ISSN: 2476-2377

Research Article

International Journal of Cancer Research & Therapy

# Molecular pathobiology of aspirin responsive erythromelalgia in thrombocythemia and incurable inherited erythermalgia in Nav1.7mutated neuropathy

**Jan Jacques Michiels***

*Multidisciplinary Internist, Scientific Investigator and Consultant Professor Blood and Coagulation Disorders, Departments of Hematology, University Hospitals Antwerp and Rotterdam, Europe*

***Corresponding author**
Jan Jacques Michiels, Multidisciplinary Internist, Scientific Investigator and Consultant Professor Blood and Coagulation Disorders, Goodheart Institute & Foundation in Nature Medicine Erasmus Tower, Rotterdam, E-mail: goodheartcenter@upcmail.nl

**Submitted**: 30 June 2017; **Accepted**: 10 July 2017; **Published**: 08 Aug 2017

## Abstract

*The original description of erythromelalgia of Mitchell has been separated into three distinct disease entities of aspirin responsive erythromelalgia in thrombocythemia, incurable congenital dominant primary erythermalgia (PE), and aspirin resistant secondary erthermalgia. Aspirin responsive platelet-mediated erythromelalgic and thrombotic processes in the end-arterial circulation of toes or fingers has been discovered as a distinct arterial thrombophilic disease entity (Sticky Platelet Syndrome) in acquired and congenital thrombocythemia due to gain of function mutations in the JAK2, TPO, MPL and CALR genes. PE is a congenital dominant incurable disease with symmetric bilateral localization of red congestion and burning pain in legs with relative sparing of the toes, which spontaneously arises in childhood or adolecence and persists life long in adults. Incurable PE has been discovered as a dominant neuropathic pain disorder caused by hyperexcitibility of the sodium channel alpha subunit Nav1.7 protein located in dorsal root ganglions and nocireceptive peripheral neurons due to gain of function mutations in the SCN9A gene on chromosome 2q coding for the Nav1.7 sodium channel. Recessive chronic insensitivity for pain (CIP) is caused by homozygous or double heterozygous loss of function mutations of the SCN9A gene and loss of Nav1.7 sodium channel excitibility.*

**Keywords:** Erythromelalgia, Aspirin, Primary erythermalgia, Thrombocythemia, Platelet cyclo-oxygenase, SCN9A gene, Nav1.7 sodium channel, JAK2 gene.

## Introduction

Mitchel reported in 1872 on bilateral symmetric burning pain and purple red congestion in the feet in four young adults, aged 21-40 years [1]. Mitchell described in 1878 sixteen cases with painful affections of the feet of various types and severity in his article "On a rare vaso-motor neurosis of the extremities and on the maladies with which it may be confounded" [2]. The complaints begin with pain in the foot or feet and may start in the ball of the foot or of a great toe with extenion to the dorsum and even leg. The burning pain and red congestion may be symmetric in both feet and legs or asymmetric limited to one or both soles toes or fingers. Mitchell stated that the foot and hand disorder might be conveniently labeled erythromelalgia, deriving it from the Greek words erythros = red, melos = extremity and algos = pain [1]. The neurologist Mitchell attributed erythromelalgia of the legs and feet to an undefined form of neuritis of the peripheral nerves or nerves within the spinal marrow.

Based on a detailed analysis of the 16 cases of Mitchel and 81 cases of his own, Brown defined in 1932 six basic criteria for the diagnosis of the primary and incurable variant of burning red congested extremities in the absent of any detectable underlying disorder [3]. In 1938 Smith and Allen substituted the term erythromelalgia for another descriptive term, erythermalgia, to denote the variability of disease manifestations and to emphasis the importance of heat = therme as a main important feature of red, burning and painful extremities [4]. Erythromelalgia patients seen by Smith & Allen noted that a single dose of acetylsalicylic acid (aspirin 500mg) produced marked relief of burning distress that persited for a few days [4]. Since than both terms are used indiscriminately as synonyms in the primary and secondary forms, irrespective of aspirin-responsivenes and solely depending on the clinical absence or presence of an associated disease. Osler in 1908 and Brown in 1932 described cases of secondary erythromelalgia in polycythemia vera (PV) in which pain in the hands and feet with the extreme red congestion was suggestive for erythromelalgia of Weir Mitchell 1878 [1,5]. The etiology of erythromelalgia remained elusive for about one century until Michiels discovered between 1975 and 1980 aspirin responsive erythromelagia in thrombocythemia [6-8]. Based on analysis of the 16 cases of Mitchell and clinical observations in 81 cases in which burning pain in the hands or feet was a prominent or disabling symptom, Brown in 1932 postulated six basic criteria for the diagnosis of primary and incurable variant of burning red congested extremities [3].

1. Attacks of bilateral or symmetric burning pain occuring in the hands and feet.
2. The attacks were iniated or aggravated by standing, or exposure to heat.
3. Relief was obtained by elevation and exposure to cold.
4. During the attacks, the affected were flushed and congested

and exhibited increased local heat.

5. The pathogenesis is unknown.
6. There is no treatment available.

Following the six postulates of Brown, Michiels recognized in 1988 the incurable and rare variant of red painfull extremities in the complete absence of any underlying disorder as a distinct clinical and congenital autosomal dominant entity and therefore labeled it aspirin resistant primary erythermalgia (PE) of unknown etiology [9,10]. Our experimental and research studies undoubtedly showed that the clinical manifestations of the primary incurable inherited erythermalgia were completely different from aspirin-responsive erthromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia of patients with essential thrombocythemia (ET) and PV (Figures 1, 2 and 3) [11-15]. In extension to the six postulates of Brown (1932), we could add in the 1990s four additional specific features of incurable inherited erythermalgia (Table 2) [3,9,10,16-22]. Incurable inherited erythermalgia spontaneously arises in children with symmetricbilateral localization in both legs and persists thoughout life [9,10]. There is relative sparing of the toes because acrocyanotic ischemia or peripheral gangrene as the result of peripheral vessel (thrombotic) obstruction in skin biopsies in aspirin responsive erythromelalgia is never seen in skin punch biopsies from cases with incurable hereditary erythermalgia [4,16-24]. The histopathologic findings in skin biopsies from erythermalgic areas of patients with incurable hereditary erythermalgia were non-specific, showed the complete absence of any underlying disorder, and did not reveal a clue to its pathophysiology (Figure 4) [25]. There are a multitude of clinical conditions without thrombocythemia associated with bilateral and symmetric red swollen and burning pain in the feet and hands. These other forms are referred to as secondary aspirin resistent erythermalgia, which originate from side effects of drugs (e.g. verapramil) or arise from various disorders including cutaneous vasculitis, vasculitis in lupus erythematodes, hypertension, rheumatiod arthritis and auto-immune diseases of undetermined significance [25-28].

## Aspirin responsive platelet-mediated erythromelalgia in thrombocythemia

The relief of burning pain and red congestion by one dose of aspirin (500 mg) for a few days appeared to be a pathognomonic diagnostic clue of erythromelalgia in thrombocythemia (Figure 1) [6-8]. At that time it was known that acetylsalicylic acid (aspirin) did inhibit arachidonic induced platelet aggregation for a few days due to irreversible inhibition of platelet cyclo-oxygenase (COX-1) activity. Double blinded clinical experiments showed that sodiumsalicylate, other analgesics and the other platelet inhibiting agents including ticlopedine and dipyridamol did not inhibit platelet COX-1 activity and had no effect on erythromelalgia (Figure 1) [7,11]. This was the very first objective hint that erythromelalgia must be caused by platelet-mediated processes in thrombocythemia of patients with ET and PV. Subsequent prospective platelet function and histopathology studies were intitiated to enravel the underlying etiopathophysiology between Erythromelalgia and Thrombocythemia in ET and PV patients [6-8,11].

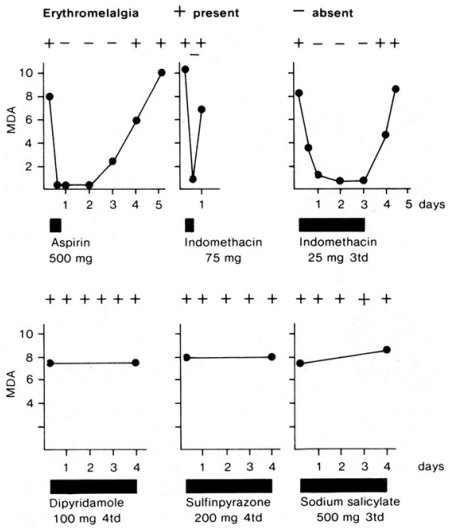



**Figure 1:** In a prospective basic research study cases with erythromelalgia in thrombocythemia were sequentially treated by sodium salicytlaat 500 mg followed by aspirin 500 mg, phenylbutazon followed by aspirin, sulfin pyrazon followed by aspirin, or ticlopedine followed by aspirin and measured MDA production after stimulation of PRP with NEM in each of the experiments to assess the degree of platelet COX) inhibition [6-8,11,12]. The longlasting analgesic effect of a single dose of aspirin is in accordance with the length of inhibition of platelet COX activity as measuered by inhibition of malondialdehyde dialdehyde (MDA) [12]. After a single dose of indomethacine 25 mg and 75 mg, the burning pain relieved for about 12 and 24 hours, which was in accordance with the lenght of inhibition of platelet MDA production. Sulfinpyrazone 800 mg, dipyridamole (platelet cyclic AMP inhibitor) 400 mg, ticlopedine (ADP receptor inhibitor) 1000 mg or sodiumslyicylate 500 mg daily for 4 days, did not alleviate the erythromelalgic signs and symptoms, but also had no effect on platelet MDA production. Thus, active platelet prostaglandin metabolism in thrombocythemic platelets is necessary for erythromelalgia to develop in ET and PVT patients [6-8].

