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Review

The impact of pharmacogenetics for migraine

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Abstract

Migraine is a paroxysmal neurological disorder affecting up to 12% of males and 24% of females in the general population. As migraine has been demonstrated to have a strong, but complex, genetic component, pharmacogenetics bears great promise in providing new targets for drug development and optimization of individual specific therapy. Better, preferably prophylactic, treatment of migraine patients is desired because the drugs now used are not effective in all patients, allow recurrence of the headache in a high percentage of patients and sometimes have severe adverse side-effects. With the recent identification of the brain-specific P/Q-type Ca^{2+} channel gene CACNA1A as a pivotal player in the pathogenesis of migraine, the first step has been taken to identify primary biochemical pathways leading to migraine. The work on migraine can also have implications for the increasing number of additional neurological episodic disorders having the common denominator of channelopathy.

Keywords: Migraine; Ca^{2+} channel; CACNA1A; Triptan; Pharmacogenetics

1. Pharmacogenetics and migraine

With the exception of the triptans, present migraine treatment formulas are based on empirical grounds. Implementation of molecular medicine in migraine is a major challenge with great potential payback. Although migraine is one of the most common disorders, affecting millions of people worldwide, the pathogenetic pathways are still poorly understood. There is even a complete lack of biochemical markers to diagnose migraine. Accordingly, diagnosis depends heavily on the interpretation of filled-out questionnaires, diagnostic interviews and physical examination by well-trained and experienced clinicians. In addition, the clinical heterogeneity of migraine symptoms makes it hard to diagnose but also complicates specific treatment of symptoms.

The episodic nature of migraine, the compelling evidence for a complex genetic etiology, and the recent indications of it being a channelopathy form a unique challenge for the development of novel, more specific treatment, preferably prophylactic, strategies. The goal of pharmacogenetics is not only to provide new drug targets, but ultimately to enable individual specific treatment. We now discuss further what we know about this disease in relation to recent developments in the field of pharmacogenetics.

1.1. Migraine—general

Migraine is a paroxysmal neurological disorder affecting up to 12% of males and 24% of females in the general population, with highest prevalence between ages 25 and 55 years (Russell and Olesen, 1996; Lipton and Stewart, 1997). The disease is characterized by recurrent attacks of disabling, mostly unilateral headache, associated with nausea, vomiting, photo- and phonophobia, and malaise (*migraine without aura*). In about one-third of the patients, the attacks are preceded or accompanied by transient focal neurological aura symptoms (*migraine with aura*). The aura can be characterized by visual, sensory or motor phenomena, and may also involve language or brainstem disturbances. Typical aura symptoms are homonymous visual disturbance, unilateral numbness or weakness, or

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aphasia. Most aura symptoms develop gradually within 5 to 20 min and usually do not last longer than 60 min. In the case of migraine with aura, the headache and associated symptoms typically start within 1 h from the end of the aura phase, and show features similar to those in migraine without aura, but may be less severe or of shorter duration. Duration of migraine attacks may vary from hours up to 3 days, but usually last about 1 day. About 25–60% of migraine patients (both migraine with and without aura) experience premonitory symptoms, which occur hours to a day or two before a migraine attack. These symptoms usually consist of changes in mood or behaviour such as hyperactivity, hypo-activity, depression, euphoria, difficulty with concentrating, craving for special food, repetitive yawning, or drowsiness. While these symptoms are quite variable among individuals, they are often rather consistent within individuals. To study migraine scientifically, there is a clear need for uniform diagnostic criteria, which were provided by the International Headache Society (IHS) in 1988 (Table 1) (Headache Classification Committee, 1988).

Anyone may suffer from one or two migraine attacks in life, indicating that the migraine attack itself is not abnormal, but the tendency to get recurrent attacks is. According to the IHS criteria, an individual can only be classified as migraine without aura if the patient has suffered from at least five attacks, whereas two attacks or more are required for a migraine with aura diagnosis (Table 1).

1.2. Threshold theory

The pathophysiology of parts of the migraine attack, notably the aura and headache phase, is only poorly understood (Ferrari, 1998). Stimuli are noticed by the brain, are interpreted, and activate the trigeminovascular system. Activation of this system gives rise not only to transmission of nociceptive information to higher central nervous system centers, but also to the release of vasoactive peptides at nerve endings that surround pial vessels. Consequently, these vessels are dilated, which gives the trobbing pulsating headache. In contrast, the aura symptoms are caused by a depolarizing wave, known as cortical spreading depression. This wave propagates across the brain cortex and causes neuronal silencing, reduced ion homeostasis, and massive efflux of excitatory amino acids. The true cause of migraine, that is why and how a migraine attack begins, is

