date nuclei of the mutant rats up to postnatal day 20. On postnatal day 30 and day 40, a few necrotic neurons were found in the caudate nuclei of some of the Slc:Wistar and the mutant rats. On postnatal days 60 and 120, numerous necrotic neurons were encountered in the caudate nuclei of the mutant rats. The number of necrotic neurons varied not only among the different mutant rats but also between right and left caudate nuclei in the same mutant rat (data not shown).

Discussion

Among many kinds of mutant rodents with movement disorders, to our knowledge only one mutant mouse, *weaver*, has been reported as having abnormalities in the striatum. In the striatum of the weaver mouse, however, a massive postnatal loss of dopamine, owing to the cell death of dopaminergic neurons in the substantia nigra pars compacta, has been reported, but no neuronal degeneration was described[2-4]. Thus, the mutant rat described in this paper, *groggy*, may be a new kind of neurological mutant displaying neuronal degeneration in the striatum.

Although the first noticeable abnormal movement appeared around postnatal day 15 in the groggy rat, the histological study of the brains during postnatal growth showed that the onset of the appearance of necrotic neurons in the striatum was around postnatal day 60. This fact suggests that the neuronal degeneration in the striatum of the groggy rat may not be the primary effect of the mutant gene, but a secondary one. At present, no obvious histological abnormalities have been observed in other regions of the brain of the groggy rat except for the striatum. Further immunohistochemical studies to identify what types of neurons degenerate in the striatum of the groggy rat may provide some clues for the elucidation of the real effect of the mutant gene.

In human beings, the occurrence of neuronal degeneration in the striatum has been reported in some extrapyramidal disorders such as Huntington's chorea, striatonigral degeneration, hereditary putaminal (striatal) necrosis, and infantile bilateral striatal necrosis, but the precise mechanism of loss of striatal neurons in these disorders remains obscure[5-8]. The groggy rat may be a useful model for studying this problem.

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Role of genetic variability in neonatal jaundice. A prospective study on full-term, blood group-compatible infants

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Abstract. A series of genetic, developmental and environmental variables have been analyzed in a prospective sample of full-term newborn babies, compatible with their mothers in the major blood group systems, in order to attempt an evaluation of the effect of these variables on serum bilirubin level during the first few days of life.

Three genetic factors (PGM_1, ACP_1 and ADA) and three non-genetic variables (rise of bilirubin level during the first day of life, a mother with a history of previous abortion, and use of alcoholic beverages by the mother) have a significant predictive value for the separation of newborns with clinically relevant jaundice from other infants.

Key words. Neonatal jaundice; genetic polymorphism; risk of hyperbilirubinemia; adenosine deaminase; acid phosphatase; phosphoglucomutase.

Neonatal hyperbilirubinemia is a relatively common symptom associated with a heterogeneous class of disorders. The role of single Mendelian genetic factors, and of developmental or environmental conditions, is well known. In most cases the mechanism underlying the development of hyperbilirubinemia in full-term, blood group-compatible infants is likely to be multifactorial [1, 2]. In the present paper we report a prospective study performed on a series of full-term newborn babies, compatible with their mothers in the major blood group systems, in order to elucidate the role of some polymorphic enzymes in the development of neonatal jaundice.

Materials and methods

A series of 236 newborn babies, born consecutively at full term, percentile class > 10, and compatible with their mothers in ABO and Rh systems, were studied.

Variables considered are reported in table 1. Clinically significant hyperbilirubinemia was defined as the need for phototherapy (serum bilirubin > 12 mg/dl).

Blood groups and the phenotype of other genetic polymorphisms were determined by standard methods [3]. Rao's discriminant analysis and regression analysis were performed according to SPSS programs on an IBM personal computer [4].

Results

A set of genetic and a set of non-genetic variables which made a significant contribution to the separation of infants who needed treatment from those who did not, were selected by the discriminant procedure (table 2). A high increment of bilirubin during the first 24 h of life, a mother with a positive history of previous abortion, use of alcholic beverages by the mother, the presence of the PGM_1^2 allele and the simultaneous presence of p^a, p^b (ACP_1) and ADA^2 alleles increased the risk of hyperbilirubinemia. Similar results were obtained by regression analysis (table 3). Overall, the variables account for 15.7% of bilirubin variance. Genetic variables contribute 9.1% and non-genetic variables 6.6%.

It is worth noting that weight and gestational length made no detectable contribution to discrimination, and these variables were rejected by the discriminant procedure. Seven newborns revealed a deficit of G-6-PD. None of them showed clinically significant hyperbilirubinemia. The distribution of discriminant variables, along with that of birth weight and gestational length, in relation to neonatal jaundice is shown in table 4.

