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# Phenotypic diversity of patients with LEOPARD syndrome carrying the worldwide recurrent p.Tyr279Cys PTPN11 mutation

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**Abstract:** LEOPARD syndrome (LS, OMIM 151100) is a rare monogenic disorder. The name is an acronym of its major features, including multiple lentigines, electrocardiographic conduction defects, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, growth retardation and sensorineural deafness. LS develops due to mutations in the protein-tyrosine phosphatase nonreceptor-type 11 (PTPN11) gene. Here, we report a 51-year-old Hungarian male patient affected by LS. Direct sequencing of the PTPN11

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| | gene revealed a worldwide recurrent missense mutation (c.836A/G; p.Tyr279Cys), which has been previously identified in 47 LS patients. Comparison of the clinical phenotypes of our patient and those reported in the literature revealed great phenotypic diversity, despite the shared genotype. |
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# Phenotypical diversity of patients with LEOPARD syndrome carrying the worldwide recurrent p.Tyr279Cys PTPN11 mutation

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**Concise title: Phenotypical diversity in LEOPARD syndrome**

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**ABSTRACT**

LEOPARD syndrome (LS, OMIM 151100) is a rare monogenic disorder. The name is an acronym of its major features such as multiple lentigines, electrocardiographic conduction defects, ocular hypertelorism, pulmonary stenosis, abnormalities of genitalia, retardation of growth and sensorineural deafness. LS develops due to mutations in the protein-tyrosine phosphatase nonreceptor-type 11, *PTPN11*. Here we have investigated a 51-year-old Hungarian male patient affected by LS. Direct sequencing of the *PTPN11* gene revealed a worldwide recurrent missense mutation (c.836A/G; p.Tyr279Cys), which has been previously identified in 47 LS patients. Comparison of the clinical phenotypes of our patient and the ones reported in the literature demonstrates great phenotypic diversity despite of the same genotype.

**Key words:** LEOPARD syndrome, *PTPN11* gene, worldwide recurrent missense mutation, phenotypic diversity, p.Y279C

## INTRODUCTION

LEOPARD syndrome (LS, OMIM 151100) - a rare monogenic disorder belonging to the family of neuro-cardiofacio-cutaneous syndromes [16] - is inherited as an autosomal dominant trait with full penetrance and variable expressivity [3, 10]. The major features of LS include multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth and sensorineural deafness, this is why the syndrome is referred with the LEOPARD acronym [4, 10]. LS develops as a consequence of mutations of the protein-tyrosine phosphatase nonreceptor-type 11 (*PTPN11)* gene encoding a cytoplasmic protein tyrosine phosphatase (SHP-2), which regulates intracellular signaling and controls several distinct developmental processes [15, 19]. In about 85% of the cases a heterozygous missense mutation is detected in the exon 7, 12 or 13 [6, 11]. Among the so far identified missense mutations, there are two (p.Tyr279Cys and p.Thr468Met), which account for about 65% of all LS cases worldwide [6, 11].

Here we report a Hungarian LS patient carrying the most common p.Tyr279Cys heterozygous missense mutation and compare his clinical features with the symptoms of previously reported LS patients (n=47), in whom the same causative mutation was identified.

## PATIENTS AND METHODS

### Patients

A 51-year-old Hungarian male patient was admitted to the cardiology unit of the Orosháza Hospital (Orosháza, Hungary) with dizziness and palpitation. On investigation, facial anomalies including ocular hypertelorism, palpebral ptosis, dysmorphic ear, slight mandibular

prognathism (Fig 1a) and pigmentation abnormalities such as multiple lentigines (Fig 1b) and café-au-lait spots (Fig 1c) were also observed. Cardiology investigations revealed third degree atrioventricular block (Fig 1d). The patient is deaf and dumb since he was born and mild growth as well as mental retardations were also present. Urological investigation revealed mild genital abnormalities such as atrophic testes. These clinical symptoms suggested LS, therefore genetic screening of the *PTPN11* gene was also performed. The patient was born out of wedlock, however the family members of his father and his mother were available for the clinical and genetic investigations (Fig 1e). All the investigated relatives were clinically unaffected suggesting the presence of a *de novo* mutation in the patient.

