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Ring 18 Molecular Assessment and Clinical Consequences

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Ring chromosome 18 is a rare condition which has predominantly been described by case reports and small case series. We assessed a cohort of 30 individuals with ring 18 using both microarray comparative genomic hybridization (aCGH) and fluorescence in situ hybridization (FISH). We determined that each participant had a unique combination of hemizygosity for the p and q arms. Four ring chromosomes had no detectable deletion of one of the chromosome arms using aCGH. However, two of these ring chromosomes had telomeric sequences detected using FISH. These data confirm the importance of molecular and cytogenetic analysis to determine both chromosome content and morphology. We failed to find dramatic changes in mosaicism percentage between cytogenetic measurements made at the time of diagnosis and those made years later at the time of this study, demonstrating that dynamic ring mosaicism is unlikely to be a major cause of phenotypic variability in the ring 18 population. Lastly, we present data on the clinical features present in our cohort, though the extreme genotypic variability makes it impossible to draw direct genotype-phenotype correlations. Future work will focus on determining the role of specific hemizygous genes in order to create individualized projections of the effect of each person's specific ring 18 compliment.

Key words: chromosome 18; ring chromosome 18; mosaicism

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INTRODUCTION

Although ring chromosomes have long been appreciated in maize [McClintock, 1932] and *Drosophila* [Morgan 1933], knowledge of their existence in human cells was limited to irradiated cell lines or tumor cells until 1962. In the next two years, several groups reported individuals with constitutional ring chromosomes [Lindsten and Tillinger, 1962; Wang et al., 1962; Genest et al., 1963]. Since then ring chromosomes have been described originating from all human chromosomes [Kosztolanyi 2009].

Ring chromosome 18 was among the first to be identified in humans. In fact, patients in two of the first three human ring chromosome reports likely had ring 18 [Wang et al., 1962; Genest et al., 1963]. The presumption was that ring chromosomes were formed by the joining of the two ends of the normally linear chromosome. Thus, a ring formation led to a net loss of material from both ends of the chromosome. As early as 1963, de Grouchy [1965] appreciated that individuals with ring 18 had a composite phenotype of 18p- and 18q- [1963]. Additionally, he noted that the variable phenotype between patients with ring 18 indicated that different individuals had different degrees of hemizygosity for each chromosome arm, even predicting that the gene for aural atresia would be on distal 18q. Forty-nine years later the gene for aural atresia was indeed identified at 18q23 [Feenstra et al., 2011].

A unique feature of ring chromosomes in comparison with other types of derivative chromosomes is their dynamic quality. In humans, as in maize and *Drosophila*, ring chromosomes appear to be unstable, generating multiple mitotically derivative cell lines within any one individual. Ring stability after successive mitoses was more recently investigated in vitro [Sodré et al., 2010]. In cultured cells from six patients with a ring chromosome, the percent of cells with a karyotype of 46,r(N) was reduced over time and the percent of cells monosomic for the ring chromosome or containing a derivative of the ring chromosome was increased at the second time point.

Conflict of interest: none.

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In addition to variable chromosome arm hemizygosity and mosaicism, it has been appreciated that the formation of the ring chromosome itself often resulted in duplications just proximal to the deletion breakpoint in 21% of ring chromosome cases [Rossi et al., 2008]. This phenomenon was first described by Ballif et al. [2003] in the formation of human terminal deletions of chromosome 1p. We have also reported this phenomenon in individuals with 18q deletions [Heard et al., 2009 Cody et al., 2014]. These data imply that the mechanism of formation of a ring chromosome and a terminal deletion may be similar.

Determining the phenotypic effects of a ring chromosome is thus complicated by multiple factors, including the variable extent and location of hemizygosity, the presence of duplications within the ring, somatic mosaicism, and ring instability.

Although there have been numerous case reports and small series of individuals with Ring 18, these individual reports use a variety of techniques and technologies to assess genotype, making these data incomplete and difficult to compile and compare. We wished to assess our large cohort of individuals with ring 18 using a high resolution array comparative genomic hybridization (aCGH) in conjunction with FISH to determine if there were unique properties of ring chromosome18 such as breakpoint clusters which might guide future work to identify dosage sensitive genes and direct clinical management.

METHODS

Participants are enrolled in a longitudinal study of individuals with chromosome 18 abnormalities at the Chromosome 18 Clinical Research Center. The study has been approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. All participants have provided informed consent for their participation. A criterion for enrollment is the submission of the original cytogenetic and/or cytogenomic diagnostic laboratory report. Additional medical records are also collected, abstracted, and entered into our database.

