Maxillary first molar extraction for orthodontic treatment
Hans Booij, Christos Katsaros, Anne Marie Kuijpers-Jagtman, Piotr Fudalej, Christos Livas
This thesis aims to study the orthodontic treatment results in patients with a Class II malocclusion (n=100) after extraction of the first upper molars. The quality of the treatment results, the influence on facial profile as well as the effect on the eruption of the upper third molars are some of the aspects that will be analysed.

Biomechanical behaviour of the tooth and surrounding structures during orthodontic force application
Solla Jónsdóttir, Jaap Maltha, Anne Marie Kuijpers-Jagtman
Aim of the study is to develop a biomechanical model to predict orthodontic tooth movement. With this model local stress and strain in the periodontal ligament can be calculated. The mechanical data should be associated then with clinical observations.

Collagen turnover during orthodontic tooth movement
Sjoerd Henneman, Hans von den Hoff, Jaap Maltha, Dries Desmedt, Anne Marie Kuijpers-Jagtman
One of the main problems in orthodontics is relapse. During relapse the teeth tend to return to their pre-treatment position. Although the exact cause of relapse is still unclear, several possible causes have been proposed. Some authors suggest that periodontal fibers are stretched during tooth movement, and pull back the teeth after treatment. Other theories propose that relapse is caused by supra-alveolar elastic fibers or the transseptal fibers between the teeth. These theories all require that fibers are present within the periodontium that remain there for a long time and therefore have a long half-life. Literature data indicate a half-life of a few weeks for PDL fibers, and a few months for supra-alveolar fibers. The aim of this study is to determine the half-life of periodontal collagen fibers in different compartments of the periodontium. In addition, the effect of tooth movement on collagen turnover will be studied. These data will show whether the cause of relapse can be found in periodontal fibers with a long half-life.

3D-imaging fusion models in orthodontics
Frits Rangel, Anne Marie Kuijpers-Jagtman, Stefaan Bergé, Thomas Maal, Rania Nada
With the introduction of Cone Beam Computed Tomography (CBCT), it became possible to obtain an accurate three-dimensional (3D) representation of the patient’s head with a much lower radiation exposure, compared to Multi Slice Computed Tomography (MSCT), and a much higher information content, compared to two-dimensional (2D) X-rays. With the introduction of digital dental models development of three-dimensional digital datasets, combining the triad bone, soft tissues and dentition, have regained interest. If it would be possible to add the dentition to the 3D-stereophotographic image and/or to the CBCT dataset, this would give a 3D dataset of the face of the patient, with the dentition in an anatomical correct position. This research focuses on the development of fusion models of the head that are useful for orthodontic purposes.

Heme oxygenase as a novel target in the prevention of vascular complications in type 2 diabetes mellitus
Douwe Dekker, Paul Smits, Frank Wagener
The prognosis of type 2 diabetes mellitus (DM) is largely determined by the development of micro- and macrovascular complications. In this project, we will investigate whether the enzyme Heme Oxygenase (HO) is a relevant molecular target for the prevention and treatment of the macrovascular complications. In DM, the production of radical oxygen species (ROS), the expression of inflammatory adhesion molecules and the resulting leukocyte binding and vascular injury have been attributed to DM. One of the protective mechanisms is the activity of the enzyme HO, which breaks down heme into the strong anti-oxidants biliverdin/bilirubin and the potent vasodilator molecule carbon monoxide. In general, the versatile HO-products strongly protect against these vascular insults by reducing oxidative stress, inflammation, proliferation and apoptosis. However, hyperglycemia itself has been observed to inhibit HO-activity, thereby impairing protective mechanisms against vascular dysfunction in DM. The present study will focus on “proof of concept” studies in the human in vivo setting. We postulate that induction of HO-activity or administration of its effector molecules will ameliorate vascular function in DM patients. Parallel to the clinical studies, cellular in vitro and animal experiments will be performed to fill in particular gaps in the pathophysiological concept, and to control for confounding mechanisms.
Mirrin Dorresteijn, Paul Smits, Peter Pickkers, Frank Wagener
Influence of inflammation/sepsis on the HO-pathway. By use of the "human endotoxemia model" and by clinical studies in patients with septic shock, we will examine the pathways by which inflammation induces modulation of HO-expression and activity. In addition, in several ex vivo experiments, we will investigate the expression and activity of HO and intracellular signalling of the HO-pathway in whole blood stimulated by several Toll-like receptor agonists. This project offers the unique possibility to comprehensively investigate the interaction between the HO-pathway and systemic inflammation in humans in vivo, potentially resulting in a more pathophysiologically directed treatment strategy against sepsis.

