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Genetic mapping of isozyme loci in *Secale cereale* L.

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Summary. The genetics and linkage relationships of several isozymatic and morphological markers have been investigated in different cultivars of rye (*Secale cereale* L.). The inheritance and the variability among cultivars of three new isozymatic zones are described: GOT2 and LAP, each of them under the control of a two-allele single locus, namely *Got2* and *Lap*, respectively; and 6PGD1 controlled by two loci, *6Pgd1a* and *6Pgd1b*, which have alleles in common. Four linkage groups have been found: *Acp2-Acp3*, *Got3-Mdh2-Lper4*, *Mdh1-6Pgd2-Pgi2*, and *Pgm-Eper2-[Eper1-Eper3]*. The assignment of these four groups to the chromosomes *7R*, *3R*, *1R*, and *4R* is discussed.

Key words: *Secale cereale* – Rye – Isozyme genes – Genetic map – Chromosomal assignment

Introduction

Rye (*Secale cereale* L.) is the only cultivated species of the genus *Secale*. Its agricultural interest is in part based on the better resistance to winter climatic conditions and other adverse environmental factors, which make it difficult to cultivate other less resistant cereals such as wheat. Furthermore, rye is one of the parentals of the triticales, allopoliploids originated from wheat × rye hybrids.

The genetics of rye have been studied less extensively than the genetics of other cereals; nevertheless, a great number of cytogenetic analyses and several molecular marker descriptions have been published. The last review by Schlegel et al. (1986) shows a compilation of data on morphological, cytogenetic, molecular, and other genetic markers. However, as can be seen in this review, there are only a few studies on genetic linkage relationships between different markers (García et al. 1982; Wehling and Schmidt-Stohn 1984; Figueiras et al. 1985; Wricke and Wehling 1985; Lawrence and Appels 1986). Another two related papers (Rebordinos and Pérez de la Vega 1987, 1988) appeared after the publication of the above-mentioned review.

According to Tanksley (1983), to investigate certain genes by linkage with certain molecular markers, knowledge on markers within intervals no wider than 20 map units is necessary. The construction of linkage maps, as complete as possible, is very important both for basic genetic research and for practical applications; thus, the existence of complete genetic maps is helpful in planning the proper crosses to combine several genes of agronomic interest in one cultivar. For instance, the introduction of resistance factors to nematode in tomato was achieved because of its linkage with an acid phosphatase marker (Rick and Fobes 1974).

In this work we have studied the inheritance of several isozymatic and morphological markers and the genetic linkage relationships between them, in order to broaden the previous data on genetic maps in rye.

Materials and methods

The materials used in this work were different cultivars of *S. cereale* L. (2n = 14) listed in Table 1.

Eight isozymatic systems from extracts of leaf tissue were analyzed by means of electrophoretic techniques: acid phosphatase (ACP, EC 3.1.3.2), glutamic oxaloacetic transaminase (GOT, EC 2.6.1.1), leucine aminopeptidase (LAP, EC 3.4.11.1), cathodal peroxidase (LPER, EC 1.11.1.7), malate dehydrogenase (MDH, EC 1.1.1.37), phosphoglucose isomerase (PGI, EC 5.3.1.9), phosphoglucose mutase (PGM, EC 2.7.5.1), and 6-

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Table 1. List of accessions

Cultivar	Origin	Abbreviation
Ailés	Zaragoza (Spain)	A
JNK	Japan	J
Lines with morphological markers	The Netherlands[a]	L
Merced	California (USA)	M
MM	León (Spain)	MM
Polycross	California (USA)[b]	P

[a] Obtained and kindly provided by Prof. J. Sybenga
[b] Obtained and kindly provided by Prof. C.O. Qualset

Table 2. Genetic markers used

Isozyme loci				Morphological markers
Leaf		Endosperm		
Acp2	(*Phos2*)[a]	*Eper1*	(*Cpx1*)[b]	*an*
Acp3	(*Phos3*)[a]	*Eper2*	(*Cpx2*)[b]	*Ps*
Got1		*Eper3*	(*Cpx3*)[b]	
Got2				
Got3	(*Got2*)[a]			
Lap				
Lper4	(*Cpx4*)[a]			
Mdh1				
Mdh2				
Pgi2				
Pgm				
6Pgd1				
6Pgd2				

