**Methods e-1 Cohort background, migraine assessment method, and sampling procedure.**

**LUMINA**

Participants of the Leiden University Migraine Neuro-Analysis (LUMINA) were recruited through a dedicated, nationwide website inviting migraine patients and non-migraine controls to participate in migraine research. In addition, patients attending the Leiden University Medical Centre (LUMC) dedicated headache clinic were invited to participate. We therefore consider this a web-based “clinic-based” cohort.

Participants recruited through the website were considered eligible after a two-step inclusion process using validated questionnaires via the dedicated LUMINA website1. A migraine diagnosis or non-migraine control status was validated by a semi-structured telephone interview, in accordance with the International Classification of Headache Disorders (ICDH-3β)2. Finally, the diagnosis was checked by the study-physician at the day of blood draw. Migraine patients recruited through the headache clinic were diagnosed with migraine by a neurologist specialized in headache. Patients with a diagnosis of probable migraine in accordance with the ICHD-3β were excluded2. Non-migraine controls were free of any primary disorder, apart from tension-type headache2. Migraine assessment was conducted on the day of sampling blood.

EDTA plasma samples were available for 317 migraine cases and 91 non-migraine controls. Samples from 279 participants were collected after overnight fasting. Blood samples were drawn by venipuncture from the median cubital vein, after centrifugation on room temperature; plasma was aliquoted and stored at -80°C. Samples were freeze-thawed no more than once prior to shipment to Nightingale Health for analysis, where samples were measured in April 2014.

**NESDA**

The Netherlands Study of Depression and Anxiety (NESDA) is an observational longitudinal naturistic cohort study on the long term course and consequences of depressive and anxiety disorders3. We consider this a mixed clinic- and population-based cohort. In total 2,981 participants were recruited between 2004 and 2007 through different settings: community, primary care and specialized mental health clinics in order to obtain a representative sample of persons with and without depressive and anxiety disorders. Using this approach NESDA created a range in psychopathology: participants (‘controls’) without symptoms or disorders, those at risk due to subthreshold symptoms or with a personal or family history, and participants with a current or remitted depressive or anxiety disorders. A more extensive description of the composition of NESDA can be found elsewhere3.

Migraine was assessed at the baseline assessment using a headache questionnaire based on the criteria for migraine listed in the ICHD-24. The migraine part of the questionnaire was preceded by one screening question (“Do you ever experience headache attacks, for instance migraine?”), participants had to answer positive in order to be required to answer the remaining detailed questions about their headaches. This provided information on the presence of symptoms and criteria present in the ICHD-2: number of experienced headache attack (≥5), pain intensity (moderate/severe), pain duration (4-72 hours), and pulsating pain (yes/no). During a headache attack: visual aura (yes/no), aggravation by physical activity (yes/no), presence of nausea and/or vomiting (yes/no), and phonophobia or photophobia (yes/no). Based on the criteria in the ICHD-2 participants were classified as: migraine patients, probable migraine patients, mild non-migrainous headache patients, and participants without headache (participants answering negative to the first screening question). In the current study only participants characterized as migraine patients using the questionnaire were included as migraine patients. Probable migraine patients were excluded. Participants characterized as mild non-migrainous headache patients, and participants without headache were included in the analysis as controls. Questionnaires were completed in close temporal relation to sample collection (on average within seventeen days ).

Baseline EDTA plasma samples were available for 444 migraine cases and 1,584 non-migraine controls. Samples from 1,944 participants were collected after overnight fasting. Samples were drawn by venipuncture from the median cubital vein, after centrifugation on room temperature; plasma was aliquoted and stored at -85°C. Samples were freeze-thawed no more than once prior to shipment to Nightingale Health for analysis. Samples were measured at Nightingale Health at two separate occasions (April and December 2014). Based on this two separate NESDA cohorts were defined, NESDA-1 (n=1,082) and NESDA-2 (n=946), respectively.