Blood samples were obtained from the affected individual and his/her biological parents for chromosome preparations, DNA

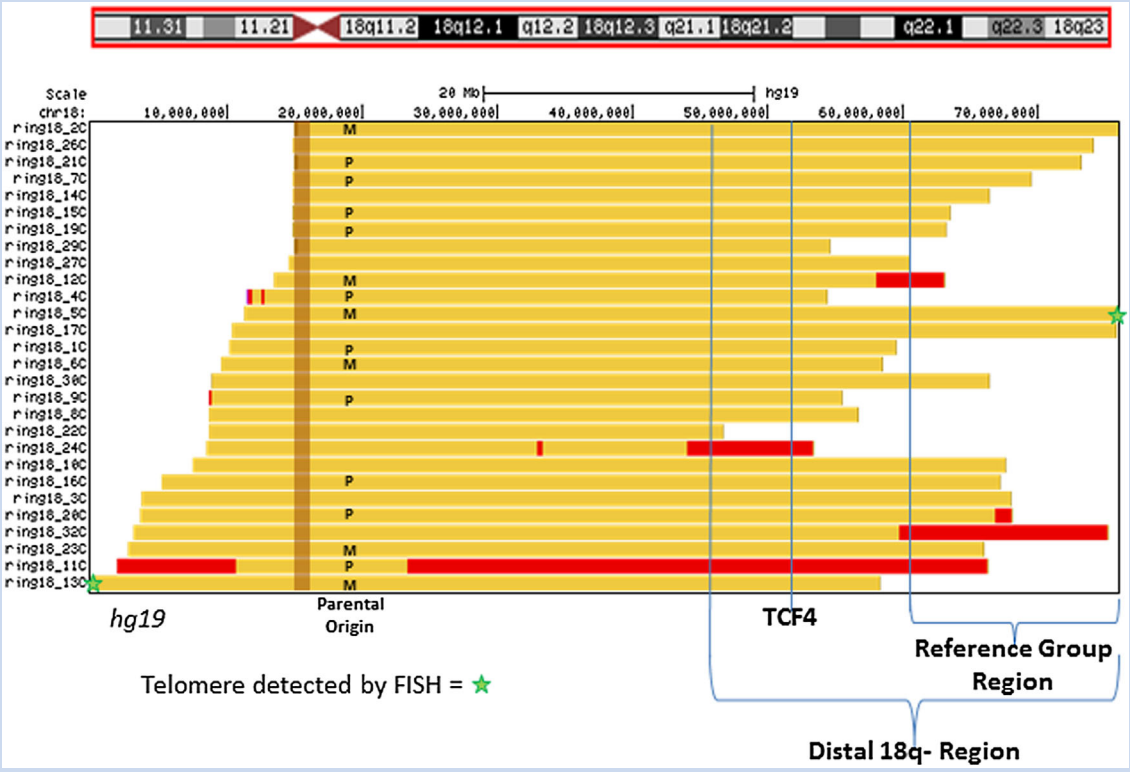

FIG. 1. Molecular analysis of the content of 30 ring chromosomes. These data are presented as custom tracks in the UCSC Genome Bowser. Across the top of the figure, the red box around the chromosome ideogram indicates that the image below includes the entire length of the chromosome. Below the ideogram, each horizontal (gold) bar indicates the intact region of each participant's chromosome 18. At the end of each bar is a darker region indicating the breakpoint region. In most cases this region is too small to be visible in this figure. Regions present in duplicate are indicated by the darker (red) bars. The participant numbers are listed down the left hand side. The thick vertical bars indicated the location of the centromere. The parental origin for those on whom we could gather the data is aligned with each individual's molecular data. Individuals whose telomeres were detected by FISH are indicated by the star at the telomere position. Several regions with clinical significance are indicated by the bracketed regions.

TABLE I. Cytogenetic and Molecular Findings

	% of cells with each type of chromosome 18 compliment								
Study #	18, 18 (normal)	18,0	18, r(18)	18, r(18)X2	18, r(18)X3	18, der(18)	Type of derivative chromosome	aCGH breakpoints determined	Parental origin of ring chromosome
1			100					x	P
2			100					x	M
3			100					x	
8			100					x	
11			100					p and q prox dups	P
12			100					q prox dup	M
19			100					x	P
21			100					x	P
22			100					x	
23			100					x	M
24			100					q prox dup	
26			100					x	
27			100					x	
29			100					x	
30			100					x	
32			100					q prox dup	
5		13	87					x	M
7		***7***	***93***					x	P
9		***6***	***94***					p prox dup	P
10		***3***	***97***					x	
13		15	85					x	M
14		***6***	***94***					x	
16		15	85					x	P
17		***11***	***82***	***4***	***3***			x	
4			90			10	18p-	p prox dup	P
6			90			10	18p-	x	M
15			81			18	9% double ring and 9% 2 double rings	x	P
20			52			48	inv18	q prox dup	P
25	11		13			76	4 cell lines/3 mar chromosomes	no identifiable deletion	
28	56	11	33					no identifiable deletion	

Mosaicism numbers in bold italic are from our studies. Other mosaicism data are from the diagnostic cytogenetic report. x indicates that hemizygosity was determined using aCGH.
The numbers in bold italics are our laboratory's data as opposed to data from our participants medical records.

isolation, and creation of immortalized cell lines. Genotyping was performed on DNA from a peripheral blood samples by microarray comparative genomic hybridization (aCGH) using the Agilent system and a custom oligonucleotide array containing 32,000 oligonucleotides across chromosome 18 and 12,000 across the remainder of the genome as previously described [Heard et al., 2009]. Parental origin of the abnormal chromosome was also determined as previously described using PCR-based polymorphic microsatellites [Heard et al., 2009].