Table 2. Discriminant analysis. Rao's V is a measure of distance between groups

Step	Variable entered	Change in Rao's V	Significance of change
1	ACP_1BA-ADA	17.9	0.0000
2	Increase of bilirubin	9.3	0.0023
3	Abortion	6.1	0.0134
4	Alcohol	3.9	0.0469
5	PGM_1	3.6	0.0581

Significance of discriminant function

χ	df	P
37.209	5	0.0000

Standardized canonical discriminant function coefficients

ACP_1BA-ADA	0.73
Increase of bilirubin	0.44
Abortion	0.44
Alcohol	0.31
PGM_1	0.30

Table 3. Regression analysis

Correlation with serum bilirubin level	
ACP_1BA-ADA	0.267
Increase of bilirubin	0.179
Abortion	0.162
PGM_1	0.113
Alcohol	0.106

Multiple R = 0.3859; R^2 = 0.1489.
Analysis of variance: F = 8.1523; Significance: 0.0000.

Equation of regression

Variable	Beta	t	Significance
ACP_1BA-ADA	0.268	4.420	0.0000
Increase of bilirubin	0.158	2.602	0.0099
Abortion	0.157	2.584	0.0104
PGM_1	0.107	1.767	0.0785
Alcohol	0.108	1.774	0.0774

Table 1. Variable examined

Mother	Labor	Fetus-newborn	
Age	Anesthesia	Sex	ABO blood group
Smoking	Analgesia	Birth weight	Rh blood group
Alcohol	Type of labor	Placental weight	Haptoglobin (Hp) phenotype
Abortion (spontaneous)		Gestational duration	Erythrocyte acid phosphatase (ACP_1) phenotype
Secretor (Se) phenotype			
ABO blood group		Birth order	Placental alkaline (P1) phenotype
Rh blood group		Bilirubin level at birth	Adenosine deaminase (ADA) phenotype
Haptoglobin (Hp) phenotype		Hematocrit at birth	Combination of ADA and ACP_1 phenotype
		Increase of bilirubin during the first 24 h	Adenylate kinase (AK_1) phenotype
			Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency
			Phosphoglucomutase locus 1 (PGM_1) phenotype

Table 4. Distribution of discriminant variables in relation to neonatal jaundice

Variable	Category	Absolute frequencies (n° of individuals) mean values and standard error of variables	
		Treated newborns	Not-treated newborns
ACP_1 and ADA	ACP_1BA carriers of ADA^2 allele	5	7
	Other genotypes	16	208
PGM_1	Carriers of PGM_1^2 allele	14	102
	PGM_1^1/PGM_1^1	7	113
Previous abortion	Positive	8	35
	Negative	13	180
Alcoholic beverages	Used	15	114
	Not used	6	101
Increment of bilirubin during the first day (mg/dl)	mean	5.44	3.90
	SE	0.43	0.17
Birth weight (g)	mean	3478	3389
	SE	94	25
Gestational length (weeks)	mean	40.00	40.19
	SE	0.39	0.08

Discussion

The identification of specific genetic factors which influence multifactorial diseases is of both theoretical and practical importance, especially in disease prevention. Research in this area should be directed to factors responsible for 'normal' variability: genetic polymorphisms appear to be the most important candidates for this type of analysis[5,6].

The polymorphic enzymes listed in table 1 were selected for study on the basis of previous clinical observations and/or information about their function in metabolism. Placental alkaline phosphatase[7] interacts with ABO blood groups and influences the susceptibility to neonatal jaundice in ABO incompatible infants[8]. In our sample, which included only compatible infants, no association was found between P1 and neonatal hyperbilirubinemia.

Phosphoglucomutase[9] catalyzes the reaction Glucose-1-P$\rightleftharpoons$Glucose-6-P. In vitro, the product of the PGM_1^2 allele is more active than that of PGM_1^1[10], and therefore genetic variability at the PGM_1 locus might influence the production of uridine-diphosphate-glucose and in turn bilirubin glucuronide formation. The data showed that the incidence of jaundice increases with the dose of PGM_1^2 (6%, 11% and 20% for PGM_1 1, PGM_1 2-1 and PGM_1 2, respectively).