We introduced the method of Brian Smith (1976) to measure platelet COX-1 activity directly by measuring malondialdehyde (MDA) after stimulation of platelets in platelet rich plasma (PRP) with N-ethylmaleimide (NEM) [12]. NEM maximizes the arachidonic acid mediated cyclo-oxygenase pathway to form prostaglandin endoperoxides, thromboxane A2 and its endproducts thromboxane B2 and MDA (Figure 2). MDA production in NEM stimulated PRP reflects the degree of platelet COX-1 inhibition by aspirin to document prospectively that the longlasting effect of aspirin on erythromelalgia indeed was due to irreversible inhibition of platelet COX-1 activity (Figure 1) [7,11]. Sodiumsalicylate and other platelet inhibiting agents which do not inhibtit platelet COX-1 and do not have any effect on erythromelalgic signs and symptoms [7,11]. Thus, active platelet COX-1 activity and the production of platelet prostaglandin products is necessary for erythromelalgia to develop in thrombocythemia of ET and PV patients [7]. Reversible inhibtion of platelet COX-1 activity by indomethacin 25 mg TID is an alternative to relief erythromelalgia (figure 1). In subsequent studies we showed that coumadin and ticlopedine (platelet ADP-receptor inhibitor) are not effective in the treatment of erythromelalgia because coumadin and ADP

receptor inhibitors do not inhibit the platelet COX-1 activity of hypersensitive platelets in thrombocythemia [7,11].

Erythromelalgic distress in thrombocythemia patients varies from prickling and 'pins and needles'sensations (acropresthesias) to burning pain and red congestion. The asymmetric and often unilateral localisation of red congested, warm and painful swelling affects one or more toe, the sole of the forefoot or sometimes the tips of the fingers (Figure 2A) [7,11]. If left untreated, blue ischemic spots in erythromelalgic areas, acrocyanosis or even digital gangrene of one or more toes often occurs in due course of time during long-term follow-up (Figure 2B). In contrast to arterioslerotic circulatory obstruction, peripheral arterial pulses usually remain normal in thrombocythemia of ET and PV patients with erythromelalgia. In the 1970s very little was known about the histopahology of erythromelalgia as the opportunity to examine tissue from recently developed erythromelalgis before ischemic complications do occur has not been reported. Michiels & Ten Kate decided in the late 1970s to perform skin punch biopsies from freshly relapsed erythromelalgia a few days to one week after discontinuation of aspirin in symptomatic cases of ET (Figures 2 and 3 left) [6]. Skin punch biopsies were taken from relapsed erythromelalgia 1 to 3 weeks after discontinuation of aspirin, and from cases of untreated erythromelalgia complicated by acrocyanotic spot or acrocyanosis but no digital gangrene (Figures 2 and 3 middle panel) [6].

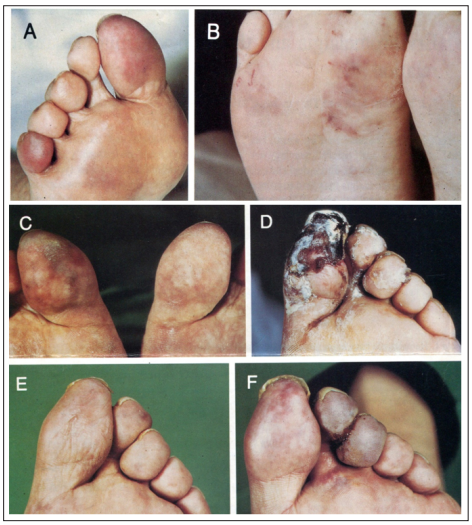



**Figure 2:** A. Severe asymmetric burning pain and red warm congestion of big and little toe, and sole of forefoot of the right foot 1 week after discontinuation of aspirin.
B. Burning, aching pain and mottled red-blue discoloration of the sole of the right foot 3 week after discontinuation of aspirin.
C. Detail of an untreated painful and bluish big toe, of which the skin surface temperature was low (cold ischemic toe) [15].
D. Progression of painful toes to 'black' big toe and peeling of the skin of second and third toe in a 6 week period of coumarin treatment.
E. Complete restoration of painful black ischemic toe within a few weeks by treatment with aspirin.
F. Relapse of painful red and blue toes 2 weeks after discontinuation od aspirin [15].

To demostrate the platelet-mediated origine of aspirin responsive erythromelalgia, we prospectively performed histopathological and platelet kinetic studies in PV and ET cases in whom erythromelalgia relapsed a few days after intended discontinuation of aspirin. A first skin biopsy was taken at day 7 to 10 in 1 PV and 2 ET cases when full blown red congested erythromelalgia (E+) (Figure 2A upper panel left) was present. A second biopsy 2 to 3 weeks after discontiuation of aspirin was taken from areas of 'advanced' erythromelalgia complicated by blue ischemic spots (figure 2B, upper panel right). In between the first and second skin biopsy, platelet kinetic studies in the presence of erythromelalgia were performed. The erythromelalgia and ischemic complications completely disappeared after re-institution of aspirin, and platelet kinetic studies were repeated. During the whole period of the prospective study design we checked the degree of platelet COX-1 inhibtion by measuring platelet derived MDA levels in PRP after NEM stimulation at times of use, discontinuation and re-institution of aspirin treatment. This protocol was prospectively executed after full informed consent of the patient and approval by the Medical Ethical Committee of the Academic Hospital, Rotterdam. The details of patient characteristics and methods are described [6].

The histopathological appearances of a skin punch biopsy from areas of recently relapsed erythromelalgia one week after aspirin discontinuation show arteriolar lesions of fibromuscular intimal proliferation without involvement of venules, capillaries or nerves (Figure 3, left pannels). The lining endothelial cells are swollen with large nuclei. The inner layer of the vessel wall was thickened by proliferation of cells, with narrowing of the lumen. With factor VIII antisera an affected arteriole shows an inner single layer of fluorescent cells, and with an antiserm aganinst smooth muscle cells strong fluorescence a multilayer of proliferated cells in the media of the arteriolar vessel wall was seen indicating that 'fibromuscular intimal thickening' is caused by increased smooth muscle cell proliferation and breaking up of the membrana elastica interna of the vessel wall. Cellular intimal proliferation and swelling of cells with cytoplasmic vacuolisation narrows the lumen and is accompanied by perivascular oedema, vascular and perivascular fibrosis and slight perivascular infiltration by inflammatory cells [6,7]. Occlusive arteriolar thrombi were not seen in the first skin punch biopsy of recently relapsed erythromelagia one week after dicontinuation of aspirin (figure 3l eft pannels)

Histopathology from a skin area of relapsed erythromelalgia complicated by blue ischemic spots (figure 2B) after 3 weeks of aspirin discontinuation showed arterioles with pronounced 'fibromuscular intimal proliferation' with narrowing of the lumen and occlusive thrombotic lesions on top of 'fibromuscular intimal proliferation' in the reticular dermis with no involvement of venules or capillaries (figure 3, middle panels, black toe due to thromboangiitis obliterans). The affected arterioles with 'fibromuscular intimal proliferation are partially or completely occluded by organised thrombus in skin areas from bluish discoloured erythromelalgic area 3 weeks after aspirin discontinuation. Immunoflurescence with IgG, IgA and IgM antisera were negative. An antiserum against complement B1A, showed granular deposits in the walls of several vessels in the subendothelial and perivasclar zones of some vessels indicative for a very non-specific inflammatory process [6,7].

