PROJECT FINAL REPORT

Grant Agreement number:	**279227**
Project acronym:	**CRESTAR**
Project title:	**Pharmacogenomic biomarkers as clinical decision making tools for clozapine treatment of schizophrenia**
Funding Scheme:	**FP7-CP-FP**
Period covered:	**from 01/11/2011 to 31/10/2015**

Name of the scientific representative of the project's co-ordinator, Title and Organisation:

Prof David Collier,
Eli Lilly and Company ltd.

Tel:	**+44 (0) 1276-4-83142**
Fax:	**+44 (0) 1276-483525**
E-mail:	**collier_david_andrew@lilly.com**

Project website address:	**www.crestar-project.eu**

Table of contents

1 An executive summary

The CRESTAR project was a four-year collaboration between centres of excellence for the treatment and study of treatment resistant schizophrenia, and consists of leaders from European academic centres, psychiatric hospitals, SMEs and industry, with advisory input from experts in Canada and the USA (please see a list of participants in Annex B). The overall aim of CRESTAR is to perform translational research that improves the identification, treatment and management of patients with treatment resistant schizophrenia, a population of high unmet need, and leads to improvement in quality of life, morbidity and mortality in this difficult to treat and neglected group of patients.

Schizophrenia is a severe neuropsychiatric disorder that affects around 0.5 - 1% of the population. Although antipsychotic medications are effective treatments, around 30% of patients fail to respond adequately, and are classified as being treatment resistant. These patients can be treated with the atypical antipsychotic clozapine, the only evidence-based pharmacotherapy for treatment-resistant schizophrenia. Clozapine is only prescribed in patients who have failed to respond to trials of two other antipsychotics due to rare but potentially severe side effects, a process that may take many years. This is problematic, since an extended duration of untreated psychosis and lack of efficacy for the initial treatment are associated with a poorer prognosis. About one third of treatment resistant patients fail to respond even to clozapine and are called extreme treatment resistant. These patients are among the most disabled of all people with mental illness, are at higher risk of suicide self-harm, and are over-represented in forensic psychiatry settings. The main limiting factor for a broader use of clozapine is the risk of adverse drug reactions (ADRs) including a rare but severe and potentially fatal ADR, clozapine-induced agranulocytosis, which selectively destroys precursors of polymorphonuclear leukocytes (neutrophils) in the bone marrow. Clozapine is required by law to be given in conjunction with weekly measurement of the white-cell count, a procedure which is inconvenient, consumes considerable healthcare resources, and is a common reason for patients declining to take clozapine, or discontinuing treatment. After an episode of neutropenia, clozapine treatment can never be resumed under current guidelines. This and other dangerous and intolerable ADRs such as weight gain and type 2 diabetes have limited its use dramatically, and consequently many treatment resistant patients who would benefit from clozapine do not have access to it. Thus very early identification of patients who will eventually require clozapine, and identification of those who will respond to it, combined with biomarkers for potential adverse reaction to the drug, are important goals for improving clinical outcome and patient safety.

The scientific aims of CRESTAR were to: identify **genetic and other factors that increase the risk** of developing treatment resistant schizophrenia and extreme treatment resistance, to investigate the genetic and environmental nature of clinical response to clozapine treatment, to identify genetic and non-genetic factors that might predict vulnerability to adverse reactions to clozapine treatment, to understand the patterns of use of clozapine in children, to perform health economic analysis of treatment resistant schizophrenia, to develop clinical tests for clozapine response and adverse drug reactions, and to disseminate these results to the wider community.

The CRESTAR team used genome-wide association analysis in a sample of over 11,000 patients with treatment resistant schizophrenia and almost 25,000 controls, to identify ten new genetic loci associated with treatment resistant schizophrenia, and clozapine treatment failure. We used epigenetic analysis (DNA methylation) to develop a model to predict clozapine use in schizophrenic patients, with good specificity and sensitivity. We assembled the largest known cohort of patients with agranulocytosis (544) and used these to identify new genetic risk loci for this rare but potentially serious side effects, including one gene (SPTA1) conferring a 15-fold increase in risk. We also searched for rare, high impact sequence variants associated by whole genome sequencing with the aim of uncovering variants useful for diagnostics. We also used polygenic risk score to show that the type 2 diabetes seen in patients on clozapine is mediated through weight gain, and that patients with a high polygenic risk score for schizophrenia were more likely to be receiving clozapine and be non-responders.

These data hold great promise for the development of molecular algorithms based on genetic and epigenetic data to predict clozapine use amongst schizophrenia patients. Our analysis of individual longitudinal population-based registry data from Denmark (population 5.6 Mio), including the Danish National Newborn Biobank was used to define treatment resistant schizophrenia, and show that people with schizophrenia had high endogenous risk for type 2 diabetes, further increased by antipsychotic treatment. We showed that patients taking clozapine has a **50% lower risk for all-cause mortality** and deliberate self-harm, and completed studies to improve the efficacy and safety of clozapine use in children and adolescents. This work was further validated by health economic modelling to show that treatment resistant schizophrenia has a high cost, and although a genetic test for response would increase costs, it could benefit quality of life. Despite the unprecedented sample sizes it is currently not feasible to genetically predict treatment resistant schizophrenia or adverse reactions to clozapine. However, in a **significant leap forward, we have produced a large body of data** that has set the stage and will continue to contribute value to the development of predictive tests for clozapine treatment and safety. We anticipate that these tests will be developed based on our data and additional data that accrues in the coming years.

2 A summary description of project context and objectives

The CRESTAR project, Pharmacogenomic biomarkers as clinical decision making tools for clozapine treatment of schizophrenia is a four year project funded under the FP7-HEALTH-2011-two-stage program "HEALTH-2011.1.1-2: Genome-based biomarkers for patient stratification and pharmacogenomics strategies".

Concepts

The primary concept of CRESTAR was to: translate genetic, biomarker, epidemiological and environmental data. We and others generated from large-scale research efforts investigating the pharmacogenetics of clozapine, using well-characterised European schizophrenia patients, into commercially viable, predictive personalised medicine tools which can be used to improve clinical trial design and the treatment of schizophrenia with clozapine.

Schizophrenia is a common, complex, mental disorder, affecting 0.5-1% of the population. It mostly presents with several episodes and tends to become chronic. It is characterized by disintegration of thought processes and of emotional responsiveness, most commonly manifesting as auditory hallucinations, paranoid or bizarre delusions, or disorganized speech and thinking, and it is often accompanied by significant social or occupational dysfunction. Symptoms are classed as positive (e.g. delusions), disorganised (e.g. speech and thinking) or negative (e.g. social withdrawal). The onset of symptoms typically occurs in young adulthood, with onset peaking in the late teens for males, and slightly later for females. Onset may also occur in childhood, and may take a more severe form (Kinros et al., 2010). Genetics (heritability is greater than 80%), early environment, neurobiology, and psychological and social processes appear to be important contributory factors; some recreational and prescription drugs (such as cannabis) appear to cause or worsen symptoms (Picchioni and Murray, 2007).

The cost of care for individuals with schizophrenia is very high, estimated to be in £6.7 billion in the UK alone in 2004/05. Approximately 30% of patients with schizophrenia require support throughout their lives. Schizophrenia ranks eighth among all the diseases in the population aged between 15- and 44 years old, contributing 2.6% of all DALYs (Disability Adjusted Life Years) in this age group (Rossler et al., 2005). Almond et al. (2004) showed that illness relapse is a major factor in generating high hospitalisation rates and costs. Its direct costs in western countries range between 1.6-2.6% of total health care expenditures (Davies, 1994) that accounts for 7-12% of the gross national product, and is the seventh most costly medical illness to western societies. Active psychosis has been ranked the third-most-disabling condition, after quadriplegia and dementia (Ustun et al. 1999), and life expectancy is reduced by 10-25 years, due to decreased physical health and a high suicide rate. Life expectancy in schizophrenia has not improved significantly in recent years, and new biomarker tools are needed to monitor overall health risks.

The concept of this project arose from the burden of treatment resistance in schizophrenia, and the disability and economic cost caused by this. About one third of patients with schizophrenia are treatment resistant (TRS), i.e. they do not respond adequately to treatment with antipsychotic medication other than clozapine, and around 10% show clozapine resistance, i.e. they do not improve at all on any medication. To date, the atypical antipsychotic drug clozapine is the only evidence-based treatment for TRS, and in addition, is also superior for negative and cognitive symptoms, as well as reducing suicidality (the InterSept study) and all-cause mortality. The role of antipsychotic polypharmacy and other augmentation strategies remains unclear, at best (Kane and Correll, 2010). Because of the adverse effects associated with clozapine, it is not usually prescribed as a first line therapy in Europe. If two trials of antipsychotics fail, then the patient can be given clozapine, the only drug shown to be effective in treatment resistant schizophrenia, but this process might take many years (Taylor et al., 2003) and it is at present not possible to predict which patients will require clozapine. To improve the treatment outcomes in schizophrenia, research efforts are urgently needed that elucidate biomarkers of the illness and of treatment response, both with respect to therapeutic (clinical response to treatment) and adverse drug reactions (ADRs).

Mortimer et al. (2010) examined a group of 150 consecutive assertive outreach and former rehabilitation inpatients, and found that almost half of this group was treatment resistant, with 54% of these treated with clozapine. Of those treatment resistant patients not receiving clozapine, half refused and half could not be treated for medical reasons including the failure of previous trials and neutropenia. Levels of ongoing clinical problems were significantly greater in treatment-resistant patients not taking clozapine. Thus, in a tertiary referral service, only just over half of the patients who need clozapine on clinical grounds were actually taking it. While half of these refuse, the rest encounter obstacles to treatment. This illustrates the difficulties of delivering clozapine to treatment resistant patients. Improvements in therapeutic drug monitoring and overall safety of clozapine, for example by reducing the risk of ADRs or more effective therapeutic concentration, could reduce the number of patients either refusing clozapine therapy, or reduce treatment failure either because of lack of efficacy or ADRs. The

development of treatment resistance should be avoided if possible, and this might also be achieved by giving clozapine to patients much sooner in their illness.

Clozapine, although a very effective therapy for TRS, can be poorly tolerated, with several unpleasant side effects and ADRs. The fact that it is still a cornerstone in the pharmacological treatment of schizophrenia is a testament to its value in clinical psychiatry, and its unique clinical profile means that it is critical that it be used to maximum clinical effect whilst minimising risk. The most notorious ADR is agranulocytosis, which has proved fatal in a few individuals. Because of this, the use of clozapine in the UK and most other countries is confined to treatment-resistant cases, with regular monitoring.

The central aim of CRESTAR was to develop tools which enable patients who need clozapine - because they are not likely to respond to the usual antipsychotics - have access to the drug as early as possible, perhaps within weeks of illness onset, and that clozapine use is as safe as possible in these patients. This is important, because the duration of untreated psychosis (DUP) is an important factor in long-term prognosis; if clozapine could be administered at an earlier stage then earlier remediation of schizophrenia would be possible in the majority of those that fail to respond to current first line antipsychotics.
There are two ways to achieve this: identifying these treatment resistant patients using pharmacogenetic methods, combined with other predictive factors, for earlier clozapine therapy, and making clozapine use safer and more acceptable, by developing better therapeutic drug monitoring tools, and better tools to assess risk of side effects. This is especially important in children and adolescents which treatment resistant schizophrenia, because of both acute safety and long-term side effect concerns.

CRESTAR is an SME-driven project, focusing on the development of pharmacogenomic biomarkers in order to stratify psychotic patients for treatment decision making. The principal objectives CRESTAR were to predict those patients who will not respond to other antipsychotics, so they can be treated with clozapine earlier, those patients who are at high risk of particular ADRs, to make clozapine safer and better tolerated and thus more widely used, and those patients who will not respond to any antipsychotic, so that clinical trials for these fully refractory patients can be designed early in their illness (i.e. before they become chronic and institutionalized).

CRESTAR examined dense genome-wide association data, exome and genome sequence, and epigenetic biomarkers in large European subject cohorts totalling up to 17000 individuals either treated successfully with the usual antipsychotics, treatment refractory patients who respond to clozapine, patients who developed agranulocytosis or granulocytopenia while taking clozapine, fully refractory patients who do not respond to any medication, and population controls without schizophrenia. CRESTAR will use these groups to:

1. Develop algorithms to predict the 50% of patients who will fail to respond to conventional antipsychotics, as a clinical decision making tool for indication of early clozapine use.
2. Develop algorithms to predict the patients who will develop potentially fatal ADRs under clozapine, namely agranulocytosis, diabetic ketoacidosis (DKA) and severe obesity.
3. Develop algorithms to predict which patients are likely to fail not only to any treatment in schizophrenia, but also to clozapine, and end up in the one-third of patients who are fully refractory to any medication (the clozapine resistant group).
4. Develop an SME-based genetic test with sufficient sensitivity and validity for i) resistance to first line antipsychotics, with an indication to use clozapine as a first line treatment ii) risk of clozapine-induced agranulocytosis and DKA, so that patients at high risk can avoid this medication, and to make it possible to prescribe clozapine more widely without lower risk and inconvenience iii) clozapine resistance for use in clinical trials, particularly the design of clinical trials aimed specifically at developing novel solutions to the problem of fully refractory schizophrenia.
5. Perform health economic research and cost effectiveness analysis on the potential benefits of tests for treatment resistance and agranulocytosis.
6. Perform ethical and patient-centered research with stakeholders, including patients and patient organizations and advocates, carers and relatives, and healthcare professional, to assess the views of these groups on the benefits and acceptability of such tests.
7. Improve the knowledge-base in relation to ADRs and efficacy the use of clozapine and other antipsychotics in both adults children (10-18 years old), leading to better therapeutic drug monitoring (for example adding monthly assessment for diabetic ketoacidosis and GIT hypomobility syndrome).

The basis for the concept of CRESTAR

Despite some advances in treatment over the past few decades, schizophrenia remains one of the most severe psychiatric disorders, and is associated with a chronic, relapsing course and significant functional impairment in a substantial proportion of patients. A better understanding of the pathophysiology of this devastating disease, and the factors that influence progression, specific symptoms, symptomatic improvement, and the development of side effects to treatment, are therefore urgently needed. This will allow the development of innovative drugs to treat the disease, and the optimisation of existing treatments, in order to reduce both its social and economic burden.

Progress in the treatment of schizophrenia: Antipsychotic drugs are the mainstay of treatment for schizophrenia. The first antipsychotics were discovered in the 1950s. Clozapine, was discovered in the 1960s and introduced clinically in the 1970s, but later withdrawn because of its propensity to cause fatal agranulocytosis. However, in a landmark study (Kane et al 1988), clozapine was found to be uniquely effective in treatment resistant schizophrenia, and was re-introduced, with compulsory neutrophil monitoring, and a subsequent wave of second-generation, or atypical antipsychotics has since been introduced. However, for reasons that are not understood, none of the other antipsychotics is as effective as clozapine. All antipsychotics block receptors in the brain's dopamine pathways, but most antipsychotic drugs, especially clozapine, are also agonists and antagonists at a wide range of other receptor targets. A number of side-effects and adverse drug reactions include, sedation, hyperprolactinaemia, tardive dyskinesia, diabetes and weight gain (metabolic syndrome), agranulocytosis, akathisia, hypersalivation, and sexual dysfunction (Flanagan, 2008). While the atypical antipsychotics show lower propensity to cause some motor ADRs, they have been associated with greater risk of metabolic syndrome. While the atypical antipsychotics show lower propensity to cause some side effects such as movement disorders, they have been associated with greater risk of metabolic syndrome. Although new antipsychotic drugs are still appearing on the market, there has been no quantum leap in their effectiveness. To date, clozapine is the only evidence-based treatment for treatment resistant patients, and reduced suicidality and all cause mortality. The role of antipsychotic polypharmacy (i.e. one patient receiving more than one antipsychotic drug) and other augmentation strategies remains unclear, at best, with a lack of evidence to support it use (Kane and Correll, 2010). Indeed, one study patients on polypharmacy were hospitalized on average 8.5 days (55 percent) longer than those on monotherapy, and experienced 56 percent more adverse events than those on monotherapy. Although, the treatment aims of clinical response, remission, and recovery have been defined more uniformly, the most appropriate drug for an individual patient requires careful consideration, as the desired, individualized treatment approach needs to consider current symptoms, comorbid conditions, past therapeutic response, adverse effects, social and lifestyle factors, as well as patient choice and expectations. In this regard clozapine is a unique drug, successful in half of treatment resistant schizophrenia patients, and demonstrated to improve cognitive function, negative symptoms and reduce suicide and all cause mortality. Clozapine is worth investing considerable resources in to optimise its use, especially in the context of withdrawal of many pharmaceutical companies from antipsychotic drug development and the decades long drought of truly novel antipsychotics (see Schizophrenia, the drug deadlock). In order to enable personalized clozapine treatment, improving efficacy and reducing side effects, the genetic and environmental mechanisms underlying illness development and progression, symptomatic improvement, and adverse drug reactions need to be elucidated.

