COFFI
Collaborative On
Fatigue Following Infection

AKERSHUS UNIVERSITETSSYKEHUS

Long-Term Effects of COVID-19 in Adolescents (LoTECA)

Study protocol

NCT 04686734

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Project description

1. State of the art, knowledge needs and project objectives

The Coronavirus Disease 2019 pandemic has high mortality; in general, children are mildly affected

The Coronavirus Disease 2019 (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is a tremendous threat to human health and welfare worldwide.[55] The clinical presentation varies from asymptomatic infection over mild upper respiratory tract illness to severe viral pneumonia causing Acute Respiratory Distress Syndrome (ARDS) and respiratory failure; old age and chronic cardiovascular morbidity are important risk factors for severe outcome.[56]

Evidence suggests that previously healthy children are usually mildly affected by SARS-CoV-2 in the acute phase. A nation-wide retrospective study in China including 2143 children with COVID-19 reported "severe" or "critical" disease among 6 % only; age < 1 year was a risk factor.[13] Two other Chinese studies of hospitalized children during the height of the pandemic in the city of Wuhan showed excellent recovery in the great majority, and only one death.[29,32] Corroborating these results, statistics from the outbreak in Italy report only 1.2% COVID-19 cases and no deaths < 18 years of age.[30]

Long-lasting complications to COVID-19 in children might be development of post-infectious chronic fatigue and chronic fatigue syndrome

Despite the relatively mild clinical presentation of COVID-19 in children, the long-lasting medical effects of is unknown at the moment. However, as fatigue seems to be a prominent symptom of COVID-19 infection,[13] it is conceivable – and in line with preliminary reports - that post-infectious chronic fatigue (PICF) might develop in vulnerable individuals, of which a subgroup may fulfill diagnostic criteria for the Chronic Fatigue Syndrome (CFS). PICF/CFS is a well-known sequel of several other infections, such as Epstein-Barr virus (EBV).[17,22] Normally, increased load of the infectious agent is not reported in PICF/CFS.[5,25] The underlying causes remain elusive; however, autoimmunity is a possible mechanism. The most consistent immune alteration is a tendency towards low-grade systemic inflammation, as reflected in significantly elevated serum C-reactive protein (CRP),[25,53] elevated pro-inflammatory cytokines,[24,39] and increased levels of innate immunity gene products in whole blood gene expression analyses.[41] Alternatively, immune disturbances might be a side-effect which is not causally involved in PICF/CFS symptoms and disabilities. Recent neurobiological studies suggest that PICF/CFS is related to misguided automatic and unconscious predictions.[26,54] This alternative model is supported by evidence of psychosocial factors (such as negative life events and trait negative affectivity) being risk factors for long-lasting post-infectious conditions.

PIFC/CFS is a significant health problem among adolescents: Disability is often severe, treatment options are limited, and there is a strong negative impact upon academic development, social security systems, and family networks.[19] The societal costs are correspondingly large.[47] The present pandemic provides a unique opportunity to further scrutinize the disease mechanisms underlying PIFC/CFS in children.

COVID-19 is associated with inflammatory enhancement; a "cytokine storm" might contribute to development of respiratory failure

At a gross level, the immunological host response to SARS-CoV-2 is characterised by increased levels of circulating proinflammatory cytokines; this inflammatory enhancement, which might escalate towards a "cytokine-storm", is thought to contribute significantly to tissue damage and clinical deterioration, including severe pulmonary pathology and ARDS.[8,46,58]

Interestingly, SARS-CoV infection is also associated with a delayed expression of type I interferon (IFN) signaling, which is a crucial part of the innate defense against viral infections.[36] Type I IFN signaling is initiated by pattern recognition receptors (such as the Toll-like receptors) in responses to viral nucleic acid; the subsequent intracellular cascade promotes the synthesis of type I interferons (IFNs), which in turn activate the JAK-STAT signal pathway promoting the expression of IFN-stimulated genes (ISGs).[40] One effect of this signaling cascade is increased transcription of the APOBEC3-family of deoxycytidine deaminases that counteract a broad range of retroviruses, including coronaviridae.[2,38] The net result is a limitation of virus spread. The N protein of SARS-CoV seems to antagonize the type I interferon (IFN) signaling by interacting with the pattern recognition receptors, thereby enabling the virus to escape this host response;[33] other viral mechanisms might contribute as well.[8]