[a] Gene symbol used by Pérez de la Vega and Allard (1984)
[b] Gene symbol used by García et al. (1982)

Table 3. Single-locus segregations for *Got2* and *Lap*

Parental genotypes		Progenies[a]		
		11	*12*	*22*
Got2	*12×11*	10	11	
	11×12	9	15	
	12×11	15	13	
	11×12	8	8	
	11×12	6	7	
		48	54	
Lap	*11×12*	15	15	
	11×12	12	15	
	11×12	10	14	
	11×12	22	25	
		59	69	
	11[b]	17	31	18
	12[b]	10	28	14
	12[b]	10	19	8
		37	78	40

[a] All the χ^2 values were not significant
[b] Self-pollinated plants

phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44). The electrophoretic, fractionation, and staining procedures of the leaf isozymes were carried out following the methods previously described by Pérez de la Vega and Allard (1984), Vences et al. (1987), and Rebordinos and Pérez de la Vega (1988), except for LAP, which was fractionated using the histidine buffer system described by Pérez de la Vega and Allard (1984) instead of the Poulik's buffer system used by these authors. The electrophoretic patterns are shown in Fig. 1. The endosperm peroxidase isozyme system (EPER, EC 1.11.1.7) was analyzed following the method used by García et al. (1982).

The morphological markers used were two of those described by De Vries and Sybenga (1984); *an* and *Ps* are, respectively, the antocyaninless recessive marker and the purple seed dominant marker.

In each of the cultivars a census was made to determine the genotype of a number of individuals; these were afterwards specifically crossed and their progenies were analyzed, and they were tested for the linkage/independence relationships between the genetic markers listed in Table 2. Recombination frequencies were calculated using the maximum likelihood method of Allard (1956).

Results

The inheritance of isozyme loci fitted the previous genetic control proposed by García et al. (1982) and Pérez de la Vega and Allard (1984) for most loci, and that proposed by Rebordinos and Pérez de la Vega (1988) for the GOT1 zone. Morphological markers also fitted the expected segregations according to the results by De Vries and Sybenga (1984).

These previous analyses did not describe the inheritance of three isozymatic zones used in this work: GOT2, LAP, and 6PGD1 (Fig. 1).

In previous works GOT2 has been described as an invariant zone, showing in all individuals a single and constant isozyme. The study of additional samples has allowed us to detect variability in some cultivars in which a three-banded pattern can be observed in a number of individuals: 5‰ in the cultivars Merced and MM, and

Table 4. Allelic frequencies for the loci *Got2, Lap,* and *6Pgd1*

Locus	Allele	Population				
		A	J	M	MM	P
Got2	*1*	1.000	1.000	0.997	0.997	0.986
	2			0.003	0.003	0.014
		N=590	*N*=590	*N*=598	*N*=597	*N*=594
Lap	*1*		0.969	0.770	0.606	0.638
	2		0.031	0.230	0.394	0.362
			N=258	*N*=141	*N*=71	*N*=174
6Pgd1	*1*	1.000	1.000	1.000	1.000	0.996
	2					0.004
		N=590	*N*=258	*N*=598	*N*=597	*N*=594