Pharmacogenetics has clear potential to profoundly influence the practice of medicine. Although there have been some spectacular development in pharmacogenetics over the last decade, at present there are relatively few clear examples where it has led to a change in practice for the use of individual drugs, such as carbemazepine (OR 2504 for SJS with HLAB*1502, OR 7 for hypersensitivity syndrome with MLN Omega). Pharmacogenetic analysis, using a one-off genetic test, has the potential to facilitate the selection of the most effective drugs at the optimal dose, and avoid many adverse drug reactions (ADRs) (Blakey and Hall, 2011). If this is combined with ongoing biomarker analysis, and environmental data such as lifestyle and demographics, it can have a profound effect on clinical management of individual patients, so called personalised medicine. ADRs in particular represent a major health problem, accounting for 6–7% of hospitalization cases and approximately 100,000 deaths a year in the USA (Lazarou et al., 1998). They are also the primary cause of drug withdrawal from the market.

The concept of CRESTAR is based around adopting all of these approaches to increase substantially the power of European researchers to identify novel genetic and other factors influencing the efficacy and adverse drug reactions of clozapine. In addition, because we recognise the importance of ensuring that these advances in molecular understanding are translated into knowledge which can impact on the health of European member states, CRESTAR is committed to a major, concerted effort by leaders in the field to understand the impact of the variants discovered at both the epidemiological and the individual level, and to exploration and implementation of methods which will facilitate clinical translation.

The **overall objectives** of CRESTAR can be summarized:

1) To **develop a framework for research into the pharmacogenetics, therapeutic drug monitoring and epidemiology of clozapine use and effects** that assembles the best researchers, the best sample and data sets for studying clozapine use in schizophrenia, the best ethical guidance and the best analytical and translational platforms.
2) To **accelerate the discovery of genetic factors and biomarkers influencing or predicting the effects of clozapine** in schizophrenia through integrated analyses across Europe and a range of experimental designs, thereby enabling predictive algorithms pharmacogenetic variants and biomarkers allowing for stratification of clinical trials, more widespread, better targeted and safer clozapine use, improved personalised medicine tools and optimal therapy.
3) To **improve the efficacy and safety of clozapine use** in children and adolescents through therapeutic drug monitoring research, epidemiology, genetics and biomarker analysis.
4) To **characterize the aetiology of treatment resistant schizophrenia**, and contribute to knowledge of the underlying aetiology of the disease.
5) Contribute to **improved clinical trial protocols** in of this heterogeneous disease by stratifying patients according to predictive genetic variants and biomarkers for treatment response, resistance, dose and side effects
6) To **develop commercially-viable array-based pharmacogenetic tests** for clozapine's effects (CLOZACHIP), which prove cost effective means of reducing the economic cost of treatment and improving quality of life for the patient, and develop methods by which genetic data can be analysed along with environmental data to better predict risks and outcomes.
7) To **translate these findings into the clinical arena**, by providing an evidence base to guide the use and monitoring of antipsychotic drugs, particularly clozapine, thus ensuring better functional outcomes and improved longevity for patients with treatment resistant schizophrenia.
8) To **disseminate research outputs** to both the scientific and non-specialist audience.
9) To **contribute to international efforts**.

References:

Almond S, Knapp M, Francois C, Toumi M, Brugha T. Relapse in schizophrenia: costs, clinical outcomes and quality of life. Br J Psychiatry. 2004 Apr;184:346-51.

Blakey JD, Hall IP. Current Progress in Pharmacogenetics. Br J Clin Pharmacol. 2011 Jan 14. doi: 10.1111/j.1365-2125.2011.03912.

Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, Venturi P, Jones LA, Lewis SW, Sham PC, Gottesman II, Farmer AE, McGuffin P, Reveley AM, Murray RM. Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. Arch Gen Psychiatry. 1999 Feb;56(2):162-8.

Davies T. Psychosocial factors and relapse of schizophrenia. BMJ 1994;309:353–4.

Flanagan RJ. Side effects of clozapine and some other psychoactive drugs. Curr Drug Saf. 2008 May;3(2):115-22.

Kane JM, Correll CU. Pharmacologic treatment of schizophrenia. Dialogues Clin Neurosci. 2010;12(3):345-57.

Kane JM, Honigfeld G, Singer J, Meltzer H. Clozapine in treatment-resistant schizophrenics. Psychopharmacol Bull 1988;24(1):62-7.

Kinros J, Reichenberg A, Frangou S. The neurodevelopmental theory of schizophrenia: evidence from studies of early onset cases. Isr J Psychiatry Relat Sci. 2010;47(2):110-7.

Lazarou J, Pomeranz BH, Corey PN: Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA 279(15),1200–1205 (1998).

Picchioni MM, Murray RM. Schizophrenia. BMJ. 2007 Jul 14;335(7610):91-5.

Rössler W, Salize HJ, van Os J, Riecher-Rössler A. Size of burden of schizophrenia and psychotic disorders. Eur Neuropsychopharmacol. 2005 Aug;15(4):399-409

Taylor DM, Young C, Paton C. Prior antipsychotic prescribing in patients currently receiving clozapine: a case note review. J Clin Psychiatry. 2003 Jan;64(1):30-4.

Ustün TB. The global burden of mental disorders. Am J Public Health. 1999 Sep;89(9):1315-8.

3 A description of the main S&T results/foregrounds

3.1 Summary of the main results/foreground of CRESTAR

The aims of CRESTAR were to identify genetic and other risk factors that increase the risk of developing treatment resistant schizophrenia and extreme treatment resistance, to investigate the genetic and environmental nature of clinical response to clozapine treatment, to identify genetic and non-genetic factors that might predict vulnerability to adverse reactions to clozapine treatment, to understand the patterns of use of clozapine in children, to perform health economic analysis of treatment resistant schizophrenia, to develop clinical tests for clozapine response and adverse drug reactions, and to disseminate these results to the wider community. In **work package (WP) 2**, we used **genome-wide association analysis** in a sample of 11077 cases with TRS and 24899 controls, which identified five new loci associated with treatment resistant schizophrenia, and a further five variants associated with clozapine discontinuation, a proxy for extreme treatment resistance and/or lack of tolerability. In **WP 3**, we developed a **classifier model to predict clozapine use from DNA methylation data** in schizophrenic patients, with good specificity and sensitivity. These data hold great promise for the development of molecular algorithms based on genetic and epigenetic data to predict clozapine use amongst schizophrenia patients.

In **WP4** we also examined **clozapine-induced neutropenia/agranulocytosis** in a CRESTAR sample of 544 cases and controls which underwent genome wide genotyping. The best results were seen for a novel locus on Chromosome 1 with a genome wide significant p-value of p=4.28E-08 and OR=15.25. This variant is located near SPTA1 gene (spectrin, alpha, erythrocytic 1). Based on this analysis we performed a replication study and meta-analysis with the CIAC sample from the US-led NIH initiative comprising 163 cases with agranulocytosis or neutropenia and replicated their main results: HLA-DQB1 (126Q) and HLA-B (Goldstein et al. 2014). Interestingly, prediction analyses based on all three samples integrating several immune diseases like Crohn's Disease, Rheumatoid Arthritis, Ulcerative Colitis and Inflammatory Bowel Disease showed that Crohn's Diseases explained the most variance of all genome wide analyses. We also analysed Polygenic Risk Score for BMI/obesity and type 2 diabetes in patients taking clozapine, to see if this could predict which patients were most vulnerable to metabolic adverse reactions. We found that patients treated with clozapine and diagnosed with Type 2 Diabetes during clozapine treatment had similar Type 2 Diabetes polygenic risk score as other patients with Type 2 Diabetes. A high polygenic risk score for Type 2 Diabetes in addition to clozapine treatment therefore does not have synergistic effects in the risk for developing Type 2 Diabetes. However the BMI polygenic risk score was higher for patients diagnosed with Type 2 Diabetes during clozapine treatment. That might indicate that the Type 2 Diabetes was mediated through weight gain caused by clozapine. In **WP5,** we also found that the **polygenic risk score for schizophrenia** was increased in patients receiving clozapine compared to those who did not. Furthermore, polygenic risk scores were higher in clozapine non-responders compared to clozapine responders. Thus, the present study is the first to provide molecular evidence that an increased genetic loading for schizophrenia is a risk factor for poor outcome.

In **WP6,** we assessed **pathways and predictors of treatment resistant schizophrenia** and extreme non-response in schizophrenia based on individual longitudinal population-based registry data from Denmark (population 5.6 Mio), including the Danish National Newborn Biobank. We developed a proxy definition for treatment resistant schizophrenia, and found that people with schizophrenia had high endogenous risk for type 2 diabetes, and their risks were further increased by antipsychotic treatment, lipid abnormalities were found at a higher frequency. However for clozapine exposure (non-clozapine-exposed periods as reference) the risk for all-cause mortality was decreased by 50%, and by 44% for deliberate self-harm. In **WP7,** we completed studies to **improve the efficacy and safety of clozapine use** in children and adolescents through therapeutic drug monitoring research, epidemiology, genetics and biomarker analysis, and this has now been published. In **WP8,** we used **health economic modelling** to show that the expected annual cost of the schizophrenia population much higher for TRS cases then treatment responsive cases, for example €8,710 per patient, €20,987 for TRS patients, and €5,020 for non-TRS cases, in Germany. We also found that a genetic test for response would increase costs but be more effective, at a cost of £34,111 per Quality-Adjusted Life Year over a 20 year horizon. In **WP9**, we **evaluated the ethical context** of offering such tests.

In **WP10,** we assembled, **genotyped and whole genome sequenced DNA** from a large sample of treatment resistant schizophrenia patients. We evaluated the impact of the confluence of common alleles on: disease, disease related phenotypes and adverse drug reaction (ADRs) including clozapine-induced agranulocytosis. We also searched for rare, high impact sequence variants associated with the same aiming for uncovering variants useful for diagnostics, but we have not yet uncovered high impact variants. Despite the unprecedented sample sizes it is currently not feasible to genetically predict treatment resistant schizophrenia or adverse reactions to clozapine. This is due to the fact that there do not appear to be any single genetic variants of large effect (which on their own would be useful for prediction) and also because genetic variants

collectively cannot effectively be used to predict treatment resistance or adverse effects to a clinically meaningful level. However, in a significant leap forward, we have produced a large body of data that has set the stage and will continue to contribute value to the development of predictive tests for clozapine treatment and safety. We anticipate that these tests will be developed based on our data and additional data that accrues in the coming years.

3.2 Main results/foreground of the different work package

WP01: Project management

Work package 1 was dedicated to project management to take care of all administrative and coordinating tasks. In order to support the coordinator in monitoring the compliance by beneficiaries with their obligations under the grant agreement, the project management office at concentris kept a close eye on the following partners' performance:

- making sure that tasks assigned to them were correctly and timely performed,
- that reports were submitted according to the guidelines and in time,
- that funds are used and claimed according to the rules,
- that the partners fulfilled their obligations regarding dissemination and funding acknowledgements,
- that any changes to the work plan were communicated to the EC swiftly,
- and compliance to ethical regulations.

The Project management office acted as a helpdesk for all participants; it was the central node of communication on a day-by-day basis and communicated with the European Commission on behalf of the Coordinator regarding administrative and managerial issues (i.e. contract, amendment, reporting etc.).

WP02: Genetic biomarkers indicative for clozapine treatment

This work package had **three broad aims**:

1. To identify genetic variants that increase risk of developing treatment resistant schizophrenia and replicate results.
2. To investigate the genetic nature of response to clozapine and if possible identify and replicate risk variants.
3. Depending on the results of the first two aims, develop predictive tools based on genetics and, with the addition of wider clinical and demographic data from other work packages, identify those with schizophrenia at increased risk of becoming treatment resistant and those that are more likely to respond to clozapine.

There is good evidence that the genetics of schizophrenia are complex and that the risk imported by individual common genetic variants are typically of small effect size though collectively are substantial. Given the number of potential factors that influence response to antipsychotic treatment we envisaged this outcome would be similarly complex and anticipated that the effect sizes of common genetic variants would typically be small. We therefore set out to recruit very large samples of those with treatment resistant schizophrenia in order to be able to detect these predicted small individual genetic effects.

Coming in to CRESTAR we had acquired the CLOZUK dataset of over 5000 samples taken from routinely collected bloods of those prescribed clozapine. This study was consistent with the UK Human Tissue Act and had UK NHS ethics approval, which was expanded for the CRESTAR sample collection. In the initial stages of CRESTAR we worked closely with partners in Leyden Delta, an SME that supplies and monitors clozapine in the UK and who formed a vital part of our work package. After a lot of hard work we were able to set up the collection of samples from over 7000 individuals taking clozapine. These samples were transferred and genotyped, by deCODE, another SME on the CRESTAR grant. We combined this genetic data with CLOZUK to give 15 000 samples from those on clozapine that we considered our treatment resistant schizophrenia (TRS) dataset. This exceeded our recruitment targets formulated at the start of the project. In order to identify genetic risk variants associated with TRS we acquired data on over 25000 healthy control samples from UK population datasets. Following strict

quality control measures we performed the genetic analysis (genome-wide association study (GWAS)) on 11077 cases and 24899 controls, an unrivalled sample size for this kind of work. This **GWAS analysis confidently identified 20 common genetic variants as increasing risk for TRS** at a level of probability very unlikely to be as a result of chance, as outlined in *WP2 Table 1*. Five of these variants were identified for the first time in this study (bold in Table 1).

WP2 - Table 1. *Genome-wide significant signals found in the CLOZUK2 TRS GWAS*

Tagged gene[1]	Index SNP[2]	P[3]	OR[4]	MAF[5]
xMHC	chr6:32198981	6.99E-27	1.3396	0.3758
TSNARE1	rs58033671	1.01E-11	1.1209	0.4717
LOC101927295	rs6424546	7.40E-11	1.1189	0.3719
MAD1L1	chr7:2027311	2.40E-10	1.1153	0.1033
CACNA1C	rs882194	4.48E-10	0.9019	0.0478
ZSWIM6	rs10223052	7.36E-10	0.8959	0.3422
CLU	rs7012010	2.05E-09	1.1180	0.2279
NPM1	**chr5:170775978**	**2.83E-09**	**1.1564**	**0.3456**
RAPGEF4	**chr2:173649656**	**5.05E-09**	**0.8307**	**0.0799**
SLC32A1	chr20:37369996	7.40E-09	1.1243	0.2712
TCF4	rs144158419	1.52E-08	1.2016	0.4205
PRSS35	rs60984498:	1.75E-08	1.1044	0.4535
CILP2	chr1919565015:I	1.85E-08	0.9064	0.1380
ZNF664	**chr12:124489100**	**2.35E-08**	**0.7411**	**0.4752**
DRD2	chr11:113392994	2.68E-08	1.1031	0.1593
ALDOA	rs35045736	2.86E-08	1.1012	0.0172
SLC39A8	chr4:103285906	2.95E-08	0.8498	0.4113
ASCL1	**chr12:103374378**	**3.15E-08**	**1.1615**	**0.3832**
DLX2	**chr2:173009067**	**3.39E-08**	**1.1428**	**0.3573**
CNTN4	chr3:2533746	3.75E-08	0.8823	0.3430

[1] One gene is reported, though most of the clumps tag multigenic loci. When possible, the gene shown was taken from the list of confident OMIM associations in PGC2.[2] rs-name taken from dbSNP build 142, when available, [3,4] p value (p) and Odds Ratio (OR) of TRS case vs control association, [5] MAF – minor allele frequency

To complete our first aim we then took these results forward to replication datasets (from CRESTAR colleagues and more widely) using polygenic analyses (many of the associated variants considered together) and demonstrated that the genetic signals that emerge in the above analyses also show **strong association in the replication datasets** of clozapine cases versus controls and also clozapine cases versus those with schizophrenia not on clozapine. This later finding is important since it suggests we are identifying genetic signals specific to TRS. This is what is required if we are to seek to predict whom of those with schizophrenia will fail to respond to initial antipsychotics.

