Assises de Génétique 2012 Marseille

These are the personal notes of Heather C. Etchevers from 2 and 3 February 2012. They may not accurately reflect the content of the talks and posters I visited. I haven't sought permission from the presenters to post my notes. They are my notes, and they presented in public at a conference of 1500 people or so. Also, the abstracts are freely available for the plenary sessions, the other oral communications (about 1/10 of the submitted abstracts) and the posters. The program is here.

First morning was in common with another professional society which was wrapping up.

Epilepsies of genetic origin – replaces the term « idiopathic » whereas unknown causes = cryptogenic. Others from metabolic causes.

Check out the DECIPHER database. And notes on **Posters**.

Christelle Depienne

discusses one type of X-linked disease in protocadherin 19 that in htz state due to somatic mosaicism, gives rise to migration and synapse establishment defects. Whereas not in itself essential (boys usually spared, girls who are naturally mosaic are affected, and the asymptomatic father transmits). Also the case for craniofrontonasal syndrome with mutations in Ephrin B1.

Most of these epilepsies are kinds of "channelopathies"

Pb is in the GABAergic bipolar interneurons – loss of inhibition on the glutaminergic pyramidal neurons, at least for the severe Dravet syndrome type (*SCN1A* mutations for ¾ of them).

Catherine Chiron

Last speaker discussing GABA receptor inhibitors like stiripentol – acts on a particular subunit, mostly expressed during development. Indeed most effective during early childhood.

Nabbout et al. in preparation all the Dravet syndrome patients respond same way to stiripentol and another medication whereas not all have the *SCN1A* mutations. Paper on ketogenic diet.

TSC1/2 treatment of pharmacoresistant epilepsies – one group responds well: the ones with infantile spasms – VGB or vigabatrin. Tried and compared Hydrocortisone – 100% responders for first and only 50% for second – it inhibits GABA transaminase and augments GABA level in the brain – better penetration of BBB or what?

TSC1/2 are anti-oncogenes and inhibit mTOR in particular via mTORC1/2. Other inhibitors of mTOR such as rapamycin but also everolimus from Novartis (cf Curran MP et al 2012).

Drug restores inhibition of cellular growth. One study still open Krueger et al NEJM 2010 – diminish the intracerebral tumors for many of the patients of tuberous sclerosis of Bourneville. Reduces also some frequency of epileptic crises. Second controlled trial, it also reduces the cutaneous and renal lesions (antimyolipomas). Effect on epilepsy still not clear.

Still studying Tsc1 WT/KO mice – spontaneous epilepsy implicating the NMDA receptors with specific inhibitors (cf UBP141) – study underway in Marseille.

Véronique Paquis (Nice)

mDNA mutations – Surveyor screens common variants that are heteroplasmic and "Mitochips" for homoplasmic variants. 743 patients in their cohort to do the epidemiology on clinical signs and subdivided by age of onset.

Nathalie Boddaert and Isabelle Desguerre

Severe epilepsy at onset (17%) or, once onset, 9 months later many of the children dead (40%) – in mtDNA mutants. Age of onset mostly before age 3. Syndrome of West (spasmoflexions) can be sign of ATPase6 mutants – problems of putamen and peak of lactates. But responsive to treatments.

21 month onset of pharmacoresistant with 7 cases mtDNA depleted, half have pb in liver, 4 POLG mutants and 3 PEO1 mutants. Cortical atrophy. Syndrome d'Alpers. Leads to death, neuronal loss is massive.

Quinone deficit – oedema during the partial continuous epilepsy.

Myoclonal epilepsy associated 45% with mtDNA mutations in many genes. Onset after age 3, similar to adult forms. Loss of energy via mitochondria enters into vicious circle with neuronal loss and epileptic crises.

Nathalie presents pheno-geno correlations. Look for lactate in the cerebrospinal fluid – mutants in complex I had problems (hypersignal) in the tronc cerebral. Many cases had also pbs in the "noyaux gris" (putamen). Leuko-encephalopathie associated always with nuclear DNA (*NDUFS1/3* etc.)

Anomalies in the colliculi mostly when patients had mtDNA mutations (90%) as opposed to nuclear DNA.

Three children with loss of visual acuity, strabisme, some with ocular nerve atrophy or without, certain images can lead to search for mtDNA mutations.

Same for Complex V (*ATP6*). In *NARP* mutants all had a sigmal in the putamen and caudate, whereas the other genes of Complex V such as *ATP6* and *m.918*5 mutations, more rare (though still frequent in ATP6) – leads to algorithms for which genes to prioritize in sequencing. The *ATP6* mutants in spectroscopie show lactate peaks but never have stroke-like lesions (acute paroxysms associated with focal and sometimes regressive neurological signs).

Why is it stroke-like? Look at microcirculation – volume of blood per voxel with gadolinium injections. Can also use ASL new method with quantitative measurements without gadolinium. And then of course diffusion. In a classic stroke, vessels are thrombotic or stenotic and less blood flow in area of brain.

However in stroke-like, flow is normal but vasodilation – volume is increased. Vasogenitc oedema = extracellular liquid, but cytotoxic oedema in real strokes – water goes into neurons and destroys them. In the stroke-like children, see both kinds of images (hyper and hypo-signals) – coefficients of diffusion. ADC – lower can induce more neurological sequels. Try to see if the ADC could be prognostic. In the stroke-like that had augmented ADC (5 patients) they all had clinical recovery on the neurological plan. In five other patients, when ADC is diminished, leads to permanent atrophy and sequels, just like in real stroke. Pathological vessels, stenosis, etc.

Now exploring further with prospective studies.

*What is the mechanism by which cerebral nevocytes can induce problems? Do the excess nevocytes mop up necessary **EDN3** to get the vasodilation needed to keep neurons nourished when they are active? Does augmented flow lead on the contrary to neural stimulation or inhibition of GABA interneurons?*

Arnold Munnich

OXPHOS deficiencies can be secondary and not necessarily primary – each gene only ever has a handful of patients, each sample is too small and genetically very heterogeneous. Makes clinical trials challenging!

No cure for primary OXPHOS deficiencies, but can combat the common consequences outside of the disease-causing gene. Impaired oxidation of reduced NADH and lowered ATP production, free-radical injuries.

Primum non nocere – shares personal errors in trying to help earlier patients. Hypercaloric diet or parenteral nutrition, GH to help height gain – these can be fatal. Don't try to make them gain weight like this. Consumption is very limited. Risk of vaccination is greater than for not being vaccinated. Correcting anemia in Friedreich ataxia by giving iron worsens the gait ataxia (can be transfused instead if need better RBCs before an operation for example). And don't give quinines to all the OXPHOS. Carnitine and vitamin B cocktails are useless. Valproate can precipitate Alpers onset.

Cannot deal with an energetic affluence. The kids are protected by the catexia. Cannot oxidize more than they do. (Thus arginine to treat hypersignal in the brain started with good clinical course but then worsening and finally neuronal necrosis.)

2% of a cohort of some 600+ patients hace a CoQuinone 10 deficiency (it's the shuttle between the complexes by trafficking electrons) – each complex is normal on its own. So test ubiquinone in fibroblasts – and correction by quinines in vitro… gives example of PDSS2 mutant patient. Giving oral CoQ10 - it doesn't easily get into the cells and mitochondria where it is needed – still helps a lot. However, COQ8 mutants not helped. And try instead to add idebenone which is a short chain quinone that is hydrophilic, this introduces a competitor for the little CoQ10 that remains.

Q10 can act either as a pro- or an anti-oxidant. This is what enables the electron transfer (pro-oxidant) – but also has an anti-oxidant effect to detoxify free radicals. When the complex I chain is deficient, then adding in quinones actually induces oxidative stress and damage. Also aggravates the iron-sulfur assembly deficiency in Freidreich ataxia. Oral idebenone helps the cardiac function, but use also deferiprone to chelate iron and permeate brain (used in beta-thalassemia) to also help the iron accumulation in the dentate nuclei. Otherwise this accumulation in the cerebellum leads to the ataxia.

