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Short Communication

Molecular Basis of Phenylketonuria in Italy

I. Dianzani, C. Camaschella*, G. B. Ferrero, L. De Sanctis, A. Ponzone and R. G. H. Cotton**

Istituto di Clinica Pediatrica, Piazza Polonia 94, 10126 Torino, Italy
* *Dipartimento di Scienze Biomediche e Oncologia Umana, Via Genova 3, 10126 Italy*
** *The Murdoch Institute, Royal Children's Hospital, Flemington Road, Parkville 3052, Australia*

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Introduction

In recent years, a lot of data have been produced to elucidate the molecular basis of phenylketonuria (PKU), the disease caused by the deficiency of the liver enzyme phenylalanine hydroxylase (PAH). The identification of the RFLP haplotypes associated to the PAH gene has offered the means for prenatal diagnosis and carrier detection, previously infeasible or not always reliable by using biochemical methods. The characterization of the causal mutations may extend both these analyses, available only for selected families by RFLP studies, to the entire population. So far more than thirty mutations have been identified (ref. in 1–7). Most of the studies have been focussed on North European populations and for many of them 50 to 70% of the PKU causal mutations have been identified. On the other hand, the information about Mediterranean populations is scanty.

In a previous paper we have observed that the distribution of RFLP haplotypes associated with the phenylalanine locus is different in Italy as compared to North European populations, suggesting the existence of different causal mutations (1). A similar RFLP haplotype pattern has been reported for another Mediterranean populations, the Turks (ref. in 1).

The aim of this study was to identify the molecular defects in Italian PKU alleles.

Material and Methods

DNA extracted by standard methods from unrelated Italian PKU patients was amplified across single exons by using the appropriate PCR primers.

The amplified exons were then screened for the presence of mutations by using different methods, including the chemical cleavage of mismatch (CCM) method (followed by direct sequencing when a mismatch was identified) (1), allele specific oligonucleotide (ASO) analysis, digestion with restriction endonucleases for mutations which abolish or create novel restriction sites. Whereas the CCM approach allowed us to screen for all the mutations (novel or known) that affect a DNA region, the other methods were used to search for specific DNA changes, already reported as a cause for PKU in other populations. In total, twenty mutations were searched for within Italian PKU alleles (Table 1) and five PAH exons (exons 7–11) were completely screened.

Results

Two novel mutations were identified: a G → A substitution at the 5′ junction splice site of intron 7 (IVS VII-1) (1) and a mutation at codon 359 (I. Dianzani, submitted).

Only four out of the eighteen known mutations were actually found in Italian PKU patients.

This search allowed characterization of less than 30% of the mutations responsible for PKU in Italy.

Discussion and Conclusion

A great heterogeneity within the molecular defects responsible for PKU in Italy was suggested by the scattered distribution of RFLP haplotypes. This pat-

Table 1. Mutations analysed in the Italian population within the present study.
The exact references for mutations 2, 3, 4, 6, 7, 10, 11, 14, 18, 19 and 20 are reported in ref. 2; mutations 1 and 15 are reported in ref. 4; mutations 5 and 9 are reported in ref. 5; mutations 8 and 13 were reported respectively in ref. 8 and 7. References for the remaining mutations have been cited in the text.

Mutation (codon)	Method	Tested alleles	Positive alleles	%
1. 39	Mbo II	32	0	–
2. 158	ASO	92	0	–
3. 243	CCM	32	0	–
4. 252	CCM	32	0	–
5. 259	CCM	32	0	–
6. 261	CCM/ASO	74	4	5.4
7. 272	CCM/BamHI	74	0	–
8. 273	CCM/BamHI	74	0	–
9. 277	CCM	32	0	–
10. 280	CCM	32	0	–
11. 281	CCM	32	0	–
12. IVS VII-1	CCM/ASO	92	4	4.3
13. 299	CCM	32	0	–
14. 311	CCM	32	0	–
15. 349	CCM	32	0	–
16. IVS X-546	CCM/ASO	130	13	10
17. 359	CCM/ASO	130	1	0.7
18. 364	CCM	32	0	–
19. 408	ASO	68	2	2.9
20. IVS XII-1	ASO	68	1	1.5

tern was strikingly diffcrent by that presented by North European populations, where two haplotypes (haplotypes 2 and 3), accounted for a large number of PKU chromosomes (2). These alleles, which in Northern Europe are in linkage disequilibrium with two specific mutations, are very rare in Italy. On the other hand, haplotype 6 frequent in another Mediterranean population (the Turks) attains a frequency of 11% in Italy and is virtually absent in Northern Europe (1).

This study, aimed at identifying the molecular changes responsible for PKU within the Italian population, confirms our previous hypotheses. First the screening for seventeen known mutations, most of them identified in North European PKU, allowed identification of less than 10% of the PKU mutations in Italian patients, confirming the existence of different molecular lesions as the cause of the majority of PKU defects in Italy as compared to North Europe. Secondly, two novel mutations (IVS VII-1 and codon 359) were identified in Italian PKU alleles. These mutations attain an overall prevalence of only 5%. The higher frequency (10%) was observed for a mutation described in Mediterranean PKU subjects (IVS X-546), in linkage disequilibrium with haplotype 6 (3). Thus, the association of the RFLP haplotype and the mutation analysis data allows to expect many mutations, each with a low frequency, within the remaining uncharacterized PKU alleles.

The exiguity of the mutations identified in Italian PKU patients obliges prenatal diagnosis and carrier detection to rely still on RFLP analysis in selected families.

In conclusion, a different molecular basis for PKU is present in Italy as compared with North European populations. This is shown by a different RFLP haplotype distribution among PKU alleles, by the rarity of the most common North European PKU mutations in Italian patients and by the identification of DNA changes which seem specific for Mediterranean PKU.

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