In order to address aim 2 we ideally would have had prospective data on whether those starting clozapine responded to the drug with symptomatic or functional improvement. Unfortunately such samples are not currently available in sufficient numbers and so we elected to identify a surrogate marker of treatment response that was available in the large CRESTAR samples described above. We identified and sought to validate 'Time on Clozapine' as an available variable that may identify those that best *tolerate* clozapine whilst recognising that this variable likely reflects a balance between response to clozapine and adverse effects/patient choice. Using a separate dataset of anonymous electronic health records, with colleagues in King's College, we demonstrated that **non-response to treatment is very rarely the primary reason for stopping clozapine**. The most common reason for doing so is adverse effects such as sedation. Of those that remained on clozapine for 24 months or over the vast majority where classed as responders following reviews of their notes. Those that stopped clozapine before this time were a combination of responders and non-responders. In this way time on clozapine cannot be considered an accurate proxy of treatment response but nonetheless we felt was still an outcome worthy of further study given how clinically informative

prediction of 'staying on clozapine' or tolerating clozapine would be. Indeed on reflection this is the outcome clinicians may find more informative.

Following on from this work we then conducted a further **GWAS using a time cut-off of 2 years for stopping clozapine** as our outcome to identify **genetic variants associated with remaining on clozapine** for at least two years. The number of cases who stopped clozapine within this time frame was fewer than anticipated, only 131 individuals within the 2 years of sample and data collection of the project, and these were compared with 6667 individuals who had remained on clozapine for at least 2 years. **Five SNPs were associated with clozapine discontinuation at genome-wide significance** (GWS) level of 5 x 10^{-8}; rs113821156 on chromosome 13 (OR=10.06, P=2.85 x 10^{-9}), rs572085671 on chromosome 1 (OR=21.05, P=6.07 x 10^{-9}), rs112647842 on chromosome 13 (OR=4.18, P=2.16 x 10^{-8}), rs545977145 on chromosome 11 (OR=20.37, P=2.87 x 10^{-8}), and rs13130722 on chromosome 4 (OR=2.08, P=3.94x10^{-8}). The most significantly associated variant, rs113821156, is intronic to 5-Hydroxytryptamine (Serotonin) Receptor 2A (*HRT2A or 5-HT$_{2A}$)* and was present in 4% of cases and 0.4% of controls. Clozapine has a high affinity for the serotonin 5-HT$_{2A}$ receptor and studies indicate that 96% of 5-HT$_{2A}$ receptors are occupied at daily doses of 300-600 mg. Thus, variants within 5-HT$_{2A}$ have previously been investigated in candidate studies of clozapine response. In 1995, Arranz and colleagues identified an association between the 102-T/C polymorphism in 5-HT$_{2A}$ and clozapine response. Although there were a number of subsequent negative findings regarding this variant, a meta-analysis, including these negative studies, revealed a significant excess of 102C allele carriers in clozapine non-responders. Additional support came from a study implicating 1438-G/A, a functional variant in the promoter region of 5-HT$_{2A}$, and in strong linkage disequilibrium with 102-T/C. Furthermore, a less common polymorphism in 5-HT$_{2A}$, 452-His/Tyr has been associated with response although again there are conflicting findings. An alternative possibility is that this variant is associated with increased risk of developing adverse effects leading to clozapine discontinuation. In this respect it is worth noting that serotonin impacts on appetite and serotonin agonists such as d-fenfluramine have been used as appetite suppressants. Furthermore, variants within 5-HT$_{2C}$ have been associated with the clozapine-induced weight gain.

Other groups within CRESTAR and external collaborators have experienced similar challenges in identifying large numbers of those who do not respond, or stop taking clozapine. Together with colleagues in work package 5 we sought **replication of the significant results in a German sample of 121 non-responders** to clozapine. A limited number of the significant variants were present in the replication sample and given this very limited sample size and the use of somewhat different definitions of clozapine response it was not surprising that we failed to find replication for any results.

We had hoped to develop predictive tests and algorithms based on the findings from the above analyses. Despite the unprecedented sample sizes it is currently not feasible to genetically predict treatment resistant schizophrenia. This is due to the fact that there do not appear to be any single genetic variants of large effect (which on their own would be useful for prediction) and also because genetic variants collectively cannot effectively be used to predict treatment resistance. We have learnt that even larger sample sizes are likely to be required for genetic prediction in this field but also that it is at least as important to recruit large numbers of well characterised samples from those that respond to treatment. Following on from the relationships and collaborations formed as part of CRESTAR we have secured funding from the UK Medical Research Council stratified medicine call for a project, STRATA that builds on what we have learnt from CRESTAR, uses the data to have arisen from CRESTAR and seeks to recruit the kind of samples that we now know are required to fully address questions of genetic (and clinical) prediction of antipsychotic treatment response and resistance.

References:

Arranz MJ, Collier DA, Sodhi M, Ball D, Roberts G, Price J, et al. Association between clozapine response and allelic variation in the 5-HT2A receptor gene. Lancet 1995;346:281-2.

WP03: The dynamic epigenome and clozapine

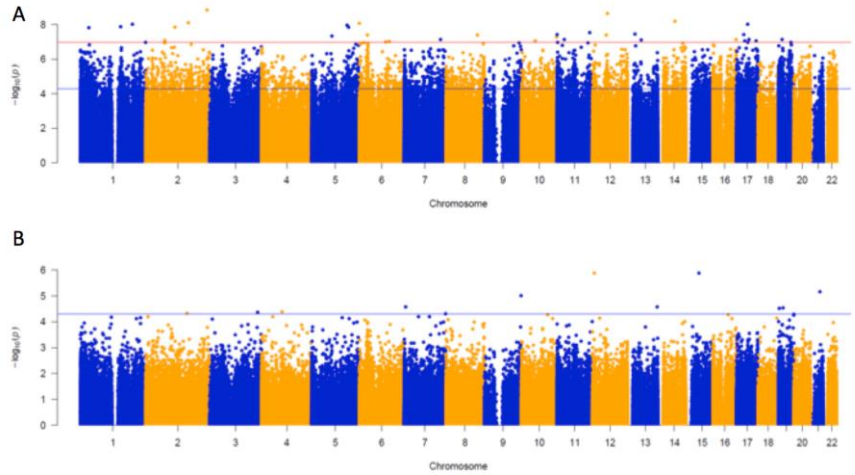
The primary objective of our work package was to explore the hypothesis that antipsychotic drugs elicit functionally-relevant epigenetic changes, and that epigenetics can act as a predictor of response or adverse events on clozapine therapy. We aimed to:

1. Determine the epigenetic and gene expression changes which occur in white blood cells upon commencement of clozapine therapy in adults and children.
2. Determine the epigenetic and gene expression changes which occur during treatment response, clozapine response, and which occur in fully treatment resistant schizophrenia in adults and children.
3. Determine the epigenetic and gene expression changes which occur during agranulocytosis or granulocytopenia.
4. Develop epigenetic and gene expression biomarkers for indication for clozapine use, clozapine response, clozapine resistance and risk of agranulocytosis or granulocytopenia.

In the first phase of WP3 we conducted a **genome-wide analysis of DNA methylation and gene expression differences** associated with clozapine use in a sample of treatment-resistant schizophrenia patients to explore functionally-relevant epigenetic changes associated with exposure, and identify epigenetic predictors of response or adverse events.

We **quantified DNA methylation at ~480,000 sites across the genome** using the Illumina 450K HumanMethylation array in whole blood samples from chronic schizophrenics. Following pre-processing, normalization and stringent quality control, an epigenome-wide association study (EWAS) was performed comparing schizophrenia patients prescribed clozapine (n = 155) to chronic schizophrenia patients on alternative medications (n = 135) while controlling for age, gender, batch, cell composition, and smoking. For a subset on clozapine (n = 48) detailed blood count data was available to identify cases of neutropenia. A second EWAS was performed comparing those classified as red or yellow (n = 22) against individuals with no evidence of neutropenia (n = 26), controlling for age, gender, cell composition and smoking. To identify differentially methylated regions (DMRs) we used the combP software programme which searches for additional signals around a seed signal ($P < 5x10^{-5}$) and calculate the Stouffer-Liptak combined p-value for the region. Region p-values were corrected for multiple testing using Sidak correction. Functional categories were downloaded from the Gene Ontology (GO) website and tested for over-representation of genes annotated to top DMPs ($P < 5x10^{-5}$) using a logistic regression modules controlling for gene size.

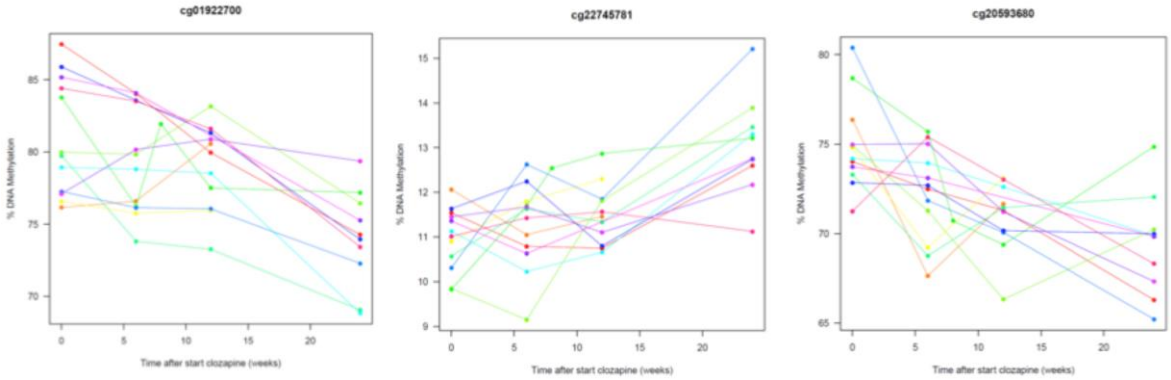
After quality control the final sample included 155 chronic schizophrenia patients on clozapine and 135 chronic schizophrenics who were not. **32 probes were identified as differentially methylated (DMPs)** with $P < 1 \times 10^{-7}$ and 4,607 at a more relaxed threshold hold of $P < 5x10^{-5}$ (*WP2 Figure 1A*), with a median difference of 1.80% (range 0.32 – 6.35%).
Using these probe level results to identify regions of co-ordinated differential methylation, 454 regions were identified with a corrected $p < 0.05$. The top-ranked DMR was located upstream of ZAP70 and included 8 sites within 1kb with both hypo and hypermethylation associated with patients who were prescribed clozapine. Of note, multiple DMRs were identified within the MHC region on chromosome 6 which contains many genes that are involved in the immune system. The top functional category identified in the GO pathway analysis DMPs associated with clozapine use was 'cellular response of catecholamine stimulus' (P = 2.48E-21). This is particularly interesting as **clozapine exposure has been associated with increased levels of catecholamines**. Other interesting pathways were related to neuronal function. A second EWAS was performed to identify DNA methylation changes in individuals on clozapine who develop neutropenia. No sites were identified at an experiment-wide significance level, but 12 sites were significant at a discovery threshold of $5x10^{-5,}$ (*WP2 Figure 1B*). Given the smaller sample and reduced power for this analysis, we used a more relaxed seed p-value of $5x10^{-4}$ in DMR analyses; three differentially methylated regions were identified with $p < 0.05$.



WP3 - Figure 1. *Manhattan plots of probe level results from A) EWAS of schizophrenia patients prescribed clozapine against those not and B) patients on clozapine who develop neutropenia against patients on clozapine with no evidence of neutropenia.*

We **profiled gene expression in 152 psychiatric patients, of whom 55 were receiving clozapine**. The effects of age, sex, ethnicity, RIN (RNA integrity number) and RNA concentration were corrected in a linear model following principal component analysis. The influence of mood-stabilizers, lithium carbonate/ lithium citrate and sodium valproate was studied to identify their possible roles as confounders, and also corrected for. An initial gene-by-gene analysis was carried out to test the association with clozapine use. Weighted Gene Correlation Network Analysis (WGCNA) is a technique that groups genes together based on their similarity. In this case each module was tested for an association with clozapine use. This study had 80% power to identify 1.3 fold differences in expression (p= 0.05) and 99% power to detect 1.5 fold changes. At the single gene level, no individual gene reached significance for association with clozapine, lithium, valproate or other antipsychotics according to Holm-Sidak threshold of 8.068e-6. No modules significantly associated with clozapine treatment, lithium, valproate or other antipsychotics. A possible confound was polypharmacy within the clozapine group. We defined a Clozapine mono-therapy group of individuals receiving clozapine as their only antipsychotic. All individuals on clozapine were receiving additional medications, including antidepressants, benzodiazepines, or mood-stabilisers. We tested for association between Module Eigengenes and Clozapine antipsychotic mono-therapy (n= 39) versus poly-therapy (n=16) with lithium and valproate as covariates. The **strongest association between clozapine mono-therapy and the purple module (p=0.002) lay just above our significance threshold** (p<0.0018). This was a down-regulation of expression. The purple module is enriched for ECM-receptor interaction (p= 1.43e-2), which included the integrin B5 and glycoprotein IX. Of the genes implicated in cell junctions, one of potential interest is YWHAH (Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide). This is an adapter protein of the 14-3-3 family, which has been previously implicated in schizophrenia.

We next assessed **longitudinal genomic changes in a sample of treatment-resistant schizophrenics newly prescribed clozapine**. Samples were recruited and first sampled at baseline (i.e. before clozapine exposure) and then sequentially over a 24-week period. At each assessment we collected blood for genetic, epigenetic and transcriptomic analyses. DNA methylation was quantified across the genome using the Illumina 450K array as described above. We performed a multilevel model controlling for age, sex and blood cell composition. We identified **highly-consistent changes associated with the onset and progression of clozapine exposure**. Examples are shown in *WP3 Figure 2*, with the ten top-ranked DMPs listed in *WP3 Table 1*.



WP3 - Figure 2. *Longitudinal changes in DNA methylation associated with clozapine exposure from baseline in treatment-resistent schizophrenics. Shown is data from 12 individuals profiled over a 24-week period highlighting highly-consistent changes at top-ranked DMPs.*

WP3 - Table 1. *Ten top-ranked longitudinal DMPs associated with clozapine treatment.*

Probe	Beta	SE	P	Chr	Pos	Annotated gene
cg01922700	-0.00296	0.000458	2.13E-07	1	197431704	CRB1
cg22745781	0.000859	0.000145	3.59E-07	7	16461244	ISPD;ISPD
cg04380955	-0.00332	0.000552	6.87E-07	17	67138637	ABCA6
cg01881899	0.00121	0.00021	1.69E-06	21	43652704	ABCG1;ABCG1;ABCG1;ABCG1;ABCG1;ABCG1
cg23508052	0.00036	6.33E-05	2.24E-06	10	102106811	SCD;SCD
cg11398158	0.000764	0.000144	3.12E-06	20	37590618	DHX35
cg20593680	-0.00222	0.00042	3.42E-06	1	94608341	
cg07103517	0.002345	0.000437	5.59E-06	7	50348485	IKZF1
cg12635783	-0.00106	0.000214	1.05E-05	1	113750056	
cg24511782	-0.00033	6.73E-05	1.09E-05	12	110841169	ANAPC7

WP04: Agranulocytosis and other severe ADRs (Adverse Drug Reactions)

The overall aim of this work package was to identify genetic biomarkers for agranulocytosis and other severe ADRs (Adverse Drug Reactions) caused by treatment with clozapine. Specifically, we aimed to:

1. Find genome-wide significant or high-risk loci for agranulocytosis/granulocytopenia using genome-wide association analysis, sequencing and meta- and mega- analysis.
2. Develop biomarker panels predictive of risk of diabetic ketoacidosis (DKA), diabetes, glucose dysregulation and obesity.
3. Develop predictive genetic marker panels for agranulocytosis, granulocytopenia risk based on findings from objective 1.
4. Develop a multi-omic biomarker risk prediction algorithm to test for susceptibility to clozapine induced agranulocytosis, granulocytopenia or DKA

Therefore, this workpackage aimed to study genetic causes of agranulocytosis as well as other side effects like Type 2 Diabetes and weight gain. The CRESTAR consortium was able to recruit over 600 samples of Schizophrenia patients developing neutropenia or agranulocytosis and controls. All these samples were characterized very well and fulfilled the following in- and exclusion criteria: Case definition ***agranulocytosis***. Inclusion criteria: (a) exposure toc, (b) absolute neutrophil count <500/mm3 (peripheral blood), (c) primary diagnosis Schizophrenia or schizoaffective disorder, (d) age 10-65 years, older subjects will be accepted subject to co-varying for age (e) any ancestry allowed, and (f) provision of written informed consent. Exclusion criteria: (a) taking medication also associated with agranulocytosis (e.g., carbamazepine), (b) medical disorder associated with agranulocytosis (e.g., autoimmune disorder, blood dyscrasias). Case definition ***granulocytopenia***. Inclusion criteria, as above but ANC < 1000. ***Control*** definition. Inclusion criteria: (a) exposure to clozapine for > 1 year, (b) absolute neutrophil count never < 2000/mm3, (c) primary diagnosis Schizophrenia or schizoaffective disorder, (d) age 18-65 years, (e) any ancestry allowed, (f) good compliance (clinical impression >80% of dosage and/or consistently documented plasma levels), and (g) provision of written informed consent. Exclusion criteria: none beyond than that implied above.

