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Numerical Chromosomal Abnormalities Detected by Atomic Force Microscopy

MEHMET ALI ERGÜN, ERDAL TAN,* FERIDE IFFET ŞAHIN, ADNAN MENEVŞE

Department of Medical Biology and Genetics, Gazi University, Ankara; *Material Research Department, Ankara Nuclear Research and Training Center, Saray-Ankara, Turkey

Summary: The numerical abnormalities of human metaphase chromosomes, fixed according to standard procedures for optical microscopy but not treated for banding, were detected by atomic force microscopy (AFM). High-resolution AFM imaging of chromosomes in trisomy 13, 21, and Klinefelter syndrome can be compared directly with the traditional optical image. The unbanded metaphase chromosomes, including the extra ones in trisomic patients showed a structural pattern very similar to G-banding. Comparison of AFM images with light microscopic data allows the identification of specific chromosomes, and images of chromosomes showing numerical and structural abnormalities can then be analysed.

Key words: atomic force microscopy, chromosome, numerical abnormalities

Introduction

Human chromosomes are routinely analysed under light microscope in cytogenetics laboratories. When they are unbanded, chromosomes have been reported to be electron dense and uniformly structured; however, banding techniques facilitate the identification of individual chromosomes (Burkholder and Duczek 1982).

Light microscopy has a low resolution when the molecular basis of chromosomes is regarded. Several different studies have focused on banded and unbanded chromosomes and consequently electron microscopy, scanning electron microscopy, and atomic force microscopy (AFM) visualisations of chromosomes were reported (Musio *et al*. 1994). Among these techniques, the AFM belongs to the recently developed family of scanning probe microscopes (SPMs). These microscopes are able to "see" single atoms, and a review of the different types of SPM, with specific reference to their biological use, can be found in Marti and Amrein (1993).

Since its introduction (Binning *et al*. 1986), the AFM has been used to image a wide variety of biological material including gap junctions (Hoh *et al*. 1991), blood cells (Butt *et al*. 1991), chromosomes (Fritzsche *et al*. 1994, Musio *et al*. 1994), DNA molecules (Mou *et al*. 1995), and living cultured cells (Ushiki *et al*. 1996). Moreover, surface topography of human chromosomes was examined by AFM during treatments for G-banding, and results indicated that AFM provides many new details of chromosome structure (Musio *et al*. 1997).

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 it is scanned across the sample (Güven *et al*. 1987).

In the present study, we used the AFM imaging to detect the numerical chromosome abnormalities of patients who consulted our cytogenetic laboratory for chromosome analysis. We analysed fields containing complete spreads imaged by AFM. The unstained observations by AFM of a complete set of chromosomes grouped together has been compared with those obtained by routine cytogenetic analysis and banding methods using light microscopy.

Materials and Methods

Chromosome Preparation

Heparinized human whole blood samples 0.5 ml were cultured at 37°C for 72 h in RPMI 1640 (Sigma Chemical Corp., St. Louis, Mo.), supplemented with 1.5% phytohemagglutinin (Sigma), 20% fetal calf serum (Seromed, Germany), 200 mM L-glutamine (Sigma), and antibiotics (Seromed).

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Address for reprints:
Adnan Menevşe
Department of Medical Biology and Genetics
Gazi University
06510, Beşevler
Ankara, Turkey
E-mail: ergun@tr-net.net.tr

Colcemid solution (0.5 μg/ml)(Sigma) was added to the cultures at 68 h of incubation for 90 min. After hypotonic treatment in 0.075 M KCl solution for 20 min at 37°C, chromosomes were fixed in methanol, acetic acid (Merck, Darmstadt, Germany) fixative in a 3:1 ratio, respectively, and metaphase spreads were prepared (Verma and Babu 1989).

After aging for 3 days, GTG banding was performed and light microscopic analyses of metaphases were made for each patient. Karyotypic abnormalities were confirmed by AFM analysis of unbanded metaphase spreads.

Atomic Force Microscopy and Analysis

The AFM used in this study is the TOPOMETRIX TMX2000 Explorer, operating in contact mode and air. Throughout the surface analysis, a standard pyramidal tip with the radius of curvature of approximately 1000 A° was also used.

The samples deposited on either polystyrene or glass surfaces (slides) were initially analysed via light microscope (Zeiss Axioskope, Jena, Germany) to determine the metaphase spreads, and by following this common procedure, AFM measurements were performed.

Various scan areas changing from 100×100 to 10×10 μm^2 were used to image the chromosomes in a good manner. The applied force and the image resolution were 0.7 nN and 400 ×400 pixels (or higher), respectively, for each image acquisition. The raw data gathered were analysed by using the software of the microscopy system in two- or three-dimensional display presentations.