**NTR**

The Netherlands Twin Registry (NTR) is composed of and collects data from Dutch twins, other siblings, their parents and partners. We consider this a population-based cohort. Participants were recruited either at birth through ‘birth felicitation’ services or when already older at the start of NTR, through city councils in 1990-1991 and through additional efforts in later years. A detailed description of NTR can be found elsewhere5.

Migraine was diagnosed using a headache questionnaire based on the criteria for migraine listed in the ICHD-24. The migraine part of the questionnaire was preceded by one screening question (“Do you ever experience headache attacks, for instance migraine?” ), participants had to answer positive in order to be required the answer to remaining detailed questions about their headaches. This provided information on the presence of symptoms and criteria present in the ICHD-2: number of experienced headache attacks (≥5), pain intensity (moderate/severe), pain duration (4-72 hours), and pulsating pain (yes/no). During a headache attack: visual aura (yes/no), aggravation by physical activity (yes/no), presence of nausea and/or vomiting (yes/no), and phonophobia or photophobia (yes/no). Based on the criteria in the ICHD-2 participants were classified as: migraine patients, probable migraine patients, mild non-migrainous headache patients, and participants without headache (participants answering negative to the first screening question). In a small number of cases (<60 cases), detailed symptom information was not available and self-reported migraine was used. In the current study only participants characterized as migraine patients using the questionnaire were included as cases. Probable migraine patients were excluded. Participants characterized as mild non-migrainous headache patients, and participants without headache are were included in the analysis as controls. If diagnosed with migraine after blood sampling, the participant was included as a migraine patient (case). If diagnosed prior to blood sampling as healthy control this was no more than 6 months prior to the sampling, otherwise the sample was excluded because of the risk of developing migraine.

EDTA plasma samples were available for 1,360 migraine cases and 1,513 non-migraine controls. Samples from 2,673 participants were collected after overnight fasting. Samples were drawn by venipuncture from the medial cubital vein during a home-visit, after centrifugation back at the laboratory at 4°C, plasma was aliquoted and stored at -20°C5. Samples were collected in two biobanking initiatives (the first between June 2004 and July 2008 and the latter between January 2011 and December 2011) and freeze-thawed once prior to shipment to Nightingale Health for analysis. Samples were measured in 2014 at Nightingale Health.

**ERF**

The Erasmus Rucphen Family (ERF) study is a large family-based cohort study, in a young genetically isolated population in the Southwest of The Netherlands. We consider this a population-based cohort. Possible participants were selected based on genealogical background. Eligible participants included three generations of living descendants, of twenty couples with at least six children living in the community in the 19th century. Participants were therefore not selected based on phenotypes of interest, and traced back through church and municipality records6.

Migraine was diagnosed using a headache questionnaire based on the criteria for migraine listed in the ICHD-24. A previously validated three-stage screening procedure was used to assess lifetime occurrence of migraine7,8. In short, stage one consisted of 5 screening questions on aura and headache symptoms. In stage two only screen-positive participants received an extensive questionnaire on headache and aura symptoms. The extensive questionnaire was based on ICHD-2 criteria for migraine headache and aura4. In stage three, screen-positive participants, or participants which did not fill in or complete the extensive questionnaire, were interviewed by telephone using a semi-structured to clarify their reported signs and symptoms of migraine headache and aura by a physician with experience in diagnosing migraine patients, and well trained supervised medical students. The final migraine diagnosis was made after this telephone interview and in consultation with a neurologist (GMT) specialized in headache. Probable migraine patients were excluded. ERF participants who were negative for migraine or probable migraine based on the aforementioned three-stage screening procedure were included as controls7,8. If diagnosed with migraine after blood sampling, the participant was included as a migraine patient (case). If diagnosed prior to blood sampling as healthy control this should not be more than 6 months prior to the sampling, otherwise the sample was excluded because of the risk participants developing migraine.

EDTA plasma samples were available for 178 migraine cases and 1,235 non-migraine controls. Samples from 1,413 participants were collected after overnight fasting. Samples were drawn by venipuncture from the median cubital vein, after centrifugation at 4°C, plasma was aliquoted in cryovials and stored at -80°C. Samples were not freeze-thawed prior to shipment to Nightingale Health for analysis. Samples were measured in the spring of 2014 at Nightingale Health.