N – Number of seedlings

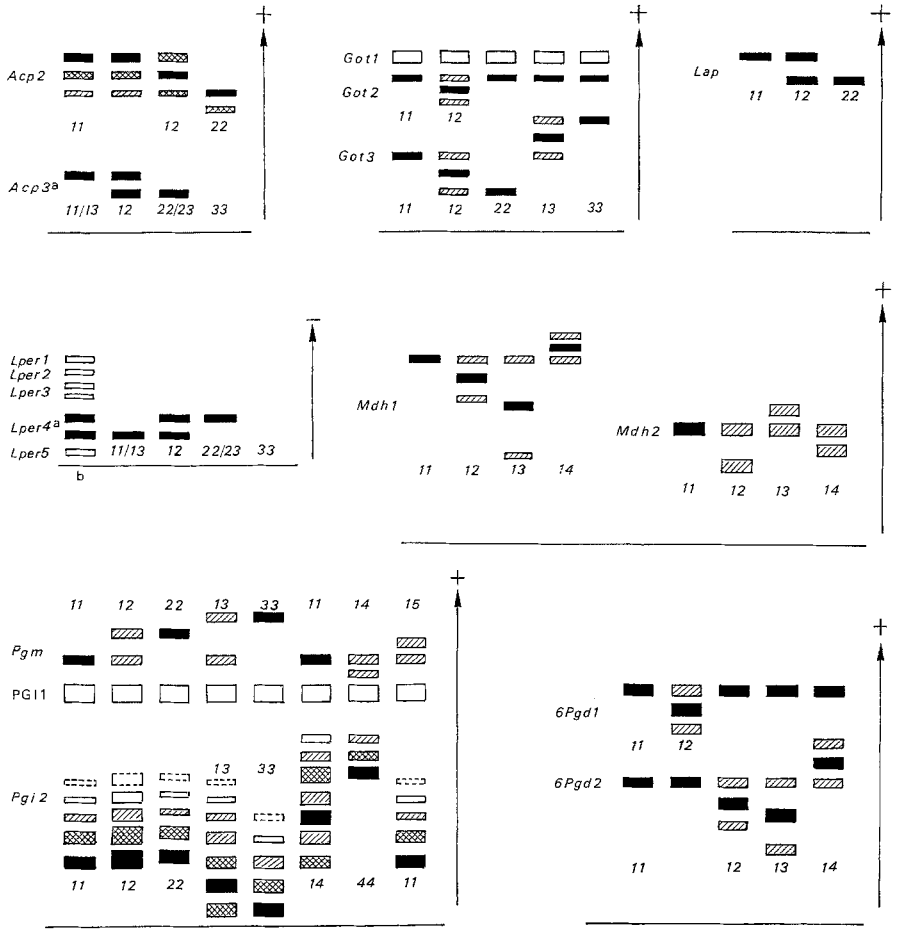

Fig. 1. Electrophoretic patterns of leaf isozyme systems. Numbers indicate the genotype attributed to each phenotype. [a] Loci with two active alleles (*1* and *2*) and one null allele (*3*). [b] Total of *Lper* isozymes

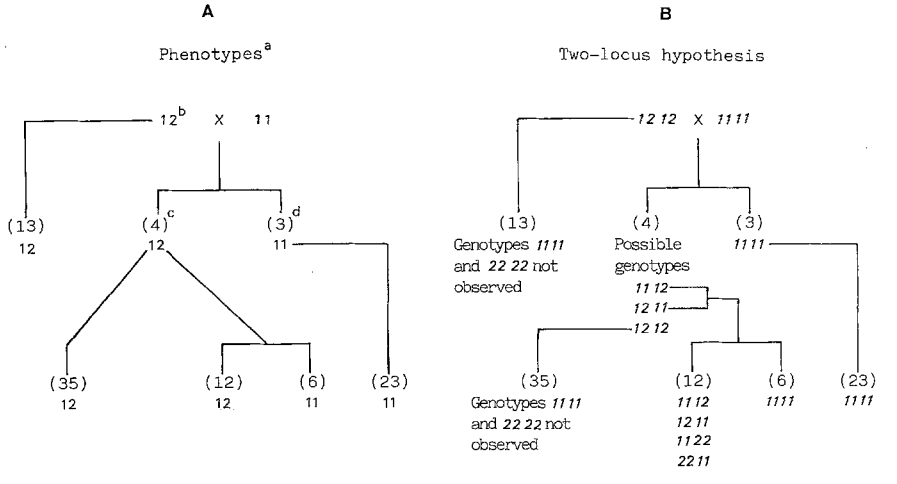

Fig. 2 A and B. Observed phenotypes and hypothetical genotypes if **A** a single locus or **B** two loci were controlling the 6PGD1 zone. Numbers in parentheses indicate the number of observed individuals. [a] 11 and 12 are, respectively, single-banded and three-banded phenotpyes and they would be the hypothetical genotypes for a single locus control. [b] A spike was emasculated and used as female in the cross by 11; a second spike was self-pollinated. [c] Two plants were self-pollinated [d] A plant was selft-pollinated

28‰ in Polycross. The hypothesis that GOT2 is a dimeric enzyme under monogenic control (locus *GOT2*) was supported, since segregations fitted a single-locus segregation (Table 3).