This "escape capability" might explain important features of COVID-19 infection as well, such as the rapid viral replication rate in infected individuals, which in turn might contribute to tissue pathology.[28] In addition, it has been hypothesized that this viral IFN antagonism plays a crucial part in promoting the "cytokine storm" in SARS-CoV infection:[7] The delayed type I IFN signaling promotes the accumulation of pathogenic inflammatory monocyte-macrophages (IMMs), which in turn are responsible for increased cytokine levels as well as direct lung tissue damage and inhibition of virus-specific T cell responses. Thus, a detailed mapping of the initial innate responses to SARS-CoV-2 might provide a clue towards better understanding of two separate causes of tissue damage, which over time might amplify each other in a vicious circle: The direct cytotoxic effect of the virus as well as the immunopathologies resulting from uncontrolled inflammatory enhancement.

This study aims to study long-term effects in adolescent COVID-19, in particular development of chronic fatigue

The overarching objective for this prospective cohort study on COVID-19 in adolescents is to study the long-term effects, with particular emphasis on post-infectious chronic fatigue. We hypothesise that children having undergone COVID-19 has a higher prevalence of PICF/CFS long after the acute infectious event; furthermore, that risk factors for PICF/CFS encompass a complex mixture of genetic vulnerability and psychosocial factors.

2. Material and methods

The design allows prospective as well as cross-sectional analyses

Adolescents (12-25 years) with confirmed SARS-CoV-2-infection are eligible for the present study (Table 1, Fig. 2). Inclusion will be based on informed consent from patients/next-of-kin. We assume a total number of 500 included cases. A follow-up control is scheduled 6 months after inclusion. This design enables prospective and cross-sectional analyses.

Risk factors for long-term complications will be analysed using regression modelling. Fatigue is defined as the primary endpoint. This sensation is operationalized using the Chalder Fatigue Questionnaire, which is known to have good validity and reliability in children.[6] Relevant independent (explanatory) variables include (but are not restricted to):

- *Background*: Sex, age, ethnicity, previous medical history, smoking habits in family, presence of allergy/atopy, etc.
- *Initial clinical findings*: Symptom load/intensity, fever, routine blood haematology and biochemistry, etc.
- *Infectious load:* SARS-CoV-2 viral counts.
- *Immune function:* Increased general inflammation
- *Genetic predisposition*: Cf. below (Tab. 2) for a comprehensive list of candidate genetic markers
- *Questionnaire results*: Emotionality (depression/anxiety, emotional awareness), personality factors (neuroticism, worrying), perceived loneliness, and negative life events.
- *Autonomic cardiovascular control*: Increased sympathetic nervous activity, decreased parasympathetic nervous activity
- *Cognitive functions*: Increased verbal memory, attention bias towards disease-related words, reduced working memory

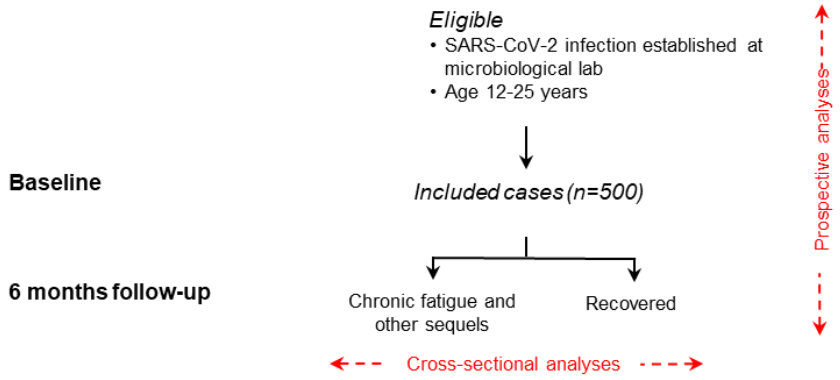

Figure 2. Design overview of the present project.

Table 1. Criteria for inclusion and exclusion

Inclusion criteria
Positive SARS-CoV-2 test
Age 12-25 years
< 28 days since onset of first symptom
Exclusion criteria
Hospitalised because of COVID-19
Pregnancy
Lack of consent from patient/next-of-kin

Investigations encompass clinical and microbiological assessments, a questionnaire, pressure pain threshold, biobanking and assessment of autonomic and cognitive functions.

