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Position effect modifying gene expression in a patient with ring chromosome 14

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Abstract The clinical phenotype of patients with ring chromosomes usually reflects the loss of genomic material during ring formation. However, phenotypic alterations can also be found in the presence of complete ring chromosomes, in which the breakage and rejoining in terminal regions of both chromosome arms result in no gene loss. Here, we present a patient with a ring chromosome 14 that lost nothing but the telomeres. Since he and other patients with a similar chromosome abnormality present certain abnormal characteristics, we investigated the gene expression of eight chromosome 14 genes to find out whether the configuration of the ring had changed it, possibly producing some of these clinical features. The expression of these eight genes was studied by quantitative real-time polymerase chain reaction (qPCR) in the patient and in seven controls matched for gender and age. Two of them were found to be downregulated in the patient compared to the controls, indicating that his phenotype might be related to alterations in the expression of genes located in the abnormal chromosome, even when the copy number is normal. Thus, the phenotypic alterations found in the presence of complete ring chromosomes may be related to changes in the chromatin architecture, bringing about a change of expression by position effect. These results may explain some of the characteristics presented by our patient.

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Introduction

Ring chromosomes usually originate from distal breakage of the short and long arms of a chromosome and rejoining of the broken ends, resulting in the loss of genetic material. Some authors have reported cases with no apparent deletion of the telomeric ends, resulting in complete ring chromosomes (Sigurdardottir et al. 1999). Based on high-resolution molecular karyotyping, other authors have proposed different mechanisms of ring chromosome formation, such as a terminal deletion and a contiguous inverted duplication due to an inv-dup-del rearrangement (Seghezzi et al. 1999; Knijnenburg et al. 2007; Rossi et al. 2008; Guilherme et al. 2011).

The most important factors determining the phenotype of patients with ring chromosomes are: the chromosome involved in the rearrangement and the extension of the deletion of genome segments containing genes that are crucial for normal development. Thus, each patient may present his (her) own features, depending on the genes deleted (Guilherme et al. 2011). Another factor that can influence the phenotype is the configuration of the ring chromosome, which can change gene expression and cause clinical abnormalities (Schlade-Bartusiak et al. 2005; Ville et al. 2009; Zollino et al. 2009).

Patients with a ring chromosome 14 usually present growth delay, dolichocephaly, epicanthal folds, downslanting palpebral fissures, large and low-set ears, short neck, psychomotor delay, hypotonia, microcephaly, abnormal retinal pigmentation, recurrent respiratory infections and seizures (Schinzel 2001). Here, we present a patient with a ring chromosome 14 lacking only the telomeric regions, who presents the main

characteristics observed in patients with a ring 14 chromosome. Array analysis revealed no gene loss during the formation of the ring chromosome. No imbalance due to ring instability was found, even though cytogenetic analysis revealed some cells with ring loss, a duplicated ring or more than one ring in the same metaphase cell. Thus, we investigated the possibility that the ring configuration affects the expression of genes located on chromosome 14, consequently influencing the carrier's phenotype.

Clinical report

The patient, a male, was previously described by Guilherme et al. (2010). Briefly, his clinical phenotype includes: short stature, dolichocephaly, downslanting palpebral fissures, slightly prominent nose, broad nasal bridge, thin upper lip, retrognathism, gynaecomastia, bilateral cryptorchidism, talovalgus deformity, muscle hypotrophy of the lower limbs and café-au-lait spots in the left gluteus region. Skeleton X-rays revealed sacrum agenesis, clubfeet, prominent calcaneus and bilateral short fourth metatarsal. A magnetic resonance imaging (MRI) examination of the brain detected a diffuse supratentorial dysplasia and asymmetry of the lateral ventricles. The patient was reported to have presented repeated episodes of generalised tonic–clonic seizures for 12 months during infancy, treated with valproic acid at the age of 36 months. He currently presents vesico-urethral reflux (grade I), recurrent urinary tract infections, primary enuresis and faecal incontinence. An ophthalmic evaluation revealed a hypopigmented area at the posterior pole of both eyes. He also has mild intellectual disability, limited verbal language repertoire, dysarthria and a docile, affectionate and cooperative personality.

Molecular studies

Quantitative real-time PCR (qPCR)

