VISUALIZING ROBERTSONIAN TRANSLOCATION WITH ATOMIC FORCE MICROSCOPY

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SUMMARY

Purpose : *Down Syndrome is the most common and best known chromosome disorder with an incidence of one in 800 births. Four percent of all Down Syndrome patients show a Robertsonian translocation in their karyotypes. A particular 21q;21q translocation has been reported to be made up of two chromosome 21 long arms. 21q;21q translocation has been studied extensively by both cytogenetic and molecular biological methods. In this study we aimed to visualize the translocation chromosome with atomic force microscopy (AFM), and to detect whether any difference from the normal chromosomes 14 and 21 occurred in the translocation chromosome.* ***Methods :*** *Two newborns were referred to our laboratory with symptoms of Down Syndrome. Cytogenetic analyses of cultured lymphocytes from peripheral blood samples resulted in 46,XY,t(21q;21q), 46,XX,t(14q;21q) karyotypes, respectively. AFM analyses were performed with Topometrix TMX2000 Explorer.* ***Results :*** *We confirmed by line measure analysis that translocation chromosomes consisted of two acrocentric chromosomes.* ***Conclusion :*** *Detailed structural examination of chromosomes will open new insights into the explanation of pathogenesis of diseases.*

Key words : *Translocation (Genetics), Down Syndrome, Atomic Force Microscopy.*

INTRODUCTION

Down Syndrome, trisomy 21, is by far the most common and best known of chromosome disorders. Among all Down Syndrome patients, 4% have 46 chromosomes, one of which is a Robertsonian translocation chromosome (a translocation between two acrocentric chromosomes by fusion at or near the centromere, with loss of the short arms) between chromosome 21q and the long arm of one of the other acrocentric chromosomes (usually chromosome 14 and 21). Clinical findings of Down Syndrome patients are similar in various karyotypes (1).

A 21q21q translocation chromosome is a chromosome made up of two chromosome 21 long arms, seen in Down Syndrome patients (1). The translocation has been studied by different methods including RFLP analysis (2).

A 14q21q translocation chromosome is a chromosome made up of chromosome 14 and chromosome 21 long arms, seen in Down Syndrome patients.

Atomic force microscopy (AFM), was

discovered by Binnig et al. and has been used in analysis of different biological materials, including chromosomes before and after trypsin-Giemsa (GTG) banding (3, 4). GTG banding is a routine protocol used in cytogenetics laboratories in order to distinguish chromosomes from each other and to detect chromosomal abnormalities (5). We visualized chromosomes by atomic force microscopy previously and detected numerical and structural abnormalities of them (6, 7).

Basically, AFM uses a probe mounted on a very small spring cantilever. When the probe is scanned across the sample, the force between the probe tip and sample changes as the surface features are encountered, causing the spring cantilever to deflect. In order to obtain the magnitude of height displacement, a laser and a quadrant detector are used to detect the motion of the spring cantilever and provide a feedback signal as itis scanned across the sample (8-10).

MATERIAL AND METHODS

Chromosome preparation. 0.5 ml heparinized peripheral blood sample from the patient was cultured at 37°C for 72 hr in RPMI 1640, supplemented with 1.5% phytohaemagglutinin, 20% fetal calf serum, 200mM L-glutamine and antibiotics. Colcemid solution (0.5 μg/ml) was added to the culture at the 68th hr of incubation for 75 min. After hypotonic treatment in 0.075M KCl solution for 20 min at 37°C, chromosomes were fixed in methanol acetic acid fixative in a 3:1 ratio respectively and metaphase spreads were prepared (11).

After aging for three days, GTG banding was performed and light microscopic analyses of metaphases were made (5). Karyotypic abnormalities were confirmed by AFM analysis of unbanded metaphase spreads.

Atomic force microscopy and analysis. In this study the AFM used, which is a member of scanning probe microscope family was Topometrix TMX2000 Explorer. The system used for imaging the samples was the one operating in contact mode and in air. The probes were the standard pyramidal ones (Topometrix 1520-00). These probes were made of Si3Ni4 and mounted on to cantilevers 200 mm long. The force spring constant of these levers was given as 0.032 N/m. The surfaces i.e. the metaphase spreads were rasterly scanned by a scanner with a 150 X 150 X 10 μm, x, y, z scan range.

Before starting the surface analysis via AFM, the metaphase spreadsiles on ordinary microscope slides, were found by using an optical microscope (Zeiss Axioscope). These places were marked by sticking a transmission electron microscope grid just the reverse side of the slide. The grid size was around 125 μm. Following this marking process the slides were placed on the xy-translational stage of the AFM system, and then the addressed area seen by the CCD camera of the microscope was scanned.