**RS**

The Rotterdam study (RS) is a large prospective population-based cohort study, among people 55 years of age or older living in the well-defined Ommoord district in Rotterdam (NL) in 1990. Later the RS was expanded in 2000 and 2006 to include people 45 years of age or older living in the Ommoord district9.

Migraine was assessed using a modified questionnaire of the questionnaire used in the Leiden Genetic Epidemiology of Migraine (GEM) study7,8, based on the ICHD-2 criteria4 as part of a face-to-face structured interview or telephone interview10. Participants were first asked if they had ever experienced a headache with severe pain that affected their daily activities. If the answer to this question was negative or if the participant clearly indicated that severe headache was due to other causes, such as a tumor, sinusitis, stroke, trauma or meningitis, no further headache related questions were asked. Participants who replied positive to the first question received further questions on headache duration and attack frequency. Participants who experienced headaches of which (1) the duration was between four and seventy-two hours (untreated) or if the participant did not know the answer to this question, because they always treated their headaches and (2) the attack frequency was two or more attacks in a lifetime, further details on the characteristics and symptoms of the headaches were asked. Participants whose duration of headache was unknown, because they always used medication to prevent or treat the attack, were considered migraine patients if they fulfilled the remaining ICHD-2 criteria10. Probable migraine patients were excluded. Participants not classified as (probable) migraine patients were included as controls10. If diagnosed with migraine after blood sampling, the participant was included as a migraine patient (case). If diagnosed prior to blood sampling as healthy control this should not be more than 6 months prior to the sampling, otherwise the sample was excluded because of the risk participants developing migraine.

EDTA plasma samples were available for 173 migraine cases and 1,252 non-migraine controls. Samples from 1,425 participants were collected after overnight fasting. Samples were drawn by venipuncture from the median cubital vein, after centrifugation at 4°C, plasma was aliquoted in cryovials and stored at -80°C. Samples were not freeze-thawed prior to shipment to Nightingale Health for analysis. Samples were measured in the spring of 2014 at Nightingale Health.

**LifeLines-DEEP**

The LifeLines-DEEP cohort is a sub-cohort of the LifeLines cohort (167,729 participants), which is a large three-generation observational follow-up study set up to investigate universal risk factors and their modifiers for multifactorial diseases11,12. Participants include a large representative sample of the Northern part of the Netherlands. We consider this a population-based cohort. At the start of the study a random sample of inhabitants aged between 25 and 50 years were invited to participate through their general practitioner. After inclusion initial participants were asked if family members (partners, parents, partners-in-law and children) might be interested to participate. Interested family members were included in the study. Additional participants, again living in the Northern part of the Netherlands, were included via the LifeLines website11,12. A subset of 1,539 participants (636 males and 903 females, age range 18–84 years) also took part in the more detailed phenotyping and omics profiling, called the LifeLines-DEEP study. For these participants, additional biological materials were collected, including additional blood (n=1,387), exhaled air (n=1,425) and fecal samples (n=1,248), and elicited responses to gastrointestinal health questionnaires (n=1,176) for analysis of the genome, epigenome, transcriptome, microbiome, metabolome and other biological levels. The overview of the different data layers in LifeLines DEEP and the baseline characteristics of the study population has been described previously13.

This study included the baseline information and plasma samples of LifeLines-DEEP participants. The migraine status was assessed using a single question, which was part of a general health questionnaire. The question “could you indicate which of the following disorders you have (had): migraine”, was used to classify migraine patients and non-migraine controls. The participants who answered “yes” were included as a migraine patient (case) and those who answered “no” were included as healthy control. If diagnosed with migraine after blood sampling, the participant was included as a migraine patient (case). If diagnosed prior to blood sampling as healthy control this should not be more than 6 months prior to the sampling, otherwise the sample was excluded because of the risk of participants developing migraine.