**Genetic investigations**

Blood sample was taken from the patient and genomic DNA has been isolated by a BioRobot EZ1 DSP Workstation (Qiagen; Godollo, Hungary). All the coding regions of the *PTPN11* gene and the flanking introns were amplified and sequenced (primers were used as displayed on the UCSC Genome Browser www.genome.ucsc.edu). The investigation was approved by the Internal Review Board of the University of Szeged. Written informed consent was obtained from the patient, and the study was conducted according to the Principles of the Declaration of Helsinki.

**RESULTS**

Direct sequencing of the coding regions and the flanking introns of the *PTPN11* gene revealed a heterozygous missense mutation (c.836A/G; p.Tyr279Cys) in the seventh exon (Fig 1f). The clinically affected patient carried the mutation in heterozygous form, while the unrelated healthy controls carried the wild type sequence (Fig 1g).

## DISCUSSION

The investigated Hungarian LS patient carries one of the most common missense mutation (p.Tyr279Cys) of the *PTPN11* gene. Functional studies demonstrated that the p.Tyr279Cys heterozygous missense mutation of the *PTPN11* gene perturbs the switching of the SHP-2 protein between its catalytically inactive and active conformation and engenders loss of SHP-2 catalytic activity [18].

The p.Tyr279Cys mutation has previously been reported in 47 different LS patients with Italian, French, Spanish, German, Estonian, Bosnian, Chinese Han, South-Korean, Japanese and Australian origin [1, 5, 8, 9, 12, 13, 14, 17, 20, 21, 22]. Thus this missense mutation is a worldwide recurrent missense mutation. Among the reported LS cases there are ones with *de novo* mutation [9, 21] and others, in which the disease show familial clustering and affects multiple family members in multiple generations [1, 5]. These data suggest a mutational hotspot on the *PTPN11* gene.

Previous reports demonstrated that the p.Tyr279Cys PTPN11 mutation is associated with short stature, deafness and hypertrophic cardiomyopathy [6, 11], the detailed comparison of the clinical symptoms of the 48 LS patients with the same causative mutation further complicated the analysis of the genotype-phenotype correlations of this mutation.

The most common symptom, which was present in 46 (96%) out of 48 patients is the presence of multiple lentigines (Fig 2). Further skin abnormality such as the development of café-au-lait spots were observed in only 22 (46%) patients. Besides the characteristic multiple lentigines, some of the facial anomalies were also very common among these patients: ocular hypertelorism was detected in 40 (83%) patients, palpebral ptosis in 32 (67%) patients and dysmorphic ears in 31 (65%) ones. Besides these common ectodermal abnormalities, patients

with the p.Tyr279Cys PTPN11 mutation have a great chance to develop cardiovascular anomalies. Hypertrophic cardiomyopathy was diagnosed in 25 (52%) patients. Although short stature was previously reported to be frequently associated with the p.Tyr279Cys phenotype, it was present only in 19 (40%) patients out of 48.

Regarding the other side of the spectrum, the p.Tyr279Cys mutation is rarely associated with deafness, which was reported in 12 (25%) patients (Fig 2). Moreover our analysis identified that certain symptoms - such as cryptorchidism, macrocephaly, horse kidney, hydrothorax, myelodysplasia and umbilical hernia - are rarely associated with the p.Tyr279Cys phenotype. In one of the 48 LS patients with the recurrent p.Tyr279Cys PTPN11 mutation, Marfan syndrome was also present, which is probably a rare independent association [17].

The observed differences in the clinical symptoms of the 48 LS patients carrying the same missense mutation clearly demonstrate the wide phenotypic diversity and the variable expressivity of the disease. In general, multiple lentigines, café-au-lait macules, ocular hypertelorism, palpebral ptosis, dysmorphic ears and hertrophic cardiomyopathy are hallmarks of the p.Tyr279Cys PTPN11 mutation related phenotype. However, there is no similar analysis investigating the most frequently associated clinical features in LS patients carrying the other common, recurrent missense mutation (p.Thr468Met) of the *PTPN11* gene, the published reports suggest that the p.Thr468Met mutation is also associated with high phenotypic diversity [2, 7].

