Brazilský příběh mutace *p53* R337H

Brazilian Story of the R337H *p53* Mutation

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Summary
The p53 tumour suppressor is an evergreen of molecular oncology. Since its discovery in 1979 it has been subjected to intensive investigation. The p53 protein is composed of "only" 393 amino acid residues and the function of almost each of them has been addressed in detail. Somatic mutations are extremely frequent, they can be found almost in each of the *p53* codons and in all types of tumours. Inherited *p53* mutations are rare but highly penetrant and they are typically associated with development of a broad spectrum of tumours. However, in 2001 the p53 research provided an unexpected discovery: the R337H allele was found in southern Brazil. This allele was atypically associated with only one type of tumour – childhood adrenocortical carcinoma – and it exhibited low penetrance. Therefore, new data on functioning and impact of the R337H mutation were highly desired. The results obtained during a few following years helped to elucidate not only this specific p53 variant but also provided insight in general principles of mutant p53 functions. It also turned out that all R337H alleles that are very frequent in southern Brazil originate from one common ancestor.

Key words
Li-Fraumeni syndrome – adrenocortical carcinoma – *p53* gene – R337H

Souhrn
Nádorový supresor p53 patří k „evergreenům" molekulární onkologie. Byl objeven v roce 1979 a od té doby je intenzivně zkoumán. Protein p53 má „pouhých" 393 aminokyselinových zbytků a funkce téměř každého jednoho z nich je pečlivě prozkoumaná a známá. Somatické mutace se vyskytují v téměř všech kodonech *p53*, jsou extrémně časté a vyskytují se téměř ve všech typech nádorů. Vrozené mutace *p53* jsou vzácné, ale výrazně penetrantní a jsou typicky spojeny s rozvojem širokého spektra nádorů. V roce 2001 však v oblasti výzkumu p53 došlo k nečekanému objevu: v jižní Brazílii byla objevena alela R337H, která byla zcela netypicky spojena s jediným typem nádoru – dětským adrenokortikálním karcinomem – a vyznačovala se nízkou penetrancí. Nastala doslova honba za dalšími informacemi o fungování a důsledcích mutace R337H. Ta během několika málo let přinesla nejenom spoustu poznatků o vlastnostech této konkrétní varianty p53, ale i obecnějších principech fungování mutovaných variant p53. Také se ukázalo, že všechny alely R337H, které jsou v jižní Brazílii masivně rozšířené, pocházejí z jediného zdroje, tj. mají společného předka.

Klíčová slova
Li-Fraumeniho syndrom – adrenokortikální karcinomy – gen *p53* – R337H

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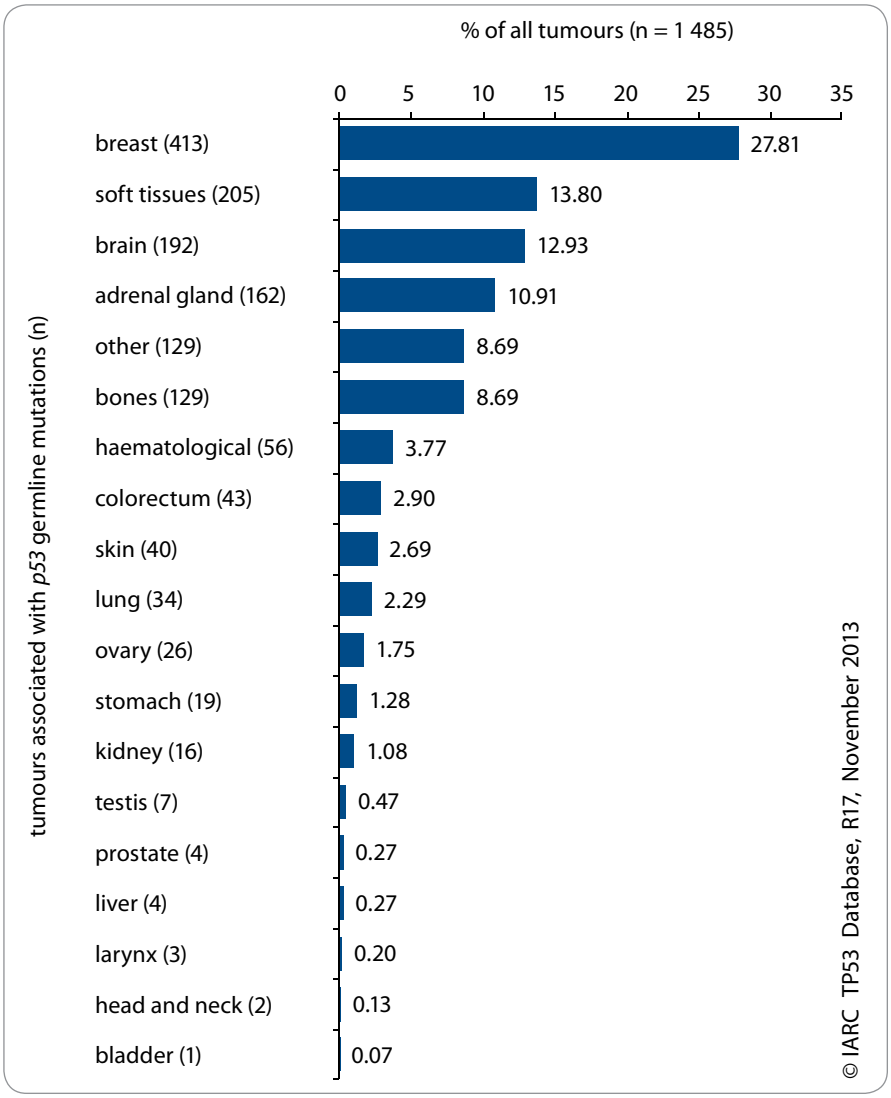
Introduction

