SPONTANEOUS CHROMOSOME ABNORMALITIES IN ATM- AND NBS1-DEFICIENT LYMPHOCYTES *IN VITRO*

Hayane Akopyan[1,2], Nataliya Huleyuk[2], Galina Bezkorovaina[2]

[1]Institute of Experimental and Clinical Medicine, University of Rzeszow, Rzeszow, Poland
[2]Institute of Hereditary Pathology of National Academy of Medical Sciences of Ukraine, Lviv, Ukraine

*Corresponding author e-mail: akopyan.h@ihp.lviv.ua

S u m m a r y. The frequency and typical features of spontaneous chromosome instability in the lymphocytes *in vitro* of patients with verified Nijmegen breakage syndrome (NBS) and ataxia-telangiectasia (A-T) were evaluated. Low mitotic index was noted in 39% of 23 cases, whereas 7 patients with NBS and 7 with A-T were karyotyped successfully with special reference to structural chromosomal abnormalities, premature chromatid separation (PCS) and polyploidy. About 12% of NBS1 and ATM deficient lymphocytes exhibited chromosomal type aberrations with particularly high incidence of inv(7)(p13:q35), t(7;14)(q35;q11), t(7;14)(p13;q11), t(7;7)(p13;q35), t(7p13;14q32), del(7)(q35), del(14)(q11). The chromosomes 1, 3, 8 and 9 were also often targeted. Each 3rd break was located in centromeric or near-centromeric regions. The significant incidence of cells with premature chromatid separation and polyploidy was also noted. The results allow suspect non-random association between increased chromosomal instability in heterochromatin regions and both PCS and polyploidy in NBS1 and A-T deficient cells with special reference to increased risk of cancer.
K e y w o r d s: Nijmegen breakage syndrome, ataxia-telangiectasia, chromosome instability

INTRODUCTION

DNA is constantly exposed to a number of exogenous or endogenous DNA-damaging agents: ultraviolet irradiation, natural and technogenic chemical mutagens, as well as peroxide compounds generated upon the action of ionizing radiation and influence of ions of heavy metals [1, 2]. Among multiple consequences of such damaging influence, generation of double strand breaks (DSBs) of DNA is among the most dangerous ones. These critical DNA lesions can result in cell death or a wide variety of genetic alterations, including deletions, translocations, loss of heterozygosity, chromosome loss or inappropriate fusions, which enhance genome instability and can trigger carcinogenesis. Presently, there are no doubts that the mentioned chromosomal translocations appear as a result of sequential combination of one or few DSBs upon the defective course of DNA repair [1–7].

According to the recent data, Nbs1 protein (p95/nibrin) is functionally related to the program of DSBs repair at the stages of its initiation and termination, where the Атм protein (Ataxia-Telangiectasia Mutated) limits incorrect end use during non-homologous end joining [4–9]. The defects in both courses on DNA repair are remarkably similar and lead to increased level of chromosomal instability with the appearance of quite similar chromosomal aberrations including translocations between chromosomes 7 and 14 pairs because of the failure of terminal stages of V(D)J recombination during immune response [8–10].

Defective course of V(D)J-recombination as well as ineffective repair of DSBs after its termination may be the causes of the development of combined congenital T and B-deficiency in hereditary diseases, called Nijmegen breakage syndrome (NBS; OMIM 251260) and Ataxia-telangiectasia (A-T; OMIM 208900). Immunodeficiency and lymphoid malignancy are the hallmarks of human diseases caused by mutations in *NBS1* (*NBN*) and *ATM* genes [3–9]. It has been supposed that the high incidence rate of leukemia and lymphoma in populations is partially linked to the great frequency of heterozygous carriers of mutated *ATM* gene (1% individuals) [9].

In the era of molecular genetic studies, less attention is paid to changes in chromosomes. It is believed that molecular genetic markers are more informative and easier to access than the results of a classic cytogenetic analysis. At the same time, in oncogenic syndromes like NBS or A-T certain chromosomal anomalies may be indications of cancer cell transformations. As already mentioned, the spectrum of chromosomal aberrations in NBS and A-T is remarkably similar. It specifically concerns the translocations between chromosomes 7 and 14 pairs, due to the failure of terminal stages of V(D)J recombination [8–10]. If recombination has occurred between the genes *TCR*□ (7q13) and *TCR*□ (7q35), i.e. inside chromosome 7, it leads to the formation of an inversion: inv(7)(p13;q35). If such recombination occurred between homological chromosomes of pair 7, the translocation t(7;7)(p13;q35) is formed. Upon recombination events, the translocations t(7;14)(q35;q11) and t(7;14)(p13;q11) are generated between chromosomes 7 and 14 [11–13]. It is considered that the presence of specific chromosomal rearrangements provides the cells with some proliferative advantage, that's why their content *in vitro* nearly10% yield in PHA-stimulated lymphocytes is observed in patients with A-T and NBS, whilst in healthy individuals they may be found less than in 1/500 cells [13].

