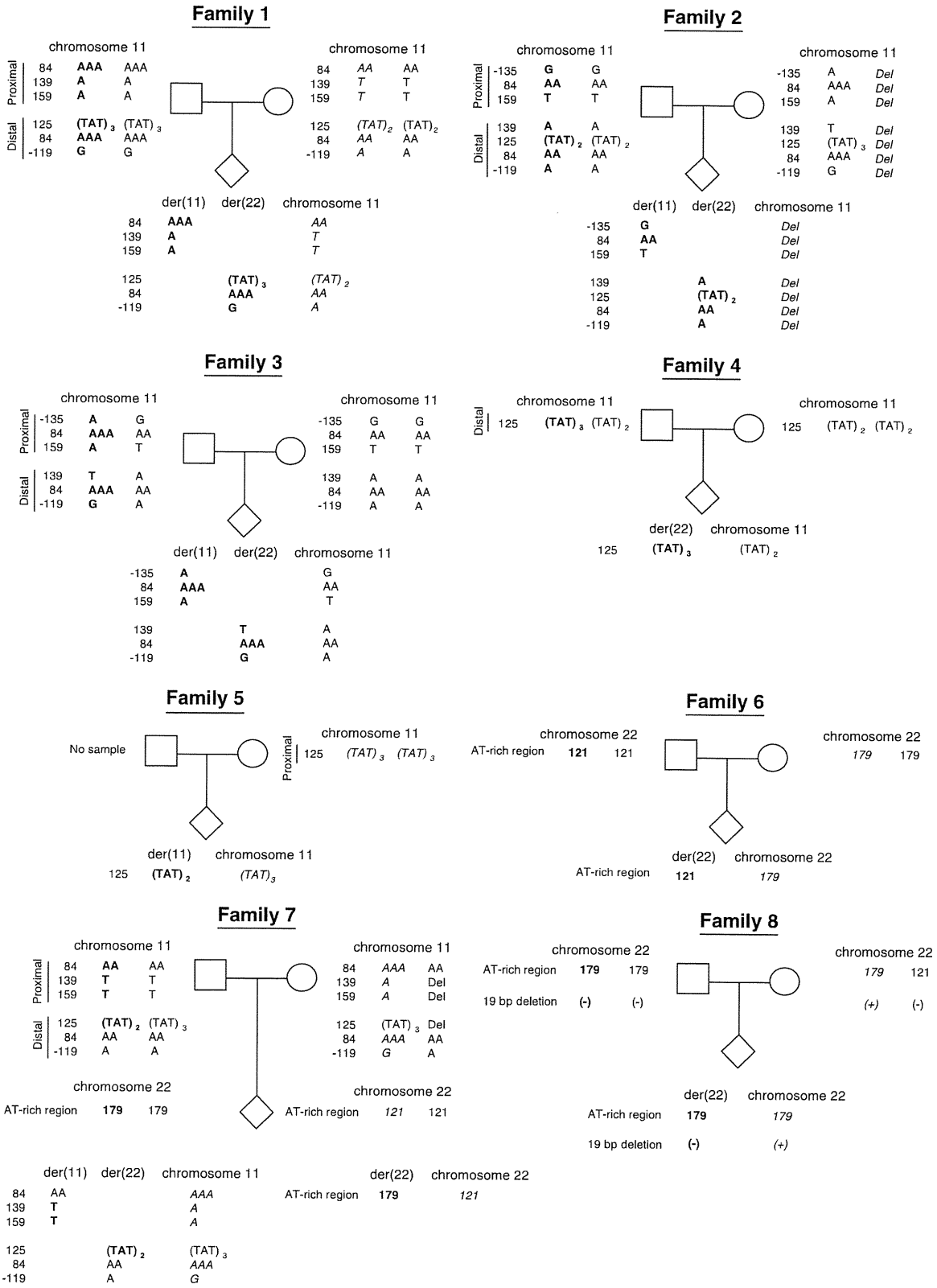

Figure 2 Summary of results for the eight families. Only informative polymorphisms to determine the parental origins were shown. Bold and italic indicate the definitive polymorphisms of paternal and maternal origin, respectively. Numbers at the left indicate the nucleotide positions from the starting point of the PATRR11. In Families 3, 6 and 7, samples from translocation carriers were obtained prenatally from chorionic villi or amniotic fluid. Family 1 was reported previously in Macville *et al*.[28], whereas families 4 and 8 were reported previously in Kurahashi *et al*.[16]

origin. In family 4, although the allele type of the proximal part of the PATRR11 was not informative among the family members, the distal part of the PATRR11 on the der(22) of the carrier corresponded with the one in the father, not with the mother. In family 5, we did not obtain a genomic DNA sample from the father. However, the proximal sequence of the PATRR11 on the der(11) corresponded with neither of the maternal PATRR11s, one of which corresponded with the carrier's normal PATRR11 on chromosome 11, suggesting that the translocation was also of paternal origin.