3D-stereophotographic study into facial growth of CLP babies and unaffected children
Sander Brons, Jeneé Meulstee, Anne Marie Kuijpers-Jagtman, Stefaan Bergé, Thomas Maal, Amir Darroudi
Stereophotogrammetry is a noninvasive technique based on the principle of photographing a 3D object with two pairs of identical cameras separated by a known base distance. The result is a stereo pair of photographs of the face taken from two different positions at the same time. These two photo images are then combined to form a 3D model. To date, a few cross-sectional studies have used stereophotogrammetry to characterize facial soft tissue features in babies. There is very little data regarding 3D facial growth for very young Caucasian children in the age range of 0–6 y with or without orofacial clefts. For longitudinal studies, facial growth can be evaluated with stereophotogrammetry by measuring changes in 3D facial volume and changes in anatomical landmark positions over time and then superimposing 3D stereophotogrammetry pictures from different time points.

Heme oxygenase-1 and its central role in the pathogenesis, prognosis and treatment of systemic sclerosis (SSc)
Lenny Geurts-Van Bon, Tim Radstake, Frank Wagener
Systemic sclerosis (SSc) is an autoimmune disease leading to severe disabilities and markedly shortened life expectancy. Currently, knowledge on the underlying pathways causing this syndrome is mostly lacking and focuses primarily on aberrant fibroblast function. However, there is growing appreciation for the role of Dendritic cells (DCs), the professional antigen-presenting cells that play a decisive role in our immune system. HO-1 protects against oxidative stress, inflammation, and apoptosis and is strongly associated with modulation of the immune response. Indeed, preliminary data from our group clearly indicated an aberrant expression of HO-1 in DCs from SSc patients, and more intriguingly the products of HO-1 function in vivo, carbon monoxide and bilirubin, are decreased in SSc patients. Hence, our observations provide strong rationale for further investigation of the role of an altered HO-1 expression in DCs from SSc patients.

Osteodistraction of the mandibular anterior segment
Christof Joss, Stavros Kiliaridis, Anne Marie Kuijpers-Jagtman
Distraction osteogenesis of the lower alveolar segment was introduced by Triaca et al. and gives the possibility to create space to align teeth and/or implant placement in patients with i.e., increased overjet and retruded alveolar process. The extraction of lower premolars for tooth alignment can thus be eliminated. It is possible to achieve overjet reduction by moving the mandibular anterior alveolar process in a more translational or rotational manner. However, so far no long term results of this treatment approach have been published. The aim of the present study is to evaluate the immediate skeletal and dental effect as well as long term effects in patients treated with distraction osteogenesis (DOG) of the mandibular anterior alveolar process, and to identify factors related to skeletal and dental stability.

Soft palate muscle regeneration
Paola Carvajal Monroy, Hans Von den Hoff, Frank Wagener, Anne Marie Kuijpers-Jagtman
Velopharyngeal competence in patients with a cleft of the soft palate can be only obtained after surgical repair. Unfortunately, surgical correction of the soft palate leads to varying degrees of functional impairment, and about 10 to 30% of the patients exhibit velopharyngeal dysfunction after surgery. Although fundamental and clinical research on the field of muscle regeneration has been extensively performed, it has been limited almost exclusively to limb muscles. Information about the regeneration process of the soft palate musculature might provide significant insight required to develop new therapeutic strategies based on tissue engineering. These treatment modalities could improve outcomes after surgical repair. Therefore, aim of study is (1) to establish a in vivo model for the study of the muscle regeneration of the soft palate, (2) to study the regeneration process of the
soft plate musculature after excisional and incisional injury, (3) to develop and evaluate new strategies from the field of tissue engineering in order to promote healing and prevent fibrosis after induced injury.