Table 1
IHS classification and diagnostic criteria for migraine without aura, migraine with aura and familial hemiplegic migraine

Migraine without aura
(A) At least five attacks fulfilling B–D
(B) Headache attacks lasting 4–72 h (untreated or unsuccessfully treated)
(C) Headache has at least two of the following characteristics:
1. Unilateral location
2. Pulsating quality
3. Moderate or severe intensity (inhibits or prohibits daily activities)
4. Aggravation by walking stairs or similar routine physical activity
(D) During headache at least one of the following:
1. Nausea and/or vomiting
2. Photophobia and phonophobia
(E) At least one of the following:
1. History, physical and neurological examinations do not suggest associated head trauma, vascular or non-vascular intracranial disorders, exposure to or withdrawal from (toxic) substances, non-cephalic infection, metabolic disorders or cranial or facial disorders
2. History and/or physical- and/or neurological examinations do suggest such disorder, but is ruled out by appropriate investigations
3. Such disorder is present, but migraine attacks do not occur for the first time in close temporal relation to the disorder
Migraine with aura
(A) At least two attacks fulfilling B
(B) At least three of the following four characteristics:
1. One or more fully reversible aura symptoms indicating focal cerebral cortical and/or brain stem dysfunction
2. At least one aura symptom develops gradually over more than 4 min or, two or more symptoms occur in succession
3. No aura symptom lasts more than 60 min. If more than one aura symptom is present, accepted duration is proportionally increased
4. Headache follows aura with a free interval of less than 60 min (It may also begin before or simultaneously with the aura).
(C) At least one of the following:
1. History, physical and neurological examinations do not suggest associated head trauma, vascular or non-vascular intracranial disorders, exposure to or withdrawal from (toxic) substances, non-cephalic infection, metabolic disorders or cranial or facial disorders
2. History and/or physical- and/or neurological examinations do suggest such disorder, but is ruled out by appropriate investigations
3. Such disorder is present, but migraine attacks do not occur for the first time in close temporal relation to the disorder
Familial hemiplegic migraine
(A) Fulfills criteria for migraine with aura
(B) The aura includes some degree of hemiparesis and may be prolonged
(C) At least one first degree relative has identical attacks

not known. It is commonly believed that there is an individual "threshold" to get migraine attacks. This threshold may be high, so migraine attacks occur seldom, for example only under extreme, unfavourable, external conditions. The threshold may also be low, so that attacks occur frequently after common triggers, or apparently spontaneously. Attacks thus seem to involve physiological mechanisms, initiated by migraine-specific triggers. Genetic factors appear to set the individual threshold; internal and environmental factors, such as hormonal fluctuations, fatigue, relaxation after stress, meteorological changes and substance misuse may modulate this set-point.

1.3. Migraine, a multifactorial disorder

Migraine frequently runs in families, suggesting that hereditary factors are involved (Russell et al., 1995). Family and twin studies have provided conflicting results with respect to the mode of inheritance of migraine. This discrepancy is at least partly a result of methodological differences and shortcomings (Haan et al., 1994). It was concluded from a large proband-oriented clinical study that migraine with aura is largely determined by genetic factors, whereas migraine without aura seems to be caused by a combination of both genetic and non-genetic factors (Russell et al., 1995). Recent studies of twins, using the IHS criteria, confirmed that genetic factors play an important role in the common types of migraine (Gervil et al., 1999; Ziegler et al., 1998). Consequently, it is now generally accepted that migraine is a genetic disorder with variable expression of clinical symptoms, a complex mode of inheritance, and is influenced by environmental factors.

1.4. Treatment of migraine

Different treatment strategies, based on empirical grounds, are currently used to treat migraine patients. Non-specific drugs, for instance analgesics such as paracetamol, and rapidly absorbable non-steroid anti-inflammatory drugs (NSAIDs) such as aspirin or naproxen, are usually sufficient to treat mild to moderate migraine attacks (Ferrari, 1998; Diener et al., 1998). In patients with a high attack frequency, prophylactic treatment may be considered. Although these various treatment strategies are far from efficient or satisfactory, their aspecific mode of action will hardly support a thorough pharmacogenetic endeavor to improve their performance. In contrast, the recent development of more specific migraine drugs, especially the triptans, is more relevant for pharmacogenetics studies. The clinical treatment of migraine attacks with aspecific and specific drugs is discussed below.