Like GOT2, LAP was described as monomorphic (Pérez de la Vega and Allard 1984) but, using the histidine buffer system, we have now found some variability in every population analyzed for this enzyme system (Table 4). Three different phenotypes were observed: two of them showed one band each (fast or slow) and the third had both bands (Fig. 1). The proposed controls were monomeric isozymes coded by a two-allele locus designated as *Lap*. The segregations for this locus are also shown in Table 3.

6PGD1 was variable only in the cultivar Polycross, in which some individuals showed three active isozymes

Table 5. Two-way test for linkage between segregating loci[a]

	Acp3	*an*	*Eper1*	*Eper2*	*Eper3*	*Got3*	*Lap*	*Lper4*	*Mdh1*	*Mdh2*	*Pgi2*	*Pgm*	*6Pgd2*	*Ps*
Acp2	+P[b]	nt	nt	nt	nt	−A,P	nt	−A	nt	−A	−A,P	nt	−A,P	nt
Acp3		nt	nt	nt	nt	−M,P	nt	−M	−M	−A	−M,P	−M	−M	nt
an			−L	−L	−L	−L,P	−L	nt	nt	nt	−L,M	−L	−L	−L
Eper1				nt	nt	−L	−L	nt	nt	nt	−L	+L	nt	nt
Eper2					nt	−L	−L	nt	nt	nt	−L	+L	nt	nt
Eper3						−L	−L	nt	nt	nt	−L	+L	nt	nt
Got1						nt	−L	nt	nt	nt	nt	nt	nt	nt
Got2						−P	nt	nt	nt	−P	−P	nt	nt	nt
Got3							−L,J	−A,M,P	−A,M	+A,M,P	−A,L,M,P	−A,L,M,P	−A,MM,P	−L
Lap								nt	−A	−A	−A,L	−L	−A	nt
Lper4									−M	+A,P	−M,P	−A,M,P	−P	nt
Mdh1										−A,M	+A,M	−A,M	+A,MM	nt
Mdh2											−A,M,P	−M	−A	nt
Pgi2												−A,L,M	+A,P	−L
Pgm													−A,L,M	−L

[a] + and − indicate genetic linkage or independence, respectively; nt – not tested
[b] Capital letters indicate the cultivar in which the analyses were carried out

Table 6. Linkage relationships

Loci pair	Chromosome	Cultivar	Recombinational distance in cM ±standard error
Acp2-Acp3	*7R*	P	25.0±5.2
Got3-Mdh2	*3R*	A M P	16.6±1.7
Mdh2-Lper4	*3R*	A P	30.2±5.8
Got3-Lper4	*3R*	A M P	Independent
Mdh1-6Pgd2	*1R*	A MM	25.9±3.4
Mdh1-Pgi2	*1R*	A M	36.7±3.6
Pgi2-6Pgd2	*1R*	A P	28.6±3.1
Pgm-[Eper-1-Eper3]	*4R*	L	36.7±3.9
Pgm-Eper2	*4R*	L	18.4±8.2

instead of the single isozyme present in most of the cultivars. These two patterns agreed with the described dimeric nature of 6PGD isozymes. The segregations obtained from a group of related plants, which were crossed with each other and/or selfed, indicate that this isozymatic zone seems to be controlled by two loci (*6Pgd1a* and *6Pgd1b*) with two alleles in common. One three-banded individual (phenotype 12) was crossed as maternal parent with a single-banded individual (phenotype 11), and the progeny showed both parental isozyme phenotypes. Another spike from the maternal plant (12) was selfed, and all 13 seedlings of the progeny showed phenotype 12. Plants obtained from the cross 12×11 were forced to self-pollinate; a selfed 11 plant yielded 23 seeds, all of them with the phenotype 11; on the other hand a 12 plant, when selfed, yielded 6 individuals 11 and 12 individuals with the phenotype 12, while a second self-pollinated 12 plant gave 35 descendents, all with the phenotype 12. Figure 2 shows the phenotypes observed and the hypothetical genotypes of these progenies if a single locus or two loci were controlling the 6PGD1 zone.

Genetic linkage relationships were analyzed on the basis of the segregations obtained from several crosses for each pair of loci. Distances between loci were jointly estimated from all the progenies segregating for each pair of loci. Since neither heterogeneity among families nor among cultivars was found for the maximum likelihood estimates of recombination, the recombination frequencies and genetic map distances should be similar in all cultivars tested.