Mosaicism studies were performed on interphase cells and metaphase nuclei from PHA-stimulated lymphocyte cultures from peripheral blood samples by fluorescence in situ hybridization (FISH) analysis using Vysis CEP 18 (α satellite), Telvysion 18p and 18q probes (Abbott Molecular Inc., Des Plaines, IL). Hybridization and post-hybridization washes were performed according to the manufacturer's protocol and the signals were enumerated using a fluorescence microscope. In order to determine the levels of mosaicism in participants without previous mosaicism studies, one hundred metaphase nuclei were analyzed using centromeric and telomeric probes. In order to determine changes in mosaicism in participants with previously established levels, one hundred interphase nuclei were scored for the number of centromeric signals. Images were captured using CytoVision 3.6 software (Applied Imaging Corporation, Santa Clara, CA).

For study participants who were at least five years of age, we also assessed several developmental parameters. Executive functioning or meta-cognition was examined using either the Behavior Rating Inventory of Executive Function Parent (BRIEF) [Gioia et al., 2000] or the Behavior Rating Inventory of Executive Function Adult Version (BRIEF-A) [Roth et al., 2005]. Parents also completed the Behavioral Assessment System for Children (BASC), Second Edition (BASC-2) [Reynolds and Kamphaus, 2004]. In order to assess social and communication skills, parents were asked to fill out the Social Responsiveness Scale, Second Edition [Constantino and Gruber, 2012]. All of the behavioral questionnaires chosen are well-normed instruments with demonstrated reliability and validity information provided by the test publishers and by post-publication validation studies [Cabrera et al., 1999; Dowdy et al., 2011].

RESULTS

The results of the molecular analysis are shown in Figure 1. Fourteen individuals were mosaic. The content of the ring chromosome was able to be determined for 12 of the 14 individuals with mosaicism using aCGH. In two individuals whose mosaicism involved a normal cell line, aCGH was not able to detect net copy number changes (see Table I). The aCGH data (Fig. 1) revealed an additional source of heterogeneity in addition to mosaicism in this cohort. While nine participants have 18p breakpoints at the centromere, each of them have unique 18q deletions. The remainder of the cohort has unique 18p as well as unique 18q deletions. Therefore each of the 28 individuals has unique regions of chromosome hemizygosity.

While the assumption has been that a ring chromosome involves the loss of material from both ends of the chromosome, aCGH identified no loss of unique sequences for one of the chromosome arms in three individuals (3/28 = 11%). Therefore FISH was employed as an additional means to assess potential telomeric sequences. Ring18_2C had no apparent deletion of 18q by aCGH but failed to have an 18q telomere FISH signal. In contrast, Ring18_5C had no loss of 18q by aCGH yet had an 18q telomere

