The 4th meeting of the International FD/MAS consortium was opened by the presentation 'GNAS spectrum of diseases' given by Giovanna Mantovani.
Giovanna Mantovani gave an overview over a spectrum of disorders, which are all related to G-nucleotide binding protein alpha subunit (*GNAS*), *Gsα*, gene. A Gsα gene mutation involves codon R201 ($Gs\alpha^{R201C}/^{201H}$) and rarely codon 227 and causes an unregulated activation of cAMP leading to FD or MAS. In addition to this specific activating mutation in GNAS, other dominantly inherited activation mutations or genetic- or epigenetic-based alterations of GNAS or related genes lead to different forms of pseudohypothyroidism (PHP). PHP indicates a group of heterogenoeous disorders whose common feature is represented by impairing signalling of various hormones (primarily PTH) that activate cAMP dependent pathways via the Gsα protein. The patients are characterized by e.g. short stature and laboratory abnormalities as hypocalemia and elevated PTH levels. Giovanna reported a recently proposed new nomenclature and classification of these disorders characterized by the term "inacticating PTH/PTHrP signaling disorder', iPPSD.

The second presentation was given by Mara Riminucci about the role of stem cells in FD initiation and maintenance. Skeletol stem cells (SSCs) participate in the regulation of marrow stem cells and are major important in FD. The number of mutated SSCs expressing the mutated allele $Gs\alpha^{R201C}/^{R201H}$ are important for the phenotype FD. Mara's group developed a mouse model for the expression of the disease by introducing the transformed gene into different mouse strains. It could be shown that a number of genes are involved in osteoclasteo-genesis as hematopoesis- and immune genes. A high turn-over of the unmineralized bone matrix has been shown in the transformed mouse strains, which has an effect on other stem cells and leads to upregulation of RANK-L. Thus, osteoclasts modulate osteoclastic differentiation, which may have an implication for FD therapy.

The third speaker, Yingzi Yang, also talked about mouse models in FD/MAS. He stated that cell-cell signalling is essential in skeletal development by controlling embryonic morphogenesis and adult physiologies. Gsα protein is responsible for G-protein coupled receptors, which are on their hand responsible for controlling both skeletal development and homeostasis. Gsα mediated signaling interacts with another signalling complex Wnt. Yang's group introduced a mutation in the Gsα gene and observed that the germ line expression of the mutant gene allele $Gs\alpha^{R201H}$ causes embryonic lethality, whereas the expression of the same mutant in stem cells leads to FD. If then the constitutive Wnt signalling is reduced, FD phenotypes can be partially rescued.

Furthermore, Yang's group wanted to answer the question how mutant cells may behave in normal tissues. A huge increase of the marrow fibrotic phenotype can be observed after the introduction of the Gsα mutant. Even if they are genetically wildtype the mice will develop FD. The reason therefore will most be probably the progressive osteoblast differentiation during cranial bone formation which can be explained by the upregulation of the Wnt signalling by $Gs\alpha^{R201H}$ in mesenchymal bone formation.

In contrast, Ed Hsiao's research on Gs signalling in FD/MAS is based on human cellular models. Potential primary cell sources for theses analyses may be e.g. bone marrow cells (BMCs) from cell lesions or skin melanocytes. His group cultured mosaic cell, normal + mutant clones of BMC cells, and then induced pluripotent stem cells (iPSC). By introducing both, iPS with the traditional allele not carrying the FD/MAS mutation and those with a mutant allele, the influence of mosaicism on the cell survival can be studied. The goal of the research is to develop a iPSC model for FD/MAS by using the new so-called CRIPR/CAS technology, which allows to "edit' DNA by introducing a modified sequence very specifically into the genome. Therefore his group used a new technique called Droplet Digital PCR, which allows to divide one PCR sample into 10.000 droplets. Thus, $Gs\alpha^{R201H}$ mutation can be introduced and selected in an immortalized cell system (HEK293 cells). CRIPR/CAS seems to be *the* method of choice to introduce the mutation into iPS cells.

The last presentation of the morning session was by Luisa de Sanctis who presented different methods for $Gs\alpha^{R201H}$ and $Gs\alpha^{R201C}$ gene mutation analysis. She reported that the detection rate of the mutant allele is only 30% in peripheral blood lymphocytes (PBMCs), whereas it increases in skin cells to 80%. Pyrosequencing, a Next Generation Sequencing (NGS) technique alone or in combination with the TaqMan method is recommended. Her group performed a meta analysis of 168 sporadic cases, in which mosaicism and underlining macroorchidism (enlarged testis) could be detected.

