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Variations in the *TRPV1* gene are associated to Exertional Heat Stroke

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1 **Abstract :**

2 **Objectives:**

3 Exertional Heat Stroke (EHS) is one of the top three causes of sudden death in athletes. Extrinsic and
4 intrinsic risk factors have been identified but the genetic causes still remain unclear. Our aim was to
5 identify genes responsible for EHS, which is a necessary step to identify patients at risk and prevent
6 crises.

7 **Design:**

8 Genetic and functional laboratory studies

9 **Methods:**

10 Whole Exome Sequencing (WES) was performed to search for candidate genes in a cohort of 15
11 soldiers who had a documented EHS episode. *In silico* and *in vitro* functional studies were performed
12 to evaluate the effect of mutations identified in the candidate gene *TRPV1*.

13 **Results:**

14 WES led to the identification of two missense variations in the *TRPV1* gene. These variations were
15 very rare or unreported in control databases and located in critical domains of the protein. *In vitro*
16 functional studies revealed that both variations induce a strong modification of the channel response to
17 one of its natural agonist, the capsaicin.

18 **Conclusions:**

19 We evidenced mutations altering channel properties of the *TRPV1* gene and demonstrated that *TRPV1*,
20 which is involved in thermoregulation and nociception, is a new candidate gene for EHS. Our data
21 provide the bases to explore genetic causes and molecular mechanisms governing the pathophysiology
22 of EHS.

23

24 **Keywords:**

25 Heat Exhaustion; Malignant Hyperthermia; Whole Exome Sequencing; Thermogenesis; In vitro

26 Contracture Test

27

28 **Introduction**

29 Exertional Heat Stroke (EHS) is a life-threatening disease that occurs in young individuals who
30 engage in prolonged and strenuous activity¹. First cases have been reported in army soldiers² but many
31 cases are now described in civilian population. Indeed, the popularity and increased competitiveness in
32 summer road races and pre-season American football are likely associated with the number of EHS in
33 athletics in recent years. The incidence of EHS is around 3 cases/10,000 person-years³ but it can reach
34 up to 2/1000 athlete-exposures⁴; it was responsible for 30 deaths over the last 10 years among
35 American football players⁵.

36 EHS is defined by a severe hyperthermia, with a body temperature above 40°C, associated with a
37 sudden neurological dysfunction, observed in healthy individuals exposed to intensive physical
38 exercise⁶. Ultimately it results in a multiple organ failure leading to a high mortality rate that can be
39 drastically reduced with early and quick cooling of the patient. The pathophysiology of EHS is based
40 on a dysfunction of thermoregulation that leads to an uncontrolled increase of internal heat production
41 exceeding compensation mechanisms. The core body temperature rises continuously and the patient
42 evolves into an uncompensable heat stress state⁷. A hypermetabolic state, especially in the skeletal
43 muscle, and the development of a systemic inflammatory syndrome are also part of the
44 pathophysiology⁶. Extrinsic risk factors such as hot and humid environment have been identified, as
45 well as intrinsic factors such as dehydration or sleep deprivation. Some studies have suggested that the
46 impact of these risk factors could be modulated by training or acclimatization, which should help the
47 patient to adapt to exercise and environment conditions⁸. However, several evidence support the
48 existence of inherited genetic mutations that predispose some patients to EHS⁹⁻¹¹, despite these
49 adaptation mechanisms.

50 EHS shares many clinical and biological similarities with Malignant Hyperthermia (MH)¹², a life-
51 threatening genetic disease triggered by volatile halogenated agents. MH is characterized by a
52 hypermetabolic state in skeletal muscle with hyperthermia and can lead to multiple organ failure¹³.
53 MH can be diagnosed by a positive *In Vitro* Contracture Test (IVCT), which quantifies the excessive
54 contraction of a skeletal muscle biopsy in response to triggering agents, or by genetic testing.
55 Mutations are detected in the *RYR1* gene in 60% of MH cases¹⁴, and rarely in the *CACNA1S* and
56 *STAC3* genes^{15,16}. All these genes encode proteins that are involved in calcium homeostasis in skeletal
57 muscle. Interestingly, half of EHS patients displays a positive IVCT which evidences a calcium
58 release disorder in these patients³. Contrary to MH, the genetic bases of EHS remain poorly
59 documented. Mutations in *RYR1* have been associated with EHS with a much lower prevalence than in
60 MH, estimated at 10%⁹, which suggests the involvement of other genes in the disease.