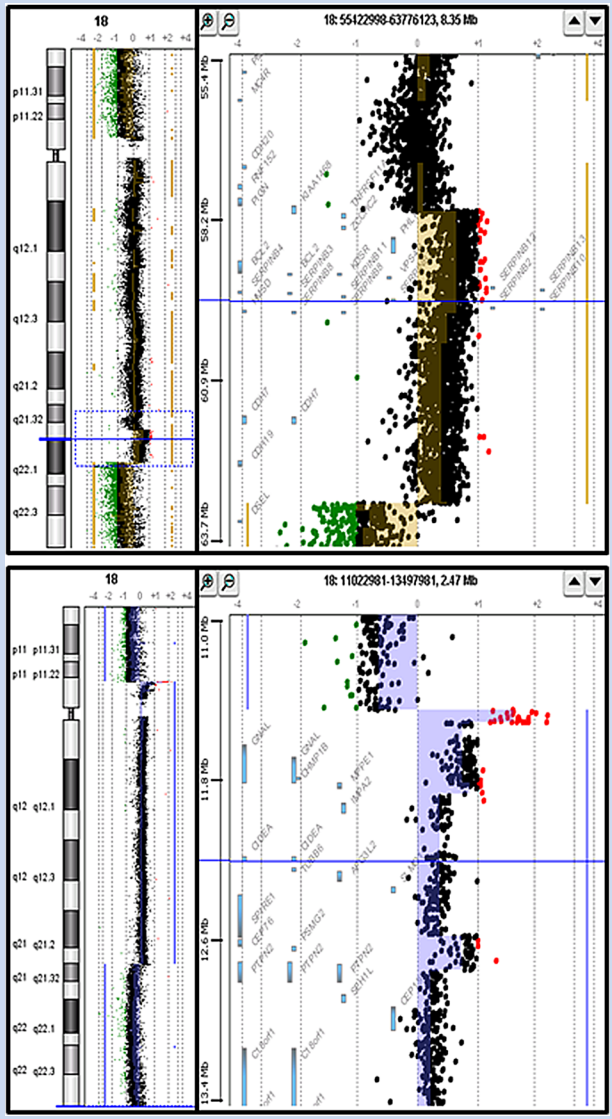

FIG. 2. aCGH data showing the breakpoints of two ring 18 chromosomes. The upper panel shows the chromosome 18 view of participant Ring18_12C with the dotted outlined box indicating the region that is shown in the section to the right. In the right-hand panel, each dot indicates a data point; those not significantly different from a $\log^2$ of zero are black, those above +1, indicating a duplication, are red and those below -1, indicating a deletion, are green. These data illustrate the presence of a duplication just proximal to the deletion breakpoint. The lower panel is similarly configured for participant Ring18_4C. These data show a discontinuous duplicated regions proximal to the deletion breakpoint as well as a region that appears to be present in more than three copies.

TABLE II. Change in Ring Chromosome Mosaicism Over Time

Study #	Years after diagnostic determination	Percent of cells with each number of chromosome 18 centromeres		
		1	2	3
4	0	0	90 r(18)/ 10 del(18p)	0
	6.00	17	83	0
16	0	15	85	0
	7.75	12	87	1
28	0	11	89	0
	9.44	8	92	0
20	0	0	52 r(18)/48 pericentric inversion	0
	12.60	7	92	1

Peri in = pericentric inversion.

FISH signal. Ring18_13C had no apparent deletion of the p arm by aCGH and also had a telomere using 18p telomere FISH. Telomeres detected by FISH are indicated by a star in Figure 1.

Six participants had ring chromosomes containing duplicated material in additional to the terminal deletions of each chromosome arm. This duplicated material is just proximal to the deletion breakpoints; as we have seen in people with 18q deletions [Heard et al., 2009]. Also as seen in 18q-, these duplicated regions vary greatly in size, from 146 Kb to 43 Mb. However the resolution of these analyses was not sufficient to detect copy neutral linkers between the duplicated regions as reported elsewhere [Hermetz et al., 2014]. Unique to these ring chromosomes, as opposed to terminal deletions, were the possible triplication or even quadruplication of sequences near the breakpoints (Fig. 2).

We determined the parental origin of the ring chromosome of those participants for whom we had parental DNA samples. Ten of 16 (62%) were of paternal origin. Because we have seen parental origin differences between the 18p centromere breakpoints (37% paternal) (Sebold et al., in press) and 18q terminal deletion breakpoints (88% paternal) [Cody et al., 1997], we indicated the parental origin of the ring chromosome associated with each participant in Figure 1. Within the group with ring 18 and centromeric breakpoints of 18p, 80% (4/5) were paternal in origin. In the group with non-centromeric 18p breakpoints, 45% (5/11) were paternal in origin.

The level of mosaicism was determined by reviewing the participant's clinical cytogenetic records and performing our own cytogenetic analysis on cells from peripheral blood samples (Table I). Fifty-three percent (16/30) had no evidence of ring 18 mosaicism, (46,XX or 46,XY, r[18]). Of the 47% (14/30) with evidence for mosaicism, there were several different types; either mosaicism for loss of the ring (46,XX or 46,XY, r[18]/45,XX or 45, XY, -18) (N = 7), or mosaicism for the presence of a double ring or derivative of the ring chromosome or mosaicism with a normal cell line (N = 7).

Ring chromosomes have been hypothesized to be mitotically unstable in vivo. Since four participants with established mosaicism enrolled in our study many years after their initial diagnosis, we wanted to know if their level of mosaicism had changed with time. Therefore we compared the level of mosaicism of the four individuals with quantified mosaicism studies performed at the time of diagnosis with cells from blood samples for our study taken at least six years later. We compared the level of mosaicism at enrollment using interphase FISH for a centromere probe and compared that data to the level identified in the initial diagnostic data (Table II). Interestingly the two individuals who had a low level of mosaicism for a monosomy 18 cell line at diagnosis maintained that same level of mosaicism 7.75 and 12.6 years later. However, the two people who had secondary cell lines containing a derivative chromosome 18 developed a monosomy 18 cell line which was not apparent at diagnosis. In one of these individuals, the initial derivative cell line was 18p- and, in the other, it was a pericentric inversion.