Discussion round:

Remark: $Gs\alpha^{R201C}$ and $Gs\alpha^{R201H}$ mutations are more important than a mutation at position 227 since they are more activating; there are also other mutations at pos 201 but far less.

Q (Mike Collins): Can you tell us something about the nature of cell-to-cell communication?

Answer: possibly Wnt ligand expression is increased

Q: How many cells have to be mutated in a lesion to make it a lesion?

A: Probably not only the number is important (but? Time of mutation in the embryonal development?)

A (Stanton): no difference in number of mutations in different ages of patients

Second morning session:

'Extrasceletal diseases in MAS'

Daniele Tessaris decribed a study about tyroid abnormalities in a cohort of MAS children and adolescents and tried to give indications for their treatment and follow-up. He reported about good clinical and endocrinal responses to low doses of methimazol in patients with hyperperthyroidism.

'Gonodal diseases in MAS' by Malgorzata Wasniewska

Aim of the study was to review data on pathophysiologic and clinical aspects of gonodal diseases in MAS. Her research group concluded that peripheral precocious puberty (PPP) is significantly more infrequent in boys than in girls. Furthermore, the most common testicular abnormality in MAS presentation is macroorchidism (enlarged testis), which is on the other hand more frequent in MAS than non-MAS patients. In girls, PPP appears in 7/9 cases as precocious mensis. Her take-at-home message was that i) MAS should be considered in the differential diagnosis of PPP in both sexes, ii) a gold standard treatment has not yet been established iii) gonodal function during post-pubertal period does not seem to be impaired in the majority of patients, and iiii) an autonomous gonodal hyperfunction may persist over time in both sexes.

'The role of inflammation in FD/MAS' by Roland Chapurlat

Roland poses the question if FD/MAS can be seen as an (auto)inflammatory disease.

As in auto-inflammatory diseases the level of IL6 and the number of neutrophils and macrophages are increased in FD/MAS but without autoantibodies or specific T cells. The increase of IL6 production is observed in cells of the bone tissue in FD/MAS patients, whereas the glucocortoid levels are decreased. However, an effect of the cAMP on IL6 production and decretion cannot be observed. Chapurlat concluded that FD/MAS may be seen as a local auto-inflammatory disease.

Natasha Appelman-Dijkstra talked about cancer risk in FD/MAS. In a cohort of 134 FD patients, 10 patients were detected with breast cancer. Of these 10 patients, 6 had polyostotic and 4 monostotic form of disease. In 9/10 patients lesions had been detected. In contrast to the average

age of cancer patients of about 61 years, the FD/MAS patients were far younger, ~ 46 years old. As Standardized Morbidity ratios show, divers other tumours can also be observed in FD/MAS patients. Mazabraud seems to be an extra risk factor. In comparison to the general Dutch population, patients with FD/MAS show an increased risk for various malignancies including breast cancer, osteosarcoma, cervix cancer, melanoma, tyroid carcinoma and prostate cancer. Whether these malignancies represent additional extra-skeletal manifestations of *GNAS* mutations in FD/MAS remains to be established. Particularly as GNAS mutations have not been identified in osteosarcoma.

The afternoon session of the first day started with a presentation of Rudolfo Capanna, who reported that MRI scan is useful to detect FD. However, the incidence of locally aggressive tumors is not known since they show the same histological features as FD. Even a PET/CT scan can be questionable, and MAS, too, can be mistaken for tumors. Furthermore, co-existence of FD and malignancies has been reported. Discrimination between FD and malignancies is therefore not easy. In addition, Mazabraud myxomas can also be mistaken for thyroid cancer. A malignant degeneration has been reported for 0.4% in monostotic FD and for 4% in MAS. 50% of theses cases appear with a fracture. PET/CT scans may be useful to follow FD and to detect malignancies. He concluded that not a single method can be recommended as guide for differential diagnosis. To be sure, a biopsy has to be taken.

Mike Collins continued the session with his talk about MAS-associated gastrointestinal (GI) pathologies. He stated that *GNAS* mutations can be considered as a mild (soft) oncogene. Somatic GNAS mutations have been reported in intraductal papillary mucinous neoplasms (IPMN). In a cohort of 54 MAS patients about the half showed IPMNS. Of these, 25 patients were further investigated and two of them got a surgical resection. Collins conclusions are that GI manifestations are common in FD/MAS, as IPMNs, hepatic adenomas, and risk for pancreatic and hepatic neoplasia. However, additional mutations seem to be necessary for the development of more aggressive pancreatic cancers. IPMNs in MAS occur at a younger age than in non-MAS patients and an appropriate history indicates a need for GI screening. An optimal care for GI findings is evolving.