1.4.1. Prophylactic treatment of migraine

The most important indications to start prophylaxis are headache frequency of more than two attacks per month, no, or inappropriate, response to acute treatment or optimal abortive therapies that have produced intolerable side-effects. Some drugs have been shown effective, for example valproic acid and some of the β-adrenoceptor antagonists (like propanolol), but their efficacy is limited: at most 55% of patients will have 50% or more reduction of the attack. Other drugs, like Ca^{2+} channel blockers (f.i. flunarizine) or serotonin ($5\text{-}HT_2$ receptor antagonists (f.i. methysergide)), seem to be effective, but efficacy has not been evaluated in randomised trials with the current methodology (Silberstein, 1998; Toda and Tfelt-Hansen, 2000). Unfortunately, these drugs have severe side-effects. Clearly, there is a great need for better prophylactic agents, and well-designed trials are needed to guide the use of these drugs. At present, the mode of action in prophylactic treatment is mostly unknown (Goadsby, 1997).

1.4.2. Acute treatment of migraine

As mentioned before, mild to moderate migraine attacks may be treated by non-specific drugs such as analgesic and rapidly absorbable NSAIDs (Ferrari, 1998; Diener et al., 1998). Especially the combination of a NSAID such as aspirin and an antiemetic compound like metoclopramide, which helps absorption of the drug, has proven highly effective in the treatment of migraine (Tfelt-Hansen et al., 1995). Aspirin and paracetamol are the most frequently used drugs for the treatment of migraine, and combined with caffeine have proved to be effective in the treatment of migraine pain and associated symptoms in several placebo-controlled trials (Lipton et al., 1998; Goldstein et al., 1999). The choice of drug, dose, and route of administration, depends on the characteristics and frequency of the attacks, and on the specific preferences and contra-indications of the patient (Ferrari, 1998). It is important to note that overuse of analgesics may lead to rebound headache, especially with combination products that contain caffeine. Therefore, to reduce the risk of rebound headache, patients should be advised to limit the use of these products to no more than 3 days per week.

Severe attacks, associated with high disability, usually respond better to specific anti-migraine drugs. The appropriate drug and dose should be selected and titrated in a stepwise fashion, starting with the lowest likely effective dose, and increasing the dose, or changing the drug, after treatment of two or three attacks, if necessary. The maximal tolerated and effective dose should then be taken in one dose, rather than using repeated smaller doses.

1.4.3. Specific drugs for the acute treatment of migraine

1.4.3.1. Ergot alkaloids. For decades, the only specific drugs for the acute treatment of migraine were ergot alkaloids (e.g. ergotamine). Ergotamine is effective because of its potent vasoconstrictor activity. Although these ergot alkaloids are widely used, their efficacy has been poorly demonstrated by placebo-controlled clinical trials. Still, ergotamine may be useful in certain patients, such as

those with prolonged attacks (> 48 h) or in whom headache recurrence is a substantial issue. Use of ergotamines may induce many side-effects such as nausea, vomiting, vertigo, gastric symptoms, dry mouth and restlessness. The drug, ergotamine, has frequently been associated with substernal chest pain, discomfort and even myocardial infarction and sudden death. In addition, overdose or chronic overuse of ergotamine may induce ergotism: cyanosis, necrosis and infarctions of the heart and brain (Tfelt-Hansen et al., 1995; Meyler, 1996). A more frequent side-effect is ergot-dependent headache that can be induced even by small doses of ergots, if taken regularly twice or more a week (Ferrari, 1998). The high occurrence of side-effects is probably due to the wide range of receptors (e.g. α-adrenoceptor, dopamine and 5-HT receptors) to which ergots display affinity (Tfelt-Hansen et al., 1995; Meyler, 1996). More detailed characterization of the ergotamine receptor pathways may provide clues to response improvement in individual cases (e.g. receptor polymorphisms) and lead to the development of more specific derivative structures to achieve better specificity and efficacy. Ergot alkaloids have low oral and rectal bioavailability and clinical response is not related to plasma concentration of the drug (Tfelt-Hansen and Paalzow, 1985).

1.4.3.2. Sumatriptan and other triptans. Some 40 years ago it was observed that administration of 5-HT could abort migraine attacks (Kimbell et al., 1960). Further evidence that 5-HT is involved in the pathophysiology of migraine was provided by the observation that 5-HT metabolism in migraine patients is disturbed: interictal systemic 5-HT levels are reduced and rise during attacks, possibly as a (failing) self-defense response (Ferrari et al., 1989). These observations prompted the development of sumatriptan; the first migraine drug for which a specific molecular basis of action is known (Humphrey et al., 1990). Sumatriptan was designed to act selectively as a vasoconstrictor at 5-HT_1 receptors in cranial blood vessels, but the drug also acts on 5-HT_1 receptors located in peripheral human blood vessels. The exact mode of action of sumatriptan, however, is still under debate. Three distinct modes of action have been suggested:

- Vasoconstriction of meningeal, dural, cerebral, or pial vessels, mediated via stimulation of vascular 5-HT_{1B} receptors.
- Inhibition of dural neurogenic inflammation, most probably mediated by presynaptic stimulation of 5-HT_{1D} and/or 5-HT_{1F} receptors.
- Central inhibition of pain transmission: inhibition of trigeminal neurons in the brain stem and upper spinal cord, mediated by 5-HT_{1B}, 5-HT_{1D} or 5-HT_{1F} receptors.