61 We report here the genetic study of a cohort of soldiers presenting with an EHS episode in whom
62 mutations in *RYR1* had been previously excluded. Whole Exome Sequencing analysis and downstream
63 functional studies led to the identification of *TRPV1* as a new gene associated with EHS. *TRPV1*
64 encodes a cationic channel implicated in thermoregulation and has been recently associated with MH
65 phenotypes, arguing in favor of an overlap between the two diseases¹⁷.

66 **Methods**

67 Samples were obtained from EHS soldiers of the French national cohort recruited between January
68 2004 and December 2006 by the Military Hospital Lavéran reference center for EHS. EHS occurred
69 during or immediately after a military exercise consisting in a timed race (8-km running with boots
70 and combat jacket) or a commando race (8-km running with complete battle dress, including an 11-kg
71 combat rucksack and weapon). These exercises were part of soldiers' sessional exams. Patients were
72 included on the basis of a characterized EHS crisis with 1) body temperature $\geq 39^{\circ}\text{C}$ recorded at the
73 time of the crisis either rectally or, when not possible in the field, by ear (less accurate method), and 2)
74 neurological dysfunction such as mental confusion or loss of consciousness¹⁸. The temperature
75 threshold used to defined a crisis was 39°C in this study in order to increase diagnostic sensitivity,
76 because of the inevitable delay between the crisis and the temperature measurement in the field and to
77 anticipate care of patients with neurological dysfunction with hyperthermia². All selected patients
78 displayed a positive *In Vitro* Contracture Test according to the European Malignant Hyperthermia
79 Group (EMHG) guidelines. IVCT was considered positive when contraction was equal to or above
80 0,2g at 2% halothane concentration or 2 mM caffeine.

81 Genetic analyses were performed after written informed consents were obtained. Whole Exome
82 Sequencing (WES) was performed (n=15) using Illumina HiSeq[®] 2000/2500 technology (Illumina,
83 USA). The Ingenuity[®] software (Qiagen, Germany) was used to filter the variations. Based on the
84 estimated prevalence³ of EHS, variants with an allele frequency of at least 0.1% in common controls
85 databases (ExAC and gnomAD) were excluded and only missense, frameshift (indels) or splice site
86 variations were analyzed. Because our cohort consisted of sporadic cases, WES data analysis was
87 performed with either autosomal recessive or dominant mode of inheritance hypotheses. Genes with 1-
88 at least two patients harboring a variant matching with previous criteria and 2- a tissue expression in
89 the skeletal muscle, were further analyzed. Variants were confirmed by Sanger sequencing.
90 Segregation studies were not performed because relatives clinical evaluation and paraclinical
91 assessment could not be performed and DNAs were not available. Additional EHS probands (n=45)
92 were analyzed by Sanger sequencing for the *TRPV1* (NM_018727.5) candidate gene.

93 *In vitro* functional studies of *TRPV1* variations identified by WES were performed with a plasmid
94 containing the human *TRPV1* sequence in fusion with the mCherry tag that has already been
95 described¹⁷. The p.Gly684Val and p.Arg772Cys mutations were introduced by site-directed
96 mutagenesis, following the instruction of the QuickChange XL Site-Directed Mutagenesis kit
97 (Agilent, USA). Plasmids encoding the Wild-Type or a mutant TRPV1 were transfected in HEK-293
98 cells according to the DharmaFECT Duo Transfection Reagent protocol (Dharmacon, USA). Cells
99 were cultured in standard conditions and experiments were performed after 48h of *TRPV1* expression.

