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N Simon Thomas, Joan K Morris, Julia Baptista, Bee Ling Ng, John A Crolla, et al.. De novo apparently balanced translocations in man are predominantly paternal in origin and associated with a significant increase in paternal age. *Journal of Medical Genetics*, 2010, 47 (2), pp.112. 10.1136/jmg.2009.069716 . hal-00552705

HAL Id: hal-00552705

<https://hal.science/hal-00552705>

Submitted on 6 Jan 2011

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De novo apparently balanced translocations in man are predominantly paternal in origin and associated with a significant increase in paternal age.

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Running title: origin of balanced translocations

Abstract

Background

Congenital chromosome abnormalities are relatively common in our species and among structural abnormalities the most common class is balanced reciprocal translocations. Determining the parental origin of *de novo* balanced translocations may provide insights into how and when they arise. While there is a general paternal bias in the origin of non-recurrent unbalanced rearrangements, there are few data on parental origin of non-recurrent balanced rearrangements.

Methods

The parental origin of a series of *de novo* balanced reciprocal translocations was determined using DNA from flow sorted derivative chromosomes and linkage analysis.

Results

Of 27 translocations, we found 26 to be of paternal origin and only one of maternal origin. We also found the paternally derived translocations to be associated with a significantly increased paternal age ($p < 0.008$).

Conclusion

Our results suggest there is a very marked paternal bias in the origin of all non-recurrent reciprocal translocations and that they may arise during one of the numerous mitotic divisions that occur in the spermatogonial germ cells prior to meiosis.

Introduction

Congenital chromosome abnormalities are relatively common in our species and using only moderate levels of banding, the frequency of structural chromosome abnormalities in an unbiased series of newborns was found to be 0.3%[1]. Higher resolution banding, molecular cytogenetics and genome wide array CGH have all substantially increased the number of detectable structural rearrangements. The parental origin of chromosome abnormalities is of considerable interest as this may provide an insight into the mechanism by which they arise.

It is comparatively easy to determine the parental origin of *de novo* unbalanced structural abnormalities and hence most studies have focussed on this type of rearrangement. Among *de novo* non-recurrent, cytogenetically visible imbalances, there appears to be a paternal bias, although there is some variation among different classes of structural rearrangements. Approximately 70% of terminal deletions are paternal and 30% maternal in origin, while interstitial deletions are 84% paternal and 16% maternal[2]. Among a small number of duplications, 58% were paternal and 42% maternal, while unbalanced translocations were shown to be 62% paternal and 38% maternal[2].

Such non-recurrent rearrangements are most likely to arise through non homologous end joining (NHEJ) [3]. In contrast, structural abnormalities with recurrent breakpoints, such as microdeletion and microduplication syndromes, generally result from non-allelic homologous recombination (NAHR) between low copy repeats[3]. In many *de novo* microdeletion syndromes, there are approximately equal numbers of maternal and paternal origin[4] suggesting that NAHR occurs during both paternal and maternal meiosis.

Balanced translocations involve points of breakage and exchange distributed throughout the genome and are found in approximately 1 in 500 individuals[1]. Direct determination of the parental origin of balanced translocations requires either physical separation of the derivative chromosomes involved in the translocation or linkage studies in large pedigrees. To date one balanced translocation has been analysed by flow sorting the derivative chromosomes and one by creating somatic cell hybrids; the origin of both translocations was paternal[5,6].

The great majority of balanced translocations manifest no discernable phenotype, and may pass through several generations without detection, only coming to attention as a consequence of subfertility or an unbalanced affected offspring. Although they appear balanced cytogenetically, up to 25% of translocations ascertained because of an abnormal phenotype have *de novo* cryptic imbalances identifiable by array CGH at one or both breakpoints[6-11]. If we assume that the parent of origin of these imbalances indicates the parent in whom the translocation originated, all nine previously reported cases were paternal[7,8,10]. X inactivation has also been used to study X-autosome translocations and, on the assumption that the normal X chromosome will be unilaterally inactivated, all 12 t(X;A) analysed were also shown to be paternal in origin[12]. Therefore, among *de novo* t(X;A) and balanced translocations with breakpoint-associated imbalances there is a clear paternal bias. However, since these constitute only a small proportion of cases, the relevance of these observations to the great majority of balanced translocations is unclear. **Furthermore, a paternal bias does not extend to all translocations as the common Robertsonian translocations are known to be predominantly maternal in origin [13].**

To our knowledge, there has been no systematic survey of the parental origin of *de novo* reciprocal apparently balanced translocations. We present parental origin studies on 27 patients with *de novo* constitutional translocations that appear cytogenetically balanced.