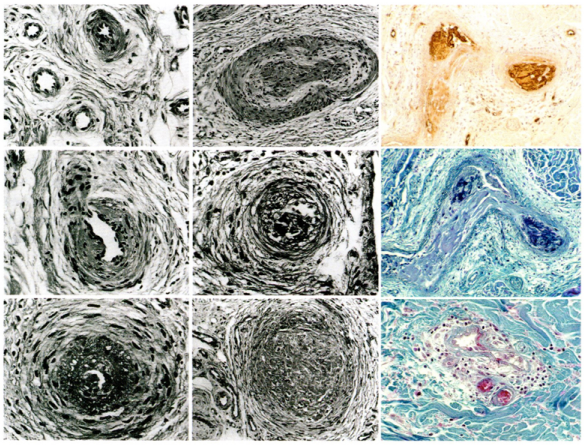


**Figure 3:** Skin punch biopsies from skin areas of recently relapsed erythromelalgia 1 week after discontinuation of aspirin (Figure 2A) in ET patients show arterioles with swollen endothelial cells and fibromuscular intimal proliferation (left) [7]. Skin punch biopsies from blue spots of skin areas with relapsed erythromelalgia 3 weeks after discontinuation of aspirin (Figure 2B) show arteriole with fibromuscular intimal proliferation with partial and complete occlusion of arteiole by fresh organized thrombus (middle upper two). Arteriole with complete occlusion of the arteriole in skin area of untreated eryhromelalgia of an acrocyanotic toe (middle bottom) [6].

An arteriole occluded by a thrombius show a strong staining for von Willebrand factor (VWF, right upper) and a very weak staining for fibrin (right middle) indicating a platelet-rich thrombus. Positive fibrin staining of an arterial thrombus in a patients with lupus anticoagulant (right lower) [40].

## The platelet mediated inflamatory component in aspirin responsive erythromelalgia

Superficial thrombophlebitis or livido reticularis is a skin manifestation of erythromelalgia in ET and PV patients [13]. The incidence of superficial thrombophlebitis was 14% in our cohort of 50 thrombocythemia (30 ET and 20 PV) patients [13]. The prostaglandin activity in fluid from artificially skin blisters can be regarded as an inflammatory component in tissues. We measured the prostaglandin activity in artificial skin blisters from areas with active erythromelalgia (2 patients), in a control person and in skin area during effective treatment with aspirin (Table 1). The total prostaglandin activity in fluid of skin blisters from area of erythromelalgic skin areas was very high as compared to control (Table 1) [14]. Effective treatment of erythromelalgic skin areas with asprin in figure 6 was associated with reduction of total prostaglandin content in skin blisters to normal (Table 1).

**Table 1:** Prostaglandin E-like (PGE) activity in fluid of artificial blisters from skin areas with erythromelalgia (E+) and control without erythromelalgia (C→E-) and from skin areas after treatment of erythromelalgia with aspirin (E+→E-)

| Blister fluid | | Total PGE | PGE/ml |
|---|---|---|---|
| 1.19 ml | E+ | 6.43 ug | 5.40 ug |
| 1.29 ml | E+ | 8.42 ug | 6.53 ug |
| 1.02 ml | C→E- | 0.82 ug | 0.80 ug |
| 1.36 ml | E+→E- | 1.35 ug | 0.99 ug |

## Molecuar etiology of incurable inherited erythermalgia

Drenth & Michiels collected blood samples from European families and the large Finley family in the United Statesn (Figure 4) with incurable inherited erythermalgia and a clear-cut autosomal dominant hereditance [24]. Drenth et al could localize the incurable inherited erythermalgia susceptibility gene on the long arm of chromosome 2 (2q24.3) [30]. Yang, et al. (2004) studied a chinese family with incurable inherited erythermalgia in three generations and discovered that heterozygous gain of function mutations in the SCN9A gene on chromosome 2q coding for the high voltage dependent sodium channel alpha subunit (Nav1.7) segregated with the affected family members [31]. Heterozygous mutations in the SCN9A gene as the cause of incurable inherited erythermalgia has been confirmed in a Flamish family by Michiels, et al. in European families by Drenth et al, and in the large USA family with autosomal dominant penetrance of PE in 5 generations (Figure 4) in which linkage of the PE susceptibility gene to chromosome 2q was reported by Drenth et al [30,32-34]. Heterozygous gain of function mutation F1449V in the SCN9A gene (Nav1.7) was found in 14 of 14 affected membersof the large USA family (Figure 4) [34]. Michiels et al investigated 10 members of a Flemish family and found a novel heterozygous S241T mutation in the SCN9A gene (Nav1.7) in all 5 investigated affected family members (figure 5) [33]. The neurologist Waxman and his team extensively studied the electrophysiological properties of mutant (SCN9A) Nav1.7 sodium channels on the basis of which primary erythermalgia became a neuropathic disorder of the small peripheral sensory and sympathetic neurons caused by hyperexcitibility of Nav1.7. Nav1.7 is located in dorsal root ganglions and in nociceptive peripheral neuron [35-39]. Untill to date the invalidating condition is incurable and refractory to any treatment, althought some subjective relief is given by analgesics, cold environment and refrain from daily activities.

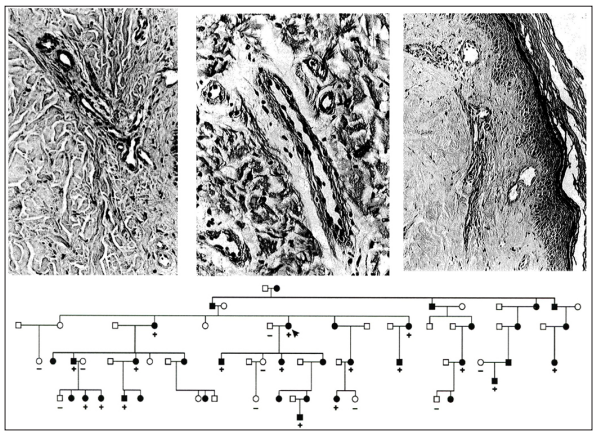


**Figure 4:** Cutaneous pathology in primary congenital and incurable erythermalgia [25]. Capillary blood vessels in the lower dermis show moderate thickenig of the wall, moderately swollen endothelial cells, minimal to moderate perivascular inflammatory cells with the absence of obvious inflammatory disease (left panel) and a lamellar appearance with perivascular edema and thickening (middle panel) in case 1 with congenital erythermalgia caused by the C1185A gain of function. mutation in the voltage-gated sodium channel alpha subunit Nav 1.7. Capillary blood vessels of the upper dermis, showing similar slight perivascular fibroplasia as well as endothelial swelling and mild aspecific inflammation (right panel) in case II 2 (figure ) with congenital erythermalgia caused by the S2417T gain of function mutation in the in the voltage-gated sodium channel alpha subunit Nav 1.7.

Family pedigree of the large USA pedigree with autosomal dominant primary erythermalgia (PE) [24,34]. Circles denote females; squares denote males. The proband is shown by arrow. Blackened symbols indicate sybjects with PE. (+) denotes subjects heterozygous for the 4393G mutation and (-) denotes subjects without the mutation [34].

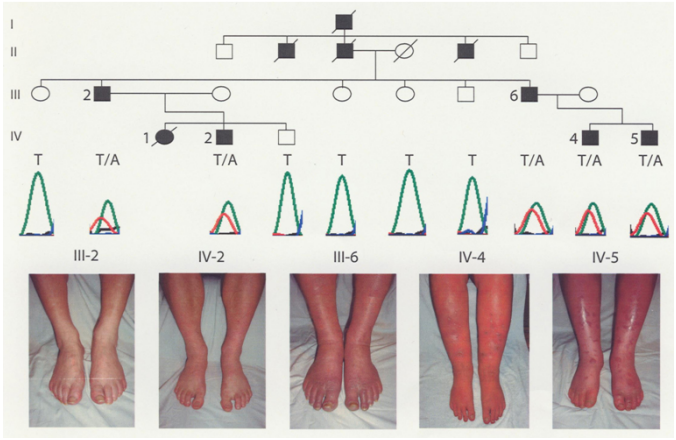



**Figure 5:** Autosomal dominant aspirin-resistant symmetric red warm and burning painfull lower legs and feet in incurable autosomal dominant erythromelalgia (familial erythermalgia) is a neuropathic disorder of hyperexcitable small peripheral and sympathetic neurons caused by the gain of function mutation S2417T (red/green) in the voltage-gated sodium channel alpha subunit Nav 1.7 (red/green) [33].

## Pathophysiology of erythromelalgia and microvascular thrombosis in thrombocythemia

Van Genderen & Michiels prospectively performed platelet kinetis studies in 10 individuals with reactive thrombocytosis, in 10 patients with thrombocythemia vera complicated by erythromelalgia (E+) and in 10 patients with asymptomatic thrombocythemia (E-) [40]. Platelet kinetic studies were performed according to the protocol during the presence of erythromelalgia for about 7 to 10 days, and after aspirin teatment several months later. The survival times in the E+ group (4.2 days) were significantly shorter than in the group with RT (7.7 days) and E- group (7.2 days). Platelet survival studies were repeated in seven E+ patients while on treatment with aspirin, which relieved the erythromelalgic symptoms completely and lastingly. The survival time in symptomatic E+ patients of 4.0 days significantly increased to 6.9 days during maintained aspirin demonstrating that aspirin-responsive erythromelalgia is a platelet-mediated microvascular thrombosis [40]. Two symptomatic E+ patients were on adequate oral anticoagulation therapy and showed shortened platelet survival times, which reversed to normal by curative treatment of erythromelalgia with aspirin [40].