Further studies are needed to identify putative genetic, environmental or life style factors, which can modify the development of the clinical symptoms and responsible for the observed phenotypic diversity. The availability of the extended clinical findings about the p.Tyr279Cys mutation carriers, as provided by this study, is critical for promoting both our understanding of the disease and the development of causative therapies that will be more specific and effective than the symptomatic treatments currently available for LS patients.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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## FIGURE LEGENDS

**Fig. 1 The clinical symptoms, the pedigree of the patient and the identified recurrent mutation of the *PTPN11* gene.** On examination facial dysmorphims including ocular hypertelorism, palpebral ptosis, slight mandibular prognathism and dysmorphic ears were observed (Fig. 1a). Pigmentational abnormalities such as multiple lentigines (Fig. 1b) and café-au-lait spots (Fig. 1c) were also present on the patient skin. On electrocardiography a third degree antrioventricular block was present (Fig. 1d). The patient was born outside marriage. The family members of his father and his mother were all clinically unaffected individuals suggesting (Fig. 1e). Direct sequencing revealed a heterozygous missense mutation (c.836A/G; p.Tyr279Cys) in the seventh exon of the *PTPN11* gene. The patient carried the mutation in heterozygous form (Fig. 1f), while the unrelated controls carried the wild type sequence (Fig. 1g).

**Fig. 2 Comparison of the frequency of the different symptoms detected in the p.Tyr279Cys phenotype.** The most common symptoms were the pigmentational abnormalities including multiple lentigines and café-au-lait spots and the mild facial dysmorphisms such as ocular hypertelorism, palpebral ptosis and dysmorphic ears. In contrast with these, cryptorchidism, mental retardation, macrocephaly, horse kidney, hydrothorax, myelodysplasia and umbilical hernia were rarely associated with the p.Tyr279Cys phenotype.

Figure 1a
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Figure 1b
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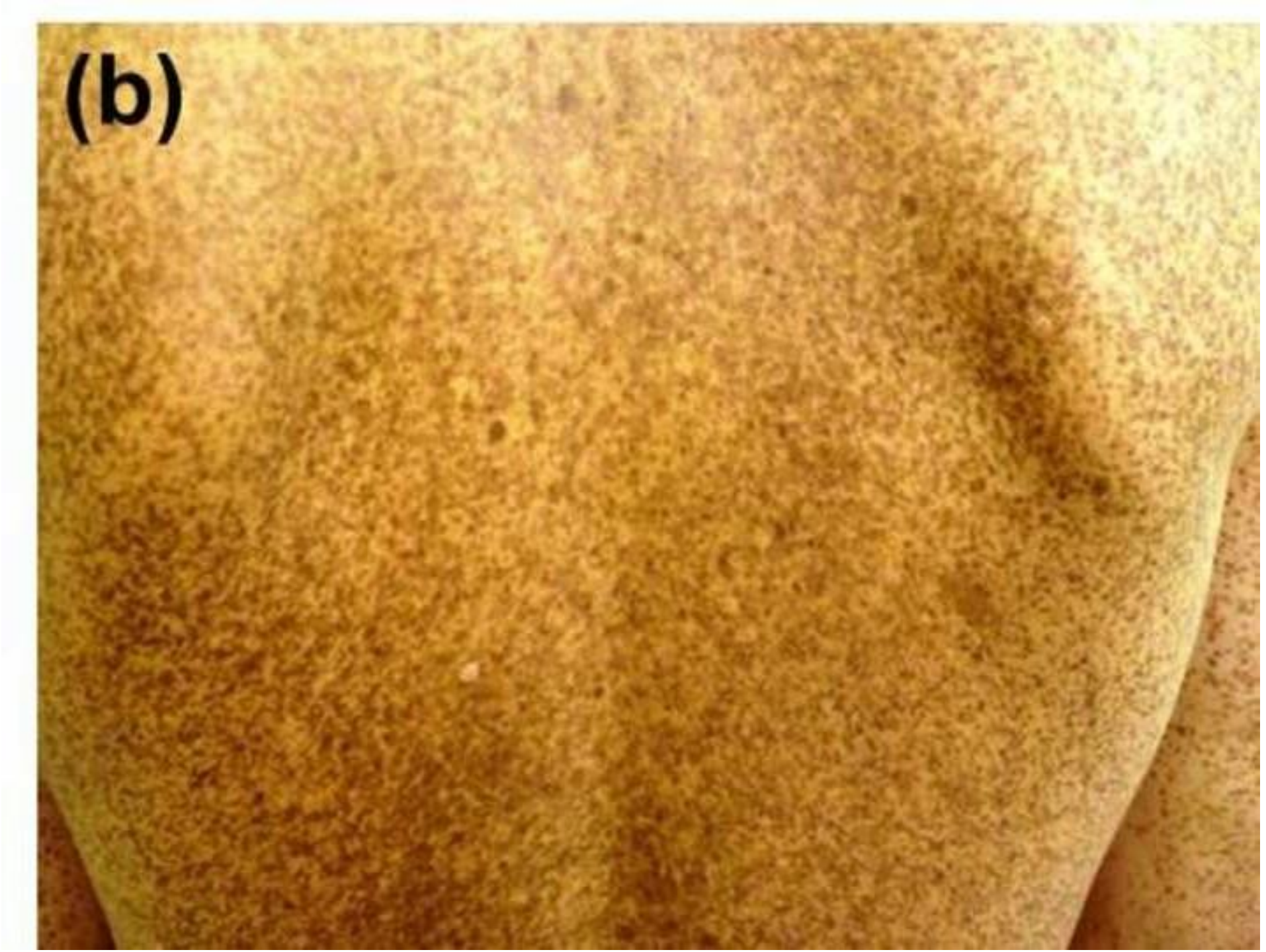