Methods

Study Population

All patients included in the study carry a *de novo* constitutional translocation that appears to be cytogenetically balanced. Flow-sorted DNA of the derivative chromosomes was available from nine of the patients described by Gribble et al[10] (numbers 1-9) and from 13 of the patients described by Baptista et al[7] (numbers 10-22), including one patient with two *de novo* translocations. Flow-sorting and DNA amplification have been described previously in these studies. Genomic DNA was available from the parents and at least one child from a further five patients described by Baptista et al[7] (numbers 23-27). Table 1

Determination of Parental origin

DNA from each flow-sorted derivative chromosome was amplified alongside the patient's genomic DNA using microsatellites selected from ensembl and the UCSC genome browser. The parental origin of the single allele amplified from the derivative chromosome was determined by comparison with genomic DNA from both parents. Figure 1A. Alternatively parental origin was determined using linkage. Microsatellites close to each breakpoint interval were selected using the UCSC genome browser and amplified from the patient carrying the *de novo* translocation and their first degree relatives. Figure 1B.

Analysis of Paternal Age

Paternal and maternal ages were known for 23 of the patients studied and these ages were compared to data on paternal and maternal ages obtained from the UK Office for National Statistics for all jointly registered births from 1964 to 2007. To adjust for both date of birth and maternal age, the national distribution of paternal ages was selected for each patient's year of birth and their mother's age. For example, if a patient was born in 1950 and the mother was 35, the distribution of paternal ages in 1950 corresponding to a maternal age of 35 was selected.

Results

Determination of Parental origin

We successfully determined the parental origin of 26 translocations from 25 patients using direct analysis. Table 1. We were unable to obtain results for the translocations in Patients 4 and 11. However for Patient 11, a breakpoint associated deletion on chromosome 5q12 was previously shown to be of paternal origin[7] and, although we cannot exclude a post-zygotic event, this translocation is also likely to be paternal. In total of 27 translocations, we found 26 to be of paternal origin (96%) and only one of maternal origin.

Analysis of Paternal Age

All 23 national data sets of paternal ages were combined and compared with the observed distribution of paternal ages. The paternal ages of the patients were found to be significantly older than the population data ($P < 0.008$). In Figure 2, the cumulative age distribution of the 23 patients lies to the right of the national distribution line which shows that there are more older fathers than expected. The odds of a balanced translocation more than doubles with every ten year increase in paternal age: (OR for 10 years = 2.19, 95%CI:1.23-3.90).

Discussion

Our series includes five cases with a breakpoint associated imbalance and two X;autosome translocations, all of which were paternal in origin. Of the remaining 20 translocations cases, which included both clinically normal and clinically abnormal individuals, 19 were of paternal origin and one of maternal origin. Therefore our results suggest that there is a very marked paternal bias in the origin of all non-recurrent reciprocal translocations, irrespective of the chromosomes involved, the ascertainment reason and whether or not they are balanced on array CGH.

Altogether, three of the translocations in our series had a breakpoint-associated deletion, three had a deletion unrelated to the translocation and two had both types of deletion. (Table 1). All imbalances were paternal in origin and occurred in the clinically abnormal group; no imbalances were seen in the six clinically normal carriers. Breakpoint associated deletions have been identified in up to a quarter of translocation carriers with an abnormal phenotype[7,8,10,11,14] and all cases tested were paternal in origin[7,8,10]. All *de novo* breakpoint independent deletions found in association with a *de novo* balanced translocation were also paternal in origin[6,7,8,10]. However, the closest equivalent class of chromosome rearrangement for which data are available, non-recurrent interstitial deletions, also show a very strong paternal bias (85%)[2]. Therefore it is unclear whether the formation of breakpoint independent deletions and reciprocal translocations are related, or represent two independent mutational events, both with a very strong paternal bias.

