

Triplet repeat in the *Repin1* 3'-untranslated region on rat chromosome 4 correlates with facets of the metabolic syndrome

Nora Klötting
Barbara Wilke
Ingrid Klötting*

Department of Laboratory Animal
Science, Medical Faculty, University of
Greifswald, Karlsburg, Germany

*Correspondence to: Ingrid Klötting,
Department of Laboratory Animal
Science, Medical Faculty, University
of Greifswald, D-17495 Karlsburg,
Germany.
E-mail: kloeting@uni-greifswald.de

Abstract

Background Congenic and subcongenic rat strains confirmed the quantitative trait loci (QTLs) for facets of the metabolic syndrome between 60.53 and 77.11 Mb on chromosome 4. The analysis of candidate genes in this region favoured the replication initiator 1 (*Repin1*) characterized by a SNP in the coding region and a triplet repeat (TTT) in the 3'-untranslated region (3'UTR).

Methods We analysed nine rat strains (BB/OK, SHR, F344, BN, DA, LEW, hHTg, WOKW, and their founders WOK-F) and four wild rats on DNA (sequencing) and RNA level (gene expression in blood, liver, subcutaneous, and epididymal adipocytes). In addition, the rats were phenotypically characterized in order to link the rat phenotype to genotype differences in the QTL on chromosome 4.

Results Wild rats were heterozygous for the SNP (C/T), whereas all the inbred strains were homozygous. The shortest triplet repeat was found in SHR (5) and the highest was found in hHTg and WOKW (11), which developed metabolic disorders. The repeat number correlated with most phenotypic traits studied. Using linear multiple regression analysis with repeat size as the dependent variable and considering all the data of this study, it was clearly demonstrated that not only VLDL cholesterol and serum insulin but also the expression of *Repin1* in the liver is significantly associated with the repeat size of the 3'UTR.

Conclusions It is concluded that the triplet repeat expansion in 3'UTR is involved in metabolic alterations as found in hHTg and WOKW rats and that the functional unknown gene, *Repin1*, could be a novel candidate gene for the development of facets of the metabolic syndrome. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords serum lipids; hypertriglyceridemia; triplet expansion; gene expression

Introduction

It is well-recognized that the metabolic syndrome, including obesity, dyslipidemia, glucose intolerance or hyperglycemia, and hypertension, has a major genetic component. However, the search for candidate genes has been very difficult since each facet of the syndrome is complex, heterogeneous, and multifactorial, resulting both from genetic susceptibility and environmental risk factors [1–3]. Therefore, the use of inbred animal



Received: 13 January 2006

Revised: 1 September 2006

Accepted: 17 October 2006

models closely resembling the human disease is an essential component of genetic investigations in this field.

There are a number of crossing studies to dissect the facets of the metabolic syndrome. In male cross hybrids of diabetes-prone BioBreeding/OttawaKarlsburg (BB/OK) and spontaneously hypertensive rats (SHR), quantitative trait loci (QTLs) for serum triglycerides, total cholesterol, and body weight were mapped on chromosome 4 some years ago [4–6]. Using the hereditary hypertriglyceridemic (hHTg) and disease-resistant Brown Norway (BN) rats, the QTL for serum triglycerides was confirmed on this chromosome. In addition, a QTL for serum insulin was mapped at the same position on chromosome 4 [7].

To study the relevance of QTLs on rat chromosome 4, several congenic and subcongenic BB rat strains introgressing a chromosome 4 segment of SHR or Wistar Ottawa Karlsburg RT1^u (WOKW) rats [8–11] developing a polygenetically inherited, complete metabolic syndrome were established [12–16]. The phenotype of congenic BB.SHR (BB.4S) and BB.WOKW (BB.4W) as well as subcongenic BB.4S rats clearly confirmed the phenotype of significantly increased serum lipids, serum insulin or leptin as well as body weight compared with rats of the parental strain, BB/OK [8–11]. All congenic and subcongenic rat strains are common at the 16.6 Mb region located between 60.53 and 77.11 Mb on chromosome 4. The analysis of additional subcongenic BB.4S rat strains narrowed down this region to 1.3 Mb located between 75.9 and 77.2 Mb [17]. This strongly suggests that facets of the metabolic syndrome that influence genes are located in this 1.3 Mb region. To identify the underlying gene/s, several genes (finger proteins *Zfp398*, *Zfp282*, *Zfp212*, *Zfp467*, *Repin1*, *Torid*, *Abp1*) were selected and sequenced in DNA from BB/OK, SHR, and WOKW rats. One of them, the replication initiator 1 (*Repin1*), located at position 76.6 Mb on chromosome 4 showed a SNP at position 449 (T/C) in the coding region and a triplet repeat (TTT) in the 3'-untranslated region (3'UTR) markedly varying between 5 and 11. This finding prompted us to analyse additional rat strains on DNA, RNA, and phenotypic level.