A review of the medical records and interviews with parents revealed the major physical features of this cohort shown in Table III. Medical conditions found in more than one individual are included in the table. All of these findings are well-known features of either 18p- or 18q-. Additionally, the behavioral characteristics of many of the individuals in this cohort are listed in Table IV.

DISCUSSION

Our data clarify the underlying molecular complexity associated with the presence of a ring 18 chromosome. The heterogeneity with regard to chromosome content is hardly surprising given that in our entire cohort of over 300 unrelated participants with deletions of 18q all have unique regions of hemizygosity [Heard et al., 2009 unpublished data]. Additionally, in our cohort of people with ring 18 chromosomes, the 18p deletions reflect the types of deletions in our 18p- cohort of nearly 100 individuals, with just under half having breakpoints at the centromere and the others, each with unique terminal deletions [Sebold et al., submitted]. Additionally, the presence of duplications just proximal to the deletion breakpoint points to a chromosome healing process similar to that found in 18q terminal deletions which have been seen with other ring chromosomes [Ross et al., 2008; Guilherme et al., 2011].

The parental origin data show somewhat different biases than the terminal deletions. The Ring 18 population has a paternal parent of

TABLE III. Ring 18 Phenotypes

Trait or characteristic	%
Neurologic/neuromuscular	
Hypotonia	92
Microcephaly	70
Abnormal white matter	31
Seizures	26
Holoprosencephaly spectrum[a]	13
Agenesis of corpus calosum	12
Endocrine/metabolic	
Growth hormone deficiency	93
Hypothyroid	41
Neonatal jaundice	37
ENT/hearing	
Hearing loss	80
Chronic otitis media	74
Aural atresia	50
Tracheomalacia	10
Laryngomalagia	10
Choanal atresia	6
Skeletal/orthodedic	
Scoliosis/kyphosis	31
Vertical talus	10
Metatarsus aductus	13
Club feet	9
Pes cavus	7
Overlapping toes	7
Pectus excavatum	4
Feeding/gastrointestinal	
Reflux	68
Neonatal feeding difficulties	61
Constipation	58
G or NG tube/dysphagia	30
Vision/eye	
Hyperopia	67
Strabismus	47
Ptosis	38
Nystagmus	20
Astigmatism	8
Amblyopia	16
Optic nerve hypoplasia	6
Coloboma	6
Cardiac	
Pulmonary stenosis	38
Atrial septal defect	25
Patent foramen ovale	17
Patent ductus arteriosus	12
Palate	
Cleft palate	19
Bifid uvula	10
Pulmonary	
Neonatal respiratory difficulties	67
Asthma	13

[a]Single central incisors or MRI findings.

origin in 62% of cases. This is in contrast to the 18q- data, in which 88% of the abnormal chromosomes are of paternal origin [Heard et al., 2009]. When taking into consideration only the p arm deletion in the ring 18 population, those with centromere breakpoints and paternal deletions are 80% compared to 37% in those with terminal 18p deletions [Sebold et al., submitted]. In the Ring 18 population with unique short arm deletions the paternal origin is 45%, which is comparable to the terminal 18p deletions of 48% (unpublished data).

The finding of a ring chromosome without a detectable deletion of one of the chromosome arms is not unique. In fact in a study of individuals with ring 14, 6 of 27 individuals had no detectable deletion [Zollino et al., 2012]. Additionally, in a study of 28 individuals with ring 20 almost half had no detectable deletion of at least one chromosome arm [Conlin et al., 2011].

There are relatively few reports with more than three individuals with rings of the same chromosome origin (Table II). Interestingly, each chromosome appears to have its own properties or spectrum of chromosome diversity. Rings originating from chromosome 13 and 15 do not appear to be present in a mosaic state [Rossi et al., 2008]. Although the authors did not specifically comment on mosaicism, they employed techniques that would have detected mosaicism had it been present, yet the existence of mosaicism was not mentioned. Interestingly, in a study of 16 individuals with Ring 14, all were mosaic for a monosomy cell line and a single ring cell line (e.g., 45,XX,-18/46,XX,r[18]) [Zollino et al., 2012]. Only in chromosome 20 cases were the rings present as double rings or absent altogether in monosomy cell lines [Conlin et al., 2011]. In our cohort, we have a lower proportion of participants with ring chromosome mosaicism than found in other studies. However, in our participants with mosaicism, we saw a greater variation in the type of chromosome content for the alternative cell lines. Additionally, only two individuals had normal cell lines indicating that the original ring formation is rarely a post-zygotic event.