"Surcharge martial".

Clinical trial in the youngest, less affected yet patients, helps the cardiac function very much, less clear effect on the brain yet. Too much actually associated with worsening ataxia.

Prestwick Chemical Library is a commercially available off-market drug panel of 1200 molecules for screening purposes. Using in a yeast screening model.

Richard Redon

Variabilité du genome

GWAS requiring large samples. 500K SNPs captures approximately 80% of 10 million common SNPs in European population. 1500 studies so far, www.genome.gov/GWAStudies.

Ignores the CNV while only common/frequent SNPs are examined. Now 11,700 CNV loci known. New tools for genotyping. Deletions are better predicted by SNPs than are duplications.

Inversion polymorphisms exist as well.

Agilent 1M chip has 200K probes for CNVs to test, and the others around the genome. Can mask the common variants and detect the others. Decipher website to associate genotype/phenotype.

Exome can look either for new variants in a population of unrelated people who have the same symptoms, or present de novo in children but not in their affected parents, but other possibilities.

Notion of how many generations for a variant frequent 1/100,000 (275) or 1/100 (many more…)

LCRs can promote structural variations. Saw there were many conserved areas of CNVs between chimps and humans thanks to this architecture.

Novembre and Ramachandran Ann Rev Genomics Human Genetics 12:245 2011 – nice discussion and figures about population genetics.

Remember Decode and Iceland. Any chance one can contact and see if anyone is affected there with one's rare disease of interest?

Cf. v43 April 2011 Nature Genetics – variant in *MYH6*, in so-called "sick sinus syndrome" affecting heart rate, for intermediately rare variants in small populations that confer increased risk. (But also see more recent http://www.ncbi.nlm.nih.gov/pubmed/22194935 for ASDs.

At Institut du Thorax - aortic retrecissement aortique calcifiée – trace back to a founder. Need good cohorts – mutualize data cf Genome of the Netherlands and UK 10K as initiatives along these lines.

Maynard Olson in Science 331:87 2011 – there just are no "wild-type" humans…

Jean-Louis Mandel

http://evs.gs.washington.edu/EVS/ is the NHLBI exome sequencing project – but even through there is a lot of variation data – there is a risk that some "common variants" are listed as mutations and vice-versa. Quality/Filtering etc. – detection of non-protein coding genes? Some success stories via whole genome sequencing.

Strasbourg strategy was to sequence 12 patients per line (up to 96 patients per run) with Illumina – cf Simultaneous Session 9 for presentation. Can now do more. This is very helpful for diagnostics.

Cf. hypercholesterolemia published in HMG 2010 Helen Hobbs and Jonathan Cohen – all normal genes excluded, proposed liver transplant – but ended up finding out the real cause and set up a pharmacological treatment instead.

Also look at Nat Rev Genet 2011 Sep by Cooper and Shendure – "needles in stacks of needles'…

Evoked "preconception genetic testing" to check on severe genetic pediatric diseases before fertilization. England has a position paper saying could make it available to general population. Would the cost of preventing such diseases – and the risk of eugenism – offset the expense to the public health insurance – and if not, would it be "two-speed" medicine only available to the wealthy?

Think digenism or oligogenism is probable as a mechanism – but diagnostic becomes difficult.

"de novo" mutations have a role in schizophrenia… Rare variants of a same gene can have opposite effects – two "faux sens" ID'd by Catherine Boileau in hypercholesterolemia. Same genes with nonsense mutations actually protect against cholesterol accumulation and are considered protector mutations wrt cardiac disease (H.Hobbs).

Jamel Chally

CNVs and the *DMD* gene.

CGH array not efficient <10kb for exonic deletions/dupl. But in fact in their hands with a custom array they can detect deletions 1.5-2 kb as CGH – helpful in diagnostics, and can't otherwise detect complex intragenic rearrangements.

Philippe Froguel

Diabetes type 2 and obesity. 20 years ago have seen that there could be monogenic contributions to frequent diseases.

Idea of "functional" genome – highly conserved regions, other regulatory elements, miRNA regions. What can we treat as far as data? Intermediate between exome and whole-genome. 12 million variants in diabetes families he has studied. What to do with them?

A non-synonymous mutation is more likely to lead to an idea of its function.

3 ug gDNA of excellent quality at 200 ng/uL can be used to make the library, which will then be sequenced. Both steps are very important. If representation (depth of coverage) insufficient, cannot hope for ID of htz mutations for example. And if did not have a way to amplify a given sequence it won't be covered at all.

Doesn't appreciate 1000 Genomes as a filter. **For diabetes tries to include gene expression data as well as co-segregation**. Filtering is quite essential and needs to be adapted to each disease. "Neonatal" diabetes is genetically heterogeneous.

Young adult diabetes is hard between type II and type I – cf www.diabetesgenes.org to try to predict which forms would be monogenic, based on clinical questions.

Targeted resequencing is doing better than sequential sequencing gene after gene (now available from Agilent and Raindance). But expects exome sequencing will succeed also. Have done this and published for neonatal diabetes – published in PLoS One in 2010. If certain diabetes forms caused by potassium channels in the pancreas, can cure the diabetes not with an insulinotherapy but rather with sulfonylurea treatment, which is quite successful.

Considers that when the workflow is in place it is much more quick for the analyses. But if look close at certain targets the coverage is not always optimal. Even when overall coverage supposed to be excellent. Considers the Agilent 50 Mb capture is superior in sensitivity to the Illumina 62 Mb.

In their 12 genes there are 100% coverage but also some at 75-80%.

GATA6 is a new gene in pancreatic diabetes, all neonatal diabetes patients also have cardiac defect. First publication showed they all were de novo. However, this group had a family where everyone had the cardiac defect, the mother not diabetic but both children also diabetic. Expression of *Gata6* – usually not expressed in beta cells of pancreas. Not important for pancreatic development, after all. They think it is more likely a vascular and intestinal defect – affecting the endoderm. No direct effect on the pancreas. Found thanks to the exome sequencing but the interpretation was a bit faulty. Turns out they also had biliary vesicle agenesis.

Melatonin receptor 1B *MTNR1B* variants increase type 2 diabetes risk. One of few of the fifty-odd genes identified by GWAS that is identified in multiple studies.

Olivier Delattre

RNA-seq and genomic profiling in Ewing sarcoma (mostly of the bone). Most frequent in adolescence. Quite specific to Caucasian/European populations. Genetic susceptibility.

Very few families for co-segregation. Try a kind of GWAS, though not so many cases – still 1500 cases!!

Good association with three loci: OR were better than often found (>1.3-1.4) – here they were over 2. Constitutional genotype and the level of expression in tumors. Loci were new but suspect interaction with the somatic modifications seen.

CD99+ small round cell tumor. A translocation is a specific marker: EWS-FLI1 fusion gene drives the oncogenesis thereafter. There are other EWS fusion partners, somewhat more rare. Other genes of the ETS family (with a DNA fixation domain). Eg ETV1, NFATC2, FEV, ERG… all impact on a variety of targets because it is an abnormal TF.

But what about Ewing sarcomas without the canonical translocations? What drove the oncogenesis, and should these cases be treated the same way as the other ones?

Did RNA-seq on translocation-negative cases. (SOLiD sequencing). Strand-specific RNA seq, select a range of sizes and then paired-end sequencing because wanted to ID overlaps/fusions.

This allowed them to ID some fragments that co-overlapped FUS-FEV – homologue of EWS and of FLI1 respectively. Another case had been described in the literature. However, had another case with a BCOR-CCNB3 fusion (BCOR represses BCL6, and CCNB3 is a cyclin in meioses).

Then showed that this fusion was a result of an inversion on chromosome X.

Went back and looked at other sarcomas as well. Turns out they also have BCOR-CCNB3 fusions, usually starting after the stop codon of BCOR. No sex bias, even though this is on chrX. Mostly bone sarcomas but not exclusively. Ewing-like, the pathologists can't determine completely. Survival is variable, and some metastatic and lethal. They are in fact rather distinct – they cluster together separately from other sarcomas. (non-supervised)

Mutations of *BCOR* known in oculofaciocardiodental and Lanz microphthalmia syndromes. It acts on HDACs. The fusion protein activates cell cycle and tumorigenesis in NIH3T3 cells.