The p53 tumour suppressor is an evergreen of molecular oncology. It was discovered in 1979 [1,2] and became the molecule of the year 2003. p53 is a transcription factor providing cells with ability to response adequately to various types of stress by control of its target genes. Upon DNA damage, starvation, oxygen deficiency, critical telomere shortening or due to non-physiological high expression of certain oncogenes, the stabilized p53 protein activates transcription of its target genes. It can induce cell cycle arrest, DNA repair, cell senescence or apoptosis. Therefore, p53 is a key tumour suppressor comprehensively protecting cells from neoplastic transformation. This is also documented by high frequency of the *p53* **somatic** mutations occurring in human tumours. They can be detected in about 40% of all tumours of various origins.

The **inherited** *p53* mutations are associated with the Li-Fraumeni syndrome (LFS). This rare autosomal dominant syndrome predisposes to development of a wide spectrum of tumours at young age. 60–80% of LFS cases result from the *p53* germline mutations. The specific cumulative risk of tumour development for the inherited *p53* mutation carriers is 18% for women aged 20 years, 49% for women aged 30 years, 77% for women aged 40 years and 93% for women aged 50 years. For men at the same age groups, the risk reaches 10%, 21%, 33% and 68%, respectively [3,4]. The most frequent tumours associated with LFS include breast tumours (27.8%), sarcomas of soft tissues (13.8%), brain tumours (12.9%), adrenocortical carcinomas (10.9%), osteosarcomas (8.7%), leukaemias (3.8%) and other tumours (Graph 1). In addition to the "classical" LFS, a Li-Fraumeni-like syndrome (LFLS) was also defined with less strict criteria in comparison with LFS both in terms of the spectrum of tumours, and the age of patients at time of diagnosis. Frequency of inherited *p53* mutations is also lower in families with LFLS than in families with LFS [4].

The latest R17 version of the *p53* mutations database of the IARC (International Agency for Research on Cancer) from November 2013 (http://www-p53.iarc.fr) [5,6] comprises 1,360 germline and 27,721 somatic *p53* mutations. It enables comparison of spectra and distributions of germline (Graph 2) and somatic (Graph 3) *p53* mutations. Missense mutations causing substitution of one amino acid for another in the affected p53 protein prevail among somatic as well as inherited mutations (73.2 and 73.5%, respectively). Most germline and somatic mutations are located in the part of the *p53* gene coding for a central DNA-binding domain. Most of the hot-spot codons, i.e. codons with the highest frequency of mutations, are identical for somatic and germline mutations. These are the codons 175, 245, 248, 273 and 282. For somatic mutations, there is one additional hot-spot codon 249, while among germline mutations it is the codon 337 – apart from the codon 248 – that is the most frequently mutated. It affects the oligomerization domain of the p53 protein.



Graph 1. Proportion of particular tumour types among patients with LFS.
Adopted from the database http://www-p53.iarc.fr; version R17 from November 2013 [5].