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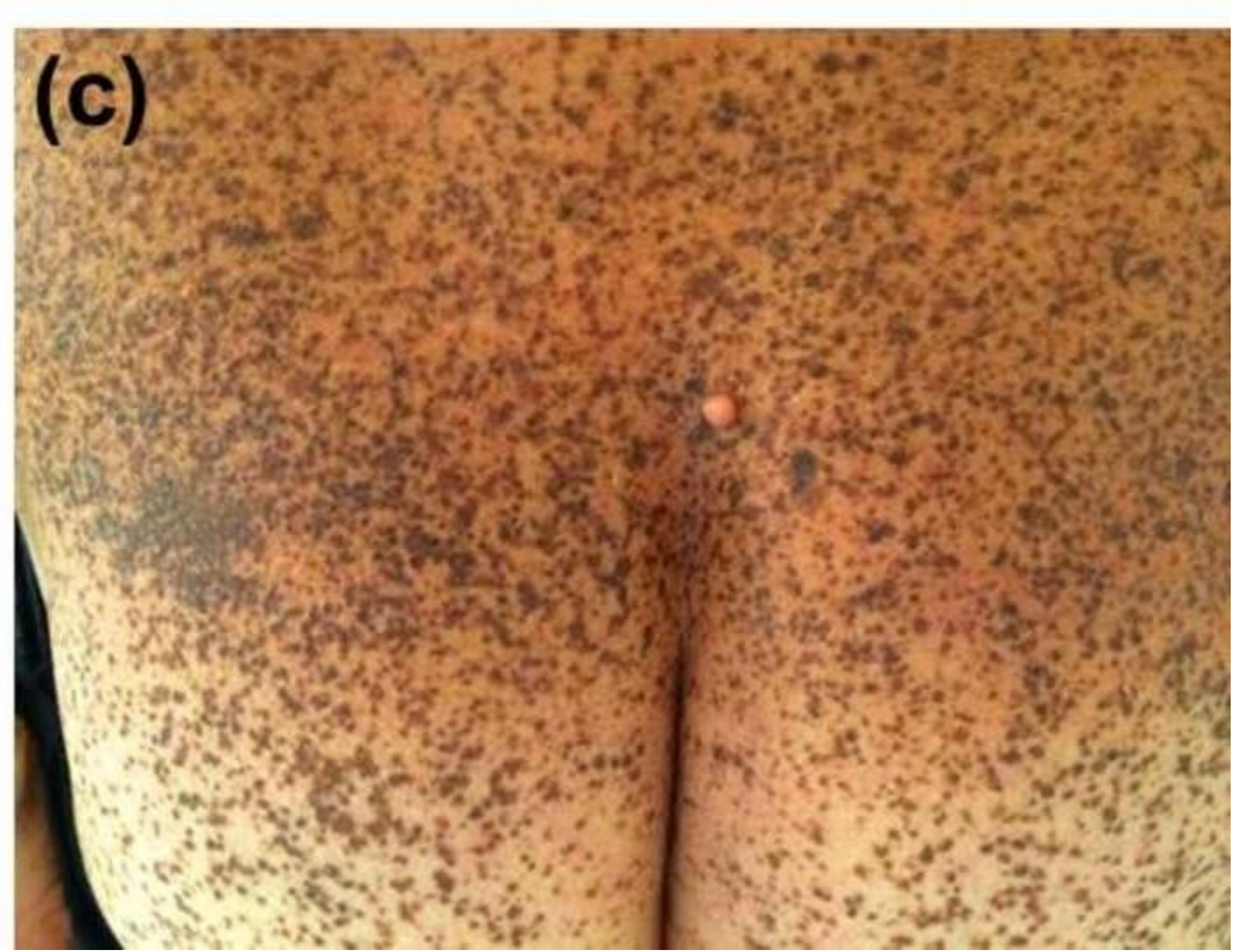