NAHR does not appear to be involved in the formation of the great majority of balanced translocations[8,9]. Instead junction fragment sequencing suggests that balanced translocations are likely to arise through the formation of random double stranded breaks (DSBs) followed by NHEJ[11,15]. Cytogenetically visible structural imbalances with non-recurrent breakpoints are similar in that the majority are also presumably mediated by NHEJ and also have a paternal bias[2]. **However, the paternal bias among unbalanced translocations (13 of 21 cases analysed) is significantly lower than for the reciprocal translocations in this study, although the reason for this discrepancy is unclear.**

Among recurrent translocations, there are certain classes which have a strong maternal bias; these are mediated by genome architecture, and presumably involve NAHR. For example, Robertsonian translocations, which presumably occur as the result of homologous recombination between shared sequences on the short arms of the acrocentric chromosomes, are predominantly the result of maternal meiotic errors[13]. Also, while the parental origin of the rare balanced $t(4;8)(p16;p23)$ has not been reported, all *de novo* unbalanced cases studied had a maternal origin shown to be mediated by a common inversion polymorphism(s) involving olfactory receptor gene clusters[16].

An overview of the breakpoint intervals defined by FISH[7], suggest that all the translocations in this study appear to be unique events, not mediated by large-scale sequence homology with the exception of the single translocation of maternal origin (Baptista and Crolla, unpublished data). Interestingly, this differs from all the others in having predisposing genome architecture underlying the rearrangement: both breakpoint intervals contained defensin gene clusters, one on 8p23 and one on 12q23, which may have undergone ectopic pairing, breakage and reunion during female meiosis (i.e. NAHR).

The strong paternal bias in our series of translocations lead us to examine paternal age. Paternal and maternal age was known for 23 patients and these data sets were combined and compared with the observed distribution of paternal ages. The paternal ages of the patients were found to be significantly older than the population data ($P < 0.008$) with more older fathers than expected.

Male gametogenesis appears particularly susceptible to the formation of apparently balanced reciprocal translocations. Although there are well documented differences between male and female meiosis with regard to timing[17] and the level of recombination[18], our observation of a significant increase in paternal age suggests that balanced translocations may arise during pre-meiotic mitosis rather than meiosis. Some classic mutations due to base pair substitutions also have a male excess, and are associated with an increase in paternal age. This is generally attributed to the much larger number of repeated cycles of premeiotic cell division undergone by male germ cells by comparison with female germ cells (for review see Crow 2000)[19].

Any mutation event occurring during premeiotic germ cell division would be expected to be more frequent in males and also to show a male parental age effect. This seems to be a plausible explanation for both the male excess in the origin of balanced translocations and their association with raised paternal age. This may also explain the large bias towards a male origin of “random” (i.e. not associated with genome architecture) *de novo* deletions detected by array CGH. If so, it is likely that this type of structural chromosome mutation will also be found to have an association with increased paternal age.

In summary, we show that non-recurrent, constitutional apparently balanced reciprocal translocations are almost exclusively paternal in origin. The translocations are associated with a significantly elevated paternal age suggesting a premeiotic mitotic origin which is likely to involve NHEJ. This highlights the complex range of mechanisms through which human chromosome rearrangements can arise.

ACKNOWLEDGEMENTS

We are very grateful for the contribution of the Wessex Regional Genetics Staff and Drs Susan Gribble, Elena Prigmore and Nigel Carter for supervising the flow sorting at the Sanger Centre and supplying amplified DNA. Work at the Sanger Centre was supported by the Wellcome Trust [grant number WT077008]. Additional DNA amplification was carried out in Salisbury by Jo Score. We also thank Dr John Barber for his expertise on defensin gene cluster locations.

