Results and conclusion: Continuous passage of K562 cells in suspension demonstrated presence of a small fraction of K562 cells (5% + 2.1%) to be adherent to culture plastic ware (K562/Adh). BCR-ABL gene amplification was present in K562 cells (approx 13 fusion signals per cell) by FISH, with no significant difference in copy number between K562/Adh and K562/NonAdh (p=0.822). qPCR analysis of genomic DNA from K562/Adh and K562/NonAdh showed an increase in BCR-ABL gene copy numbers in both relative to the Jurkat cells, with no statistical significance (p=0.117). RT-qPCR showed upregulation of BCR-ABL mRNA in K562/Adh cells compared to K562/NonAdh in both single and bulk cells (p<0.0001). PLA and flow cytometric assays displayed higher expression of phospho-BCR-ABL protein in K562/Adh cells than K562/NonAdh cells. These results demonstrate, at both the single and bulk cell level, existence of an adherent subpopulation of K562 cells with higher level of heterogeneity in mRNA and protein expression of BCR-ABL which are resistant to Imatinib, suggesting the possibility that a similar subpopulation of cells in CML may cause clinical resistance.

P4.18

Association of the A1330V polymorphism of the low-density lipoprotein receptor-related protein 5 gene with bone mineral density and fracture risk in Maltese postmenopausal women

M. Formosa, A. Xuereb Anastasi
Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida

Introduction: The Low-density lipoprotein receptor-related protein 5 (LRP5) is involved in osteoblast differentiation, making it an important determinant of bone mass and strength. The single nucleotide polymorphism, SNP (C>T) at position 1330 in exon 18 results in a missense substitution of alanine to valine (A1330V) which has been associated with low bone mineral density (BMD) and increased fracture risk.

Aim: To investigate the influence of the A1330V polymorphism on BMD and different low-trauma fractures in Maltese postmenopausal women.

Methodology: 1043 women between 40 and 79 years were recruited and their BMD measured by dual-energy X-ray absorptiometry. Subjects without a history of a fragility fracture were subdivided in three groups: normal (n=223), osteopenic (n=271), and osteoporotic (n=282) according to their BMD. The remaining 267 were fracture cases who had a normal (n=12), osteopenic (n=107) or osteoporotic BMD (n=148). Genotyping was performed by polymerase-chain reaction and restriction enzyme digest.

Results: The genotype distributions were as follows: normal controls CC (78%), CT (21%), TT(1%); osteopenic subjects CC (78%), CT (20%), TT (2%); osteoporotic subjects CC (68%), CT (28%), TT (4%); and fracture cases CC (69%), CT (27%), TT (4%). In the total study group, the A1330V SNP was associated with reduced lumbar spine BMD (TT: age-adjusted Odds ratio, OR 3.7 [95% Confidence Interval 1.2-11.0], p=0.02; CT: OR 1.6 [1.1-2.4], p=0.01) and to a lesser extent reduced femoral neck BMD (TT: OR 2.8 [0.9-8.7], p=0.07; CT: OR 1.7 [1.2-2.6], p=0.01). Women without a fracture history had an increased risk of osteoporosis when carrying one or both copies of the minor allele T (CT: OR 1.6 [1.0-2.4], p=0.04; TT: OR 10.6 [1.4-80.5], p=0.03). No significant difference was observed between osteopenic subjects (without fractures) and normal controls. When comparing fracture cases to normal controls, women carrying CT/TT genotypes had an increased fracture risk than women with the CC genotype (CT: 1.6 [1.0-2.4], p=0.05; TT: OR of 8.3 [1.0-67.0], p=0.05). All fracture cases homozygous for the T allele had an osteopenic or osteoporotic BMD; fracture risk was partly attenuated by BMD adjustment (TT: OR 2.5 [0.2-27.7], p=0.45) and remained unchanged in carriers of the A1330V SNP (CT: OR 1.4 [0.7-2.8] p=0.31). The TT genotype was the most common among subjects with a wrist, humerus or hip fracture; however the difference was not significant (p>0.05).

Conclusion: The results indicate that the A1330V polymorphism is associated with reduced BMD and increased fracture susceptibility in Maltese postmenopausal women.

P4.19

Inflammatory cytokines in maternal circulation and placenta of chromosomally abnormal first trimester miscarriages

J. Calleja-Agius[1], E. Jauniaux[2], Sanu. Muttukrishna[3]
[1]Department of Obstetrics and Gynaecology, Mater Dei Hospital, Msida; Department of Anatomy, University of Malta, Msida, [2]Institute for Women's Health, University College London, London, [3]Anu Research Centre, UCC Department of Obstetrics and Gynaecology, Cork University Maternity Hospital, Cork

The impact of abnormal placental karyotype on the inflammatory response within the villous tissue and peripheral circulation of women with miscarriage was evaluated. Villous (n=38) and venous blood samples (n=26) were obtained from women with missed miscarriage. Tissue chromosome analysis indicated 23 abnormal and 15 normal karyotypes. Concentration of tumour necrosis factor alpha (TNFα), TNF-R1 and TNF-R2, and interleukin (IL)-10 were measured using flow cytometric bead array in fresh villous homogenate, cultured villous extracts, culture medium, maternal whole blood, and plasma. Plasma TNFα/IL-10 ratios were significantly (p<0.05) lower in miscarriages with abnormal karyotype. In the abnormal karyotype group, there were significantly higher levels of TNFα (p<0.01), IL-10 (p<0.01), TNF-R1 (p<0.001), and TNF-R2 (p<0.001) in the villous extracts and culture-conditioned medium compared to normal karyotype group. In miscarriage with abnormal karyotype, there is an exacerbated placental inflammatory response, in contrast to miscarriage of normal karyotype where maternal systemic response is increased. Our data illustrate that the mechanisms leading to amiscarriage may depend on the karyotype of the conceptus. We suggest that there is a local functional disturbance in the karyotypically abnormal placental tissue while, in the case of a normal karyotype miscarriage, rejection occurs due to a maternal systemic inflammation.

P5.01

Audit of suspected breast cancer referrals to breast clinic

J. Vassallo, N. Cassar, D. Micallef, D. Gamoudi

Introduction: The latest NICE guidelines published in April 2011, set standards about features suggestive of possible breast malignancy and referral timelines for different breast complaints. It divides referrals into urgent and non-urgent; urgent cases should be seen within two weeks of referral. In Malta, patients presenting with breast problems are usually referred to the breast clinic, which has been set up more than ten years ago to provide specialist surgical assessment of such patients.

Aim: The primary aim of this audit was to assess whether referrals for suspected breast cancer were in line with the referral guidelines issued by the NICE in April 2011.

Methodology: 113 random referral tickets of women reviewed at the breast clinic between July and December 2011 were analysed.

Results: Two of the patients referred were male and the mean age of the sample was 50.5 years. 60.2% of suspected breast cancer referrals were found to have a history or examination findings indicative of possible breast malignancy. Of these, 4 patients (6.7%) were seen within the