The discovery of sumatriptan was a major improvement in the acute treatment of migraine. The drug is highly effective and well tolerated (Ferrari, 1991). However, sumatriptan has some shortcomings such as low oral bioavailability and recurrence of headache within 24 to 48 h after initial headache relief in up to 40% of the patients with an initially good response (Ferrari and Saxena, 1993, 1995). Still, up to 15% of patients never respond to subcutaneous sumatriptan. Importantly, up to about 40% of the patients report chest-related symptoms, when specially asked about these (Visser et al., 1996). Other adverse events that have frequently been reported are, for example, tingling, paraesthesias, and warm sensations in the head, neck, chest and limbs. In exceptional cases, more severe side-effects have been reported, such as myocardial infarction and cardiac arrest. This is the main reason why the drug is contraindicated in patients with coronary artery disease because of its potential to constrict coronary arteries (MaassenVanDenBrink et al., 1999). Based on the success of sumatriptan, second-generation triptans have been developed with similar pharmacodynamic characteristics. The main differences between these compounds are determined by their different pharmacokinetics. Compared to that of sumatriptan, the oral bioavailability is much higher (45–75%) and thus more consistent and the therapeutic plasma levels are reached more rapidly (30–60 min). In addition, the second generation triptans show increased lipophilicity and brain penetration. However, the main characteristic of all triptans available for acute treatment remains the ability to contract blood vessels and to prevent secondary symptoms.

The development of new generation triptans with improved characteristics and specificity can be guided by the rapidly increasing knowledge about the heterogeneity and function of the 5-HT receptor superfamily (Peroutka, 1998). Equally, identification of functional polymorphisms in these receptors may help to explain inter-individual differences in response and side-effects. Relevant published data on the use of pharmacogenetics in migraine was reported by MaassenVanDenBrink et al. (1998) who studied the possible relation between 5-HT_{1B} polymorphisms (G861C and T-261G) and the clinical response to sumatriptan in various groups of migraine patients, but did not observe a significant difference. Interestingly, two polymorphisms in receptor 5-HT_{1B} (F124C) and 5-HT_{2C} (C23S) have been shown to negatively affect, for instance, binding of dihydroergotamine, sumatriptan and serotonin, respectively (Brüss et al., 1999; Lappalainen et al., 1995) and therefore might be good starting points for future pharmacogenetic studies in migraine patients.

2. Pharmacogenomics in the Human Genome Project era?

With the realisation of the shortcomings of present migraine treatment strategies, and in view of the potential of identifying molecular pathways by genetic approaches,

thereby providing novel targets for drug development, we initiated genetic studies to identify genes playing a pivotal primary role in the pathogenesis of migraine. Genetic dissection of migraine should not only provide an insight into (inter-related) pathways but also automatically indicate potential target genes affecting drug performance.

2.1. P / Q-type Ca^{2+} channel as new drug target?

The complex nature of the genetics of the common types of migraine has, however hampered the identification of an underlying genetic factor. The migraine spectrum comprises the common types of migraine (with and without aura) as well as rare autosomal dominant variants of migraine, such as familial hemiplegic migraine. Patients with familial hemiplegic migraine suffer from attacks of migraine with aura, associated with a transient hemiparesis or hemiplegia (one-sided weakness or paralysis of the body) in addition to other aura symptoms. In some families, symptoms of familial hemiplegic migraine are also associated with (progressive) permanent ataxia (disturbance of coordination of movements). The symptoms of headache and aura phase of familial hemiplegic migraine and "normal" migraine attacks are very similar, and both types may alternate within individuals and co-occur within families. These observations suggest strongly that familial hemiplegic migraine is part of the migraine spectrum and that familial hemiplegic migraine can be used as a model to study the complex genetics of the common types of migraine. Importantly, familial hemiplegic migraine exhibits a clear autosomal dominant inheritance pattern, allowing conventional parametric linkage analysis to identify the chromosomal localization of genes causal to the syndrome in the specific family.