Get co-expression of normal *BCOR* and fusion *BCOR*. Expression is ubiquitous usually. One single allele can code for both. The importance is not loss of function of BCOR. The stop codon is included in a 5' cryptic splice donor site, and even in the males the WT *BCOR* is expressed.

There are already drugs against cyclin B3/CDK complexes to now try out. Epidrugs such as HDAC inhibitors could be effective, too. And then see that Hh and Wnt pathways could be overactivated. See Pierron G, Tirode, Lucchesi soon to appear in Nature Genetics.

Did not take notes for the following talks. Dr. Mattei's talk will be published as an essay but I asked if I could e-mail Marie-Génévième.

Chris Gordon

2nd family with rib phenotype and PRS, small deletion.

3rd family with deafness and contracture de Dupuytren - deletion very close to *SOX9* between it and the RevSex element. Craniofacial enhancer possibly.

ChIP Seq for p300 on CF tissues from murine embryo branchial arches. 9 peaks between SOX9 and KCNJ2 at 5'. One on proximal promoter, 5 concentrated in region with families 1 (our publication) and 2. One is particularly conserved to the zebrafish level, others at least to chicken.

Transitory transgenic mice being made in collaboration with Len Pennachio at Berkeley. Some peaks give mandibular expression. Trying to sequence them in 80 isolated SPR patients. A few variants in the promoters but some seem to be transmitted so either irrelevant or incomplete penetrance.

Question of transgenic mice – expression in the testicle? Would be important to dissect and find out.

What about the deafness? Otic vesicle – perhaps the mandibular expression gives way and is under the same enhancer control as the

Laurence Heidet

2-10% interruption of pregnancies due to severe renopathies.

CAKUT is English acronym with malformations of urinary tracts or hypoplasic kidney – complex hereditary but some can be monogenic and these tend to be syndromic. Even these can appear to be isolated kidney issues.

Major screened genes = *HNF1b*, *PAX2*, *EYA1* and *SIX1* – branchio-oto-renal.

Retrospective study on 93 fetuses. 31 had family antecedants. 84 had oligoamnios or anamnios.

11 also had uterine anomalies, 3 others with testicular issues. 12 had pancreatic anomalies (these would be good candidates for *HNF1b* for example).

35 fetuses tested for *PAX2* only if there was something in eye. 3 tested for *EYA1*. 9 *HNF1b* mutations but the lesions were bilateral. Of these 2 had no pancreas and 1 had partial agenesis. When familial, other renal disease but not always obvious – incomplete penetrance (variabilité intrafamiliale plutôt).

Hypoplasia and aplasia is significantly more found in mutated group than in non-mutated group.

Eye is always implicated when it's *PAX2* (in the fetal series).

EYA1 found in 2 fetuses – frameshift and inherited. One fetus also had macrocrania, cardiopathie, other signs. Inherited from father who had branchial issues.

Stanislas: Can uterine phenotype be like a Rokitansky phenotype? Answer: never complete agenesis. Many had been tested for RET and they had no mutants. Tested PAX2 and HNF before RET though.

Echographie > diagnostic moleculaire pour le conseil genetique.

PAX2 postnatal case no apparent ocular phenotype mentioned – and Laurence confirms that the anomalies can be quite minor but 20% of them have really nothing at all even with ID'd mutation. Correlation genotype-phenotype?

Bilal El Waly (Marseille - Laurent Villard)

NHEJ1 after "organogenesis" during the "histogenesis" phase. AKA Cernunnos and XLF.

46,XX,t(2;7)(q35;p22) interrupts this gene, strongly expressed in human embryonic brain. Non-homologous end-joining to repair double-stranded DNA breaks. Mutations either in this or in ligase IV leads to microcephaly and immune deficit. The immune deficit studied by A. Fischer (brought up by Arnold) but apparently no MRI images available for those patients.

Leslie Ratié (Rennes – Veronique David, Sylvie Odent)

DLL1 in a deletion. ISH in chicken. Would be nice to redo *N1* hybridization but also *DLL1* of course.

Connolly et al 1995, Chapman et al.

Inhibit? Or stimulate? NOTCH pathway with DAPT + DMSO or DMSO alone. Used Genespring to study the transcriptome differences. "Enrichment" is unfortunate choice – they were green in the clustering – apparent DOWNregulation of Notch but also Shh pathway genes. Hes5, Hey1, other targets. Ascl1 usually not Tgln3 (transgelin3) both upregulated in presence of DAPT, loss of Shh in the midline all the way back through the optic vesicles. 100% penetrance.

Notch phenotype in heart via Shh also?

"Here we show that either absence of the Notch ligand Jagged1 or inhibition of Notch signaling in second heart field tissues results in murine aortic arch artery and cardiac anomalies. In mid-gestation, these mutants displayed decreased Fgf8 and Bmp4 expression. **Notch inhibition within the second heart field affected the development of neighboring tissues.** For example, faulty migration of cardiac neural crest cells and defective endothelial-mesenchymal transition within the outflow tract endocardial cushions were observed." From High et al 2009. "We have previously observed the expression of multiple Notch signaling components in the cardiac OFT myocardium, including the receptor **Notch2**, the ligand **Jagged1** (Jag1), and downstream target genes Hrt2 and Hrt3 (16)."

Embryos treated after gastrulation to avoid effects on somitogenesis.

Fgf8 downregulated (Valérie's other paper) – think to electroporate NICD of Notch into prosencephalon rather than to add in Fgf8-impregnated beads.

Chloe Quélin

Smith-Lemli-Opitz – 28 fetal cases.

DHCR7 on 11q13,4 for cholesterol metabolism problems. Postnatal data cf Porter et al., 2008 EJHG.

Goldenberg et al AmJMG 2004 for prenatal signs. Looked to report more malformations: 16-34 SA. @) boys and 8 girls.

Biochemical of cholesterol by mass spec. All reported cases had intrauterine growth retardation. Hypertelorism but narrow intertemporal distance. Low-implanted ears, short neck. Low, gping mouth. Some clefts or HPE.

Mostly syndactyly II-III (with duplication of the digit). Brachymesophalangy of 1st and 5th digits. Some club feet (29%). Postaxial polydactuly in 71%. Anomalies of external genitalia in boys – cholesterol needed for testosterone synthesis. 2 girls had bicornate uterus. Still an issue with the hormone synthesis.

Four malformations more represented – hypo/aplasia of kidneys. 79% AVC (atrioventricular canal) isolated or associated but lots of other cardiopathies – too quick to enumerate. One was atrial valve dysplasia, another pulmonary. One commentator has a referral for an IUGR and an apparent external female genitalia but is 46,XY – discrete phenotype

4 fetuses had anomalies of sacral vertebrae. 1/3 had placental hypoplasia.

"Holomyelie" – anterior horns of spinal cord were fused. Hypothalamic hamartoma like in Pallister-Hall but the digit anomalies were different. DHCR7 with most common splice mutations in all fetuses of this series.

Many spontaneous abortions in these families as well.

There has been a description of a normal cholesterol dose in a mutated patient (cf literature).

Marine Legendre (Paris – Tania Attié-Bitach)

CHD7-mutated 38 fetal cases.

Nice slides for the diagnostic criteria of CHARGE syndrome (#2 in particular) and explain why the #3 from Damien's paper J Med Genet 2006 is better for prenatal diagnosis.

Confirms malformations of external ears and SCC agenesis. 89% arhinencephaly. (*Saw in poster that can also be the case in 22q11 deletion*. Would be nice to know where in the region CHD7 binds. Check the literature?) (This paper says that it generally maps to H3K4 methylated sites. The same authors published in PLoS Genetics shortly after to say that "CHD7 functions at enhancers as a transcriptional rheostat to modulate, or fine-tune the expression levels of ES-specific genes." Which might be *TBX1* but could also well be *HIRT*.)