Materials and methods

Animals

Disease-prone BB/OK (F63), SHR/K (F?F21), and WOKW rats (F68) and disease-resistant Lewis (LEW; F > 80), Brown Norway (BN/K; F? > 15), Dark Agouti (DA/K; >F80), and Fischer 344/K (F344; F > 15) rats, and four wild rats captured in North Germany were studied [18–20]. In addition, DNA of one male and one female rat from the parental species that founded the WOKW rats (WOK-F) more than 20 years ago [21,22] and DNA of three male hHTg rats characterized by an incomplete metabolic syndrome were analysed (the rats were kindly

provided by Iwar Klimes, Institute of Experimental Endocrinology, Slovak Academy, Bratislava, Slovakia) [23,24]. Except the hHTg rats, all the other animals used were bred in our own animal facility; the animals were kept in Macrolon cages (Size 3, Ehret GmbH, Emmendingen, Germany) under strict hygienic conditions and were free of major pathogens, as described previously [25]. They had free access to food (Ssniff R, Soest, Germany) and water and were maintained on a 12-h light/dark cycle (5 A.M./5 P.M.). The animals were killed with an overdose of Sevofluran (Abbot, Germany) in order to remove their liver for extraction of genomic DNA.

DNA analysis

Genomic DNA was extracted from liver tissue using a commercially available DNA isolation kit (Wizard, Genomic DNA Purification Kit, Promega, Mannheim, Germany). To identify the genetic variants in *Repin1*, the genomic DNA of at least two rats of each strain was sequenced according to the standard protocol of the ABI PRISM BigDye Terminators v3.0 (Applied Biosystems, Foster City, CA, USA), as recently described [26]. Primers were designed to perform PCR experiments based on the published *Repin1* sequence (GenBank Acc. No. AY691175; exon primers: forward 5' TGC ATC TTC AGG TTC ATG CTT CT 3' and reverse 5' GCA TAC ATA CCG GCG AGA AGC 3') and on *Rattus norvegicus* chromosome 4 genomic contig (GenBank Acc. No. NW_047691; triplet primer: forward 5' CGT TCT ATC CTC TGG TGT TAA CGC 3' and reverse 5' TAG TAA ATG AAT CCC TGG GCA GG 3').

Phenotypic characterization

Twelve BB/OK, SHR, WOKW, LEW, BN, DA, and F344 males were monitored for body weight, non-fasting blood glucose, serum insulin, leptin, triglycerides, total cholesterol, HDL-, LDL-, and VLDL cholesterol at an age of 30 weeks. Blood samples were obtained between 7 and 9 A.M. by puncturing the ophthalmic venous plexus after anaesthesia following inhalation exposure with isoflurane (Abbott, Wiesbaden, Germany). Blood glucose was determined using a glucose analyzer (ESAT 6660-2, Medingen, Germany). Serum leptin and insulin were determined using a radioimmunoassay kit (Rat Leptin RIA Kit; Linco Research, St. Charles, MO, USA) or ELISA (Rat Insulin ELISA, Mercodia, Uppsala, Sweden). Serum lipids were determined with lipid electrophoresis using the SAS-3 cholesterol profile kit according to the manufacturer's instructions (Helena BioSciences Europe, Sunderland, UK).

Gene expression analysis

Five of the 12 males from each strain that were phenotypically characterized were used for gene expression analysis at an age of 32 weeks. At the time of euthanasia, blood,

liver, and subcutaneous and epididymal fat pads were removed. Fat pads were used to isolate the adipocytes by collagenase (1 mg/ml) digestion, as described [27]. The total RNA of blood, liver, and subcutaneous and visceral adipocytes was isolated, transcribed in cDNA, and used for real-time PCR as detailed before [28]. Each quantitative PCR was performed in triplicate. The target cDNA was amplified by a primer set of *Repin1* (GenBank Acc. No. AY691175; forward 5' ATC TGG GCT CTG TTT AGG AAT GG 3' and reverse 5' CCT GAC TAG CTC AAA CCC AGA TC 3').