At baseline as well as at 6 months follow-up, included individuals will be exposed to a standardised investigational program, lasting about 1,5 hours. All participants will be instructed to fast overnight and abstain from tobacco products and caffeine at least 48 hours. Participants will bring morning spot urine in a sterile container as well as a fecal sample (Bio-Me, Oslo, Norway), and are to apply a local anesthetic ointment (EMLA®, AstraZeneca) on both antecubital areas one hour before arriving. Then, the following investigations are to be carried out in a fixed sequence:

- *Clinical assessment*: A comprehensive recording of exposure, previous medical history, symptoms and findings, and routine blood tests.
- *Biobanking*: Biobanking of blood samples (whole blood, plasma, serum, RNA, viable Peripheral Blood Mononuclear Cells (PBMC)) as well as urine, hair and fecal samples, for subsequent analyses (cf. below). Blood sampling will be obtained in a fixed sequence from antecubital venous puncture.
- *Questionnnaire*: A composite questionnaire will chart clinical symptoms, as well as background, psychological and social variables, and will be used for diagnostic categorization according to different case definitions of CFS/ME. The Chalder Fatigue Questionnaire (CFQ) charts subjective experience of physical and mental fatigue, and has been extensively used in CFS research. In this study, the CFQ total linear score (i.e. the sum across all 11 items, each item scored on a zero to three Likert scale) is applied as the primary endpoint in prediction modelling. Furthermore, fatigue caseness is defined as a CFQ total dichotomous score of 4 or higher (each item scored 0-0-1-1). The Hospital Anxiety and Depression Scale (HADS) is a validated questionnaire for charting symptoms of depression and anxiety. It consists of 14 items rated zero to three on Likert scales, allowing computation of sub-scores for depression and anxiety symptoms. In the present study, these sub-scores as well as total sum score will be applied. The Life Event Checklist (LEC) is a validated questionnaire for assessing both positive and negative life events. The inventory lists 45 events; the respondents are asked to mark each of them as positive or negative, and grade their impact on four-point Likert scales. The Brief Pain Inventory (BPI) is a validated questionnaire for assessing pain. In this study,

Table 2. Candidate genes for gene sequencing

Gene	*Protein*
ACE	Angiotensine converting enzyme
ADRA1A	$\alpha 1_A$ adrenergic receptor
ADRA2A	$\alpha 2_A$ adrenergic receptor
ADRB2	β2 adrenergic receptor
BDNF	Brain-derived neurotrophic factor
CCL27	Chemokine (C-C motif) ligand 27
CCR4	C-C chemokine receptor type 4
COMT	Catechol-O-methyltransferase
CXCL16	Chemokine (C-X-C motif) ligand 16
DBH	Dopamine beta-hydroxylase
DRD2	Dopamine receptor D_2
DRD4	Dopamine receptor D_4
HTR1A	5-HT1b receptor
HTR1B	5-HT1a receptor
HTR2A	5-HT receptor 2A
HTR2C	5-HT_4 receptor
HTR4	5-HT_{2C} receptor
IFNG	Interferon gamma
IL10	Interleukin 10
IL12B	Subunit beta of interleukin 12
IL17B	Interleukin 17 B
IL17F	IL-17F
IL18	Interleukin-18
IL1A	Interleukin 1 alpha
IL1B	Interleukin 1 beta
IL1RN	Interleukin 1 receptor antagonist
IL2RB	Interleukin-2 receptor subunit beta
IL4	Interleukin 4
IL6	Interleukin 6
IL6ST	Glycoprotein 130
IL8	Interleukin 8
IRAK4	Interleukin-1 receptor-associated kinase 4
IRF7	Interferon regulatory factor 7
LTA	Lymphotoxin-alpha
MAOA	Monoamine oxidase A
MAOB	Monoamine oxidase B
NR3C1	Glucocorticoid receptor
NRAMP1	Natural resistance-associated macrophage protein 1
P2RX7	P2X purinoceptor 7
SLC18A2	Vesicular monoamine transporter 2
SLC6A2	Norepinephrine transporter
SLC6A3	Dopamine active transporter
SLC6A4	5-HT transporter
TH	Tyrosine hydroxylase
TIRAP	Adapter molecule associated with toll-like receptors
TLR4	Toll-like receptor 4
TNF	TNF
TPH2	Tryptophan hydroxylase 2

the four BPI items assessing pain severity on ten-point Likert scales will be used to compute a total sum score; in addition, scores on a single item (average pain) will be reported. Cf. supplement for further information on questionnaire instruments.