Two cases (family 6 and 8), whose allele types of the PATRR11 were not informative, were analyzed for the segregation of the PATRR22, instead. Because of the difficulties in sequencing the PATRR22, we used the size polymorphism of the AT-rich region adjacent to the proximal side of the PATRR22[7] (Figure 1a). In family 6, the proband carried the 121 bp-allele on the der(22) and the 179 bp-allele on the normal chromosome 22, respectively. The father was found to be a homozygote for the 121 bp-allele, whereas the mother was homozygous for the 179 bp-allele, indicating that the translocated chromosome of the carrier was derived from the father (Figure 1b, 2 and Supplementary Table 2). Similarly, the proband in family 7 was found to carry the 179 bp-allele on the der(22) that was derived from the father. In addition, the information of the PATRR11 also supported the paternal origin in this case (Supplementary Table 1).

In family 8, because even the genotyping of the flanking polymorphism was not informative, we finally performed sequence analysis of the PATRR22. The PATRR22 on the der(11) of the translocation carrier corresponded with one of the PATRR22s of the father but with neither of the mother. The PATRR22 on the normal chromosome 22 of the carrier harbored a small deletion at the center of the PATRR22. The mother, but not father, was also found to carry the deleted PATRR22, indicating that the translocated chromosomes are also of paternal origin (Figure 2 and Supplementary Table 3).

All eight of the translocations were found to be of paternal origin. The difference in paternal origin was significant (Fisher's exact test, $P=0.00016$). The break points of the eight families were within both the PATRRs but not identical to each other. Thus, we concluded that the paternal origin of *de novo* t(11;22) in these families can be applicable to other cases of t(11;22).

DISCUSSION

It is well documented that *de novo* numerical chromosomal abnormalities are preferentially of maternal origin, whereas structural abnormalities arise predominately in paternal germ cells.[19,20] One exception to this generalization is the Robertsonian translocation. Although it is one of the best known non-random constitutional translocations, ~95% of *de novo* Robertsonian translocation cases originate in maternal germ cells.[21] An oogenesis-specific mechanism has been assumed for Robertsonian translocations. The centromeres of acrocentric chromosomes are brought in close proximity during formation of the nucleolar organizer regions during the prolonged prophase of female meiosis I. Thus, homologous recombination between centromeric repetitive regions may be involved in the generation of Robertsonian translocations. The t(11;22)(q23;q11) is another example of a recurrent constitutional translocation in humans. Despite the similarity between the two PATRRs with regard to AT-richness, no substantial homology is observed between the PATRR11 and the PATRR22.[7] Thus, homologous recombination does not appear to be responsible for the t(11;22). In this study, all eight of the *de novo* t(11;22)s were found to be exclusively of paternal origin. Our result implicates a novel mechanism for sperm-specific generation of the t(11;22).

One way to account for this observation is the difference in the number of cell divisions between spermatogenesis and oogenesis. The number of cell divisions in oogenesis is relatively constant with approximately 22 divisions throughout the female lifetime. In contrast, spermatogenesis reaches roughly 150 divisions by the age of 20 years, with a linear increase of about 23 cell divisions per year. There is a positive relationship between paternal age and *de novo* gene mutations by replication errors.[22] The genomic instability of palindromic DNA appears to be primarily mediated by stalling of the DNA replication fork at a region that forms a hairpin DNA structure.[23] The secondary structure-mediated replication errors during the numerous cell divisions in pre-meiotic spermatogenic cells might contribute to male-specific formation of *de novo* t(11;22)s. Indeed, a positive relationship between paternal age and *de novo* occurrence of non-recurrent translocation has been reported,[24] although such a relationship has not been observed for t(11;22).[25]

On the other hand, experimental data suggest that a replication-independent DNA cruciform can potentially be a target for a structure-specific nuclease and contribute to palindrome-mediated translocations in humans.[26] Cruciform extrusion is energetically prohibited in genomic DNA under standard conditions, because sufficient negative supercoiling is a prerequisite for the formation of a cruciform DNA structure.[27] However, successive transitions of chromatin components from histones to protamines might cause dynamic changes in DNA superhelicity. DNA dissociation from histones may involve accumulation of free negative supercoiling that potentially induces cruciform extrusion at the PATRR leading to male-specific formation of *de novo* t(11;22)s. Thus, conformational changes of the DNA during chromatin remodeling in post-meiotic stages of spermatogenesis might also account for the general fact that structural chromosomal aberrations predominantly originate in paternal gametogenesis. This work implicates a possible novel mechanism of sperm-specific generation of palindrome-mediated chromosomal translocations in humans.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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WEB RESOURCES