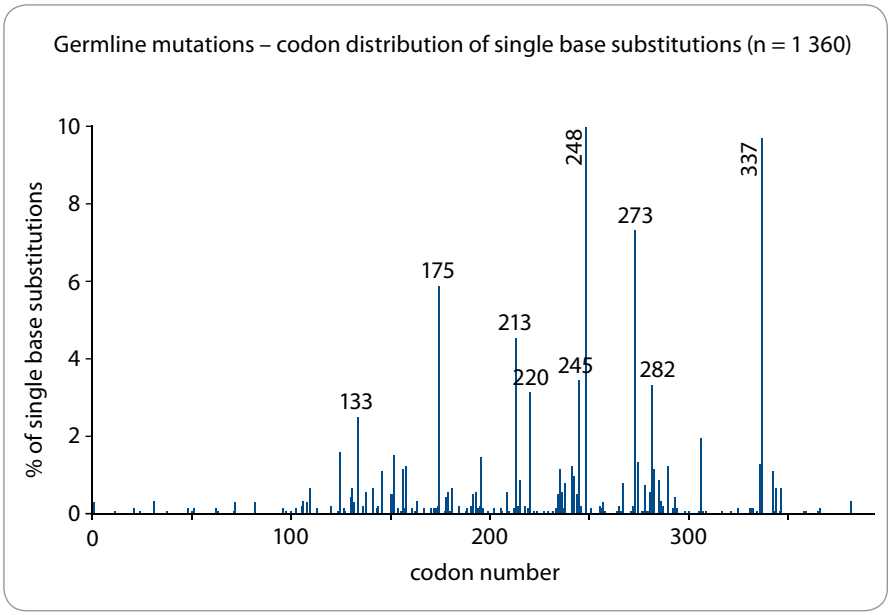
Germline p53 mutations – is there any association between the genotype and phenotype of LFS?

One of the first studies highlighting a possible link between the type of the *p53* germline mutation and the LFS phenotype – i.e. the spectrum of tumours and patients' age at which the tumours develop – was performed by Varley et al (1998). The authors described

a rare silent mutation in the codon 125 leading to aberrant splicing of exon 4 and expression of three abnormal transcripts. The spectrum of tumours in the affected family was unusual: no sarcoma, no childhood tumour and an occurrence of adrenocortical carcinoma at an adult age of 45 years, which is not common for LFS [7]. At the same time, the authors found the data described for another family with the same mutation and noticed the striking similarity of the phenotype: although childhood tumours (brain and adrenocortical) occurred in this family, there was no sarcoma, and, again, an adult (woman aged 33) was diagnosed with adrenocortical tumour [8].

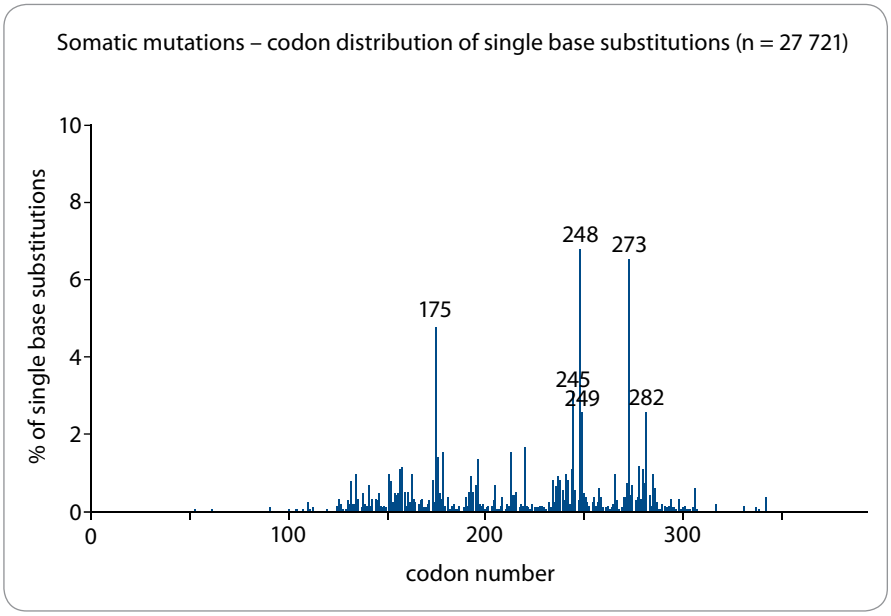
With regard to the "Brazilian story" of the R337H mutation, there is yet another interesting study performed by the same team. The study presented the results of analysis of the *p53* gene in 14 unrelated paediatric patients with adrenocortical carcinoma. Strikingly, in 11 of them (78.6%), the *p53* germline mutation was detected. Even more surprisingly, the *p53* mutation spectrum was very narrow; only two mutations, P152L and R158H, were found in nine children. In the affected children's families, occurrence of any other tumours among the *p53* mutation carriers was quite rare, indicating that these mutations are relatively low penetrant [9].