If two parental plants are heterozygous for different pairs of alleles, the number of expected genotypic classes in the progeny is high and, therefore, progenies should have a high number of individuals to avoid empty or almost-empty classes, which would interfere with the maximum likelihood estimations. Since it is not always possible to get numerous progenies, we predominantly carried out test crosses (i.e., diheterozygous × dihomozygous) in order to reduce the number of expected phenotypic classes. To save space, the complete two-locus segregations' list is not included in this paper, but it can be obtained from the authors on request.

A total of 68 combinations of loci has been tested for genetic linkage; 9 of these loci appeared to be linked (Table 5) in four different linkage groups. A recompila-

tion of the linkage relationships results can be found in Table 6.

Discussion

The genetic control of zones GOT2, LAP, and 6PGD1 was elucidated by means of different specific crosses (Table 3 and Fig. 2).

GOT2 behaved as a monogenic-controlled zone. The *Got2* gene, probably located in the long arm of chromosome *6R* (Tang and Hart 1975), would have at least two active alleles and would control dimeric isozymes, since the heterozygotes have three electrophoretic bands. Five 12×11 crosses were made and all the χ^2 tests for the 1:1 segregation were not significant; there was no heterogeneity among these five crosses. The dimeric nature of GOT2 isozymes was expected, since such is the described structure for rye GOT1 and GOT3 isozyme zones (Pérez de la Vega and Allard 1984; Rebordinos and Pérez de la Vega 1988). Isozymes of different zones do not form hybrid bands.

LAP was analyzed on the basis of four 12×11 crosses and three 12 selfed plants. Chi-square tests for 1:1 and 1:2:1 segregations, respectively, were not significant and heterogeneity was not detected. Consequently, the data fit the hypothesis of a monomeric enzyme controlled by one locus with two active alleles. As in rye, LAP is a monomer in different plant species including other cereals such as maize, wheat, rice, etc. (Tanksley and Orton 1983). Two loci controlling LAP isozymes have been described in rye, one of them located in chromosome *4R* and the other in the short arm of chromosome *6R* (Tang and Hart 1975). Our electrophoretic method revealed only these two allozymes, and no evidence of gene duplication has been found. *Lap* behaved as genetically independent from some markers located in chromosome *4R* (*Pgm* and *EPer1*). On the other hand, we could not analyze any family to study the existence (or absence) of genetic linkage between *Lap* and the markers located in the chromosome *6R* (one of the *6Pgd1* loci and *Got2*). Therefore, it is possible that the locus we have worked with was the locus located in *6R*, named *Lap1* by Schlegel et al. (1986).

6PGD1 is most likely controlled by two loci. In fact, if we assume a monogenic control, for this zone, the probability of some segregations showed in Fig. 2 is very low, especially the absence of homozygous phenotypes as a result of the selfing of heterozygous plants ($P < 0.0003$ for the progeny of 12 seedlings and $P < 3\ 10^{-11}$ for the progeny of 35). On the other hand, to be able to explain every progeny if we assume a digenic control, the phenotypically heterozygous maternal plant must be heterozygous at both loci (12 12) (Fig. 2). The existence of two loci for 6PGD1 was previously described on the basis of the location data obtained from the analysis of wheat-rye addition lines (Schlegel et al. 1986). Since the two loci share the same alleles, they probably originated by a duplication, but their present location is at independent loci since they were related to the chromosomes *4R* and *6R*, respectively.

In relation to the linkage relationships between loci, we have detected four linkage groups (Table 7). The first one includes the two phosphatase loci, *Acp2* and *Acp3*. This result is not surprising because genes for phosphatases were located on chromosome *7R* by Hart (1979); Tang and Hart (1975) and Salinas and Benito (1984a) located these two genes in chromosome arm *7RS*. However, no previous data on recombination frequencies between them have been reported. Although we could not analyze families segregating simultaneously for the phosphatase markers and for the *Got1* and *an* loci, it is interesting to note here that one of the loci controlling *Got1* and the *an* locus were related to chromosome arm *7RL* (Rebordinos and Pérez de la Vega 1988).