The URLs for data presented herein are as follows:
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim

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Polymorphisms of the 22q11.2 breakpoint region influence the frequency of *de novo* constitutional t(11;22)s in sperm†

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The constitutional t(11;22) is the most frequent recurrent non-Robertsonian translocation in humans, the breakpoints of which are located within palindromic AT-rich repeats on 11q23 and 22q11 (PATRR11 and PATRR22). Genetic variation of the PATRR11 was found to affect *de novo* t(11;22) translocation frequency in sperm derived from normal healthy males, suggesting the hypothesis that polymorphisms of the PATRR22 might also influence the translocation frequency. Although the complicated structure of the PATRR22 locus prevented determining the genotype of the PATRR22 in each individual, genotyping of flanking markers as well as identification of rare variants allowed us to demonstrate an association between the PATRR22 allele type and the translocation frequency. We found that size and symmetry of the PATRR22 affect the *de novo* translocation frequency, which is lower for the shorter or more asymmetric versions. These data lend support to our hypothesis that the PATRRs form secondary structures in the nucleus that induce genomic instability leading to the recurrent translocation.

INTRODUCTION

The constitutional t(11;22) is the most frequent recurrent non-Robertsonian translocation in humans. Carriers of this balanced translocation usually have no clinical symptoms and are often identified after the birth of offspring with an unbalanced form of the translocation, the supernumerary-der(22)t(11;22) syndrome (Emanuel syndrome). Patients with the supernumerary-der(22) syndrome have a distinctive phenotype, which consists of severe mental retardation, preauricular tag or sinus, ear anomaly, cleft or high-arched palate, micrognathia, heart defects and genital abnormalities in the male (1–3).

The t(11;22) translocation represents a good model for studying the molecular mechanisms that contribute to genomic rearrangements. There are several reports describing multiple cases of t(11;22) balanced carriers including *de novo* cases (1,2,4). The recurrent nature of this translocation prompted the examination of the t(11;22) breakpoints for a specific genomic structure, culminating in the identification of palindromic AT-rich repeats at both breakpoint regions on chromosomes 11q23 and 22q11 (PATRR11 and PATRR22) (5–8). All the breakpoints were located within the PATRR11 and PATRR22, near the center of the PATRR but different at a nucleotide level among individual families, confirming that the rearrangement is recurrent (9). Indeed, translocation-specific PCR using the sequences flanking the translocation junction fragments from both derivative translocation chromosomes detected multiple *de novo* t(11;22)s in sperm derived from normal healthy males (10).

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†The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers: AB533274, AB533275, AB533276, AB533277, AB538236, AB538237, AB538238 and AB538239.

In our previous study, we demonstrated that the nucleotide sequence of the PATRR11 was hypervariable and varied among individual alleles. We calculated the *de novo* translocation frequency based on the occurrence of positive PCRs and reported that the polymorphisms of the PATRR11 affected the *de novo* translocation frequency (11). It is reasonable to hypothesize that PATRR22 polymorphisms might be another important factor in the generation of the t(11;22) translocation, although the data also showed that the PATRR22 has little influence on the frequency. Our recent studies have indicated that the PATRR22 also manifests variation at the nucleotide sequence level, but we did not find any large-scale size polymorphism similar to the insertion/deletion polymorphism seen for the PATRR11 (6). Indeed, the translocation frequency varies moderately among individuals with the same PATRR11 genotype, promoting the speculation that subtle polymorphisms in the PATRR22 affect this variation.

In this study, we analyzed the *de novo* t(11;22) translocation frequency as a function of the PATRR22 allele type. It is well documented that the PATRR22 is located at one of the chromosome 22-specific low-copy repeats (LCR22-B), which have been identified at multiple loci on 22q11 (12–14). The duplicated sequences share 97–98% sequence homology with each other, preventing genotyping of the PATRR22. This is due to competing sequence that produces excessive background during PCR amplification (6). In the current study, we utilized the genotypes of PATRR22-flanking marker to determine whether the *de novo* t(11;22) translocation frequency could be associated with variation of the PATRR22. Further, we optimized PCR conditions for amplification of the PATRR22 using new LCR-specific primers. This approach identified rare PATRR22 variants facilitating the ability to analyze the association between PATRR22 variation and t(11;22) formation.