In 50% of the familial hemiplegic migraine families reported, the causative gene encodes the brain-specific voltage-gated Ca^{2+} channel α1A subunit (CACNA1A) (Joutel et al., 1994; Ophoff et al., 1994, 1996) located on the short arm of chromosome 19. A number of the remaining families have a locus on chromosome 1q21–q23 (Ducros et al., 1997; Gardner et al., 1997), whereas other familial hemiplegic migraine families are not linked to either chromosome 19p or 1q (Ducros et al., 1997; Ophoff, not published). These results clearly show a genetic heterogeneity for familial hemiplegic migraine with at least three different genes involved only one of which has been yet identified.

At present, at least 13 familial hemiplegic migraine mutations in the CACNA1A gene have been reported (Ophoff et al., 1996; Ducros et al., 1999a,b; Carrera et al., 1999; Battistini et al., 1999; Montagna, 2000) (Fig. 1, Table 2). All these mutations are missense mutations, single base-pair changes that lead to substitution of a single amino acid residue in the predicted protein. The fact that particularly missense mutations are associated with familial hemiplegic migraine suggests a molecular mechanism similar to what is found in other human channelopathies. Both alleles are likely to be expressed with the mutated allele resulting in gain-of-function variants of the α1A Ca^{2+} channel subunit. Such mutations have been described in the α subunit of the skeletal muscle sodium channel, resulting in hyperkalemic periodic paralysis, paramyotonia congenita, and the sodium channel myotonias (Hudson et al., 1995; Cannon, 1996).

As the main functional role of the P/Q-type Ca^{2+} channel is to mediate neurotransmitter release, its involvement in migraine pathophysiology is highly intriguing. A wealth of data suggests a disturbed transmitter homeostasis in the pathogenesis of migraine (Ferrari et al., 1989, 1990). Accordingly, it is of importance to understand how familial hemiplegic migraine mutations cause the disease, and one has to study the functional consequences of the mutations for Ca^{2+} channel function. Disturbances in electrophysiological parameters can be measured, using patch clamp techniques, on *Xenopus* oocytes or human embryonic kidney (HEK) 293 cells transfected with mutated CACNA1A cDNA constructs. Although certain mutations cause a similar phenotype in patients, and one would expect similar effects on electrophysiological parameters, this does not seem to be the case. Two research groups have shown very complicated patterns for various mutants (Kraus et al., 1998, 2000; Hans et al., 1999). All mutants studied are opened even at weak membrane depolarisations, but differ considerably as to channel inactivation and recovery from inactivation. Also, the expression level of functionally active channels on the plasma membrane varied considerably among the mutants. From these studies it can be concluded that some mutations, like the T666M mutation, probably result in a decreased Ca^{2+} influx, whereas other mutations, like the R192Q mutation, are expected to increase Ca^{2+} entry. For other mutations such as V714A and I1811L the situation is less clear, and they might result in either an increased or a decreased influx of Ca^{2+} ions into the cell. Therefore, the variability in channel dysfunction (more or less Ca^{2+} influx) combined with the genetic heterogeneity (a large number of mutations have been described) and clinical variation (f.i. the presence or absence of ataxia in the patient) clearly exemplifies the complexity of familial hemiplegic migraine.

2.2. Mouse mutants of the P / Q-type Ca^{2+} channel

Natural mutant mouse strains that have been shown to harbor mutations in the same P/Q-type Ca^{2+} channel subunit (Fig. 1, Table 2) are available. In *tottering* and *Rolling Nagoya* mice, missense mutations were identified, whereas in *leaner* mice, the causative mutation is a splice mutation, giving rise to products of aberrant length (Fletcher et al., 1996; Mori et al., 2000). In all three strains, the mutations result in different recessively inherited neurological phenotypes, with symptoms including absence and focal motor seizures, mild to severe ataxia,

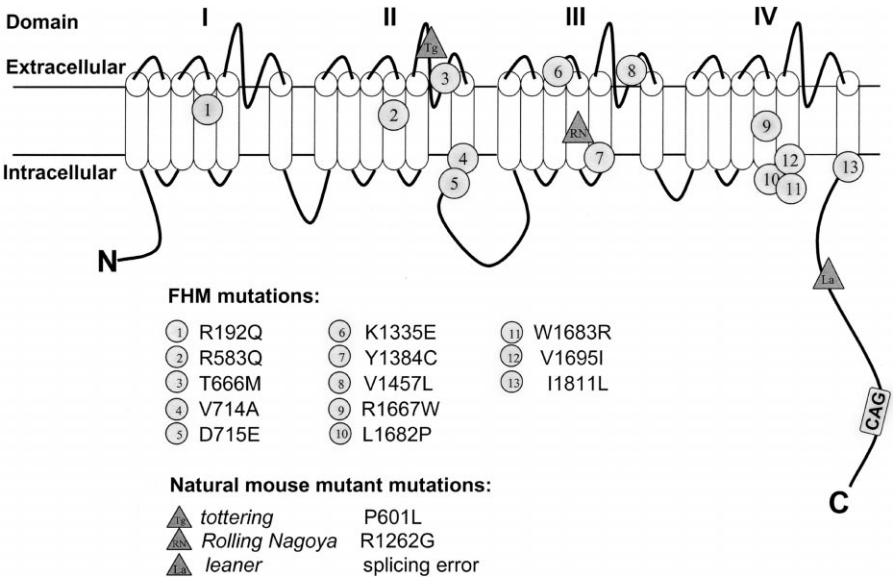

Fig. 1. Schematic representation of FHM and natural mouse mutations in the CACNA1A gene.