A second group included *Got3*, *Mdh2*, and *Lper4*. The estimated distance betwen *Got3* and *Mdh2* is 16.6 ± 1.7 centimorgans (cM). This result agrees with the distance of 13 ± 3 cM reported by Figueiras et al. (1985). *Mdh2* and *Lper4* are separated by 30.2 ± 5.8 cM, while *Got3* and *Lper4* behaved independently. In fact if we add the two segments (16.6 ± 30.2), the distance between the two farthest loci must not be significantly different from the independence condition. Figueiras et al. (1985) also studied segregational progenies for *Mdh2* and *Lper4*, but they did not find any linkage relationships. Allard (1956) suggested that the different values for recombination observed in some cases could have been the result of seasonal influences upon recombination values, differences among the parental strains used, different recombination values in the sexes, or a combination of these factors. The difference in the recombination distance between the results of Figueiras et al. (1985) and our work could be mainly due to the different rye cultivars used.

This linkage group is related to chromosome arm *3RL* because *Got3* and *Mdh2* were located in it (Schlegel et al. 1986) and, consequently, *Lper4* should also be in chromosome *3R*. However, other works have found genetic linkage between *Lper4* and *Lper3* (Figueiras et al. 1985) and related both to chromosome arm *2RS* (Salinas and Benito 1984b). This disagreement about the location of these loci is difficult to explain, but if we consider the frequent appearance of reciprocal translocations in some rye cultivars it could be understood. It is known that the genus *Secale* has evolved by reciprocal translocations (Stutz 1972) and that in some populations of *S. cereale* several translocations are maintained (Candela et al. 1979; Figueiras et al. 1979). Thus, it is possible that in some cultivars there is a translocation that could be mod-

ifying the standard linkage groups and/or the distance between markers.

A third group is integrated by *Mdh1, 6Pgd2,* and *Pgi2*. Locus *6Pgd2* would be the central one because the greatest distance (36.7 ± 3.6 cM) was obtained for *Mdh1* and *Pgi2*. Loci *Mdh1* and *6Pgd2* were also described as being linked by Figueiras et al. (1985) but these authors did not study *Pgi2*. Since in a previous work (Salinas and Benito 1983) *6Pgd2* was related to the long arm of chromosome *2R*, Figueiras et al. concluded that *Mdh1* would be in it; otherwise, *Pgi2* was located by Salinas (1982) in the short arm of chromosome *1R* in agreement with Chojecki and Gale (1983) (in this last work the name of the locus is *Gpi1*, as listed by Schlegel et al. 1986), and a further work (Lawrence and Appels 1986) indicated that *6Pgd2* and *Pgi2* were on chromosome *1R* separated by 22 ± 3.7 cM. This distance is comparable to the estimated 28.6 ± 3.1 cM obtained by us. Consequently, we assume that this linkage group is most probably related to the *1R* chromosome, and the discrepancy on the location of *6Pgd2* can be attributed to a mislocation or to differences in translocated segments between the cultivars analyzed in different works.

The last linkage group consisted of *Pgm, Eper2, Eper1,* and *Eper3*. The loci *Eper1, Eper2,* and *Eper3* are closely linked (Rebordinos and Pérez de la Vega 1987). *Pgm* was located in *4RS* (Figueiras et al. 1985) and *Eper1* in the long arm of the same chromosome, being *Eper3* related to the *7RS* chromosome arm; since *Eper2* was found to be linked to *Eper3* it was also located in *7RS* (Salinas and Benito 1984b). We do not know if locus *Eper3*, which we analyzed, is the same on that Salinas and Benito (1984b) located, because we have being working with endosperm of mature seed, while they studied isozymes during seed formation. We propose that this group is associated with chromosome *4R*, although the possible existence of differences in reciprocal translocations are in this case especially remarkable. In fact, chromosomes *4R* and *7R* seem to have evolved from *S. montanum* chromosomes after a reciprocal translocation (Koller and Zeller 1976).

To sum up, we have offered further data about leaf isozyme inheritance in rye and linkage relationships between a collection of markers widely used in different works. The assignment of genes to linkage groups and chromosomes can be disturbed, as stated Schlegel et al. (1986), by the reported existence of structural differences between cultivars and, in some of them, between individuals. Therefore, further studies, including cytogenetic analysis, will be needed to clarify the controversy of some of these results.

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