Compared to other studies of ring chromosome 18 who report more than three participants [Stankiewicz et al., 2001 Rossi et al., 2008 Conlin et al., 2011 Guilherme et al., 2011 Zollino et al., 2012 Spreiz et al., 2013] our much larger cohort confirms the presence of the individual variation between cell line types in the individuals with mosaicism as well as the variety of derivative cell lines. Given the diverse types of ring mosaicism possible with ring chromosome 18, one might predict that because so many variations are viable that a greater proportion of individuals with ring 18 might be mosaic. However, in actuality, the opposite is true; fewer people with ring 18 are mosaic than with the other chromosomes studied (Table V).

Ring instability has been hypothesized to be a cause of phenotypic variability between people with presumably identical ring chromosomes. Our data do not reveal extensive ring instability over time in the blood of our study participants. However, only a few participants had initial diagnostic reports that identified and quantified mosaicism. This is in contrast to previous studies reporting ring instability [Sodré et al., 2013]. It is important to recognize, however, that those studies employed time courses in cell culture as a proxy for patient aging. This may or may not represent what happens in vivo. Our data instead support the hypothesis that the variability in levels of mosaicism likely stems from the unique

TABLE IV. Behavioral Characteristics

Behavior (BASC-2): N = 14	**%**
Inability to express ideas and communicate effectively	86
Odd behaviors	79
Inability to perform basic living tasks safely	71
Easily distractible and unable to concentrate	64
Poor social skills	57
Evading others to avoid social contact	36
Inability to work with others	36
Overly active and acting without thinking	29
Hostile threatening behaviors	29
Inability to adapt to change	21
Overly sensitive and complaining about minor problems	7
Nervous, fearful or worried	7
Anti-social and rule-breaking behaviors	0
Executive Functioning (BRIEF and BRIEF-A): N = 9	
Moving from one situation to another	67
Managing current and future oriented tasks	56
Remembering information in order to complete a task	44
Keeping track of own problem solving successes or failures	44
Modulate emotional responses	44
Acting on impulse	44
Begin a task independently	22
Keep work and living spaces orderly and organized	22
Understanding their effect on others	0
Social Impairment (SRS-2): N = 3	
Interpreting social cues	100
Social communication	100
Repetitive behaviors and obsessing same routines	100
Motivated to engage in social behavior	67
Picking up on social cues	67

individual nature of each person's ring chromosome content and dynamic ring stability plays little if any role.

Our data emphasize two points with regard to the diagnosis of the chromosome 18 conditions. First, the ability to detect a deletion by aCGH does not rule out mosaicism. We were able to determine breakpoints using aCGH for 12 of 14 participants who were mosaic. The two mosaic individuals whose breakpoints could not be determined by aCGH were the two who had cells with a normal chromosome compliment. Therefore, an identifiable copy number change on aCGH does not preclude mosaicism and cytogenetic studies also need be performed. Second, an individual may in fact have a ring chromosome even though, by aCGH, a single p-arm or q-arm deletion was detected. We have had several recently enrolled participants whose referring diagnosis was made using only aCGH indicating a terminal deletion without cytogenetics to confirm chromosome morphology. Thus, a diagnosis of an 18p or 18q deletion cannot be made using molecular techniques alone without a cytogenetic analysis showing the chromosome to be linear since ring chromosomes may have undetectable deletions of either chromosome arm. The ability to differentiate between mosaicism and non-mosaicism as well as between a terminal deletion and a ring chromosome has significant genetic counseling implications. Thus, these points reinforce the need to utilize a cytogenetic approach in addition to molecular techniques when considering a diagnosis of any chromosome 18 condition.

As can been noted from the list of clinical features of our cohort with ring 18 (Table III), the list is long with three quarters of the features seen is less than half of the individuals. The wide variety of clinical features present in our cohort is likely attributable to the molecular heterogeneity of this group. As such, this list should not be considered to be a syndrome description predicting the possibility of any of these features in a single individual. For example, someone with a ring 18 chromosome would only be at a higher than population risk for congenital aural atresia if they were hemizygous for the *TSHZ1* gene on 18q [Feenstra et al., 2011] and not the 50% risk as indicated in the table. Thus, genetic counseling should be tailored based on the breakpoint locations and not solely on the identification of a ring chromosome.

For this reason, several clinically relevant designations are indicated in Figure 1. The maximum region of hemizygosity for distal 18q- is indicated to show that the full range of 18q terminal deletions are also found in people with ring 18 chromosomes. A gene with a major impact on clinical outcome is the *TCF4* gene, whose location is indicated in the figure. People with inactivating mutations in or deletions of this gene have Pitt Hopkins syndrome which includes severe intellectual disability. People with terminal deletions of 18q