EDTA plasma samples from participants were collected after overnight fasting. Samples were drawn by venipuncture from the median cubital vein, placed at 4°C and transported from the research site to the LifeLines laboratory in Groningen, under tightly controlled and continuously monitored conditions. After preparation plasma was aliquoted and stored at -80°C11. Samples were freeze-thawed two times prior to shipment to Nightingale Health for metabolome analysis. With some sample drop-off, this study finally included 1,319 LifeLines-DEEP participants, including 249 migraine cases and 1,070 migraine controls.

**TMS**

The Maastricht Study (TMS) is a prospective observational population-based cohort study enriched with individuals suffering from type 2 diabetes mellitus. We therefore consider this a mixed clinic- and population-based cohort. Eligible participants were selected based on age (between 40 and 75 years) and place of residence (the Southern part of the Netherlands). Recruitment proceeded through mass media campaigns, municipal registries and the regional Diabetes Patient Registry via mailings14. In the current study only subjects suffering from diabetes were included.

Migraine status was assessed via web-based questionnaires. First, a screening questionnaire based on the screening questionnaire used in the GEM study was offered to all participants7. Second, only screen-positive participants received an extensive questionnaire on headache and aura symptoms based on the ICHD-2 criteria4. Based on the results from the extensive questionnaire participants were classified as definite, probable or possible migraine patients. Participants classified as probable migraine patients were excluded from the current study. TMS participants who were negative for definite or probable migraine based on the screening or extensive questionnaire were included as controls7,8. Migraine questionnaires were taken within 3 months after plasma samples were collected.

EDTA plasma samples were available for 79 migraine cases and 608 non-migraine controls. Samples from all participants were collected after overnight fasting. Plasma was collected via venipuncture in EDTA tubes on ice, separated after centrifugation, and stored at −80°C until the assays were performed. The time between collection and storage was less than 2 hours. Samples were collected from April 2010 until April 2013 and not freeze-thawed prior to shipment to Nightingale Health for analysis. Samples were measured in April 2014 at Nightingale Health.

**References**

1. van Oosterhout WPJ, Weller CM, Stam AH, et al. Validation of the web-based LUMINA questionnaire for recruiting large cohorts of migraineurs. Cephalalgia. 2011;31:1359–1367.

2. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia. 2013;33:629–808.

3. Penninx BWJH, Beekman ATF, Smit JH, et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res. 2008;17:121–140.

4. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders: 2nd edition. Cephalalgia. Blackwell Publishing Limited; 2004;24 Suppl 1:9–160.

5. Boomsma DI, Geus EJC De, Vink JM, et al. Netherlands Twin Register : From Twins to Twin Families. Twin Res Hum Genet. 2006;9:849–857.

6. Sleegers K, de Koning I, Aulchenko YS, et al. Cerebrovascular risk factors do not contribute to genetic variance of cognitive function. The ERF study. Neurobiol Aging. 2007;28:735–741.

7. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology. 1999;53:537–542.

8. Stam AH, de Vries B, Janssens ACJW, et al. Shared genetic factors in migraine and depression Evidence from a genetic isolate. Neurology. 2010;74:288–294.

9. Hofman A, Van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. 2011;26:657–686.

10. Loehrer E, Vernooij MW, van der Lugt A, Hofman A, Ikram MA. Migraine and cerebral blood flow in the general population. Cephalalgia. 2014;0:1–9.

11. Scholtens S, Smidt N, Swertz MA, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. Int J Epidemiol. 2015;44:1172–1180.

12. Stolk RP, Rosmalen JGM, Postma DS, et al. Universal risk factors for multifactorial diseases: LifeLines: A three-generation population-based study. Eur J Epidemiol. 2008;23:67–74.

13. Tigchelaar EF, Zhernakova A, Dekens JAM, et al. Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics. BMJ Open. 2015;5:e006772.

14. Schram MT, Sep SJS, Van Der Kallen CJ, et al. The Maastricht Study: An extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29:439–451.