Van Genderen & Michiels performed histopathology of skin biopsies derived from erythromelalgic skin areas of 2 ET patients and showed that erythromelalgic thrombi stained strongly positively for von Willebrand factor opposed to no or weak fibrin staining (Figure 3 right pannels), which is indicative for platelet-rich thrombi formation in the endarterial circulation [41]. To demonstrate that erythromelalgia in thrombocythemia is characterized by platelet activation and endothelial cell damage as the cause of fibromuscular intimaproliferation (Figure 3 left pannels) but not by thrombin generation Van Genderen & Michiels measured in vivo platelet, endothelial and coagulation activation markers in ET patients with recently relapses erythromelalgia after discontinuation of aspirin. In a cross-section study we compared plasma levels of endothelial cell marker thrombomodulin (TM), platelet activation markers platelet factor 4 (PF-4), bêta thromboglobulin (beta TG), and coagulation markers prothrombin fragment 1+2 (F1+2) and total degradation products of fibrin (TDP) in 5 ET patients with erythromelalgia (E+), in 16 asymptomatic ET patients, and 20 control persons (Figure 6) [41]. The results before and after aspirin treatment were characterized by significant higher beta-TG and TM levels, but no significant differences were detected in either F1+2 or TDP levels (Figure 6). Disappearance of erythromelalgia with aspirin was paralled by a significant decrease of beta-TG and TM. In another study Van Genderen & Michiels showed that within 10 days after withdrawal of aspirin, 3 ET-patients developed endarterial (microvascular) von Willebrand factor rich platelet- thrombi in skin biopsies from erythromelalgic areas, which was associated with a 3-30-fold increase in urinary TXB2 excretion. Both the increased urinary TXB2 excretion and clinical signs of erythromelalgic microvascular thrombosis could be corrected with a platelet-specific very low dose aspirin regimen of 50 mg daily, which is sufficient enough to inhibit platelet cyclo-oxygenase (COX) for the prevention of platelet thrombi in symptomatic thrombocythemia patients [42]. We concluded that erythromelalgia (E+) in thrombocythemia of ET and PV patients was featured by increased plasma levels of platelet factor 4 (PF4), beta-thromboglobuline (ß-tg), platelet derived growth factor (PGDF) and increased thromboxane B2 (TXB2) excretion in urine and that the generation of thrombin is not essential for platelet thrombi formation in thrombocythemia patients with erythromelalgia and its ischemic complications [42]. This explains the observed inefficacy of coumadin derivatives in the prevention and treatment of erythromelalgia, erythromelalgic acrocyanotic ischemia and migraine-like atypical transient ischemic attacks (MIAs), in ET and PV patients [11,42-47].

If erythromelalgia is left untreated, ET and PV patients not on aspirin are at high risk of digital ischemia (black toe, figure 2), migraine-like atypical transient ischemic attacks (MIAs), stroke or acute coronary syndrome during long-term follow-up [11,42-47]. The presence of erythromelalgia in ET patients is characterized by significant higher plasma levels of platelet activation markers PF4, btg, and PDGF (Figure 6).

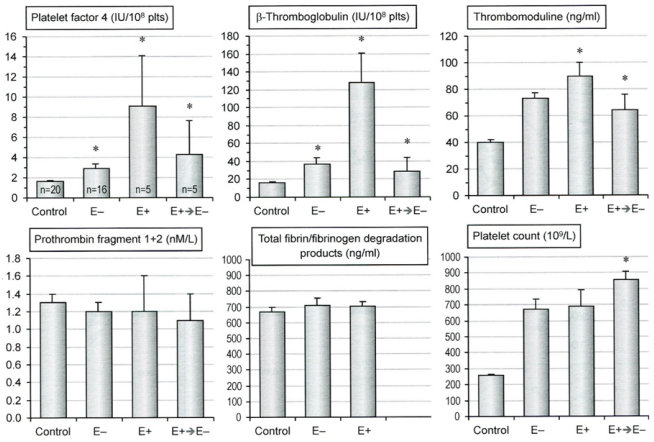



**Figure 6:** The effects of intervention with aspirin on platelet activation markers platelet factor 4 and beta-thromboglobulin, on the endothelial cell activation marker thrombomodulin, on the

coagulation activation markers prothrombin fragment 1+2 and fibrin/fibrinogen degradation products, and on platelet counts in controls asymptomatic thrombocythemia (E-), thrombocythemia complicated by erythromelalgia (E+), and cure of erythromelalgia by aspirin (E+ → E-) [41-46].

Increase of PDGF released from platelet and the endothelial cell (EC) activation marker TM refers to increased platelet-EC-vessel wall interactions underlying the etiology of fibromuscular intimal proliferation (Table 2, figures 2 and 3) [41]. Erythromelalgia is provoked by (spontaneous) intravascular activation and aggregation of thrombocythemic platelets at high shear stress rate in the end-arterial circulation of the peripheral, cerebral, ocular and coronary circulation [6-8,11,42-46]. Erythromelalgia is never seen in reactive thrombocytosis and thromboctosis in Ph-positive CML. This clearly indicates that not only a quantitative (platelets >350x9L), but also a qualitative 'hypersensitive' functional platelet defect ('sticky' platelets') is the underlying etiology of platelet mediated arterial thrombophilia in myeloproliferative thrombocythemia. A gain of platelet function (Sticky Platelet Syndrome : SPS) is postulated by Michiels & Van Vliet in the early 1990s as the underlying pathophysiology of platelet-mediated arteriolar microvascular erythromelalgic thrombotic complications in thrombocythemia [6-9,40-45]. If left untreated, the erythromelalgic microvacular circulation disturbances (including the migraine-like atypical TIAs) indeed do progress to major vascular complications in particular in thrombocythemia associated with PV due to increased leukocyte, hematocrit and erythrocyte counts. Venesection aiming at a hematcrit around 0.40 in PV patients significantly reduced the major arterial thrombotic complications [46-50]. The erythromelalgic and cerebral-ocular microvascular ischemic manifestations persist if the PV is brought into hematological remission by phlebotomy alone and no aspirin, because the microvascular syndrome of thrombocythemia at platelet count above $350x^{9}/L$ persists [50].

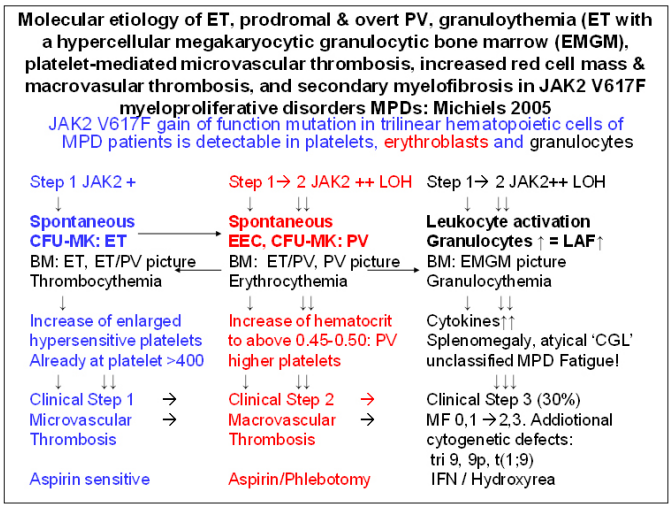
**Table 2:** Diagnostic and differential diagnostic criteria of aspirin responsive erythromelalgia and incurable erythermalgia (Michiels 1997)

| *Erythromelalgia* | *Erythermalgia* |
|---|---|
| 1. Attacks of unilateral or bilateral but asymmetric burning pain and red, warm congestion of footsole, handpalm, or one or more toes or fingers. | *Attack of bilateral and symmetric burning pain and red, warm congestion in the feet, lower legs, or hands. |
| 2. The attacks aggravate by standing, exercise, and/or exposure to heat. | *The attacks are initiated or aggravated by standing, exercise, or exposure to heat. |
| 3. Relief is obtained by rest, by elevation of the involved extremity, and by exposure to cold. | *Relief is obtained by rest, by elevation of the extremities, and by exposure to cold. |
| 4. During the attacks, the affected parts are red and congested and exhibit local heat. | *During the attacks, the affected parts are red, congested, and exhibit local heat. |
| 5. Erythromelalgia is causally linked to thrombocythemia in myeloproliferative disorders. Symptoms are caused by platelet-mediated thrombosis and inflammation in the endarterial circulation. | *The pathogenesis of erythermalgia is unknown. |
| 6. Low-dose aspirin cures erythromelalgia. | *There is no treatment available. |
| 7. Erythromelalgia arises at adult age. | Erythermalgia spontaneously arises in children or adolescents and persists throughout life. |
| 8. Erythromelalgia in thrombocythemia is an acquired condition. | A hereditary basis of primary erythromalalgia has become evident. |
| 9. There is preferential involvement of forefoot sole and one or more toes or fingers and it usually progresses in acrocyanotic ischemia or even peripheral gangrene. | There is relative sparing of the toes and acrocyanotic ischemia. Peripheral gangrene is never observed. |
| 10. The histologic findings in skin biopsies from erythromelalgic areas are specific, showing fibromuscular intimal proliferation and thrombotic occlusion of arterioles or small arteries in the absence of preexistent vascular disease.[7] | The histologic findings in skin biopsies from erythermalgic areas are nonspecific showing the absence of an underlying disorder and do not reveal a clue to its pathophysiology.[15] |