Another contribution to the question of the association between the type of the *p53* germline mutation and phenotype of LFS was brought by Olivier et al (2003). They analysed data from the IARC database concerning 265 families and 226 *p53* mutations. They found that brain tumours occurred more frequently in families with the mutation causing an amino acid substitution in the p53 DNA-binding domain involved in contact with the small groove of DNA, while adrenocortical tumours were significantly more often associated with changes in amino acids located at the opposite side of the p53 protein outside the DNA-binding domain. The *p53* mutations causing a complete loss of the p53 protein and its function are significantly associated with an early development of brain tumours [10].



Graph 2. Spectrum of the germ-line *p53* mutations.

Adopted from the database http://www-p53.iarc.fr; version R17 from November 2013 [5].



Graph 3. Spectrum of the somatic *p53* mutations.

Adopted from the database http://www-p53.iarc.fr; version R17 from November 2013 [5].

Monti et al (2007) used another approach to answer the same question. The authors classified the *p53* mutations according to the level of the p53 transcriptional activation function. They found that the extent of the p53 transactivation activity affects the risk rate, i.e. the penetrance, the spectrum of tumours and the age at which they develop [11].

Adrenocortical carcinomas

Adrenocortical carcinomas (ACC) are malignant tumours that develop from the cells of adrenal cortex. They occur at any age, mainly in childhood and later in adulthood in the 4th to 5th decade of age. They are frequently (in up to 50% of cases) hormonally active. Their incidence in the Czech Republic reaches approximately 1–2 cases per 1 million

Fig. 1. Map of Brazil with marked historic area of "Tropeiro route".

individuals per year [12], corresponding to the global incidence of ACC in adults: 1.7–2.0 per 1 million individuals per year [13].

ACC in children typically occur by the fifth year of life, with more frequent incidence in girls. Patients with small operable tumours have overall survival about 80%, patients with inoperable tumours have only a low chance of survival, and patients with large operable tumours have medium survival prospects. Operable tumours are detected in about two-thirds of patients. ACC in children are very often associated with mutations in the *p53* tumour suppressor [14,15], while in adult ACC, the incidence of *p53* mutations is less frequent [16–18].

Adrenocortical carcinomas are very rare in children with the incidence about 0.3 cases per 1 million children under age of 20 in the USA [19]. The paediatric tumour registry in Manchester, UK, recorded 12 paediatric ACC cases in the period 1954–1986, and the incidence was set to 0.38 cases per 1 million children under 15 years of age [20]. Estimated incidence of ACC in France is 0.2 cases per million children [21]. In contrast, it was estimated that in southern Brazil (in the states of Sao Paulo, Paraná and Minas Gerais; Fig. 1) the incidence of paediatric ACC was significantly higher – reaching 3.4 to 4.2 cases per 1 million children. A more accurate determination of incidence was carried out in 2006 for the State of Paraná based on the ACC deaths reported in the state hospital in Curitiba in 1998–2003. The mortality rate reached 1.6 cases per 1 million children under 15 years of age. Based on the proportion of surviving individuals (0.542), the incidence was estimated at 3.5 cases per 1 million children under 15 [22], thus exceeding the incidence in other regions of the world more than 10-times! The population in southern Brazil is very heterogeneous with a high proportion of Europeans (Portuguese, Italian, Spanish, German), along with Brazilian Indians and Africans. The disease occurs in all ethnic groups. The source of such a frequent occurrence of paediatric ACC and more general family genetic predisposition to the tumour development has been unclear for a long time.

Brazilian story of the R337H mutation

Beginning of the story – 2001

The story of the R337H mutation began in 2001. A group of authors [23] had collected the data available for 92 children with ACC treated in a hospital in Curitiba between 1966 and 1999. Based on these data, the authors contacted 55 families and invited them to participate in genetic study. Later, 36 probands with ACC, 29 girls and 7 boys, and a total of 186 family members, including 21 parents and 29 siblings were examined. The results were literally shocking. 35 of the 36 probands (97%) had the same germline mutation of *p53*: R337H! This mutation was also found in parents, siblings and other relatives, but without the occurrence of other tumours in the family history. The tumour was diagnosed in only one relative of the first degree. Only one family (out of 25) met the criteria for LFS, the remaining 24 families had no evidence of increased predisposition to tumour development. Multiple occurrence of ACC was observed in four families. It seemed that the potential of the R337H mutation to cause tumours was low in general, exhibiting low penetrance and significant organ-specificity, which is an exception in the *p53* world. Perhaps, the only exception from the rule of diversity and "non-specificity" of the *p53* mutations is the somatic mutation R249S associated with hepatocellular carcinoma and aflatoxin- and hepatitis B exposure. However, this is a somatic mutation associated with a specific carcinogen. The R337H mutation in children with ACC was the germline mutation and the authors have not identified any known mutagen in the affected area [23].