WEB RESOURCES

Ensembl	http://www.ensembl.org/
Genome database	http://www.gdb.org/

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TABLE 1

No	Reference	Phenotype	Method	Karyotype	Origin	Breakpoint-associated Imbalances	Unrelated Imbalances	Paternal Age	
1	10	A1	Abnormal	Flow sorting	t(6;10)(q13;q21.2)de novo	PATERNAL	Deletion	None	42
2	10	A2	Abnormal	Flow sorting	t(6;17)(p23;q25)de novo	PATERNAL	None	None	27
3	10	C1	Abnormal	Flow sorting	t(17;22)(q21.1;q12.2)de novo	PATERNAL	None	None	37
4	10	C2	Abnormal	Flow sorting	t(2;7)(q37.2;q36.3)de novo	No Result	None	None	Not Known
5	10	C3	Abnormal	Flow sorting	t(3;11)(q21;q12)de novo	PATERNAL	None	None	37
6	10	B2	Abnormal	Flow sorting	t(2;5)(q31.1;q23.2)de novo	PATERNAL	None	Deletion	25
7	10	A3	Abnormal	Flow sorting	t(11;12)(q21;p13.33)de novo	PATERNAL	Deletion	None	31
8	10	C4	Abnormal	Flow sorting	t(7;13)(q31.3q21.3)de novo	PATERNAL	None	None	36
9	10	B3	Abnormal	Flow sorting	t(4;9)(q25;q22.3)de novo	PATERNAL	None	Deletion	34
10	7	16	Abnormal	Flow sorting	t(10;22)(q24.3;q13.31)de novo	PATERNAL	None	None	31
11	7	20	Abnormal	Flow sorting	t(2;5)(q33;q12)de novo	No Result	Deletion	None	Not Known
12	7	45	Abnormal	Flow sorting	t(X;19)(q21;p13.11)de novo	PATERNAL	None	None	31
13	7	48	Abnormal	Flow sorting	t(4;6)(q33;q22.2)de novo	PATERNAL	None	None	30
14	7	50	Abnormal	Flow sorting	t(X;8)(q22.1;q24.13)	PATERNAL	None	None	Not Known
15	7	51	Abnormal	Flow sorting	t(4;20)(p15.2;p11.23)de novo	PATERNAL	None	None	39
16	7	52	Abnormal	Flow sorting	inv ins (11;4)(q22.2;q13.2q21.3)de novo	PATERNAL	Deletion	Deletion	Not Known
17	7	53	Abnormal	Flow sorting	t(4;8)(q21.1;p12)de novo	PATERNAL	Deletion	Deletion	51
18	7	54	Abnormal	Flow sorting	t(14;15)(q23;q26.3)de novo	PATERNAL	None	None	28
19	7	55	Abnormal	Flow sorting	t(19;20)(q13.43;q11.1)de novo	PATERNAL	None	None	36
20	7	56	Abnormal	Flow sorting	t(6;21)(q16.2;q11.2)de novo	PATERNAL	None	None	36
21	7	57	Abnormal	Flow sorting	t(2;5)(p23;q11.2)de novo	PATERNAL	None	Deletion	35
					t(18;22)(q11.2;p13)de novo	PATERNAL			
22	7	11 / 2I	Normal	Flow sorting	t(2;7)(p23.3;p22.3)de novo	PATERNAL	None	None	39
23	7	2H	Normal	Linkage	t(11;21)(p15.4;p12)de novo	PATERNAL	None	None	28
24	7	1B	Normal	Linkage	t(3;9)(p26.2;p22.3)de novo	PATERNAL	None	None	30
25	7	2B	Normal	Linkage	t(1;13)(q32;q32)de novo	PATERNAL	None	None	29
26	7	3B	Normal	Linkage	t(8;12)(p23;p13)de novo	MATERNAL	None	None	35
27	7	3C	Normal	Linkage	t(6;9)(q22;p22)de novo	PATERNAL	None	None	32