## Molecular etiology of thrombocythemia and platelet thrombophilia: Sticky Platelet Syndrome

With the advent of the $JAK2^{V617F}$ mutation as the driver cause of ET and PV it became evident that $JAK2^{V617F}$ mutated megakaryocytes are constitutively activated with increased hypersentivity to TPO as the production of hyperreactive sticky platelet as the cause of platelet mediated arteriolar inflammation (platelet thrombophilia) in thrombocythemia (Table 3, Figures 2 and 3) [51-53]. Heterozygous $JAK2^{V617F}$ mutation is associated with no or slight increase of erythropoiesis in ET and prodromal PV) (table 3). Homozygous $JAK2^{V617F}$ mutation in PV preferentially leads to pronounced constitutive trilinear activation in the bone marrow of megakaryo/erythro/granulopoiesis due to a more pronounced hypersensitivity to EPO and GCSF (Table 3) [51-54].

**Table 3:** Model for disease heterogeneity of $JAK2^{V617F}$ Myeloproiferative Neoplasms. The $JAK2^{V617F}$ occurs in the hematopietic cells and gives rise to the onset of disease. The kinase activity of heterozygous $JAK2^{V617F}$ mutation is impaired by the presence of a normal JAK2 wild type allele. The disease generated by this low constitutivey activated kinase activity in ET or forme fruste PV. A second event leading to an increased kinase activity occurs in a hematopietic stem cell already carrying the mutation. The second event is caused be an amplification of the $JAK2^{V617F}$ activity locus through mitotic recombination resulting in homozygous $JAK2^{V617F}$ mutated MPN with 9p loss of heterogeneity. Sustained high levels of JAK2 kinase activity could lead to progression to PV from its pletoric stage to spent phase complicated by splenomegaly and secondary myelofibrosis.




The sequential occurrence of erythromelagic microvascular ischemic symptoms in heterozygous $JAK2^{V617F}$mutated ET and major arterial and venous thrombosis on top of microvascular disturbances in homozygous $JAK2^{V617F}$ mutated PV is related to increase of low JAK2 mutation load in ET into high JAK2 mutation load in homozygous PV [53]. About 30 to 40% of MPN cases with masked ET and PV (not meeting the crude PVSG criteria of ET and PV) do initially present with splenomegaly and subsequently will develop myeloid metaplasia of the spleen and advanced secondary myelofibrosis (post-PV myelofibrosis) [53]. The high risk of erythromelalgic peripheral, cerebral and coronary microvascular events has been observed in $JAK2^{V617F}$ positive thrombocythemia vera patients, much less frequent in $JAK2^{V617F}$ negative thrombocyhtemia, and has never been reported in reactive thrombocytosis and thrombocytosis associated with CML [54-56]. Vannucchi, et al.retrospectively compared the clinical profile and the incidence of major thrombotic events in 188 $JAK2^{V617F}$ homozygous MPN patients ($JAK2^{V617F}$ mutation more than 50% in 104 PV and 14 ET) with 587 heterozygous ($JAK2^{V617F}$ mutation less than 50% in 219 PV and 257 ET) and 257 JAK2 wild type ET patients (Table 4) [57]. Homozygous $JAK2^{V617F}$ positive patients (mutation load above 50% in ET and PV) were older, had higher leukocyte counts, hematocrits and larger spleen volumes. Major thrombotic

events were recorded after diagnosis in the preceding years during follow-up when not on aspirin. Major thrombotic events included ischemic stroke, transient ischemic attacks, myocardial infarction, angina pectoris, deep vein thrombosis, abdominal vein thrombosis, and pulmonary embolism. Symptoms due to microvessel disorder migraine-like headache, acral paresthesia, erythromelalgia, transient neurological and visual disturbances were neglected and excluded in this retrospective analysis. One hundred seventy-six patients (18.3%) had a major thrombotic event not on aspirin at diagnosis with a similar frequency in PV (19.2%) and ET (17.8%). During longterm follow-up, major thrombosis (not on aspirin) occurred in 122 patients (12.7%), corresponding to 14.9% in PV and 11.6% in ET patients (Table 4). Hemorrhages at diagnosis manifested in 55 (5.7%) patients, 5.3% in PV and 6.0% in ET. Hemorrhages during follow-up was recorded in 45 (4.7%) ET/PV. The frequency of bleeding was higher in JAK2V617F homozygous PV patients (21.4%) than in wild type or heterozygous ET patients (3.1%) and 3.8%. There were no significant differences in the overall rate and type of major thrombosis in heterozygous PV and homozygous PV (Table 4).

**Table 4:** Major cardiovascular events at diagnosis or during long-term follow-up in 323 PV and 639 ET patients according to the $JAK2^{V617F}$ mutation status in the retrospective study of Vannucchi. Only major thrombotic events were retrospectively recorded not including the erythromelalgic peripheral and migraine like cerebral ischemic events.

| **Patients** | **PV N= 323** | | **ET N= 639** | |
|---|---|---|---|---|
| **JAK2V617 mutation status** | **hetero** | **homozygous** | **hetero** | **wild type** |
| Number of patients | 219 | 104 | 368 | 257 |
| **At diagnosis** | | | | |
| Major arterial events | 21% | 15,4% | 21.7% | 10.5% |
| Venous events | 6.4% | 2.9% | 7.9% | 4.7% |
| **During 10 years follow-up (no aspirin)** | | | | |
| Major arterial events | 10.1% | 12.5% | 6.3% | 5.8% |
| Venous events | 4.1% | 7.7% | 6.3% | 2.7% |
| **Total during life time follow-up** | | | | |
| **Major arterial** | **31.1%** | **27.9%** | **28%** | **16.3%** |
| **Venous** | **10.5%** | **10.6%** | **14.2%** | **7.4%** |

## Expert opinion

In 1878 Mitchel defined erythromelalgia as a rare vaso-motor neurosis of the extremities [1]. Brown postulated in 1932 six basic criteria of the idiopathic variant of burning pain and red congestion in the absence of any detectable underlying disorder [4]. In 1938, Smith and Allen substituted the term erythromelalgia for erythermalgia to denote the importance of heat-therme, and first reported that a single dose of acetylsalicylic acid (aspirin 500mg) produced marked relief of burning distress and red congestion that persisted for a few days [5]. Since than both terms are used indicriminately as synonyms in the primary and secondary forms, irrespective of aspirin-responsiveness and solely depending on the absence or presence of a detectable disease. Between 1975 and 1985 Michiels et al discovered aspirin-responsive erythromelalgia causally related to thrombocythemia in ET and PV patients to be clearly distinct from aspirin resistent primary erythermalgia (PE) [6-10]. Following the six postulates of Brown (Table 2), Michiels discovered between 1988 and 1990 that incurable PE in the absence of any detectable underlying disease in skin biopsy (Figure 4) is a novel distinct clinical entity and could add four additional features of PE (Table 2) [9-10]. PE as a novel dominant and incurable disease has been confirmed in 1992 by the publication of a large family with autosomal dominant PE (Finley et al, Figure 4) [24].

**Table 5:** Nosologic classification and molecular etiology of aspirin responsive erythromelalgia in acquired and congenital thrombocythemia caused by gain of function mutations in the TPO, MPL and JAK2 genes and incurable primary erythermalgia (PE) a congenital dominant neuropathy caused by gain of function mutations in the SCN9A gene; from Mitchell 1878 to Michiels 2016.

| **Discoverer** | **Year** | **Disease** | |
|---|---|---|---|
| Mitchel [2] | 1878 | Erythromelalgia | |
| Brown [4] | 1932 | Primary erythromelalgia: PE | |
| Smith & Allen [5] | 1938 | Erythermalgia versus | Aspirin responsive Erythromelalgia in thrombocythemia |
| Michiels [6-8] | 1985/2013 | → | Aspirin responsive erythromelalgia and migraine-like ischemic attacks: Sticky Platelet Syndrome (ET & PV) |
| Michiels [9] | 1988 | Incurable inherited Erythermalgia is a | |
| Michiels [10] | 1990 | congenital dominant disease | |
| Drenthet al [30] | 2001 | erythermalgia susceptibility gene on chromosome 2q | |
| Molecular etiology | 2004 | Gain of function mutation SCN9A gene in PE. Yang et al 200431 | |
| | 2005 | | Gain of function mutation JAK2 gene in acquired thrombocythemia (ET & PV) thrombocythemia (ET & PV) |
| | 2006 | | Germline gain of function mutation in |
| | 2013 | | TPO, MPL and JAK2 genes in congenital dominant thrombocythemia |