Functional and structural analysis of the R337H mutation

The functional form of the p53 protein is a tetramer, i.e. a dimer of dimers. As noted above, most of the germline and somatic *p53* mutations result in amino acid substitutions in the central DNA-binding domain. Several mutations disrupting the structure of the oligomerization domain (defined by the codons 310–360), thus impairing the tetramer formation and the p53 protein function, are also known. The R337H mutation falls within the oligomerization domain. Functional analysis of the mutant was already performed by the authors of the original paper published in 2001. Using the transcriptional activation assays they found that the R337H mutant exhibited almost the same activity as the standard variant of p53 retaining the ability to induce apoptosis and suppress colony formation by osteosarcoma SAOS-2 cells [23]. The high transcriptional activation ability of the R337H mutant was confirmed also by other authors [24].

A detailed analysis revealed that the structure of tetramerization domain with histidine at position 337 is very similar to the standard variant, but it is less stable. In particular, it is very sensitive to pH. The R337H mutant loses activity already at physiological pH levels above 7.7 [25]. In addition, the p53 protein with histidine at position 337, compared to the standard protein, strongly tends to form the amyloid-like fibrils [26]. The authors speculated that it might be the extreme sensitivity of the R337H mutant to pH that explains its significant association with ACC. The adrenal glands undergo extensive remodelling during prenatal and postnatal development, requiring an exact induction of apoptosis. During apoptosis, the cellular pH increases to around 7.9, a level destabilising the R337H mutant. In the developing adrenal gland, it may create a microenvironment which disrupts the tumour suppressor function of p53 and may contribute to tumour development [13,25].

Specific functional impact of the R337H mutation was later revealed also in relation to the increased variability in the copy number. Copy number variability (CNV) is one of the forms of polymorphism in the mammalian genome. CNV represents imbalances – gains and losses – of the DNA that change the diploid status of the affected locus. They usually range from about 50 bp to about 1 Mbp. As estimated, the CNV can affect about 15% of the human genome and every individual contains about 12 CNV in his/her genome on the average [27]. An increased CNV number in the genome (both the germline and somatic one) may reflect increased genetic instability and may be closely related to the development of tumours [28,29]. Persons with the germline *p53* mutation possess about 3-fold increase in the CNV number compared to the healthy controls. This may increase genetic instability and the risk of developing cancer and may also explain the anticipation of the *p53* mutations [30]. Although the latter study did not confirm the increase in the total CNV number in individuals with *p53* mutations compared to controls without mutations, a significant (more than 5-fold) increase in the so-called rare CNV was detected. Comparison of the CNV spectra in healthy controls and carriers of different types of *p53* mutations demonstrated that the spectrum of CNV in the R337H mutation carriers differs greatly from the spectrum of the carriers of the hot-spot mutations in the DNA-binding domain, resembling the control samples. This would correspond to an overall moderate impact of the R337H mutation [31].

Another contribution to the potential specific effect of the R337H mutation was the study by Maceda et al (2012). They confirmed higher levels of oxidative stress indicators in the blood of the R337H mutation carriers compared to controls lacking the *p53* mutation. This suggests that increased oxidative stress might be the cause of the oncogenic character of this p53 variant. On the other hand, it is not clear yet, whether this is a specific effect of this particular variant or whether other p53 mutants act similarly [32].

Penetrance of the R337H allele – part I

The original study on the R337H mutation strongly suggested that this *p53* allele had a low penetrance [23]. At the same time, a study by other authors performing a similar kind of analysis in the same area of Brazil (Sao Paulo) was published. They examined 55 patients, 37 adults and 18 children, with benign or malignant ACC with no other family history of cancer, 21 asymptomatic close relatives and 60 unrelated healthy controls. The R337H mutation was found in 19 patients, 14 of them were children (14/18 – 77.7%) and 5 adults (5/37 – 13.5%). The mutation was detected also among the healthy relatives of the first degree, but it was not detected among the controls [16]. Another similarly-designed study from the same area supported these results [33].