100 To evaluate the calcium release in the cytoplasm after TRPV1 activation, cells were loaded with Fluo-
101 4 AM dye for 30 min at 37°C and next maintained in a calcium-free medium containing 1 mM EGTA,
102 what allowed to assess exclusively calcium release from intracellular stores. The stimulation with the
103 agonist (capsaicin 20 µM, Merck, Germany) was performed at room temperature and the emission of
104 fluorescence of the Fluo-4 AM dye was followed by microscopy on a DMI6000 Leica FRAP (Leica
105 Microsystems) with a 10x objective. Images were taken every second during 80 sec and stimulation
106 was performed at 10 sec. Data were analyzed with the ImageJ software to select regions of interest and
107 acquire the fluorescence intensity that was normalized to baseline fluorescence (F₀). An average of 20
108 cells was analyzed for each condition and experiments were repeated 3 times.

109 To evaluate the resting calcium in the cytosol, cells were loaded with Fura-2 (2.5 µM) for 30 min at
110 37°C in Tyrode buffer. After loading, cells were washed and maintained in Ca²⁺-free Tyrode buffer.
111 Fura-2 measurements were performed at room temperature using a wide field Leica DMI6000B
112 microscope equipped with a 40x oil-objective and an Orca-Flash4.0 digital camera (HAMAMATSU).
113 Using a Lambda DG-4+ filter (Sutter instruments), Fura-2 AM was excited at 340 and 380 nm and
114 their respective emitted fluorescence lights were measured at wavelength 510 nm. Images were taken
115 with 5 seconds interval. Fluorescence ratios were analyzed with MetaFluor 6.3 software (Universal
116 imaging).

117 Data were analyzed with GraphPad Prism 6.0 (USA) using a one-way ANOVA after checking for
118 Gaussian distribution. Normally-distributed data were analyzed through parametric ANOVA followed
119 by Tukey's post-hoc tests while non-normally-distributed data were analyzed through non-parametric

120 Kruskal-Wallis ANOVA followed by Dunn's post-hoc tests. Differences were considered significant
121 when $p < 0.05$.

122 **Results**

123 Among the 15 patients studied by Whole Exome Sequencing (WES), two possibly damaging
124 heterozygous variations were identified in the *TRPV1* gene in two patients: the c.2051G>T;
125 p.Gly684Val and the c.2314C>T; p.Arg772Cys variations (table A, figure A). Sequencing of the entire
126 *TRPV1* coding sequence in 45 other patients did not reveal additional potentially deleterious
127 variations.

128 Patients harboring a variation in *TRPV1* were young men, with body temperature recorded at the time
129 of the crisis above 40°C. They both presented neurological signs resulting in a loss of consciousness.
130 Both patients experienced rhabdomyolysis and patient 2 developed hepatorenal failure. Interestingly,
131 patient 2 also harbored elevated CPK at rest (three times the normal level). According to ICVT result,
132 patient 1 was diagnosed as MHS (susceptible to Malignant Hyperthermia) and patient 2 was MHSh
133 (susceptible to Malignant Hyperthermia only to halothane stimulation). The patients did not report any
134 others symptoms. These clinical features were similar to the other patients of the cohort analyzed by
135 WES (table A, supplemental table A).

136 The c.2051G>T; p.Gly684Val variation identified in patient 1 was very rare with a Minor Allele
137 Frequency (MAF) of 0.000004063 in the ExAC database and the c.2314C>T; p.Arg772Cys variation
138 identified in patient 2 was not reported in genomic databases. Both variations were also predicted
139 damaging by four different software (CADD¹⁹, SIFT²⁰, Polyphen2²¹, Mutation Taster²²) and modified
140 highly conserved amino acids in functional domains of the protein (table A, figure A).