Four sequential discoveries have contributed to the elucidation of the molecular basis for PE. Linkage analysis in 2001 by Drenth & Michiels of 5 European families and one large USA family with documented incurable dominant PE located the PE susceptibility gene on chrmosome 2q32-32 [30]. In 2004 Yang et al first found two mutations within this locus leading to single acid substitutions in the SCN9A gene for the human Nav1.7 sodium channel in patients with familial PE [31]. The finding of gain of function mutations in the SCN9A gene for the human Nav1.7 sodium channels in the 5 European PE families and the large USA PE family are completely in line with the dominant inheritence of PE as first delineated by Michiels [9,10,32-34]. Incurable PE is the first inherited painful neuropathy and has become a model disease that could held lessons for other painfulll conditions and for the development of rational, mechanism-based treatments for pain [31-39]. The Nav1.7 gain of function mutations are expected to increase the excitability the nociceptors of peripheral sensory neurons and sympathetic ganglion neurons [34-39]. Functional analysis using patch clamp recording

showed that these Nav1.7 mutations cause a hyperpolarizing shift in activation of the sodium channel and a slowing of deactivation. This is accompagnied by an enhanced response to small depolarizing changes that should confer hyperexitability on nerve cells, which express the mutant sodium channels. The Nav1.7 sodium channels are not globally present within all neurons but are selectively expressed within peripheral sensory neurons in dorsal root ganglia (DRG). There are about a dozen SCN9A mutations in multiple families identified causing PE (Reviewed by Drenth & Waxman Figure 7) [58]. All PE mutations detected are missense mutations that change important and highly conserved amino acid residues of the Nav1.7 protein [58]. The majority of the gain of function PE mutations are located in cytoplasmic linkers of the Nav1.7 channel, and some (F216S and N395) are located in transmembrane domains of the channel (Reviewed by Drenth & Waxman, Figure 7) [58].PE mutations contribute to the hyperexcitability of pain-signalling DRG neurons thus causing extreme sensitivity to pain (hyperalgesia). Genotype-phenotype analysis of the SCN9A gene showed that smaller effects on sodium channel activation are related to a smaller degree of DRG neuron excitability and later onset of clinical PE signs and symptoms [58].

Cox, et al. discovered SCN9A mutations that cause a loss of Nav1.7 function in 6 patientss from 3 consanguineous Japanese families with a congenital insensitivity to pain (CIP) [59]. The mutations were identified in exon 10 (S495X), exon 13 (I767X, and exon 15 (W897X) Goldberg (2007) and Ahmed (2007) performed studies in 9 Western European and North and South American families and a third large Canadian family used linkage analysis is search for homozygous halotypes identified the same gene and detected 10 truncating SCN9A mutations [60,61]. The majority of affected patients in these two studies were homozygous for SCN9A mutations and 2 patients were compound heterozygous for different SCN9A nonsense mutations. The Nav1.7 channelopathy-associated insensitivity to pain (CIP) is an autosomal recessive disorder. There is intact ability to distinguish between sharp and dull stimuli and to detect differences in temperature. CIP individuals are insenstive to pain beginning in infancy that leads to frequent painless injuries, fractures, burns, ultimately resulting in a shortened life expectancy. Affected individuals are either homozygous or compound heterozygous for loss of function (nonsense) mutations of the Nav1.7 protein. All loss of function mutations are randomly distributed across the SCN9A gene [58]. The mechanism behind this profound insensitity to pain may stimulate the search for novel painkillers by targeted inhibition of impulses-travel from nociceptive (pain-signalling) dorsal root ganglia (DRG) neurons.

Sudden attacks of rectal, ocular, and submaxillary pain, first described in 1958, has been named as paroxysmal extreme pain disorder (PEPD) [58,62,63]. PEPD is an autosomal dominant disorder charactetized by paroxysmal episodes of sudden onset pain at different body sites, accompanied by skin flushing. There are four well defined types of painfull episodes in PEPD patients [62,63]. A genome wide-linkage search in one large pedigree with PEPD led to the PEPD susceptibility gene on chromosome 2q24.3 [64]. As this region contained SCN9A, the investigators sequenced the gene and found 8 heterozygous missense mutations in 8 families [64]. Each family possessed a unique PEPD mutationand one individual was compound heterozygous for R996C and V1298D (Figure 7) [58]. This individual was more severly affected than his heterozygous father. Functional analysis of 3 mutations (I1461T, T1464I and M1627K) were reported to impair inactivation of the Nav1.7channel. The effects in response to provoking stimuli (such as stretching and exposure to cold temperature) of PE mutations enhance channel inactivation [58].The effects in response to provoking stimuli of PEPD mutations impair channel inactivation. Such differences are predicted to contribute at least in part to the different clinical symptomatology in these two congenital pain disorders PE and PEPD due to gain of function

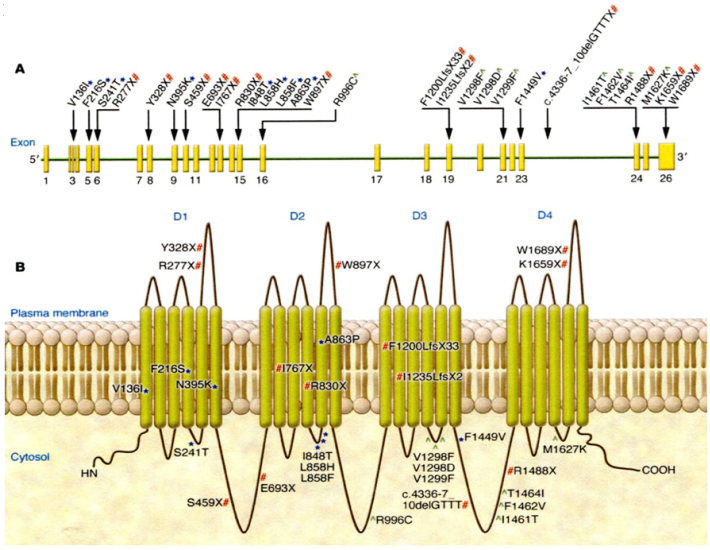



**Figure 7:** Heterozygous gain of function mutations in the sodium-channel subunit Nav1.7 that are associated with autosomal dominant pain disorders of primary erythermalgia (PE) (*) and paroxysmal extreme pain disorder (PEPD) (^), and homozygous or double heterozygous loss of function mutations in the sodium-channel subunit Nav1.7 that is associated with chronic insensitivity to pain (CIP) (#) (A). Sodium-channel subunit Nav1.7 is encoded by the SCN9A gene comprising 26 coding exons (A).The mutations of PE and CIP are distributed over the entire gene sequence (A and B), but the mutations linked to PEPD are located closer to the 3'end of the SCN9A gene (A and B). The sodium-channel subunit Nav1.7 consists of 4 domains (D1-D4) each with 6 transmembrane segments. The locations of known mutations associated with genetic pain disorders PE (*), PEPD (^) and CIP (#) are shown. Source Drenth & Waxman [58].

## Aspirin responsive erythromelalgia in congenital dominant thrombocythemia

The first well documented report on autosomal dominant congenital ET due to a gain of function mutation in the TPO gene was first described in a large Dutch family and confirmed in a Polish family [65-67]. Analysis of the TPO gene in the Dutch family was recommended by Drs Michiels and van der Maas and performed by Dr Skoda [66]. The C→G transversion in the splice donor of intron 3 of the TPO gene on chromosome 3q27 co-segregated with the affected autosomal dominant hereditary ET (HET) in the Dutch and Polish families [66-68]. This mutation destroys the splice donor site in intron 3 and results in exon 3 skipping. The shortened 5'UTR gene resulted in a gain of function which leads to overproduction of thrombopoietin (TPO) by a mechanism of increased efficiency of the TPO mRNA translation. The *in vivo* increased TPO levels are responsible for the etiology of congenital ET by stimulating megakaryocyte production both in vitro and in vivo. Congenital gain of function mutation in the TPO gene in the Dutch and Polish families results in increased plasma TPO

levels, which induce a physiological activation of the TPO→MPL signalling pathway [66-68]. This results in hyperproliferation of large mature megakaryocytes and persistant increased platelet counts. The marked increased TPO levels in affected family members as the cause of increased platelet count (congenital thrombocythemia) is associated with microvascular circulation disturbances including erythromelalgia and atypical transient ischemic attacks similar to platelet-mediated thrombophilia in acquired thrombocythemia in ET patients as first discovered by Michiels et al in 1984/1985 [6,7,68].