Alison Boyce continued the session with her presentation about imaging modalities in FD/MAS. In order to diagnose the disease, a total skeleton bone scan is needed. For further diagnosis two methods are described using radioactive elements, either ^{18}F-NaF Pet/CT scan or Tc99 MDP. ^{18}F-NaF Pet/CT has the advantage of an improved bone uptake, a superior resolution, and the CT allows a morphologic characterization of FD lesions. A disadvantage of this method is that it requires specialized software and nuclear medicine expertise. Tc99 MDP in contrast, is less expansive and uses slightly less radiation. Furthermore, it is reliable, reproducible, and validated

with mobility status of the patient. However, this method is semi-quantitative and does not measure FD lesions. Radiographs (scans) allow morphological characterization, to monitor age-related changes, to show deformities and fracture visualization. CT scans on the other hand are superior to radiographs in details and visualization of bone changes and fractures, and to demonstrate age related changes. MRI scans on contrast show better the cellularity and changes in haemorrhagic cysts, whereas aneural bone cysts can be shown by MRI *plus* CT scans, and optical canal imaging can also be reached by using both techniques.

The fourth session in the afternoon was about surgical therapy.

The first two presentations were about craniofacial FD. Barbara Franceschini (?) stated that surgical strategies need a lot of different specialities. In children and adolescents, resorbable material is often used; other techniques are the splitting bone technique and the use of 3D printed plates. A functional outcome is desirable but as quick as possible. A radical surgery is mostly not necessary.

Giuseppe Spinelli reported (in Italian!) that there are two forms of craniofacial FD, the aggressive and the non-aggressive form. The aggressive form has to be operated as quick as possible, whereas the non-aggressive one can be treated conservative and without hurry. For reconstruction, autologous bone can be used or material from the bone bank. He mentioned new technologies as the hololens.

Robert Stanton talked about scoliosis in FD/MAS. He reported that serial x-rays are necessary to determine progression. Most forms are mild, and only severe forms need surgical intervention. Within the NIH cohort 12 of the 18 severe cases needed surgery, and 10/12 needed a follow up. Hypothyroidism and Hypophosphatemia are correlated with scoliosis progression. A scoliosis of more than 40 degrees will progress. Scoliosis is more common than appreciated: Look and you will find it!

Giovanni Beltrami reported about different orthopaedic practices, as plates and screws, divers nails, structural allografts, and their pros and cons. He then mentioned open questions as the use of Bone Marrow aspirate (BMA) and plates in paediatrics, which may lead to bone resorption. Multidisciplinary approaches are also mentioned, as e.g. the local use of Zolodronic acid (Zol) and Denosumab - the latter of which had no effect - or Zol together with autologous bone grafting. Another approach is the use of bioglass, which appears to be safe and well tolerated but there are not results yet, and the use of cryotherapy, which shows reduced bleeding and is used for giant cell tumors. There are also unfortunately no results yet.

Ernesto Ippolito reported about 20 years of experience in surgical treatment of bone lesions in PFD and MAS. Plates should only be used in exceptional case, whereas intramedullary nailing (IN) is often achieved with good results. IN is successfully performed for femural and tibial FD, and for the lower limb excellent and good long- term results could be reported. Poor results were observed, however, in patients, which were temporarily lost for follow-up. Follow-up showed that pain had disappeared or markedly decreased in more than 90% of their patients.

The last presentation was reserved for the winner of the Charles Harles Award for excellence in FD/MAS research. The award was given by the US FD foundation to Kristin Pam for her research on craniofacial FD about potential mechanisms and monitoring of optic neuropathy in FD. To observe compression or contraction and avoid intra–operative injuries she worked on a novel technique, namely optical coherence tomography (OCT). OCT is used to monitor the retina and the optic nerve, the retinal nerve fibre layer RNFL. This measurement of the RNFL thickness by OCT accurately identifies optic neuropathy and can be used to monitor for disease progression.

The last part of the afternoon session was occupied by a presentation of the 'FD/MAS international consortium Terms of reference Writing group comprised of FD/MAS patient advocates and clinicians/researchers'.