THE AIM OF THE STUDY

The aim of the present work was to study typical features of chromosomal instability in the short-term culture of *ATM*- and *NBS1*-deficient lymphocytes in Ukrainian patients with Nijmegen breakage syndrome and Ataxia-telangiectasia.

MATERIALS AND METHODS

Cytogenetical study was carried out on 11 patients with typical clinical features of Nijmegen breakage syndrome and homozygous state of *NBS1*657del5 mutation, 12 children with clinical and laboratory features of A-T, and 23 controls. The chromosomal preparations were obtained from short-term PHA-stimulated culture of lymphocytes [14]. In addition, in one case of NBS complicated by the development of lymphoma, the short-term culture of bone marrow cells was analyzed. Chromosomal preparations were stained homogeneously as well using GTG and CTG banding, and analyzed with reference to cytogenetic nomenclature, and the recommendations on registration of chromosomal aberrations [14, 15].

The fact of premature chromatid separation (PCS) was observed when at the stage of metaphase the sister chromatids were divided and lost the typical X or V-like pattern. If the number of prematurely divided chromosomes equaled 23-46, the phenomenon was considered total PCS, whereas partial PCS was diagnosed in cases of premature separation of 1 to 20 chromosomes [16]. The frequency of total and partial PCS was registered separately, according to their own algorithm of chromosome division [16–18].

RESULTS AND DISCUSSION

Only in one out of 11 investigated NBS cases it was possible to analyze 100 m.p. in both blood and bone marrow cultures, probably due to lymphoma development. In other 10 NBS cases the pronounced difference in mitotic indexes of cultured lymphocytes was observed: in 3 cases not less than 100 m.p., in 4 cases – not less than 50 m.p., but in 3 cases - less than 10 m.p. Finally, the experimental sampling of NBS cases with no oncological complications consisted of 500 m.p. In 7 out of 12 examined patients with A-T, we successfully analyzed up to 100 m.p., while in 4 cases quite low mitotic index (9-25 m.p.), and total absence (1 case) of lymphoblast proliferation was noted. In the case of A-T the analysis of 700 m.p was possible.

Table 1 presents the frequency of spontaneous aberrations in the cultured lymphocytes from patients with diagnosed NBS or A-T. These data show a significantly higher frequency of the cells with chromosomal aberrations in the cultures of patients with NBS and A-T compared to the control group: 11.3 ± 3.3%, 5.3 ± 1.8%, 1.7 ± 0.2% respectively ($p < 0.001$). The rearrangements of chromosomes 7 and 14 typical for NBS and A-T syndromes were not found in the controls, in 2/7 cases of NBS and in 5/7 cases of A-T. On the other hand, in 5/7 patients with NBS typical rearrangements of chromosomes 7 and 14 were observed in 4-12% m.p. The structure of mentioned specific chromosomal rearrangements (8% of all studied m.p.) comprised 3% cases of inv(7)(p13;q35), 2% – t(7;14)(q35;q11), 1% – t(7;14)(p13;q11), 2% – t(7;7)(p13;q35), t(7p13;14q32), del(7)(q35), del(14)(q11), which is in accordance with the published data [11–13]. In 10% of cells bearing translocations, the additional marker chromosomes – possibly derivatives of chromosomes 7 and/or 14 were

found (Figure 1). In ATM-deficient lymphocytes, contrary to NBS, not inter-chromosomal rearrangements, but mainly the deletions in chromosomes 7 and 14 were observed.