Results

Atomic force microscopy has been used to examine the chromosomal structures previously (Fritzsche *et al*. 1994, Musio *et al*. 1994); however our study is a somewhat different work, carried out on patients who were referred to our department for routine chromosome analysis. Four patients with chromosome number abnormalities, consisting of trisomy 21, 13, and Kleinefelter syndrome, and two individuals with normal karyotypic findings as control samples were included in the study.

After detection of the numerical chromosome abnormalities by routine cytogenetic analysis and banding methods using light microscopy, the untreated samples were subjected to AFM analysis.

Figure 1a shows a typical two-dimensional image of the metaphase with trisomy 13, obtained from air-dried samples with AFM. The image is magnified 2000 times, which is higher than that of a ×100 objective in light microscopy. Images in Figure 1b, 1c, and 1d are magnified about 6000 times in three dimensions.

Longitudinal variations in the thickness of the chromosome 13 that display a banding pattern are clearly visible (Fig. 1d), as it is shown in a typical case (Fig. 1c) in which chromosome 3 is partially superimposed on chromosome 13; hence, the identity of chromosomes has become very difficult to solve by optical microscopy. In this respect, the high-z proximity feature of AFM is valuable, and with the aid of the software and its rotation, the chromosome images could be rotated and therefore could be identified more easily. Also, measurements of the three-dimensional structure of the chromosome are available by this feature (Fig. 1e). In air-dried samples, the average length of the D-group chromosomes is about 3.86 μm. The banding pattern also causes an average diversity of 20 nm in the z-direction (Fig. 1e). As shown in Figure 1f, the average width of the chromosome is about 1 μm and the height is 0.06 μm.

Figure 2a shows a metaphase of the patient with trisomy 21. Within this spread chromosomes are discrete with defined morphology, including the G-group chromosomes (Fig. 2a–f). The comparison with G-banding by optical microscopy allowed us to identify the five images of the G-group chromosomes (Fig. 2b–f).

Metaphase spreads of two controls and of Klinefelter syndrome showed similar structural images of chromosomes as those obtained for patients with trisomy 13 and trisomy 21. In Figure 3, the metaphase spread from the patient with 47, XXY karyotype is shown, and 47 chromosomes are easily visible.

Discussion

The application of AFM to the imaging and analysis of chromosomes has been proved very useful. This relatively new technique has been used to provide high-resolution, three-dimensional images of uncoated and unstained human chromosomes in which surface features < 50 nm have been resolved (De Grooth and Putman 1992, Fritzsche *et al*. 1994).

In this study we have presented a brief overview of some of our recent results on patients with numerical chromosome abnormalities. The topographic features of the trisomic chromosomes within their respective metaphase spreads, disclosed by AFM in this paper, are novel and presumably indicate the potential usefulness of AFM in the numerical and structural chromosome abnormalities.

McMaster *et al*. (1994) suggested that staining materials might have an effect on chromosome topography and structure and worked on native, untreated metaphase spreads. In our study we also worked on untreated chromosomes and observed banding patterns similar to G-banding. On the other hand, Fritzsche *et al*. (1994) worked on chromosomes under different loading forces of the scanning tip and under different liquid media. Their findings were similar to those of De Grooth and Putman (1992). Recently, another report on analysis of chromosomes by AFM was carried out by Musio *et al.* (1997); they detected changes in chromosomes during different phases of G-banding, and in each phase chromosomes had different structural changes. As this study is a preliminary report, we chose to work on samples of patients, and we preferred to observe chromosomes in their native