Congenital dominant ET due to a germline gain of function mutation S505N in the MPL gene has been described in 2004 [69,70]. Teofili et al reported the clinical and hematological features of 41 patients with a congenital germline gain of function mutation S505N in the MPL (thrombopoietine = TPO receptor) gene in seven Italian families (21 ET patients with a proven MPL S505N mutation and 20 relatives with thrombocythemia) [70]. In 41 affected congenital ET patients with a proven or suspected MPL S505N mutation, 15 major thrombotic episodes in 14 members (34%) were reported as Budd-Chiari syndrome, deep vein thrombosis, ecclampsia, stroke and myocardial infarction at ages between 31-81 years, median 52 years. Fourteen out of 21 well documented MPL S505N mutated ET patients had no splenomegaly and were free of major thrombosis during follow-up at ages between 2 and 76 years (mean 31 years) [70,71].

There are three novel molecular variants of a germline gain of function mutation in the JAK2 gene for congenital dominant ET: dominant ET caused by a heterozygous gain of function mutation JAK2V617I, JAK2R564Q and JAK2H698N in the JAK2 gene [72-75]. Mead et al described the germline mutation JAK2V617I as the sole genetic abnormality, sufficient to induce the ET phenotype of MPN in a family with congenital dominant ET complicated by microvascular ischemic events in some of the them [72,73]. Another novel heterozygous JAK2R564Q mutation has been identified by Etheridge et al in another unrelated family with congenital dominant ET [74]. The investigatos demonstrated that JAK2V617I and JAK2Q564W heterozygous germline mutation are the sole driver for congenital ET in JAK2V617I-positive and JAK2Q564W-positive individuals. The clinical investigators nicely documented that peripheral blood and bone marrow histology in these two families were consistent with normocellular ET without features of PV [72-74]. The heterozygous JAK2V671I and JAK2Q564W mutated congenital ET patients had completely normal values for haemoglobin, haematocrit, erythrocytes, TPO and EPO levels [72-74]. Heterozygous JAK2V671I or JAK2Q564W gain of function mutation in affected congenital ET patients is sufficient to induce cytokine hyperresponsivenees of hematopoietic stem cells to stimulate megakaryopiesis and platelet production with the induction of a ET clinical phenotype. Congenital dominant ET caused by a heterozygous gain of function mutation in the JAK2 gene R564Q and V617I[72-74] did present with typical manifestations of dominant aspirin responsive Sticky Platelet Syndrome (SPS) similar as seen in acquired JAK2V617F mutated ET [6,7,54,72-74].

## Acknowledgement

The author is a founder of the Goodheart Intitute and Foundation in Nature Medicine and Health, Rotterdam, The Netherlands, Freedom of Science and Education, International Free University Network. JJ Michiels serves as consultant professor in the Bloodcoagulation, Hemostasis Research Laboratory at the department of Hematology University Hospital, Antwerp; as consultant professor in Hematology and Bloodcoagulation, Comenius University, Bratislava, consultant to the Dutch Society of Internal Medicine and Ministery of Public Health; consultant of Industrial and Pharmaceutical Medicine; as Editor in Chief of 2 Medical Journals and as a Guest Editor on request and by self initiation.

## References

1. Mitchell SW (1872) Clinical lecture on certain affections of the feet. Phil Med Times 3: 81-82 and 113-115.
2. Mitchel SW (1878) On a rare vaso-motor neurosis of extremities and on the maladies with which it may be confounded. AmerJ Med Sc 76: 2-36.
3. Brown GF (1932) Erythromealgia and other disturbances of the extremities accompanied by vasodilatation and burning. Am J Med Sci 183: 468-485.
4. Smith LA, Allen FV (1938) Erythermalgia (erythromelalgia) of the extremities. A syndrome characterized by redness, heat and pain. Amer Heart J 16: 175-188.
5. Osler W (1908) A clinical lecture on erythraemia. Polcythemia vera with cyanosis. Maladie du Vasques. Lancet 1: 143-145.
6. Michiels JJ, Ten Kate FWJ, Vuzevski VD, Abels J (1984) Histopathology of erythromelalgia. Histopathology 8: 669-678.
7. Michiels JJ, Abels J, Steketee J, van Vliet HHDM, Vuzevski VD(1985) Erythromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia. Ann Intern Med 102: 466-471.
8. Michiels JJ (1997) Erythromelalgia and thrombocythemia: a disease of platelet prostaglandin metabolism. Thesis Rotterdam 1981. Sem Thromb Hemostas 23: 335-338.
9. Michiels JJ, Van Joost (1988) Primary and secondary erythermalgia: a critical review. Neth J Med 33: 205-208.
10. Michiels JJ, Van Joost TH, Vuzevski VD (1989) Idiopathic erythermalgia: a congenital disorder. Amer Acad Dermatol 21: 1128-1130.
11. Michiels JJ (2014) Erythromelalgic microvascular disturbances, major thrombosis and hemorrhagic manifestations of thrombocythemia in patients with essential thrombocythemia and polycythemia vera: original observations and therapeutic implications. J Hematol Thromb Dis 2: 5
12. Smith JB, Ingerman CM, Silver MJ (1976) Malondialdehyde formation as an indicator of prostaglandin production by human platelets. J Lab Clin Med 88: 167-172.
13. Michiels JJ, Van Genderen PJJ, Lindemans J, Van Vliet HHDM (1996) Erythromelalgic, thrombotic and hemorrhagic manifestations in 50 cases of thrombocythemia. Leuk Lymph 22: 47-56.
14. Michiels JJ, Zijlstra FJ (1993) Prostaglandin cyclooxygenase products but not thromboxabe A2 is involved in the pathogenesis of erythromelalgia in thrombocythemia. Mediators Inflammation 2: 385-389.
15. Michiels JJ, Van Joost Th (1990) Erythromelalgia and thrombocythemia: a causal relation J Amer Acad Dermatol 22: 107-111.
16. Michiels JJ (1990) Erythromelalgia versus erythermalgia. Lancet 336: 183-184.
17. Van Genderen PJ, Michiels JJ, Drenth JP (1993) Hereditary