The average frequency of cells with spontaneous nonspecific (except pairs 7 and 14) chromosomal aberrations were significantly higher in the patients with Nijmegen breakage syndrome (5.6 ± 2.2% *vs.* 1.2% m.p. in the controls, $p < 0.001$), and did not differ in the case of controls and A-T (Table 1). The nonspecific chromosomal aberrations in NBS cases mostly presented as deletions, paired fragments and marker chromosomes, along with translocations: t(1;3)(q22;p22), t(3;7)(p21;q11), t(2;10)(q21;p10), t(X;5)(q26;qter), t(5;12)(q13;qter). In the metaphase cells of NBS patients about 75% of nonspecific chromosomal aberrations were detected in chromosomes 1, 3, 8 and 9 pairs, and each third break localized in pericentromeric regions, including heterochromatin region of chromosome 7. In addition, the rare polymorphic variant of larger near-centromeric heterochromatin on chromosome 7 (7q11-12) was found in one NBS patient and his father. Along with significant frequency of specific and nonspecific chromosomal aberrations, the examined lymphocytes of the patients with NBS and A-T demonstrated polyploidy and centromeric instability which manifested mainly as total or partial premature chromatid separation of metaphase chromosomes (Table 2, Figures 2 and 3).

The cells with total PCS (Figure 2) were found in 12 out of 14 patients with A-T and NBS, and their average content yielded 2.9 ± 0.9% and 2.3 ± 1.0% of metaphase cells respectively *vs.* nearly total absence in the controls upon the analysis of 100 m.p. in each case ($p < 0.001$). In single NBS cases complicated by lymphoma, total PCS was found in 54% of bone marrow cells, and in 40% of cultured lymphocytes. Upon the analysis of 100 m.p. per individual, no polyploid cells were found in the control samples of cultured lymphocytes, whereas their frequency was significantly elevated among A-T and NBS samples (0.9 ± 0.4 and 1.2 ± 0.7% m.p. respectively, $p < 0.01$) (Table 2).

The phenomenon of partial PCS (Figure 3, Table 2) was noted in the majority of cases of the control and experimental groups with nearly equal frequency: 1-3 per 100 m.p. In the control group, the majority of PCS-cells demonstrated PCS of one chromosome, in some cases – PCS of 2 or 3 chromosomes, and never – of 4 and more chromosomes. Partial PCS in the A-T and NBS patients was markedly associated with premature division of chromosomes 2 or 3 along with the appearance of representative pool of cells with PCS of more than four chromosomes (Table 3).

In the control group, premature chromatid separation was detected only in the chromosomes of group C (6, X, 11-12) and E (18), and only in single cases the premature division of chromosome 3 (A group) was registered (Table 4). In the cultured lymphocytes of the A-T and NBS patients, PCS significantly influenced all known chromosomes, except of group E (Table 4). The frequency of PCS-chromosomes in the group B (4-5) and D (13-15) in the cases of A-T, and the groups A (2-3) and B (4-5) in the cases of NBS was markedly elevated. So, the increased induction of premature division of chromosomes in group B was found to be a common feature of cytogenetic phenotype of A-T and NBS.

The principally distinctive difference between NBS and A-T cases was the total absence of PCS-chromosomes in group A in A-T cases compared to its significant prevalence among NBS-deficient cells, as well as the absence of PCS group D chromosomes in NBS cases *vs* its significant increase among ATM-deficient lymphocytes *in vitro* (Table 4). Earlier, paradoxically high rate of cells with PCS of 3-38 chromosomal set and non-occasionally frequent PCS of D-group chromosomes was described in 2 A-T cases [19]. Our results confirm the significant elevation of PCS-D in the cells of patients suffering from A-T. Moreover, for the first time we described the significant induction of PCS in B-group chromosomes in ATM-deficient lymphocytes, and selective increase in PCS of both A- and B-group chromosomes in NBS-deficient lymphocytes.

The results of our study found increased frequency of non-specific chromosomal aberrations, which allows to suggest that cytogenetic phenotype of ATM- and NBS1-deficient lymphocytes *in vitro* is not limited by the formation of specific translocations with preferable involvement of chromosomes 7 and 14. In the studied cases of A-T and NBS, a wide spectrum of additional cytogenetic failure was found, including selective increase in the chromosomal-type aberrations, elevated chromosomal fragility in centromeric and near-centromeric regions, significant number of the cells with premature division of all chromosomes (total PCS) and polyploidy, markedly increased incidence of partial PCS in 2-3 chromosomes, occurrence of PCS in 4 and more chromosomes of the set, absent

in the control group. Along with the significant increase of PCS of all known chromosome groups, the selective elevation of specific PCS content targeting D and B chromosomes in A-T, and A and B chromosomes in NBS cases was shown.