Figure 1A Patient 18 t(14;15)(q23;q26.3) D15S219

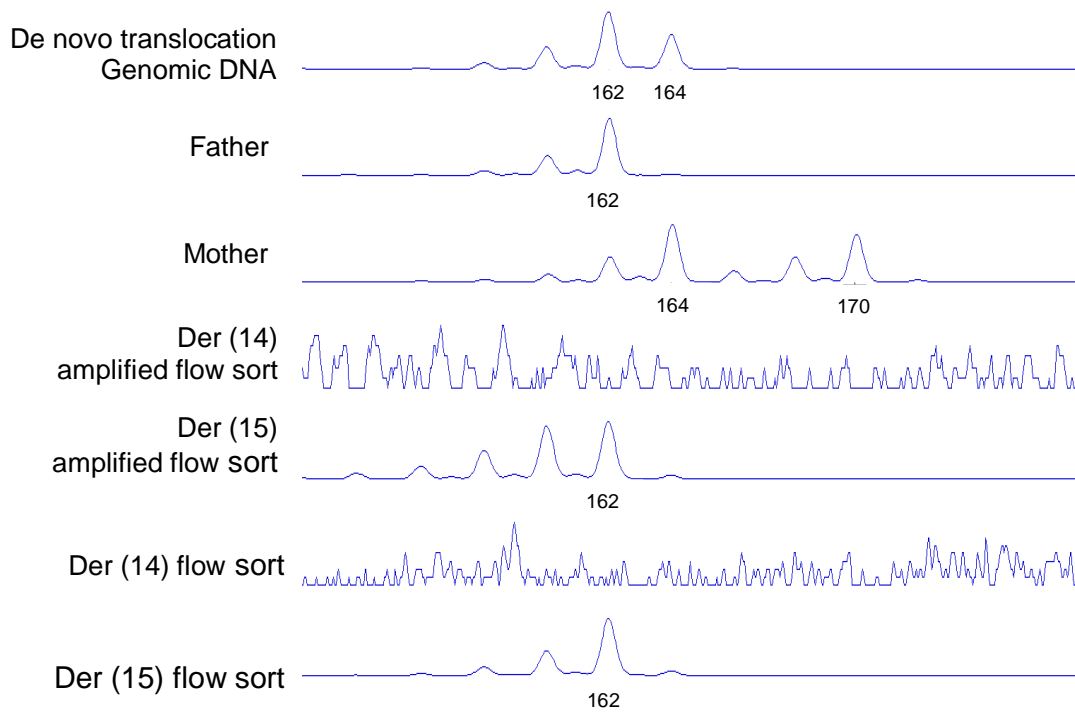


Figure 1B Patient 24 t(3;9)(p26.2;p22.3) D3S1297

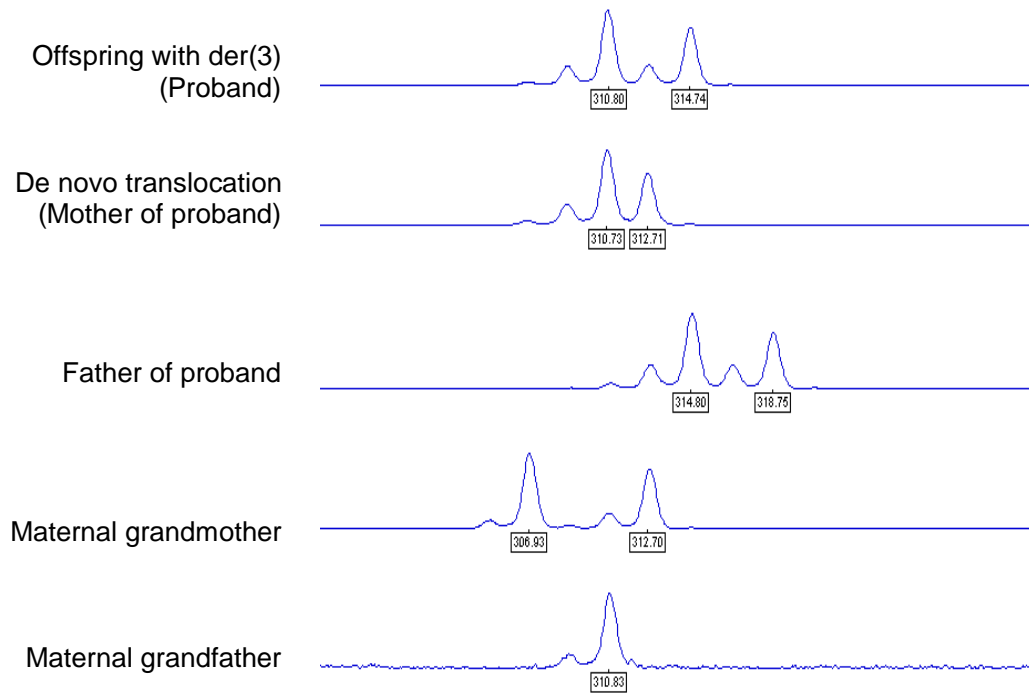


Figure 2

Cummulative age distribution of the 23 balanced translocations compared with national paternal ages adjusted for year of birth and maternal age.