and behavioral arrest. The most prominent effect of the mutations in Purkinje cells of *tottering*, *leaner* and *Rolling Nagoya* mice is a reduction in Ca^{2+} currents through P-type channels to about half (Lorenzon et al., 1998; Wakamori et al., 1998; Mori et al., 2000), which is accompanied by diverse changes in activation and inactivation kinetics of Ca^{2+} channel functioning in the different strains. Recent studies have also indicated that neurotransmitter release is impaired in hippocampal neuronal slices and in neuromuscular junctions of *tottering* mice (Qian and Noebels, 2000; Plomp et al., 2000). Although it is still unclear whether these mice are suitable models for migraine, the natural models together with future transgenic knock-in mouse models, harbouring familial hemiplegic migraine mutations, will provide interesting study material for future work. For instance, with these models, alterations in gene expression profiles can be analysed using novel micro-arraying techniques, or one can use these models to genetically map modifying genes.

2.3. Involvement of chromosome 19p13 locus in common forms of migraine

Knowing that the CACNA1A gene is implicated in familial hemiplegic migraine, the next step is to study whether the same gene is also involved in the common types of migraine. A direct way to investigate such an involvement is direct sequencing of the entire gene, including the regulatory regions. However, the gene is rather large with almost 50 exons distributed over 350 kb of genomic sequence (Ophoff et al., 1996), which makes direct sequencing very labor intensive. In addition, locus heterogeneity of the common types of migraine with and without aura is likely to exist, meaning that only a fraction of the patients may carry a variant in this gene associated with migraine susceptibility. A study by Hovatta et al. (1994) showed no linkage to 19p in four Finnish families with migraine with aura and positive family history of migraine, probably because of the stringent criteria used to find linkage (one gene assumption, large contribution of the locus). In contrast, Nyholt et al. (1998) did find positive linkage in an Australian family with migraine with aura. This genetic heterogeneity also impedes a case-control study for migraine if the intragenic polymorphisms of the CACNA1A gene are used: extremely large sample sizes will be required. A useful but indirect approach for gene mapping is the affected sib-pair analysis. In the affected sib-pair analysis method, the observed sharing of alleles among affected siblings is compared with the expected sharing for a random locus. Since a pair of affected siblings has the same disease, these patients are expected to share a chromosomal region around the gene involved in the etiology of the trait. Tentative evidence was found in a first affected sib-pair analysis migraine study showing that the familial hemiplegic migraine containing region on chromosome 19p13 is involved in the common types of migraine (May et al., 1995). However, the results were inconclusive as to the magnitude of the involvement and the relative importance of migraine with aura and migraine without aura. A second affected sib-pair study was performed in an independent sample of 36 extended Dutch families with the common types of migraine (Terwindt et al., 1997). Significant increased sharing was found for migraine with aura. No such sharing was observed for migraine without aura. These two studies provided independent evidence of the involvement of the chromosome 19p13 region containing the P/Q-type Ca^{2+} channel gene in the etiology of migraine, especially migraine with aura. The exact nature of this involvement, however, remains to be elucidated. Ultimately, the involvement of the

Table 2
CACNA1A mutations in familial hemiplegic migraine patients and natural mouse models