TABLE V. Summary of Ring Chromosomes Studies

Chr.#	% of participants with each typeof chromosome compliment						Type of derivative chr.	% with mosaicism	Parental origin of ring chr.	Reference
	Normal (2 copies)	nl,0	nl, R	nl, R, R	nl, R, R, R	nl, der chr.				
13			100% (9/9)				Prox dup (3/9)	Not determined		Rossi et al. [2008]
14		100% (16/16)	100% 16/16)					100%	12 pat4 mat	Zollino et al. [2012]
15			100% (9/9)				Prox dup (3/9)	Not determined		Rossi et al. [2008]
20	75% (21/28)	46% (13/28)	100% (28/28)	21% (6/28)				96% (27/28)		Conlin et al. [2011]
18			71% (5/7)			29% (2/7)	inv dup 18 + ring 18	29%(2/7)		Stankiewicz et al. [2001]
18			100% (6/6)				Prox dup (3/6)	Not determined	3 pat/1 mat	Rossi et al. [2008]
18		100% (3/3)	100% (3/3)			100% (3/3)		100%(3/3)		Guilherme et al. [2011]
18			100% (9/9)				Prox dup (3/9)	0%	4 pat/2 mat	Spreiz et al. [2013]
18	7% (2/30)	30% (9/30)	100% (30/30)	3% (1/30)	3% (1/30)	17% (5/30)	(Table I)	47%(14/30)	10 pat/6 mat	Present study

nl, a single normal chromosome; R, ring chromosome; der chr, derivative chromosome.

that do not include this gene have only moderate intellectual disability [Hasi et al., 2011]. Although as can been seen in Figure 1, only one individual is hemizygous for TCF4. Therefore, determining if this gene is hemizygous in someone with a ring 18 chromosome has important clinical implications. Also important for informing clinical care is the 18q- reference group, whose region of hemizygosity is also indicated in Figure 1. The clinical and developmental characteristics of individuals with these similar 18q- deletions are described previously [Cody et al., 2014]. These landmarks help to relate the ring 18 genotypes to those seen in people with 18q-.

As the role of additional dosage sensitive genes becomes known (e.g., *TCF4*) knowledge of the molecular breakpoints of the ring chromosome will be of increasing relevance. Additionally, since the presence of an inverted duplication at the site of the breakpoint may play a role in the phenotype, it is critical to identify patients who have a duplication in addition to a deletion, necessitating the use of aCGH in the diagnostic process. Thus, our data support the use of cytogenetics to identify the presence of a ring chromosome as well as the level of mosaicism and aCGH to determine the extent of the deletion(s) as well as to identify regions of duplication.

CONCLUSIONS

This study demonstrates that individuals with ring 18 each have unique chromosome content, caused by variable breakpoints and subsequent differences in the region and extent of hemizygosity as well as possible mosaicism of various cell lines. Comprehensive diagnosis of an individual with a ring chromosome requires both a molecular diagnostic approach such as aCGH as well as a cytogenetic approach in order to determine a specific individual diagnosis. We also show preliminary data that with regard to chromosome 18, there is little evidence of dynamic ring mosaicism. These studies help to define the path forward for the identification of dosage sensitive genes and potential clinical management. That path cannot rely on defining a uniform clinical description of a "ring 18 phenotype" due to the underlying molecular diversity of people with ring chromosome 18. Instead, progress toward clinical management can take advantage of work done on 18p- and 18q- and apply that knowledge to ring 18.

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REFERENCES

Ballif BC, Yu W, Shaw CA, Kashork CD, Shaffer LG. 2003. Monosomy 1p36 breakpoint junctions suggest pre-meiotic breakage-fusion-bridge cycles are involved in generating terminal deletions. Hum Mol Gene 12:2153–2165.

Cabrera P, Grimes-Gaa L, Thyer BA. 1999. Social work assessment of adaptive functioning using the vineland adaptive behavior scales: Issues of reliability and validity. Journal of Human Behavior in the Social Environment 2:33–50.

Cody JD, Pierce JF, Brkanac Z, Plaetke R, Ghidoni PD, Kaye CI, Leach RJ. 1997. Preferential loss of the paternal alleles in the 18q–syndrome. Am J Med Genet 69:280–286.

Cody JD, Hasi M, Soileau B, Heard P, Carter E, Sebold C, O'Donnell L, Perry B, Stratton RF, Hale DE. 2014. Establishing a reference group for distal 18q-: Clinical description and molecular basis. Hum Genet 133:199–209.

Conlin LK, Kramer W, Hutchinson AL, Li X, Riethman H, Hakonarson H, Mulley JC, Scheffer IE, Berkovic SF, Hosain SA, Spinner NB. 2011. Molecular Analysis of ring chromosome 20 syndrome reveals two distinct groups of patients. J Med Genet 48:1–9.

Constantino JN, Gruber CP. 2012. Social responsiveness scale. 2nd edition. Torrance, CA: Western Psychological Services.

de Grouchy J. 1965. Chromosome 18: A topologic approach. J. Pediatr 66:414–431.