The rat *18S rRNA* gene (eukaryotic 18S rRNA endogenous control; FAM Dye/MGB Probe, Applied Biosystems) served as the endogenous reference gene. The standard curve method was used for relative quantification. For each experimental sample, the amounts of target and endogenous reference, *18S rRNA*, were determined from the calibration curve. The target amount was then divided by the endogenous reference amount to obtain a normalized target value.

Statistical analysis

Data are given as mean \pm SD. Differences were assessed by one-way analysis of variance corrected by Bonferroni-Holm. Correlation analysis and linear multiple regression analysis (stepwise integration of variables) were carried out using the statistical analysis system SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL USA).

Results

As shown in Table 1, there were two variants in exon SNP in all the rat strains studied. Wild rats are heterozygous for the SNP (449 C/T) and all inbred strains are homozygous, whereby most strains possess the C at position 449. The shortest triplet repeat was found in SHR with 5, followed by BN, DA, and F344 with 7, followed by BB/OK, LEW, and WOK-F with 9, followed by hHTg and WOKW with 11 repeats, respectively. Interestingly, the repeat number for WOK-F founder rats increased from 9 to 11 during inbreeding. Three of the four wild rats were heterozygous

Table 1. SNP and repeat number in the 3'UTR of *Repin1* in different rat strains

Strains	SNP (449)	Number of repeats
SHR	C	5
DA, BN, F344	C	7
Wild rats	C/T	7/9
BB/OK, LEW	T	9
WOK-F	C	9
hHTG, WOKW	C	11

for repeat number 7/9 and one rat was homozygous for repeat number 7.

Comparing the phenotype of rat strains that depend on repeat size as summarized in Table 2, the body weight, serum insulin, leptin, total cholesterol, HDL, and VLDL generally showed an increase with the repeat numbers of 7 to 11, whereas serum triglycerides increased from repeat sizes 5 to 11. The connection between repeat size and phenotypic traits is supported by significant correlations. No significant effect was observed in blood glucose and LDL, although LDL decreased with an increase in the repeat size.

As shown in Table 3, *Repin1* is expressed in all tissues studied. The relative expression is low, ranging from 0.02 to 0.89, and showed no connection between expression values and repeat size.

The simple correlations found between repeat size and phenotypic traits and the values of relative gene expression (Tables 2 and 3) were further analysed by multiple linear regression. The repeat number was chosen as the dependent variable, and the phenotypic traits of the animals used for expression studies and the values of relative expression of *Repin1* were used as covariates. As summarized in Table 4, VLDL cholesterol, serum insulin, and the relative gene expression of *Repin1* in liver were significantly associated with the repeat size.

Discussion

Repin1 encodes a protein that contains 15 C₂H₂ zinc finger (ZF) DNA binding motifs organized in three clusters, termed *hand Z1* (ZFs1-5), *hand Z2* (ZFs6-8), and *hand*

Table 2. Phenotype of males (mean \pm SD) dependent on the repeat size and the correlations between repeat size and phenotype (*r*)

Phenotypic trait	Repeat size				Sign.	<i>r</i>
	5 (<i>n</i> = 12)	7 (<i>n</i> = 36)	9 (<i>n</i> = 24)	11 (<i>n</i> = 12)		
Body weight (g)	370 \pm 20	334 \pm 33	451 \pm 27	476 \pm 30	<i>p</i> < 0.001	0.77***
Blood glucose (mg%)	91 \pm 9	98 \pm 17	94 \pm 16	97 \pm 15	<i>p</i> < 0.05	0.04
Serum insulin (ng/ml)	0.92 \pm 0.6	0.87 \pm 0.7	1.8 \pm 1.7	3.7 \pm 1.5	<i>p</i> < 0.001	0.61***
Serum leptin (ng/ml)	6.0 \pm 1.3	4.2 \pm 1.5	7.5 \pm 1.9	12.0 \pm 2.4	<i>p</i> < 0.001	0.75***
Triglycerides (mmol/l)	1.0 \pm 0.2	1.4 \pm 0.7	2.4 \pm 0.8	3.1 \pm 0.7	<i>p</i> < 0.001	0.70***
Cholesterol (mmol/l)	2.1 \pm 0.2	2.0 \pm 0.6	2.8 \pm 0.4	2.9 \pm 0.4	<i>p</i> < 0.001	0.57**
HDL (mmol/l)	1.7 \pm 0.2	1.6 \pm 0.6	2.3 \pm 0.3	2.3 \pm 0.3	<i>p</i> < 0.001	0.50**
LDL (mmol/l)	0.30 \pm 0.07	0.23 \pm 0.13	0.22 \pm 0.08	0.14 \pm 0.06	<i>p</i> < 0.003	-0.36
VLDL (mmol/l)	0.12 \pm 0.04	0.11 \pm 0.05	0.31 \pm 0.16	0.46 \pm 0.21	<i>p</i> < 0.001	0.68***