89% cardiopathy as well which is somewhat more than the postnatal frequency, also about ½ cases have variable clefting, also a bit more than in postnatal. Cardiopathy is "non-specific" such as hypoplastic left heart (lethal). Pauline Parisot helped out with diagnosis.

Coloboma 14/31 in histology, sometimes microphthalmia. (Binding site on PAX2?)

Hypo/agenesis of thymus in 16 cases, anomalies urinary tract or hydramnios in 9-10 cases. As many skeletal problem. No growth retardation.

8 cases see "lateralization" issues: also occasional omphalocoele, hypertrophy of salivary glands and lack of thyroid isthmus; mesencephalic hamartoma…

Other CNS malformations can be added as minor signs.

Concludes that absence of *CHD7* mutation (only in 60% so far) is not exclusive of the diagnosis.

Mains fendues in one case. Feet not affected. An old case of Marie Gonzales. Though this excludes *DLX5* it doesn't mean that part of the phenotype wouldn't be the regulation of *DLX5* by CHD7.

For *Dlx5* mice, "Targeted disruption of either *Dlx5* or *Dlx6* does not cause a limb phenotype"

Celine Poirsier-Violle

Rhabdomyome – congenital heart tumor usually pretty benign, often regress before birth or just at birth. Problem is that it might be part of spectrum of tuberous sclerosis of Bourneville. Hamartin and tuberin = TSC1/TSC2. Mutations lead to overexpression of *MTOR*.

55 fetuses studied.

3 had "taches achromiques" as well as warty hamartomas.

Means I can ask the SOFFOET if they have not seen prenatal cases of giant NCM or multiple NCM.

7 (max, did not examine mRNA) had no mutation in *TSC1* or *TSC2*, mostly the latter. Fetal MRI was able to detect anomalies. If MRI is normal only 15% likely to have TSC instead of 80% before (with rhabdomyomas).

Louise Devisme

Isolated cerebral malformations – 295 cases. She is based in Lille.

21% hemorrhagie among the ventriculomegaly (two glioblastomes), Lissencephaly type II 10%. One XLAG syndrome confirmed, with , one Fowler a bit unusual, confirmed *FLVCR2*. Bickers-Adams syndrome with hydrocephalus for *L1CAM* in 5 cases

62 cases with agenesis of corpus callosum. 4% problems of midline. 1 deletion qqter, 11% anomalies in CGH array.

Some HPE – one mutation *SHH*, one *SIX3*.

Microcephalies. One had *ASPM* with gyration that was "simplified".

Dandy-Walker isolated for 50% others problem with ischemia, LISS II, anomalies chromosomiques.

Some septal agenesis, other malformations.

Posters

P606:

MEF2C is mutated in Rett syndrome along with *MECP2*. Cardiopathies? (No.) See Edmondson et al., 1994: "We first detected MEF2C expression at day 7.5 postcoitum (p.c.) in cells of the cardiac mesoderm that give rise to the primitive heart tube, making **MEF2C one of the earliest markers for the cardiac muscle lineage yet described**. By day 8.5, MEF2A, MEF2C and MEF2D mRNAs are all detected in the myocardium. By day 9.0, MEF2C is expressed in rostral myotomes, where its expression lags by about a day behind that of myf5 and several hours behind that of myogenin. Expression of each of the MEF2 transcripts is observed in muscle-forming regions within the limbs at day 11.5 p.c. and within muscle fibers throughout the embryo at later developmental stages. The expression of MEF2C in the somites and fetal muscle is distinct from that of MEF2A, MEF2D and the myogenic bHLH regulatory genes, suggesting that it may represent a distinct myogenic cell type. **Neural crest cells also express high levels of MEF2 mRNAs between days 8.5 and 10.5 of gestation**. After day 12.5 p.c., MEF2 transcripts are detected at **high levels in specific regions of the brain** and ultimately in a wide range of tissues." (So why is effect only Rett?

A.-C. Noel: P695

74 fetuses with 22q11 deletions. 92% had common arterial trunk (19/74), T4F (13), pulmonary atresis qnd interventricular canal (9), interruption of aortic arch (7), coarctation of the aorta (7) and some unspecified other vascular anomalies (37/68).

Five of these had arhinencephaly, and four had facial clefting. (Not so frequent as all that.)

Has anyone looked in other 22q11 for arhinencephaly? No, but a description of DiGeorge with extremely mild HPE spectrum anomalies including solitary incisor, and another where DiGeorge patients have impaired olfaction (another here).

Khaddour-Issa: P595

With B. Gérard.

NTDs after lower folate. 10 fetuses: 8 myelomeningocoele, 2x exencephaly, 1x encephalocoele between 14-28 SA. Vs 10 late fetal deaths as controls.

Hypothesis is that problems in methylation that happened early would stay constant between tissues of two different origins and if happened after ecto-meso separation could be filtered out.

35 genes that were hypomethylated were relevant in development (present in heart and skin tissue):

FGF2, SMAD1, BMP7, MSX1, GLI1, NKX2-2, GATA6, CDX2, FRAT2, DVL2, WNT3, RARB, PTEN, SNAI1, KLF10, PRDM1, ENC1, TGIF2, RING1.

E. Alix and D. Sanlaville: P597

PHRCs for NTDs, microcephaly and so forth (with L Devisme?) on 100 fetuses. On a CGH array 4x180K. For NTDs for example, 41% were normal, as many had chromosomal aberrations confirmed but need more confirmation, and 18% not yet done.

Ratbi and Sefiani: P598

A case of Johnson-McMillin. Child is balding but not bald; anosmia; deafness; small, malformed ears that make you think a little of CHARGE ears (somewhat triangular); hypergonadotrophic hypogonadism. Café-au-lait spots on the lower back and butt specific to this patient, and a sacral dimple (think of people you know; when isolated this is never a problem as far as spina bifida occulta but in syndromic form can be). Untreated unspecified dental caries (deficient in dentine?) and the mouth hangs open, somewhat slack. Normal mental development.

MP Roth: P598

Micro and anophthalmias? – see paper appeared in Birth Defects Research pt A. (don't have open access to it) http://onlinelibrary.wiley.com/doi/10.1002/bdra.22877/abstract

Would still like to see the following posters:

P117 INTRAGENIC EHMT1 MUTATIONS SPECTRUM IN PATIENTS WITH KLEEFSTRA SYNDROME.S Drunat

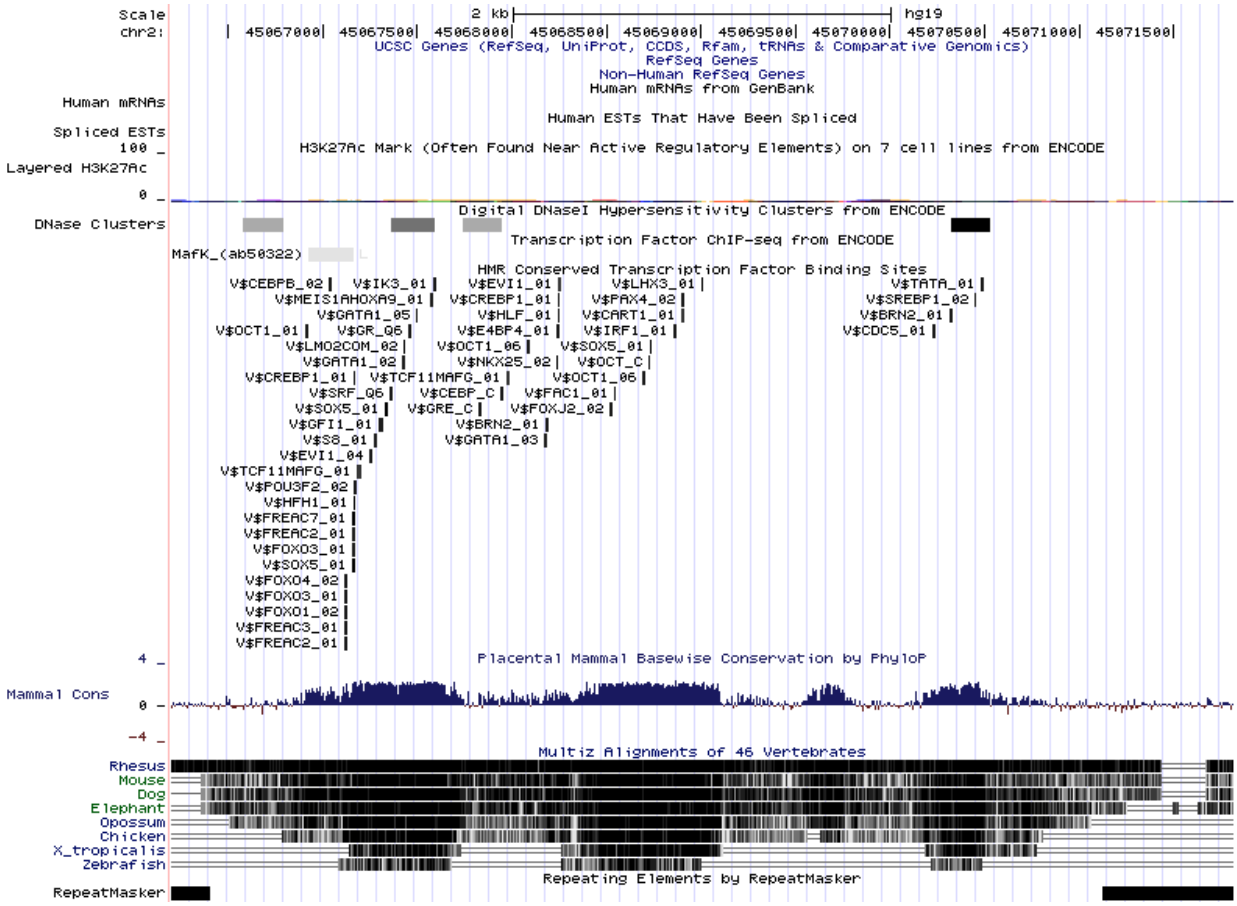
« déficience intellectuelle, une hypotonie et une dysmorphie faciale caractéristique. Des anomalies rénales ou cardiaques, des troubles du comportement et une épilepsie ont également été rapportés de façon plus variable chez ces patients. Il s'agit soit d'une délétion submicroscopique de la région 9q34 (75% des cas) soit de mutations intragéniques inactivatrices du gène codant pour l'Euchromatine Human Methyl Transférase 1 (*EHMT1*). » Apparently more cardiac pbs in 9q34 than in *EHMT1* mutants. Go see? I must have misunderstood – exactly 30% for both, and no specifications about what anomaly.

P133 ETUDE DU GENE SLC29A3 DE L'HISTIOCYTOSE DE ROSAIDORFMAN A LA SURDITE : L'EXPERIENCE FRANÇAISE. L Jonard

'H syndrome' is characterized by cutaneous **hyperpigmentation** and hypertrichosis, hepatosplenomegaly, heart anomalies, and hypogonadism; hearing loss is also found in about half of patients, and many have short stature ('low height'). An allelic disorder designated 'PHID' is characterized by predominantly antibody-negative insulin-dependent diabetes mellitus associated with pigmented hypertrichosis and variable occurrence of other features of H syndrome, with hepatosplenomegaly. For heart defects: pulmonary stenosis, pericarditis. In particular, the patient 2 in abstract has only the pericarditis predisposition, as well as a number of ocular issues.

P349 DELETION INTERSTITIELLE DE LA BANDE CHROMOSOMIQUE 2P21 AVEC DES ANOMALIES OCULAIRES. INSTERTITIAL DELETION OF CHROMOSOMAL BAND 2P21 WITH EYE ANOMALIES. ***A Schneider***

275kb away from SIX3 possible regulatory element? Between CAMKMT and the 5' end of SIX3. For the largest peak of conserved TF binding sites just 3' to a H3K27Ac binding peak from the ENCODE data, supposedly near active regulatory elements.



P300 PATERNAL MALSEGREGATION OF A TRANSLOCATION T(11;13)(Q25;Q14) IN TWO PATIENTS WITH INTELLECTUAL DISABILITY, ***C METAY***

Le premier frère (patient 1) présente un retard psychomoteur modéré, une légère dysmorphie faciale et un déficit immunitaire. L'examen de son frère (patient 2) montre un retard psychomoteur sévère, une microcéphalie, une dysmorphie faciale (malformations du crâne, épicanthus, nez court, philtrum bombé, petites oreilles, oreilles basses), une cryptorchidie et une malformation cardiaque (*it's a inter-ventricular communication*, also has overlapping fingers). Chez le patient 1, le caryotype puis l'analyse chromosomique sur puce à ADN Agilent 105K ont détecté la présence de deux trisomies partielles, (1) localisée au niveau de la région 11q25qter de taille 780Kb et (2) en 13q11q14.2 de 28Mb. Le patient 2 est porteur d'une monosomie partielle localisée en 11q24 et une trisomie partielle en 13q13. **(*CDON* is**

at 11q24, which is a Patched receptor interactor involved in HPE11). They evoke Jacobsen Syndrome for the second child even in the absence of thrombocytopenia. In this disorder it can be ASD or VSD.

"Grossfeld et al. (2004) previously identified an approximately 7-Mb cardiac critical region in distal chromosome 11q that contained a putative causative gene(s) for human congenital heart disease. Ye et al. (2010) used chromosomal microarray mapping to characterize 3 patients with congenital heart defects and interstitial distal 11q deletions that overlap the 7-Mb cardiac critical region. The 1.2-Mb region of overlap contains 6 genes, including the *ETS1* (164720) gene, which is expressed in the endocardium and neural crest during early mouse heart development. Gene-targeted deletion of Ets1 in C57/B6 mice caused large membranous ventricular septal defects and a bifid cardiac apex, and less frequently a non-apex-forming left ventricle. Ye et al. (2010) proposed an important role for ETS1 in mammalian heart development and suggested that hemizygosity for this locus may be responsible for the cardiac lesions seen in **Jacobsen syndrome**."

P336 ANOMALIES CHROMOSOMIQUES ET RETARD DE CROISSANCE INTRAUTERIN : BILAN DE 11 ANS D'ETUDE ***M kammoun***

« un cas d' inversion péricentrique 5(p13;q11) découvert devant un RCIU associé à une malformation cardiaque et une artère ombilicale unique » - what kind of malformation ? breakpoints known ? No answer to either question – pure karyotyping.

P348 THE 1Q42Q44 DELETION : A NEW SYNDROME ASSOCIATED WITH AGENESIS OF THE CORPUS CALLOSUM? ***S Dimassi***

"Il présente une dysmorphie faciale évocatrice la délétion 1q terminale, un hypospadias, un retard psychomoteur, des troubles du comportement, une malformation cardiaque à type de **communication interventriculaire périmembraneuse restrictive associée à un rétrécissement pulmonaire modéré** et une agénésie totale du corps calleux. La CGH array a montré une délétion télomérique de 11,67Mb avec des points de cassure à 235500506 pb et à 247179291pb» - gènes ? *Ha – it WAS hg18 and not the most recent hg19 version. Of the genes in the interval, GREM2 (gremlin2, BMP antagonist) and AKT3 are particularly interesting. Can there be too MUCH BMP signaling in the heart? I imagine so.*

P601 DIAGNOSTIC PRENATAL D'UNE TRISOMIE 15 EN MOSAÏQUE : APPORT DES PUCES OLIGONUCLEOTIDIQUES SNP DANS LA DETERMINATION DU MECANISME DE FORMATION ***S Chantot-Bastaraud***

STRA6 is on chromosome 15. « Le phénotype est hétérogène et associe de façon variable dysmorphie faciale (*short neck, narrow eyelid openings, hypertelorism, wide and shallow nasal bridge*), malformations cardiaques (CAV, Hypoplasie du ventricule droit) et rénale (duplication rénale) , hypoplasie pulmonaire et malrotation intestinale" « retard de croissance intra-uterin et d'une

malformation cardiaque complexe (canal atrio-ventriculaire et ventricule droit à double issue) chez un foetus de sexe apparemment féminin (*no orifice, the anus doesn't look normal – no gluteal crease* ?) au caryotype foetal masculine"

P619 INTERACTION ENTRE SOX10 ET LES INTEGRINES B1 AU COURS DU DEVELOPPEMENT DU SYSTEME NERVEUX ENTERIQUE V BARAL

No notes to take. There will be a nice Dev Biol paper someday. Mice with htPA-Cre and Sox10 or Itgab1 and genetic interaction between the two as far as cell migration into the distal enteric area.