141 Mapping of the Glycine 684 onto the crystal structure of TRPV1 (PDB number: 5IRZ, residues 335 to
142 751²⁶) showed that this residue is localized in the critical pore domain of the TRPV1 channel. The *in*
143 *silico* structure prediction showed that replacement of the Glycine 684 by a Valine induces a major
144 steric hindrance in the pore of the channel (supplemental figure A).

145 The p.Arg772Cys variation affects a residue located in a regulatory domain of the C-terminal region of
146 TRPV1, whose structure is so far unavailable.

147 To further explore the impact of both variations, calcium release of mutated TRPV1 after stimulation
148 with the specific agonist capsaicin was performed. The TRPV1-Arg772Cys mutant induced a

149 modification in the kinetics of calcium release resulting in an increase in the total calcium released as
150 compared to TRPV1-WT (figure B.1 and B.2). The TRPV1-Gly684Val mutant did not respond to
151 capsaicin stimulation. Ectopic expression of TRPV1 in HEK-293 cells induced an elevation of the
152 resting cytosolic calcium concentrations even in absence of TRPV1 stimulation (figure B.3) due to a
153 calcium leak from intracellular stores. The expression of the TRPV1-Arg772Cys induced a similar
154 elevation of basal cytosolic calcium concentration (figure B.3). On the contrary, the expression of the
155 TRPV1-Gly684Val mutant did not change the resting concentration of calcium (figure B.3),
156 confirming this mutant channel is not functional. The expression and localization of recombinant
157 TRPV1 in HEK-293 cells were not altered by variations according to Western-Blot and
158 immunofluorescence results (supplemental figure B), and we conclude that both genetic variations
159 alter *in vitro* the global channel function of TRPV1, at rest or after specific agonist stimulation.

160 **Discussion**

161 Our work led to the identification of variations in the *TRPV1* gene in association with Exertional Heat
162 Stroke phenotype in two patients. The *TRPV1* gene encodes a non-selective cation channel with a
163 broad spectrum of tissue expression. In the skeletal muscle, TRPV1 is localized in the sarcoplasmic
164 reticulum membrane and is especially involved in calcium transport²⁴, which is consistent with the
165 hypothesis of a calcium release disorder in patients who display a positive *In Vitro* Contracture Tests.
166 TRPV1 is a nociceptor that can be activated by pungent ingredients like capsaicin, the active molecule
167 of chili pepper, or heat and acidic environment^{25,26}, which are produced during exercise and muscle
168 contraction. TRPV1 is also stimulated by halogenated anesthetics²⁷, and interestingly a recent study
169 reported mutations in the *TRPV1* gene in patients with Malignant Hyperthermia¹⁷.

170 The two variants that we report present the standard criteria of pathogenicity. They are unreported or
171 very rare (MAF <0.0001%) in control databases while we chose to keep variants with a MAF <0.1%,
172 which is compatible with a rare and triggered disease such as EHS. They are also predicted as
173 pathogenic *in silico* and located in critical functional domains of the protein. The p.Gly684Val
174 mutation is located in the S6 transmembrane domain (residues 559 to 687 according to Uniprot
175 website: <https://www.uniprot.org/uniprot/Q8NER>.) which is part of the highly conserved pore domain
176 of TRPV1 and is crucial for the channel function. The region including the pore domain is implicated
177 in selectivity, opening and closure of the channel but is also implicated in conformational change in
178 capsaicin-induced activation and heat-induced desensitization²⁸. The steric hindrance resulting from
179 the replacement of the small amino acid by a larger one in the pore is likely to impact the channel
180 function as shown in X-ray structure (supplemental figure A). The absence of response observed after
181 capsaicin stimulation, as well as its lack of effect on calcium levels at rest, strongly suggest that the
182 p.Gly684Val mutation blocks the channel function of TRPV1. The p.Arg772Cys mutation is located in
183 the C-terminal region of TRPV1²³, which is responsible for the interaction with calcium binding
184 proteins, such as calmodulin and the S100A1 protein. Previous work demonstrated that the Arg772
185 residue is involved in binding of the S100A1 molecule that plays a role in the regulation of TRPV1,
186 particularly in capsaicin-induced desensitization^{29,30}. Together with our results showing modified

187 kinetics of calcium release in ectopic expression, this strongly suggests that the p.Arg772Cys mutation
188 alters the regulation of the channel function of TRPV1. These data suggest that both variations induce
189 a modification of the function of TRPV1 and classify these variations as likely pathogenic according
190 to the recommendations of the American College of Medical Genetics and Genomics (ACMG)³¹.