As there was a parallel NIH funded Clozapine-Induced Agranulocytosis Consortium (CIAC) led by Pat Sullivan we tried to be as comparable as possible with this consortium.

The current **CRESTAR sample for neutropenia/agranulocytosis consists of 544 cases and controls** which underwent genome wide genotyping and passed all quality checks. The best results were seen for a loci on Chromosome 1 with a genome wide significant p-value of p=4.28E-08 and OR=15.25. This variant is located near SPTA1 gene (spectrin, alpha, erythrocytic 1). Spectrin is an actin crosslinking and molecular scaffold protein that links the plasma membrane to the actin cytoskeleton, and functions in the determination of cell shape, arrangement of transmembrane proteins, and organization of organelles. Spectrin is the predominant component of the membrane skeleton of the red cell. It is essential in determining the properties of the membrane including its shape and deformability. Mutations in this gene result in a variety of hereditary red blood cell disorders like Hereditary Spherocytosis (also known as Minkowski–Chauffard syndrome), Elliptocytosis 2, and Pyropoikilocytosis.

Based on this analysis the CRESTAR consortium performed a **replication study and meta-analysis** including the CIAC sample from the NIH initiative comprising 163 cases with agranulocytosis or neutropenia. The mains results from CIAC comprised two loci in the major histocompatibility complex independently associated with CIAG: i) a single amino acid in HLA-DQB1 (126Q) (P=4.7 × 10(-14), odds ratio (OR)=0.19, 95% confidence interval (CI)=0.12-0.29) and ii) an amino acid change in the extracellular binding pocket of HLA-B (158T) (P=6.4 × 10(-10), OR=3.3, 95% CI=2.3-4.9) (Goldstein et al. 2014). The association of HLA-DQB1 (126) was replicated also within CRESTAR for agranulocytosis only and neutropenia. The meta-analysis combining the CRESTAR sample and the CIAC sample for HLA-DQB1 (126) improved the p-value. The best result of the genome wide association study of the CIAC sample was HLA-B rs41549217. This SNP was replicated in the CRESTAR sample. The second best association was within the HLA-B (158) region and meta-analysis including CRESTAR and CIAC provided also genome wide association. The next steps are to add to that meta-analysis the results from CLOZUK, a third sample of several thousand cases and controls.

Interestingly, prediction analyses based on all three samples integrating several immune diseases like Crohn's Disease, Rheumatoid Arthritis, Ulcerative Colitis and Inflammatory Bowel Disease showed that Crohn's Diseases explained the most variance of all genome wide analyses.

Beside agranulocytosis and neutropenia our focus was on side effects like **Type 2 Diabetes** and weight gain. Polygenic risk scores were further used to predict risks for subgroups with Schizophrenia. Correlation between the scores and Type 2 Diabetes was estimated using logistic regression. The score for Type 2 Diabetes derived from the DIAGRAM meta-analysis of Type 2 Diabetes including the Metabochip of 34,840 cases and 114,981 controls (Morris et al. 2012). In all regressions we used the control group of 85,019 people aged 50 or higher from the deCODE database. Patients treated with clozapine and diagnosed with Type 2 Diabetes during clozapine treatment had similar Type 2 Diabetes polygenic risk score as other patients

with Type 2 Diabetes. A high polygenic risk score for Type 2 Diabetes in addition to clozapine treatment therefore does not have synergistic effects in the risk for developing Type 2 Diabetes.

Clozapine-induced weight gain was mainly analysed within the Islandic sample. Polygenic risk scores for those patients with schizophrenia or schizoaffective disorder who developed Type 2 Diabetes after commencing on clozapine (follow-up time from 1 month to 25 years) based on 288 independent variants associated with high Body Mass Index (BMI) / Obesity (each at a $p<0.00001$). Similar studies were done for post-clozapine glucose dysregulation / Type 2 Diabetes. Polygenic risk scores for BMI were based on meta-analysis data from the GIANT consortium (Locke et al. 2015). 85,019 control people from the deCODE database were included. The BMI polygenic risk score was higher for patients diagnosed with Type 2 Diabetes during clozapine treatment. That might indicate that the Type 2 Diabetes was mediated through weight gain cause by clozapine.

In summary, this workpackage provided the **world wide largest sample on clozapine induced agranulocytosis and neutropenia**. Own genome wide association studies detected very interesting new genes to be followed up in future. Furthermore, results from the large CIAC consortium were replicated and improved by combining them in meta-analyses.

References:

Goldstein JI et al. Clozapine-induced agranulocytosis is associated with rare HLA-DQB1 and HLA-B alleles. Nat Commun. 2014 Sep 4;5:4757.

Morris AP et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 2012 Sep;44(9):981-90. doi: 10.1038/ng.2383.

Locke AE et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015 Feb 12;518(7538):197-206. doi: 10.1038/nature14177.

WP05: Extreme non-responders

The overall objective of our work package was to identify patients with schizophrenia who are resistant to clozapine therapy, and use them to identify genetic risk factors (common SNPs, rare CNV and point mutations) and non-genetic risk factors in order to characterise the aeitology of this trait. This information will be used to develop tests and algorithms which can be used to predict which patients will be clozapine resistant. In the long term will lead to improved therapies (for example by the design of clinical trials aimed at discovering drugs effective in clozapine-resistant schizophrenia). Specifically we aimed to:

1. Find genome-wide significant or high risk loci for extreme treatment resistance using genome-wide association analysis and genome sequencing.
2. Develop predictive genetic tests for TRS based on findings from objective 1.
3. Develop a multi-omic biomarker risk prediction algorithm to test for extreme treatment resistance.

Thus very early identification of patients who will eventually require clozapine, and ideally identification of those who will respond to it are important goals for improving clinical outcome.

Research trying to identify factors associated with poor treatment outcome has most consistently shown an association between poor treatment response and poor social functioning before disease onset. Furthermore, reports of an association between a family history of psychosis and an unfavourable treatment response suggest the influence of genetic factors. So far no genome-wide association study (GWAS) trying to explore the genetic background of clozapine response and non-response has been conducted.

Thus the main objective for WP5 (in cooperation with WP2) was i) to perform a GWAS of clozapine response and non-response ii) investigate association with clinical factors and outcome and iii) combine these data for an algorithm to predict treatment resistance and extreme treatment resistance.

To do so, two samples were available: a) a large sample (termed: Leyden Delta sample) with patients receiving clozapine with no phenotype information but duration of treatment with clozapine, and b) a small sample with detailed phenotype characterization (termed: CIMH sample). We sought to combine information obtained from both samples. As no clinical information about treatment response was available in the large Leyden Delta sample, we tested in the CIMH sample whether duration of treatment correlates with treatment response. As this was the case we used clozapine discontinuation within 24 months of treatment as proxy for extreme treatment resistance.

GWAS analysis in the Leyden Delta sample

In cooperation with WP2 we obtained DNA samples from 6798 schizophrenia patients treated with clozapine. Of those we jointly defined 131 as extreme treatment resistant. Genome wide genotyping was performed on these samples at deCODE using Illumina BeadChips, and GWAS analysis was eventually performed at Cardiff University.

GWAS analysis revealed five genome wide significant loci. The most significant variant associated with extreme treatment resistance was rs113821156 on chromosome 13, intronic to 5-HT2A, and present in 4% of cases and 0.4% of controls. Clozapine has a high affinity for the serotonin 5-HT_{2A} receptor and studies indicate that 96% of 5-HT_{2A} receptors are occupied at daily doses of 300-600 mg. Thus, variants within this gene have previously been investigated in candidate studies of clozapine response. A previous meta-analysis revealed a significant association between a variant in this gene and clozapine non-response.

Analyses in the CIMH sample

The CIMH sample consists of two different schizophrenia patients who have received clozapine. The first sample comprises 716 thoroughly assessed schizophrenia patients with clozapine treatment in whom response to clozapine was assessed in detail. In those patients clinical attributes were screened for their association with treatment and extreme treatment resistance. Selected premorbid clinical features comprised age and mode of onset, poor premorbid work and social adjustment, premorbid personality disorder, alcohol and drug abuse, family history of schizophrenia and other psychiatric disorders and psychosocial stressors. Genome wide genotyping could only be performed in a fraction of this sample (n=123) as for the rest no DNA was available. The second sample comprises 681 schizophrenia patients with and without clozapine treatment also with detailed phenotype information, however, lacking response data. Together this eventually yielded an extensively assessed sample of 434 patients receiving clozapine and 370 patients without history of clozapine treatment available for genome wide analysis performed at CIMH. Genotyping was performed at the University of Bonn.

Findings from the CIMH samples

A **correlation between duration of clozapine treatment and response** was shown. However, this correlation is modest, as stopping clozapine is not only due to non-response but also due to side-effects, as it was also shown for the sample at King's College (WP2). We could show in the CIMH samples that **stopping clozapine treatment due to side effects mainly occurs during the first three months of treatment**. It was however not possible to replicate our finding based on 716 patients as our cooperation partners had no data available with sufficient documentation of reasons for discontinuation. The most common reason given in the charts for stopping treatment was "unknown". Poor treatment response was associated with premorbid social functioning, an insidious disease onset and an early age-at-onset.

As sample sizes were too small to obtain reliable association signals for GWAS analysis of extreme treatment resistance, genetic risk was eventually assessed by using the **polygenic risk score for schizophrenia**, which is based on the aggregated number of risk loci previously identified from genome-wide association studies. The polygenic risk score for schizophrenia was increased in patients receiving clozapine compared to those who did not. Furthermore, polygenic risk scores were higher in clozapine non-responders compared to clozapine responders. Thus, the present study is the first to provide molecular evidence that an increased genetic loading for schizophrenia is a risk factor for poor outcome.

A prediction model including genetic data and premorbid clinical variables achieved a predictive power of 63% (AUC). A replication in an independent sample is necessary to validate this finding.

WP06: Epidemiology of antipsychotic use, resistance and ADRs

The aim of work package 6 was to assess pathways and predictors of treatment resistant schizophrenia and extreme non-response in schizophrenia based on individual longitudinal population-based registry data from Denmark (population 5.6 Mio), including The Danish National Newborn Biobank.
Specifically we aimed to:

1. Identify pathways and patterns of antipsychotic use and determinants of treatment resistant schizophrenia, clozapine (non-) response and clozapine related adverse events in the Danish population.
2. Integrate this data with data from other centres to produce a population, epidemiological and clinical predictive algorithm for antipsychotic use and adverse events.
3. Contribute to generate genetic data on selected cases (agranulocytosis, other severe ADRs, complete treatment resistance) obtained from Guthrie cards in order to contribute to genetic models for these ADRs and traits.

The general approach for this work package was to identify candidate predictors of treatment resistant schizophrenia (TRS) at first hospital contact with a schizophrenia (SZ) diagnosis. People had to be older than 18 years, and been born in Denmark after 1955 to enable assessment of family history of psychiatric disease among parents and siblings.
We chose to look at **known risk factors for schizophrenia and other patient and treatment related factors** that could influence the course of treatment and outcome of the patient and that could be identified at the time of the contact with the health care system when the individual was diagnosed with schizophrenia for the first time. We identified information on more than 30 selected factors.
We developed **a proxy definition for treatment resistant schizophrenia** that was based on prescription data and hospitalization data. According to pertinent guidelines of treatment of schizophrenia during the years of the studies we looked at the treatment pathways after the first diagnosis of schizophrenia and defined treatment resistant schizophrenia as the earliest instance of either (i) clozapine initiation or (ii) hospitalization for schizophrenia after having had two periods of different antipsychotic monotherapy trials. Thereby we were able to identify and include patients with potential TRS though not receiving clozapine.

- We identified 8624 people with schizophrenia,
- 21.1% fulfilled the TRS proxy definition during follow-up. Fifty percent of these people were followed for more than 9 years, 25% for up to 6.3 years, and 25% for more than almost 12 years.
- Younger age, living in a less urban area, higher education, previous psychiatric hospitalization, paranoid subtype, comorbid personality disorder, psychotropic drug use, and previous suicide attempt, were all significantly associated with an increased rate of TRS.
- Further evaluation of the **association between urbanicity and TRS** confirmed its robustness by measuring urbanicity at various points in time during follow up with regard to the patient's age thus at birth, during the first years of life etc, and in relation to schizophrenia diagnosis. The different direction of urban-rural differences regarding TRS and schizophrenia risk may indicate systematic differences in treatment practices across different levels of urbanicity, or indeed urban-rural different etiologic types of schizophrenia.
- We evaluated **functioning** in people with schizophrenia with regard to risk of re-hospitalization, TRS and clozapine treatment response using the general assessment of functioning (GAF-F) scale ranking a patient from 1 (lowest score) to 100. The lower the GAF-F scores at first diagnosis the higher hospitalization risk among men with an up to more than 2-fold increased risk for those with GAF scores below 20 indicating high risk for suicide and neglected self-care. GAF-F was not associated with re-hospitalization in women. Low GAF scores among men were also associated with increased TRS. During a follow up of 2 years among clozapine users, full response to clozapine defined as improvement of GAF above 50 points and an increase in 20 GAF points was detected in 29 (5.8%); and 96 (19.1%) achieved partial response of a 20 point GAF increase. Clinical response defined as no hospitalization within the 2 years of follow up was detected in 232 (46.2%). Insufficient treatment response/extreme treatment resistance defined as discontinuation of CLZ treatment within 2 years occurred in 25% patients after the first prescription of clozapine.

Insufficient treatment response/extreme treatment resistance defined as re-hospitalization as a proxy for relapse within two years after CLZ initiation was detected in 53.8%.