Dowdy E, Chin JK, Twyford JM, Dever BV. 2011. A factor analytic investigation of the BASC-2 behavioral and emotional screening system parent form: Psychometric properties, practical implications, and future directions. J Sch Psychol 49:265–280.

Feenstra I, Vissers LELM, Pennings RJE, Nillessen W, Pfundt R, Kunst HP, Admiraal RJ, Veltman JA, van Ravenswaaij-Arts, Brunner HG, Cremers CWRJ. 2011. Disruption of teashirt zinc finger homeobox 1 is associated with congenital aural atresia in humans. Am J Hum Genet 89:813–819.

Genest P, Leclerc R, Auger C. 1963. Ring chromosome and partial translocation in the same cell. Lancet 281:1426–1427.

Gioia GA, Isquith PK, Guy SC, Kenworthy L. 2000. Behavior rating inventory of executive function. Lutz, FL: Psychological Assessment Resources, Inc.

Guilherme RS, Meloni VFA, Kim CA, Pellegrino R, Takeno SS, Spinner NB, Conlin LK, Christofolini DM, Kulikowski LD, Melaragno MI. 2011. Mechanism of ring chromosome formation, ring instability and clinical consequences. BMC Med Genet 12:171.

Hasi M, Soileau B, Sebold C, Hill A, Hale DE, O'Donnell L, Cody JD. 2011. The role of the TCF4 gene in the phenotype of individuals with 18q segmental deletions. Hum Genet 130:777–787.

Heard PL, Carter EM, Crandall AC, Sebold C, Hale DE, Cody JD. 2009. High resolution genomic analysis of 18q–using oligo-microarray comparative genomic hybridization (aCGH). Am J Med Genet 149A:1431–1437.

Hermetz KE, Newman S, Conneely KN, Martin CL, Ballif BC, Shaffer LG, Cody JD, Rudd MK. 2014. Large inversted duplications in the Human Genome form via a fold-back mechanism. PLoS Genet 10:-e1004139.

Kosztolanyi G. 2009. The genetics and clinical characteristics of constitutional ring chromosomes. J Assoc Genet Technol 35:44–48.

Lindsten J, Tillinger K-G. 1962. Self-perpetuating ring chromosome in a patient with gonadal dysgenesis. Lancet 279:593–594.

McClintock B. 1932. A correlation of ring shaped chromosome with variegation in Zea mays. Proc Natl Acad Sci 18:677.

Morgan LV. 1933. A closed X chromosome in Drosophila melanogaster. Genetics 18:250–283.

Reynolds CR, Kamphaus RW. 2004. Behavior assessment system for children. 2nd edition. Circle Pines, MN: AGS Publishing.

Rossi E, Riegel M, Messa J, Gimeli S, Maraschio P, Ciccone R, Stroppi M, Riva P, Perrotta CS, Mattina T, Memo L, Baumer L, Kucinskas V, Castellan C, Schinzel A, Zuffardi O. 2008. Duplications in additional to terminal deletions are present in a proportion of ring chromosomes: Clues to the mechanisms of formation. J Med Genet 45:147–154.

Roth RM, Isquith PK, Gioia GA. 2005. Behavior rating inventory of executive function ? adult version. Lutz, FL: Psychological Assessment Resources, Inc.

Sebold C, Soileau B, Heard P, Carter E, O'Donnell L, Hale DE, Cody JD. 2014. Whole Arm Deletions of 18p: Medical and Developmental Effects. Am J Med Genet in press.

Sodré CP, Guilherme RS, Meloni VFA, Brunoni D, Juliano Y, Andrade JAD, Belangero SIN, Christofolini DM, Kulikowski LD, Melaragno MI. 2010. Ring chromosome instability evaluation in six patients with autosomal rings. Genet Mol Res 9:134–143.

Spreiz A, Guilherme RS, Castellan C, Green A, Rittinger O, Wellek B, Utermann B, Erdel M, Fauth C, Haberlandt E, Kim CA, Kulikowski LD, Meloni VA, Utermann G, Zschocke J, Melaragno MI, Kotzot D. 2013. Single-nucleotide polymorphism array-based characterization of ring chromosome 18. J Pediatr 163:1174–1178. e3 e3.

Stankiewicz P, Brożek I, Hélias-Rodzewicz Z, Wierzba J, Pilch J, Bocian E, Balcerska A, Woźniak A, Kardaś I, Wirth J, Mazurczak T, Limon J. 2001. Clinical and Molecular-cytogenetics studies in seven patients with ring chromosome 18. Am J Med Genet 101:226–239.

Wang H-C, Melnyk J, McDonald LT, Uchida IA, Carr DH, Goldberg B. 1962. Ring chromosomes in human beings. Nature 195:733–734.

Zollino M, Ponzi E, Gobbi G, Neri G. 2012. The ring 14 syndrome. Euro J Med Genet 55:374–380.