The area of interest was primarily scanned in 150 X 150 μm^2 and with a resolution of 200 X 200 pixels. After obtaining a good image of the metaphase, the area was zoomed down to 50 μm for t21q21q and 60 mm for t14q21q with 400 X 400 or (500 X 500) pixels resolution.

RESULTS

Prior to the AFM analysis, the karyotype analysis was performed with light microscopy. We have analyzed 20 metaphases from each patient, and found a karyotype of 46,XY,t (21q;21q), and 46,XX,t(14q;21q), respectively showing a translocation type Down Syndrome. The samples were then spread on glass slides and AFM analysis was carried out. Figure 1 shows a two-dimensional image of one of the metaphases.

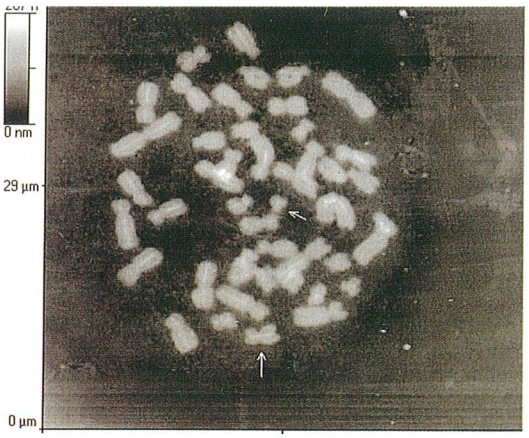
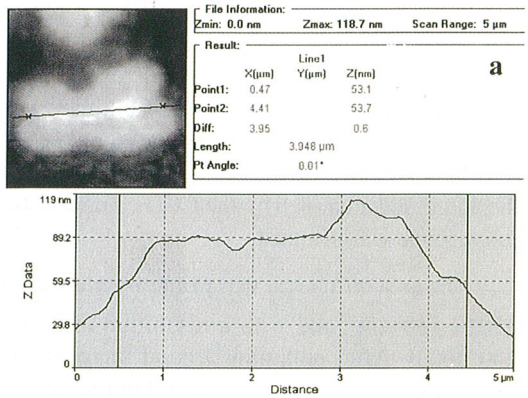


Fig - 1: Metaphase of the translocation patient; t(21q;21q). The vertical arrow indicates the translocation chromosome and the oblique arrow indicates chromosome 21.



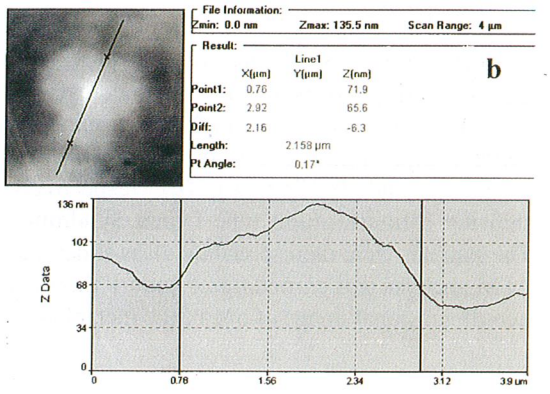


Fig - 2 : (A) Line measure analysis of the translocation chromosome. (B) Line measure analysis of chromosome 21.

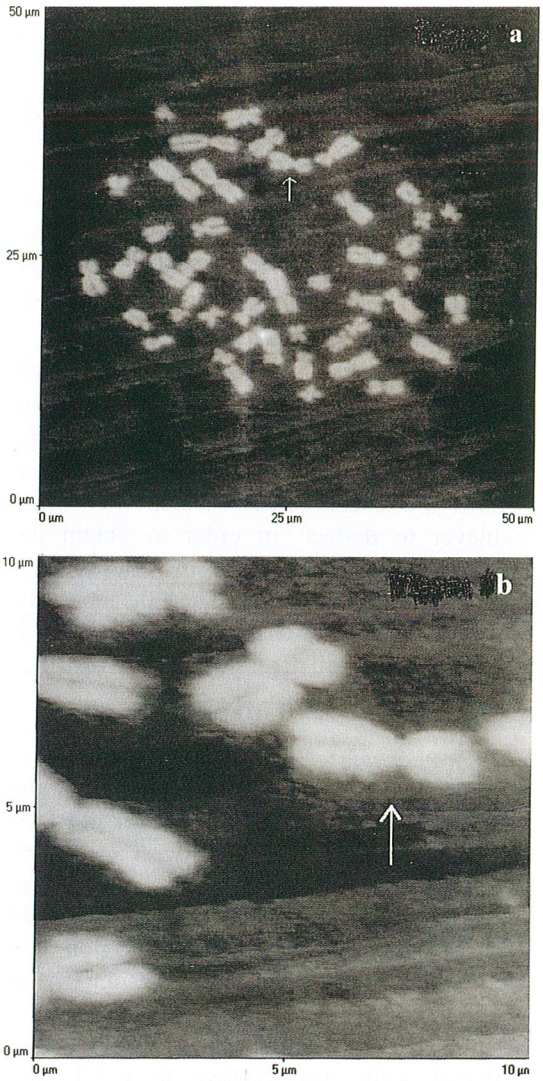


Fig - 3: (A) Metaphase of the translocation patient; t(14q;21q). (B) Two-dimensional image of the translocation chromosome.