- **Any somatic hospital contact** with any somatic disease prior to first schizophrenia diagnosis influenced the risk of hospitalization due to schizophrenia during the first years after first schizophrenia contact. In particular, infectious diseases (HRR=1.15; 95%-CI=1.04; 1.28), cardiovascular diseases (HRR=1.33; 95%-CI=1.09; 1.62), musculoskeletal diseases (HRR=1.13; 95%-CI=1.1.03; 1.25), epilepsy (HRR=1.23; 95%-CI=1.05; 1.44), and brain injury (HRR=1.11; 95%-CI=1.01; 1.21) significantly increased re-hospitalization risks.
- **Diabetes mellitus (DM)** was evaluated among all people with schizophrenia in comparison with the general population. Antipsychotic naïve people with schizophrenia had a threefold increased risk of DM compared with antipsychotic naïve people without schizophrenia. Antipsychotic related risk for DM after starting any antipsychotic drug was three times increased compared with people with schizophrenia not treated with antipsychotics. The risk increase was the same for first-line treatment with either first or second generation agents. Starting clozapine was associated with a 4-fold increased risk for DM compared with people with schizophrenia, not treated with clozapine. We concluded that people with schizophrenia had high endogenous risk for DM, and their risks were further increased by both frist and second generation antipsychotics. Early detection and effective treatment of DM should be an integral part of multidisciplinary management of schizophrenia regardless of antipsychotic exposure.
- **Metabolic screening** in people with schizophrenia at their first diagnosis of schizophrenia was assessed based on routine laboratory measurements available for a subgroup of patients during the years 2000 until 2010. Less than half of the patients with a first-time schizophrenia diagnosis were screened for abnormal baseline metabolic parameters, although the proportion increased throughout the study period. Abnormal lipid profiles were found in more than half of the persons tested with a higher frequency among persons having previously redeemed prescriptions for antipsychotics. The increased metabolic abnormalities already present in the early phase of illness emphasize the need for screening and monitoring of this group of patients.
- We evaluated **mortality** among clozapine users compared with patients fulfilling the TRS criteria to account for confounding by indication and channelling, which have been major methodological issues in previous observational studies. For time-varying clozapine exposure (non-clozapine-exposed periods as reference) the risk for all-cause mortality was decreased by 50%, and by 44% for deliberate self-harm. The decreased rate of deliberate self-harm during clozapine use may suggest a potential pathway of effect of clozapine in the prevention of deaths in patients with treatment-resistant schizophrenia.
- Finally we explored the association between **polygenic risk score for schizophrenia and TRS**. Higher polygenic risk score for schizophrenia may be associated with a higher rate of treatment-resistant schizophrenia in patients with schizophrenia. However, the association in this study was not statistically significant, and the effects were more pronounced when restricting the outcome to clozapine initiation only. For future research on genetic prediction for TRS and potential implementation in clinical practice larger genetic samples in combination with clinical data are needed.

WP07: Paediatric use of clozapine

The overall objective of this work package was to provide a pharmacovigilance study by prospectively following 100 children and adolescents being treated with clozapine, in order to monitor adverse drug reactions (ADRs) associated with clozapine therapy in children seen in secondary- and tertiary-care settings. Specifically we wanted to:

1. To examine whether there are unique patterns of heterogeneity or subgroups of individuals based on their ADRs profile to clozapine.
2. To determine whether clozapine level, demographic and clinical characteristics, and genotypic variables predict cluster membership (i.e. membership to unique subgroups).
3. To identify changes in biological and observed side-effect markers that may predict the occurrence of severe adverse outcome specifically metabolic syndrome (including type 2 diabetes and weight gain), agranulocytosis or persistent neutropenia or discontinuation due to intolerance.

Management of early onset schizophrenia

Schizophrenia begins in childhood or adolescence for 1 out of 20 patients. Early onset schizophrenia (EOS) is associated with greater clinical and psychosocial morbidity and with the need for long-term antipsychotic treatment. Optimal pharmacotherapy is vital to help, and where possible avoid, chronic symptoms and the ensuing human toll to patients and family and economic costs to healthcare systems and society. Our current results outline, summarize and augment available knowledge regarding treatment optimization in EOS, especially in clozapine.

Use of antipsychotics in early onset schizophrenia

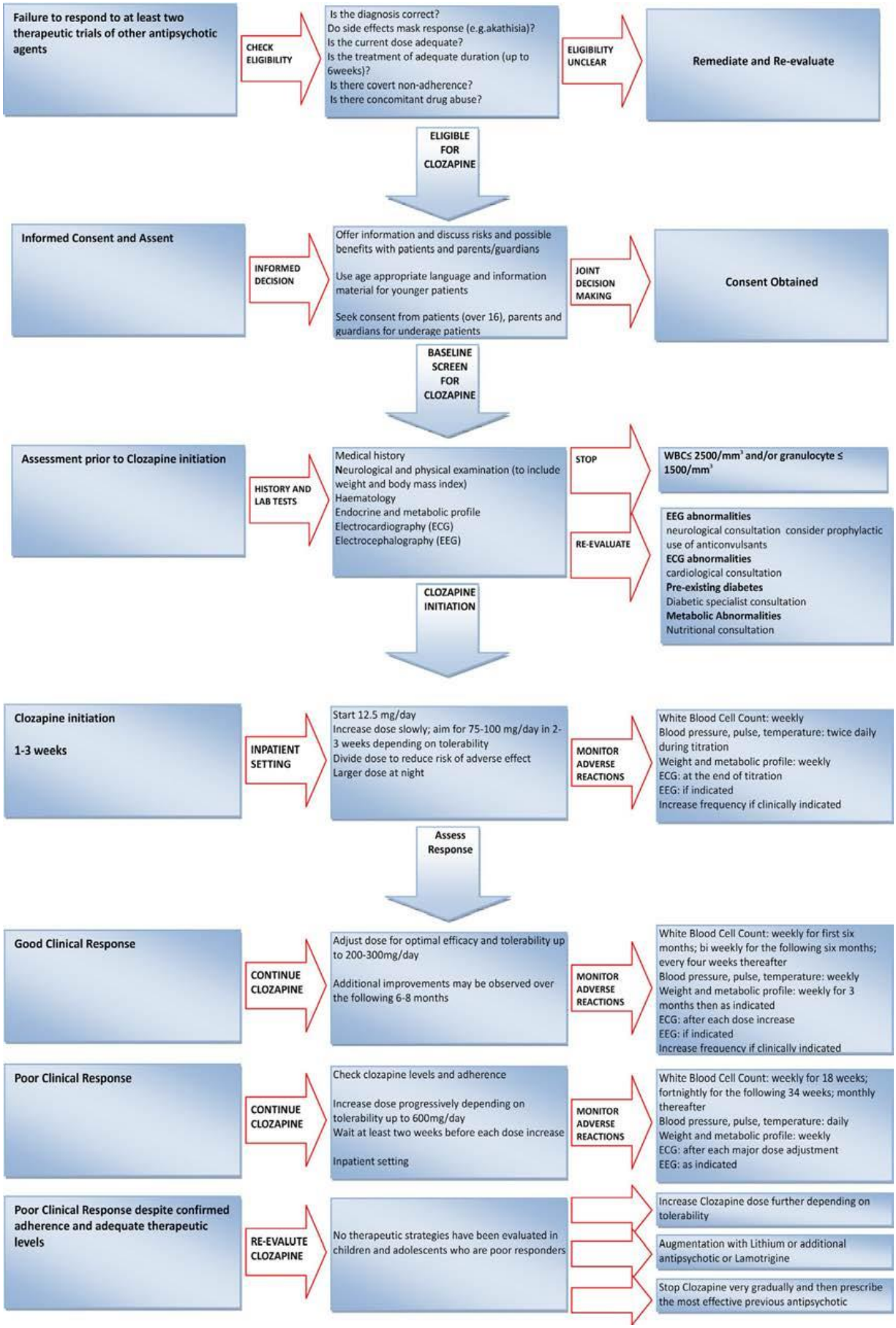
The use of antipsychotics (APs) in youth with psychotic but also neurodevelopmental, behavioral and psychiatric disorders has significantly increased while the age of prescription has decreased. In our paper (Schneider et al., 2014a) we reviewed the available literature and described the still existent gap in gathering and measurement of statistically significant analysis in relation to the efficacy between different APs, their adequacy for various pathologies and their long-term effects on children. However nowadays it is widely accepted that all APs improve symptoms of EOS and bipolar disorder and prevent recurrence, becoming their treatment of choice. AP treatment along with psychotherapy, family, social and cognitive interventions, allows many impaired children and their families to acquire a better outcome and quality of life.

Use of clozapine in early onset schizophrenia

Systematic review of the evidence regarding the efficacy and tolerability of clozapine (CLZ) in EOS confirmed the superior efficacy of CLZ in patients that had failed to respond to two previous trials of antipsychotic medication. Most patients experienced multiple ADRs but life-threatening events were infrequent and the discontinuation rate was low. We demonstrated that clozapine CLZ is effective and generally safe in the treatment of refractory EOS provided patients are regularly monitored. As EOS is associated with poor treatment response, timely use of CLZ may be beneficial (Schneider et al. 2014b). *WP7 Figure 1* below synthesizes the available information on screening and monitoring patients during CLZ treatment.

As shown in our paper (Schneider et al., 2015) the nationwide population-based study in Denmark, found that approximately 1 in every 5 patients with EOS was prescribed CLZ within 3-4 years from illness onset following on average 3 failed trials with other APs. The majority where considered as having had a favorable response since CLZ was prescribed to them for more than 6 months from the initiation of the treatment. The initiation of treatment with CLZ was associated with diagnosis at an older age, a history of suicide attempts and positive family history of schizophrenia.

In naturalistic clinical London setting we collected data from 100 patients treated with APs in order to assess the efficacy and tolerability of different agents and their specificity to EOS as opposed to a broader spectrum of psychotic disorders. At 3 month follow-up the data indicated that all the APs used in routine care were effective in improving overall function in children and adolescents. Approximately 10% of the sample did not achieve substantial remission and remained inpatients over the entire 12-month observation period. However, only three patients received CLZ at 12 months follow up. This might suggests that CLZ was underused in this population and there was reluctance in following the current guidelines that recommend CLZ treatment initiation after the failure of at least two other APs.



WP7 - Figure 1. *Overview of available information on screening and monitoring patients during CLZ treatment*

References:
Schneider C, et al. 2014a. Antipsychotics use in children and adolescents: An on-going challenge in clinical practice. J Psychopharmacol. 28(7):615-623.
Schneider C, et al. 2014b. Systematic review of the efficacy and tolerability of clozapine in the treatment of youth with early onset schizophrenia. Eur Psychiatry. 29(1):1-10.
Schneider et al., 2015. Clozapine use in childhood and adolescent schizophrenia: a nationwide population-based study. Eur Neuropsychopharmacol. 25(6): 857-63.

WP08: Health economics of response prediction

The overall objective of this work package was to analyse the health economics of predictive testing and personalised medicine in relation to clozapine use in schizophrenia. Economic evaluation is increasingly recognised as essential. Healthcare commissioners, providers and policy makers want to know not only whether a treatment or service arrangement is effective, but also whether it is cost-effective. They want to know if it is it worth paying for. Specifically we aimed to:

1. Estimate economic impact of treatment resistance in schizophrenia for UK, Iceland, Germany and Denmark.
2. Assess the cost effectiveness of the introduction in the UK National Health Service of a genetic test that will predict patients who will be benefit from the early use of clozapine, and patients who may be refractory to all treatment.
3. Assess the cost effectiveness of the introduction in the UK National Health Service of genetic testing before initiating clozapine treatment in paediatric population.

Economic impact of treatment resistance in schizophrenia: systematic literature review

The first systematic review on the economic evidence of treatment resistance in schizophrenia (TRS) identified five relevant studies, which showed that (i) first-generation antipsychotics might be more cost-effective than second-generation antipsychotics, (ii) cognitive behavioural therapy combined with standard care was more cost-effective than standard care, and (iii) olanzapine led to lower costs and fewer acute admissions. All studies confirmed previous research, showing that hospitalisation was the main contributor to total costs, with one study suggesting a shift of resources from inpatient to outpatient care. The second review, assessing the economic evidence on the use of clozapine in schizophrenia, identified twelve studies, the majority of which suggested that clozapine is a cost-effective option. Only a few studies were based on decision analytic modelling and the results were sensitive to a number of parameters. The majority of studies used a mirror-image design to assess the treatment costs and effects. Due to variability in study methodologies, in definitions of treatment resistance and in findings, cross-country comparisons were difficult to make. The economic evidence for TRS, and the cost-effectiveness of clozapine in TRS was, in general, of moderate quality, based on short- or medium-term studies and was difficult to generalise to the whole population of people with TRS.

Economic impact of treatment resistance in schizophrenia for UK, Germany, Iceland and Denmark

Comparing the service utilisation and costs per patient before and after clozapine initiation in a UK sample of clozapine users (n=307), patients had more outpatient contacts and fewer hospitalisations, but with increased length of stay, following clozapine treatment. Total costs were higher by approximately £22,600 per person in the post-clozapine period. Responders (much improved and mildly ill according to CGI scores) incurred lower annual costs of approximately £21,600 per patient than non-responders. Costs in the pre-clozapine period were predicted to have a minimal but significant impact on post-clozapine costs. Ethnicity was significant, with Black patients having costs that were increased by about 40%.

Results from the simulation models showed that: In the UK, the total annual expected costs of schizophrenia were estimated to be £10,068 per patient. For patients treated with clozapine (defined as TRS patients), the expected annual cost of treatment was £21,712 per patient while for non-TRS cases the figure was £6,582. In Germany, the expected annual cost of the schizophrenia population was €8,710 per patient, €20,987 for TRS patients, and €5,020 for non-TRS cases. In Iceland, the expected cost of schizophrenia was €12,830, €28,329 for TRS patients, and €9,580 for non-TRS cases. In all countries, the costs of non-TRS patients were approximately one third of the costs of TRS patients. Parameters with the highest impact on costs across all countries included the cost of non-relapse, the cost of relapse and the probability of relapse. Varying the costs of non-relapse and relapse by 25% in both directions, would change the total annual cost by 10%-13%, and 7%-11%,

respectively. At a lower probability of relapse, the total annual costs would decrease by 7%-22%, while at a higher probability of relapse; the total annual cost would increase by 4%-17%.

In analyses of the Danish data, TRS was defined as clozapine initiation or eligibility for clozapine, but never been treated with clozapine. During the whole study period (from TRS date until 2011), TRS patients (n=1,819) had significantly higher emergency room and outpatient costs per patient than non-TRS patients (n=6,791). Inpatient costs per patient were significantly higher for the TRS group by €56,489. Significant reductions in total psychiatric costs were associated with being non-TRS, the year and age of schizophrenia diagnosis, not living in the capital city, suffering from undifferentiated schizophrenia or post-schizophrenia depression, and having received antidepressants one year before diagnosis. Significant increases in total psychiatric costs were associated with being hospitalised in the year prior to diagnosis, being an outpatient at diagnosis, previous diagnosis of unspecified schizophrenia, and having attempted suicide. In a mirror-image analysis of 1-year costs, emergency room costs were lower for clozapine patients compared to those eligible for clozapine in the year before TRS, and significantly lower in the year after TRS. Outpatient costs were significantly lower in the year before and significantly higher in the year after TRS for those on clozapine. Clozapine patients had higher total costs in the years before and after TRS compared to those eligible for clozapine. However, the between groups difference was significant only in the year after TRS. Significantly lower costs in the year before TRS were associated with the year of schizophrenia diagnosis, living in a provincial city or rural area, and having catatonic or residual schizophrenia. Significantly higher costs were associated with: being eligible for clozapine, female, hospitalised in the year prior to schizophrenia diagnosis, being an outpatient at diagnosis, and previously diagnosed with unspecified schizophrenia, other affective disorders, depression, substance abuse, personality disorder, attempted suicide, and received antipsychotics or benzodiazepines in the year prior to diagnosis. Significantly lower costs after TRS were associated with year and age of diagnosis, place of living other than capital or capital suburbs, having undifferentiated, and post-schizophrenia depression, and having been prescribed antidepressants in the year before diagnosis. Significantly higher costs were associated with being eligible for clozapine, hospitalised for more than 30 days in the year before diagnosis, and being an outpatient at diagnosis.

Cost effectiveness of genetic testing to assist pharmacological treatment in schizophrenia

The base case results from the Markov model showed that the genetic test was more expensive and more effective, producing more Quality-Adjusted Life Years (QALYs) than no test, over the 20-year time horizon. The incremental cost-effectiveness ratio (ICER) for the genetic test strategy was £34,111 per additional QALY. This result was sensitive to the proportion of clozapine responders, the distribution of patients to different subpopulation groups according to the test efficacy, the test efficacy, and the costs of relapse and remission. These parameters were tested in sensitivity analysis showing that the genetic test becomes a cost-effective option (i) when the proportion of clozapine responders increased by 1% (from 24% to 25%), with an ICER of £14,583 per QALY, and (ii) when sensitivity of the test was increased above 75%, with an ICER of £26,531 per QALY. Response rates were a critical determinant of the utility and cost-effectiveness of the test, and therefore represent an important parameter in reporting and comparing pharmacogenetics tests.