An association between the ACP_1 phenotype[11] and neonatal jaundice has been previously observed by our group[12]. Recently it has been suggested that ACP_1 may act in vivo as a flavin-mononucleotide-phosphatase[13–15]. The enzyme may regulate glutathione reductase, a flavoenzyme which exerts a key role in the maintenance of red cell integrity[13]. It has also been shown that the enzymatic activity of ACP_1 is modulated (activated or inhibited) by purines and inhibited by folic acid[13–15]. There is a quantitative variation in the ACP_1 enzymic activity in different phenotypes, with the contribution of the alloenzymes increasing in the order $p^a < p^b < p^c$[11]. However, when the effect of purine and folic acid modulation is considered, the contribution of the alloenzymes is either in the order $p^b < p^a < p^c$, or $p^c < p^a < p^b$. This difference in the order indicates that these two characteristics of the enzyme are independent of each other[14].

Adenosine does not influence ACP_1 activity, whereas its derivative inosine is a strong activator. The deamination of adenosine to inosine is catalyzed by ADA, another polymorphic enzyme which shows quantitative variations of enzymatic activity among phenotypes[16,17]. ADA polymorphism could therefore influence the relation between ACP_1 and neonatal jaundice by affecting the inosine level. In the present sample, ACP_1-BA newborns carrying ADA^2 showed a significantly higher incidence of the need for phototherapy, suggesting an interaction at the clinical level between the two polymorphic systems.

Adenylate kinase (AK_1)[18] catalyzes the reaction $2ADP \rightleftharpoons ATP + AMP$, and may influence the metabolic pathway controlling glucuronide formation at several stages. AK_1 could also influence ACP_1 in a way similar to that suggested for ADA. In our sample, no significant association was found between hyperbilirubinemia and this enzyme.

In conclusion, the results of the present study indicate that some of the enzymes included in the analysis may have a significant role in influencing the development of clinically significant jaundice in full-term, compatible infants.

The six variables identified as significant predictors of jaundice account for a relatively small part of the variance of bilirubin level, therefore it appears that many more factors are involved in neonatal hyperbilirubinemia.

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Cyercenes, novel pyrones from the ascoglossan mollusc *Cyerce cristallina*. Tissue distribution, biosynthesis and possible involvement in defense and regenerative processes

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Abstract. The extraordinary regenerative phenomena in the marine ascoglossan mollusc *Cyerce cristallina* are described here for the first time. The possible correlation between cyercenes, pyrones recently isolated from the mollusc regenerating dorsal appendages (cerata), and the processes of chemical defense and regeneration in the mollusc, was investigated by studying the tissue distribution, biological activity and biogenesis of cyercenes. Differences in distribution between the *C. cristallina* mantle, cerata and mucous secretion were found. Cyercenes showed activity in the *Hydra vulgaris* regeneration assay and the mosquito fish ichthyotoxicity assay. The de novo biosynthesis of cyercenes from propionic acid was demonstrated by means of in vivo adsorption experiments with radiolabeled propionate.
Key words. Pyrones; polypropionates; marine molluscs; regeneration; growth factors; ichthyotoxins; marine toxins; *Hydra*.

Marine gastropod molluscs have provided many attractive models for the study of marine biochemistry, physiology, neurobiology and ecology[1] and, from this point of view, the species belonging to the ascoglossan genus *Cyerce* are no less interesting. Ecological studies conducted on the Australian species *Cyerce nigricans* have shown, for example, that the live mollusc is repellent to coral reef fish, as is its organic crude extract[2], although a recent chemical study conducted on the same species did not succeed in correlating the substances isolated from the mollusc mantle with this chemical deterrence[3]. Morphologically very similar to *C. nigricans* is the Mediterranean species *Cyerce cristallina* (Trinchese, 1881), whose body volume is mainly due to the presence of aposematically colored dorsal appendices (cerata)[4]. When the animal is attacked by predators, the cerata are detached from the mantle and exhibit prolonged contractions while secreting large amounts of a supposedly toxic mucous secretion (Perrone[4], and unpublished observations). In the laboratory, this typical defensive behavior, known as autotomy, can be induced either by pinching the cerata with pliers or by slightly raising the temperature of the seawater containing the mollusc (unpublished observations). After the occurrence of the autotomic process, the mollusc provides a striking example of regeneration by completely reproducing the cerata within only 7–10 days (fig. 1); to the best of our knowledge, this phenomenon, which is probably very unusual for such a complex organism, has not been reported before.

With the aim of characterizing some of the chemical signals which are, at least in part, responsible for either the chemical defense or the regenerative processes of *C. cristallina*, we very recently conducted a chemical study on the mollusc cerata and isolated the seven novel py-