Premature chromatid separation, isolated or combined with mosaic variegated aneuploidy or structural chromosomal abnormalities were detected in the cells of patients suffering from different oncological diseases [16, 20–23, 26]. Our own results are also pointing to the expediency of considering the phenomenon of total and partial PCS and polyploidy of karyotype of lymphocytes *in vitro* in practical cytogenetic study of Ataxia-Telangiectasia and Nijmegen breakage syndrome. Such approach seems to be perspective for the prognosis of oncogenic risk in cases of heterozygous carriers of mutations in *ATM* and *NBS1* genes suggesting high probability of induced centromere chromosomal instability. The cells with total PCS may be a source of oncogenic transformation *via* their ability to polyploidization of karyotype in the next mitotic cycles [16–18, 24–25]. We assume that this mechanism may be due to an increased susceptibility of the patients with A-T and NBS to the development of lymphoproliferative processes. Easy identification of PCS phenomenon and centromeric fragility allows to reveal the limits of chromosomal instability after which an oncogenic transformation of the cells may be initiated.

CONCLUSIONS

The significant incidence of mitotic cells with signs of centromeric (pericentromeric) fragility, premature chromatid separation and polyploidy was found in ATM- and NBS1-deficient lymphocytes, along with the appearance of specific rearrangements of chromosomes 7 and 14. The phenomena of premature chromatid separation of all chromosomes (total PCS) and polyploidy are not characteristic of cultured lymphocytes of healthy individuals, however they significantly appeared in the cells of homozygous and heterozygous carriers of mutated *NBS1* and *ATM* genes. The increased frequency of the cells with premature division of 3 and more chromosomes of the karyotype is a typical feature of *in vitro* ATM- and NBS1-deficient lymphocytes compared with the cells from healthy individuals. Selective induction of premature chromatid division of group B chromosomes is a common pattern of cytogenetic phenotype of ATM- and NBS1-deficient lymphocytes, whilst the selective induction of PCS-chromosomes from group D is associated with ATM-deficiency, and PCS-chromosomes from group A – with NBS1-deficiency. An increased induction of total and partial PCS in the case of Nijmegen breakage syndrome complicated by the development of non-Hodgkin's malignant lymphoma suggests possible relation of premature chromatid separation in mitotic cells with the formation of genome's instability upon oncogenic transformation.

REFERENCES

1. Hoeijmakers JHJ (2001) Genome maintenance mechanisms for prevention cancer. Nature 411: 3466-74.
2. Ceccaldi R, Rondinelli B, D'Andrea AD (2016) Repair Pathway Choices and Consequences at the Double-Strand Break. Trends Cell Biol 26: 53-64.
3. Pennisi R, Antoccia A, Leone S, Ascenzi P, di Masi A (2017) Hsp90α regulates ATM and NBN functions in sensing and repair of DNA double-strand breaks. FEBS J 284: 2378-95.
4. Varon R, Vissinga C, Platzer M, Cerosaletti KM, et al (1998) Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell 93: 467-76.
5. Carney JP, Maser RS, Olivares H, Davis EM, et al (1998) The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell 93: 477-86.
6. Berardinelli F, di Masi A, Antoccia A (2013). NBN Gene Polymorphisms and Cancer Susceptibility: A Systemic Review. Curr Genomics 14: 425-40.
7. Bennardo N, Stark JM (2010) ATM limits incorrect end utilization during non-homologous end joining of multiple chromosome breaks. PLoS Genet 6: doi: 10.1371/journal.pgen.1001194.
8. Saidi A, Li T, Weih F, Concannon P, Wang ZQ (2010) Dual functions of Nbs1 in the repair of DNA breaks and proliferation ensure proper V(D)J recombination and T-cell development. Mol Cell Biol 30: 5572-81.
9. Lantelme E, Turinetto V, Mantovani S, Marchi A, et al. (2003) Analysis of secondary V(D)J rearrangements in mature, peripheral T cells of ataxia-telangiectasia heterozygotes. Lab. Invest 83: 1467-75.
10. Fugmann CD, Lee AI, Shockett PE, Villey IJ, Schatz DG (2000) The RAG proteins and V(D)