**The role of oxytalan fibres in orthodontic relapse**

Hardus Strydom, Jaap Maltha, Hans Von den Hoff, Anne Marie Kuijpers-Jagtman

Orthodontic relapse remains one of the most challenging obstacles the orthodontic team faces. Its etiology is still unknown, but it has been suggested that the oxytalan fiber network plays a role. Some *in vivo* data from studies evaluating the effect of orthodontic tooth movement on the oxytalan fiber network within the periodontium are available, but these data are mostly descriptive and lack quantification. The exact function of the oxytalan fiber network remains unclear. The aim of this project is to elucidate the physiology of oxytalan fibers and ultimately determine its function and possible role in orthodontic tooth movement and relapse.

**Heme oxygenase and stem cells as novel strategy to prevent fibrosis and excessive scar formation**

Niels Cremers, Frank Wagener, Carine Carels

Aberrant wound healing, oxidative and inflammatory stress, are important down-stream complications in CLP patients and contribute to the progression towards excessive or hypertrophic scarring, which interrupts normal midfacial growth and development. Bone marrow stem cells are thought to mediate tissue repair. Following injury, stem cells can migrate towards the injury, where these normally quiescent cells start to multiply, and differentiate into the cells that are needed. However, stem cell functioning is sometimes impaired in an oxidative environment. Therefore we aim to overexpress cytoprotective proteins to protect the stem cell, and in addition, to create an environment that prevents exacerbation of injury, fibrosis, and hypertrophic scarring. *Heme Oxygenase* (HO) is an important cytoprotective protein and functions as an adaptive response against injurious processes, including oxidative stress, inflammation, apoptosis, and fibrosis. We postulate here that stem cell therapy alone and in combination with induction of the cytoprotective gene HO provides protection in CLP against oxidative and inflammatory insults, fibrosis, and hypertrophic scarring. Therefore, we are confident that this study will give us a better understanding in the mechanisms involved in wound healing and may provide novel therapeutic strategies for CLP patients.

**Treatment outcome after one and two stage palatal repair**

Raj Reddy, Anne Marie Kuijpers-Jagtman, Stefaan Bergé, Ann Kummer

This study compares one-stage versus two-stage palatal closure in patients with a complete UCLP in a prospective design. We have started with a systematic review into the topic. In the clinical evaluation we concentrate on dental arch relationships, speech evaluations, and nasometry.

**The smile**

Akhter Husain, Anne Marie Kuijpers-Jagtman, Stefaan Bergé

This project is about smiles: posed smile and spontaneous smile and the role of dimples and easy in smile attractiveness. See content of the planned articles

**Identification of novel genetic variations by exome sequencing and in non-coding regulatory elements associated with orofacial clefts (OFC)**

Kriti Khandelwal, Jo Zhou, Hans van Bokhoven, Carine Carels

Cleft lip/palate (CL/P) is one of the most common birth defects in man (1 in 700 live births) and arises through a combination of genetic susceptibility and influences of / exposures to environmental risk factors in utero. In the identification of genetic causes of CL/P , only a limited number of cases is expected to be explained by mutations in the coding regions. These are presumed to be present in families with multiple OFC affected members. In these families exome sequencing in 2-3 affected family members will be performed and the found variants will be genetically validated in the other affected and unaffected family members. For the families where no variants are found in the coding regions, the non-coding parts of the genome will be interrogated by checking the variation (like SNPs) in regulatory DNA elements, like motifs in DNA binding sites (bs) of transcription factors. We will specifically look at binding sites for the transcriptional regulator p63 either in the proximity of known cleft genes (like has been shown upstream of the enhancer gene IRF6) or in area’s which are in linkage equilibrium with significant SNPs in GWAS area’s. The sequence variations within p63 binding sites upstream of IRF6 are associated with increased risk of CLP. p63 binding sites in non-
coding inter- and intragenic regions might also contribute to the etiology of CL/P. We will also look at the 8q24 region which has been shown to be significantly associated with nsCLP in several GWAS studies.