- *Autonomic cardiovascular control*: A 5-minute ECG recording will be performed applying The Bittium Faro 360® device (Bittium Corporation, Oulu, Finland), enabling later heart rate variability analyses. In the frequency-domain, vagal (parasympathetic) activity is the main contributor to high-frequency (HF) variability of heart rate, whereas both vagal and sympathetic activity contributes to low-frequency (LF) variability. The LF/HF ratio is considered an index of sympathovagal balance.
- *Spirometry:* The EasyOne Air® spirometer device (NDD Medical Technologies, Andover, Massachusetts, USA) is used to obtain standard ventilation variables.
- *Cognitive functions.* Participants are exposed to the Hopkins Verbal Learning Test-Revised (HVLT-R), assessing verbal learning, delayed recall, and recognition; the Digit Span forward and backward test, assessing working memory; an Attention Bias test of automatic biases towards disease-associated words; and the Function Acquisition Speed Test assessing cognitive fusion.

Ahus will host the biobank and database for the present study.

Considerations on power estimation and statistical analyses

The novelty of COVID-19 and the lack of comparable studies among adolescents precludes a formal power analyses. However, it should be noted that a prospective cohort of 500 patients is comparable to similar cohort studies in the field. Based upon previous post-infective studies, about 10 % of included patients will adhere to diagnostic definitions of CFS after 6 months; in the present study, that will leave about 50 patients for cross-sectional analyses, which is also considered sufficient to detect at least medium effect sizes.

All statistical analyses are carried out with SPSS statistical software, and a $p < 0.05$ are considered statistically significant. Missing values are to be handled by multiple imputation techniques.

Ethics

Participation is based upon informed consent, and thorough information will be provided orally as well as in writing to the participants and (if younger than 16 years) to their parents/next-of-kin. All data will be treated and stored without personal identifying information, and in accordance with national directives. Generally, investigational methods are neither harmful nor painful (local anaesthetic ointment will be given prior to venous puncture).

Gender perspectives

Severe COVID-19 appears to be more common in males than in females.[56] Post-infectious complications, however, such as PICF/CFS, are more common among females than males, the ratio being about 3:1. Thus, such complications strongly impact on women's health, but have traditionally received low attention. In this project, possible gender differences will be scrutinized in the analyses.

The projects benefits from a strong relationship to well-established research networks

The projects builds upon two collaborating networks that have been firmly established through previous research project: a) An international consortium of researchers on post-infectious complications (Collaborative of Fatigue Following Infection, COFFI), encompassing highly reputed research groups from six countries (US, UK, Australia, New Zealand, The Netherlands and Norway), and with the lead co-PI of this proposal as the chair of the consortium; b) An in-house collaborative with the EpiGen Molecular Research Laboratory and the Dept. of Microbiology, which are equipped with state of the art technology and manages the local biobank facilities. There are several co-publications within all these networks already.

The present project will guide clinical care and priorities in acute disease, contribute to development of therapeutics and prophylaxis, and help understand mechanisms behind long-term complications

The on-going COVID-19 pandemic represents a global threat towards health and welfare. The present project aims to mitigate some of the detrimental consequences. First and foremost, it will provide increased knowledge on long-term effects including a better understanding of PICF/CFS and other sequels, and might guide clinical services and development of prophylactic measures. To the best of our knowledge, no similar study on adolescents has been performed – or is ongoing – at present.

Primary and secondary stakeholders
Given the seriousness of COVID-19 infections and the urgent need for scientific knowledge, rapid and continuous dissemination or project results will receive high attention. The target for dissemination is not restricted to the scientific community, but includes a wide range of health-care workers, politicians, policy makers, health care administrators, and the general public.

Results will be disseminated widely; scientific dissemination is prioritized
Several means of dissemination will be exploited, including scientific papers; popular science newspaper articles; giving interviews; meetings with politicians, public health providers and other stakeholders; social media. Dissemination to researchers and the scientific community includes publications in international, peer-reviewed medical journals. The main results will be offered to journals of high impact. Negative findings will also be reported.

3. Implementation

Research group, project managers, and in-house resources
The present project is hosted by the PAEDIA research group at Akershus University Hospital (Ahus), which has extensive experience in translational immunology as well as clinical research, and a strong track record of result dissemination and implementation. The group has established a well-working infrastructure for patient flow and data acquisition, and a large collaborative network with national and international experts on various methodological aspects.

The PAEDIA research group is headed by Prof. Vegard Wyller, who is also the PI of the present project, and Chair of the COFFI collaboration. Prof. Wyller is the most published and cited PICF/CFS researcher in Norway. Other members of PAEDIA include two postdoctoral fellows, six PhD fellows, two master students, and three clinical consultants. The local research support staff consists of one laboratory engineer, two research nurses and two research secretaries. An internal learning program for PhD fellows has been established, encompassing a) one-to-one supervision sessions; b) regular in-group discussions; c) invited lecturers; d) oral presentation training and practice; e) seminars on selected topics (statistics, ethics). A total of five PhD fellows have successfully graduated over the past four years.