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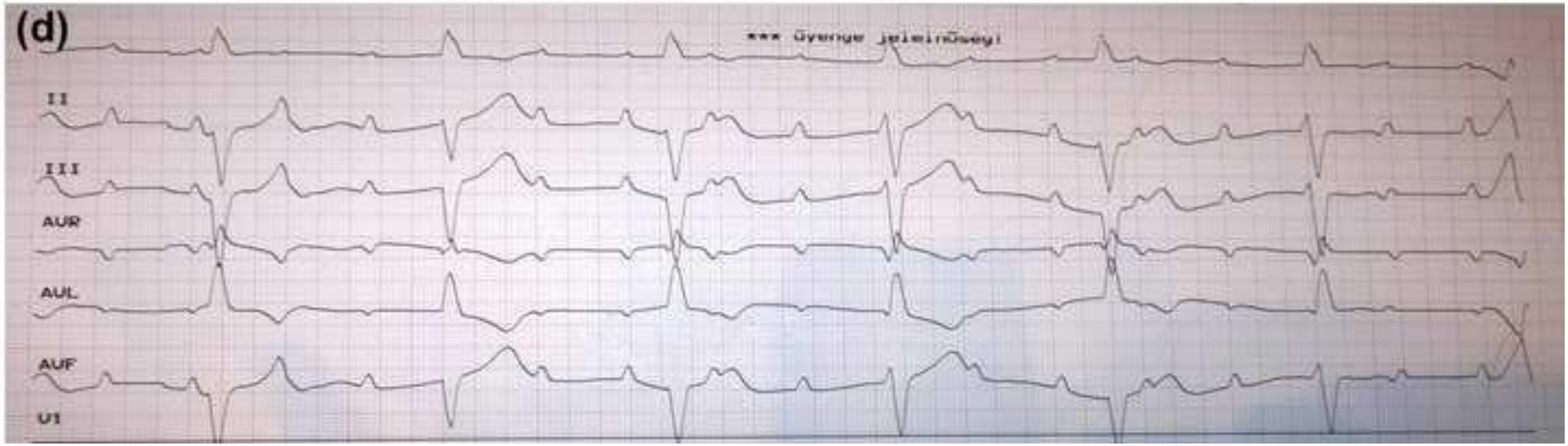