191 Although the pathophysiology of EHS is still largely unclear, results obtained in the recent years on
192 TRPV1 function could provide new research avenues. First, the fact that EHS patients show abnormal
193 response to the MH *in vitro* contracture test may rely on calcium channel properties of TRPV1 in
194 muscle. Pioneer work demonstrated that heat stress activates TRPV1 and induces calcium
195 accumulation in myocytes during muscle contraction³². Recent studies suggested that TRPV1 may
196 crosstalk with RyR1, amplifying calcium release in skeletal muscle²⁴. Therefore, an abnormal function
197 of TRPV1 could modify calcium release from RyR1, explaining the massive cytosolic calcium release
198 that occurs during an EHS or MH crisis due to TRPV1 mutations. This functional relationship with
199 RyR1 and the demonstrated sensitivity of TRPV1 to halothane¹⁷ may explain the fact that EHS
200 patients with TRPV1 mutations have a positive IVCT. However, this hypothesis may only partially
201 explain our results since the TRPV1 mutant p.Gly684Val does not release calcium after capsaicin
202 stimulation. Further experiments with other stimuli such as halogenated anesthetics, and muscle
203 specific expression system, would help to clarify the patient positive *in vitro* contracture test.
204 However, *RYR1* independent mechanisms could also explain the pathophysiology of EHS due to
205 *TRPV1* mutations.

206 The link between the mutations identified in *TRPV1* and EHS could be based on a dysfunction of the
207 thermosensitive role (heat detection) or thermoregulatory role (the control of core body temperature)
208 of TRPV1 during exercise. *In vivo*, the thermosensitive role of TRPV1 has been largely described with
209 TRPV1^{-/-} mice models presenting reduced sensitivity to noxious heat^{33,34}. The effect of TRPV1
210 deficiency on thermoregulation in knock-out mice is more debated, but several studies have
211 demonstrated abnormal thermogenesis in response to the agonist capsaicin^{34,35} and distinct
212 thermoregulatory phenotypes in terms of behavior and metabolism in mice lacking *TRPV1*³⁶.

213 Recently, the molecular role of TRPV1 in thermosensation was dissected. In most mammals, the heat-
214 induced activation of TRPV1 is rapidly followed by a desensitization of the channel, in order to

215 protect cells against damages if noxious heat persists²⁸. This desensitization is regulated by the heat-
216 induced interaction between the N and C-terminal regions of TRPV1 which is coupled to the outer
217 pore domain, inducing a conformational change of the channel and closing the pore²⁸. Point mutations
218 in key residues in these regions can abolish this interaction, and the desensitization phenomenon. The
219 p.Arg772Cys mutation we described here is located in the C-terminal region of TRPV1 delineated by
220 the authors of this work and it could alter the physiological interaction between C- and N-terminal
221 parts of TRPV1, thus impacting the desensitization mechanism. This hypothesis fits with the delay
222 observed in the kinetics of calcium release in our calcium imaging experiments with capsaicin
223 stimulation. Inefficient heat-induced inactivation of the TRPV1 channel during a strenuous exercise
224 due to a mutation such as p.Arg722Cys could therefore participate in the pathophysiology of EHS.