In the ***GS Roche seminar***, presenters said that get best results when analyze with another program in addition to the one furnished with the sequencer: for diagnostics this was Seq(Something) and the other was something like CGC Genome Walker.

Sophie Thomas

KIF7 not just acrocallosal syndrome and hydrolethalus but also hypomorphic alleles act in trans with the BBS loci.

Transcriptome with patient fibroblasts showed upregulation *GLI1/2/3* and leads in one AC patient to longer cilia, similar to the *KIF7* mutant mice.

Role in the maturation of GLI3?

Putoux et al., Nat Genet 2011 paper.

One fs mutation with Grieg syndrome and no *GLI3* mutation. Sylvie Odent asks if he had molar tooth sign – apparently not. But the acrocallus ones did. How many patients Pallister-Hall and Grieg (6)? Perhaps other genes to find.

Caroline Schluth-Bolard

With Damien Sanlaville – cloning breakpoints in patients with abnormal phenotypes but no apparent disequilibrium in CGH array. Included some known abnormal patients.

Subcontracted out to Integragen.

Chen et al 2008 discusses the technique of cloning breakpoints without using FISH, PCRs etc. (Also see http://genome.cshlp.org/content/21/10/1720.abstract .)

10-12x suffice for this. >2 mismatch, then duplicated pairs, eliminated. Finally, selecting abnormally mapped pairs for further examination: eg reverse primers that map to the + strand in the same

orientation as the forward primer. Must ask for this on purpose because otherwise these are eliminated as a filter in the quality control.

Able to focus down into 90-400 pb for all 10 tested – 4 SINE, 4 LINE and 1 repeated sequence in the DNA. 9/10 were confirmed by Sanger sequencing.

Logiciel IGV for visualization. Was able to show interruptions of certain genes in mental retardation children.

Quite quick and can also detect CNV, meaning not so much need to do a CGH array. Still expensive and still need to know which regions to study.

Sophie Saunier

Ciliopathy diagnosis (Filhol et al including Mohammed Zahrate)

Have with Valerie Cormier-Daire, Jean-Michel Rozet, Sophie Saunier and Tania Attié-Bitach, use SureSelect Agilent to target the ciliome, and SOLiD for sequencing in 600 patients that haven't been molecularly diagnosed already, among whom MKS, ACL, pilot on 45 fetuses.

5.3 Mb captured over 32,146 exons for 1666 candidate genes: known protein interactants, structure and function of cilium, and other candidates.

Pipeline Patrick Nietschke: Analysis led to the ID of *TTC21B*. They have local internal variant databases also if the variant is found more than a certain number of times.

OLL and *TCNT3* were identified as new genes, and found mutations in 32/45 patients so far, one already known, and others in ciliary genes.

Coverage on first run was moderate. a few patients had very many patients and still in analysis.

IFTA genes often found in isolated nephronophthisis with or without bone anomalies. New gene for RP – Isabelle Perrault paper submitted. Saldino Mainzer syndrome with cone-shaped epiphyses of fingers, NPH and ocular sympromes. Patient fibroblasts have short cilia and IFT46 which ought to be in the cilium is mislocated in the cell.

NPHP9 mutations found in a renal hypo/dysplasia (NEK8) also cardiomegaly, situs inversus and interstitial fibrosis. There is a KO of this gene in the mouse which has a similar phenotype. So, also a ciliopathy.

Ciliome 2 library being developed to multiplex more sample, cover fewer genes but better.

Corinne Antignac

(replace Cécile Jeanpierre) – new genes of renal hypodysplasia.

CAKUT congenital abnormalities of the kidneys and the urinary tract. Catch-all term. Affects 1/500 births, but it kills 1/2000 at birth.

Maternal diabetes and malnutrition can act on kidney development. Familial and syndromic forms with very variable expressivity. Htz mutations of *RET* can lead to fetal diagnosis with renal hypodysplasia. The mutation in *RET* does not suffice to explain any given phenotype.

Over 700 patients without known mutation 22 recessive transmission families and 39 families with a dominant form, among which 200 fetal cases. The idea is that the renal phenotype is isolated, not syndromic.

Exome SureSelect 50Mb.

Will discuss 5 families. Among which, FGF20 mutation c.337delG leading to a stop. However, no other mutations identified in 100 fetuses.

Excluded variants based on benign variant in Sift/Polyphen, intronic variant, gene expression profile not relevant… cf GoodMap database for renal expression.

One was ITGA8 and the other ITGA3 (integrin alpha 3 and 8). Were able to confirm that in renal tissue the first one was a splice mutation that led to a deletion of an exon needed for function. The other missense mutation in the ITGA3 was in the interaction domain with alpha chain. Want to do functional studies. No other mutations in the other fetuses.

But all three genes expressed in the kidney. *FGF20* gene is Barak et al., submitted. *Itga8* features in Muller et al., Cell, 1997 and in subsequent papers pointing out stereocilia defects in inner ear. (Osteopontin is its ligand.) And *Itga3* in a KO in Kreidberg et al., Development 1996. Tania thinks maybe should now look in syndromic cases eg. hypoplasia + deafness like in the *Fgf20* mutant mouse. Corinne wonders if there are a lot of less severe cases or perhaps oligogenism.

David Génévième

For C. Collet. Treacher-Collins Franceschetti syndrome = mandibulo-facial dysostoses. Microtia. No RM. No eyelashes, sometimes lower lid coloboma. Inter and intrafamilial variability. *TCOF1* is dominant, and 80% of cases.

Why affect specifically 1er and 2nd arch? Also POLR1D and POLR1C. All needed for ribosomal RNA – needed for proliferation and differentiation of these CN.

Doesn't explain the microtia? Maybe it does – 2nd arch derivatives, but the external ear isn't really a CN thing – but the cartilage is. Sometimes the ear is hardly affected.

Two separate patients with a deletion that also took *CAMK2A*, that had cognitive difficulties. Apparently the mouse also does.

"in the absence of one *Tcof1* allele in the mouse, upregulation of p53-related apoptotic genes in neural crest progenitors leads to severe craniofacial defects (Jones et al., 2008)." Rinon et al 2011

Apparently used antibodies to study expression. POLR1D at C13 not C15 and opposite for POLR1C, ubiquitous; TCOF1 expressed in neural folds and apparently in PA mesenchyme.

Rinon et al 2011 "p53 is expressed in the developing brain and in cranial neural crest (CNC) cells migrating into branchial arches 1-2." *Tcof1* mutations would actually STABILIZE p53. Too much of it would repress cell proliferation. But they must have fewer tumors.

> Question for David at some point, discussed with Patrick Calvas:
>
> Response of Treacher-Collins patient fibroblasts, which may have a protective effect on proliferation mediated by p53, against induced transformation, for example? Patrick says they often die young so can't see if they are kind of protected against carcinogenesis.

B. Bressac-de Paillerets

Corinne Bertolotto and Robert Ballotti in Nice.

1/12/11 480:94-98 perhaps Nature? SUMOylation-defective MITF germline mutation predisposing both to melanoma AND renal carcinoma.

Rare phenotype, 71% males, age of onset 56-58 years. Cf. Eve Maubec et al Cancer 2010 116:5716-24.

MITF isoform M/4 in melanoma, but also in renal cells, part of family including TFE3 and TFEB in juvenile renal cancer. Isoform A. Acts not only on MET but also HIF1A, which is a kidney cancer gene target.