RESULTS

PATRR22 genotype did not affect the total frequency of *de novo* t(11;22)s

To investigate the effect of PATRR22 polymorphisms on the frequency of *de novo* t(11;22) formation in sperm from normal healthy males, the translocation frequency in individuals with various PATRR22 genotypes was determined. To avoid the potential effects introduced by PATRR11 polymorphisms, individuals homozygous for the L-PATRR11, the allele most commonly seen in the Japanese population, were selected (11). Among these subjects, the frequency of translocation ranged from 6.01×10^{-6} to 1.65×10^{-4}, a relatively narrow range when compared with variation of greater than three orders of magnitude induced by polymorphisms of the PATRR11. The AT-rich region flanking the PATRR22 manifests size polymorphisms (A allele, 355 bp; B allele, 179 bp; C allele, 121 bp) (6). The initial analyses of eight PATRR22 alleles from four individuals suggested that these polymorphisms appeared to be linked to the PATRR22 polymorphisms. This linkage disequilibrium was confirmed by further analyses of additional 16 alleles (3 A alleles, 6 B alleles and 7 C alleles; data not shown). Thus, we utilized these polymorphisms as a surrogate for the genotype of the PATRR22, which is difficult to determine. We identified three major genotypes, A/C, B/C and C/C. Although the total translocation frequency varies among individuals (Table 1), we found no statistical difference among the A/C, B/C and C/C groups ($P = 0.14$).

Table 1. Association between the type of PATRR22 polymorphism and total frequency of *de novo* translocations

Case	Genotype of AT-rich region	PATRR11 genotype	Template DNA (ng/μl)	Positive PCR	Translocation frequency[a]
1	A/C	L/L	100	23/44	2.24×10^{-5}
2[b]	A/C	L/L	50	30/50	5.55×10^{-5}
3	A/C	L/L	100	10/50	6.76×10^{-6}
4[b]	B/C	L/L	50	39/50	9.18×10^{-5}
5	B/C	L/L	100	35/51	3.51×10^{-5}
6[b]	B/C	L/L	10	21/50	1.65×10^{-4}
7	C/C	L/L	100	33/71	1.89×10^{-5}
8	C/C	L/L	100	17/26	3.21×10^{-5}
9	C/C	L/L	100	18/39	1.88×10^{-5}
14[b]	A/A	L/AS	10	7/50	4.57×10^{-5}

[a]The total frequency was calculated based on the total number of positive PCR.
[b]The first two examinations of these samples showed positive results in almost all of the aliquots, precluding the estimation of the exact frequency of the translocation. We therefore used 50 ng (Cases 2 and 4) and 10 ng (Cases 6 and 14) of DNA as a template in these cases.

PATRR22 allele type affects the frequency of *de novo* t(11;22)s

To circumvent the effect of various factors that potentially affect translocation generation, we determined the allelic origin of each translocation product and determined the allele-specific translocation frequencies in each individual (Fig. 1). In the case of A/C heterozygotes, the translocation was more frequently generated from the C allele than from the A allele ($P = 0.0033$; Table 2). The PATRR22, regardless of the genotype of the AT-rich region, manifests an almost perfect palindromic structure, showing >98% homology between the proximal and distal arms (Table 3). However, the size of the A allele PATRR22 is 583 bp, which is 14 bp shorter than that of C allele PATRR22 (597 bp). The A allele PATRR22 carries a short asymmetric region at its center, whereas the C allele of the PATRR22 does not. Thus, short size or central asymmetry might influence the relatively low translocation frequency of the A allele.

Although the percentage of translocations from the C allele was marginally greater than that from the B allele in B/C heterozygotes, statistical analysis indicated no significant difference ($P = 0.06$). The size difference between the B and the C alleles is only 2 bp (595 bp versus 597 bp) and both are similar in their symmetry (Table 3). Since allelic preference varied among individuals, it is hypothesized that subtle nucleotide alteration among the same allele type might influence the variation of the translocation frequency. We sequenced both the B and the C allele PATRR22s in all six B/C heterozygotes. All the sequences from the B and C allele PATRR22s were identical to one another except one case that manifested a two-nucleotide substitution in the