J recombination: complexes, ends and transposition. Annu Rev Immunol 18: 495-527.
11. Meyn MS (1993) High spontaneous intrachromosomal recombination rates in ataxia-telangiectasia. Science 260: 1327-30.
12. Kleier S, Herrmann M, Wittwer B, Varon R, et al (2000) Clinical presentation and mutation identification in the NBS gene in a boy with Nijmegen breakage syndrome. Clin. Genet 57: 384-87.
13. Taylor AMR (2001) Chromosome instability syndromes. Best Practice Research Clin Hematol 14: 631-44.
14. Human cytogenetics: a practical approach Vol. I Constitutional Analysis Second edition (1992). Eds. Roony D.E. & B.H.Czepulkowski NY: "Oxford University Press": 211-17.
15. Methods for analysis of chromosomal aberrations in man (1975) Bacton K, Evans G, Eds. WHO, Geneva: 64 p.
16. Akopian GR, Sirenko AG, Hnateiko OZ, Polischuk RS, et al (1999) Premature centromere division of the metaphase chromosomes in children's acute lymphoblastic leukemia. Oncology [Ukr.]: 283-89.
17. Akopyan H, Sirenko A, Sedneva I, Kluchevska O, et al (2001) Cytogenetic assay in apoptosis investigations. Folia Histochemica et cytobiologica: 39: 158-60. 18. Akopyan HR (2001) Perspectives of premature anaphase's molecular evaluationю Proceedings of the Second Euroconference on Quantitative Molecular Cytogenetics: 31-4.
19. Mehes K, Bühler EM (1995) Premature centromere division: a possible manifestation of chromosome instability. Am J Med Genet 56: 76-9.
20. Shimada K, Yanagisawa R, Kubota N, Hidaka E, et al (2017) Wilms tumor accompanied by premature chromatid separation. Pediatr Blood Cancer 64. doi: 10.1002/pbc.26255.
21. Sari N, Akyuz C, Aktas D, Gumruk F, et al (2009) Wilms tumor, AML and medulloblastoma in a child with cancer prone syndrome of total premature chromatid separation and Fanconi anemia. Pediatr Blood Cancer 53: 208-10.
22. Rio Frio T, Lavoie J, Hamel N, Geyer FC, et al (2010) Homozygous BUB1B mutation and susceptibility to gastrointestinal neoplasia. N Engl J Med 363: 2628-37.
23. Petković I (2007) Premature chromatid separation in a woman with carcinoma in situ of the uterine cervix and in her son with keratoacanthoma. Cancer Genet Cytogenet 179: 66-8.
24. Yih LH, Lee TC (2003) Induction of C-anaphase and diplochromosome through dysregulation of spindle assembly checkpoint by sodium arsenite in human fibroblasts. Cancer Res 63: 6680-8.
25. Mailhes JB, Hilliard C, Fuseler JW, London SN (2003) Vanadate, an inhibitor of tyrosine phosphatases, induced premature anaphase in oocytes and aneuploidy and polyploidy in mouse bone marrow cells. Mutat Res 538:101-7.
26. Dudzińska E, Gryzińska M, Kocki J. (2018). Single nucleotide polymorphisms in selected genes in inflammatory bowel disease. Biomed Res. Int. vol. 2018 art. ID 6914346

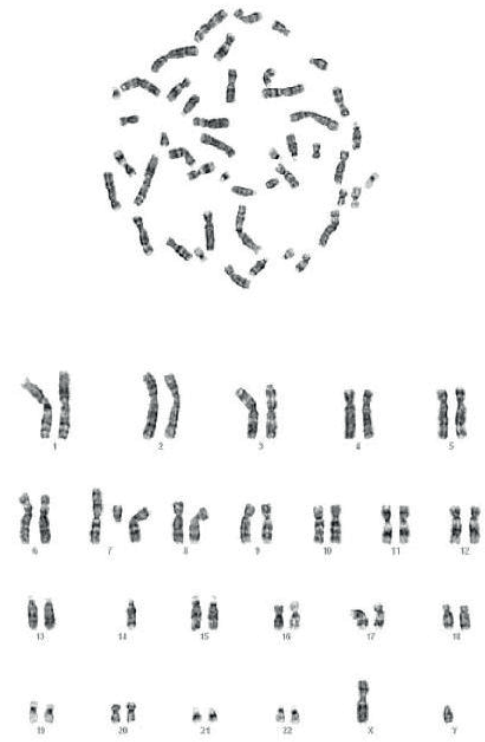

Fig. 1. Specific chromosomal rearrangement *in vitro* in lymphocytes of the patients with Nijmegen breakage syndrome and ataxia-telangiectasia: translocation between 7th and 14th chromosomes: t(7;14)(p13;q11). GTG-staining. Mag. x 100.