The morning session of the second workshop day was about pain issue.

Diego Fornasari talked about the pathogenic mechanisms of bone pain. A central role in bone pain have the neurons, which transfer high forms of energy. This holds true for skin but also for bone. The spinal cord is very important for synapsis, which then may lead to pain. Different sorts of pain are described a) pain induced by stimuli as heat or cold, b) inflammatory pain, caused by peripheral inflammation and tissue damage leading to peripheral sensitization, and c) neuropathic pain leading to the injury of fibres and spinal sensitization. Chronical pain of the bone is in principal the same as pain elsewhere. Osteoclastic production as in FD can produce pain; bisphosphonates can reduce the osteoclast production (*but do not reduce pain!!???)*. Sprouting (*het vormen van nieuwe uitlopers van neuronen)* can be observed in malignant and non-malignant bone, which causes bone pain. No general differences of pain can be defined. Generally speaken, drugs may help.

Renato Vellucci talked about the clinical evaluation of pain. Kids have in general less pain than adults. An assessment should be made since pain is *subjective* but *patterns of pain are objective for a given tissue of origin*. For an accurate diagnosis pain mapping is necessary, as well as a tracking of the outcomes. Additionally, patient management tools should be developed and have to be followed by a documentation for third parties. The assessment ("Who?") has first to discriminate between, acute, persistent and chronic conditions to get an idea about the evolution of pain and second ("How?") validated tools have to be used as a prerequisite of a good pain management. Unidimensional (One-dimensional?) and multidimensional pain scales are useful for the measurement of pain intensity and the extent to which pain interferes with life activity and emotional functioning. An approach has been developed, the so called 'PQRST' approach, taking into account Provokes and Palliates (P), Quality (Q), Region and Radiation (R), Severity (S), and Time (T). They have also developed the components of taking a pain history. They gave a description of a mono-dimensional *versus* multi-dimensional categorization of pain. He presented additionally a brief pain inventory, and finished with the presentation of the sensitivity and specificity of divers neuropathic pain screening tools and with a quality of life assessment.

The next speaker, Ronald Chapurlat tried to answer the question: What are we doing to answer patient's needs? He, too, mentioned the importance of the evaluation of (bone pain for the management of pain. There are different pain inventories, the 'red flag' especially for night pain and a pain detect questionnaire for neuropathic pain. He reported about general principles for the treatment of (bone) pain, such as the evaluation of possible complications as impending fractures or aneurysmal bone cysts. In this cases, surgical corrections have to be considered. Additionally, sleep hygiene interventions can be proposed as cognitive and physical therapies. Furthermore, pharmacological management of the pain has to be considered as the correction of hypophosphatemia. Then, there are 1) the first line drugs as paracetamol and acetaminophen, 2) the non-steroid anti-inflammatory drugs (NSAIDS), 3) bone therapies before opioids, and 4) drugs against neuropatic pain as amitriptyline and others. Before bisphosphonates are given, you have to ensure that an adequate calcium and Vitamin D uptake is guaranteed. Oral bisphosphonates do not reduce bone pain more than placebos do, whereas intraveneously (IV) given bisphosphonates as Pamidronate and Zoledronate are equally recommended. Several doses of the bisphosphonates may be necessary for pain relief. Furthermore, the patients should be counselled about short and long term risks of bisphosphonates, bone turn over and density should be monitored, and a dental evaluation should be performed. If nevertheless an inadequate clinical improvement is reached, Denosumab should be considered as medication instead but only in a setting of a clinical trial.

In summary, Chapurlat suggested first to try simple analgesics, then NDAIDS, consider IV bisphosphonates, consider surgery, and treat osteomalacia. Furthermore, one should not forget the psychological aspects, and delayed diagnosis and limited therapies are an issue. Multidisciplinary approaches may be an outcome.

During the discussion round, the question was raised how to treat multiple pain. The answer was given by two experts, who stated that it is important to make a good diagnosis first and then, dependent on the outcome of the diagnosis, to treat mixed pain by different combined drugs. One patient recorded that she was treated successfully by a psychiatrist. It was remarked that chronic pain is a disease itself, what not all doctors seem to know. As an answer to the question which doctors should be consulted, it was proposed to consult pain centres. However, pain centres are often full and it was also noticed that pain centres are often full and the treatment can be rough according to the motto "For everything that looks like a nail, use a hammer". The take at home message was to try to sensitize the doctors/specialists.