Significant correlations at 1% (**) or 0.1% (***) level.

Table 3. Relative expression of *Repin1* in blood, liver, subcutaneous (sub-Ad), and visceral adipocytes (vis-Ad) dependent on repeat size, and the correlations between gene expression and repeat size

Tissue	Repeat size				p-value	r
	5 (n = 5)	7 (n = 15)	9 (n = 10)	11 (n = 5)		
Blood	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.018	-0.05
Liver	0.43 ± 0.11	0.61 ± 0.19	0.59 ± 0.13	0.58 ± 0.21	n.s.	0.17
sub-Ad	0.25 ± 0.26	0.37 ± 0.26	0.09 ± 0.07	0.44 ± 0.10	0.012	-0.07
vis-Ad	0.89 ± 0.51	0.51 ± 0.50	0.33 ± 0.29	0.55 ± 0.22	n.s.	-0.20

Table 4. Multiple linear regression analysis with repeat size as the dependent variable

Variable	b ^a	SE ^a	Sign.
VLDL	5.79	0.95	0.0000033
Serum insulin	0.44	0.12	0.0008
<i>Repin1</i> in liver	2.61	0.99	0.015

^aRegression coefficient (b) and its standard error (SE), based on the phenotypic traits of those animals used for expression studies and the values of relative gene expression as covariates.

Z3 (ZFs9-15). A proline-rich region is located between hands Z2 and Z3. Plasmid replication assays have shown that *Repin1* has weak replication enhancing activity. It is assumed that it may act as an accessory factor in identification of the origin prior to the assembly of pre-initiation complexes. Like many other polydactyl ZF proteins, the cellular function of *Repin1* is unknown [29,30].

The genetic analysis of *Repin1* revealed obvious differences between rat strains; however, this was seen in the triplet repeat of the 3'UTR but not in the SNP. Assuming that wild rats represent the wild type triplet, number 7 and number 9 should be considered 'normal'. If so, number 5 in SHR and number 11 in WOKW and hHTg rats should be mutants. This hypothesis is supported by the fact that the founder animals of WOKW (WOK-F) were characterized by a triplet number of 9, which was also found in the LEW and BB/OK rats. Like WOK-F, BB rats also descended from the same outbred Wistar rat strain of the BioBreeding Laboratories, Ottawa, Canada, and were transferred to Karlsburg more than 20 years ago [21,22]. Therefore, the repeat number of 11 in WOKW must be a mutation that took place during inbreeding. WOKW rats were phenotypically striking after 35 inbred generations. The reproduction of this strain obviously decreased. Because infection could be excluded as a cause, metabolic traits were investigated to examine the reason for low reproduction. Significantly higher gain in body weight, serum insulin, triglycerides, and impaired glucose tolerance were the first abnormalities observed [14].

The same number of triplets was found in hHTg rats. Both strains, hHTg and WOKW, are characterized by hypertriglyceridemia, hyperinsulinemia, and glucose intolerance [12,15,16,23,24]. Using a hHTg × BN cross population to identify the primary genetic lesions for metabolic disorders, a significant linkage was found for serum triglycerides and insulin to the loci on chromosome

4 flanked by markers *D4Mit5* and *D4Mit17* (*Tacr2*) [7]. This region spans from 72.2 to 116.8 Mb on chromosome 4 and the *Repin1* maps within this region at position 76.6 Mb. Considering the highly positive correlation between repeat sizes in the 3'UTR of *Repin1* and most phenotypic traits studied, the enlarged repeat in hHTg and WOKW rats may be the key element of some facets of the metabolic syndrome.