PAEDIA's primary collaborating unit at Ahus is the EpiGen Molecular Research Laboratory, headed by prof. Hilde Nilsen, which is equipped with state of the art technology and manages the local biobank facilities. Profs. Nilsen and Wyller have collaborated closely for several years, enjoying complementary expertise and ensuring a translational perspective.

National collaborative network and the COFFI collaborative
Over the last decade, Prof. Wyller has established a multidisciplinary national scientific collaborative network, covering a large area of expertise. Several co-publications exist. For the present project, the collaboration with Profs. B. Fevang and T.E. Mollnes at Oslo University hospital provides expertise in basic and applied immunology, the collaboration with Prof. H.L. Nilsen provides expertise in molecular immunology.

The COFFI consortium was founded by principle investigators following 12 post-infection cohorts in six countries.[21] As of March 2019, the consortium is chaired by Prof. Wyller; basic infrastructure (data managing, biobanking, statistical support) has been established, and will be made available for the present project.

Important measures to ensure integration between all collaborators encompass a) Shared responsibility for PhD supervision, b) Regular meetings (video link for international partners) to discuss scientific and strategic challenges; c) Hosting of seminars; and d) Face-to-face meetings.

Infrastructure and activities
Necessary research infrastructure is primarily related to a) immunological and molecular laboratory facilities, b) biobanking facilities, and c) data management facilities. All these facilities are available through the established collaborative networks, in particular the COFFI collaborative which is already hosted at Ahus.

Personnel and funding
Study personnel (paediatrician, research nurse and research secretary) necessary for initiation of the study and primary data acquisition is available within the PAEDIA research group. For data analyses, writing of papers, etc. a full-time PhD fellow will be needed, and funding will be sought from relevant funding institutions during the coming months. Other running costs in the project will be covered by the Section for Research and Development at Dept. of Pediatrics, Ahus.

An advisory board of two user representatives is the most important user involvement
For the present project, an advisory board of users will formally established, consisting of a) One representative from the "Youth council" at Ahus, consisting of adolescents suffering from chronic disorders; and b) One representative from the organization Recovery Norway, consisting of individuals having recovered from post-infectious chronic fatigue and similar conditions. The advisory board will meet quarterly with the PI; important themes for discussions are results' interpretation and practical impact, ethical issues, considerations regarding further research, and dissemination/implementation strategies.

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COFFI Collaborative On Fatigue Following Infection

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Long-Term Effects of COVID-19 in Adolescents (LoTECA)

Supplement

Late effects after COVID-19 in adolescents – overview of inventories

Category	*Inventory*	*Characteristics*	*References*	*No. in questionnaire*
BACKGROUND/DEMOGRAPHICS				
Household, socioeconomic level	Self-invented	Household members; parents work/education; chronic disease in family		1-6
Smoking, alcohol, drugs	Self-invented	Alcoholic beverages, illicit drugs, smoking; 5-point Likert scale (never to each day)		7-12
SYMPTOMS AND FUNCTIONS				
Fatigue	Chalder Fatigue Scale	11 items; 4-point Likert scale; each item scored 0-3; total sum score range 0 to 33.	Chalder T, Berelowitz G, Pawlikowska T, et al. J Psychosom Res 1993;37:147-53.	14
Chronic Fatigue Syndrome diagnosis and related symptoms	CDC symptom inventory for CFS Self-invented	28 items addressing frequency of symptoms on 5-point Likert scale; each item scored 1-5 (never to each day/always).	Wagner D, et al. Popul Health Metr 2005;3:8	13
Post-exertional malaise (PEM)	PEM items from DePaul Symptom Questionnaire	5 items addressing frequency of symptoms on 5 point Likert scale; each item scored 0-4 (never to each day/always). Average, then multiply with 25 to get a 100 point scoring scale.	Bedree H, et al. Fatigue 2019; 7: 166-79	13
Sleep disturbances	Karolinska sleep questionnaire (KSQ)	12 items addressing frequency of sleep related problems; 6-point Likert scale scored 1 – 6; lower score means *more* symptoms. Indexes for insomnia, awakening problems, and sleepiness	Akerstedt T, Ingre M, Broman JE, Kecklund G. Chronobiol Int 2008;25:333-48	16

