

#3094 Rapid and accurate determination of CAG repeat lengths in androgen receptor gene using PCR and automated fragment analysis. Bharaj, B.S., Vassilikos, E., & Diamandis, E.P. *Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada, M5G 1X5.*

The androgen receptor (AR) gene contains two polymorphic trinucleotide segments that code for polyglutamine (CAG) and polyglycine (CGG) tracts in the N-terminal domain of the AR in exon 1. Variation in the lengths of these repeats have been associated with an increased risk of pathological conditions such as prostate cancer, low virilisation, oligospermia, azoospermia, testicular atrophy and are also important features of neurological diseases such as Huntington's disease and spinal muscular bulbular atrophy. These polymorphisms can therefore, act as genetic markers. However, the potential use of microsatellites as genetic markers has been severely hampered by practical problems associated with the means of detection. In this paper we have improved the methodology for determining the lengths of CAG repeats. The method is based on optimising the PCR using Cy5.5 fluorescently labelled primers. The PCR product is then run on a sequencing gel on a newly developed automated DNA sequencer with appropriate molecular weight markers. Analysis of 18 DNAs isolated from normal males indicated that they were all hemizygous, as expected. CAG repeat lengths ranged from 20-30 with a mean of 22 and a median of 21. Among 60 normal females 27% were homozygotes with repeat lengths which ranged from 19-25 (a mean of 22 and a median of 22) and 73% were heterozygotes with repeat lengths ranging from 17-30 (with a mean of 22 and a median of 21). Both males and females show a bimodal distribution of CAG repeat lengths. These data demonstrate that the new method has the capability to provide accurate, precise and reproducible results in a fraction of time as compared to conventional methods.