225 The role of TRPV1 in the control of body temperature is less understood. It was evidenced during
226 pharmacological screening to search for new analgesics targeting TRPV1^{25,34}. Clinical trials
227 established that some TRPV1 antagonists tested as analgesics can cause thermogenesis dysfunction
228 (either hyperthermia or hypothermia) in patients, depending on their ability to selectively inhibit
229 capsaicin-, heat- or proton-induced TRPV1 activity. These results suggested that TRPV1 could
230 participate in the regulation of the systemic thermogenesis pathways and reflected the complexity of
231 the thermoregulation function of TRPV1, ^{36,37}. Interestingly, compounds able to block TRPV1
232 channel function after any type of stimulation (capsaicin, heat or acidic pH) trigger hyperthermia.
233 Along with this result, one can hypothesize that a complete block of TRPV1 function by a loss of
234 function mutation such as the p.Gly684Val may also result in an abnormal regulation of thermogenesis
235 during strenuous muscle activity.

236

237 **Conclusions**

238 In conclusion, our study involves *TRPV1* mutations as a new cause of Exertional Heat Stroke. The
239 *TRPV1* gene, as well as *RYR1*, may both be linked to Exertional Heat Stroke and Malignant
240 Hyperthermia, which supports the hypothesis of a continuum between the two diseases³⁸. We
241 identified two missense mutations in EHS patients that presented positive IVCT and demonstrated that

242 these mutations impact capsaicin-induced calcium release. However, precise mechanisms by which
243 TRPV1 participates in MH and EHS pathophysiology and alters calcium homeostasis in muscle
244 inducing altered response in IVCT need further explorations. It is possible that each of the mutations
245 of *TRPV1* we describe here impairs one of the functions TRPV1 is involved in, thermosensitivity or
246 whole body thermoregulation.

247 Nevertheless, mutations found so far in *RYR1* and *TRPV1* show a low prevalence. It can be first
248 explained by the fact that EHS is a multifactorial disorder, and not solely caused by genetic
249 predispositions. It can also reflect that mutations in other genes may be involved in EHS.

250 **Practical implications**

- 251 • *TRPV1* is a new candidate gene in Exertional Heat Stroke.
- 252 • The identification of molecular bases of EHS will help to propose efficient genetic screening
253 to individuals at risk of EHS.
- 254 • Because of the identification of variations in *TRPV1* and *RYR1* genes both in Exertional Heat
255 Stroke and Malignant Hyperthermia, the cross-risk between the two diseases should still be
256 considered.

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361

Patients	Patient 1	Patient 2
Gender	Male	Male
Age	31	25
Personal and family history	NA	No
Exertional Heat Stroke crisis		
Trigger	timed race	commando race
Corporal temperature during crisis	41°C	40°C
Date of crisis	December (French Guyana)	February
Neurological signs	Spatial and temporal disorientation, loss of consciousness	Loss of consciousness
Hepatorenal failure	No	Yes
Rhabdomyolysis	Yes	Yes
CPK at crisis	232 UI/L	NA
CPK at rest (N: 55-170 UI/L)	76 UI/L	427 UI/L
In Vitro Contracture Test		
Status	MHS	MHSh
halothane (2%)	positive (0,9g and 0,2g)	positive (0,3g and 0,2g)
caffeine (2mM)	positive (0,4g and 0,3g)	negative (0,1g)
Genetic features		
Gene	<i>TRPV1</i>	<i>TRPV1</i>
cDNA change	c.2051G>T	c.2314C>T
Amino acid change	p.Gly684Val	p.Arg772Cys
Status	heterozygous	heterozygous
RS number	rs759094783	rs1217651219
Prevalence in databases		
ExAC frequency	0.000004063	absent
GnomAD frequency	absent	absent
Prediction tools		
CADD phred score	25,9	34
SIFT	Damaging	Damaging
PolyPhen	Damaging	Damaging
MutationTaster	Damaging	Damaging
Orthologue conservation	Conserved across sarcopterygians species	Conserved across tetrapods species
Protein localization	Pore domain	S100A1 binding domain

362 **Table A:** Clinical and genetic features of the patients with variations in the *TRPV1* gene.
363 MHS: Malignant Hyperthermia Susceptible with a positive *In Vitro* Contracture Test to halothane and
364 caffeine. MHSh: Malignant Hyperthermia Susceptible with a positive *In Vitro* Contracture Test to
365 halothane only. NA: not available.