erythermalgia and acquired erythromelalgia. Am J Med Genet 45: 530-532.
18. Drenth JPH, Michiels JJ (1992) Clinical characteristics and pathophysiology of erythromelalgia and erythermalgia. Am J Med 93: 111-112.
19. Drenth JP, Michiels JJ (1994) Erythromelalgia and erythermalgia: diagnostic differentiation. Int J Dermatol 33: 393-397.
20. Michiels JJ, Drenth JP (1994) Erytromelalgia and erythermalgia; lumpers and splitters. Int J Dermatol 33: 412-413.
21. Michiels JJ, Drenth JP, Van Genderen PJJ (1995) Classification and diagnosis of erythromelalgia and erythermalgia. Int J Dermatol 34: 97-100.
22. Drenth JP, Michiels JJ (1994) Erythromelalgia and erythermalgia: diagnostic differentiation Int J Dermatol 33: 393-397.
23. Michiels JJ (1997) Erythromelalgia and vascular complications in polycythemia vera. Sem Thromb Hemostas 23: 441-454.
24. Finley WH, Lindsey JR, Fine JD, Dixon GA, Burbank MK(1992) Autosomal dominant erythromelalgia. AM J Med genet 42: 310-315.
25. Drenth PJH. Vuzevski VD, Van Joost Th, Casteels-Van Daele, Vermylen J, et al. (1996) Cutaneous pathology in primary erythermalgia. Amer J Dermatol 18: 3034.
26. Drenth JHP, Michiels JJ, Van Joost T, Vuzevski VD (1993) Secondary erythermalgia in systemic lupus erythematodes. J Rheumatol 20: 144-146.
27. Drenth JHP, Michiels JJ, Van Joost T, Vuzevski VD (1995) Secondary erythermalgia associated with an autoimmune disorder of undetermined significance. Dermatology 190: 232-234.
28. Drenth JHP, Michiels JJ, Ozsoylu S (1995) Acute secondary erythermalgia and hypertension in children. Eur J Pediatr 154: 882-885.
29. Drenth JHP, Michiels JJ, Van Joost T, Vuzevski VD (1922) Verapamil-induced secondary erythermalgia. BritJ Dermatol 127: 292-294.
30. Drenth JP, Finley WH, Breedveld, Testers I, Michiels JJ, et al. (2001) The primary erythermalgia-susceptibilty gene is located on chromosome 2q31-32.Am J Hum Genet 68: 1282.
31. Yang Y, Wang Y, Li S, Xu Z, Li H, et al. (2004) Mutations in SCN9A, encoding a (Nac1.7) sodium channel alpha subunit in patients with primary erythermalgia. J Med Genet 41: 171-174.
32. Drenth JPH, Morsche RH, Guillet G, Taieb A, Kirby RL, (2005) SCN9A mutations define primary erythermalgia as a neuropathic disorder of voltage gated sodium channels. J Invest Dermatol 124: 13330-1338.
33. MichielsJJ, Morsche HM, Jansen JBMJ, Drenth JPH (2005) Autosomal dominant erythermalgia associated with a novel (SCN9A) mutation in the voltage-gated sodium channel alpha subunit Nav1.7. Arch Neurol 62: 1587-1590
34. Dib-Hajj, Rush AM, Cummins TR, Hisama FM, Novella S, et al. (2005) Gain of function mutation in (SCN9A) Nav1.7 in familial erythermalgia induces bursting of sensory neurons. Brain 128: 1847-1854.
35. Cummins TR, Dib-Hajj SD, Waxman SG (2004) Electrophysiological properties of mutant (SCN9A) Nav1.7 sodium channels in a painful inherited neuropathy. J Neursci 24: 8232-8236.
36. Han C,Rush AM,Dib-Hajj, Li S, Xu Z, et al. (2006) Sporadic onset of erythermalgia: a gain of function mutation in Nav1.7. Ann Neurol 59: 553-558.
37. Burns TM, Te Morsche RHN, Jansen LBMJ, Drenth JPH (2005) Genetic heterogeneity and exclusion of a modifying locus at 2q in a family with autosomal dominant primary erythermalgia. BritJ Dermatol 153: 174-177.
38. Waxman SG, Dib-Haij SD (2005) Erythermalgia: molecular basis for an inherited pain syndrome. Trends Mol Med 11: 555-562.
39. Waxman SG, Dib-Haij SD. Erythromelalgia: a hereditary pain syndrome enters the molecular era. Ann Neurol 57: 785-788.
40. Van Genderen PJJ, Michiels JJ, van Strik R, Lindemans J, van Vliet HHDM (2005) Platelet consumption in thrombocythemia complicated by erythromelalgia; reversal by aspirin. Thromb Haemostas 73: 210-214.
41. Van Genderen PJJ, Lucas IS, van Strik R, Vuzevski VD, Prins FJ, et al. (1996) Erythromelalgia in essential thrombocythemia is characterized by platelet activation and endothelial cell damage but not by thrombin generation. Thromb Haemostas 76: 333-338.
42. Van Genderen PJJ, Prins FJ, Michiels JJ, Schroer K (1999) Thromboxane-dependent platelet activation in vivo precedes arterial thrombosis in thrombocythemia: a rationale for the use of low-dose aspirin as an antithrombotic agent. Brit J Haematol 104: 438-441
43. Michiels JJ, Koudstaal PJ, Mulder AH, Van Vliet HHDM (1993) Transient neurological and ocular manifestations in primary thrombocythemia. Neurology 43: 1107-1110.
44. Michiels JJ, Van Genderen PJJ, Jansen HP, Koudstaal PJ (1999) Atypical transient ischemic attacks in thrombocythemia of various myeloproliferative disorders. Leukemia Lymphoma 22: 65-70.
45. Scheffer MG, Michiels JJ, Simoons ML, Roelandt JRTC (1991) Thrombocythemia and coronary artery disease. Am Heart J 122: 573-576.
46. Michiels JJ, Berneman Z, Schroyens W, Koudstaal PJ, Lindemans J, et al. (2006) Platelet-mediated erythromelalgic, cerebral, ocular and coronary microvascular ischemic and thrombotic manifestations in patients with essential thrombocythemia and polycythemia vera: a distinct aspirin-responsive and Coumadin-resistant arterial thrombophilia. Platelets 17:528-544.
47. Michiels JJ, Berneman Z, Gadisseur A, Lam KH, De Raeve H, et al. (2015) Aspirin-responsive migraine-like transient cerebral and ocular ischemic attacks and erythromelalgia in JAKV617F-positive essential thrombocythemia and polycythemia vera. Acta Haematol 133: 56-63.
48. Pearson TC, Weitherley-Mein G (1978) Vascular occlusive episodes and venous hematocrit in primary polycythemia. Lancet 2: 1219-1222.
49. Thomas DJ, Marshall J, Ross Russel, Weitherley-Mein, et al. (1972) Cerebral blood-flow in polycythemia vera. Lancet 2: 161-163.
50. Messinezy M, Pearson TC, Prochanzka A (1985) Treatment of primary proliferative polycythemia vera by venesection and low dose buslfan: retrospective study from one center. Br J Haematol 61: 657-666.
51. James C, Ugo V, Vainchenker W (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythemia vera. Nature 434: 1144-1148.
52. Villeval JL, James C, Pisani D, Casadevall N, Vainchenker W

(2006) New insights into the pathogenesis of JAK2V617F-ositive myeloproliferative disorders and consequences for the management. Sem Thromb Hemostas 32: 341-351.
53. Michiels JJ, Berneman Z, Van Bockstaele D, van der Planken M, De Raeve H, et al. (2006) Clinical and laboratory features, pathobiology of platelet-mediated thrombosis and bleeding complications and the molecular etiology of essential thrombocythemia and polycythemia vera: therapeutic implications. Sem Thromb hemostas 32: 174-207.
54. Michiels JJ, Ten Kate FWJ, Koudstaal PJ, Van Genderen PJJ (2013) Aspirin responsive platelet thrombophilia in essential thrombocythemia and polycythemia vera. World J Hematol 2: 20-43.
55. Finazzi G, Rambaldi A, Guerini V, Carobbo A, Barbui T(2007) Risk of thrombosis in patients with essential thrombocythemia and polycythemia vera according to JAK2V617F mutation status Haematologica 92: 135-136.
56. Anonioli E, Guglielmelli P, Pancrazzi A (2005) Clinical implications of the JAK2V617F mutation in essential thrombocythemia. Leukemia 19: 1847-1848.
57. Vannucchi A, Antonioli E, Guglielmomelli P (2007) Clinical profile of homozygous JAK2V617F mutation in patients with polycythemia vera or essential thrombocythemia. Blood110: 840-846.
58. Drenth JPH, Waxman SG (2007) Mutations in sodium-channel gene SCN9A cause a spectrum of human genetic pain disorders. J Clin Invest 117: 3603-3609.
59. Cox JJ (2006) An SCN9A channelopathy causes congenital inability to experience pain. Nature 444: 894-898.
60. Goldberg Y (2007) Loss-of-function mutations in the Nav1.7 gene underlie congenital indifference to pain in multiple human populations. Clin Genet 71: 311-319.
61. Ahmad S (2007) A stop codon mutation in SCN9A causes lack of pain sensation. Hum Mol Genet 16: 2114-2121.
62. Koopmann TT, Bezina CR, Wilde AAM(2006) Voltage-gated sodium channels: action platers with many faces. Ann Med 38: 472-482.
63. Brunklaus A, Ellis R, Reave E, Semsarian C(2014) Genotype phenotype associations across the voltage-gated sodium channel family. J Med Genet 51: 650-658.
64. Fertleman CR (2006) SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. Neuron 52: 767-774.
65. Schlemper RJ, van der Maas APC, Eikenboom JCJ (1994) Familial essential thrombocythemia: clinical charateristics of 11 cases in one family. Ann Hematol 68: 153-158.
66. Wiestner A, Schlemper RJ, van der Maas APC, Skoda RC (1998) An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia. Nat Genet 18: 49-52.
67. Liu K, Kralovics R, Ridzli Z, Grabovsky B, Buser AS, et al. (2008) A de novo splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia in a Polish family. Haeatologica 93: 706-714.
68. Michiels JJ, Jan Stasko J, Peter Kubish P, Achille Pich A, De Raeve H (2014) Hereditary Essential Thrombocythemia due to a gain of function mutation in the thrombopoietin (TPO) and JAK2 gene as the cause of congenital autosomal dominant Essential Thrombocythemia and Aspirin-responsive Sticky Platelet Syndrome, J Hematol Thrombo Dis 2-6
69. Ding J, Momutsa H, Wakita A, Kato-Uranishi A (2004) Familial essential thrombocythemia associated with dominant-positive activation mutation of the MPL gene, which encodes for the receptor for thrombopoietin. Blood 103: 4198-4200.
70. Teofili L, Giona F, Torti L (2010) Hereditary thrombocytosis caused by MPL S505N is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis. Haematologica 95: 650-670.
71. Michiels JJ, Pich A, De Raeve H, Campr V, Schwarz J (2014) WHO clinical, molecular and pathological (WHO-CMP) features of congenital MPL S505N and acquired MPLW515L/K mutated essential thrombocythemia and myelofibrosis. J Hematol Thrombo Dis 2: 6
72. Mead AJ, Rugless S, Jacobson SER, Schuh A (2012) Germline JAK2 mutation in a family with hereditery thrombocytosis. New Eng J Med 366: 967-968.
73. Mead AJ, Chowdhury O, Pecquet C, Dusa A, Woll P, Atkinson D, et al. (2013) Impact of isolated germline JAK2V617I mutation on human hematopoiesis. Blood 121: 4156-4165.
74. Etheridge SL, Cosgrovel ME, Sangkhael V, Corbol L, Rohl M, et al. (2014) A novel, germline JAK2 mutation, JAKR564Q, causes familial essential thrombocythemia. Blood december 123: 1059-1063.
75. Rumi E, Harutyunyan A, Casetti I, Pietra D (2014) A novel germline JAK2 mutation in familial myeloproliferative neoplasms. Am J Hematol 89: 117-118.