WP09: Ethics, dissemination and public engagement

The overall objective of this work package was to understand the ethical views of stakeholders and the public on pharmacogenetic testing, and train, educate and disseminate the findings of CRESTAR to a broad audience. Specifically we wanted to:

1. Perform ethical and patient-centred research with stakeholders, including patients and patient organizations and advocates, carers and relatives, and healthcare professional, to assess the views of these groups on the benefits and acceptability of such tests.
2. Disseminate the results of the project at all levels of stakeholder, including healthcare professionals, scientists, regulators, managers, commercial organisations, patient support groups and patients, carers, families, and the public.

3. Initiate a Task Force between the National Drug Safety Organisation, and a collaboration with the Pharmacovigilance directive to help in the extension of the EU-wide system of reporting and monitoring of ADRs, the EU's "Eudravigilance" database.

One aim of this WP was therefore to assess the **ethical and regulatory issues with respect to the implementation of genetics test for therapeutic drug monitoring** and assessment of response and adverse reaction risk to clozapine. We performed a literature review of the ethical and regulatory issues surrounding pharmacogenetic testing. Our key findings include: a consideration of the probabilistic results that a pharmacogenetic test may return; the impact on drug licensing; and the potential for pharmacogenetic tests for clozapine being used without consent under the UK's legal framework (see summary below).

- Research in pharmacogenetic testing for clozapine is underway given that it is the only antipsychotic with proven efficacy in treatment-resistant schizophrenia, but it is associated with serious but rare adverse events, such as agranulocytosis. Mandatory haematological monitoring is currently part of the terms of the license to reduce this risk in clinical practice.
- A pharmacogenetic test for clozapine is likely to return probabilistic data, posing complex challenges for interpretation and communication. An alternative is to provide results via a heuristic algorithm with the issues of disempowerment and threshold setting. This produces difficult questions regarding who could, or should, design these and set the thresholds.
- We recommend that any changes to the licensing of clozapine based on a pharmacogenetic test should still allow for treatment under the current licensing criteria if a pharmacogenetic test is refused by the patient.
- Patients with a mental disorder can be considered to belong to one of the most vulnerable groups in clinical care, and there are complex legal issues surrounding the use of this testing, including the possibility of testing without consent. We would recommend that clozapine pharmacogenetic testing be regulated under section 58A of the Mental Health Act 1983 by the Care Quality Commission.
- There are substantial ethical issues surrounding the use of clozapine pharmacogenetic testing in the clinic, and we would recommend further research into the views of relevant consumer groups and stakeholders (including clinicians, patients and carers).

The findings were published in:

Spencer BW, Prainsack B, Rujescu D, Giegling I, Collier DA, Gaughran F, MacCabe JH, Barr CL, Sigurdsson E, Stovring H, Malhotra AK, Curran SR; CRESTAR Consortium. Opening Pandora's box in the UK: a hypothetical pharmacogenetic test for Clozapine. Pharmacogenomics. 2013 Nov;14(15):1907-14. doi: 10.2217/pgs.13.182.

Another goal of this work package was to assess the **views of patients and physicians on the benefits and acceptability of genetic tests** for clozapine. Therefore we designed questionnaires on the topic of genetic testing with clozapine. The English version was developed especially for physicians including questions like:

- If a pharmacogenetic test to determine treatment efficacy and side effects from a therapeutic agent was available would you use it in your clinic?
- If tests like these give information regarding your patient's risk of developing a rare side-effect that is possibly life threatening, which information would you find more useful?
- Would you be concerned that your patient's genetic information could be used to discriminate against them?

The questionnaires were sent out to physicians and finally 81 physicians participated. The questionnaire for patients was translated into German. 52 patients with a psychotic disorder with the potential possibility to receive clozapine or who already received it filled in the questionnaire. Interestingly, ca. 2/3 of them would wish to have such a test prior medication. About the half would be concerned that such a test could discriminate them and about 50% would wish that a specialized physician in genetics would perform such a test instead of nurses or the current doctor. We will combine these views of the patients with the views of the physicians and summarize these results in a manuscript for publication in a peer-reviewed journal in early 2016.

A third major aspect was **to disseminate the results of the project at all levels of stakeholder**, including healthcare professionals, scientists, regulators, managers, commercial organisations, patient support groups and patients, carers, families, and the public. We had been very successful in doing so. Besides local dissemination within Departments and Clinics of the single Collaborators we performed 11 national and international symposia and workshops. Please see *WP9 table 1* presenting a list of events.

WP9 Table 1: *List of CRESTAR dissemination events*

Type of activities	Title	Date	Place
Course / Summer School	MRC SGDP Centre - 13th Annual SGDP Summer School	18 - 22/06/2012	London, UK
Course / Summer School	MRC SGDP Centre - 14th Annual SGDP Summer School	17 - 21/06/2013	London, UK
Workshop	Clozapine Therapeutic Drug Monitoring	28/06/2014	London, UK
Workshop	Workshop on personalized medicine in psychiatry, SIRS Conference	9/4/2014	Florence, Italy
Symposium	SESSION II: UPDATE ON CRESTAR PROJECT, PIP Meeting	15/6/2014	Hollywood-Miami, USA
Educational Workshop	Understanding modern genetics, CINP congress	24/6/2014	Vancouver, Canada
Symposium	CRESTAR: Personalized Medicine in Schizophrenia- Development of Pharmacogenomics Biomarkers, EPA	31/03/2015	Vienna, Austria
Symposium	Optimal use of clozapine in treatment-resistant schizophrenia, NCP congress	20-23/9/2015	Kopenhagen, Denmark
Symposium	Clozapine: the art of prescribing, ESCR congress	24-26/9/2015	Berlin, Germany
Symposium	The CRESTAR consortium: Development of biomarkers for schizophrenia, PIP Meeting	15/10/2015	Toronto, Canada
Symposium	CRESTAR symposium, WCPG 2015	17/10/2015	Toronto, Canada

We are happy to say that due to the success of CRESTAR we had the opportunity to organize a satellite session on the largest conference on psychiatric genetics at the WCPG meeting in Toronto in October 2015. This meeting was of high success with a broad audience.

A last objective was to initiate a Task Force between the National Drug Safety Organisation, and collaboration with the Pharmacovigilance directive to help in the extension of the EU-wide system of reporting and monitoring of ADRs, the EU's "Eudravigilance" database. The Halle site successfully became a member of the German Society for Pharmacovigilance. As Eudravigilance is the umbrella organization for national initiatives we will next connect to the Eudravigilance.

In sum, this work package was very successful in the dissemination of the results of the CRESTAR consortium at all levels of stakeholder, including healthcare professionals, scientists, commercial organisations, patient support groups and patients, carers, families, and the public.

WP10: Development of clinical genetic tests

The overall aim of work package 10 was to develop personalised medicine biomarker tests for use as decision making tools in the clinic as an adjunct to clinical information for indication of clozapine therapy, optimisation of clozapine dosage, ascertaining the risk of clozapine-induced severe and chronic adverse drug reactions, as well as estimating the overall risk-benefit ratio of clozapine treatment. Specifically we wanted to:

1. Develop algorithms for predicting disease risk, drug response and ADRs for schizophrenia.
2. Develop a panel of predictive genetic markers for clozapine use in schizophrenia.
3. Develop a clinical genetic test alongside a tool for interpreting the genetic tests results in the clinic.

CRESTAR has assembled, **genotyped and whole genome sequenced DNA from a large sample of treatment resistant** schizophrenia patients. CRESTAR evaluated the impact of the confluence of common alleles on; disease, disease related phenotypes and adverse drug reaction (ADRs) including clozapine-induced agranulocytosis. CRESTAR furthermore searched for rare, high impact sequence variants associated with the same aiming for uncovering variants useful for diagnostics.

Common variants

deCODE genetics isolated DNA from 8,400 samples from treatment resistant schizophrenia patients and genotyped the DNA using the Illumina OmniExpress SNP arrays. This array is most suitable for assessing common variants in all blocks of linkage disequilibrium in the genome at reasonable cost.

Currently well over 100 common sequence variants have been associated with schizophrenia[1] as well as at least 11 rare copy number variants. Variants associated with clozapine drug response and adverse drug reactions are, however, few and none are high-impact variants.

To evaluate whether common variants have an important role *en masse*, hence directly testing the classic theory of polygenic inheritance, polygenic risk scores (PRS) were tested for association with drug response and adverse drug reactions. CRESTAR collaborated with the CLOZUK and CIAC consortia's working on treatment resistant schizophrenia. PRS were derived from the CLOZUK and CIAC meta-analysis, as well as the PGC. Relevant phenotypes were predicted in the CRESTAR sample including:

- Schizophrenia PRS predict schizophrenia in an independent sample (validation)[2]
- Schizophrenia PRS predict bipolar disorder (validation)[2]
- Schizophrenia PRS predict creativity[2]
- Schizophrenia PRS don't predict reduced fecundity in a large sample[3]
- Schizophrenia PRS don't predict treatment resistance in a validation sample (unpublished data)
- T2D PRS predict T2D in schizophrenia patients with T2D, both those that developed T2D during clozapine treatment and also those who developed T2D but were not treated with clozapine (unpublished data)
- BMI PRS predict BMI in schizophrenia patients with T2D, both those that developed T2D during clozapine treatment and also those who developed T2D but were not treated with clozapine (unpublished data)
- Agranulocytosis PRS (CIAC, CLOZUK) don't predict agranulocytosis in the CRESTAR sample (unpublished data)

Thus, while common variants explain a fair fraction of the heritability for disease risk there is little evidence suggesting the same for agranulocytosis.

Rare sequence variants conferring high-risk of agranulocytosis and for clozapine use in schizophrenia

1. Reported rare variants predictive for clozapine use in schizophrenia

Currently the only rare variants, conferring modest risk and recommended for individual genotyping are those reported by the CIAC consortium[4]. Although replicated in CRESTAR samples, the odds ratios (ORs) for the associated variants don't suggest immediate clinical application in predictive testing.

2. A search for rare variants predictive for clozapine use in schizophrenia

CRESTAR has assembled the largest Agran/neutropenia (re-challenged) cohort to date (265 cases). All investigators in the area were contacted and samples from two consortia, CIAC and CLOZUK, were included.

deCODE has whole genome sequenced (WGS) the 265 agran samples and 265 matched (age, gender and ethnicity) control samples from treatment resistant schizophrenia patients. The clozapine-treated controls had received clozapine for over two years with no documented ANC < 1,500mm^{-3} and no medical condition with an increased risk of agranulocytosis. The large sample was sequenced to a dipath of 30X as described in more detail below (i-iv).

i) Whole-genome sequencing sample preparation

Paired-end libraries for sequencing were prepared according to the manufacturer's instructions (Illumina, TruSeq™). In short, approximately 1 µg of genomic DNA, isolated from frozen blood samples, was fragmented to a mean target size of 300 bp using a Covaris E210 instrument. The resulting fragmented DNA was end repaired using T4 and Klenow polymerases and T4 polynucleotide kinase with 10 mM dNTP followed by addition of an 'A' base at the ends using Klenow exo fragment (3' to 5'-exo minus) and dATP (1 mM). Sequencing adaptors containing 'T' overhangs were ligated to the DNA products followed by agarose (2%) gel electrophoresis. Fragments of about 400–500 bp were isolated from the gels (QIAGEN Gel Extraction Kit), and the adaptor-modified DNA fragments were PCR (Polymerase Chain Reaction) enriched for ten cycles using Phusion DNA polymerase (Finnzymes Oy) and a PCR primer cocktail (Illumina). Enriched libraries were further purified using AMPure XP beads (Beckman-Coulter). The quality and concentration of the libraries were assessed with the Agilent 2100 Bioanalyzer using the DNA 1000 LabChip (Agilent). Barcoded libraries were stored at −20 °C. All steps in the workflow were monitored using an in-house laboratory information management system with barcode tracking of all samples and reagents.

ii) Whole-genome sequencing (WGS)

Template DNA fragments were hybridized to the surface of flow cells (GA PE cluster kit (v2) or HiSeq PE cluster kits (v2.5 or v3)) and amplified to form clusters using the Illumina cBot. In brief, DNA (2.5–12 pM) was denatured, followed by hybridization to grafted adaptors on the flow cell. Isothermal bridge amplification using Phusion polymerase was then followed by linearization of the bridged DNA, denaturation, blocking of 3' ends and hybridization of the sequencing primer. Sequencing-by-synthesis (SBS) was performed on Illumina GAIIx and/or HiSeq 2000 instruments. Paired-end libraries were sequenced at 2×101 (HiSeq) or 2×120 (GAIIx) cycles of incorporation and imaging using the appropriate TruSeq™ SBS kits. Each library or sample was initially run on a single GAIIx lane for QC validation followed by further sequencing on either GAIIx (≥4 lanes) or HiSeq (≥1 lane) with targeted raw cluster densities of 500–800 k mm−2, depending on the version of the data imaging and analysis packages (SCS2.6-2-9/RTA1.6–1.9, HCS1.3.8–1.4.8/RTA1.10.36–1.12.4.2). Real-time analysis involved conversion of image data to base-calling in real-time.

iii) Whole-genome alignment

Reads were aligned to NCBI Build 36 (hg18) of the human reference sequence using Burrows-Wheeler Aligner (BWA) 0.5.7–0.5.9. Alignments were merged into a single BAM file and marked for duplicates using Picard 1.55 (http://picard.sourceforge.net/). Only non-duplicate reads were used for the downstream analyses. Resulting BAM files were realigned and recalibrated using GATK version 1.2–29-g0acaf2d.

iv) Whole-genome SNP and INDEL calling

Multi-sample calling was performed with GATK version 2.3.9 using all the available BAM files at deCODE together.
Genotype calls made solely on the basis of next generation sequence data yield errors at a rate that decreases as a function of sequencing depth. Thus, for example, if sequence reads at a heterozygous SNP position carry one copy of the alternative allele and seven copies of the reference allele, then without further information the genotype would be called homozygous for the reference allele. To minimize the number of such errors, we used information about haplotype sharing in the Icelandic sample, taking advantage of the fact that all the sequenced individuals had also been chip-typed and long range phased. This effort helped identifying reliable calls in the out-bread CRESTAR sample.

Analyses of the WGS data have this far not resulted in identification of high-impact variants for clozapine related traits. Table 1 contains the most promising signals from whole-genome sequencing of 530 treatment resistant schizophrenia patients. Analysis will be continued and most promising signals tested for association in a replication samples. Unfortunately high-impact sequence variants predictive for clozapine use in schizophrenia have not been uncovered. Hence, neither previously reported associations nor variants uncovered though CRESTAR efforts suggest immediate clinical application in predictive testing.

Analyses of the WGS data have this far not resulted in unequivocal identification of high-impact variants for clozapine related traits. *WP10 Table 1* contains the most promising signals from whole-genome sequencing of 530 treatment resistant schizophrenia patients. Analysis will be continued and most promising signals tested for association in a replication samples. Unfortunately high-impact sequence variants predictive for clozapine use in schizophrenia have not been uncovered. Hence, neither previously reported associations nor variants uncovered though Crestar efforts suggest immediate clinical application in predictive testing. The variant in the UHRF1 gene is though promising and has to be tested in a replication sample. The associated allele, chr19:4944193_G, was only found in WGS agran cases and none of the controls. The protein UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1) is required for maintaining DNA methylation. Epigenetic inheritance in mammals relies in part on robust propagation of DNA methylation patterns throughout development. UHRF1 colocalizes with the maintenance DNA methyltransferase protein DNMT1 throughout S phase. UHRF1 appears to tether DNMT1 to chromatin through its direct interaction with DNMT1. UHRF1 contains a methyl DNA binding domain, the SRA (SET and RING associated) domain, that shows strong preferential binding to hemimethylated CG sites, the physiological substrate for DNMT1. It has been suggested that UHRF1 may help recruit DNMT1 to hemimethylated DNA to facilitate faithful maintenance of DNA methylation5. This is particularly interesting since clozapine but not haloperidol or olanzapine activate brain DNA demethylation6.