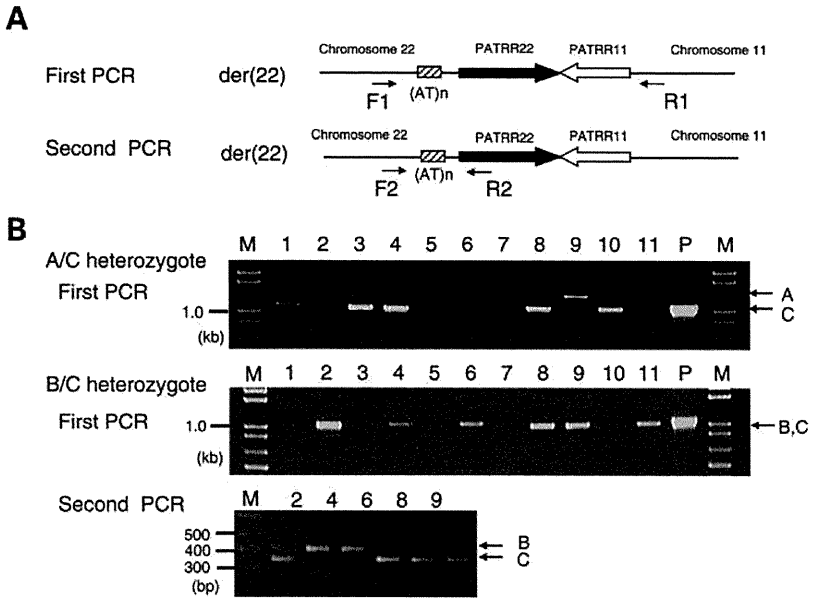

Figure 1. Analysis of the allelic origin of *de novo* t(11;22) translocations. (**A**) Nested PCR strategy for determining allelic origin of the der(22) of the t(11;22). PATRRs are shown by thick arrows, and the AT-rich region flanking the PATRR22 is indicated by a hatched box. PCR primers are shown by thin arrows to indicate the location and orientation. (**B**) Representative results of the nested PCR. For an A/C heterozygote, the first PCR can distinguish the allelic origin of the PCR products based on product size (upper panel). For a B/C heterozygote, the size of the first PCR product is similar to one another (middle panel). The second PCR can clearly distinguish between the allelic origins (lower panel). Two bands were observed in lane 2, suggesting that the template DNA includes two or more translocations. M, size markers; P, DNA from t(11;22) translocation carrier served as positive controls.

Table 2. Association between allele type of the PATRR22 polymorphism and frequency of *de novo* translocation

Case	Genotype of the AT-rich region	PATRR11 genotype	Frequency	Positive PCR	Allelic origin A	B	C
1	A/C	L/L	2.24×10^{-5}	23/44	1 (4%)	—	22 (96%)
2	A/C	L/L	5.55×10^{-5}	30/50	5 (17%)	—	25 (83%)
3	A/C	L/L	6.01×10^{-6}	9/50	2 (22%)	—	7 (78%)
10	A/C	L/AS	1.45×10^{-5}	19/50	1 (5%)	—	18 (95%)
4	B/C	L/L	9.18×10^{-5}	39/50	—	20 (51%)	19 (49%)
5	B/C	L/L	2.55×10^{-5}	35/51	—	17 (49%)	18 (51%)
6	B/C	L/L	1.65×10^{-4}	21/50	—	9 (43%)	12 (57%)
11	B/C	L/S	1.35×10^{-5}	18/50	—	7 (39%)	11 (61%)
12	B/C	L/S	1.60×10^{-5}	30/73	—	14 (47%)	16 (53%)
13	B/C	L/AS	8.72×10^{-6}	22/88	—	10 (45%)	12 (55%)

L, long PATRR; S, symmetric short PATRR; AS, asymmetric short PATRR.

Table 3. Characterization of the polymorphic PATRR22 alleles

PATRR22	Size (bp)	Accession number	Homology between proximal and distal arms (%)	ΔG (kcal/mol)	ΔG/nt (kcal/mol)
Standard A allele	583	AB261997[a]	98.6	12.57	0.022
Standard B allele	595	AB538236	98.7	9.66	0.016
Variant B allele (Case 5)	595	AB538238	98.0	14.03	0.024
Standard C allele	597	AB538237	98.3	13.24	0.022
Variant B1 allele (Case 15)	553	AB533274	100.0	2.71	0.005
Variant C1 allele (Case 15)	509	AB533275	98.8	5.47	0.011
Variant C2 allele (Case 16)	539	AB533276	99.6	5.67	0.011
Variant C3 allele (Case 16)	457	AB533277	96.9	19.90	0.044
Flanking AT-rich region (A allele)	355	AB261997[a]	89.3	49.35	0.139

[a]AB261997 includes sequence information of both PATRR22 and flanking AT-rich region of A allele.