Sequenced isoform 4 in 5 patients finding E318K in heterozygous state, germline. Also found in the general population at 0.003 frequency (10 out of 1649).

Data here: http://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-1198

Found mutations in melanoma alone and renal carcinoma alone. Familial melanoma co-segregates. One cousin with an ocular melanoma had the WT allele.

Cf schematic in *Teri Manolio et al Nature 2009* and *Bertolotto C Nature 2011* and *Yokoyama S Nature 2011*. For the distribution of strong-weak effect on Y axis and rare-frequent on X axis.

E318K is located +2 of a SUMO acceptor lysine. In WB showed defect in HEK293 cells.

The mutant is actually more efficient in activating some of its target promoters, eg *HIF1A* and melanoblast *ABCB5*, but not so much or at all for *MET*, *TYR* or ***p16INK4A***.

Binds to more sites in the genome, though. Upreg of *CCR7* and *GADD45G* transcripts in addition to *ABCB5*.

Higher migration, invasion and clonogenicity phenotypes.

Because this is a rare variant perhaps it was eliminated in our cases because present in the databases?

In accompanying paper the English/Australian group sees **high nevus count**. This group hasn't looked. Neither group has examined *MC1R* status (which I find a little surprising for the other group. Need to read the papers.)

Falchi et al 2009 NG

"Linkage to 9p21 has been found in two genome-wide analyses of nevus count, suggesting that shared genetic factors might be involved in melanoma susceptibility and nevogenesis." "We identified strongly associated variants in *MTAP*, a gene adjacent to the familial melanoma susceptibility locus *CDKN2A* on 9p21 (rs4636294) and *PLA2G6* on 22q13.1 (rs2284063)"

(I asked question during this talk.)

E. Pasmant - U745 unit.

BDD *NF1* cf genotype by Dominique Vidaud at Cochin and for the phenotype, Pr Wolkenstein at the Ctr de Reference Maladies Rares at Henri Mondor.

Modifying genes specifically for plexiform neurofibromas. Made primary cultures of Schwann cells. 22 neurofibromas had deletions of 9p21.3 locus **INK4** with ARF-ANRIL-CDKN2A/B.

Have a look at our NF1 patient?! (her only CNV is on 9p21: 44362584-44770712 which is quite far away.) **The MTAP gene can be fused to ANRIL on 9p21** cf Falchi et al 2009, removing **p16INK4A.**

Remember that **beta-catenin usually suppresses p16INK4A cf Delmas et al., 2007.**

Did a GWAS highly linked to a SNP within ANRIL. More NFs. Less transcript. SNP rs2151280.

Pasmant E JNCI 2011 or so.

Also look at *SUF12* which is often rearranged just next to *NF1*. Part of Polycomb group. This is on chr 17. Not implicated either.

Questions:

Cardiovascular papers on *ANRIL*. But it's not the same paper. More associated with basocellular carcinoma. Is the SNP associated with other kinds of neurofibromas? Only number of plexiforms. No association with cutaneous NFs or age of onset.

C Vincent-Delorme

Microduplications of most frequently 3 Mb between recombination points 2-3 or 2-4 in the DiGeorge critical region. Triangular like ears a bit like CHARGE ("mal ourlée") sometimes. Slightly dysmorphic, heavy but not bushy eyebrows. Slightly bifid tip of nose. Not so many cardiopathies. Definite mild to moderate mental retardation. Referenced a paper from 2007 in which a 5' variant in *TBX1* led to an effective double dose (duplication like) and this was in a patient with MR.

Laurence Colleaux

Presents the *MED23* paper about the Mediator complex which comprises a kinase, CDK8. One mutation has opposing effects on *JUN* as on *FOS*. And surprised at precision of the phenotype in something so ubiquitous.

Transcription initiation by RNA pol II passes through a step of chromatin remodeling, which assembles TFII1, B, D, E, F, H cf Malik and Roeder 2010 for a review.

JUN-P and AP1 normal binding but mutation brings ELK3 instead of ELK1 to the promoter of FOS. Leads to over-expression.

Isolated mental retardation. Get specific effects because of more tissue-specific

MED12 mutant patient – MR and dysmorphie, corpus callosum agenesis, etc. Get similar JUN/FOS response but deregulation of response by RAR. Perhaps more genes are deregulated.

Similar effect also on XPD mutant (subunit of one of the TFII protein bits). However XPC (TFIIH) other disease and in those cells, no effect on FOS and JUN, necessary for the formation of synapses (regulation of "immediate-early genes").

David Génévième

Kabuki syndrome presentation. Described in 1980. 700 patients now described. AD with mutations *de novo*. *MLL2* and *KDM6A*.

Diagnostic based on face and extremities.

MLL2 is a H3K4 methyltransferase. The other one used to be known as *UTX*. They interact and are implicated in regulating *HOX* genes and *BMP2*.

Poster 170 presented by *S Nassereddine* shows a 17 year old girl with **right ventricle double chamber** (not double outlet).

Lee et al., 2007 Science "during retinoic acid signaling events, the recruitment of the UTX complex to *HOX* genes results in H3K27 demethylation and a concomitant methylation of H3K4."

Extra symptoms: 47% cardiac defects, 38% renal anomalies, and 31% palate cleft but much fewer lip clefting. About ¼ has immune defects.

A couple patients have de novo splice defects in MLL2, leading to an anomalous copy.

No easy genotype-phenotype correlation. The KDM6A patients seem to have a longer first digit of the foot (the thumb).

Cf. Miller et al., 2008 about potential effects on the action of *TBX* gene targets. Without MLL2 the positive mark could not be made to permit transcription of targets.

"In this study, we define two overlapping, but physically separable regions within the conserved T-box DNA-binding domain that are required for the coordinated interaction with enzymatic activities that both remove nonpermissive H3K27me2/3 modifications and establish the permissive H3K4me2 epigenetic state. A mutagenesis analysis examining the T-box domain demonstrated the essential nature for these epigenetic-mediated events because mutations associated with several different T-box protein-dependent human developmental genetic diseases, when placed within the context of T-bet, abolish these activities and the functional induction of target gene expression."

(Saturday morning)

Eric Streichemberger

from Mike Mitchell's group on novel gene in Lebanese family.

Their OZF1 is expressed in the testis. Mouse homologue also, upregulated during spermatocyte-generating stages – impact on meiosis but essentially cytoplasmic. ID'd mutations using homozygosity-mapped region and then large scale sequencing.

H. Cavé

Check out http://ras-pathway-syndromes.com/

GM-CSF stimulates RAS-MAPK pathway via its receptor: SHP2 repressor (most), NF1 repressor 4% usually somatic activating but also in Noonan syndrome. 35% Ras GTP

LOH in 11q: acquired disomie uniparental. *CBL* candidate gene showed hmz missense or splice. Loh et al 2009. Recurrent mutations.

Germline mutations cf Perez -similar to Noonan syndrome. CALMs, microcephaly. Htz mutation in th syndrome. Can be present in asymptomatic carriers. Cf. Niemeyer Nat Genet: 2010. Only point mutations have been described at germinal region.

Juvenile MM leukemia v rare, strikes <2 years. Look at other RASopathies: 0/7 NF1- and SPRED1-patients; 2/214 NS and PTPN11- Noonan patients and two other studies Martinelli et al Am J Hum Genet 2010 and unpublished results mean approx 1% of NS patients actually have *CBL* mutations.

Molecular adaptor with a ubiquitin ligase E3 catalytic domain. Interactome published Schmidt and Dikic 2005 (Nat Reviews Mol Cell Biol). (Same authors wrote Bioessays. 2010 Jun;32(6):481-7. "Notch: Implications of endogenous inhibitors for therapy.")

Hotspot mutations.

Pérez et al Br J Haematol 2010 : when assemble all activities Syndromic JMML with mutated parents (eg. they don't themselves have a leukemia) Y368 this tyrosine needed in particular and hotspot. No hotspot seen in the more Noonan-like CBL syndrome. ¼ cardiac defects, as much MR, cryptorchidism in boys.