In Figures 2a and 2b, measurements of translocation chromosome and chromosome 21 from this metaphase are given.

Figures 3a, b show a two-dimensional image of the other metaphase and the translocation chromosome. In Figures 4a, 4b, and 4c, measurements of translocation chromosome, chromosome 14, and chromosome 21 are given.

DISCUSSION

In Figure 1, the translocation chromosome and chromosome 21 are marked by arrows. In Figures 2a and 2b, the measurements of the translocation chromosome and the normal chromosome 21 are given. In order to exclude the other chromosomes, first of all, the short and the long arms of chromosome 21 and the translocation chromosomes were measured and measurements were profiled by the AFM software. They were then compared with each other to confirm that the translocation chromosome is made up of two chromosome 21 long arms. The findings were similar, confirming that the translocation chromosome consists of two chromosome 21 long arms. In this respect, asymmetric arms of translocation chromosome have been found to be in accordance with the optical microscope results.

In the other patient we have measured chromosome 14 and 21 as well as the translocation chromosome. In figure 4b and 4c, we measured chromosome 14 and 21; the lengths

of their long arms were 1.87mm: 1.2 mm and their centromere lengths were found to be 0.55 mm and 0.55 mm, respectively. Then the line measure analysis of the translocation chromosome (Fig. 4a), revealed three peak regions, similar to the analysis of chromosome 14 and 21. Besides, we measured the translocation chromosome, and calculated the length of the centromere to be 0.7 mm (Figure 4a). Among these calculations, we found that each chromatid of the translocation chromosome is made up of the long arms of chromosome 14 and 21. Another interesting point was that the centromere length of the translocation chromosome was different from the summation of the other two chromosomes' centromere lengths (0.7 mm with respect to 0.55 + 0.55 mm). These data showed us the process of losing the short arms of each acrocentric chromosome.

In the light microscopy analyses, the translocation chromosome seemed to be somewhat asymmetric. This finding was consistent also in AFM analysis. Different studies have been reported regarding the nature of t (21q;21q) and t (14q;21q) translocation in Down Syndrome patients (2).

Although majority of t (21q;21q) chromosomes are isochromosomes derived from a single parental chromosome 21; in our case, because of the asymmetric structure of the translocation chromosome, it is difficult to conclude that the chromosome is an isochromosome without molecular biological analysis.

The results showed the success of AFM in detecting the translocation chromosome. AFM as a new tool in biology, is shedding light on all of the chromosomal abnormalities. Our aim is to detect the chromosomal abnormalities at atomic resolution to give clues in understanding the complex structure of chromosomes. Previously we visualized the structure of DNA interactions with fluorescent dyes by scanning tunneling microscopy which also belongs to the family of scanning probe microscopes AFM (12). As a result we believe that visualization of biological materials in atomic dimensions will give new insights into the explanation of pathogenesis of diseases.

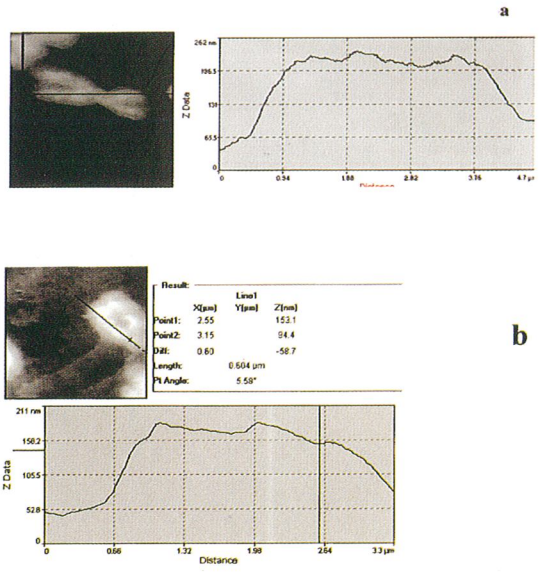


Fig - 4: (A) Line measure analysis of the translocation chromosome (B) Line measure analysis of the chromosome 14.

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