This idea is supported by the relative gene expression. In contrast to the positive correlation between repeat size in the 3'UTR and most phenotypic traits, there was no correlation between relative expression of *Repin1* in blood, liver, subcutaneous as well as visceral adipocytes and repeat size. However, using linear multiple regression analysis with the repeat size as the dependent variable and considering all data of this study, it was clearly demonstrated that not only the VLDL cholesterol and serum insulin but also the expression of *Repin1* in the liver is significantly associated with the repeat size of the 3'UTR.

Our findings clearly indicate that with the increase in TTT in 3'UTR of *Repin1*, the VLDL cholesterol and serum insulin also increase, as observed in the metabolic syndrome of rats and human beings. This increase is associated with an increased expression of *Repin1* in the liver. It could be concluded that the TTT expansion in 3'UTR is involved in metabolic disorders, as described in the hHTg and WOKW rats; a phenomenon known for several other diseases like fragile X syndrome, myotonic dystrophy, or Friedreich ataxia [31]. However, the triplet repeat may also influence trans-regulating genes, an idea that cannot be explained. Although the gene expression does not necessarily reflect the activity of the gene product, our findings are a potent stone in this mosaic to elucidate the genetics of the metabolic syndrome facets. The functional unknown gene, *Repin1*, could be a novel candidate gene in the development of metabolic disorders.

Acknowledgements

We thank Silvia Sadewasser, Susanne Schuldt, Edeltraut Lübke, and Kathrin Stabenow for expert technical assistance. This research is supported by Grant No. KL 771/11-2 of Deutsche Forschungsgemeinschaft.

References

- Clement K, Boutin P, Froguel P. Genetics of obesity. *Am J Pharmacogenomics* 2002; 2: 177–187.