A detailed analysis of R337 allele penetrance was performed in extended cohort. The families of 30 probands with ACC bearing the R377H mutation were examined. A total of 927 individuals, including 695 probands' family members were analysed. None of the analysed families met the criteria for LFS, seven families were included in the group of LFLS. Out of the 695 individuals from the probands' families, 240 (34.5%) carried the R337H mutation, while the mutation was not found in any of the control subjects. Among parents and grandparents there were 50% mutation carriers; therefore not a single *de novo* R337H mutation was detected. Among the relatives of the first and second degree another 31 tumours were found. The overall penetrance was estimated at 9.9%. In other words, every 10th carrier of the R337H mutation develops cancer, most likely just the childhood adrenocortical carcinoma, because ACC cases represent 57% of the R377H mutation carriers having tumour, while in case of the classical LFS (whose lifetime penetrance reaches 80%) ACC represents a significantly smaller percentage – 10.9% (Graph 1) of all tumours [34].

Is the R337H mutation associated with LFS and LFLS? Penetrance of the R337H allele – part II

Achatz et al (2007), who used a different strategy, came up with quite different findings than the authors of previous studies. They examined 45 Brazilian

(Sao Paulo, Porto Alegre, Rio de Janiero) unrelated individuals from families meeting criteria of LFS or LFL. They found a germline *p53* mutation in 13 cases (28.9%). Within this group, 6 patients (46.1%) carried the R337H mutation. Families with the R337H mutation developed a broad spectrum of tumours including breast cancer (30.4%), brain tumours (10.7%), sarcomas (10.7%) and ACC (8.9%). Among the 57 individuals with no history of cancer, not a single *p53* mutation was found. The conclusion made by the authors was clear and significantly different from the findings by Figueiredo et al (2006): the R337H mutation predisposes to the development of a wide range of tumours, and thus it does not differ from other *p53* mutations [35].

The conclusions of the study, however, were quickly attacked and challenged. The authors of the study by Ribeiro et al (2007) were convinced that the studied families [35] met the LFLS, but not LFS criteria. Furthermore, they pointed out that the study was differently designed and the aim was defined in a different way. Using the same criteria, the original study [34] would include only seven probands and their families out of the 30 enrolled; the other 23 children/families showed no signs of increased predisposition to other tumours than ACC. In addition, they pointed to other differences in the spectra of tumours present in the R337H mutation carriers and carriers of other *p53* mutations than just different ACC frequencies. In this context, the question arises whether the altered disposition to tumour development might be determined by a combination of R337H and some other co-operating genetic factor [36].

Three other studies further extended the depiction of the R337H allele penetrance, its relationship to the spectrum of tumours to which it predisposes, and also the frequency of its occurrence in southern Brazil. In the first study, 123 women with breast cancer and 223 healthy controls were screened for the R337H germline mutation. The mutation was found in three women with cancer (2.4%) but in none of the controls. The case history of two of the three women met the criteria for LFLS. What conclusions were made by the authors? The R337H allele can significantly increase the risk of breast cancer, i.e. not only of ACC, although probably in connection with some other genetic factors [37]. A similarly designed study in Rio de Janeiro conducted in 390 patients with breast cancer found two carriers of the R337H mutation (0.5%), while the mutation was not found in any of the 324 healthy women. Both carriers of the mutation were diagnosed with breast cancer at a young age (under 40 years) and reported more cases of breast cancer among their relatives [38]. The third study analysed 750 healthy women – volunteers (aged 40–69 years) from Porto Alegre. The R337H mutation was found in two patients (0.3%), who were the fourth-degree relatives and reported a history of occurrence of various tumours in their families. The families did not meet the criteria for LFS or LFLS. Further family examination identified three other R337H mutation carriers, of which one woman developed breast cancer at the age of 36. Thus, the low penetrance of the R337H allele was repeatedly demonstrated. The incidence of this allele in southern Brazil was set to 0.0013, i.e. about 10 to 20 times higher than the incidence of other p53 mutations associated with LFS or LFLS [39]. Some sources indicate that the incidence is even higher [40].

The debate on the penetrance of the R337H allele and the spectrum of associated tumours was later joined by authors whose research was primarily focused on other problems (see below). Based on an analysis of 12 families with an inherited R337H mutation they concluded that the spectrum of tumours associated with this mutation is wide and not limited to the ACC, and the rate of lifetime penetrance of the R337H allele is comparable with other *p53* mutations. But they pointed to the specific dynamics of the penetrance, reaching about 15% at the age of 10–15, followed by a plateau. Later, a leap increase in the tumour incidence occurs after the 30th year of life. The authors claim that the lifetime risk of cancer is therefore probably similar as in case of other *p53* mutations associated with LFS and LFLS. However, the tumours tend to develop at a relatively advanced age [41].