Mutation	Associated clinical phenotype	Localization in CACNA1A protein	Predicted overall effect of mutation on calcium influx?	Key references
Patients				
R192Q	FHM	IS4	Increased	(Ophoff et al., 1996; Kraus et al., 1998; Hans et al., 1999)
R583Q	FHM + ataxia	IIS4	Decreased	Battistini et al., 1999; Ducros et al., 1999b
T666M	FHM + ataxia	IIS5-S6 linker	Decreased	(Ophoff et al., 1996; Ducros et al., 1999a; Kraus et al., 1998; Hans et al., 1999)
V714A	FHM	IIS6	Increased or decreased[a]	(Ophoff et al., 1996; Kraus et al., 1998; Hans et al., 1999)
D715E	FHM + ataxia	Loop II–III	Decreased	(Ducros et al., 1999a; Kraus et al., 2000)
K1335E	FHM	IIIS3-S4 linker	Unknown	Ducros et al., 1999b
Y1384C	FHM + ataxia	IIIS5	Unknown	(Ducros et al., 1999b; Vahedi et al., 1999)
V1457L	FHM	IIIS5-S6 linker	Increased or decreased[a]	(Carrera et al., 1999; Kraus et al., 2000)
R1667W	FHM	IVS4	Unknown	Ducros et al., 1999b
L1682P	FHM + ataxia	IVS4-S5 linker	Unknown	Montagna, 2000
W1683R	FHM + ataxia	IVS4-S5 linker	Unknown	Ducros et al., 1999b
V1695I	FHM	IVS5	Unknown	Ducros et al., 1999b
I1811L	FHM + ataxia	IVS6	Increased or decreased *	(Ophoff et al., 1996; Kraus et al., 1998; Hans et al., 1999)
Natural mouse mutants				
P601L (*tottering*)	Seizures + ataxia	IIS5-S6 linker	Decreased	(Fletcher et al., 1996; Wakamori et al., 1998)
R1262G (*Rolling Nagoya*)	Ataxia	IIIS4	Decreased	Mori et al., 2000
splicing error (*Leaner*)	Seizures + ataxia	C-terminus	Decreased	(Fletcher et al., 1996; Lorenzon et al., 1998; Wakamori et al., 1998)

Specific amino acid substitutions are indicated; positions are according to the author that described the mutation first. Ataxia is included in the clinical phenotype when it is a prominent symptom observed in patients with the respective mutation.

[a]Various electrophysiological parameters (channel activation, channel inactivation, recovery of inactivation, channel density) might give rise to either increased or decreased calcium influx.

CACNA1A gene has to be demonstrated by mutational or functional studies.

2.4. Other (modifying) genes; dopamine D2 receptor (DRD2)?

A number of association studies of migraine have been published, many of them with conflicting results (for review see Montagna, 2000). Most consistent, however, is the association between migraine and the dopamine D2 receptor. Clinical and pharmacological evidence supports the hypothesis that dopamine could be involved in the pathogenesis of migraine (Peroutka, 1997). In a case-control study, association between the DRD2 *Nco*I C allele and migraine with aura was observed, but not in migraine without aura (Peroutka et al., 1997, 1998). This finding failed to be replicated in a small uncontrolled association study published by Dichgans et al. (1998). In a family-based association study in a population isolate of Sardinia, evidence was found, using the transmission disequilibrium test (TDT), that the allelic distribution of DRD2 differed significantly in a subgroup of 'dopaminergic' patients with migraine without aura, suggesting that hypersensitivity of the dopaminergic system could be involved in at least a subgroup of patients (Del Zompo et al., 1998). Dopaminergic migraine patients were selected based on the presence of both nausea and yawning immediately before or during the pain phase of migraine. The fact that two independent studies have shown an association between molecular vari-

ations within the DRD2 gene and migraine suggests that genetic variation within dopaminergic system function may underlie the clinical susceptibility to migraine. It is clear, however, that additional genetic, biochemical and clinical studies are needed to assess the (putative) role of dopamine in the pathophysiology of migraine.

3. Future developments, conclusion

Pharmacogenetics in migraine still is in its infancy, but might be helpful in an integrated route from patient to gene and therapy (Fig. 2). Two areas are of great potential importance; further exploration of the existing 'triptan world' and the terra incognita of pathways and targets to be revealed by the identification of genetic (predisposing) factors. The triptans will largely set the scene for the coming decade as novel treatment strategies based on genetics defined pathways are at best at the drawing table stage. It is conceivable that the specificity and efficacy of triptan derivatives can be improved on the basis of detailed functional dissection of 5-HT receptors and downstream pathway key determinants. The development of completely new drugs will depend on dissection of the molecular pathways contributing to migraine. The heterogeneity and multitude of interacting pathways can only be speculated upon.

Our recent work on the role of the CACNA1A gene in FHM and the common forms of migraine with aura, supporting the hypothesis that migraine is a channelopathy, has set the stage for evidence-based thinking and direction of future work to improve the resolution of the molecular portrait of migraine. The generation and evaluation of various cellular and (transgenic) mouse models are well underway to study the relevance of the CACNA1A gene pathway in the etiology of migraine. The versatility of (conditional) knock-out, and in particular of unique knock-in models, whereby the endogenous mouse gene is modulated to contain "human" causative mutations, are theoretically promising but must indeed prove their value for the study of this type of neurogenic disorder with the additional feature that it shows an episodic character. Obviously, such models will provide unprecedented opportunities, not only for testing new drugs but also for devising models to test the molecular mechanism of (anticipated) migraine triggers and modulating genetic and environmental factors.