Fig. 2. Total premature division of chromosomes (total PCS). Part of the chromosomes demonstrate a non-parallel placement of sister chromatids (splayed chromatids), that are placed at a significant distance one from other. Homogenous staining. Mag. x 100.

Fig. 3. Partial PCS of more than 4 chromosomes of chromosomal set. Parallel and tight placement of sisterly chromatids in prematurely divided chromosomes. CTG-staining. Mag. x 100.

Table 2. Frequency of metaphase plates (m.p.) with premature chromatid separation or polyploidy in lymphocytes *in vitro* in patients with NBS and A-T

	Number of cases/ m.p.	% m.p.		
		Total PCS	Polyploidy	Partial PCS
Control	23/2300	0.04 ± 0.04	0	1.4 ± 0.3
NBS	7/500	2.3 ± 1.0	1.2 ± 0.7	2.8 ± 1.5
A-T	7/700	2.9 ± 0.9	0.9 ± 0.4	1.4 ± 0.9
p1		< 0.001	< 0.01	> 0.05
p2		< 0.001	< 0.05	> 0.05

Notes: *p1*– significant difference between NBS cases and controls
p2 – significant difference between A-T cases and controls

Table 3. Frequency of metaphase plates (m.p.) with partial PCS of different chromosome number in lymphocytes *in vitro* in the patients with A-T and NBS

	Number of m.p.	% m.p. with partial PCS	% m.p. with PCS of certain chromosome number			
			1	2	3	>4
Control	2300	1.4 ± 0.3	1.1 ± 0.2	0.2 ± 0.1	0.04 ± 0.04	0
NBS	450	2.8 ± 1.5	0.8 ± 0.8	0.2 ± 0.2	0.2 ± 0.2	1.6 ± 1.3
A-T	700	1.4 ± 0.9	0.1 ± 0.1	0.4 ± 0.3	0.6 ± 0.5	0.3 ± 0.3
p1		> 0.05	> 0.05	> 0.05	> 0.05	< 0.01
p2		> 0.05	< 0.05	> 0.05	< 0.05	< 0.05

Notes: *p1*– significant difference between NBS cases and controls
p2 – significant difference between A-T cases and controls

Table 1. Frequency of metaphase plates (m.p.) with spontaneouschromosomal aberrations in lymphocytes *in vitro* in patients with A-T and NBS

	Number of cases/m.p.	% m.p. with chromosomal aberrations					
			Chromosomes 7 and 14	Other	chromosome type (I)	chromatidtype (II)	Ratio I/II
Control	23/2300	1.2 ± 0.2	0	1.2 ± 0.2	0.2 ± 0.1	1.0 ± 0.2	1 : 5
NBS	7/500	11.3 ± 3.3	7.8 ± 1.4	5.6 ± 2.2	10.0 ± 3.1	1.7 ± 0.7	5.9 : 1
A-T	7/700	5.3 ± 1.8	4.0 ± 2.6	1.3 ± 0.5	4.3 ± 2.1	1.0 ± 0.3	4.3 : 1
p1		0.001	< 0.001	< 0.001	< 0.001	> 0.05	
p2		0.001	< 0.001	> 0.05	< 0.001	> 0.05	

Notes: *p1*– significant difference between NBS cases and controls
p2 – significant difference between A-T cases and controls

Table 4. The frequency of PCS chromosomes of different chromosomal groups in lymphocytes *in vitro* in the patients with A-T and NBS

	Frequency of PCS-chromosomes in the groups (per 100 m.p.)							
	Total	A	B	C	D	E	F	G
Control	1.9	0.1	0	0.9	0	0.9	0	0
NBS	7.0	1.2	1.2	3.0	0	0.8	0.4	0.4
A-T	5.8	0	0.6	2.6	0.4	1.4	0.6	0.2
p1	<0.05	<0.05	<0.001	<0.05	>0.05	>0.05	<0.05	<0.05
p2	>0.05	>0.05	<0.001	<0.05	<0.01	>0.05	<0.05	<0.05

Notes: *p1*– significant difference between NBS cases and controls
p2 – significant difference between A-T cases and controls