Do the mutation carriers share a common ancestor?

The discussion on a possible founder effect, i.e. the possibility of a common ancestor of the R337H allele, was initiated immediately by the authors of the original study by Ribeiro et al (2001). Their study included 17 unrelated carriers of germline R337H mutation and their 10 relatives. They examined four polymorphic (intergenic and intragenic) markers and concluded that the founder effect is unlikely and that at least some of the analysed alleles developed independently [23].

In contrast, another study showed the opposite results. Pinto et al (2004) analysed two highly polymorphic intragenic markers in 22 patients – unrelated R337H mutation carriers and 60 controls. And they concluded that it is very likely that there was a common ancestor of all R337H mutation carriers [42]. The result got the strong support by the study of Garritan et al (2010) performing a detailed haplotype analysis of 12 unrelated families (a total of 48 carriers of the R337H allele) using 29 polymorphic markers. Eleven of the families originated from southern Brazil; one family with Portuguese roots was found in France [24]. The conclusion of the study was unambiguous. All R337H mutation carriers have the same haplotype (A3). Thus, the founder effect was confirmed clearly. In addition to this, the authors concluded that the common ancestor most likely originated in Europe, most likely in Portugal [41].

Many towns and villages of southern Brazil were founded in the 18th and 19th centuries along the axis known as "the Tropeiro route". Tropeiros were merchants, mostly of Portuguese origin, who travelled between Sao Paulo and Porto Alegre at that times and significantly contributed to the settlement in this area (Fig. 1). Therefore, these specific historical and demographic factors might contribute to a massive expansion of the R337H allele. The low penetrance or the specific penetrance dynamics might

contribute to the long-term persistence of the R337H allele in the population [41].

The founder effect is also strongly supported by the number of observations indicating that the germline R337H mutation has never occurred *de novo*, but it has always been detected in one of the proband's parents [16,33,34]. Furthermore, a case of a proband – homozygote – having the R337H mutation in both alleles of the *p53* gene as both of his parents were heterozygotes was reported [16].

Beyond the Brazilian story of R337H

Brazilian story is a story of an association between the R337H mutation and ACC in children occurring in southern Brazil. This constricted, narrowly defined and surprising story brings other questions exceeding its original assignment.

Is ACC the only type of tumour tightly associated with the R337H mutation?

One of them is the question, whether ACC is the only type of tumour predisposed by the R337H mutation, or whether there are also other types of tumours tightly associated with this particular mutation. Besides the already mentioned association with the development of breast cancer [37], yet another type of tumour has been found to be even more significantly associated – comparable to the ACC – with the germline R337H mutation. It is the cancer of choroid plexus in children, which is also found in southern Brazil with an increased incidence and more than 60% cases of this tumour are associated with this mutation [43,44]. In addition, the above-mentioned findings by Achatz et al (2007) indicating that the R337H allele is (possibly in connection with some other genetic conditions) associated with the development of a broad spectrum of tumours typical for LFS and LFLS in many families are of course also valid [35].

Is the R337H mutation the only allele of *p53* significantly predisposing to ACC?

Answering the question whether the R337H mutation of the *p53* tumour suppressor is the only one that significantly predisposes to ACC, and if there are other mutations, what they have in common, is more complex. In Introduction, we refer to the study of Varley et al (1999) describing the detection of the germline P152L and R158H mutations that repeatedly occurred in paediatric ACC. Both the mutations are temperature-dependent, and therefore only partially inactivating [45], and they exhibit low penetrance [9]. Later, more cases of germline *p53* mutations having low penetrance and being associated with paediatric cases of ACC, but not with LFS or LFLS were published. They included R175L [46] or splice-site mutation leading to production of the p53 protein with an altered C-terminus [47]. These studies fit into the idea that just partial loss of function and low penetrance of the *p53* mutations might be the link preferentially connecting these mutations with the predisposition to the childhood CCA [48]. Based on the deep knowledge of the R337H mutation story, and other published data, Zambetti designed a model of the gradient effect of germline *p53* mutations. In this model, *p53* mutations form gradient according to the degree of the p53 function they retain. The level of the residual activity of p53 correlates with the phenotype, i.e. with the degree of susceptibility to cancer. Nevertheless, the resulting impact of the germline *p53* mutation is significantly modified by other genetic factors, especially the *MDM2* polymorphism (SNP309) and several intragenic polymorphisms of the *p53* gene itself (Fig. 2) [48–51].