To complete the molecular portrait of migraine pathogenesis, additional genes have to be identified and their function determined. It is likely that novel candidate genes will surface in the next several years. Already, novel familial hemiplegic migraine loci have been identified on chromosomes 1q and X. One can, of course, only speculate as to what the next migraine gene might look like. Likely candidates are Ca^{2+} channel subunits, genes implicated in Ca^{2+} metabolism or neurotransmitter release mechanisms, thereby reinforcing the concept that migraine is a channelopathy. Of course, such a gene can also have a completely different role, revealing a completely new insight into migraine pathophysiology. Complementary strategies to identify such genes are being followed in our, and in other, laboratories. The most straightforward approach is to map genes by parametric linkage analysis in families with a Mendelian inheritance pattern as demonstrated by our work in familial hemiplegic migraine. This approach has also been applied to mapping migraine with aura

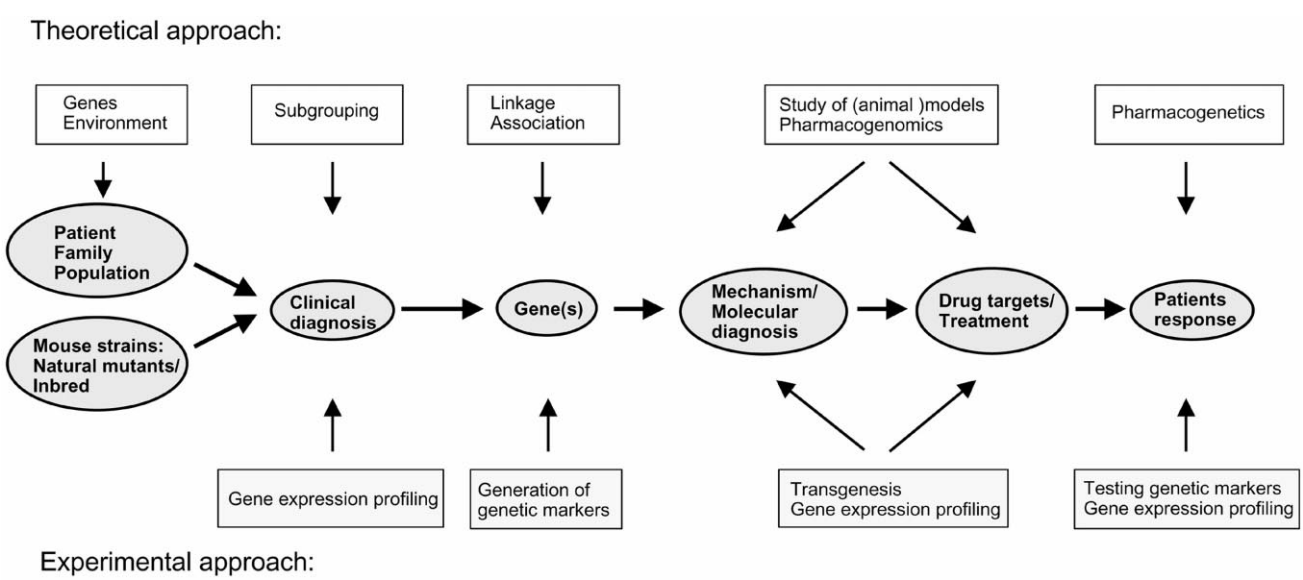

Fig. 2. From patient to gene and therapy. The scheme depicts the main steps (either theoretical (upper part) or experimental (lower part)) in the identification and evaluation of a new drug target based on positional cloning of disease-causing genes. The starting material can be either patients/families or natural mouse mutants or mouse strains with a quantitative trait. In this scheme, pharmacogenomics means drug target identification, while pharmacogenetics is restricted to genetic variation in drug response.

genes. However, considering the high prevalence of migraine, the clinical heterogeneity of migraine and available population-based estimates of heritability based on segregation and twin studies, more large-scale non-parametric linkage and association methods have to be applied. The rapid progress of the Human Genome Project with dense genetic maps, high-throughput genotyping technology, the use of common DNA variations named single-nucleotide polymorphisms in combination with advanced biostatistical methods will vastly facilitate such approaches (for review see, McCarthy and Hilfiker, 2000). In spite of the unprecedented capacity from the laboratory side, success will, however, be dependent on the quality of well-characterized patient material; families as well as patient cohorts. As part of such a multidisciplinary approach, our research group is also evaluating the power of gene identification in isolated populations that have arisen through rapid expansion of a small founder population during a period of several decades (Wright et al., 1999).

The coming decades will show whether these pharmacogenetic approaches have yielded more specific and efficient treatment of migraine patients with fewer side-effects.

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