**Clefts in 3D**
Mette Kuijpers, Piotr Fudalej, Carine Carels, Stefaan Bergé, Thomas Maal, Dries Desmedt, Rania Nada
Incidental findings on CBCT scans in CLP patients are compared with controls. Bony structures and asymmetry are studied and compared with the facial findings using CBCT scans and stereophotogrammetry. It looks into how we can use this information in the clinic and if we can use these methods to evaluate and predict outcomes and use them in the clinic for explaining treatment to our patients and evaluate our treatment goals.

**A genes and proteins network for orofacial clefting (OFC)**
Michelle Thonissen, Hans Von den Hoff, Iris van Rooij, Geert Poelmans, Carine Carels
Since the introduction of genome-wide association studies (GWASs), our knowledge about genetic loci implicated in the etiology of complex traits such as non-syndromic orofacial clefting (nsOFC) has increased significantly.

To date, 5 GWASs of nsOFC have been published (Birnbaum et al. 2009, Grant et al. 2009, Mangold et al. 2010, Beaty et al. 2010 and Camargo et al. 2012), in which a number of genetic loci for nsOFC were identified.

The first aim of this PhD-project is to build an integrated molecular network for nsOFC, based on the top findings from the five published GWASs of nsOFC. For the genetic validation of the novel OFC network, we will conduct a novel GWAS of nsOFC and subsequently use the results of this GWAS to conduct a meta-analysis of the combined results from three nsOFC GWASs, and genetically test our molecular network.

In addition, we will investigate the role of environmental factors in the etiology of OFC and their interaction with our molecular network. Questionnaires completed by parents of children with nsOFC and controls will be used for epidemiological purposes.

**Vitamin A and palatogenesis**
Aysel Mammadova, Hans Von den Hoff, Jo Zhou, Carine Carels
Orofacial clefts are among the most common birth defects in humans. CLP can be syndromic and non-syndromic. One of the main syndromic forms occurs in the transcription factor p63-related syndromes, in which CLP appears as the cardinal feature together with ectodermal dysplasia and limb malformations. Vitamin A is an important regulator of embryonic proliferation, differentiation and apoptosis. Its active metabolite retinoic acid (RA) regulates the specification of cell identity and gene expression through the activation of nuclear receptors, which bind to specific regulatory regions of target genes called retinoic acid response elements The main goal of this project is to investigate the interaction between RA and p63 in congenital ectodermal disorders. In this project, we further aim to (1) investigate the effects of vitamin A on the functional properties of keratinocytes obtained from non-syndromic CLP and syndromic (p63) CLP patients; (2) analyze the effects of RA on the in vitro fusion of isolated mouse palatal shelves; (3) validate our findings in vivo, after the administration of RA to pregnant mice. The results will contribute to the understanding the etiology of clefts and development of their diagnosis and prevention.

**Phenotypes and gene variants in 79 patients with syndromic and non-syndromic hypodontia and their families**
Karoline Dreesen, Carine Carels, Hans van Bokhoven, Koen Devriendt, Tjitske Kleefstra, Jo Zhou
Although hypodontia is the most common congenital developmental anomaly of the human dentition, its etiology still is poorly understood. Syndromic as well as isolated forms occur, and both forms are running in families.

From expression studies in animals (mostly mice) it is estimated that around 300 genes are involved in different phases of tooth formation; also for the initiation phase several genes need to cooperate for starting the development of a tooth.

Despite this knowledge it is estimated that the (genetic) etiology of around 50% of the ageneses is still not discovered.

The question has been raised whether tooth agenesis would be a monogenic, digenic, oligogenic or rather a multifactorial defects, caused eventually by the additional effects of (eventually) many genes as well as environmental factors.
In this thesis it is aimed to unravel the complex etiology of common tooth agenesis, on the one hand by building a gene and protein network for hypo/oligodontia and on the other hand to search for novel genetic causes in families with (apparent/ probable) Mendelian segregation of the hypo/oligodontia phenotype.