The sixth session was about pharmacological therapies and was opened by Alison Boyce with a presentation about the treatment of children and adolescents with antiresorptive medications in FD/MAS. First, she reported that bone remodelling rates are much higher in children than in adults. Two processes exist in bone modelling, namely bone formation and bone resorption. Generally, bone formation is higher than resorption. Antiresorptive therapeutic effects on bone modelling can be visualized by decreased metaphysical bands (e.g. Palmidronate, Denosumab) and improved by bisphosphonates. Post discontinuation, however, fracture risk is improved, so the advice would be not to stop the medication. On the other hand, an overtreatment can have negative effects as osteopetrosis. The use of Alendronate in children is far better than in adults. Unfortunately, bisphosphonates appear not to prevent FD lesions or their expansion. Denosumab has the disadvantage that after stop of the drug the osteoclast activity is higher and there is a decrease in the calcium level. Children are of higher risk after Denusomab stop because of their bone turnovers. So, Alison concluded that i) current evidence does not support routine use of anti-resorptives for children with FD for indication other than pain management, ii) Anti-resorptive treatment in children must consider effects on both, FD and non-FB bone, iii) Denosumab is a promising therapy, however safety concerns related to bone turnover rebound impact use in FD, especially in children, iiii) Research is needed to determine if and how these effects can be mitigated.

Roland Chapurlat presented results about recent clinical trials. His background information was that the only randomized placebo-controlled trial comparing alendronate versus placebo has not shown a significant effect on bone pain and imaging but a reduction in bone turnover. Chapurlat presented a second, randomized study comparing Residronate and placebo, which showed virtually the same outcome as the first study. He discussed that the substantial reduction in bone pain in both groups may result from the placebo effect, and that thus oral bisphophphanates may not be potent enough to alleviate bone pain. He then introduced another study, the TOCIDYS trial, using Tocilizumab, an anti-IL-6 monoclonal antibody, and its comparison to placebo. There are no results yet.

Laura Masi talked about osteoporosis treatment in special patient populations. She reported that *FGF-23* is an important factor in FD together with *IL-6*. Here, Denosumab appears to be more effective than placebo. However, the side effects of Denusomab is that it can cause osteonecrosis of the jaw.

As last speaker of the session, Neveen Hamdy gave an overview on the Denosumab trials. Her first topic was FD-related bone pain, which remains elusive although there is some evidence for its association with local increased bone turnover. Overexpression of *RANK-L* and *IL-6* increases the number and activity of osteoclasts in and around FD-lesions and the resulting acidic bone microenvironment may directly stimulate specific sensory neuron receptors, which are also potentially stimulated by mechanical stress and periosteal expansion of a lesion. *FGF-23* induced renal phosphate wasting and low levels of a certain *D3* vitamin exacerbate demineralization and osteomalacia and increase pain also by increased deformaties and fracture risk. About Denosumab she reported a dramatic reduction in FD-related bone pain, bone turnover markers (BTMs) and tumor growth rate over 7 months of treatment. No impaired healing of a femoral fracture that occurred during treatment. Furthermore, BTMs showed rapid and sustained suppression. Initiation of treatment was associated with the development of hypophosphatemia and secondary hyperparathyroidism necessitating supplementation with phosphorus, calcium and calcitrol. Discontinuation of treatment was associated with rapid and dramatic rebound of BTMs with osteoclast activity exceeding pretreatment levels and severe hypercalcemia. Hamdy summarized a study of 12 FD/MAS patients not reacting to bisphosphanates and reported that Denusomab administered for at least on year and with a certain concentration was effective in achieving a sustained reduction in pain and bone turnover markers. These data are promising but safety issues still need to be addressed before this antiresorptive drug could be advocated to widespread use in FD/MAS treatment. As with bisphophsphonates one has to be cautious with Denusomab with vitamin deficiency and hypophosphatemia, which has to be corrected and hyperactive endocrinopathies should be treated beforehand. Therefore, the use of Denusomab in FD/MAS should be restricted to expertise centers.

The meeting was closed with a panel discussion on prospects of the future with the title "Bringing together research across borders" with the panel members Michael Collins, Roland Chapurlat, Natasha Appelman-Dijkstra, Alison Boyce, Kassim Javaid and Marie Luisa Brandi. Every panel member showed briefly his core business and ideas for the future. Together with the participants, ideas were exchanged about how to find the patients all over the world and how patients could find their doctors. Osteoporosis centres were named as possible first point of contact.