Pain	Brief Pain Inventory (BPI)	4 items; 10 point Likert scale score 1 - 10 (no pain to worst pain possible)	Klepstad P, et al. J Pain Symptom Manage 2002;24:517-25.	15
Depression/Anxiety symptoms	Hospital Anxiety and Depression Symptoms (HADS)	14 items; 4 point Likert scale scored 0 – 3 (8 items with reversed scoring). Indexes for anxiety symptoms and depressive symptoms, also total sum score range 0 to 42.	Zigmond AS, Snaith RP. Acta Psychiatr Scand 1983;67:361-70.	17
Negative affect	Positive and Negative Affect Schedule (PANAS-SF)	5 items addressing negative affects (ashamed, anxious, nervous, hostile, upset); 5-point Likert scale scored 1-5. Total sum score range 5-25	Thompson ER. J of Cross-Cultural Psychology 2016; 38: 227-42.	18
Illness perception	Brief Illness Perception Questionnaire	8 items; 10 point Likert scale, scoring 1 – 10.	Broadbent E, et al. J Psychosom Res 2006;60:631-7	20
Quality of life	Pediatric Quality of Life (PedsQL)	23 items; 5 point Likert scale scored 0 – 4, and then multiplied with 25 to get a 100 point scale; average score reported. Four subdomains: health related, emotional, social, school.	Varni JW, et al. Health and quality of life outcomes 2007;5:9.	19
Interoception	Body Vigilance Scale (BVS)	4 items; 11 point Likert scales 0 – 10. Score on item 3 should be devided by 10. Scores on item 4: average among 15 sensations. Total sum score range 0 to 40.	Schmidt NB, et al. J Consult Clin Psychol 1997; 65: 214-20.	21
Miscellaneous/hypotheses generating	Self-invented	a) 1 item addressing avoidance behavior: "To what degree do you avoid everything that may worsen your symptoms"? b) 1 item addressing school absenteeism.		20/22
CONSTITUTIONAL				
Neuroticism	NEO-FFI-30	6 items making up the neuroticism axis; 5-point Likert scale scored 0 – 4. Total sum score range 0 – 24.	Körner A, et al. Psychother Psychosom Med Psych. 2008; 58: 238-245	24

Worrying tendencies	Penn State Worry Questionnaire (PSWQ)	16 items; 5-point Likert scale scored 1 – 5 (reversed scoring of item1,3,8,10,11). Total sum score range 16 to 80	Pallesen, S, et al. Scandinavian Journal of Psychology 2006, 47, 281–291	25
Emotional awareness	Toronto Alexithymia Scale (TAS-20)	7 items making up the index of Difficult identifying feelings. 5-point Likert scale scored 1-5, total sum score range 7 to 49.	Bagby RM, et al.. J Psychosom Res 1994;38:33-40.	23
Loneliness	UCLA loneliness scale	20 items; 4-point Likert scale scored 1 – 4 (reverse scoring of item 1,5,6,9,10,15,16,19,20). Total sum score from 20 to 80.	Russell, D., et al. Journal of Personality and Social Psychology 1980, 39(3), 472–480	26
Self-efficacy	General Self-Efficacy Scale, short form	6 items; 4-point Likert scale scored 1 – 4. Total sum score from 6 to 24.	Romppel M, et al. Psychosoc Med 2013; 10: Doc01.	27
Adverse life events	Life Events Checklist (LEC)	48 prespecified life events (good and bad). 4-point Likert scale addressing subjective impact.	Johnson JH, McCutcheon, S.M. Assessing life stress in older children and adolescents. In I.G. Sarason & C.C. Spielberger (Eds), Stress and anxiety 1980;7:111-25.	30/31
Miscellaneous/hypotheses generating	Child-Adolscent Perfectionism Scale (CAPS); Highly Sensitive Person Scale (HSP); Parentign Dimension Inventory (PDI); self-invented	a) 1 item addressing socially prescribed perfectionism (from CAPS): “Others always expect me to be perfect”, 1-5 Likert scale b) 2 items from HSP: Startling tendencies and affected by other people’s feeling tendencies c) 2 items from the PDI, both addressering parental control. d) 4 items self-invented (interoceptive awareness and positive expectancies): “The intensity of symptoms tells how dangerous a disease is”; “if you have a bodily complaint, every effort should be taken not to make it worse”; “it is important		25/28/29

		to be attentive to bodily signals in order to discover early signs of disease"; "things often turns out to be more easy than I anticipated".