WP10 - Table 1. *Association results from the whole genome sequencing of samples from 265 agran/re-challenged neutropenia subjects and 265 control subjects. The clozapine-treated controls had received clozapine for over 1 years with no documented ANC < 1,500mm-3 and no medical condition with an increased risk of agranulocytosis. The large sample was sequenced to a depth of 30X.*

Variant	*P*-value	Impact	Gene
chr3:36857500_T_C	0.000277596	MODERATE	TRANK1
chr14:95204467_C_CA	0.000580717	MODERATE	CLMN
chr4:190082345_C_T	0.000856616	MODERATE	DUX4L4
chr15:42730965_G_C	0.002854241	MODERATE	CDAN1
chr16:69963025_G_A	0.002854241	MODERATE	CLEC18A
chr2:107861192_A_G	0.002854241	HIGH	RGPD4
chr6:29943450_G_C	0.002967966	MODERATE	HLA-A
chr9:15978_C_T	0.003471871	MODERATE	WASH1
chr19:4944193_G_A	0.003709921	MODERATE	UHRF1
chr16:768452_G_A	0.004631574	MODERATE	MSLN
chr16:90011614_T_C	0.005085894	HIGH	DBNDD1
chr6:29943334_ACC_A	0.005346416	HIGH	HLA-A
chr19:39886558_C_G	0.005919625	MODERATE	FCGBP
chr11:5454368_G_A	0.005989688	MODERATE	OR51I2
chr11:62984380_G_A	0.005989688	MODERATE	SLC22A6
chr14:58396333_G_A	0.005989688	MODERATE	TOMM20L
chr14:91297376_G_A	0.005989688	MODERATE	CCDC88C
chr19:21423588_G_A	0.005989688	MODERATE	ZNF493
chr6:32584351_TCCC_T	0.005989688	MODERATE	HLA-DRB1
chr9:132670608_G_A	0.005989688	MODERATE	GTF3C4

In sum WP10 assembled, genotyped and whole genome sequenced DNA from an unprecedented large sample of treatment resistant schizophrenia patients. We evaluated the impact of the confluence of common alleles on: disease, disease related phenotypes and adverse drug reaction (ADRs) including clozapine-induced agranulocytosis. While we also searched for rare, high impact sequence variants associated with the same aiming for uncovering variants useful for diagnostics, those were not uncovered yet because there do not appear to be any single genetic variants of large effect. Nevertheless and most importantly we produced a wealth of data which will continue to contribute value to the development of predictive tests for clozapine treatment and safety.

References:

1 Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421-427, doi:10.1038/nature13595 (2014).

2 Power, R. A. *et al.* Polygenic risk scores for schizophrenia and bipolar disorder predict creativity. *Nat Neurosci* **18**, 953-+, doi:10.1038/nn.4040 (2015).

3 Niamh Mullins, J. E., Andrés Ingason, Heather Porter, Alexandra Gillette, Sigurgeir Ólafsson, Daniel F. Gudbjartsson, Cathryn M. Lewis, Engilbert Sigurdsson, Agnar Helgason, G. Bragi Walters, Omar Gustafsson, Hreinn Stefansson, Kari Stefansson. Genetic risk for psychiatric disorders and reproductive fitness in the general population. *Submitted manuscript* (2015).

4 Goldstein, J. I. *et al.* Clozapine-induced agranulocytosis is associated with rare HLA-DQB1 and HLA-B alleles. *Nature communications* **5**, 4757, doi:10.1038/ncomms5757 (2014).

5 Bostick, M. *et al.* UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* **317**, 1760-1764, doi:10.1126/science.1147939 (2007).

6 Dong, E., Nelson, M., Grayson, D. R., Costa, E. & Guidotti, A. Clozapine and sulpiride but not haloperidol or olanzapine activate brain DNA demethylation. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 13614-13619, doi:10.1073/pnas.0805493105 (2008).

4 The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

4.1 Socio-economic impact and the wider societal implications of CRESTAR

4.1.1 Background

In European and other similarly economically developed countries, patients with schizophrenia die 12 to 25 years prematurely (Crump et al., 2013; Fazel et al., 2015) .This reduced life expectancy is thought to be caused by a combination of increased smoking rates, increased rates of obesity and diabetes, suicides and accidental deaths (Olfson et al., 2015; Davis et al., 2015). Studies from Finland and UK demonstrate that treatment with clozapine is associated with a reduction in mortality rates compared to treatment with other antipsychotics and also compare to no treatment, and that family contact is important in reducing mortality (De Hert et al., 2010; Hayes et al., 2015; Reininghaus et al., 2015).

Using a summary measure of population health, called the disability-adjusted life year or DALY (a time-based measure that combines in a single indicator years of life lost from premature death and years of life lived with a disability), the recent estimates from the Global Burden of Disease study indicate that neuropsychiatric disorders contribute to more than 10% of lost years of healthy life and over 30% of all years lived with disability (WHO: The world health report 2001. Mental health: new understanding, new hope.). Globally, neuropsychiatric disorders account for 168,304,000 DALYs, and of that schizophrenia accounts for 11,642,000.

Evidence gathered during the course of the CRESTAR collaboration has demonstrated **that treatment resistant schizophrenia has a very high economic burden**. This is estimated to be between three and 11 times greater than the economic burden of treatment responsive schizophrenia. The work conducted in WP8 estimated a threefold increase. Treatment resistant schizophrenia is estimated to consume US$35 billion indirect healthcare costs alone. It is estimated that this translates to approximately 35 billion euros in the European economic Area. In CRESTAR we also showed that **patients taking clozapine have a 50% lower risk for all-cause mortality.** Furthermore, we found that **people with schizophrenia had high endogenous risk for type 2 diabetes, further increased by antipsychotic treatment,** and that the increased type 2 diabetes found in patients with schizophrenia **is mediated by the weight gain caused by clozapine use.** We also found one gene (SPTA1) **conferring a 15-fold increase in risk for agranulocytosis.**

At the only evidence based treatment for treatment refractory schizophrenia, the correct and timely use of clozapine in patients who will benefit from it, while minimising the risk of adverse effects, will be the best way to reduce the burden of treatment resistance in schizophrenia.

Taken together with our discovery of genetic biomarkers for TRS, we expect the findings of CRESTAR to have long term public health benefit by reducing mortality in schziophenia, through making clozapine safer and more accessible.

References:

Olfson M, Gerhard T, Huang C, Crystal S, Stroup TS. Premature Mortality Among Adults With Schizophrenia in the United States. JAMA Psychiatry. 2015 Dec 1;72(12):1172-81. doi: 10.1001/jamapsychiatry.2015.1737.

Davis WA, Starkstein SE, Bruce DG, Davis TM. The interactive effects of type 2 diabetes mellitus and schizophrenia on all-cause mortality: The Fremantle Diabetes Study. J Diabetes Complications. 2015 Nov-Dec;29(8):1320-2. doi: 10.1016/j.jdiacomp.2015.08.019. Epub 2015 Aug 28.

Crump C, Winkleby MA, Sundquist K, Sundquist J. Comorbidities and mortality in persons with schizophrenia: a Swedish national cohort study. Am J Psychiatry. 2013 Mar;170(3):324-33. doi: 10.1176/appi.ajp.2012.12050599.

De Hert M, Correll CU, Cohen D. Do antipsychotic medications reduce or increase mortality in schizophrenia? A critical appraisal of the FIN-11 study. Schizophr Res. 2010 Mar;117(1):68-74. doi: 10.1016/j.schres.2009.12.029. Epub 2010 Jan 12.

Fazel S, Wolf A, Palm C, Lichtenstein P. Violent crime, suicide, and premature mortality in patients with schizophrenia and related disorders: a 38-year total population study in Sweden. Lancet Psychiatry. 2014 Jun;1(1):44-54.

4.1.2 Impacts on treatment of schizophrenia and clinical trial design

The results of the study have paved the way for the introduction of stratified medicine schizophrenia. In future, we expect that initially patients will undergo a clinical and/or genetic tests, either at the onset illness or following the first failure on an antipsychotic, and in future indeed undergo routine genotyping or genome sequencing as a routine part of their health care. This will then determine whether the patient should be treated with a standard antipsychotic or fast tracked to clozapine. This new algorithm will help to minimise the 4-5 year delay that currently occurs before patients are initiated on clozapine. Work done as part of WP9 has shown that clinicians are very receptive to this approach and would be willing to use genetic testing in making treatment choices for their patients. Service users themselves were surveyed by members of the CRESTAR team as part of a related project, STRATA, and also have a positive attitude to this approach.

The selection of participants for clinical trials of treatment resistant schizophrenia will be more accurate as a result of this work. For example, it will be possible to select groups of individuals with a genetic predisposition to treatment resistant schizophrenia to undertake trials of alternative treatments other than clozapine. It will also be possible to select patients who are more likely to be treatment responsive, to maximise power and minimise costs of clinical trials. This will have the added benefit of ensuring that patients who are unlikely to respond to antipsychotics are not needlessly exposed to adverse effects as part of the clinical trial. The risk of specific adverse effects could also be minimised by screening out any patients with genetic risk factors for these adverse events.

4.1.3 Impacts on economic activity in the European economic area

CRESTAR includes two SMEs, deCODE and Leyden Delta. DeCODE and other genetic testing companies will benefit from the data and knowledge gathered by the consortium. By advancing the field of stratified medicine the project will have wider benefits to diagnostics companies in the European economic area and beyond. The adoption of personalised medicine with regard to clozapine will benefit the manufacturers of clozapine including Leyden Delta. More generally, the dissemination activities of CRESTAR will raise awareness of clozapine and improve the uptake of this treatment.

4.1.4 Impacts on health economics

We expect CRESTAR to have a significant health economic impact, i.e. reducing healthcare costs through the better treatment of schizophrenia, and a social impact, i.e. reducing the burden on partners, relatives and carers. The figures for the impact of schizophrenia on society are stark: In economically developed countries, people with schizophrenia die 12–25 years prematurely, from **preventable diseases** such as smoking-related cancers and lung disease, cardiovascular and type 2 diabetes and its complications. CRESTAR has given us a greater biological understanding of the relationship between these disorders and schizophrenia which will inform clinical practice on these areas and will have its impact by increasing the uptake and early use of clozapine, thus minimising the mortality gap in schizophrenia. Improvements in drug safety will bring health economic benefits, by reducing the incidence of severe and chronic ADRs and thus the cost associated with hospitalisation and augmentary treatment, for example for agranulocytosis and type 2 diabetes.

For example, because of CRESTAR we now know that **patients with schizophrenia are more vulnerable to type 2 diabetes**, and this risk is increased by **weight gain caused by clozapine treatment**. This indicates a clear way forward in reducing mortality in schizophrenia: early and more aggressive treatment of metabolic disorder combined with behavioural management aimed at improving diet and exercise, and reducing smoking. Likewise we have identified genetic risk factors for agranulocytosis and replicated previous finding, bringing us much closer to a **genetic test to make clozapine a safer drug so that patients can realise its benefits.** Thus, for time-varying clozapine exposure (non-clozapine-exposed periods as reference) the risk for all-cause mortality was decreased by 50%, and by 44% for deliberate self-harm. **The decreased rate of deliberate self-harm during clozapine use may suggest a potential pathway of effect of clozapine in the prevention of deaths in patients with treatment-resistant schizophrenia.**

CRESTAR's health economic modelling showed that the expected annual cost of the schizophrenia population much higher for TRS cases then treatment responsive cases, and although a genetic test for response would increase costs, it would be more effective in improving quality of life. Health economic impacts will include reducing the need for hospitalisation (inpatient stays) by optimising and advancing the treatment for refractory schizophrenics. Thus, patients who need the benefits of schizophrenia can be given the drug in weeks or months instead of years (the mean time to clozapine is over 5 years; this could be reduced to months for patients who would benefit from it, reducing the duration of unsuccessfully treated illness and therefore their need for inpatient stays and other interventions such as social care. The overall healthcare cost of antipsychotic medication will be decreased both improving and reducing the costs of therapeutic drug monitoring in schizophrenia (for example by pharmacogenetic risk assessment and point of care monitoring), especially with respect to haematological monitoring, and also allowing more widespread use of a relatively cost effective drug, clozapine.

In the conduct of clinical trials for antipsychotic medication, costs will be saved by stratifying patients according to illness type, or likelihood of treatment resistance or the development of ADRs, with consequent reductions in the size, duration and adverse effect burden of clinical trials.

By reducing the overall economic impact of schizophrenia on society, timely treatment with the correct antipsychotic will reduce the need for state benefits and social care, increasing the employment levels in people with schizophrenia, and increasing life-expectancy, especially productive years of life.

4.1.5 Impacts on schizophrenia patients and their families

CRESTAR will have significant impacts on schizophrenia patients and their families by making clozapine safer and more accessible which will lead to:

- **Guidance that will improve the physical health of patients**, i.e. the knowledge that patients are more vulnerable to type 2 diabetes and at further risk from clozapine-induced weight gain, so that their vulnerability can be effectively managed.
- **Reduction in the rates of suicide and self-harm**, which are severely traumatising and disrupting for patients and carers.
- **Improving overall quality of life**, including economic life such as the ability to work, and to have fulfilling relationships with family, friends and partners,
- **Making clozapine safer**, reducing the risk of severe adverse events and the need for so many blood tests.
- **Removing stigma**, through education and dissemination.

CRESTAR has sparked new collaborations with users and carers groups. In work following on from CRESTAR we have conducted focus groups on stratified medicine in four centres, spreading awareness of the potential of personalised medicine. Our dissemination activities have hopefully made a substantial contribution in reducing the stigma toward subjects suffering from schizophrenia and their families, and improving quality of life for schizophrenic patients, their partners, relatives and carers. In particular, by reducing disability caused by schizophrenia, this will improve quality of life for people affected by this illness, including carers and individual patients, though increasing economic and social wellbeing.

4.1.6 Impacts on the understanding of the aetiology of schizophrenia

Our work with CRESTAR has added weight to the hypothesis that treatment refractory schizophrenia is a biologically distinct entity, with different risk factors than treatment responsive schizophrenia. The development of the first polygenic risk score for treatment resistant schizophrenia (WP2) is a landmark achievement – and the fact that we have discovered loci that are not implicated in treatment responsive schizophrenia is especially important in this regard.

We are sharing our CRESTAR GWAS data from schizophrenia patients who are responders and non-responders to clozapine, so that these can be incorporated into meta- and mega- GWAs analysis, through the Psychiatric Genetics Consortium (PGC).

Our greater than expected sample sizes, for treatment resistant patients and for neutropenia, have further increased this contribution. Another outcome of the consortium is the increasing appreciation of the role of the glutamate system in schizophrenia. Data from CRESTAR has contributed to the development of new methods of gene pathway analysis, which have uncovered an excess of abnormalities in glutamate systems in people with treatment refractory schizophrenia. Thus has improved our understanding not only of the mechanisms underlying treatment resistance, but in the understanding of schizophrenia more generally.

4.1.7 Impacts on European Networks working on schizophrenia research

CRESTAR had and continuously has impact on the scientific community in schizophrenia research through wide-spread dissemination activities but by CRESTAR researchers actively contributing to international projects and networks. CRESTAR has established collaborations with US investigators looking at the genetics of agranulocytosis (Pat Sullivan, CIAC consortium) and Bio-X Shanghai (Yongyong Shi) as well as cooperation with the US Psychiatric Genomics Consortium (Mark Daly, Mick O'Donovan and Pat Sullivan). We have been undertaking joint genetic analysis in collaboration with these groups and will continue to do so including meta-analysis and cross replication of agranulocytosis risk and understanding how risk of TRS overlaps with general schizophrenia risk.

In addition CRESTAR researchers work closely with the EU-funded OPTiMiSE project, which is working on generating a scientifically-validated decision tree that could inform clinical decisions and improve outcomes for patients. The OPTiMiSE project was extended. Hence, they are not ready yet with their results and the predicative ability of decision making in OPTiMiSE trial data can therefore not be evaluated. However we will proceed with this analysis after the end of the CRESTAR project and as soon as the OPTiMiSE data is ready for collaboration.

Moreover, a formal partnership with the iPsych consortium has been established which was not envisaged at the start if the consortium. This has been very important as it has allowed CRESTAR to obtain high quality clinical and genetic data in the same patients simultaneously.

During the course of CRESTAR, several significant and important links have been established internationally. The most significant new network to have been formed is STRATA. This UK-wide consortium is funded by the UK medical research council and co-led by James MacCabe, the project coordinator for CRESTAR. The Consortium board is co-chaired by David Collier, the scientific coordinator of CRESTAR. The consortium also included James Walters and Michael O'Donovan from Cardiff University. This consortium aims to continue the work on stratified medicine in schizophrenia and will build on the results of CRESTAR over the next four years, culminating in a clinical trial of stratified medicine on schizophrenia.