A gene expression study was performed in the patient and compared with seven controls matched for gender and age (25 years). Eight genes located on chromosome 14 that are expressed in blood were selected for the study, considering their function and possible role in the patient's phenotype. Two reference genes (*GAPDH* and *ACTB*) were also selected for normalisation. RNA from the patient and controls was isolated from peripheral blood using the PAXgene Blood RNA MDx kit (PreAnalytiX), according to the manufacturer's instructions. For reverse transcription qPCR (RT-qPCR), 1 μL of RNA (100 ng/μL) was reverse-transcribed to cDNA, using a high-capacity cDNA kit (Applied Biosystems®, Life Technologies, Paisley, UK). The expression level of the selected genes (Table 1) was monitored with qPCR for the patient and controls, using TaqMan Gene Expression Assays (Applied Biosystems®). Each qPCR assay was performed in 12 μL of reaction solution (6 μL of master mix, 0.6 μL of probe and 5.4 μL of cDNA) per well on 96-well Fast Thermal Cycling (Life Technologies®) plates, in a Fast Real-Time PCR System (Applied Biosystems®). The cycling conditions were: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All cycle ramps were adjusted to 2.5 °C/s. All qPCR assays were run in triplicate, normalised by the two reference genes, using a 96-well plate for each gene studied. A negative control without cDNA was also run in each assay. The qPCR tests were carried out in a 7500 Fast Real-Time PCR System following the manufacturer's instructions, and the data were analysed using the 7500 software v2.0.6. The target mRNA was quantified by measuring the threshold cycle (Ct), in order to determine the relative expression, normalised to *GAPDH* and *ACTB*. Relative quantities between different samples were determined as ΔΔCt ($\Delta Ct_{control} - \Delta Ct_{calibrator\ sample\ (patient)}$), and the gene expression was evaluated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) (Table 1). The patient sample was used as the calibrator ($2^{-\Delta\Delta Ct}=1$) and the 95 % confidence interval for the relative expression of the controls was calculated using the resampling test with bootstrap based on 1000 samples. Genes were considered downregulated in the patient if the distribution of the $2^{-\Delta\Delta Ct}$ value among controls and the patient did not overlap. The GeneMANIA website (http://www.genemania.org) was used to verify the interaction network of the genes studied.

Results

G-banding, SNP-array, FISH (fluorescent in situ hybridisation) and MLPA (multiplex ligation-dependent probe amplification) results were described previously by Guilherme et al. (2010). Figure 1 shows the distribution of the $2^{-\Delta\Delta Ct}$ values in the patient (calibrator sample) and controls for the eight genes studied. Six out of the eight genes analysed, *PYGL*, *PTGER2*, *ARID4A*, *GPHN*, *RPS6KA5* and *VRK1*, presented normal expression levels in the patient compared to the controls. The other two genes (*RCOR1* and *ACTN1*) were found to be downregulated in the patient compared to the controls (Table 1).

Discussion

Several mechanisms have been proposed to explain the clinical phenotype of patients with complete ring chromosomes: (1) somatic mosaicism due to ring instability, which may vary in degree in different tissues; (2) telomere position effect

Table 1 Genes studied, with their localisation in chromosome 14, efficiency, $2^{-\Delta\Delta Ct}$ mean in controls and confidence interval

Gene symbol	Gene name	Chromosome band	Efficiency (%)	$2^{-\Delta\Delta Ct}$ mean (SD)	95 % CI	
					Lower	Upper
PYGL	Glycogen	14q22.1	95.34	1 (0)	0.99	1.00
PTGER2	Prostaglandin E receptor 2	14q22.1	93.49	1 (0)	1.00	1.00
ARID4A	AT rich interactive domain 4A	14q23.1	93.65	2.11 (1.08)	1.45	2.92
GPHN	Gephyrin	14q23.3	98.69	1.50 (0.40)	1.19	1.77
ACTN1	Actinin alpha 1	14q24.1	94.66	2.15 (0.35)	1.94	2.42
RPS6KA5	Ribosomal protein S6 kinase polypeptide 5	14q32.11	96.48	1 (0)	1.00	1.00
VRK1	Vaccinia-related kinase 1	14q32.2	96.27	0.92 (0.25)	0.74	1.08
RCOR1	REST co-repressor 1	14q32.31	93.05	2.24 (0.80)	1.73	2.82
Reference genes						
GAPDH	Glyceraldehyde-3-phosphate Dehydrogenase	12p13.31	96.14			
ACTB	Beta actin	7p22.1	93.04			

The expression values are presented as the mean $2^{-\Delta\Delta Ct}$ of three replicates

SD standard deviation; *CI* confidence interval

leading to silencing of neighbouring genes; and (3) silencing of genes due to spreading of the heterochromatin-inactivated state of the DNA, due to position effect variegation (PEV) (Kleinjan and van Heyningen 1998; van Karnebeek et al. 2002). The downexpression of the *ACTN1* and *RCOR1* genes, found in two copies in our patient, may explain some of his clinical features. The gene *ACTN1* is expressed in the retinal pigment epithelial cell line, leucocytes, dermal fibroblasts and retinal microvascular endothelial cells. The *RCOR1* gene, responsible for neuronal cell differentiation and expressed in the skin and kidney, encodes a protein that binds to the C-terminal domain REST (repressor element-1 silencing transcription factor), which acts in gene transcriptional regulation. Taken together, this information helps us to understand some characteristics of the clinical phenotype presented by our patient, such as seizures, vesico-urethral reflux, hypopigmented area at the posterior poles of both eyes, mild intellectual disability and café-au-lait spots. Seizures and retinal pigmentation abnormalities are features commonly found in individuals with a ring 14 chromosome and have been attributed to genes proximally located at 14q11q13 and 14q32.2, respectively (Howard et al. 1988; Zollino et al. 2009).