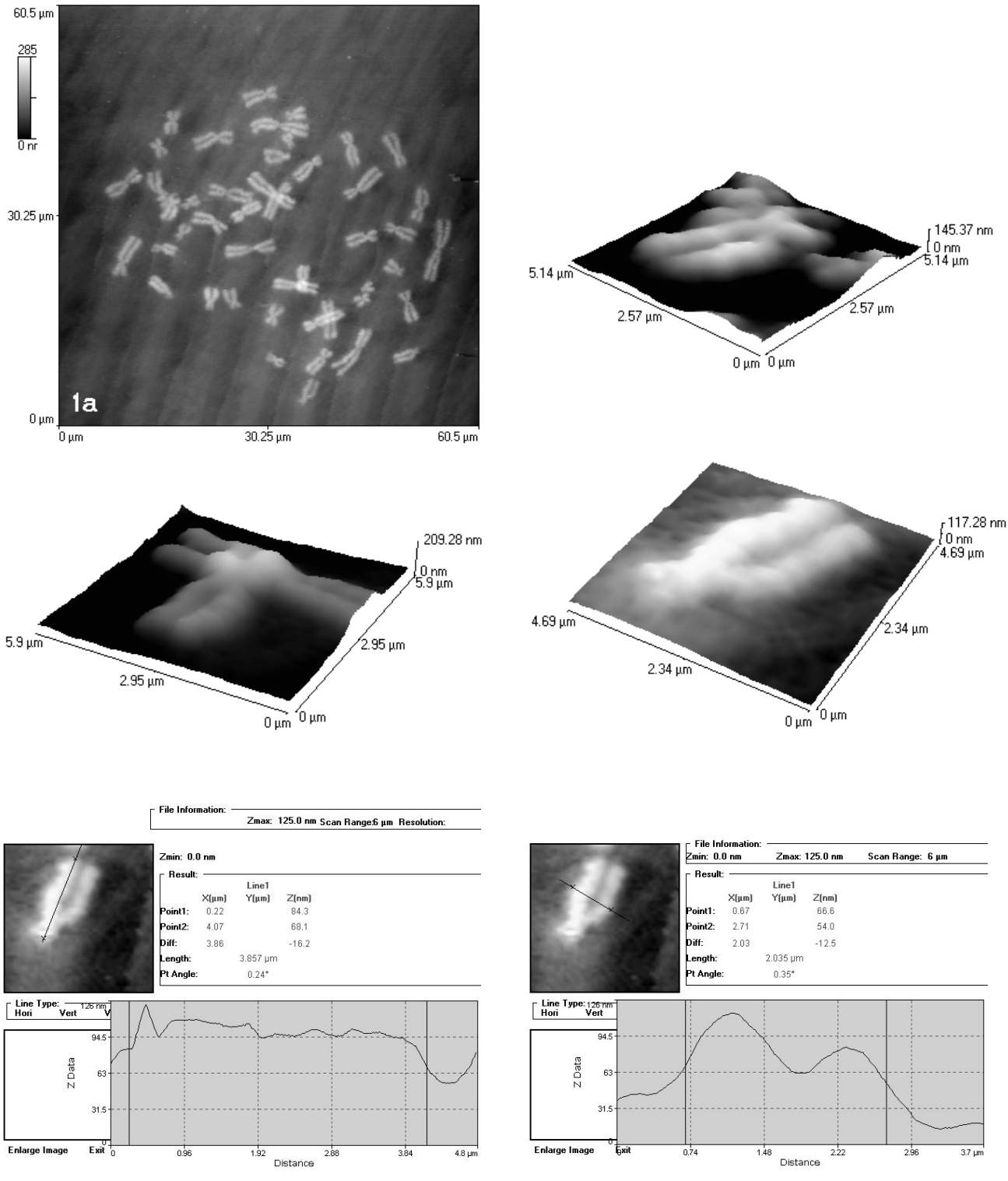


FIG. 1 (a) Untreated metaphase chromosomes including trisomy 13 analysed by atomic force microscopy (AFM). (b–d) Three-dimensional AFM images of the same metaphase showing three individual chromosomes 13. (e, f) Line measure analysis of Figure 1d.