An international registry and tissue bank for the paediatric cases of ACC has been established recently. In the tumour tissues of 48 cases stored in the bank, 23 independent germ line *p53* mutations were detected. Most mutations occurred in the typical hot-spot codons and only three mutations occurred repeatedly: R175H, R273C and R337H. Overall, it seems that the mutated *p53* alleles with low penetrance significantly predispose to the development of ACC in children, but at the same time the paediatric cases of ACC often occur in families with LFS or LFLS having a germline *p53* variant with a high penetrance [52].

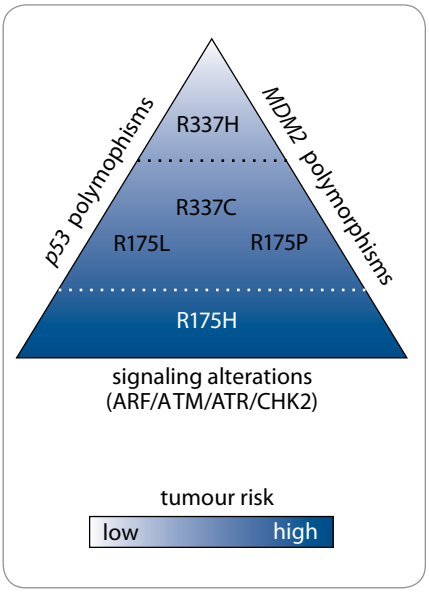


Fig. 2. Gradient effect of the *p53* impact.
Some p53 mutants are fully inactive (R175H), while others keep partial activity, or their activity almost reaches the standard p53 variant (R337H). The level of the p53 activity corresponds to the level of tumour susceptibility. Final impact of the *p53* mutation is modified by other genetic factors, especially *MDM2* and *p53* genes polymorphisms. Adopted from [48].

Conclusions I

Discovery of the close link between the R337H mutation and development of paediatric adrenocortical carcinomas was an extraordinary event in the field of *p53*. Although this tumour suppressor is frequently mutated in human tumours, and although we know many different mutations of this gene, a tight relationship between one specific type of mutation and specific tumour, such as the relationship between R337H and ACC in children, is very unusual. The main conclusions of this story can be summarized in several points:

1. The incidence of ACC in children in southern Brazil is more than 10-times higher than in other regions worldwide.
2. The incidence of ACC in children in southern Brazil is extremely often associated with the germline R337H mutation of the *p53* tumour suppressor.

3. The frequency of the R337H allele incidence in southern Brazil is 0.0013, i.e. about 10- to 20-times higher than the incidence of other *p53* mutations associated with LFS or LFLS.
4. The variant of the p53 protein carrying histidine instead of arginine at position 337 (R337H) retains high transcriptional activation capability, but is extremely sensitive to pH.
5. The penetrance of the R337H allele is low.
6. All carriers of the R337H allele share a common ancestor; the germline R337H mutation does not appear *de novo*.

Conclusions II

The conclusions summarized in the previous paragraph resulted from a great effort of several outstanding scientific teams working in the field for many years. The conclusions themselves are stunning. And yet, further conclusions outreaching the boundaries of one specific research area and bringing a profound lesson can be made.

Carefully collecting and studying the published papers, the reader is drawn into a dramatic story revealing the connections and network on the one hand. On the other hand, the reader can be confused and puzzled how "the things really are", and where the truth is. At the end, one can discover that validity of the obtained answers is substantially influenced by the formulation of the question, design of the study and accuracy of the data interpretation. This is certainly a lesson applicable in every field of human activity. Point of view matters!

There is yet another equally generally valid experience related to this particular story. At the international workshop on *p53* mutants and Li-Fraumeni syndrome held in Lyon in 2007, two Brazilian authors, Marie-Isabel Achatz and Patricia Ashton-Pröll, gave a lecture dealing with some aspects of the Brazilian story, including the founder effect. The lecture brought about not only somewhat romanticized consideration of whether the common ancestor of all current carriers of the *p53* mutation R337H was a successful and charming tropeiro or a brave, strong and equally charming (or violent?) conquistador, but especially the opportunity to learn the details directly from the epicentre of the events, and triggered spark and passionate debate that would never end. The lecture was fortunately the last one in its section, which was the last one of the meeting day, and thus the never-ending avalanche of questions and the willingness of both women to answer them caused that the conference was closed with a 2-hour delay! It was undoubtedly a stimulating, motivating and charming experience for all participants. And it also belongs to history and to the contribution of the Brazilian story of the R337H *p53* mutation.

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