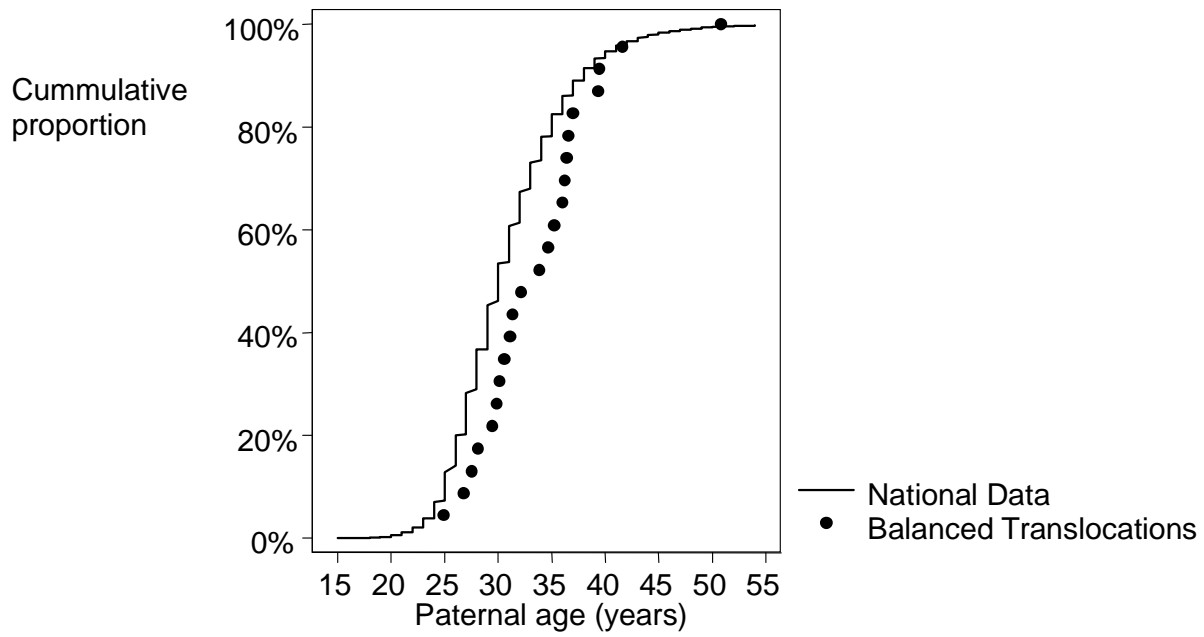


Figure Legends

Figure 1A

Patient18 carries a translocation between the long arms of chromosomes 14 and 15. D15S219 maps to 15q12, proximal to the chromosome 15 translocation breakpoint, and is therefore amplified from the flow sorted der(15) and not the der(14). The *de novo* translocation carrier has inherited alleles of 162 bp (paternal) and 164 bp (maternal). Since only the 162 bp allele is amplified from the flow sorted derivative DNA, the translocation is paternal in origin. Note that products from amplified DNA produced more stutter bands. Where results were obtained from both sources, the parental origin was always in agreement.

Figure 1B

Patient 24 carries a translocation between the short arm of chromosome 3 and the short arm of chromosome 9. Only the der(3) has been passed on to the unbalanced offspring, who is monosomic for distal 3p and trisomic for distal 9q. The chromosome 3 breakpoint interval is between 0.6 and 1.0 Mb from 3pter. D3S1297 maps to 2.0 Mb, proximal to the breakpoint interval, and so will be amplified from the der(3). The *de novo* translocation carrier has inherited alleles of 311 bp (paternal) and 313 bp (maternal). Since the 311 bp allele has been transmitted to the offspring on the der(3), the translocation is paternal in origin.

Figure 2

For each patient the national distribution of paternal ages was selected corresponding to the patient's date of birth and the maternal age. For example if a patient was born in 1950 and the mother was 35, then the distribution of paternal ages in 1950 corresponding to a maternal age of 35 was selected. All the 23 national data sets of paternal ages were combined and compared with the observed distribution of paternal ages. This procedure adjusts for both date of birth and maternal age.