2. Groop L, Orho-Melander M. The dysmetabolic syndrome. *J Int Med* 2001; **250**: 105–120.
3. Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J* 2001; **15**: 312–321.
4. Kovacs P, Klötting I. Mapping of quantitative trait loci for body weight on chromosomes 1 and 4 in the rat. *Biochem Mol Biol Int* 1998; **44**: 99–405.
5. Kovacs P, Klötting I. Quantitative trait loci on chromosomes 1 and 4 affect lipid phenotypes in the rat. *Arch Biochem Biophys* 1998; **354**: 139–143.
6. Kovacs P, van den Brandt J, Klötting I. Effects of quantitative trait loci for lipid phenotypes in the rat are influenced by age. *Clin Exp Pharmacol Physiol* 1998; **25**: 1004–1007.
7. Klimes I, Weston K, Kovacs P, *et al.* Mapping of genetic loci predisposing to hypertriglyceridemia in the hereditary hypertriglyceridemic rat: analysis of genetic association with related traits of the insulin resistance syndrome. *Diabetologia* 2003; **46**: 352–358.
8. Klötting I, Kovacs P, van den Brandt J. Congenic BB.SHR (D4Mit6-Npy-Spr) rats: a new aid to dissect the genetics of obesity. *Obes Res* 2002; **10**: 1074–1077.
9. Klötting N, Wilke B, Klötting I. Phenotypic and genetic analysis of subcongenic BB.SHR rat lines shorten the region on chromosome 4 bearing gene(s) for underlying facets of the metabolic syndrome. *Physiol Genomics* 2004; **18**: 325–330.
10. Klötting N, Wilke B, Klötting I. Alleles on rat chromosome 4 (D4Got41-Fabp1/Tacr1) regulate subphenotypes of obesity. *Obes Res* 2005; **13**: 589–595.
11. Kovács P, van den Brandt J, Bonn  ACM, van Zutphen LFM, van Lith HA, Kl tting I. Congenic BB.SHR rat provided evidence for effects of a chromosome 4-segment (D4Mit6-Npy ~1 cM) on total serum and lipoprotein lipid concentration and composition after feeding a high-fat, high-cholesterol diet. *Metabolism* 2001; **50**: 458–462.
12. Kl tting I, Kovacs P, van den Brandt J. Sex-specific and sex-independent quantitative trait loci for facets of the metabolic syndrome in WOKW rats. *Biochem Biophys Res Commun* 2001; **284**: 150–156.
13. Kl tting N, Bl her M, Kl tting I. The polygenetically inherited metabolic syndrome of WOKW rats is associated with insulin resistance and altered gene expression in adipose tissue. *Diabetes Metab Res Rev* 2006; **22**: 146–154.
14. Kovacs P, Voigt B, Berg S, Vogt L, Kl tting I. WOK.1W rats: a potential animal model of the insulin resistance syndrome. *Ann N Y Acad Sci* 1997; **827**: 94–100.
15. van den Brandt J, Kovacs P, Kl tting I. Metabolic features in disease-resistant as well as in hypertensive SHR and newly established obese Wistar Ottawa Karlsburg inbred rats. *Int J Obes* 2000; **24**: 1618–1622.
16. van den Brandt J, Kovacs P, Kl tting I. Metabolic syndrome and ageing in Wistar Ottawa Karlsburg W rats. *Int J Obes* 2002; **26**: 1–4.
17. Kl tting I, Wilke B, Kl tting N. Chromosome 4 congenic and subcongenic BB.SHR rats as tool to identify underlying genes. *Diabetologia* 2005; **48**(Suppl. 1): A108.
18. Kl tting I, Nitschke C, van den Brandt J. Impact of genetic profiles on experimental studies: outbred versus wild rats. *Toxicol Appl Pharmacol* 2003; **189**: 68–71.
19. Kl tting I, Voigt B, Kov cs P. Comparison of genetic variability at microsatellite loci in wild rats and inbred rat strains (*Rattus norvegicus*). *Mamm Genome* 1997; **8**: 589–591.
20. van den Brandt J, Kovacs P, Kl tting I. Metabolic variability among disease-resistant inbred rat strains and in comparison with wild rats (*Rattus norvegicus*). *Clin Exp Pharmacol Physiol* 2000; **27**: 793–795.
21. Kl tting I, H bner R, Stark O. Major histocompatibility complex (MHC)-independent differences between diabetes-prone BB rats and their parental Wistar rat strain in some hematologic and metabolic traits. *Res Exp Med* 1983; **182**: 231–236.
22. Kl tting I, Voigt B, Vogt L. Molecular analysis of diabetes-prone BB rat sublines and derivatives of their common ancestor as a tool to search for candidate loci causing different phenotypes in BB rats. *Diabetes Res* 1995; **29**: 65–71.
23. Klimes I, Seb kova E, Vrana A. The hereditary hypertriglyceridemic rat, a new animal model of insulin resistance syndrome. In *Lessons from Animal Diabetes*, Shafrir VE (ed). Smith-Gordon: London, 1995; 271–283.
24. Vrana A, Kazdova L. The hereditary hypertriglyceridemic non-obese rat: an experimental model of human hypertriglyceridemia. *Transplant Proc* 1990; **22**: 2579.
25. Kl tting I, Vogt L. BB/O(ttawa)K (arlsburg) rats: features of a subline of diabetes-prone BB rats. *Diabetes Res* 1991; **18**: 79–87.
26. Kl tting N, Kl tting I. Genetic variation in the multifunctional transcription factor Yy1 and type 1 diabetes mellitus in the BB rat. *Mol Genet Metab* 2004; **82**: 255–259.
27. Bl her M, Michael MD, Peroni OD, *et al.* Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev Cell* 2002; **2**: 25–38.
28. Kl tting N, Kl tting I. Congenic mapping of type 1 diabetes-protective gene/s in an interval of 4 Mb on rat chromosome 6q32. *Biochem Biophys Res Commun* 2004; **323**: 388–394.
29. Houchens CR, Montigny W, Zeltser L, Dailey L, Gilbert JM, Heintz NH. The dhfr ori -binding protein RIP60 contains 15 zinc fingers: DNA binding and looping by the central three fingers and an associated proline-rich region. *Nucleic Acids Res* 2000; **28**: 70–581.
30. Montigny W, Houchens CR, Illenye J, Coonrod E, Chang YC, Heintz NH. Condensation by DNA looping facilitates transfer of large DNA molecules into mammalian cells. *Nucleic Acids Res* 2001; **29**: 1982–1988.
31. Clearly JD, Pearson CE. The contribution of cis-elements to disease-associated repeat instability: clinical and experimental evidence. *Cytogenet Genome Res* 2003; **100**: 25–55.