**Role of microRNAs in palatogenesis and orofacial clefts**

Christian Schoen, Armaiz Aschrafi, Hans von den Hoff, Hans van Bokhoven, Carine Carels, Geert Poelmans

Palatogenesis requires a precise spatiotemporal regulation of gene expression, which is controlled by an intricate network of transcription factors and their corresponding DNA motifs. Even slight perturbations of this network may cause cleft palate, the most common congenital craniofacial defect in humans. MicroRNAs (miRNAs), a class of small non-coding RNAs, have elicited strong interest as key regulators of embryological development, and as etiological factors in disease. MiRNAs function as post-transcriptional repressors of gene expression and are therefore able to fine-tune gene regulatory networks. Several miRNAs are already known to be involved in congenital diseases. In this project, current research into the role of miRNAs in cleft palate will first be reviewed. Secondly, a small RNA sequencing study will be undertaken to identify specific differential miRNA expression between CLP patients and age matched controls. Thirdly, and following Michelle Thonisens’s article, I will perform an experimental validation study of a possible nsCLP associated novel miRNA. Lastly, I will analyze the possible link between miR-17-92, vitamin A, and cleft palate.

**Genotype/phenotype studies in craniofacial disorders**

Charlotte Ockeloen, Carine Carels, Hans van Bokhoven, Tjitske Kleefstra

The aim of my PhD project is to identify new genetic causes of craniofacial developmental anomalies. I focus on several disorders comprising orofacial clefting (syndromic and nonsyndromic), cranioynostosis, and/or abnormalities in tooth development. The genetic cause of these craniofacial disorders is only identified in a small number of patients. I use next generation sequencing (NGS) techniques to identify new genetic causes of these rare craniofacial disorders and my aim is to combine study not only the genotype but to combine this with phenotypic data. Accurate phenotyping of patients remains important, especially for the interpretation of variants detected with NGS. Therefore, one of my projects is 3D stereophotogrammetry analysis of patients with rare craniofacial syndromes (such as KBG syndrome). This 3D phenotyping can be used for future clinical as well as research purposes in craniofacial patients. As part of my project I also set up a diagnostic exome sequencing panel for craniofacial disorders and will evaluate the outcome of this diagnostic test in a clinical setting.

**Syndromic and non-syndromic tooth anomalies**

Gercheon Wicomb, Carine Carels, Celeste van Heumen, Hans von den Hoff, Edwin Ongkosuwito

The genetics of syndromic and non-syndromic dental anomalies, so also every patient with any hypo- or oligodontia and a syndromic malformation.

**Genetics of syndromic and non syndromic orofacial clefting in Sri Lanka**

Deepthi De Silva, Carine Carels, Hans van Bokhoven, Tjitske Kleefstra

Novel genes for craniofacial disorders including syndromic and non-syndromic orofacial clefting

**Function of CLP genes in mouse palatogenesis**

Laury Roa Fuentes, Hans Von den Hoff, Frank Wagener, Carine Carels

Several signaling pathways are known to be involved in palatogenesis and cleft palate. One of the main pathways is the wnt-signaling. The wnt pathway can be divided into a canonical pathway involving β-catenin, and at least two other non-canonical pathways. Initially, this project will focus on finding marker genes for these three wnt pathways in cell lines, organ culture of mouse embryo palate, and ex-vivo embryo palates. The project will then investigate the interactions between known cleft palate genes and wnt signalling in cell lines and mouse embryo palates. Gene function will be inhibited by siRNA or by pharmacological agents. These experiments will involve known genes discovered in human studies as well as in animal studies (mouse). In addition, candidate genes from our own research will be studied such as LRP6.
Investigating craniofacial development by targeting candidate genes in zebrafish models
Liesbeth Gebuijs, Hans Vonden Hoff, Frank Wagener, Carine Carels
Using exome sequencing of patient DNA it is possible to decipher what genes are involved in disturbed palatogenesis and orofacial clefting. Candidate genes found with this technique in our laboratory including LRP6. The zebrafish model enables investigation to putative genetic and environmental factors involved in craniofacial development. In the present project, we aim to better understand the molecular mechanisms involved in palatogenesis and clefting by translating human craniofacial malformations to zebrafish models. Hereto, we will pharmacologically or genetically (using e.g. morpholinos) target zebrafish embryos and investigate its' effects on craniofacial development. We expect that establishing craniofacial zebrafish models in our laboratory allows development of preventive and/or therapeutic strategies.