366 **Figure A:**

367 **1.** TRPV1 sequences with heterozygous c.2051G>T; p.Gly684Val (patient 1) and c.2314C>T;
368 p.Arg772Cys variations (patient 2).

369 **2.** Multiple sequence alignment of TRPV1 protein regions surrounding the p.Gly684Val and
370 p.Arg772Cys residues (red) in various species. An asterisk (*) indicates a position which has a single,
371 fully conserved residue. A colon (:) indicates conservation between groups of strongly similar
372 properties. A period (.) indicates conservation between groups of weakly similar properties.

373 **3.** Locations of c.2051G>T; p.Gly684Val and c.2314C>T; p.Arg772Cys variations on a TRPV1
374 schematic representation. The p.Gly684Val variation is located in the conserved transmembrane
375 segment 6 of the pore domain and the p.Arg772Cys variation is located in the C-terminal part of the
376 protein that includes regulatory domain and interacts with ligands and proteins such as PIP2,
377 calmodulin or protein S100³⁵.

378 **Figure B:** Calcium imaging studies in HEK-293 cells after TRPV1 mutants expression.

379 **1,** Representative time course of cytosolic calcium concentrations using the fluorescence ratio of the
380 Fluo4 indicator dye ($\Delta F/F_0$, arbitrary units) after injection of 20 μ M capsaicin ; minimum of 40 cells
381 for each condition (mean +/- SEM).

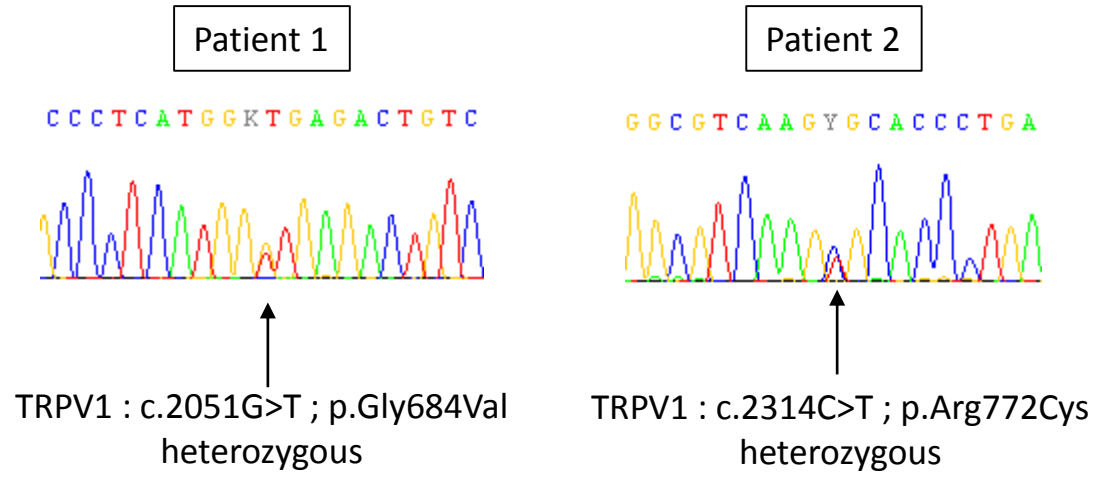
382 **2,** Corresponding Areas Under the Curve (AUC) (median +/- interquartile ranges).