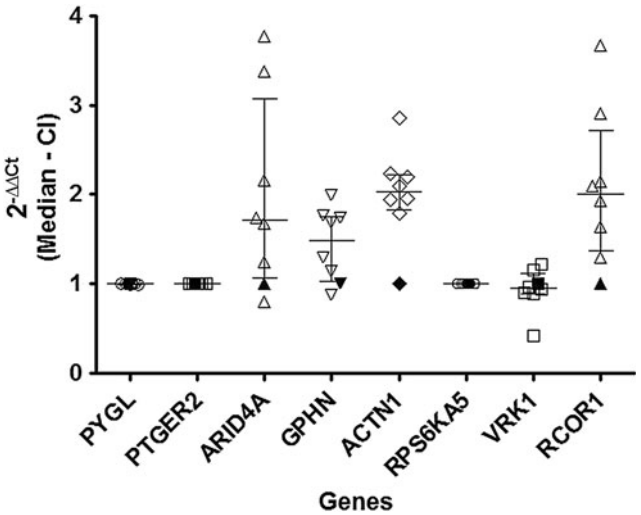

Fig. 1 Gene expression data ($2^{-\Delta\Delta Ct}$) of the eight genes studied: $2^{-\Delta\Delta Ct}$ values and confidence interval for each control (*open symbols*) and for the patient (*solid symbols*) used as a calibrator sample with the value 1

In the patient described here, the ring chromosome 14 lost only the telomeric regions, once, according to previous FISH-BAC experiments performed by Guilherme et al. (2010), the subtelomeric regions are present. These regions are heavily methylated (Brock et al. 1999), probably influencing the gene expression. It has been suggested that the interaction of genes with the nuclear lamina at the nuclear periphery may promote gene silencing (Egecioglu and Brickner 2011). A structural rearrangement can shift chromosomes from their usual positions within the nucleus to the periphery. This relocation of chromosomes disrupts the normal interactions and affects the expression not only of genes located on the chromosomes involved in the translocation, but also on others, in a genome-wide fashion (Willcocks et al. 2008). There is also evidence that the relocation of derivative chromosomes can have a knock-on effect on the organisation and placement of other chromosomes within the nucleus (Feuk 2012). Therefore, not only local *cis* interactions, but also *trans* interactions throughout the genome may be potentially disrupted by the chromosomal rearrangement. This could explain why

the large-scale gene expression changes seen in the cells of translocation-carrying individuals involve genes located on nearly every chromosome, not just those directly involved in the translocation (Feuk 2012). This phenomenon can also occur when a chromosome turns into a ring, changing the position of the aberrant chromosome within the nucleus toward the periphery and affecting the expression of its genes.

In addition to the genes studied in this work, the *AMISYN* gene was also found to be silenced due to ring 14 formation in a patient with autism and coloboma, suggesting a position effect (Castermans et al. 2008). Position effects caused by the disruption of *cis*-regulatory elements have been reported for distances of up to 1.5 Mb, both telomeric and centromeric to the gene (Benko et al. 2009).

Finelli et al. (2012) described a patient with a balanced translocation t(15;16)(p11.2;q12.1)dn. The gene expression study showed overexpression of heterochromatic genes (*VPS35* and *SHCBP1*) located at 16q11.2, underexpression of a euchromatic gene (*NETO2*/*BTCL2*) located at 16q12.1 and no epigenetic silencing in the der(15) chromosome. The expression profiles and epigenetic findings consistently revealed the presence of an alteration only on one side of the translocation breakpoint, on the der(16) chromosome. Concluding that the re-organisation of chromosomal territories may be an important step in the process of gene silencing (Harewood et al. 2010), these overall findings suggest that the different behaviour of the two derivative chromosomes may be caused by the different nuclear re-localisation of the der(16).

Surace et al. (2014) studied two patients with r(17) chromosomes, one of them complete (with telomeres), to evaluate the telomere shortening and telomere position effect. In the patient with the r(17) lacking telomeres, a group of downregulated noncontiguous genes was observed, all within an interval of 1.5 Mb from the telomeres on both arms. The upmodulation of gene *NF1* and the downregulation of *LIS1* reveal that the observed effects are not linearly correlated with the distance from the telomeres.

Conclusion

In conclusion, ring formation may change the chromatin architecture of the chromosome and lead to an alteration of the gene's environment, modifying its expression. In our patient, the juxtaposition of the short-arm DNA and the inactive centromere close to the active long-arm euchromatin may have silenced the *RCOR1* gene and others in this area by spreading of the inactivation, resulting in a position effect. Yet, the *ACTN1* gene may have been downregulated by other mechanisms, such as the repositioning of the ring chromosome in the nucleus, which could change *cis* and/or *trans* interactions. Epigenetic factors also need to be taken into account in the evaluation of the genetic consequences and in the attempt to reach a better understanding of the genotype–phenotype correlations. Incomplete penetrance or the influence of other genes involved in the multiple signal transduction pathways, as well as environmental causes, may also be responsible for the clinical heterogeneity observed in the ring chromosome cases described in the literature. Further studies on ring chromosomes are needed for a better understanding of the behaviour of genes and their interactions in these chromosomes.

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