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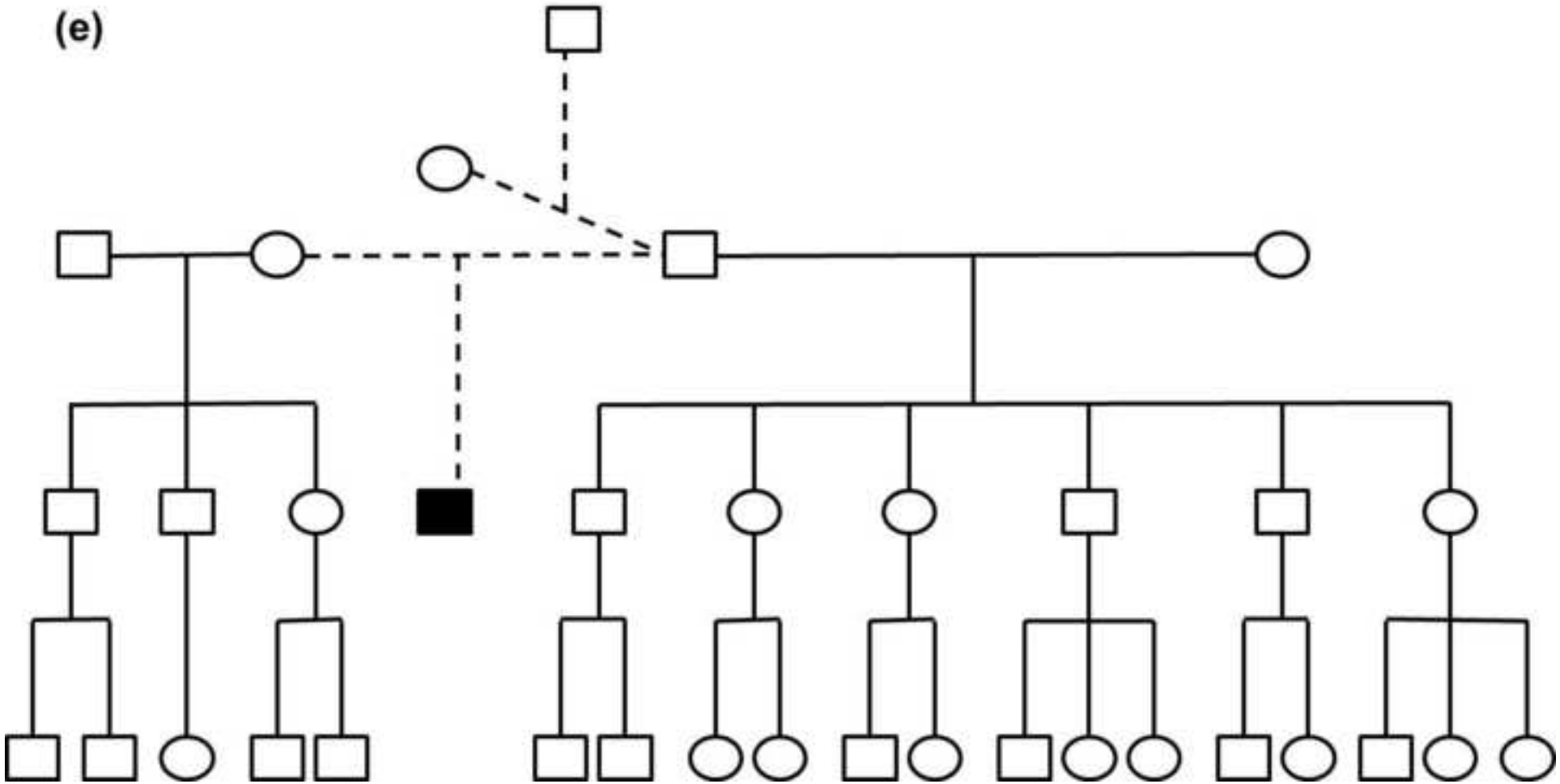