383 **3,** Measurement of cytosolic calcium concentration at rest using the fluorescence ratio of the Fura2-
384 AM indicator dye (F340/F380, arbitrary units) (median +/- IQR). Non transfected cells (NT) were
385 used as control. Non parametric ANOVA followed by post-hoc test were performed: *p < 0.05, **p <
386 0.01, ***p < 0.001, ****p < 0.0001

387

Figure A

1



2

Human amino acid	684	772
H. Sapiens	LLNMLIALMGETV	CEGVKRTLSFSL
P. anubis	LLNMLIALMGETV	CEGVKRTLSFSL
R. norvegicus	LLNMLIALMGETV	CEGVKRTLSFSL
E. caballus	LLNMLIALMGETV	CEDIKRTLSFSL
O. anatinus	LLNMLIALMGETV	SEGIKRTLSFSL
G. gallus	LLNMLIALMGETV	SGDLKRNPSYCI
A. sinensis	LLNMLIALMGETV	YGDLKRNSSF
P. sinensis	LLNMLIALMGETV	STGLKRNLSFSF
	*****	.:** . * :.:

3

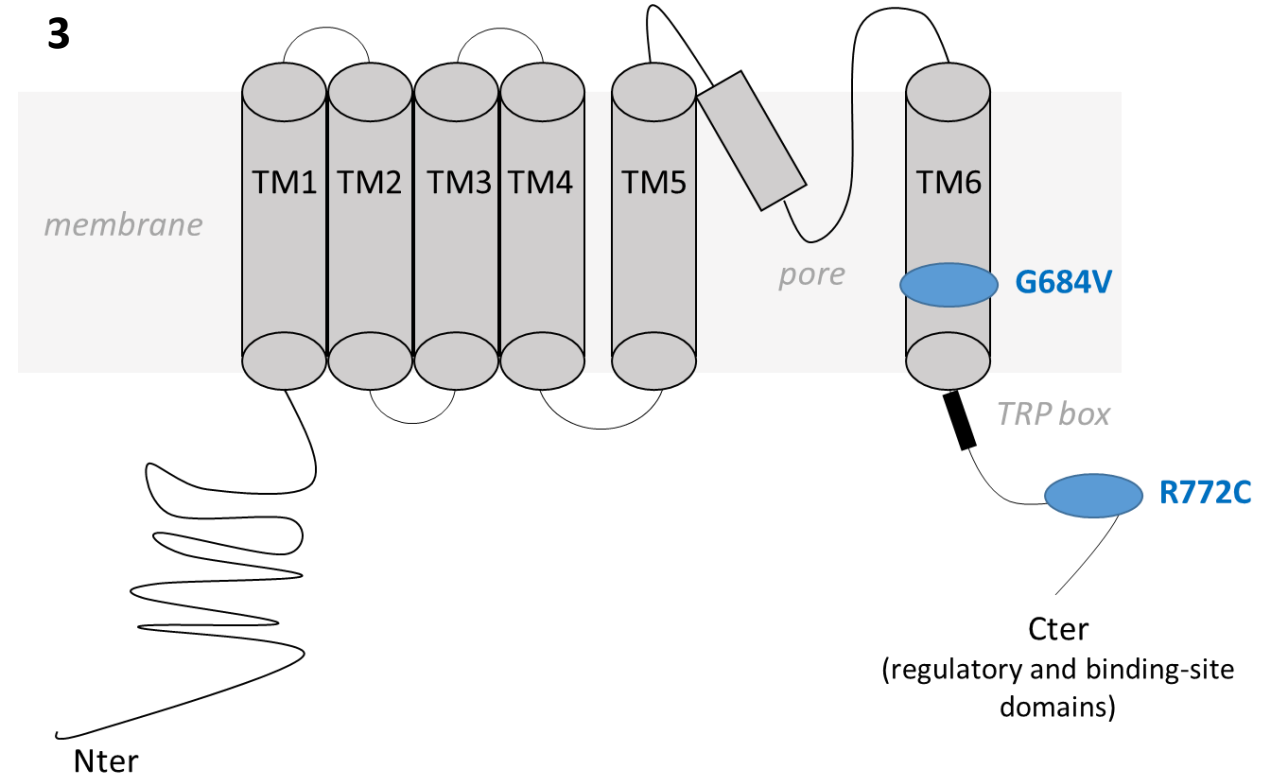


Figure B