4.2 The main dissemination activities of CRESTAR

Organisation of conferences, audience: scientific community			
Lead	**Title**	**Date**	**Place**
KCL	Demographic and clinical characteristics prior to clozapine initiation in early onset schizophrenia	21.04.2013	Jerusalem, Israel
LMU	Genetics of schizophrenia	26.04.2012	Kassel, Germany
LMU	Pro-Con-Debate: How useful is genetics and pharmacogenetics in drug discovery & response prediction	03.06.2012	Stockholm, Sweden
LMU	Pharmacogenomics and personalised medicine in psychiatry: Prospects for clinical implementation	03.06.2012	Stockholm, Sweden
LMU	Symposium des Referates Genetik & Neurobiologie der Deutschen Gesellschaft für Biologische Psychiatrie	14.09.2012	Heidelberg, Germany
LMU	The Contribution of Genetics to Drug Development and Personalized Medicine	01.10.2012	Munich, Germany
LMU	Wo steht die Genetik psychischer Erkrankungen?	22.11.2012	Berlin, Germany

Organisation of conferences, audience: scientific community			
Lead	**Title**	**Date**	**Place**
LMU	Genetics of psychosis	14.02.2013	Marrakesh, Marocco
LMU	Genetics of schizophrenia	11.04.2013	Bucharest, Romania
Decode	The genetics of common diseases	11.11.2015	Umea, Sweden

Organisation of workshops, audience: scientific community			
Lead	**Title**	**Date**	**Place**
LMU	Genetics of schizophrenia and intermediate phenotypes	24.04.2012	Rostock, Germany
LMU	Genetics of schizophrenia and intermediate phenotypes	03.07.2012	Dresden, Germany
LMU	Bedeutung von Erbfaktoren für die Entwicklung psychiatrischer Erkrankungen a.B. der Schizophrenie	11.07.2012	Magdeburg, Germany
LMU	Genetics of schizophrenia and intermediate phenotypes	17.07.2012	Ulm, Germany
LMU	Genetics of schizophrenia and intermediate phenotypes	19.10.2012	Halle, Germany
MLU	Crestar: Personalized medicine in schizophrenia - Development of pharmacogenomics biomarkers	31.03.2015	Vienna, Austria
MLU	Genetics of schizophrenia and intermediate phenotypes	15.06.2015	Athens, Greece
MLU	Genome wide association studies for typical antipsychotics	21.09.2015	Copenhagen, Denmark
MLU	PIP: Pharmacogenomics of agranulocytosis	15.10.2015	Toronto, Canada
MLU	WCPG: Pharmacogenomics of agranulocytosis	17.10.2015	Toronto, Canada

Organisation of workshops, audience: civil society			
Lead	**Title**	**Date**	**Place**
LMU	Genetics of psychiatric diseases	27.10.2012	Halle, Germany
LMU	Psychose	20.02.2013	Halle, Germany

Oral presentations, audience: scientific community			
Lead	**Title**	**Date**	**Place**
LHS	Treatment of refractory schizophrenia with clozapine in Iceland	27.09.2014	Akureyri, Iceland
LHS	Treatment of refractory schizophrenia with clozapine in Iceland - and new guidelines on follow up for patients on antipsychotic medication. Development of a semi-automated flagging system for outpatients with long-term severe mental illness	07.02.2014	Reykjavík, Iceland
MLU	Genetics of schizophrenia and intermediate phenotypes	03.05.2013	Munich, Germany
MLU	Genetics of schizophrenia	04.05.2013	Copenhagen, Denmark
MLU	Genetics of schizophrenia and intermediate phenotypes	23.05.2013	Desssau, Germany
MLU	Genetics of psychosis	31.05.2013	Bonn, Germany
MLU	Genetik psychiatrischer Erkrankungen	06.06.2013	Halle, Germany
MLU	Plenary: Genetics of Schizophrenia	13.06.2013	Luhacovice, Czech Republic
MLU	Pro Con Debate: Genetics of Psychiatric Diseases	25.06.2013	Kyoto, Japan
MLU	Genome wide association studies in schizophrenia	26.06.2013	Kyoto, Japan
MLU	Metabolomics of Schizophrenia	03.07.2013	Glasgow, Scottland
MLU	Schizophrenia, Cognition and Genetics	27.08.2013	Magdeburg, Germany
MLU	Genetik der Schizophrenie und intermediärer Phänotypen	14.09.2013	Jena, Germany

Oral presentations, audience: scientific community			
Lead	**Title**	**Date**	**Place**
MLU	Genetics of psychiatry	17.09.2013	Vienna, Austria
MLU	Pharmakologie der Schizophrenie	18.09.2013	Munich, Germany
MLU	Systemische Genetik der Schizophrenie	20.09.2013	Munich, Germany
MLU	Neue Erkenntnisse zur Pharmakogenetik der Schizophrenie	20.09.2013	Munich, Germany
MLU	Genetics of psychiatry	23.09.2013	Hohenkammern, Germany
MLU	Brain networks	06.10.2013	Barcelona, Spain
MLU	Biomarkers of Disease Risk and Treatment Response	18.10.2013	Boston, USA
MLU	Gene expression analysis of psychiatric disorders	08.11.2013	San Franscisco, USA
MLU	Animal Model of Schizophrenia	28.11.2013	Berlin, Germany
MLU	Genetic of schizophrenia	29.11.2013	Berlin, Germany
MLU	CNVs in Schizophrenia	30.11.2013	Berlin, Germany
MLU	Genetics of schizophrenia and intermediate phenotypes	21.01.2014	Irvine, USA
MLU	New results on copy number variants in schizophrenia	02.03.2014	Munich, Germany
MLU	Pro & Con Debate: Did Genetics in Psychiatry Hold its Promise?	03.03.2014	Munich, Germany
MLU	Genetics of psychiatric disorders	18.09.2014	Aarhus, Denmark
MLU	Genetik der Schizophrenie und intermediärer Phänotypen	27.09.2014	Aachen, Germany
MLU	New genetic findings in schizophrenia	19.10.2014	Berlin, Germany
KCL	Clozapine Therapeutic Drug Monitoring	28.06.2014	London
MLU	Personalized medicine in psychiatry	09.04.2014	Florence, Italy
MLU	SESSION II: Update on CRESTAR project	15.06.2014	Hollywood, Florida, USA
MLU	Pharmacogenetics in Psychiatry	24.06.2014	Vancouver, Canada
AU	Predictors of treatment resistant schizophrenia	28.08.2013	Montreal, Canada
AU	Predictors of treatment resistant schizophrenia	12.08.2013	Aarhus, Denmark
AU	Epidemiology of clozapine use in Denmark	27.08.2014	Groningen, Netherlands
AU	Epidemiology of clozapine use in Denmark	23.10.2014	Taipei, Taiwan
AU	Epidemiology of Treatment resistant schizophrenia	11.04.2014	Florence, Italy
AU	Comparative effectiveness and safety of antipsychotics in combination with NSAIDs or paracetamol	27.08.2014	Taipei, Taiwan
AU	Comparative effectiveness and safety of antipsychotics in combination with NSAIDs or paracetamol	27.08.2014	Groningen, Netherlands
CU	Genome wide association study of treatment resistant schizophrenia	18.10.2013	Boston, USA
CU	Genetic basis of treatment resistant schizophrenia	08.04.2014	Florence, Italy
CU	Genetic basis of treatment resistant schizophrenia	07.04.2014	Florence, Italy
MLU	Genetik der Schizophrenie und intermediärer Phänotypen	12.11.2014	Ulm, Germany
MLU	Kognitive Endophänotypen in der Schizophrenie	28.11.2014	Berlin, Germany
MLU	Genetik der Schizophrenie und intermediärer Phänotypen	22.01.2015	Bern, Switzerland
MLU	Genetik der Schizophrenie und intermediärer Phänotypen	27.01.2015	Mannheim, Germany
MLU	Genetik psychiatrischer Erkrankungen	05.09.2015	Leipzig, Germany
MLU	Glutamate in schizophrenia	25.09.2015	Munich, Germany
CU	GWAS in 11000 clozapine cases	17.10.2015	Toronto, Canada
CU	The Genetics of Treatment Resistant Schizophrenia	15.06.2014	Florida, USA

Oral presentations, audience: scientific community			
Lead	**Title**	**Date**	**Place**
CU	Comparison of Model-Based Estimators of Population Structure in a GWAS Framework	15.10.2014	Copenhagen, Denmark
CU	Glutamate and immune System Genetics in Schizophrenia	27.03.2015	Colorado Springs, USA
CU	Comprehensive genetic analysis implicates novel mechanisms for clozapine-associated neutropenia	20.10.2015	Toronto, Canada

Posters, audience: scientific community			
Lead	**Title**	**Date**	**Place**
KCL	Pharmacogenomic biomarkers as clinical decision making tools for clozapine treatment of schizophrenia	15.10.2014	Copenhagen, Denmark
CIMH	Genetic and Non-Genetic Risk Factors for Treatment Resistant Schizophrenia	21.10.2013	Boston, MA, USA
CU	Exome array analysis of clozapine-associated neutropenia	14.10.2014	Copenhagen, Denmark
CU	Comprehensive genetic analysis implicates novel mechanisms for clozapine-associated neutropenia	15.10.2015	Toronto, Canada
KCL	Pharmacogenomic biomarkers as clinical decision making tools for clozapine treatment of schizophrenia	15.10.2014	Copenhagen, Denmark
KCL	Establishing the characteristics of an effective pharmacogenetic test for clozapine-induced agranulocytosis	18.10.2015	Toronto, Canada
KCL	Pharmacogenomic biomarkers as clinical decision making tools for clozapine treatment of schizophrenia	15.10.2014	Copenhagen, Denmark

Flyers, audience: scientific community			
Lead	**Title**	**Date**	**Place**
LMU	Flyer to introduce the project	01.05.2013	Halle, Germany
CIMH	Flyer to introduce the project in German		Mannheim, Germany
LMU	Flyer to promote CRESTAR dissemination event in Toronto, Canada: Pharmacogenomics of agranulocytosis	15.10.2015	Halle, Germany

Publications, audience: scientific community

1. C. Schneider et al. (2014). *Systematic review of the efficacy and tolerability of Clozapine in the treatment of youth with early onset schizophrenia*. In: Elsevier Masson SAS, European Psychiatry, Vol. 29/Issue 1, Italy, p. 1-10. doi: http://dx.doi.org/10.1016/j.eurpsy.2013.08.001
2. C. Schneider et al. (2014). *Antipsychotics use in children and adolescents: An on-going challenge in clinical practice*. In: SAGE Publications Ltd., Journal of Psychopharmacology, Vol. 28/Issue 7, United Kingdom, p. 615-623. doi: 10.1177/0269881114533599.
3. J. Frank et al. (2014). *Identification of increased genetic risk scores for schizophrenia in treatment-resistant patients*. In: Nature Publishing Group, Molecular Psychiatry, 2014, United Kingdom, p. 1-2. doi: 10.1038/mp.2014.56.
4. T. Wimberley et al. (2014). *Poster #S251 PROGNOSTIC FACTORS FOR TREATMENT RESISTANCE IN SCHIZOPHRENIA*. In: Elsevier, Schizophrenia Research, Vol. 153, p. 180-181. doi: http://dx.doi.org/10.1016/S0920-9964(14)70530-4.
5. O. Donovan et al. (2014). *Biological insights from 108 schizophrenia-associated genetic loci*. In: Nature Publishing Group, Nature, Vol. 511, p. 421-427. doi: 10.1038/nature13595.
6. B. Spencer et al. (2013). *Opening Pandora's box in the UK: a hypothetical pharmacogenetic test for clozapine*. In: Future Medicine Ltd., Pharmacogenomics, Vol. 14/Issue 15, United Kingdom, p. 1907-1914. doi: 10.2217/pgs.13.182.
7. S. M. Meier et al. (2015). *High loading of polygenic risk in cases with chronic schizophrenia*. In: Nature Publishing Group, Molecular Psychiatry, 01. Sep 15, United Kingdom. doi: 10.1038/mp.2015.130.

8. C. Schneider et al. (2015). *Clozapine use in childhood and adolescent schizophrenia: A nationwide population-based study*. In: Elsevier, European Neuropsychopharmacology, Vol. 25/Issue 6, Netherlands, p. 857-863. doi: http://dx.doi.org/10.1016/j.euroneuro.2015.02.003.
9. M. Verbelen et al. (2015). *Establishing the characteristics of an effective pharmacogenetic test for clozapine-induced agranulocytosis*. In: Nature Publishing Group, Pharmacogenomics Journal, Vol. 15/Issue 5, United Kingdom, p. 461-466. doi: 10.1038/tpj.2015.5.
10. M. Verbelen et al. (2015). *How close are we to a pharmacogenomic test for clozapine-induced agranulocytosis?* In: Future Medicine Ltd., Pharmacogenomics, Vol. 16/Issue 9, United Kingdom, p. 915-917. doi: 10.2217/pgs.15.67.

Submitted manuscripts, audience: scientific community

11. J.E. Niamh Mullins et al. (2015). *Genetic risk for psychiatric disorders and reproductive fitness in the general population*. Submitted to: Nature genetics. Manuscript reference number: NG-BC42375 Stefansson.
12. O. Ingimarsson (2015). *Clozapine treatment and discontinuation in Iceland: a national longitudinal study using electronic patient records*. Submitted to: Nordic Journal of Psychiatry. D.O.I. not yet available.
13. O. Ingimarsson (2015). *Neutropenia and agranulocytosis during treatment with clozapine and other antipsychotics in a well-described schizophrenia sample in Iceland*. Submitted to: Acta Psychiatrica Scandinavica. D.O.I. not yet available.

4.3 Exploitation of results of CRESTAR

The main goal was to develop a market-ready custom array based genetic tests for clozapine therapy. First, the CRESTAR team defined algorithms for use in risk prediction. Second, we collected data to allow the development of a commercially-viable array-based pharmacogenetic tests for clozapine's effects. We have also identified methods by which genetic data can be analysed along with environmental data to better predict risks and outcomes.

In the final stage of CRESTAR we evaluated the development of a test which may prove a cost effective means of reducing the economic cost of treatment and improving quality of life for the patient. Although schizophrenia polygenic risk scores were higher and predictive for groups of individuals (treatment resistant, agranulocytosis etc.) the effects are small and taken together neither the odds ratios for associated variants, individually or *en masse*, suggest immediate clinical application in predictive testing, because the positive and negative predictive values of the tests derived from this data would not yet be clinically useful.

Hence, a clinical genetic test will not be marketed based on CRESTAR findings although: 1) assays for variants conferring highest risk of agranulocytosis have been validated and 2) a user friendly web interface to allow physicians and research investigators to enter information on subjects, which samples are being shipped to deCODE for analysis has been developed.

Nevertheless, the CRESTAR contribution, predictions of clozapine ADRs, using PRS, is a milestone in the search for useful diagnostic variants for clozapine therapy.

5 The address of the project public website, if applicable as well as relevant contact details

http://www.crestar-project.eu/

Please see Annex A for project logo.

Annex A: CRESTAR project logo

Annex B: List of CRESTAR partners with contact names

Nr.	Legal entity (acronym)	Contact	Comment
01	King's College London (KCL)	**James MacCabe**	Coordinating institution
02	Islensk Erfdagreining EHF (Decode)	Hreinn Stefansson	
03	Landspitali University Hospital (LHS)	Engilbert Sigurdsson	
04	Ludwig-Maximilians-Universität München (LMU)	n/a	Termination: 31 March 2012
05	Cardiff University (CU)	James Walters	
06	Aarhus Universitet (AU)	Preben Mortensen, Christiane Gasse	
07	Zentralinstitut für Seelische Gesundheit (CIMH)	Marcella Rietschel	
08	concentris research management GmbH (concentris)	Ameli Schwalber, Barbara Heißerer	
09	Leyden Delta BV (Leyden Delta)	Karel Joli	
10	Martin Luther Universität Halle (MLU)	Dan Rujescu	Accession: 1 January 2013
11	Eli Lilly and Company ltd. (Lilly)	**David Collier**	Scientific coordinator, Accession: 1 May 2012