Mice and humans have lymphocyte phenotype perhaps disposing toward auto-immune reactions eg. arteritis.

All transforming variants lose their E3 activity – abolishing RTK endocytosis and proteosomal degradation. And since the mutated allele is always the one that is retained, and there is a phenotype of the htz mutation, there is probably an active function – cf Kales et al Cancer Research 2010. Without the catalytic activity still activates for example the PI3K pathway.

Work in collaboration with Marco Tartaglia (speaker from Alain Verloes's group at Robert Debré).

Therapeutic basis for treating the leukemia by reducing the RTK transmission? But this is the initiating event. Rapamycin has already been tried because authorized in children.

Alexandre Buffet

(HEGP) Paragangliomas and pheochromocytomas. 10% malignant (defined by metastatic).

Somatic mutations *RET*, *NEM2*, *NF1*. …*VHL TMEM127*, *MAX*, *SHDA/B/C/D*, *SDHAF2* as constitutional germline mutations.

Diagnosis paraganglioma in ear. Familial notion in about 8% patients. Mean age of diagnosis is 45+/-17 years.

Test sporadic as well as familial cases. Sporadic less likely to have mutation in predisposing gene (most of cohort mutated in *SDHB*, *SDHD* (37, 25%) and *VHL*.

Collaborate with Pierre Rustin to do biochemical analysis of succinate dehydrogenase activity. *SDHD* almost always multiple tumors and in head/neck, contrary to *SHDB* where majority single and all over body (also 2/3 of the sporadic paragangliomas). For *RET*, 2/3 had multiple and all were syndromic with always pheochromocytomas. *TMEM127* older patients, mostly single, also all PC.

These observations helped design a decisional tree for which analyses to conduct first.

NF1 and *RET* mutant PCs mostly secrete urinary metanephrines. The *SDHBD* and *VHL* mostly normetanephrines in the urine instead.

Franck Bourdeaut

Beckwith pathologist described rhabdoid tumors in the kidney and then seen in other CNS aggressive tumors: ATRT ("atypical") – variable localizations. Quite early onset, around 20 months. Biallelic inactivations or deletion of SMARCB1 on chr22 (aka BAF27, SNF5). Olivier Delattre's group also has seen germline mutations on occasion families (across one generation). *Sévenet et al Am J Hum Genet 1999*.

When germline mutation, diagnosis very early: 6 months. Two families over two years – also very early onset. Three other families addressed from abroad. Same. But second child in a Spanish family had a tumor at 6 yo and a second British one where 2nd kid onset at 7.5 years. Carry splice mutation from asymptomatic mother. But penetrance is quite strong and early, overall. Still, on an individual basis worth testing for in later onset.

Determining characteristic of prognostic is really age of onset. No missense mutations.

Michel Goossens wonders about the original cells? "Stemness" markers. Wonders if the rhabdoid characteristic is not something that can't also be acquired in carcinoma or chondrosarcomas.

SMARCB1 responsible pour familial schwannomatosis. Have seen in literature 4-5 families with both schwannomas and rhabdoid tumors. In their cohort, have none. Perhaps because they are mutations in exon 1, splice tumors and missense mutations for the familial schwannomas.

F. Caux

Hospital Avicenne

Sebacious hyperplasia in *MUTYH* mutant – also colic polyposis, and then 6 patients / 10 develop a colorectal cancer. 7 patients have stop or missense mutations that are 7 patients; 3 patients composite htz. Treat with isotretinoine "Accutane" (now Curacne) – 3 months, low dose treatments, clear these up. Cutaneous lesions late onset. 4 melanomas, 8 adenomas, other lesions.

Often labeled Muir-Torre but not correct – not sebaceous adenomas only

Get sebaceous hyperplasias in people having been grafted and are treated with immunosuppressors. Age or immunodepression permit these bumps to develop.

Do htz patients have cutaneous signs? Has been reported with melanoma in the literature. Need to supervise the skin of biallelic mutants normally. Prevalence not really well known. Perhaps around 1% with late onset. Look for mutations in the younger patients because

Vitamin A treatment is oral – does it affect colic problems? Parallel to *PTEN* – similar treatment, and for Cowden syndrome if you look at number of polyps after treatment after 1-2 years, not easy to make a comparison – would need to make a tattoo in order to photo the same zone to be able to evaluate.

John Boudjarane

La Timone - cytogenetics

11q13.3-11q23.3 interstitial deletion in SMD and SMP – dysmegakarocytopoiesis frequent.

Syndromic Myelodysplasias (SMD). Sometimes include *ATM* and *MLL*, sometimes only *MLL*, critical zone appears to be between *MLL* and *ZNF259*, a zone of about 1.5 Mb with 50 genes therein. Past *MLL* there is the gene *CBL* (see Mme Cavé's presentation above) which in other SMDs (7 of their 50-odd) is comprised in the deletion and the other allele is mutated.

CBL mutations have been described in other syndromes – some – are they really deleterious? But in the same domain impacting the activity of E3 ubiquitin ligase. In the mutants for CBL there are no platelet problems whereas in the non-mutated ones, 44% show a thrombopenia. Past CBL there is FLI1 but they have no evidence for its implication.

The RaDiCo project – Serge Amselem

– presentation same as available online currently here, from March 2011.

As someone remarked to me, wouldn't the government have invested its money better by conferring the same mission to Orphanet? But it's done, and now there are three structures with overlapping missions that are each just somewhat underfunded perhaps (Institut Maladies Rares and Orphanet being the other two).

131 Centres de reference Maladies Rares and 501 centres de competences. LCMN would depend on the fourth "filière" of "Maladies dermatologiques rares" (there also exist #2, Maladies Cardiovasculaires, and #17, Maladies rares de la tête et du cou, and #18 which is a catch-all but includes spina bifida).

The person in charge of the groupe de travail registres is **Dr J Donadieu, Service d'hémato oncologie pédiatrique, Hôpital Trousseau 75012 Paris, Tel 01 44 73 53 14, Mail : jean.donadieu@trs.aphp.fr**. (off the Internet). For the système d'information médicale the people on the working group were Christophe Béroud, M.-C. Jaulent and Paul Landais. It's called ISy-rare. (Orphanet : "La deuxième réunion du Comité de suivi et de prospective du deuxième plan national maladies rares 2011-2014 a eu lieu Mardi 24 janvier au ministère de la Santé. […]Le Pr. Véronique Paquis a ainsi précisé les financements mis en œuvre dans le cadre des investissements d'avenir (64 M€ pour l'Institut hospitalo-universitaire « maladies génétiques » à l'hôpital Necker, 27 M€ pour les infrastructures nationales en biologie et santé (projet Phenomin), 20 M€ pour un démonstrateur préindustriel en biotechnologie (projet PGT Généthon-Inserm), et 10 M€ pour le projet de cohorte RADICO (RAre DIsease COhort), porté par le Pr. Serge Amselem (Inserm). Celui-ci a indiqué qu'il pensait mettre en œuvre un Système d'Informations partagées (ISy Rare) de données avec le Pr. Paul Landais, en lien avec la Fondation de recherche maladies rares dont le Pr. Nicolas Lévy a annoncé la création imminente et les principaux axes de travail. Nous aurons l'occasion d'y revenir."

Objectives include defining the “phenomics” of a disease, among other more far-reaching ambitions. All these cohorts will undergo prospective followup and not be retrospective.

Ambition to link clinical databases with imagery and biological information. Also to support research programs, including assisting in making iPS cells from as many rare diseases as possible.

The scientific advisory board will only consider making cohorts for certain diseases – all, is impossible.

Even so, the reference centers will provide only the most minimal data to RaDiCo, to enable this prioritization for more detailed phenotypes. 10 floating clinical research assistants will aid with data entry at given centers.

There exists a kind of federation of people representing these cohort projects, if I understand right. They are all facing the same sorts of issues about housing the databases, making commercial use of the data, establishing contracts with the hospitals involved, and reglementary issues with the CNIL, CCTIRS and CPPs.