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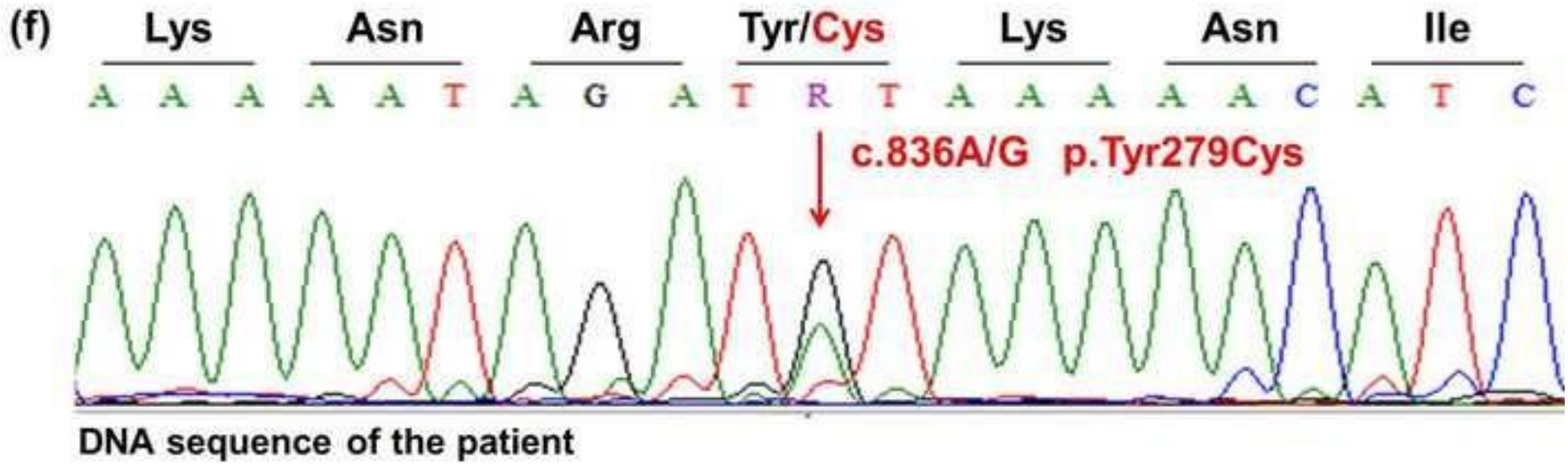

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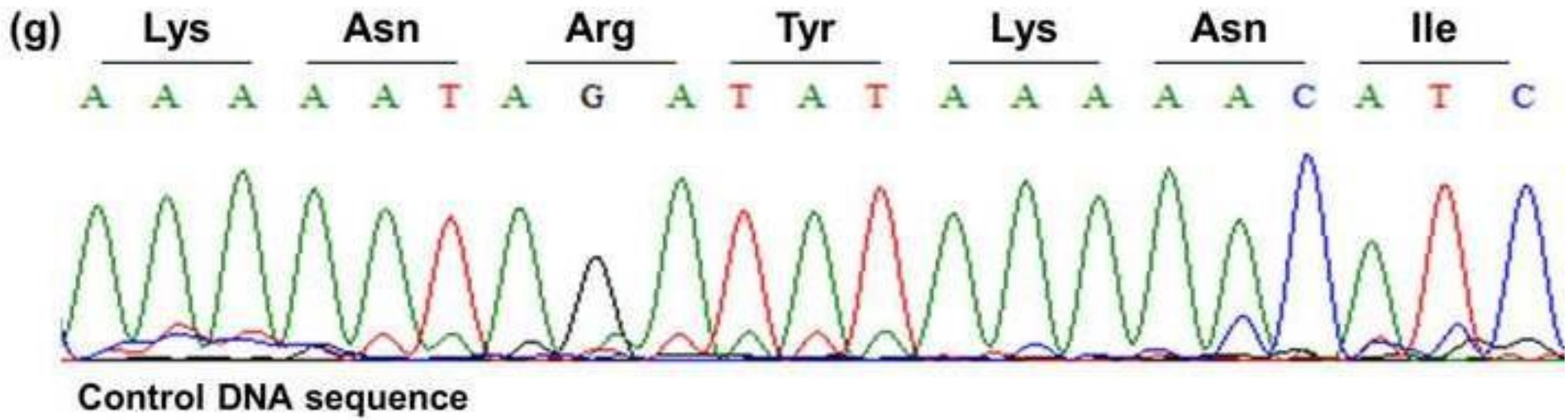

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