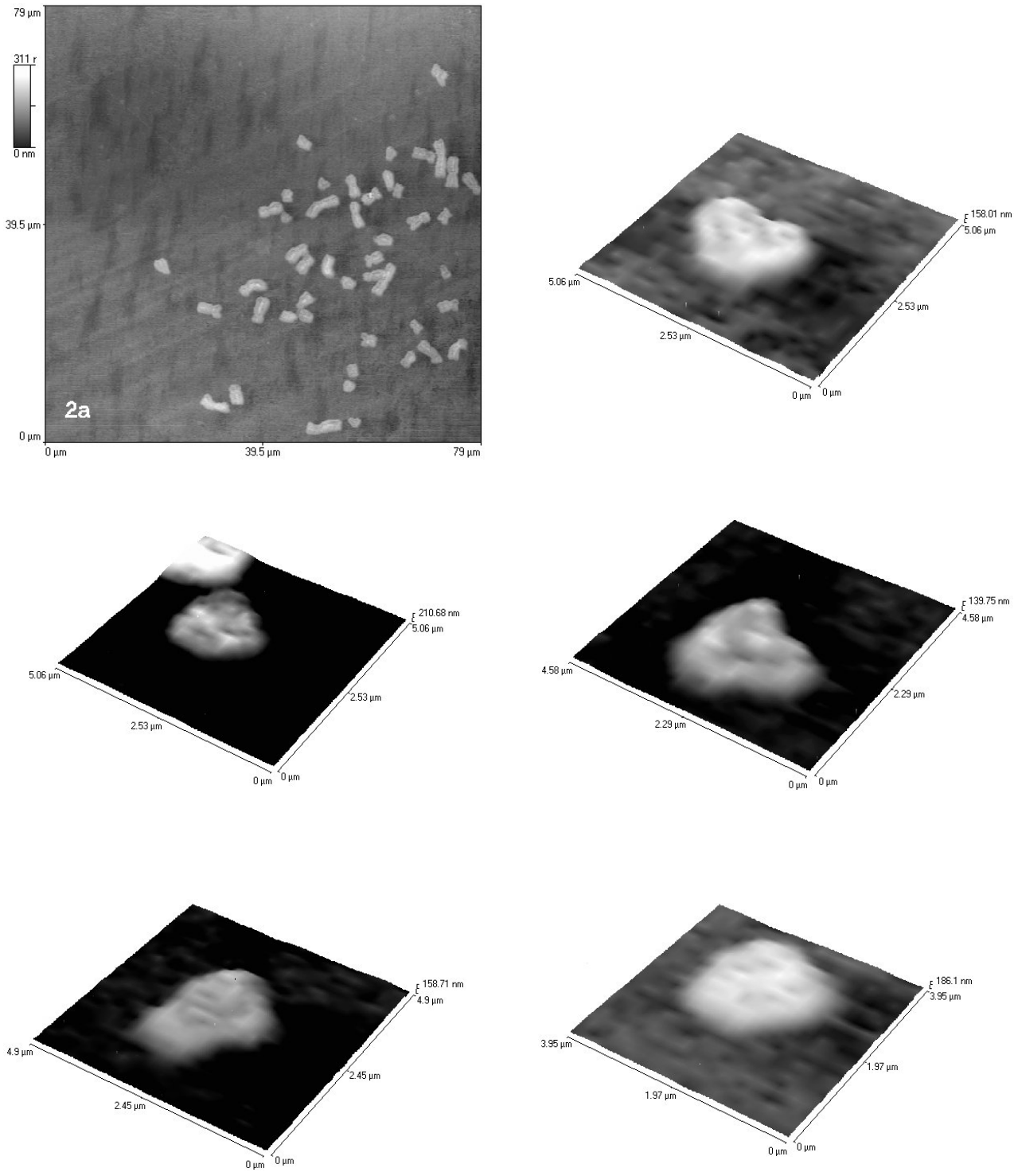


FIG. 2 Atomic force microscopy (AFM) image of a metaphase belonging to the patient with the trisomy 21. This "AFM karyotype" shows a three-dimensional surface image of the metaphase. (b–f) Three-dimensional AFM images of the same metaphase showing G-group chromosomes.

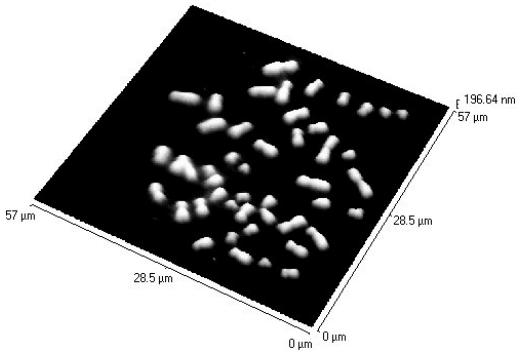


FIG. 3 Three-dimensional image of the patient's Kleinefelter syndrome metaphase.

configurations to detect any deviation from normal structure. We observed that AFM as a three-dimensional technique revealed more detailed surface topographical structure of chromosomes, including their banding patterns, when compared with the other optical devices. In high magnifications, the chromosome surface showed a granular appearance of about 20 nm in the z direction (Fig. 1e, f), and this finding was similar to the results of others (Haberle *et al*. 1991, Ushiki *et al*. 1996).

Metaphase chromosomes, which are split into separate chromatids, are clearly visualized by AFM (Fig. 1e,f). The width of the chromatids is about 1 µm and their height in this image is about 0.06 µm. These findings are in accordance with those reported by McMaster *et al*. (1994) who have recorded the dimensions of chromosomes by AFM imaging that was carried out in the contact mode, as we did in our study.

Atomic force microscopy imaging has the advantage of containing quantitative information on the sample height, indicating the importance of cicumferential grooves in the surface of chromatids similar to the "G-banding pattern."

This research is in progress. We are now working on different banded chromosomes and FISH performed metaphases to detect their different configurations. With the development of technology and the use of these facilities in laboratories, we acknowledge that AFM will illuminate many mechanisms underlying chromosomal defects.

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